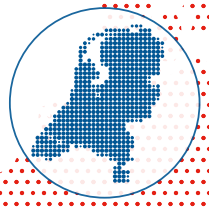
NethMap 2023

Consumption of antimicrobial agents and antimicrobial resistance among medically important bacteria in the Netherlands



National Institute for Public Health and the Environment
Ministry of Health, Welfare and Sport

SWAB Stichting Werkgroep Antibioticabeleid

NethMap 2023

Consumption of antimicrobial agents and
antimicrobial resistance
among medically important bacteria
in the Netherlands
in 2022

November 2023

Synopsis

NethMap/MARAN report

Every year for different types of bacteria is determined which percentage is resistant. The latter means that a bacterium has become insensitive to antibiotics. In the Netherlands, the percentage of resistant bacteria in 2022 was around the same as in 2021. For some types of bacteria, the resistance percentage has dropped slightly over the past five years. On the other hand, the resistance percentage for other types of bacteria has increased over the same period. These bacteria often cause mild infections, including skin infections.

The number of antibiotic prescriptions issued in the Netherlands fell in 2020 and 2021. The most likely reason for this is that fewer people consulted a hospital or their GP due to the coronavirus pandemic. This effect of the pandemic on antibiotic use is on the wane. In 2022, GPs and hospitals prescribed more antibiotics than during the coronavirus pandemic. In hospitals, antibiotic use was roughly the same as in the years before the pandemic, while GPs prescribed them slightly less often than before the pandemic. Furthermore, hospitals and nursing homes reported more outbreaks of infections with resistant bacteria than during the coronavirus pandemic, but the number of outbreaks was still lower than in the years before the pandemic.

Despite the stable figures, resistant bacteria require constant vigilance. Particularly, people who have spent time in a hospital abroad may be carrying bacteria that have become resistant against multiple antibiotics simultaneously. Adequate measures, such as thorough hand washing and other hygiene measures, are needed to prevent these bacteria from spreading. In addition, incorrect and unnecessary antibiotic use should be prevented as much as possible ('antimicrobial stewardship').

The measures that have already been taken in the Netherlands to combat antimicrobial resistance go beyond human healthcare (one health). This is because resistant bacteria are also carried by animals and are present in food and the environment.

Since 2009, the resistance in gut bacteria found in pigs, cows and chickens kept for food production (livestock) has steadily decreased. This is in line with the decreasing use of antibiotics for livestock. In 2022, fewer antibiotics for all kinds of animals were sold than in 2021. Compared to the baseline year of 2009, sales have fallen by more than 77 per cent. Nowadays, antibiotics that are crucial for the treatment of infections in humans are only used for livestock (and other kinds of animals) in extremely rare cases.

These are the findings of the annual report NethMap/MARAN 2023. In this report, a number of organisations present data about antibiotic use and antimicrobial resistance in the Netherlands for both humans and animals.

Keywords: one health, AMR, antimicrobial stewardship, antibiotic use, bacteria, infection

Publiekssamenvatting

NethMap/MARAN-rapport

Elk jaar wordt per bacteriesoort geteld welk percentage resistent is. Een bacterie is dan ongevoelig voor antibiotica. In Nederland was in 2022 het deel van de resistente bacteriën ongeveer even groot als in 2021. Bij sommige bacteriesoorten kwam resistentie in de afgelopen 5 jaar iets minder vaak voor. Toch zijn enkele soorten bacteriën vaker resistent dan vijf jaar geleden. Deze bacteriën veroorzaken vaak milde infecties van onder andere de huid.

In 2020 en 2021 zijn in Nederland minder antibiotica voorgeschreven. Dat kwam waarschijnlijk doordat er door de coronapandemie minder mensen in het ziekenhuis lagen of bij de huisarts kwamen. Dit effect van de pandemie op het gebruik van antibiotica begint af te nemen. In 2022 hebben huisartsen en ziekenhuizen meer antibiotica voorgeschreven dan tijdens de coronajaren. In ziekenhuizen was het gebruik ongeveer hetzelfde als in de jaren vóór de pandemie; huisartsen schreven ze iets minder vaak voor. Verder zijn er in ziekenhuizen en verpleeghuizen weer meer uitbraken gemeld van infecties door resistente bacteriën dan in de coronajaren. Maar dit aantal is nog altijd lager dan in de jaren vóór de pandemie.

Ondanks de stabiele percentages blijft alertheid op resistente bacteriën nodig. Vooral mensen die in het buitenland in het ziekenhuis hebben gelegen, kunnen bacteriën bij zich dragen die resistent zijn tegen verschillende antibiotica tegelijk. Goede maatregelen om infecties te voorkomen, zoals handen wassen en andere hygiënemaatregelen, zijn nodig om te voorkomen dat deze bacteriën zich verspreiden. Ook moet onjuist en onnodig gebruik van antibiotica zo veel mogelijk worden voorkomen (*antimicrobial stewardship*).

De maatregelen die nu al in Nederland zijn genomen om antibioticaresistentie te bestrijden, reiken verder dan de gezondheidszorg bij mensen (one health). Resistente bacteriën komen namelijk ook voor bij dieren, in voeding en in het milieu.

Sinds 2009 worden steeds minder antibioticaresistente darmbacteriën gevonden bij varkens, koeien en kippen die voor de voedselproductie worden gehouden (landbouwhuisdieren). Dat past bij het nog steeds verder afnemende gebruik van antibiotica bij landbouwhuisdieren. In 2022 zijn ook minder antibiotica voor álle diersoorten verkocht dan in 2021. Ten opzichte van 2009, het referentiejaar, is de verkoop met meer dan 77 procent gedaald. De antibiotica die cruciaal zijn om infecties bij de mens te behandelen, worden alleen nog bij hoge uitzondering gebruikt voor (landbouw)huisdieren.

Dit blijkt uit de jaarlijkse rapportage NethMap/MARAN 2023. Hierin presenteren diverse organisaties samen de gegevens over het antibioticagebruik en -resistentie in Nederland, voor mensen en dieren.

Kernwoorden: one health, ABR, antimicrobial stewardship, antibioticagebruik, bacteriën, infectie

Colophon

This report is published under the acronym NethMap by the SWAB, the Dutch Foundation of the Working Party on Antibiotic Policy, in collaboration with the Centre for Infectious disease control (CIb) of the RIVM, the National Institute for Public Health and the Environment of the Netherlands. SWAB is fully supported by a structural grant from CIb, on behalf of the Ministry of Health, Welfare and Sports of the Netherlands. The information presented in NethMap is based on data from ongoing surveillance systems on the use of antimicrobial agents in human medicine and on the prevalence of resistance to relevant antimicrobial agents among medically important bacteria isolated from healthy individuals and patients in the community and from hospitalized patients.

NethMap can be ordered from the SWAB secretariat, c/o Secretariaat SWAB p/a Leids Universitair Medisch Centrum (LUMC), afdeling Infectieziekten C5-P t.a.v. SWAB, Postbus 9600, 2300 RC Leiden or by email to secretariaat@swab.nl.
NethMap 2023 and earlier versions are also available from the website of the SWAB: www.swab.nl and www.rivm.nl.

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Centres contributing to the surveillance of antibiotic consumption
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Contents

1 Introduction

This is NethMap 2023, the SWAB/RIVM report on the use of antibiotics, trends in antimicrobial resistance and antimicrobial stewardship programmes in the Netherlands in 2022 and previous years. NethMap is a cooperative effort of the Dutch Working Group on Antibiotic Policy (SWAB; Stichting Werkgroep Antibiotica Beleid) and the Centre for Infectious Disease Control Netherlands (CIb) at the National Institute for Public Health and the Environment (RIVM). NethMap is issued back-to-back together with MARAN, reporting on trends in antimicrobial resistance and antimicrobial use in animal husbandry.

In 1996, SWAB was founded as an initiative of The Netherlands Society for Infectious Diseases, The Netherlands Society of Hospital Pharmacists and The Netherlands Society for Medical Microbiology. SWAB is fully funded by a structural grant from the CIb, on behalf of the Ministry of Health, Welfare and Sports. The major aim of the SWAB is to contribute to the containment of the development of antimicrobial resistance and provide guidelines for optimal use of antibiotics, taking into account results obtained from resistance surveillance and antibiotic use surveillance. Based on the national AMR surveillance system (ISIS-AR) performed by the CIb-RIVM, trends in antimicrobial resistance are monitored using routine antibiotic susceptibility testing data from microbiology laboratories in the Netherlands. Furthermore, the CIb subsidizes surveillance programs that focus on the monitoring of specific pathogens, or even specific resistance mechanisms. Finally, the CIb coordinates the Early warning and response meeting of Healthcare associated Infections and AntiMicrobial Resistance (SO-ZI/AMR) which aims to mitigate large-scale outbreaks of AMR in hospitals and longterm care facilities and to prevent spread to other healthcare facilities through early warning and reporting. Together these constitute the basis of the surveillance of resistance reported in NethMap and are used by CIb to monitor and inform the general public, professionals and policy makers about potential national health threats with regard to antimicrobial resistance.

NethMap 2023 extends and updates the information of the annual reports since 2003. Each year, we try to further improve and highlight the most important trends. The trends and developments in highly resistant microorganisms (HRMOs) receives attention in separate chapters. The reader is encouraged to visit www.isis-web.nl for tailored overviews of resistance development. Likewise, the Antimicrobial Stewardship Monitor program is gaining footage in an increasing number of hospitals that participates, making the results and conclusions more robust.

The pandemic of COVID-19 which started in 2020 had a major impact on healthcare systems and could therefore also influence, both on the shorter and the longer term, antimicrobial use and resistance; this warrants extra vigilance and analyses of data from the various AMR surveillance systems. We report on this in the present and coming NethMap reports and – if relevant – in separate reports and/or (scientific) papers.

NethMap parallels the monitoring system of antimicrobial resistance and antibiotic usage in animals in The Netherlands, entitled MARAN – Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in The Netherlands. Jointly, NethMap and MARAN provide a comprehensive overview of antibiotic usage and resistance trends in the Netherlands in humans and in animal husbandry and therefore offer insight into the ecological pressure associated with emerging resistance.

We believe NethMap/MARAN continues to contribute to our knowledge and awareness regarding the use of antibiotics and the resistance problems that are present and may arise in the future. We especially thank all those who are contributing to the surveillance efforts, and express our hope that they are willing to continue their important clinical and scientific support to NethMap/MARAN and thereby contribute to the general benefit and health of the people.

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2 Extensive summary

This chapter provides a summary of the findings described in this report and relevant conclusions with respect to antimicrobial use, policy and resistance surveillance in both humans (NethMap 2023) and the veterinary sector (MARAN 2023). The pandemic of COVID-19 had a major impact on healthcare systems in 2020 and 2021 and could therefore also influence, both on the shorter and the longer term, antimicrobial use and resistance; this warrants extra vigilance and analyses of data from the various AMR surveillance systems.

2.1 Most important trends in antimicrobial use

Outpatient use

Total outpatient use of antibiotics increased from 7.61 DDD/1,000 inhabitant days (DID) in 2021 to 8.32 DID in 2022. As compared to 2019 (pre-COVID) total outpatient use is slightly lower. During the years 2020 and 2021 outpatient antibiotic use may have been lower as a result of less health seeking behavior and the measures taken to prevent the spread of the virus. There is an overarching downward trend in outpatient antibiotic use in the Netherlands.

The consumption of β-lactamase sensitive penicillins and β-lactamase resistant penicillins increased over the past years, whereas use of tetracyclines decreased. Macrolides make up 14% of total consumption.

Inpatient use

2021

The inpatient use of systemic antibiotics decreased (-5.4%) from 85.8 to 81.1 DDD/100 patient-days compared to 2020. Total inpatient use of systemic antibiotics, expressed as DDD/100 admissions, decreased to 303.7 (-8.8%). In parallel, total use of systemic antibiotics, when calculated as DDD/1,000 inhabitant-days (DID), decreased from 0.760 in 2020 to 0.700 in 2021 (-7.9%).

2022

Compared to 2021, inpatient use of systemic antibiotics increased by 13.5% to 92.1 DDD/100 patient-days. Expressed as DDD/100 admissions, total inpatient use of systemic antibiotics increased to 338.1 in 2022 (+11.3%). In line, when calculated as DDD/1,000 inhabitant-days (DID), total use of antibiotics for systemic use increased to 0.747 (+6.7%).

In 2022 the use of most antibiotics returned to pre-COVID levels. With some notable exceptions:

- flucloxacillin: 36% higher as compared to 2019;
- meropenem: usage has increased year after year;
- vancomycin: upward trend for several years;
- aminoglycoside: decreased every year.

The downward trend of overall antibiotic use in hospitals continued (0.747 DID) in 2022. The same applies to the consistent upward antibiotic usage trend when evaluated per patient and use per bed-day. The most likely explanation is that less patients are being treated with antibiotics. However, there has been an intensification of treatment for individual patients who are receiving antibiotics.

Long-term care facilities (LTCFs)

The antibiotic use in LTCFs decreased from 50.4 to 38.1 DDD/1,000 residents/day in 2021 compared to 2020. In 2022, this recovered to 43.9 DDD/1,000 residents/day. Most common used antibiotics are as before the combination of penicillins (amoxicillin with clavulanic acid), nitrofurantoin and fluoroquinolones.

2.2 Most important trends in antimicrobial resistance and implications for treatment

In the Netherlands, in the Infectious disease Surveillance Information System on Antibiotic Resistance (ISIS-AR), antimicrobial resistance is monitored for a wide range of pathogens in different settings. In addition, a number of surveillance programs exist that focus on the monitoring of specific pathogens, or even specific resistance mechanisms. These programs often include confirmation and susceptibility testing of important resistance mechanisms and molecular typing in national reference laboratories. In Table 2.2.1 an overview is provided of surveillance programs that are included in NethMap 2023.

Table 2.2.1 Overview of antimicrobial resistance surveillance programs included in NethMap 2023

Surveillance program	Origin of isolates	Availability	Sources 2022	Central or decentral susceptibility testing	Method of susceptibility testing
Surveillance program aimed at resistance surveillance in a wide range of pathogens					
ISIS-AR	GP, Hospital, LTCF	2008-	47 laboratories	Decentral testing	Various methods used in routine susceptibility testing
Surveillance programs aimed at resistance surveillance in specific pathogens					
Neisseria meningitidis	Hospital	1994-	Nationwide	Central testing	Gradient testing
Neisseria gonorrhoeae	SHC	2006-	16 out of 24 SHC	Decentral testing	Gradient testing
Mycobacterium tuberculosis	General population	1993-	Nationwide	Primarily central testing	Whole genome sequencing, additional phenotypic testing
Influenza antiviral drugs	Community, GP, LTCF, hospital	2005-	NIVEL GP sentinels, SNIV LTCF sentinels, hospital/ regional laboratories	Central testing (RIVM, NIC-ErasmusMC, WHO-CC London)	Whole genome Nanopore sequencing, or site-specific PCR; Neuraminidase enzyme inhibition assay
HIV	Hospital	2003-	Nationwide	Decentral testing	Sequencing with viral mutation characterization for reverse transcriptase, protease, or integrase resistance-associated mutations
Resistance among anaerobic pathogens	Hospital	2010-	UMCG (since 2010) and 8 laboratories (2021)	Central testing	Gradient testing, agar dilution, whole genome sequencing
Clostridioides difficile	Hospital, LTCF	2005-	5 hospitals	(de)central testing	No susceptibility testing
Azole resistance in *Aspergillus fumigatus*	Hospital	2011-	5 university hospitals + 5 teaching hospitals	Central testing	EUCAST microbroth dilution methodology
CPE	GP, hospital, LTCF	2011-	Nationwide	Central testing	Gradient testing, Carba-PCR, next generation sequencing, Nanopore long-read sequencing
MRSA	GP, hospital, LTCF	2008-	Nationwide	Central testing	MLVA typing, whole genome sequencing
CPPA	GP, hospital, LTCF	2020-	Nationwide	Central testing	Gradient testing, Carba-PCR, next generation sequencing, Nanopore long-read sequencing

SHC = Sexual Health Centres; GP = general practice; LTCF = long-term care facility

Over the last years, resistance rates in the Netherlands are mostly stable. Noteworthy, 2020 and 2021 have been exceptional years due to the COVID-19 pandemic. In 2022, the resistance rates did not increase for most pathogens and antibiotics, and for many pathogen-antibiotic combinations there even has been a further decrease compared to 2019 and 2020. This could perhaps be (partly) explained by the different patient population and/or by the decrease in the total antibiotic consumption (at least for outpatient antibiotic use) during the COVID-19 pandemic.
In the summary below, the most important trends of 2022 and implications for therapy are provided. As implications differ by category of patient and indication of use, the summary is organized as such. There are significant differences in susceptibility by patient category. In particular for ICU patients, resistance levels are generally higher and routine culturing with susceptibility testing remains mandatory to tailor therapy to the individual patient. If broad spectrum therapy is initially chosen, susceptibility test results should be used to narrow down antimicrobial therapy to prevent further emergence of resistance and cultures have to be repeated when indicated.
Of importance, resistance rates reported in NethMap are based on data on the first isolate per patient. Resistance of bacteria in the individual patient, especially in those patients staying longer in the hospital, is often higher than reported here. On the other hand, resistance may be overestimated in GP (general practice) and LTCF (long-term care facility) patients, since cultures are usually only performed after failure of initial therapy. In 2019, EUCAST has redefined the category 'I' from a definition of 'intermediate or uncertain therapeutic effect' to the definition 'susceptible, increased exposure'. In 2021, the Dutch Society of Medical Microbiology (NVMM) has encouraged all laboratories in the Netherlands to use this new definition. At present, most Dutch laboratories have redefined the category 'I' according to recommendations by EUCAST. Nevertheless, because the percentage of resistant isolates ('R') was calculated in the analyses for this report, the new definition did not influence the presented resistance percentages or trends.
It should be borne in mind that the majority of conclusions below are based on agents used as intravenous therapy, except for agents that are available as oral drugs only or have a specific indication such as urinary tract infections (UTI).

In GPs

Urinary tract infections

- In *Escherichia coli*, resistance levels for nitrofurantoin and fosfomycin, first and second choice antibiotics for the treatment of uncomplicated urinary tract infection (UTI) in adults in primary care, were stable and low (2%).
- Resistance levels for ciprofloxacin, first choice antibiotic for the treatment of complicated UTI in adults in primary care, was stable at or below 10% for all Enterobacterales. Resistance levels for co-amoxiclav, second choice antibiotic for the treatment of complicated UTI in primary care, decreased to 25% in *E. coli* and was stable at 18% in *Klebsiella pneumoniae*. Resistance levels for co-trimoxazole, third choice antibiotic for this indication, were stable at 18% in *E. coli* and 7% in *K. pneumoniae*.
- Combined resistance for co-amoxiclav, ciprofloxacin, and co-trimoxazole in all Enterobacterales was low (≤3%).

Skin and soft tissue infections

- Clindamycin (including inducible) resistance and resistance to macrolides in *Staphylococcus aureus* was 13% in 2022, which limits its usefulness in empiric therapy for those infections possibly caused by *S. aureus*.
- MRSA was found in 3% of isolates of primary care patients which was stable over the previous 5 years.

Respiratory infections

- Resistance to doxycycline/tetracycline (15%) and macrolides (13%) in *Streptococcus pneumoniae* was higher in GP patients than in hospital patients.

In hospital departments

Inpatient hospital departments (excl. ICU)

Enterobacterales and Pseudomonas aeruginosa

- For all Enterobacterales, resistance to second and third generation cephalosporins seemed to have plateaued or slightly decreased over the past five years. In 2022, resistance to cefuroxime was 11% in *E. coli* and 13% in *K. pneumoniae*. Resistance to cefotaxime/ceftriaxone was 6% in *E. coli* and 7% in *K. pneumoniae*. This is encouraging but nevertheless, patients that are infected with *K. pneumoniae* or *E. coli* have a considerable risk of non-adequate empiric treatment with a second or (to a lesser extent) third generation cephalosporin. In case of severe infection, empiric combination therapy with aminoglycosides, reducing likelihood of resistance to 3% or less, might be a suitable option.
- For the three most important oral antibiotics, co-amoxiclav, co-trimoxazole and ciprofloxacin, a similar or decreasing trend was found as observed in isolates from primary care and OPD (outpatient departments) patients. In *E. coli*, resistance to co-amoxiclav decreased to 29% and was stable at 20% in *K. pneumoniae*. Resistance to co-trimoxazole decreased to 10% in *K. pneumoniae* and was 20% in *E. coli*. Resistance levels for ciprofloxacin were 13% in *E. coli*, 11% in *K. pneumoniae* and 10% in *P. aeruginosa*.

Staphylococcus aureus, β-haemolytic Streptococcus spp. groups A, B and C/G

- In *S. aureus* resistance was high for clindamycin (15%), erythromycin (17%) and fusidic acid (7%). MRSA was found in 2% of *S. aureus* isolates of hospital patients, which remained stable over the previous 5 years.
- In β-haemolytic *Streptococcus* spp. group A, resistance to clindamycin and erythromycin remained stable at 6% and 8%, respectively. Resistance to doxycycline/tetracycline showed an overarching increasing trend over the last five years with a peak level of 36% in 2021, but a substantially lower resistance percentage of 26% in 2022. It remains to be seen if the upward trend holds on in the coming years.
- In β-haemolytic *Streptococcus* spp. group B and C/G, resistance levels for clindamycin (17% group B, 15% group C/G), and erythromycin (20% group B, 15% group C/G) were higher than for group A.

Streptococcus pneumoniae and Haemophilus influenzae

- In *S. pneumoniae*, resistance to doxycycline/tetracycline and macrolides was 10% and 9% in hospital patients, respectively.
- In *H. influenzae*, resistance to amoxicillin and co-amoxiclav was 27% and 10%, respectively. Resistance to ciprofloxacin (4%) and doxycycline/tetracycline (2%) was lower.

Intensive Care Units

Enterobacterales, Pseudomonas aeruginosa and Acinetobacter spp.

- For *E. coli*, resistance levels for all tested antibiotics in ICU patients were comparable to resistance levels in *E. coli* from non-ICU patients.
- For *K. pneumoniae* and *P. aeruginosa*, resistance levels were much higher in ICU patients than in isolates from non-ICU patients.
- In *K. pneumoniae*, resistance to piperacillin-tazobactam and cefuroxime was almost 20% and remained stable over the last five years. Resistance to third generation cephalosporins was 14% for cefotaxime/

ceftriaxone and 12% for ceftazidime. This means that ICU patients with infections due to *K. pneumoniae* had considerable risk of non-adequate empiric treatment with a second or a third generation cephalosporin. In case of severe infection, empiric combination therapy with aminoglycosides, reducing likelihood of resistance to 7% or less, might be a suitable option.
- In *P. aeruginosa* isolates from ICU patients, resistance to piperacillin-tazobactam and ceftazidime, the two first choice agents for the treatment of severe *P. aeruginosa* infections, increased to 16% for piperacillin-tazobactam and 10% for ceftazidime over the last five years. This might complicate empirical treatment of severe infections due to *P. aeruginosa*.
- Resistance in *Acinetobacter* spp. in ICU patients was higher than for non-ICU patients but still remained low for all suitable antibiotics at 7% or less.

Staphylococcus aureus
- MRSA percentage in ICU patients increased to 4% over the last five years. The MRSA level was higher than in non-ICU patients.

Urology services
- Resistance levels in Enterobacterales from patients in urology services traditionally have been higher than in Enterobacterales from non-urology patients.
- Resistance to ciprofloxacin (23%) and co-trimoxazole (24%) in *E. coli* from admitted patients remains a problem.
- However, resistance in *E. coli* and *K. pneumoniae* to antibiotics (such as cefuroxime, ceftriaxone, ciprofloxacin, co-trimoxazole, and co-amoxiclav) that are used to treat complicated urinary tract infections showed a decreasing trend or remained stable over the last five years.

In long-term care facilities
- Resistance levels in *E. coli*, *K. pneumoniae* and *P. aeruginosa* urine isolates from long-term care facilities (LTCF) patients were higher than resistance levels in GP patients and comparable to resistance levels in OPD and hospital patients.
- Resistance levels in *S. aureus* isolates from LTCF patients were higher than resistance levels in GP patients and comparable to resistance levels in OPD and hospital patients, with the exception of resistance to ciprofloxacin (16%), which was much higher in *S. aureus* from LTCF patients than in *S. aureus* from OPD (4%), hospital (4%) and ICU patients (3%).

Specific pathogens and situations

Helicobacter pylori
- Although probably biased towards higher resistance levels, resistance was high for levofloxacin (32%), clarithromycin (51%), and metronidazole (52%), and relatively low for amoxicillin (7%) and tetracycline (2%).
- Over the last years, an increasing trend in resistance was seen for most antimicrobial agents. This means that treatment failures are expected to be more common. Therapy after treatment failure therefore should be guided by culture and susceptibility testing.

Carbapenem-resistant and carbapenemase-producing Enterobacterales
- The prevalence of CRE/CPE confirmed isolates among *E. coli* was 0.03%, among *K. pneumoniae* 0.29%, *E. cloacae* complex 0.81% and other Enterobacterales 0.25%.

- In 2022, the number of carbapenemase-producing Enterobacterales isolates submitted to the RIVM was considerably higher than in previous years and was succeeding pre-COVID-19 totals. The increase is partially attributable to the transfer of Ukrainian patients to the Netherlands.
- The most frequently identified carbapenemase encoding genes in Enterobacterales were bla_{OXA-48}, bla_{OXA-48}-like, bla_{NDM-1} and bla_{NDM-5}.
- In 51% of patients, there is a relation with hospitalization abroad for more than 24 hours during the preceding two months, which was higher than previous years. Turkey, Ukraine, and Morocco are the countries that are most often reported.

Vancomycin-resistant *Enterococcus faecium*

- The number of reported hospital outbreaks with vancomycin resistant *E. faecium* (VRE) in 2022 was comparable to 2021, but still lower compared to 2019, which was probably due to the COVID-19 pandemic.
- The proportion of VRE in infection-related isolates with *E. faecium* in various healthcare settings varies marginally below 1% and has not changed in the previous five years, although an increase of the proportion of VRE was seen in intensive care units after several years of decrease.
- The absolute number of positive screening VRE isolates is substantially higher than the COVID-19 years 2020 and 2021 and comparable to the pre-COVID-19 years.

Methicillin-resistant *Staphylococcus aureus*

- The overall proportion of routinely collected diagnostic *S. aureus* isolates that were MRSA positive in 2018-2022 was 2%. A proportion of 3% was seen for *S. aureus* isolates that were obtained from material collected by GPs and in intensive care units.
- Percentages MRSA were quite stable, except in intensive care units in which the prevalence increased from ~2% in 2018-2019 to ~3% in 2020-2022.
- LA-MRSA MC0398 remains the predominant MRSA clade constituting 22% of all screening isolates and 12% of all diagnostic isolates.
- In 2022, 37% of the diagnostic MRSA-isolates carried the PVL-encoding genes, whereas 21% of the screening isolates were PVL-positive.

Carbapenem-resistant and carbapenemase-producing *Pseudomonas aeruginosa*

- Forty percent of the CPPA isolates (n = 20) in the Type-Ned CPPA surveillance in 2022 were from samples of Ukrainian patients.
- The predominant (33%) carbapenemase-encoding allele in carbapenemase-producing *P. aeruginosa* was bla_{NDM-1} in contrast to 2021 when the dominant carbapenemase encoding allele in CPPA was bla_{VIM-2}.

Extended spectrum β-lactamases

- From 2018 to 2022, the proportion of ESBL *E. coli* was stable in general practice (GP), outpatient departments and inpatient departments. In the ICUs the proportion increased until 2021, and dropped in 2022.
- The percentages of ESBL have been stable or have slightly decreased for *K. pneumoniae* from 2018 to 2022 in most healthcare settings. In contrast, since 2020 the ESBL percentage for *K. pneumoniae* in ICUs increased from around 12 to 15%.

Anaerobes

- Applying the new species-specific EUCAST breakpoints version 12.0 resulted in an increase of resistance levels for clindamycin in most anaerobes.
- As in previous years, metronidazole resistance in *Bacteroides* spp. and *Clostridium* spp. remained low.

Neisseria meningitidis

- Penicillin and rifampicin resistance in *N. meningitidis* isolates is rare in the Netherlands.

Neisseria gonorrhoeae

- No resistance to ceftriaxone, the current first-line treatment for gonorrhoea, has been reported. However, the MIC distribution has shifted towards higher MICs since 2019.
- Resistance to ciprofloxacin yearly increases and more than doubled since 2016, to 61.5% in 2022, despite the fact that ciprofloxacin is not recommended for gonorrhoea, according to guidelines.
- Azithromycin resistance levels increased from 2.1% in 2012 to more than 25% in 2022.

Mycobacterium tuberculosis

- Resistance to the first-line antibiotics to treat tuberculosis (TB) remained almost stable over the last years (any form of resistance in 10.9% of the isolates tested), although there may be somewhat larger fluctuations in isoniazid resistance.
- The number of multi-drug resistant (MDR) isolates remained low in 2022 with a total of only 8 MDR-TB cases detected in the Netherlands.

Clostridioides difficile

- No CDI outbreaks occurred in 2022.
- There is a continued increase in severe CDI cases in the Netherlands.

Aspergillus fumigatus

- Triazole resistance frequency in 2022 was 10.6% in UMCs and 4.8% in teaching hospitals, which represents a resistance level similar to 2020.
- Overall, 82.5% of azole-resistant isolates harbored a TR-mediated resistance mechanism, with TR-variants especially frequent in TR_{46} isolates.

SO-ZI/AMR

- In 2022, 36 outbreaks were reported to the SO-ZI/AMR. This number is lower compared to the pre-COVID-19 era, but higher compared to 2021.
- Most outbreaks were due to MRSA, of which the majority were reported by LTCFs. VRE was the most frequent cause of notified HRMO outbreaks in hospitals.
- Modifications to the SO-ZI/AMR workflow introduced in 2022 will improve the surveillance and overview of healthcare-associated HRMO outbreaks.

2.3 Antibiotic use and resistance in animals

It can be concluded that antibiotic reduction policies in the Netherlands have resulted in more than 77% reduction of sales of Antimicrobial Veterinary Medicinal Products for veterinary use since 2009. Antimicrobial resistance has decreased simultaneously in isolates from most livestock species. In spite of the antimicrobial use (AMU) reduction continuous high levels of resistance are observed for fluoroquinolones and tetracycline in *Campylobacter* isolates from humans and poultry. ESBL and colistin resistance remain present at low levels, while no CPE was detected in samples from livestock or meat.

Antimicrobial use

- In 2022, a total of 112 tons of Antimicrobial Veterinary Medicinal Products (AVMPs) were sold, which is a decrease of 22.9% compared to 2021. A decrease in sales by 77.5 % over the years 2009-2022 is attained (with 2009 considered a reference year by the Dutch Government).
- The decreased sales of AVMPs in the Netherlands in 2022 is supported by an overall decrease in antimicrobial use (AMU) as observed in the use monitoring data. The calculation of consumption is based on national conversion factors (DDDAs) of authorized veterinary medicinal products.
- The use of antibiotics of critical importance to human health care (especially cephalosporins of the 3rd and 4th generation) is low, even in sectors which are not monitored for use. Use and sales of polymyxins decreased in 2022, for which the overall decrease since 2011 is 82.6% in sales. Of the fluoroquinolones, 52% is applied in sectors currently not yet monitored; an overall decrease 93.1% since 2011 is observed.

Antimicrobial resistance

Salmonella from livestock, meat and humans

- In 2022, over all *Salmonella* isolates, the highest resistance levels were observed for, ciprofloxacin (26%), ampicillin (23%), sulfamethoxazole (23%), tetracycline (22%), trimethoprim (10%) and chloramphenicol (7%).
- Over all sources, the highest levels of resistance were observed for *S.* Heidelberg, *S.* Infantis, *S.* Paratyphi B var. Java, monophasic *S.* Typhimurium and *S.* Typhimurium. Among *S.* Typhimurium, no substantial changes in resistance were observed compared to previous years.
- Among human clinical *S.* Enteritidis isolates, an increase in the resistance against ampicillin (7% in 2021, 18% in 2022) and ciprofloxacin (21% in 2021, 38% in 2022) was observed.
- In total, 14 (1.8%) ESBL-producing human clinical *Salmonella* isolates were detected, which is more than previous years. The prevalence of ESBL-producing *Salmonella* from domestic meat is considered low. The highest frequency of ESBL-producing *Salmonella* isolates were detected in imported meat from outside the EU.
- No carbapenemase-producing *Salmonella* were found in 2022.

Campylobacter jejuni and *C. coli* from livestock, meat and humans

- In 2022, resistance proportions in *Campylobacter jejuni* isolates from caecal samples of broilers and meat thereof remained at a high level for quinolones and tetracycline.
- Resistance to erythromycin was not detected in *C. jejuni* isolates from broilers and poultry meat, but was present at low levels in *C. jejuni* in veal calves (3.0%) and *C. coli* in broilers (4.2%) and chicken meat (5.0%). A notably higher level of erythromycin resistance was observed in *C. coli* from veal calves (31.7%).
- In humans, resistance proportions were higher among *C. coli* than in *C. jejuni* isolates. Resistance levels of human *Campylobacter* isolates increased in 2022 compared to 2020 and 2021, when resistance most likely

dropped due to a substantial reduction of travel-related campylobacteriosis as a result of the COVID-19 travel restrictions. However, resistance levels in 2022 were still lower than before the COVID-19 pandemic.
- Ciprofloxacin resistance in *Campylobacter* isolates from humans was high again in 2022 (62.9%), which is a concern for public health. It was, however, lower compared to 2019, before the COVID-19 pandemic. Resistance to erythromycin, first choice antibiotic in human medicine for campylobacteriosis, remained low with 4.8%.

Indicator *E. coli* from livestock and meat products

- Amongst indicator *E. coli* from animals, resistance levels to ampicillin, tetracycline, sulfamethoxazole and trimethoprim were still relatively high in broilers, pigs, and (white) veal calves.
- In broilers, pigs and veal calves, levels of resistance stabilised for most antibiotics, whereas resistance in dairy cattle remained traditionally low. Resistance to third generation cephalosporins was low or absent amongst (randomly isolated) indicator *E. coli* from caecal samples of all animal species.
- Resistance to fluoroquinolones was still commonly present in indicator *E. coli* from caecal samples of broilers (28.0%), in contrast to the lower prevalence observed in pigs (0.3%) and white veal calves (9.3%) and the complete absence in rose veal calves and dairy cattle.
- For most antibiotics tested, levels of resistance in *E. coli* from caecal samples of rosé veal calves were substantially lower than those from white veal calves.
- In fresh retail meat from bovine and chicken, resistance proportions in *E. coli* were similar to isolates from caecal content. In imported poultry meat, resistance proportions were substantially higher compared to fresh domestic retail meat.
- For the first time, resistance monitoring was performed in rabbits, showing high levels of resistance to tetracycline (56.0%), sulfamethoxazole (52.0%) and trimethoprim (54.7%), while resistance to other antibiotics was low or absent.

Specific resistances in Enterobacterales (ESBL/pAmpC/CPE/*mcr*) from various livestock sources

- The prevalence of extended-spectrum cephalosporin (ESC) resistance in randomly selected *E. coli* has been steadily low for several years in all livestock species.
- While the prevalence of selectively isolated ESC-resistant *E. coli* remained stable or decreased in most livestock sectors, in dairy cattle, an increase was measured compared to previous years. The inclusion of imported meat to the monitoring has shown that the prevalence of ESC-resistant *E. coli* on imported chicken and turkey meat is substantially higher than for domestic meat products.
- As in 2021, Whole Genome Sequencing of ESC-resistant *E. coli* shows that over 20% of isolates are clonally related. In 2022, clones were also detected that are shared between livestock sectors.
- In 2022, no carbapenemase-producing Enterobacterales (CPE) were detected in livestock, but on one occasion an OXA-48-producing *E. coli* was identified in a faecal sample of a dog.
- As in former years, the prevalence of mcr genes encoding for colistin resistance, in *E. coli* was low in livestock and meat.

MRSA from livestock and meat

- In 2022, over 25% of the investigated veal calf farms were tested positive for MRSA. The incidence of MRSA on farms rearing white veal calves was significantly higher than that of those rearing rosé veal calves.

2.4 Antimicrobial stewardship

Since 2014, all hospitals have established antimicrobial stewardship teams (A-teams) responsible for implementing an antimicrobial stewardship program, as recommended by the Dutch Health Care Inspectorate (IGJ) in response to the SWAB's statement to contain antimicrobial resistance.

The most important developments concerning stewardship in 2021 are:

- All hospitals participating in the stewardship monitor have a formal A-team, but in 20% of hospitals, the financial support for their A-team is insufficient compared to the standard staffing recommendation.
- Nineteen (~25%) acute care hospitals extracted structured data from the electronic medical records and provided these to the interactive dashboard of the antimicrobial stewardship monitor.
- Cefazolin was used as backbone of surgical antimicrobial prophylaxis in all hospitals.
- Based on prescriptions started on the day of surgery as a proxy for surgical prophylaxis, on average 82% (range 76-93%) of surgical antimicrobial prophylaxis courses were discontinued at the day of surgery or the day after.
- In 59% (range 48-72%) of the patients that received cefuroxime/ceftriaxone as empiric treatment upon admission, antibiotics were discontinued without starting another course.

Further validation studies and the linking of indications to antibiotic use will provide greater insight into the interpretation of this data. We will then be able to assess the quality of antimicrobial use using structured data extracted from the electronic medical record.

2.5 Implications for public health and health policy

In 2022, the impact of the COVID-19 pandemic and its associated measures slowly extinguished.
The overall use of antimicrobials in humans increased in 2022, compared to 2020 and 2021, and for most antibiotics the use in hospitals returned to pre-pandemic levels.
Some highly-resistant micro-organisms (HRMO) were more prevalent again in 2022, after a substantial reduction during 2020 and 2021. The absolute number of carbapenemase-producing Enterobacterales isolates that were submitted to the RIVM was considerably higher than in previous years and was comparable to pre-COVID-19 totals. The increase in 2022 is partially attributable to the transfer and influx of Ukrainian persons to the Netherlands due to the Ukraine/Russia war. Still, carbapenem resistance among Enterobacterales remains rare. The percentage of carbapenem-resistant/carbapenemase-producing isolates among *E. coli* and *K. pneumoniae* in 2022 was low at 0.03% and 0.29%, and there was no significant increase in the resistance percentage in the last years.
Also the absolute number of methicillin-resistant *S. aureus* (MRSA) isolates in 2022 was higher compared to 2020 and 2021 but still lower than in the years 2018 and 2019. We did, however, notice an increase in the proportion of MRSA in ICUs from 2020 onwards. The explanation of this finding is currently unclear. There were no large MRSA outbreaks in hospitals, including ICUs, reported to the Early warning and response meeting of Healthcare associated Infections and AntiMicrobial Resistance. In general, the total number of 36 reported outbreaks in healthcare settings in 2022 was higher than the number in 2021 (27), but still remarkably lower than in 2017-2019, when around 60 outbreaks were reported each year.

Overall in 2022, the prevalence of resistance of most pathogens was stable or even declining. Resistance percentages among Gram-negative micro-organisms in general practice, outpatient departments and inpatient departments were stable or declining in the previous five years, while resistance percentages on the intensive care units were generally higher and sometimes even still increasing. Notably, resistance among other groups of micro-organisms are on the rise in the Netherlands, such as clindamycin resistance among β-haemolytic *Streptococci* and *S. aureus*, which warrants special attention for antibiotic stewardship programs and surveillance of these pathogens as well.

Worldwide and in Europe, antibiotic resistance continues to be a serious threat to public health, leading to increased healthcare costs, prolonged hospital stays, treatment failures and sometimes death. In June 2023, the Council of the EU adopted a Council Recommendation on stepping up EU actions to combat AMR using a One Health approach, which recommends targets to be achieved by the EU by 2030. These include three AMR targets to reduce the total EU incidence of bloodstream infections with MRSA, third-generation cephalosporin-resistant *E. coli* and carbapenem-resistant *K. pneumoniae*, by 15%, 10% and 5%, respectively, by 2030 compared to baseline year 2019. While the EU incidence of bloodstream infections with both MRSA and third-generation cephalosporin-resistant *E. coli* showed encouraging decreasing trends, the EU incidence of carbapenem-resistant *K. pneumoniae* increased by almost 50% between 2019 and 2022.

Data from the European Antimicrobial Resistance Surveillance Network (EARS-Net) show that in Europe in 2022 wide variations in the occurrence of antimicrobial resistance across the EU/EEA exist.[1] There are specific AMR issues that remain concerning. A significantly decreasing trend in the EU/EEA population-weighted mean percentage of MRSA was reported during 2018-2022. Still, MRSA remains an important pathogen in the EU/EEA, as the levels of MRSA were still high in several countries, and combined resistance to other antimicrobial groups was common.

The global rise of carbapenem-resistant Enterobacterales (CRE) is alarming and represents an increasing threat to healthcare delivery and patient safety. Carbapenem resistance remained rare in *E. coli*, but almost one third of EU/EEA countries reported carbapenem resistance percentages above 10% in *K. pneumoniae*. In addition, the EU incidence of carbapenem-resistant *K. pneumoniae* bloodstream infections increased by almost 50% between 2019 and 2022, which indicates the need to rapidly strengthen prevention and control actions, in the EU and in Member States. There are widely varying estimated incidences and AMR percentages among countries, suggesting that there are further opportunities for reduction. The options for action are aimed at timely and appropriate diagnosis, high standards of infection prevention and control and antimicrobial stewardship.

In contrast to the Netherlands, also combined resistance to different antimicrobial groups was high for *K. pneumoniae*, with almost 40% of the clinical isolates reported to EARS-Net for 2022 being resistant to at least one antimicrobial group under surveillance, and combined resistance to several antimicrobial groups was a frequent occurrence with more than 20% of *K. pneumoniae* being resistant to at least three of the surveyed antimicrobial groups. In *E. coli*, combined resistance to at least three antibiotic groups was lower with a percentage of just above 10% in 2022.

[1] https://www.ecdc.europa.eu/en/publications-data/surveillance-antimicrobial-resistance-europe-2022

Conclusions and discussion

The data presented in NethMap/MARAN 2023 demonstrate that ongoing attention is needed to combat antibiotic resistance and optimize antimicrobial use in humans and animals. 2022 was the first year after the COVID-19 pandemic in which the incidence of many other infectious diseases returned to the levels of the pre-COVID-19 years, which also accounted for some bacteria under surveillance with a special focus on AMR. Still, the interpretation of the data is complicated by the wide variety of changes that took place during the pandemic years. It remains to be seen what will be the long-term impact of COVID-19 on the prevalence of AMR in the Netherlands and worldwide.

For now, it is encouraging to see that there is an overarching downward trend in use of antimicrobials in humans when compared to the years before 2019, in spite of an increase in 2022 compared to 2020 and 2021. Antimicrobial resistance is not rising and sometimes even going down in many important species. The total use of antimicrobials in animals has decreased with over 77% compared to 2009 and antimicrobial resistance has decreased simultaneously among most livestock species. Worldwide, resistance and multidrug resistance in Enterobacterales (most notably *K. pneumoniae*) is of major concern, and needs ongoing close attention. In addition, vigilance is warranted for high resistance percentages among other groups of micro-organisms as well.

Antimicrobial stewardship programs and A-teams have been implemented universally in Dutch hospitals to further optimize antibiotic prescription practices. In addition, with adequate surveillance systems the impact of measures to control the prevalence and spread of antimicrobial resistance in human healthcare as well as the open population, the environment, food-producing animals and the food chain, can be monitored and if necessary adjusted. In 2024 a new Netherlands Actionplan AMR will be implemented aiming to continue and further improve current practices to control AMR.

3
Use of antimicrobials

3.1 Outpatient antibiotic use

Methods
Data from the SFK (Foundation for Pharmaceutical Statistics, the Hague) were used to gain insight in outpatient antibiotic use in the Netherlands for the year 2022. Antibiotic usage was described as the Defined Daily Doses (DDD) for each ATC-5 code. The SFK collects dispensing data from ±90% of Dutch outpatient pharmacies, serving 93% of the Dutch population, and extrapolates the data to 100% coverage. These data include prescriptions from general practitioners, as well as prescriptions from outpatient service in hospitals, outpatient clinics and dentists. Data is presented as DDD per 1,000 inhabitants per day (DID). In 2019 the World Health Organization (WHO) implemented changes with respect to DDD definition for two major drug classes: penicillins with extended spectrum and penicillins with β-lactamase inhibitors.[1] From 2019 onwards, SWAB processed data using these updated DDD definitions. To enable comparison collected pre- and postsituation before 2019, the 2018 data are presented using both definitions for 2018, as well as using the new 2019 DDD definitions.

Results
Total outpatient use of antibiotics increased from 7.61 to 8.32 DID in 2022 (table 3.1.1, figure 3.1.1).
As compared to 2019 (pre-COVID) total outpatient use is slightly lower. The consumption of β-lactamase sensitive penicillins and β-lactamase resistant penicillins increased over the past years (figure 3.1.2), whereas use of tetracyclins decreased. Macrolides make up 14% of total consumption.

Discussion
Systemic outpatient antibiotic use in 2022 increased compared to 2020 and 2021 (COVID-19 pandemic years). This increase may have been driven by changes in illness presentation. During the years 2020 and 2021 outpatient antibiotic use may have been lower as a result of less health seeking behaviour and the measures taken to prevent the spread of the virus. After the pandemic, antibiotic use increased, but still not to the pre-pandemic level. However, when compared to the years before 2019, there is an overarching downward trend in outpatient antibiotic use in the Netherlands. However, specific antibiotic classes have

striking levels of consumption, such as macrolides, particularly azithromycin. Macrolides are considered 1st or 2nd choice antibiotics for only a few medical indications according to the Dutch guidelines for general practice (NHG). Possible explanations could be incorrectly prescribing macrolides for their usage, ease of use, over-registration of penicillin allergies and GP continuation of macrolide therapy for chronic diseases iniatied by medical specialists, such as the treatment of COPD.

Table 3.1.1 Ten years data on the use of antibiotics for systemic use (J01) in outpatients (DDD/1,000 inhabitant-days), 2013-2022 (source: SFK)

								DDD including changes as of 2019 (source: WHO)				
ATC Group*	Therapeutic group	2013	2014	2015	2016	2017	2018	2018	2019	2020	2021	2022
J01AA	Tetracyclines	2.33	2.23	2.25	2.10	1.98	1.94	1.94	1.83	1.54	1.42	1.54
J01CA	Penicillins with extended spectrum	1.99	1.94	2.13	2.08	1.94	2.02	1.35	1.26	0.98	0.98	1.22
J01CE	β-lactamase sensitive penicillins	0.31	0.30	0.23	0.24	0.22	0.07	0.07	0.16	0.12	0.13	0.20
J01CF	β-lactamase resistant penicillins	0.41	0.44	0.43	0.46	0.46	0.49	0.49	0.48	0.47	0.48	0.53
J01CR	Penicillins + β-lactamase-inhibitors	1.67	1.55	1.56	1.52	1.42	1.42	0.95	0.93	0.81	0.81	0.92
J01D	Cephalosporins & carbapenems	0.04	0.04	0.04	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
J01EA	Trimethoprim and derivatives	0.17	0.16	0.14	0.14	0.13	0.13	0.13	0.12	0.12	0.12	0.12
J01EE	Sulphonamides + trimethoprim	0.29	0.28	0.28	0.28	0.29	0.30	0.30	0.33	0.33	0.33	0.35
J01FA	Macrolides	1.22	1.18	1.20	1.17	1.17	1.22	1.22	1.22	1.13	1.07	1.14
J01FF	Lincosamides	0.17	0.18	0.19	0.20	0.21	0.23	0.23	0.23	0.23	0.24	0.27
J01GB	Aminoglycosides	0.03	0.03	0.03	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
J01MA	Fluoroquinolones	0.76	0.79	0.77	0.75	0.73	0.73	0.73	0.67	0.64	0.64	0.67
J01XE	Nitrofuran derivatives	1.37	1.40	1.40	1.39	1.36	1.35	1.35	1.30	1.24	1.24	1.23
J01XX01	Fosfomycin	0.02	0.03	0.04	0.05	0.05	0.07	0.06	0.06	0.07	0.07	0.07
	Others	0.04	0.04	0.04	0.02	0.05	0.04	0.04	0.03	0.03	0.03	0.03
J01	**Antibiotics for systemic use (total)**	**10.83**	**10.58**	**10.72**	**10.44**	**10.06**	**10.06**	**8.90**	**8.68**	**7.77**	**7.61**	**8.32**

** From the 2022 edition of the Anatomical Therapeutic Chemical (ATC) classification system*

Figure 3.1.1 Use of antibiotics for systemic use (J01) in outpatients at ATC-4 level, 2013-2022 (source: SFK)

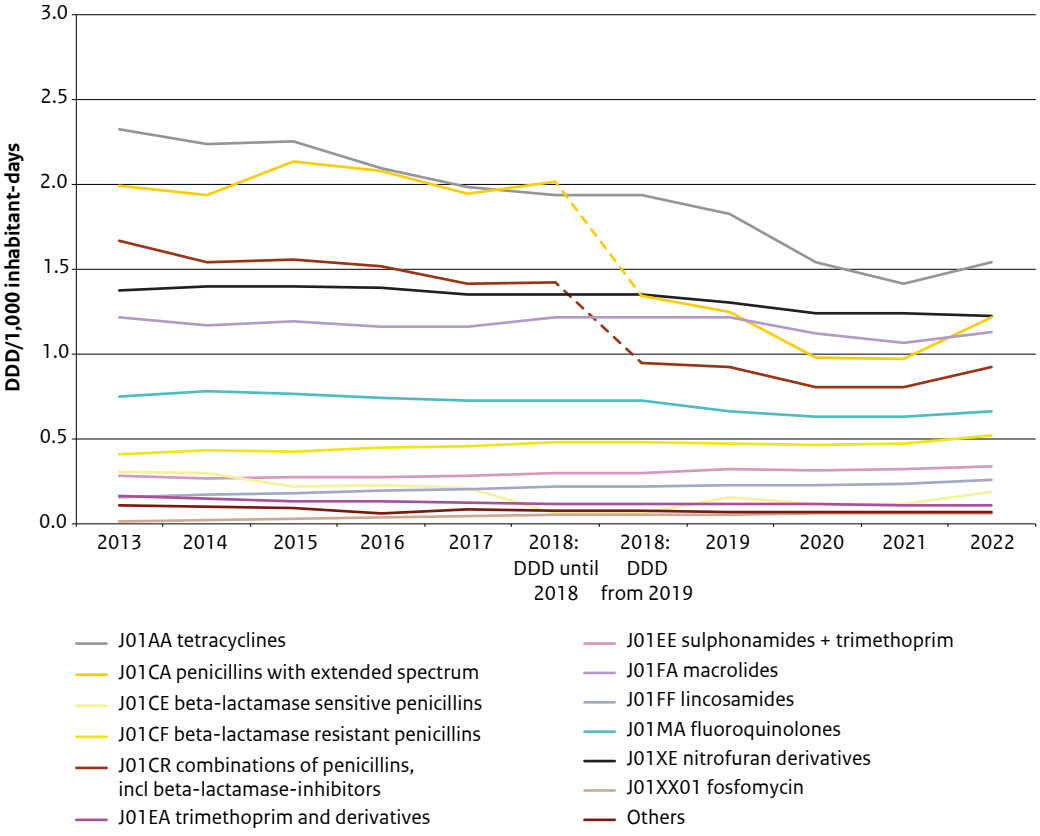
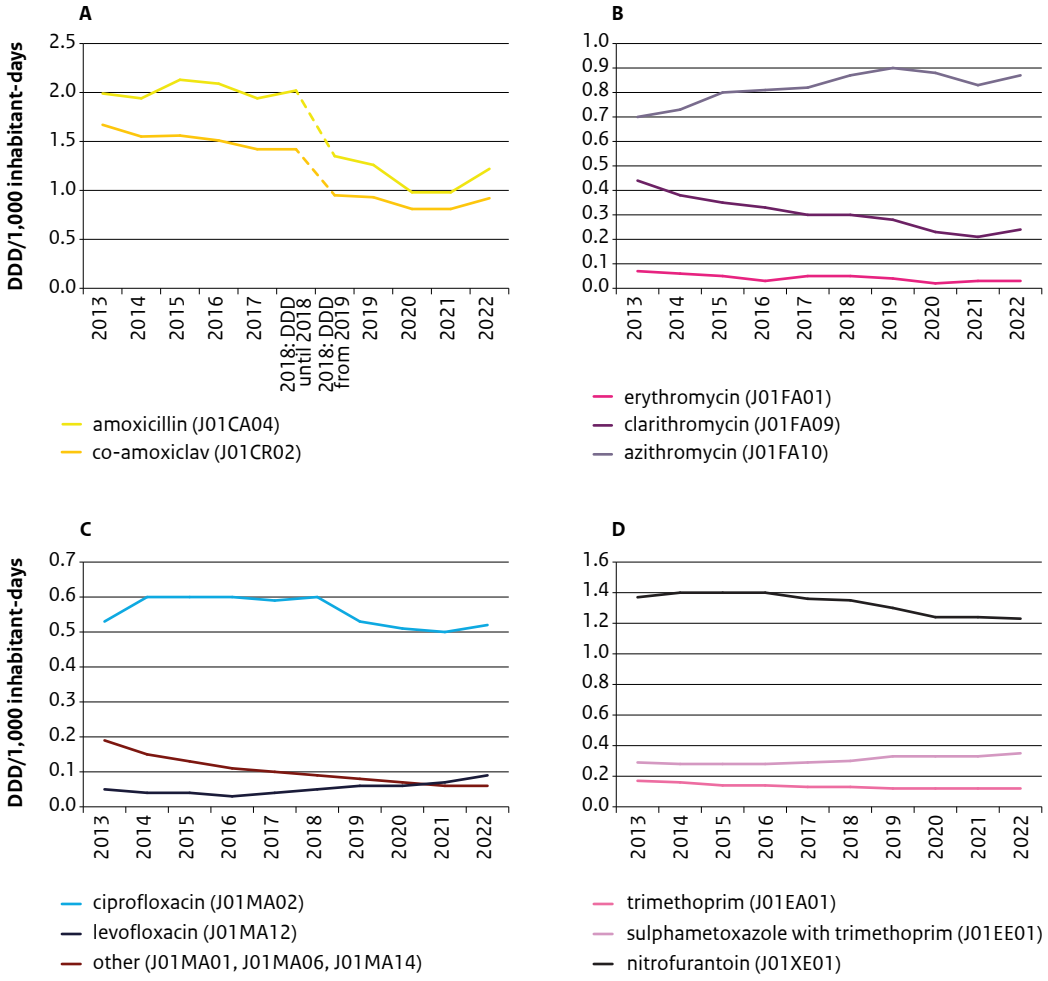


Figure 3.1.2 Use of antibiotics for systemic use (J01) in outpatients at ATC-5 level, 2013-2022 (source: SFK)



Antibiotic prescribing quality indicators in primary care

Introduction

To assess the antibiotic prescribing quality at general practitioner (GP) practice level, quality indicators (QIs) were identified in the SABEL project (Spiegelinformatie Antibiotica Eerstelijn)[1,2]. These QIs link antibiotic prescribing to clinical indications (ICPC-coded patient consultations derived from GP practices' electronic medical records). This is the second time NethMap reports these QI outcomes.

Methods

From 2023 on, the RIVM receives the QI outcomes from anonymized routinely collected health care data from GP practices in the Netherlands. Data are retrieved directly from the electronic medical records of GP practices who agree to collaborate with "Stichting Informatievoorziening voor Zorg en Onderzoek" (STIZON) and contribute to the anonymized dataset. Data are available from 2018 to 2022. For the analyses of 2022, GP practices with data for the complete year 2022 were included. The median score for each QI was calculated with the inter quartile range (IQR; 25 – 75 percentiles). For the trend analysis over time, GP practices with complete data for the period 1 January 2018 – 31 December 2022 were included. We excluded GP practices with a ≥20% non-explainable difference in number of registered patients between two consecutive years from 2018 to 2022. The median score per year for each QI was calculated.

The fourteen QIs for primary care are:
General QIs:

- Total number of systemic antibiotic prescriptions/1000 registered patients
- Percentage of amoxicillin/clavulanic acid prescriptions from total
- Percentage of macrolide prescriptions from total
- Percentage of quinolone prescriptions from total
- Percentage of amoxicillin/clavulanic acid + macrolide + quinolone prescriptions from total

Antibiotic prescribing percentages (episodes of specified diagnoses treated with antibiotics/episodes of specified diagnoses) for:

- Otitis media
- Upper respiratory tract infection (URTI)
- Lower RTI (LRTI)
- Impetigo

1st choice antibiotic prescribing percentages (episodes with first choice antibiotic prescribed/episodes with any antibiotic prescribed) for:

- Otitis media (amoxicillin)
- Tonsillitis (pheneticillin or phenoxymethylpenicillin)
- Pneumonia (amoxicillin or doxycycline)
- Cystitis in women (nitrofurantoin or fosfomycin)
- Impetigo (flucloxacillin)

Results

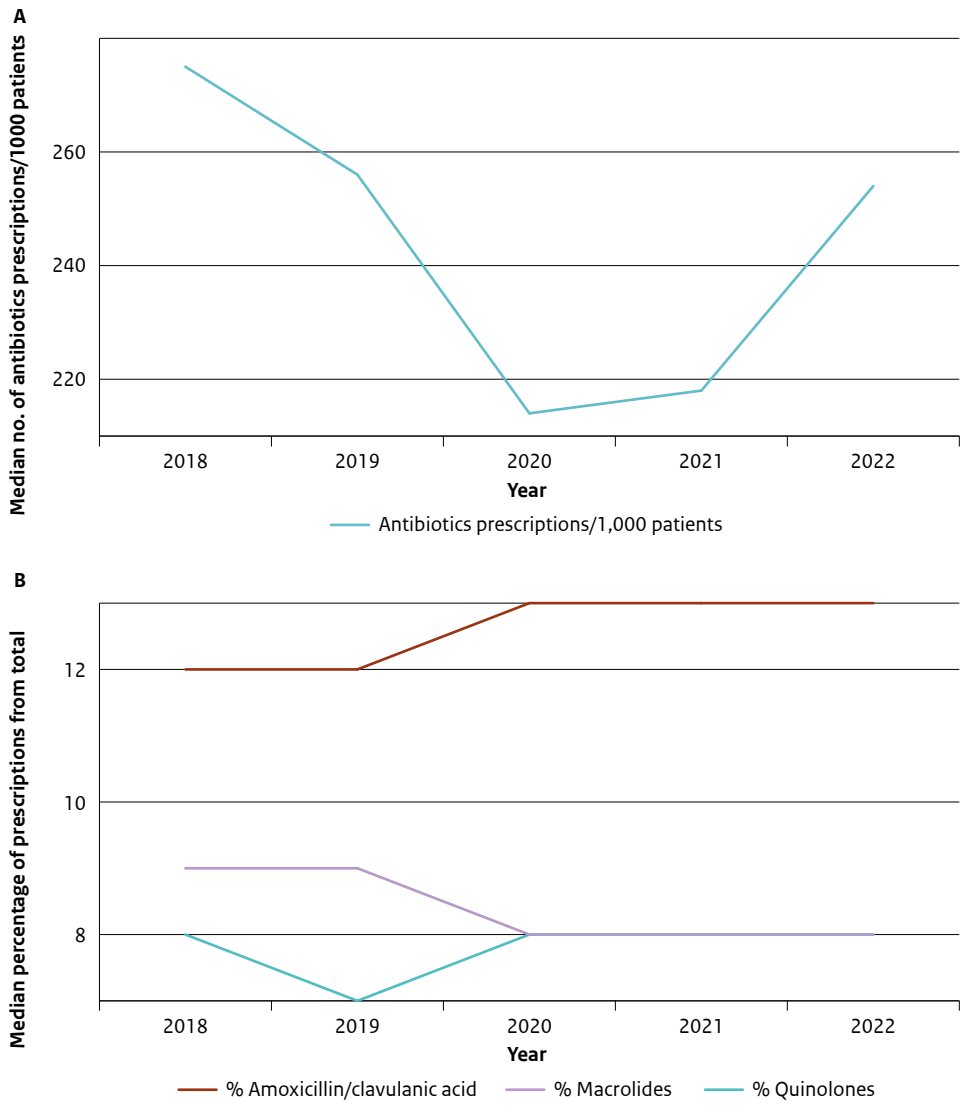
In 2022, data from 695 GP practices were included, with a median number of registered patients per practice of 3042 (IQR 2460 – 4400). The median number of antibiotics prescriptions per 1,000 patients per year was 245 (IQR 210 – 288), of which 32 (IQR 21 – 40) were attributed to chronic usage (Table 3.1.2). The QI outcomes show high variability between individual practices with respect to numbers of prescribed antibiotics (QI1) and prescribing quality (other QIs).

Table 3.1.2 Outcomes of the antibiotic prescribing QIs GP practices (N=695) in 2022, STIZON

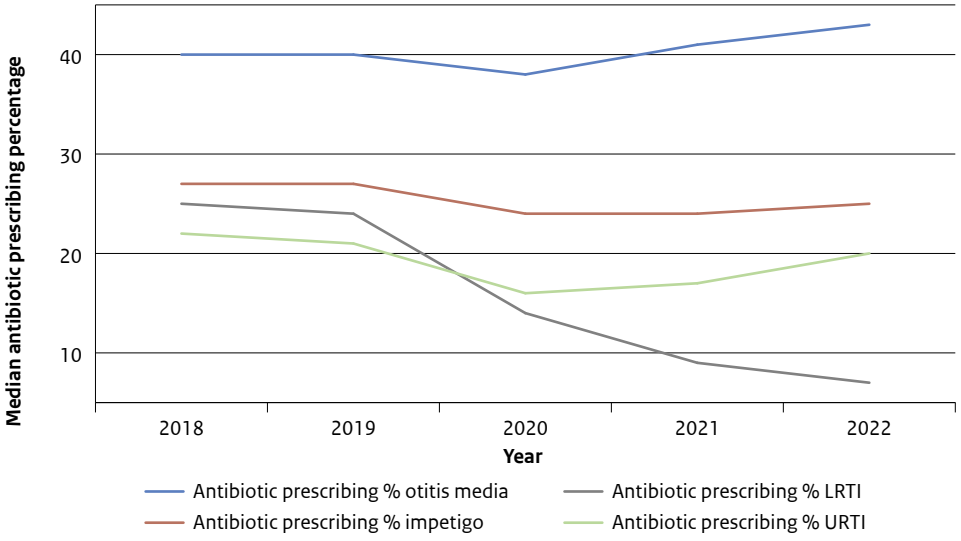
Quality Indicator	Median	IQR (25 - 75%)
Antibiotics prescriptions/1,000 patients	245	210 - 288
Number of chronic antibiotic users/1,000 patients	32	21 - 40
% Amoxicillin/clavulanic acid	13	11 - 14
% Macrolides	8	6 - 10
% Quinolones	8	6 - 9
% Amoxicillin/clavulanic acid + Macrolides + Quinolones	29	26 - 31
Antibiotic prescribing % otitis media	45	37 - 54
Antibiotic prescribing % URTI	21	16 - 26
Antibiotic prescribing % LRTI	7	6 - 10
Antibiotic prescribing % impetigo	25	19 - 32
% 1st choice antibiotic prescribing otitis media	87	80 - 92
% 1st choice antibiotic prescribing tonsillitis	55	40 - 67
% 1st choice antibiotic prescribing pneumonia	80	71 - 87
% 1st choice antibiotic prescribing cystitis (women)	86	83 - 89
% 1st choice antibiotic prescribing impetigo	70	50 - 83

For the trend analyses from 2018 to 2022, data from 464 GP practices were included (Figure 3.1.3). A sharp decline in the median number of antibiotic prescriptions per 1000 registered patients per year was seen in 2020 and 2021 during the COVID-19 pandemic (Figure 3.1.3A). In 2022, the median number of antibiotic prescriptions increased again to the level observed in 2019. The median percentages of amoxicillin/clavulanic acid, macrolide, and quinolone prescriptions were quite stable during the 2018 – 2022 period (Figure 3.1.3B). The median antibiotic prescribing percentage for otitis media slightly increased, while for LRTI a sharp decrease was seen during the COVID-19 pandemic which continued to decrease in 2022 (Figure 3.1.3C). The medians of QIs on 1st choice antibiotic prescribing per clinical indication were stable, or increased over time (Figure 3.1.3D), particularly for impetigo and tonsillitis. First choice prescribing for pneumonia decreased during the COVID-19 pandemic years but increased in 2022 to 80%.

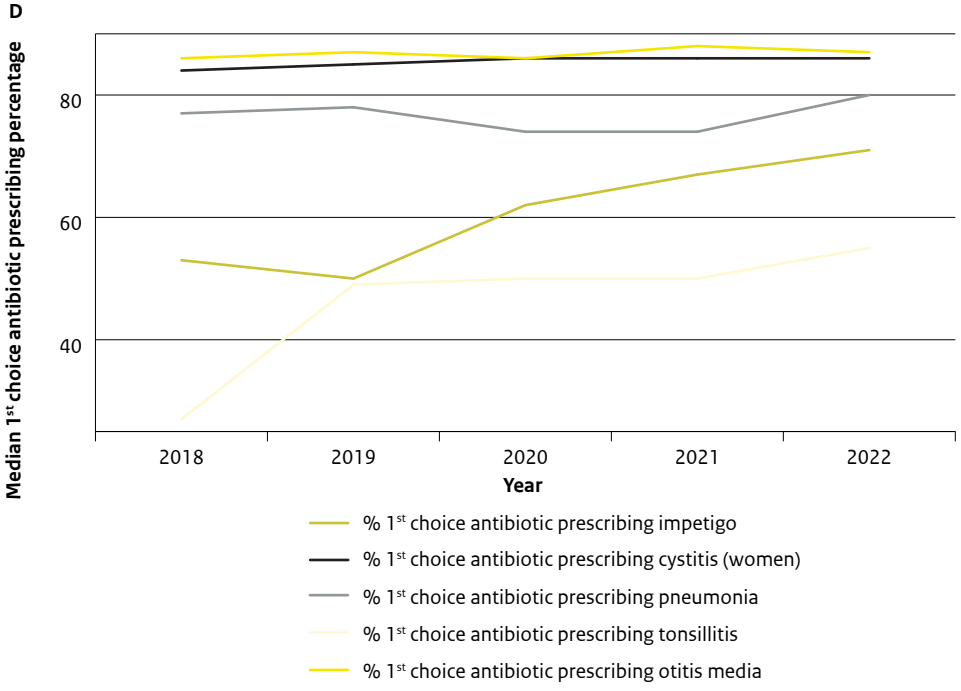
Figure 3.1.3 Trends in outcomes of the antibiotic prescribing QIs in primary care (N=464 GP practices), 2018 – 2022, STIZON. Median of the total number of systemic antibiotic prescriptions/1,000 registered patients/year (A), median percentage of amoxicillin/clavulanic acid, macrolide and quinolone prescriptions (B), median antibiotic prescribing percentages for otitis media, URTI, LRTI and impetigo (C), and the median 1st choice antibiotic prescribing for otitis media, tonsillitis, pneumonia, cystitis in women and impetigo (D). Please note that the Y-axis shows different median percentages for Figures B, C, and D.



C


Median antibiotic prescribing percentage
40
30
20
10
2018
2019
2020
2021
2022
Year
Antibiotic prescribing % otitis media
Antibiotic prescribing % LRTI
Antibiotic prescribing % impetigo
Antibiotic prescribing % URTI

D


Median 1st choice antibiotic prescribing percentage
80
60
40
2018
2019
2020
2021
2022
Year
% 1st choice antibiotic prescribing impetigo
% 1st choice antibiotic prescribing cystitis (women)
% 1st choice antibiotic prescribing pneumonia
% 1st choice antibiotic prescribing tonsillitis
% 1st choice antibiotic prescribing otitis media

Discussion

These QI outcomes provide insight into the antibiotic prescribing quality by GPs in the Netherlands. Data will also be made available to the individual participating GP practices to benchmark their own prescribing behaviour. Compared to last year's edition of NethMap, we were able to include a larger number of GP practices. For next year, numbers are expected to further increase with data from a second database, making the benchmark results even more robust and enabling stratification of results by, for example, Regional Care Network and practices with relatively more/less children.

Conclusions

- In 2022, a median of 245 antibiotics prescriptions per 1000 registered patients per year were prescribed by GP practices for acute infectious diseases.
- From 2018 to 2022, a sharp decline in the number of antibiotic prescriptions per 1000 registered patients per year was seen in 2020 and 2021 due to the COVID-19 pandemic, which increased again in 2022 to the level observed in 2019.

References

1 AW van der Velden, MI van Triest, AF Schoffelen, TJM Verheij. Structural Antibiotic Surveillance and Stewardship via Indication-Linked Quality Indicators: Pilot in Dutch Primary Care. Antibiotics (Basel). 2020;9(10):670.

2 SEJD van den Eijnde, PD van der Linden, AW van der Velden on behalf of SWAB's Working Group on Surveillance of Antimicrobial Use. Indication-linked antibiotic prescribing quality indicators using practice-based routine primary care data: feasibility, reliability and validity. 33rd ECCMID 2022, Copenhagen, Denmark, Posterpresentation P2290.

3.2 Inpatient antibiotic use

Methods

Data on inpatient use of antibiotics in Dutch hospitals in 2021 and 2022 was collected by means of a questionnaire and data-specification format for data-extraction, distributed to all Dutch hospital pharmacies. DDDs were calculated using the Dutch drug database 'Z-index' per ATC-code and route of administration and at the unit and product level using WHO definitions from 2020.[2] Several changes in DDD definitions were implemented by the WHO in 2019.[1] For these antibiotic groups, both DDDs calculated with the previous (until 2018) and new WHO definitions (starting from 2019) are depicted for the year 2018 in the tables and figures (as a dashed line), to enable long-term comparison of surveillance data. Since this year's NethMap 2023 report was published in November, it was possible to report the data of two consecutive years (2021 and 2022) at once.

Use of antibiotics is expressed as DDD/100 patient-days and DDD/100 admissions. The number of patient-days was estimated by subtracting the number of admissions from the number of bed-days to compensate for the fact that in bed-days statistics, both the day of admission and the day of discharge are counted as full days. Hospital consumption data and corresponding hospital statistics were used to estimate total inpatient consumption in the Netherlands. Methods are further described by Kwint *et al*.[3]
Hospital extrapolated data are expressed in DDD/1,000 inhabitants per day (DID), as is used in the international antibiotic consumption surveillance of the European Centre for Disease Prevention (ECDC). Data on the annual number of inhabitants in the Netherlands were obtained from Statistics Netherlands (CBS).

Results

66 hospital locations reported data for 2021 including the annual number of bed-days and admissions. The inpatient use of systemic antibiotics decreased (-5.4%) from 85.8 to 81.1 DDD/100 patient-days compared to 2020 (table 3.2.1). Total inpatient use of systemic antibiotics, expressed as DDD/100 admissions, decreased to 303.7 (-8.8%; table 3.2.1). In parallel, total use of systemic antibiotic use, when calculated as DDD/1,000 inhabitant-days (DID), decreased from 0.760 in 2020 to 0.700 in 2021 (-7.9%) (table 3.2.2).

63 hospital locations reported data for 2022 including the annual number of bed-days and admissions. Compared to 2021, inpatient use of systemic antibiotics increased by 13.5% to 92.1 DDD/100 patient-days (table 3.2.1). Expressed as DDD/100 admissions, total inpatient use of systemic antibiotics increased to 338.1 in 2022 (+11.3%; table 3.2.1). In line, when calculated as DDD/1,000 inhabitant-days (DID), total use of antibiotics for systemic use increased to 0.747 (+6.7%) (table 3.2.2).

Most antibiotic use decreased in 2021, followed by increased use in 2022 (figure 3.2.1). Exceptions to this trend are first-generation cephalosporins, carbapenems, glycopeptides and imidazole derivatives. The consumption of these four groups of antibiotics has increased consistently year after year. Third-generation cephalosporins use increased in 2021 and decreased in 2022, however usage is still higher than pre-COVID period and has now become the most used generation of cephalosporins.

Use of flucloxacillin also demonstrated a consistent upward trend, despite a slight decrease in 2021 (-5.8%, 2022 +28.2%) (figure 3.2.2). This also applies to vancomycin (+4.3% in 2021, +2.8% in 2022). Consumption of intravenous use of gentamicin (-15.4% in 2021, -4.7% in 2022) and tobramycin (-8.7% in 2021 and -15.6% in 2022) is decreasing.

As seen in previous years, antibiotic use in university hospitals was higher than in general and large teaching hospitals, as shown in figure 3.2.4.

Increase in meropenem and vancomycin use was seen mainly in university hospitals (figure 3.2.6).

Total use of antimycotics decreased to 5.63 DDD/100 patient-days in 2021 and 4.97 DDD/100 patient-days in 2022. These are still mainly used in university hospitals (12.7 DDD/100 patient-days). Two-third of use is explained by triazole derivatives such as fluconazole and voriconazole (table 3.2.3).

Discussion

Overall inpatient antibiotic usage declined in 2021 compared to 2020, most likely due to altered illness presentations and prescribing practices during the COVID-19 pandemic. In 2022 the use of most antibiotics returned to pre-COVID levels. Most notable exceptions are:

- Flucloxacillin, where consumption in 2022 was 36% higher as compared to 2019 (pre-COVID). As mentioned in previous reports, this is likely a result of clinicians employing higher treatment dosing regimens.
- Meropenem, where usage has increased year after year. The likely explanation is increasing background antimicrobial resistance in specific groups of patients or in part due to its substitution for aminoglycosides.
- Vancomycin usage has shown an upward trend for several years, likely due to the implementation of higher target through dosing levels.
- Aminoglycoside use has decreased every year. This may be due to alterations in empiric treatment guidelines for sepsis and increasing awareness of aminoglycoside toxicity.

The downward trend of overall antibiotic use in hospitals continued (0.747 DID) in 2022. The same applies to the consistent upward antibiotic usage trend when evaluated per patient and use per bed-day. The most likely explanation is that less patients are being treated with antibiotics, however, there has been an intensification of treatment for individual patients who are receiving antibiotics.

The large variation in antibiotic use between Dutch hospitals remains difficult to explain. Local practice is likely influenced by guidance from culture-results versus choice of empiric antibiotic regimens, also depending on the patient population served by the hospital. Location of hospitals and cross-border collaborations may also impact local practices.

Table 3.2.1 Ten years use of antibiotics for systemic use (J01) in hospitals (DDD/100 patient-days), 2013-2022 (source: SWAB)

ATC group*	Therapeutic group	2013	2014	2015	2016	2017	2018	2018‡	2019‡	2020‡	2021‡	2022‡
J01AA	Tetracyclines	1.75	1.90	1.89	1.96	1.97	2.05	2.05	2.10	2.00	1.87	2.83
J01CA	Penicillins with extended spectrum	7.95	8.42	9.24	10.88	10.22	11.08	5.26	4.92	5.01	4.86	6.02
J01CE	β-lactamase sensitive penicillins	1.86	2.40	2.39	2.55	2.50	2.26	2.26	2.49	2.60	2.40	2.95
J01CF	β-lactamase resistant penicillins	8.09	8.67	7.74	8.73	9.59	10.76	10.76	10.64	11.97	11.28	14.45
J01CR	Combinations of penicillins, incl. β-lactamase-inhibitors	14.84	14.48	14.31	14.62	14.73	14.48	11.98	10.13	10.60	8.84	10.03
J01DB	First-generation cephalosporins	3.71	4.35	4.59	4.63	5.29	6.43	6.43	6.68	6.55	7.13	7.58
J01DC	Second-generation cephalosporins	4.68	4.98	5.33	5.75	5.87	7.99	7.99	7.99	8.48	6.77	7.63
J01DD	Third-generation cephalosporins	5.04	5.67	5.49	5.95	6.39	6.88	6.88	7.73	9.93	11.41	10.94
J01DH	Carbapenems	1.65	1.65	1.74	1.83	1.98	1.93	1.32	1.41	1.53	1.63	1.69
J01EA	Trimethoprim and derivatives	0.30	0.26	0.26	0.25	0.27	0.23	0.23	0.20	0.23	0.17	0.19
J01EE	Combinations of sulfonamides and trimethoprim, incl. derivatives	1.92	1.89	1.76	2.13	2.38	2.15	2.15	2.41	3.00	2.59	2.96
J01FA	Macrolides	2.64	2.88	2.74	2.97	2.82	2.66	2.66	2.75	3.18	2.50	2.72
J01FF	Lincosamides	2.30	2.30	2.35	2.45	2.43	2.54	2.54	2.36	2.34	2.08	2.71
J01GB	Aminoglycosides	3.55	3.57	3.66	3.70	3.62	3.76	3.76	3.34	2.97	2.83	2.84
J01MA	Fluoroquinolones	8.65	9.02	8.39	9.15	8.65	8.45	7.67	6.99	7.39	6.57	7.69
J01XA	Glycopeptides	1.49	1.59	1.60	1.62	1.72	1.73	1.73	1.99	2.39	2.46	2.52
J01XB	Polymyxins	0.23	0.19	0.23	0.23	0.24	0.14	0.11	0.15	0.14	0.16	0.19
J01XD	Imidazole derivatives	2.55	2.60	2.58	2.80	3.00	3.20	3.20	3.21	3.28	3.44	3.72
J01XE	Nitrofuran derivatives	1.30	1.55	1.42	1.67	1.73	1.63	1.63	1.40	1.77	1.70	2.00
J01XX	Other antibacterials **	0.10	0.09	0.12	0.13	0.28	0.24	0.24	0.28	0.31	0.31	0.29
	Others***	0.08	0.07	0.07	0.07	0.08	0.10	0.10	0.13	0.10	0.12	0.15
J01	**Antibiotics for systemic use (total)**	**74.68**	**78.55**	**77.89**	**84.05**	**85.68**	**90.71**	**80.98**	**79.29**	**85.79**	**81.13**	**92.10**
	expressed in DDD/100 admissions:											
J01	**Antibiotics for systemic use (total)**	**307.8**	**326.0**	**330.1**	**326.1**	**340.2**	**339.7**	**303.2**	**318.5**	**333.1**	**303.7**	**338.1**

* *From the 2022 edition of the Anatomical Therapeutic Chemical (ATC) classification system*

** *fosfomycin, methenamine, linezolid, daptomycin*

*** *J01BA, J01DE, J01DF, J01DI, J01EC and J01XC*

‡ *DDD including changes as of 2019 (source: WHO)*

Table 3.2.2 Ten years data on the use of antibiotics for systemic use (J01) in in-patient hospital care (DDD/1,000 inhabitant-days), 2013-2022 (source: SWAB)

ATC group*	Therapeutic group	2013	2014	2015	2016	2017	2018	2018‡	2019‡	2020‡	2021‡	2022‡
J01AA	Tetracyclines	0.022	0.023	0.025	0.022	0.021	0.023	0.023	0.021	0.019	0.016	0.022
J01CA	Penicillins with extended spectrum	0.099	0.101	0.118	0.125	0.117	0.110	0.052	0.063	0.050	0.044	0.048
J01CE	β-lactamase sensitive penicillins	0.023	0.028	0.028	0.029	0.029	0.033	0.033	0.024	0.022	0.021	0.024
J01CF	β-lactamase resistant penicillins	0.100	0.105	0.097	0.102	0.103	0.105	0.105	0.104	0.103	0.097	0.116
J01CR	Combinations of penicillins, incl. β-lactamase-inhibitors	0.199	0.187	0.186	0.171	0.159	0.153	0.128	0.109	0.098	0.078	0.083
J01DB	First-generation cephalosporins	0.047	0.052	0.055	0.053	0.065	0.070	0.070	0.066	0.056	0.061	0.060
J01DC	Second-generation cephalosporins	0.055	0.058	0.065	0.066	0.067	0.070	0.070	0.077	0.073	0.063	0.062
J01DD	Third-generation cephalosporins	0.062	0.066	0.067	0.068	0.067	0.072	0.072	0.074	0.085	0.093	0.093
J01DH	Carbapenems	0.020	0.019	0.021	0.020	0.021	0.020	0.014	0.014	0.013	0.014	0.013
J01EA	Trimethoprim and derivatives	0.004	0.003	0.003	0.003	0.003	0.003	0.003	0.002	0.002	0.002	0.002
J01EE	Combinations of sulfonamides and trimethoprim, incl. derivatives	0.024	0.022	0.021	0.024	0.023	0.022	0.022	0.022	0.024	0.021	0.023
J01FA	Macrolides	0.034	0.034	0.034	0.034	0.030	0.030	0.030	0.026	0.027	0.021	0.022
J01FF	Lincosamides	0.032	0.028	0.030	0.028	0.027	0.026	0.026	0.024	0.022	0.018	0.022
J01GB	Aminoglycosides	0.045	0.044	0.046	0.043	0.037	0.037	0.037	0.033	0.027	0.025	0.024
J01MA	Fluoroquinolones	0.116	0.112	0.112	0.106	0.097	0.087	0.079	0.071	0.066	0.057	0.061
J01XA	Glycopeptides	0.018	0.018	0.019	0.019	0.019	0.018	0.018	0.018	0.019	0.020	0.020
J01XB	Polymyxins	0.003	0.002	0.003	0.002	0.001	0.002	0.001	0.001	0.001	0.001	0.001
J01XD	Imidazole derivatives	0.030	0.030	0.032	0.032	0.034	0.033	0.033	0.033	0.031	0.031	0.030
J01XE	Nitrofuran derivatives	0.016	0.018	0.018	0.018	0.019	0.017	0.017	0.015	0.016	0.014	0.017
J01XX	Other antibacterials**	0.002	0.001	0.002	0.002	0.003	0.003	0.003	0.003	0.003	0.003	0.002
	Others***	0.000	0.000	0.001	0.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001
J01	**Antibiotics for systemic use (total)**	**0.950**	**0.953**	**0.982**	**0.968**	**0.942**	**0.934**	**0.836**	**0.799**	**0.760**	**0.700**	**0.747**

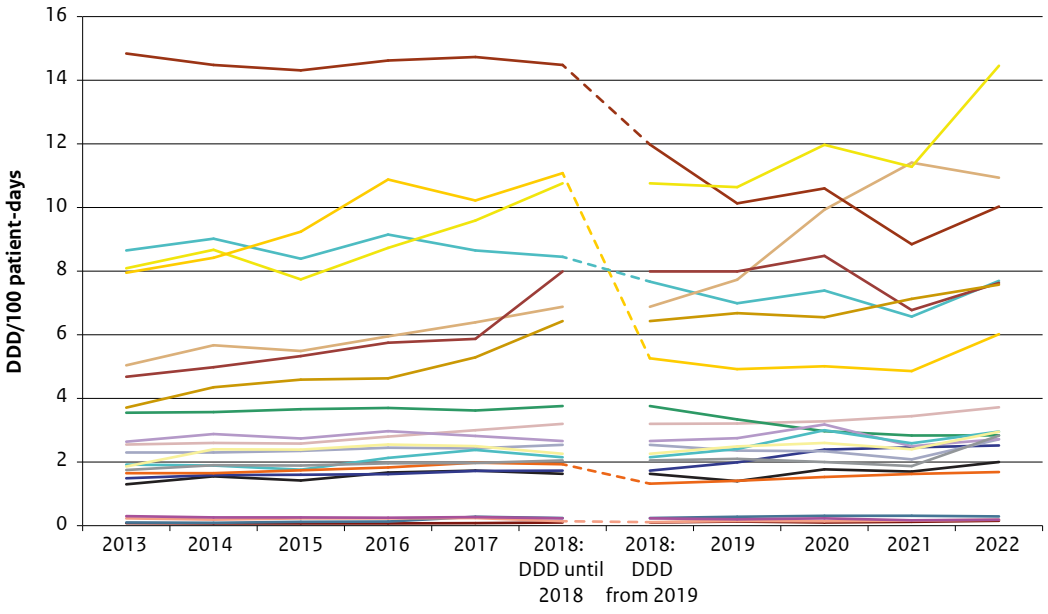
* *From the 2022 edition of the Anatomical Therapeutic Chemical (ATC) classification system*

** *fosfomycin, methenamine, linezolid, daptomycin*

*** *J01BA, J01DE, J01DF, J01DI, J01EC and J01XC*

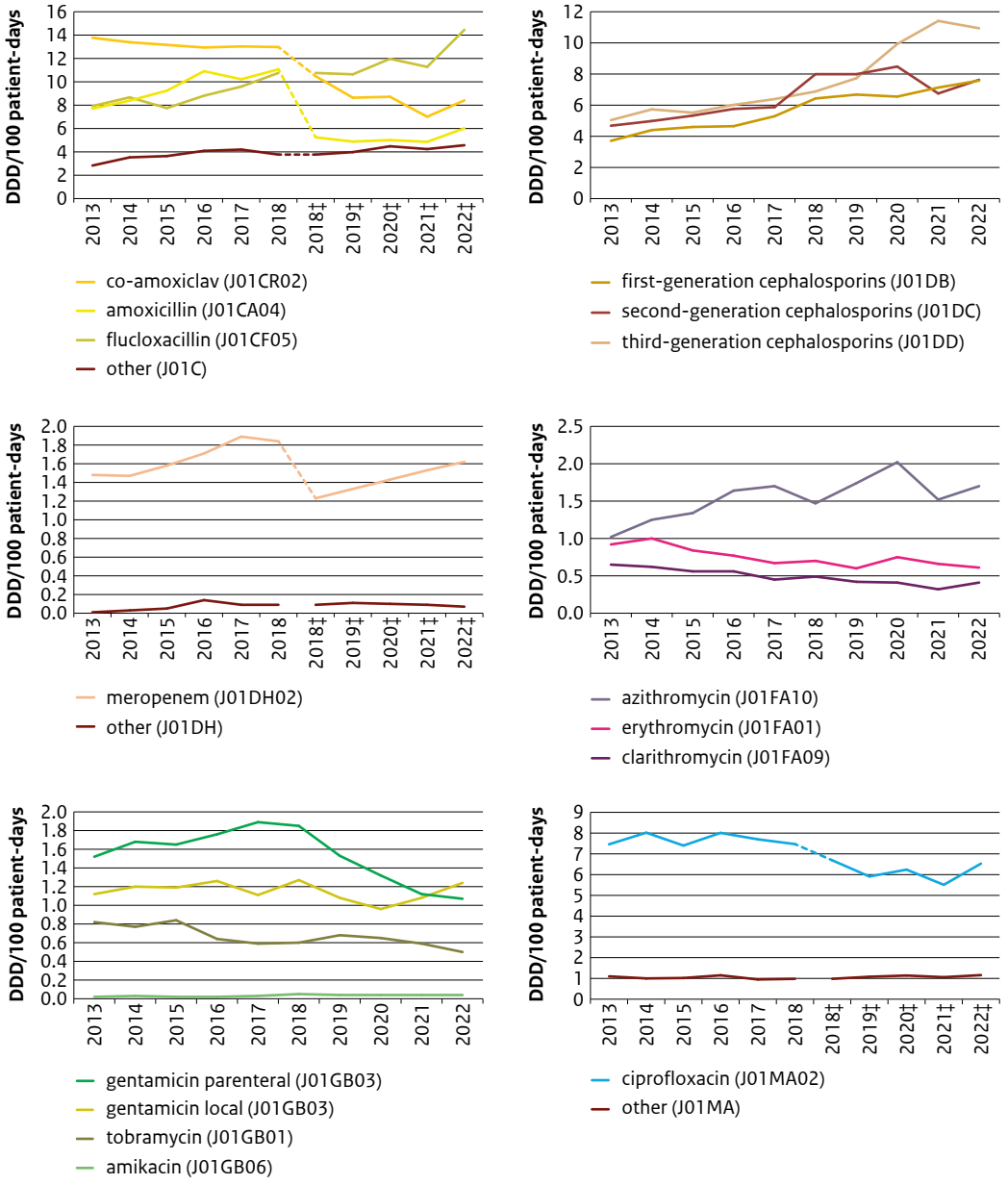
‡ *DDD including changes as of 2019 (source: WHO)*

Figure 3.2.1 Use of antibiotics for systemic use (J01) in hospitals (DDD/100 patient-days) at ATC-4 level, 2013-2022 (source: SWAB)



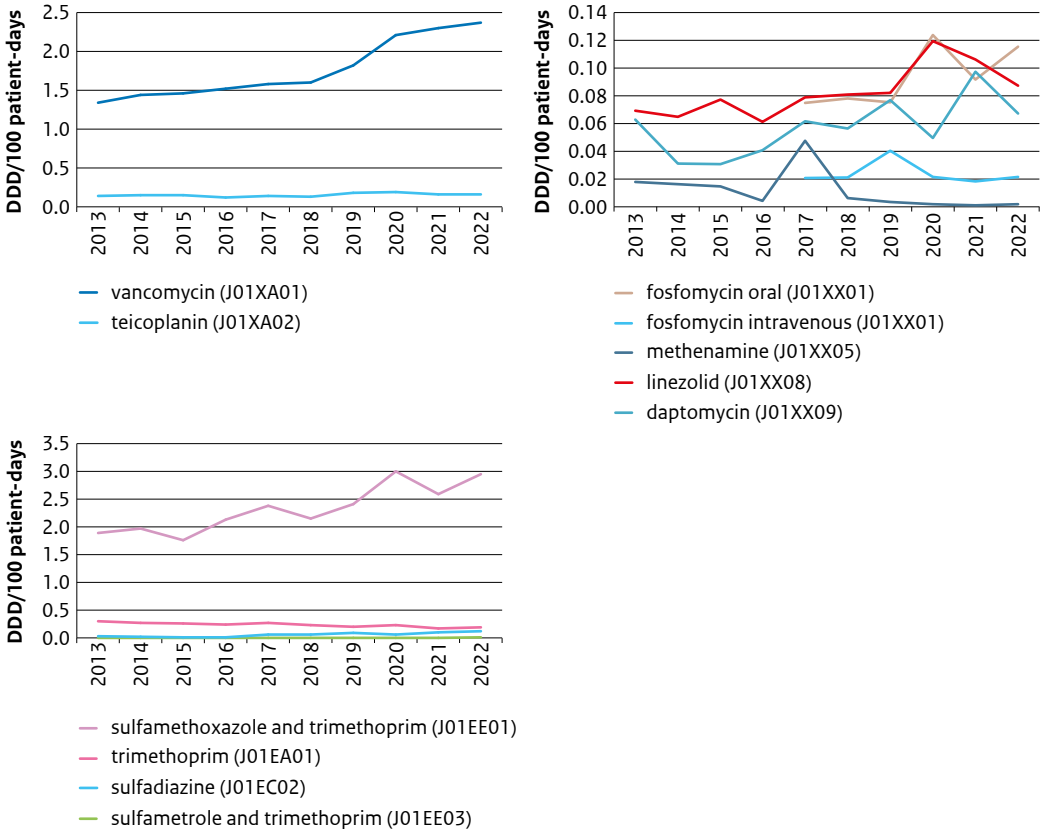
- J01AA tetracyclines
- J01CA penicillins with extended spectrum
- J01CE β-lactamase sensitive penicillins
- J01CF β-lactamase resistant penicillins
- J01CR combinations of penicillins, incl. β-lactamase-inhibitors
- J01DB first-generation cephalosporins
- J01DC second-generation cephalosporins
- J01DD third-generation cephalosporins
- J01DH carbapenems
- J01EA trimethoprim and derivatives
- J01EE combinations of sulfonamides and trimethoprim, incl. derivatives
- J01FA macrolides
- J01FF lincosamides
- J01GB aminoglycosides
- J01MA fluoroquinolones
- J01XA glycopeptides
- J01XB polymyxins
- J01XD imidazole derivatives
- J01XE nitrofuran derivatives
- J01XX other antibacterials
- Others (J01DF, J01DI, J01EC and J01XC)

Figure 3.2.2 Use of β-lactams, macrolides, aminoglycosides, fluoroquinolones, glycopeptides and other antibiotics in hospitals expressed as DDD/100 patient-days 2013-2022 (source: SWAB)



For antibiotics where the DDD was changed by the WHO in 2019, a dashed line is depicted from the DDD/100 patient-days in 2018 calculated using the DDD until 2018 to the DDD/100 patient-days in 2018 calculated using the DDD from 2019.
‡ DDD including changes as of 2019 (source: WHO)

Figure 3.2.2 (continued) Use of β-lactams, macrolides, aminoglycosides, fluoroquinolones, glycopeptides and other antibiotics in hospitals expressed as DDD/100 patient-days 2013-2022 (source: SWAB)



For antibiotics where the DDD was changed by the WHO in 2019, a dashed line is depicted from the DDD/100 patient-days in 2018 calculated using the DDD until 2018 to the DDD/100 patient-days in 2018 calculated using the DDD from 2019.
‡ DDD including changes as of 2019 (source: WHO)

Figure 3.2.3 Comparison of the total systemic antibiotic drug use (J01) across Dutch hospitals in 2022 (source: SWAB)

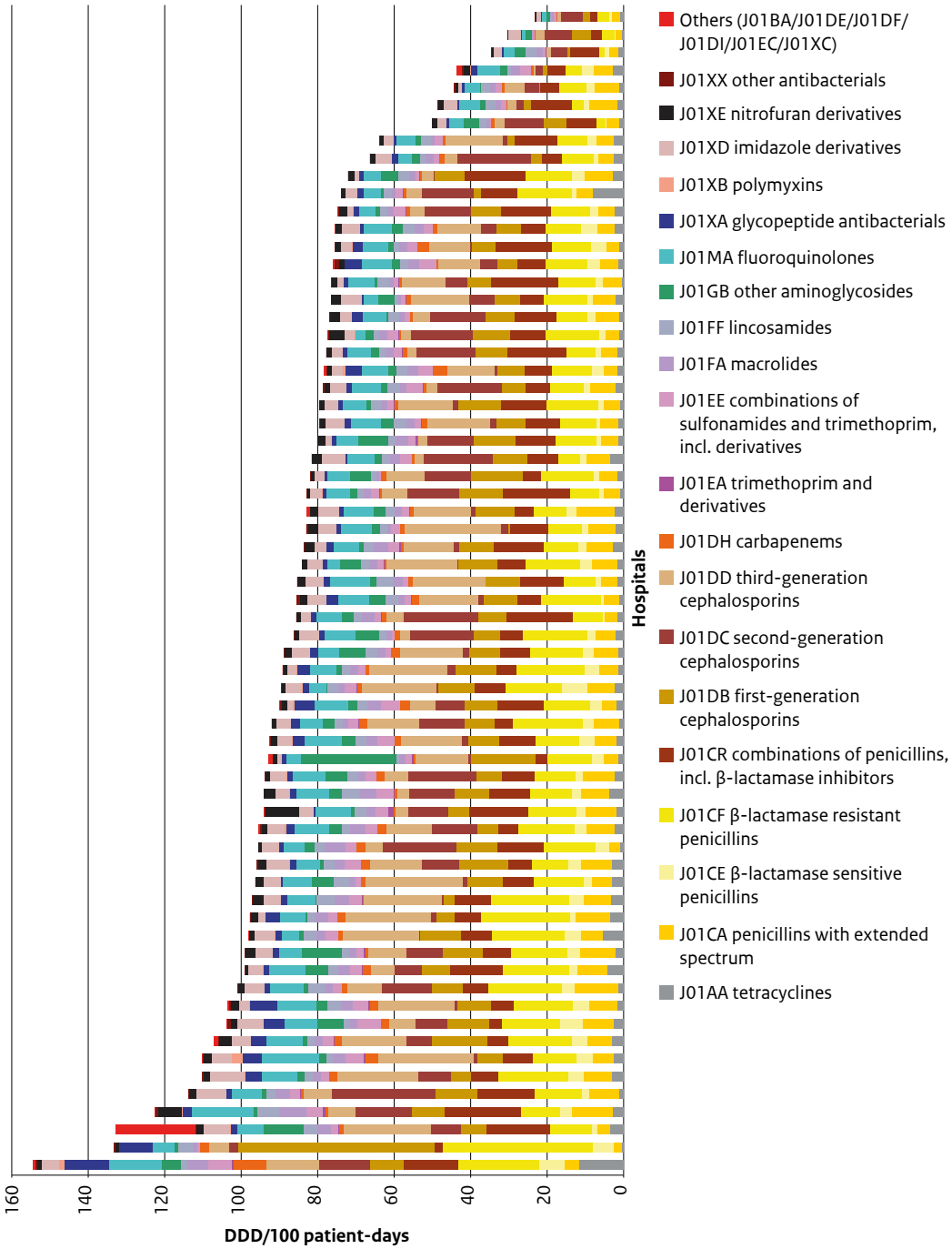
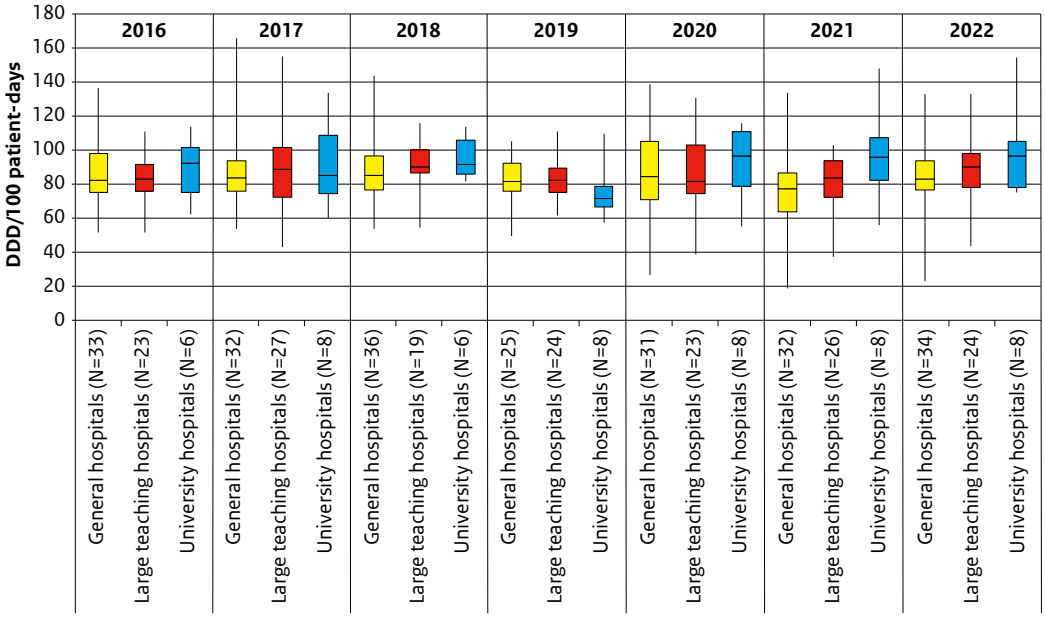


Figure 3.2.4 Seven years use of total systemic antibiotic use (J01) and comparison across university, large teaching and general hospitals (source: SWAB)



boxplot shows minimum - P25 - median - P75 - maximum

Figure 3.2.5 Distribution (%) of the use of antibiotics for systemic use (J01) in hospitals, 2022 (source: SWAB)

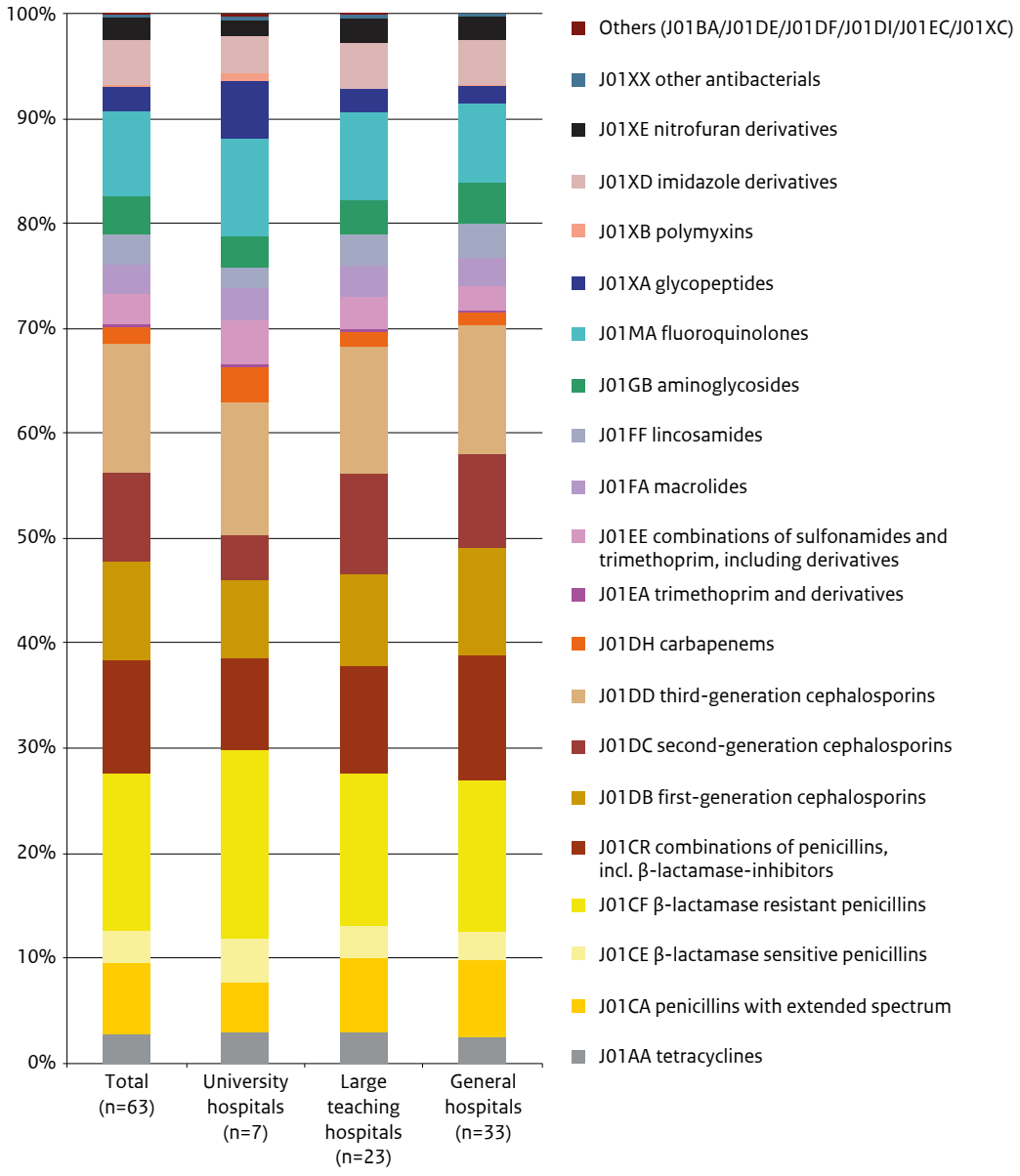
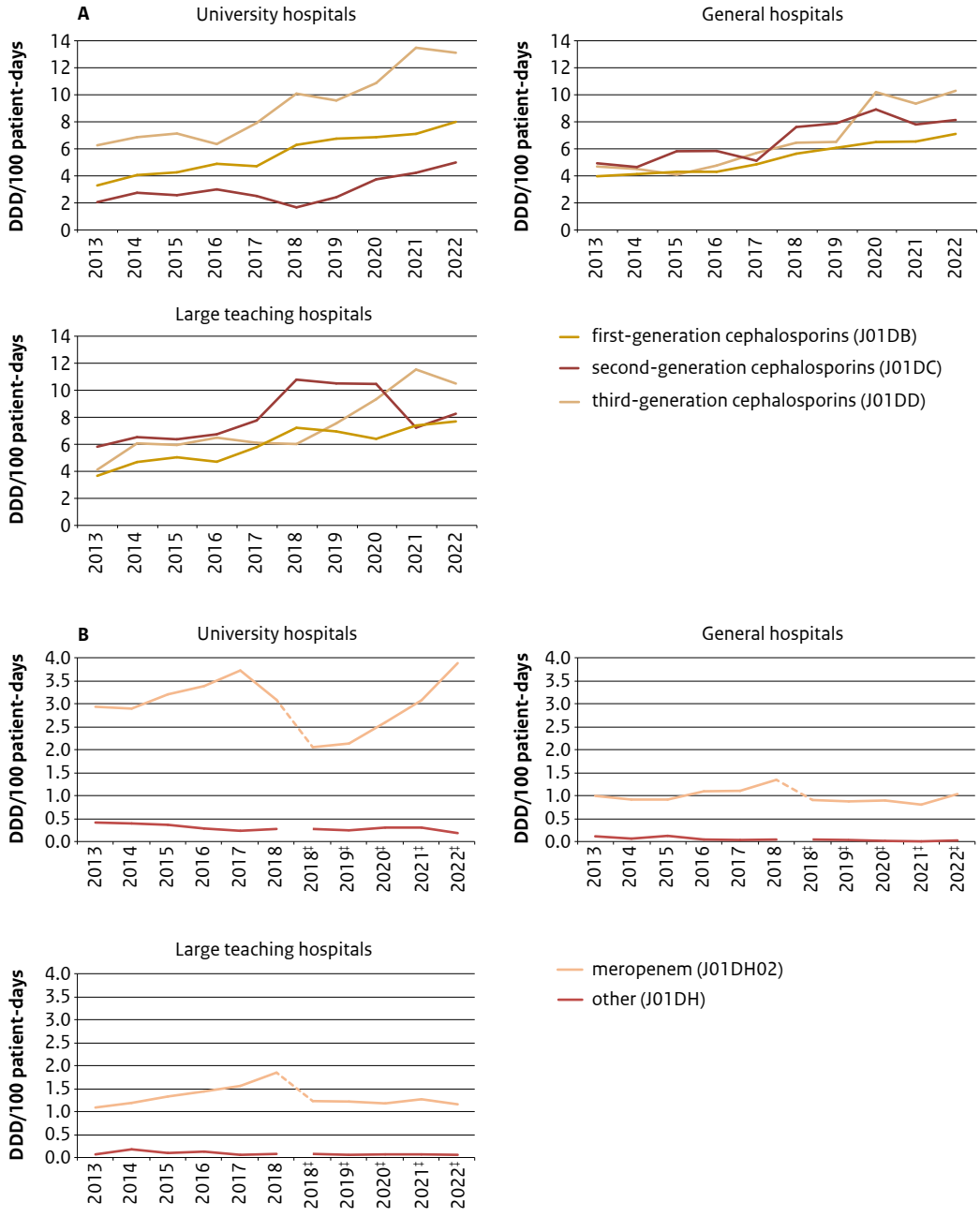
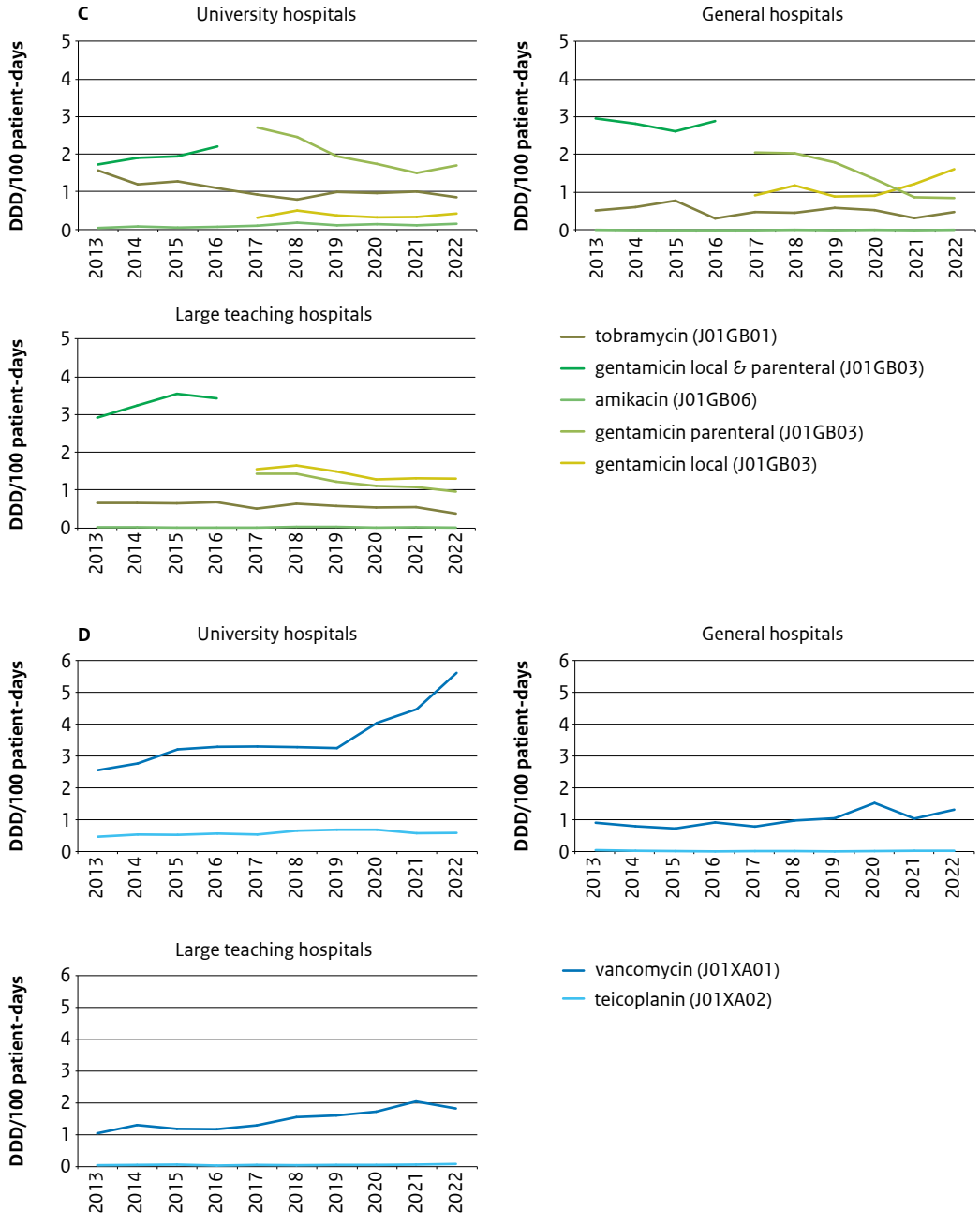


Figure 3.2.6 Use of cephalosporins (A), carbapenems (B), aminoglycosides (C), glycopeptides (D) and fluoroquinolones (E) in hospitals broken down by type of hospital, expressed as DDD/100 patient-days (2013-2022, source: SWAB)



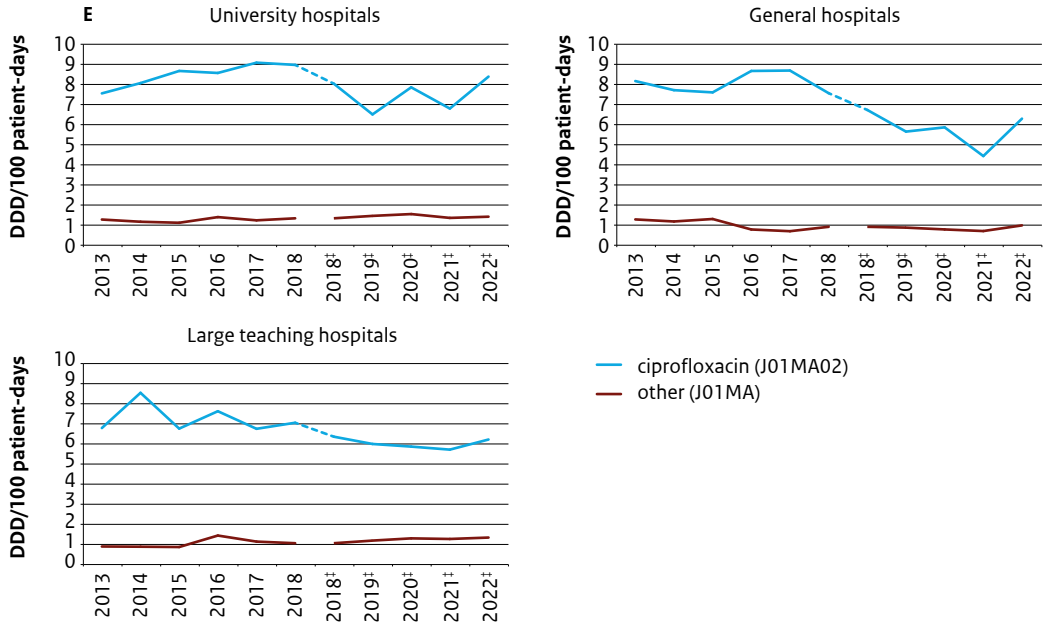
‡ *DDD including changes as of 2019 (source: WHO)*

Figure 3.2.6 (continued) Use of cephalosporins (A), carbapenems (B), aminoglycosides (C), glycopeptides (D) and fluoroquinolones (E) in hospitals broken down by type of hospital, expressed as DDD/100 patient-days (2013-2022, source: SWAB)



‡ *DDD including changes as of 2019 (source: WHO)*

Figure 3.2.6 (continued) Use of cephalosporins (A), carbapenems (B), aminoglycosides (C), glycopeptides (D) and fluoroquinolones (E) in hospitals broken down by type of hospital, expressed as DDD/100 patient-days (2013-2022, source: SWAB)



‡ *DDD including changes as of 2019 (source: WHO)*

Figure 3.2.7 Use of 1st, 2nd and 3rd generation cephalosporins in university, large teaching and general hospitals at ATC-5 level (2013-2022) (source: SWAB)

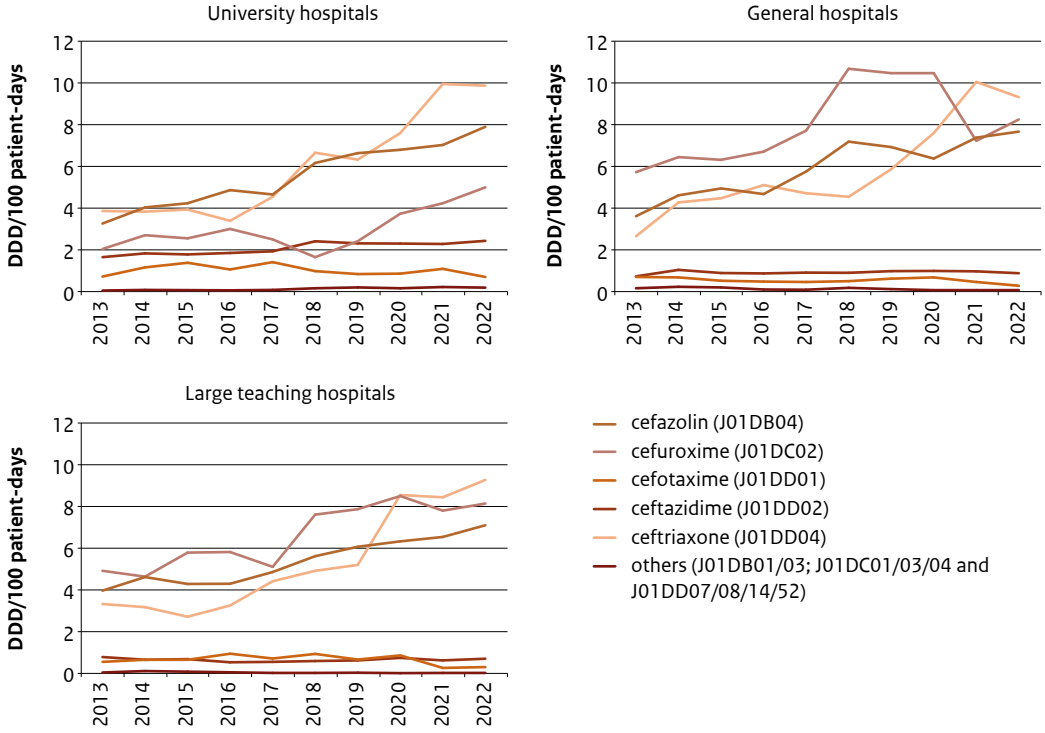


Table 3.2.3 Use of antimycotics (J02) in hospitals (DDD/100 patient-days), 2017-2022 (source: SWAB)

ATC group*	Therapeutic group	2017				2018				2019			
		total N=67	academic hospitals N=8	large teaching hospitals N=27	general hospitals N=32	total N=61	academic hospitals N=6	large teaching hospitals N=19	general hospitals N=36	total N=57	academic hospitals N=8	large teaching hospitals N=24	general hospitals N=25
J02AA01	Antibiotics (amphotericin B)	1.28	4.80	0.38	0.19	1.04	4.36	0.48	0.26	1.12	3.10	0.68	0.29
J02AB02	Imidazole derivatives (ketoconazole)	0.02	0.08	0.02	0.00	0.01	0.02	0.01	0.00	0.03	0.06	0.04	0.01
J02AC	Triazole derivatives	3.12	7.80	2.04	1.47	3.03	7.84	2.48	1.66	3.26	7.12	2.42	1.63
J02AX	Other antimycotics for systemic use (mainly echinocandines)	0.38	0.96	0.24	0.20	0.44	1.03	0.35	0.30	0.53	1.08	0.43	0.27
J02	**Antimycotics for systemic use (total)**	**4.81**	**13.6**	**2.67**	**1.86**	**4.52**	**13.2**	**3.33**	**2.22**	**4.95**	**11.4**	**3.57**	**2.20**

ATC group*	Therapeutic group	2020				2021				2022			
		total N=62	academic hospitals N=8	large teaching hospitals N=31	general hospitals N=23	total N=66	academic hospitals N=8	large teaching hospitals N=26	general hospitals N=32	total N=63	academic hospitals N=7	large teaching hospitals N=23	general hospitals N=33
J02AA01	Antibiotics (amphotericin B)	1.36	3.42	0.95	0.33	1.23	3.03	0.90	0.41	1.00	3.40	0.55	0.30
J02AB02	Imidazole derivatives (ketoconazole)	0.01	0.03	0.00	0.01	0.02	0.05	0.01	0.01	0.02	0.07	0.00	0.00
J02AC	Triazole derivatives	3.72	8.16	2.77	1.63	3.64	7.00	3.09	1.95	3.27	7.49	2.63	1.79
J02AX	Other antimycotics for systemic use (mainly echinocandines)	0.63	1.03	0.54	0.44	0.74	1.08	0.71	0.54	0.68	1.73	0.49	0.37
J02	**Antimycotics for systemic use (total)**	**5.71**	**12.6**	**4.27**	**2.42**	**5.63**	**11.2**	**4.71**	**2.91**	**4.97**	**12.7**	**3.67**	**2.46**

3.3 Long-term care facilities

Methods

All hospital pharmacists participating in the SWAB surveillance of antibiotic use in hospitals were asked to provide antibiotic consumption data of long-term care facilities (LTCFs) their pharmacy is serving. For each facility the amount of DDD/1,000 residents/day assuming occupancy of 100% and their weighed mean, capacity based, are calculated.

Results

The data of 2021 originates from 21 LTCFs or organizations serving 16,170 residents. The size of LTCFs varied from 55 to 2,455 residents per home or organization, with a median of 700 residents. The data of 2022 also originates from 21 LTCFs or organizations serving 11,155 residents. The size of LTCFs varied from 54 to 2,000 residents per home or organization, with a median of 860 residents.

The antibiotic use in LTCFs decreased from 50.4 to 38.1 DDD/1,000 residents/day in 2021 compared to 2020. In 2022 this recovered to 43.9 DDD/1,000 residents/day. Most common used antibiotics are as before the combination of penicillins (amoxicillin with clavulanic acid), nitrofurantoin and fluoroquinolones (table 3.3.1).

Discussion

The discussion of 2021 data is challenging due to the impact of the COVID-19 pandemic, with potential effects also extending to the data of 2022. The assumption of 100% bed occupancy is likely incorrect and constitutes a significant factor contributing to the sharp decrease observed in 2021. Despite minor shifts in usage patterns, amoxicillin with clavulanic acid, fluoroquinolones, and nitrofuran derivatives persist as the most extensively utilized systemic antibiotics in LTCFs. The notable prevalence of these antibiotics is unsurprising, given that urinary tract infections rank among the most common infections affecting elderly patients. Concerning broad-spectrum antibiotics, the persistently high usage of fluoroquinolones remains a cause for concern.

Table 3.3.1 Distribution of the use of antibiotics for systemic use (J01) in long-term care facilities, (expressed as weighted mean) DDD/1,000 residents/day, 2013-2022 (source: SWAB)

ATC group*	Therapeutic group	2013	2014	2015	2016	2017	2018	2018‡	2019‡	2020‡	2021‡	2022‡
J01AA	Tetracyclines	6.2	4.7	3.9	4.9	4.0	5.0	5.0	3.7	2.9	2.6	2.3
J01CA	Penicillins with extended spectrum	4.3	5.1	5.0	5.6	4.6	3.8	2.4	2.6	4.8	2.4	2.9
J01CE	β-lactamase sensitive penicillins	0.5	0.5	0.7	0.3	0.6	0.4	0.4	0.5	0.4	0.3	0.3
J01CF	β-lactamase resistant penicillins	1.7	1.4	2.3	1.8	2.2	3.3	3.3	3.0	2.5	2.4	2.9
J01CR	Combinations of penicillins, incl. β-lactamase-inhibitors	19.5	16.3	17.9	16.1	15.5	18.0	12.1	12.0	10.2	7.7	10.7
J01DB	First-generation cephalosporins	0.0	0.1	0.1	0.0	0.2	0.1	0.1	0.0	0.2	0.3	0.1
J01DC	Second-generation cephalosporins	0.2	0.1	0.2	0.1	0.3	0.1	0.1	0.2	1.0	0.1	0.1
J01DD	Third-generation cephalosporins	0.6	0.6	0.8	0.4	0.5	0.4	0.4	0.4	0.5	0.4	0.6
J01DH	Carbapenems	0.0	0.0	0.1	0.0	0.1	0.1	0.1	0.3	0.1	0.0	0.1
J01EA	Trimethoprim and derivatives	2.4	1.9	1.4	1.6	1.6	1.2	1.2	0.8	1.2	1.0	0.7
J01EE	Combinations of sulfonamides and trimethoprim, including derivatives	1.7	1.5	1.6	1.1	1.2	1.9	1.9	3.0	2.6	1.9	1.7
J01FA	Macrolides	1.8	1.8	2.1	2.4	2.8	2.7	2.7	2.7	3.0	2.3	3.3
J01FF	Lincosamides	2.4	2.0	2.6	3.7	2.9	3.0	3.0	2.9	2.2	1.8	2.2
J01GB	Aminoglycosides	0.0	0.2	0.2	0.1	0.3	0.1	0.1	0.0	0.0	0.0	0.1
J01MA	Fluoroquinolones	8.3	8.4	8.9	8.2	6.9	8.7	8.7	7.3	9.1	5.5	6.7
J01XA	Glycopeptides	0.1	0.1	0.2	0.1	0.2	0.2	0.2	0.4	0.1	0.2	0.1
J01XB	Polymyxins	0.0	0.0	0.1	0.2	0.0	0.1	0.1	0.0	0.1	0.0	0.1
J01XD	Imidazole derivatives	0.0	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.0
J01XE	Nitrofuran derivatives	11.1	10.4	11.4	9.6	8.3	11.3	11.3	9.5	8.2	8.1	8.0
J01XX	Other antibacterials**	0.4	0.2	0.5	0.8	0.8	0.7	0.7	0.9	1.4	0.8	0.8
	Others***	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0
J01	**Antibiotics for systemic use (total)**	**61.1**	**55.3**	**60.0**	**57.2**	**52.9**	**61.4**	**53.9**	**50.4**	**50.4**	**38.1**	**43.9**

* *From the 2021 edition of the Anatomical Therapeutic Chemical (ATC) classification system*

** *fosfomycin, methenamine, linezolid, daptomycin*

*** *J01DF, J01DI, J01EC and J01XC*

‡ *DDD including changes as of 2019 (source: WHO)*

References

1 WHO Collaborating Centre for Drug Statistics Methodology. DDD alterations from 2005-2020. Available from: https://www.whocc.no/atc_ddd_alterations__cumulative/ddd_alterations/ [Accessed March 7, 2022]

2 WHO Collaborating Centre for Drug Statistics Methodology. ATC index with DDDs 2020. WHO Collaborating Centre; Oslo, Norway, 2020.

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4
Surveillance of resistance

4.1 Methods and description of data from the Infectious Diseases Surveillance Information System for Antimicrobial Resistance (ISIS-AR)

4.1.1 Methods

Since 2008, routinely available antimicrobial susceptibility data of all isolates from medical microbiology laboratories in the Netherlands, including minimal inhibitory concentration (MIC) values and disk zone diameters, are collected in the Infectious Diseases Surveillance Information System for Antimicrobial Resistance (ISIS-AR). This surveillance system is a combined initiative of the Ministry of Health, Welfare and Sport and the Dutch Society of Medical Microbiology (NVMM), and is coordinated by the Centre for Infectious Disease Control at the National Institute for Public Health and the Environment (RIVM) in Bilthoven.

In 2022, 47 laboratories were connected to ISIS-AR, all performing antimicrobial susceptibility testing (AST) according to EUCAST guidelines. Out of these 47 laboratories, 37 provided complete data on the last five years (2018 to 2022). Five of these 37 laboratories exclusively served university hospitals; 29 laboratories served non-university hospitals, general practices, and long-term care facilities; and three laboratories exclusively served general practices and long-term care facilities. For the analyses in sections 4.2, 4.3, 4.5, and 4.6 we selected only data from these 37 laboratories to avoid bias in time trends due to incomplete data.

Because no time trends were calculated for resistance by regional cooperative network[1] in section 4.2 and for resistance percentages for long-term care facilities in section 4.4, we used for those analyses data from 34 non-university laboratories for which at least complete data on 2022 were available (31 serving non-university hospitals, general practices, and long-term care facilities; and three serving general practices and long-term care facilities only).

All data provided to ISIS-AR are carefully validated[2]. Data with confirmed or probable technical errors are, after consultation with the laboratory that provided the data, corrected or excluded from the analyses in this report.

Selection of isolates

We calculated resistance levels and, if applicable, time trends by setting of care, i.e., general practices, outpatient departments, inpatient departments (excl. intensive care units, incl. emergency departments), intensive care units, urology departments (inpatient and outpatient separately), and long-term care facilities. For general practices (section 4.2) and long-term care facilities (section 4.4), we selected urine isolates for analysis of resistance in Enterobacterales and *Pseudomonas aeruginosa* (in accordance with age categories used in the guidelines of the Dutch College of General Practitioners (NHG) for urinary tract infections, resistance levels and five-year trends for urine isolates in general practice patients were calculated separately for patients aged ≤12 years and patients aged >12 years), wound or pus isolates for analysis of resistance in the *Staphylococcus aureus* complex, wound or pus, respiratory, and genital isolates for analysis of resistance in β-haemolytic *Streptococcus* group A, and urinary and genital isolates for analysis of resistance in β-haemolytic *Streptococcus* group B. For analyses on data from outpatient departments (section 4.3.1), inpatient departments (excl. intensive care units, section 4.3.2), and intensive care units (section 4.3.3), we selected isolates from blood, cerebrospinal fluid, urine, lower respiratory tract, and wound or pus. Additionally, we conducted a separate analysis for blood isolates from inpatients (incl. patients from intensive care units, section 4.3.4). For urology departments (section 4.3.5), we selected only urine isolates. In section 4.5, we performed a separate analysis on respiratory pathogens (*Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis*), separately for general practitioners' patients and hospital patients. We selected isolates from the upper and lower respiratory tract for the analysis on general practitioners' patients. For the analysis on hospital patients, we additionally selected isolates from blood and cerebrospinal fluid. Finally, in section 4.6 we performed a separate analysis on *Helicobacter pylori*, for which we selected isolates from gastro-intestinal mucosa, pus, and normally sterile tissue or liquid as we could not distinguish gastric specimens specifically.

The category 'wound or pus isolates' comprises isolates from deep and superficial wounds, pus (including pus from abscesses), but also skin (excluding perineal swabs), normally sterile sites or taken using a sterile procedure (i.e., biopsy, aspiration), synovial fluid, peritoneal cavity fluid and fluid for continuous ambulatory peritoneal dialysis (CAPD), eyes (both normally sterile and non-sterile sites), amniotic fluid, and samples from- or related to medical implants. The category 'lower respiratory isolates' comprises respiratory isolates from below the glottis, whereas 'upper respiratory tract isolates' originate from respiratory samples that were taken above the glottis.

Since the number of *Staphylococcus argenteus* and *Staphylococcus schweitzeri* isolates was too small for separate analyses, the data for *S. aureus*, *S. argenteus* and *S. schweitzeri*, all belonging to the *S. aureus* complex, were analysed together and further referred to as *S. aureus*. In all sections 4.2 through 4.4, *S. argenteus* comprised 0 to 0.03% of the isolates from this complex. *S. schweitzeri*, the third member of the *S. aureus* complex, was found once in a patient from an outpatient department, and once in a patient from a non-ICU hospital department.

For each analysis, we selected the first isolate per species per patient per year to avoid repeated sampling causing bias in the calculation of resistance levels and time trends. We included only data on

diagnostic samples, and only calculated resistance levels for pathogens for which at least 100 isolates in each year were available for analysis. Furthermore, to avoid bias due to selective testing of agents, for each pathogen-agent combination, we included only data from laboratories that tested at least 50% of isolates for that specific agent in each year. Finally, for sufficient representativeness of the results, we only calculated the resistance level and time trend of a pathogen-agent combination if the data from at least 50% of the selected laboratories could be included.

Calculation of resistance levels

We calculated the percentage of resistant isolates ('R'). To avoid bias due to differences in (versions of) breakpoint guidelines and expert rules used in the participating laboratories, we first reinterpreted all crude test values according to EUCAST breakpoints version 12.0 (2022). Since 2019, EUCAST has defined an area of technical uncertainty (ATU) for several pathogen-agent combinations. These ATUs are warnings to laboratory staff that there is an uncertainty that needs to be addressed before reporting the susceptibility results to clinical colleagues. EUCAST specifically states that "the ATU is not a susceptibility category and does not prevent the laboratory from interpreting the susceptibility results". Laboratories are encouraged (but not obliged) by EUCAST to perform an alternative test (e.g., an MIC-test instead of disk diffusion) when the test value is within the ATU. Therefore, we reinterpreted all test values according to the EUCAST breakpoints version 12.0, including the test values that were within the ATU, trusting that laboratories conducted and reported re-tests if indicated. Nevertheless, this policy might have resulted in some misclassification if laboratories did not perform an alternative test, resulting in an interpretation of the test value that lies within the ATU to 'R', whereas the isolate is in reality susceptible or vice versa. However, we do not expect that this misclassification has strongly influenced resistance percentages, since the proportion of isolates with test values in the ATU is low. Also in 2019, EUCAST has redefined the category 'I' from a lumped definition of 1) uncertain therapeutic effect, 2) susceptible only for treatment in specific body sites or with high dosing regime, and 3) a buffer zone for technical laboratory uncertainties, to the definition 'Susceptible, increased exposure. From then onwards, the technical uncertainty was covered by the ATU, as described before, and the number of pathogen-agent combinations for which an I-category was defined in the breakpoints decreased. Nevertheless, because we calculated the percentage of resistant isolates ('R'), and reinterpreted all test-values, including those from previous years, according to EUCAST breakpoints version 12.0 this did not influence resistance percentages or trends.

We included data from all laboratories for which at least 80% of test values could be reinterpreted each year. Where reinterpretation was not possible, this was due to missing crude data or test values that were not compatible with EUCAST breakpoints.

For several pathogen-agent combinations EUCAST has specified breakpoints that apply only to a specific diagnosis or treatment strategy. For Enterobacterales, the co-amoxiclav MIC breakpoint for uncomplicated urinary tract infection could not be used to reinterpret MIC values because the maximum test value of >16 mg/L that can be measured by the VITEK2 system does not reach the breakpoint of 32 mg/L. Therefore, in sections 4.2 through 4.4, for Enterobacterales, we only present resistance to co-amoxiclav and all combinations of agents that include co-amoxiclav according to the breakpoint for indications other than uncomplicated urinary tract infections. Likewise, in *Escherichia coli*, the fosfomycin MIC breakpoint for oral administration in uncomplicated urinary tract infection could not be used to reinterpret MIC values, because the minimum test value of ≤16 as measured by both the VITEK2 system and the Phoenix system do not reach the breakpoint of 8 mg/L. To approach resistance percentages for oral administration as close

as possible, we reinterpreted mic-values according to the lowest cut-off that was possible; which was 16 mg/L, whereas we reinterpreted diameters according to the EUCAST breakpoint for oral administration (24 mm).

For both cefotaxime/ceftriaxone in Enterobacterales and meropenem in Enterobacterales, *P. aeruginosa*, and *Acinetobacter* spp. EUCAST has defined separate breakpoints for meningitis and indications other than meningitis. In the current report, for cefotaxime/ceftriaxone, meropenem and all empirical therapy combinations that include one of these agents we present only resistance percentages for indications other than meningitis. Likewise, EUCAST has defined separate breakpoints for screening for erytromycin in *S. aureus*, and tetracycline in *S. aureus*, β-haemolytic *Streptococcus* spp., and *S. pneumoniae*. In the current report we only show resistance percentages according to this screening breakpoint.

Because data on inducible clindamycin resistance tests were often not available in ISIS-AR, we calculated resistance levels for clindamycin including inducible resistance in *Staphylococcus* spp. and *Streptococcus* spp. based on re-interpretation of raw test values for clindamycin resistance, unless there was a positive test on inducible clindamycin resistance. In that case we considered the isolate as resistant to clindamycin. If no data on a test for inducible clindamycin test were available, but the laboratory reported the isolate resistant we assumed that results of the inducible resistance test was taken into account and considered the isolate as resistant.

For part of the laboratories no data were available on cefoxitin to screen for MRSA, and flucloxacillin results based on cefoxitin screening methods were reported. Therefore, we estimated the percentage MRSA among *S. aureus* based on positivity of MRSA confirmation tests (presence of *mecA* or *mecC* gene or pbp2), whereas if these tests were lacking, prevalence was based on laboratory S/R interpretation for cefoxitin or, if no data on a cefoxitin test was available, for flucloxacillin/oxacillin.

To estimate resistance to (benzyl)penicillin in *S. pneumoniae*, results of the oxacillin screening were taken into account. If the oxacillin zone diameter was ≥20 mm, we estimated resistance based on reinterpretation of oxacillin test values, or, if the oxacillin zone diameter was <20 mm, on reinterpretation of test values for (benzyl)penicillin. However, available gradient tests (Etest™ and MTS™) systematically underestimate (benzyl)penicillin MIC values in *S. pneumoniae*[5]. Therefore, resistance percentages for (benzyl)penicillin in *S. pneumoniae* may be biased towards a lower level.

To test resistance of *H. influenzae* to β-lactam antibiotics EUCAST has specified a flowchart with testing steps based on testvalues for (benzyl)penicillin, β-lactamase, co-amoxiclav, and the β-lactam antibiotic of interest. To resemble this flowchart we used an algorithm to estimate resistance to amoxicillin/ampicillin and co-amoxiclav as depicted in figure 4.1.1.1.

For some antimicrobial agents presented in this report, comparable resistance mechanisms exist, namely benzylpenicillin/penicillin, amoxicillin/ampicillin, cefotaxime/ceftriaxone, meropenem/imipenem (except for *P. aeruginosa* and *Proteus mirabilis*), and doxycycline/tetracycline, and often the laboratories report results for either one. For these combinations, we calculated the percentage of isolates that was resistant to at least one of both agents. Additionally, for Gram-negative bacteria except *Enterobacter cloacae* complex and *Acinetobacter* spp., we calculated resistance to specific combinations of agents that are frequently used for empiric therapy (for Enterobacterales: co-amoxiclav + gentamicin, cefuroxime + gentamicin, cefotaxime/

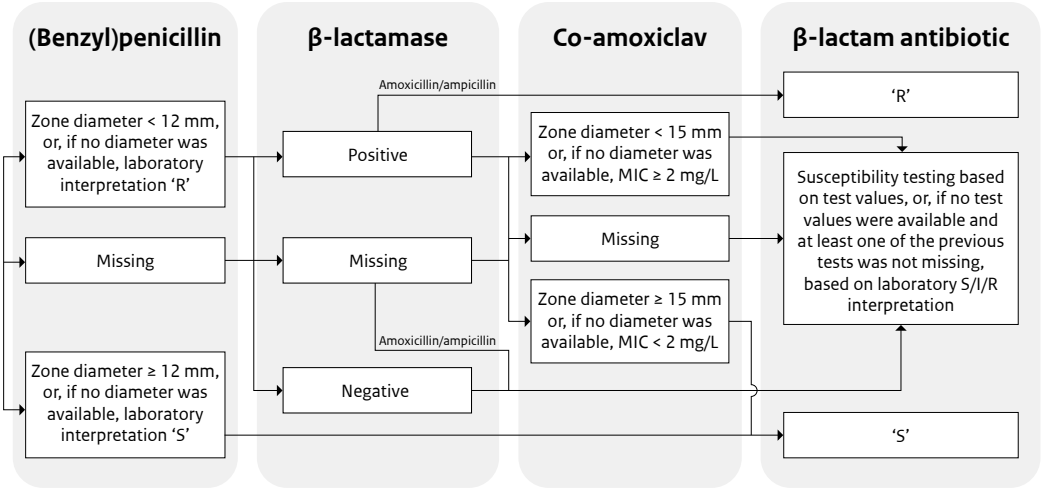
ceftriaxone + gentamicin, co-amoxiclav + ciprofloxacin, cefuroxime + ciprofloxacin, and cefotaxime/ceftriaxone + ciprofloxacin; for *P. aeruginosa*: ceftazidime + tobramycin and ciprofloxacin + tobramycin; for *H. pylori* clarithromycin + metronidazole). For these combinations, we defined resistance as resistance to both agents.

For *S. aureus*, no data on levofloxacin were available for a large part of laboratories. Therefore, we calculated resistance to ciprofloxacin as a class indicator for resistance to fluoroquinolones. However, ciprofloxacin should not be considered as a first choice for treatment of infections with these pathogens.

For Enterobacterales isolates, we calculated the percentage of isolates that was multidrug resistant to oral therapy (MDOT), which we defined as resistance to the oral agents co-amoxiclav (according to the breakpoint for indications other than uncomplicated urinary tract infections), ciprofloxacin, and co-trimoxazole combined.

For *E. coli*, *Klebsiella pneumoniae*, and *S. aureus* isolates from general practitioners' patients, we conducted an extra analysis to calculate resistance to a selection of agents in 2022 by regional cooperative network[1]. We compared resistance levels in general practitioners' patients within the regional cooperative networks with the resistance percentage in all regions combined, with a two-sided p-value of <0.05 being statistically significant and a difference that was larger than the square root of the national resistance percentage being microbiologically relevant. In the corresponding figures, differences in resistance percentages that were both statistically significant and microbiologically relevant are indicated by an asterisk.

Figure 4.1.1.1 Flowchart depicting the algorithm used to calculate resistance to β-lactam antibiotics in *H. influenzae*



Calculation of time trends

In addition to resistance levels in 2022, we calculated for sections 4.2, 4.3, 4.5, and 4.6 time trends over the last five years (2018 to 2022) using logistic regression models, except when data in one or more years before 2022 did not meet criteria for calculation of resistance levels. Because adoption of new guidelines or changes in breakpoints can have a substantial effect on resistance levels, we only analysed trends for resistance levels that were based on reinterpretation of crude test values from all five years according to EUCAST breakpoint guidelines version 12.0. We made an exception for trends in resistance for flucloxacillin and clindamycin including inducible resistance in *S. aureus*, which we based on laboratory S/I/R interpretation. However, we do not expect spurious time trends in resistance for these two pathogen-agent combinations because EUCAST breakpoints for these combinations were not changed between 2018 and 2022.

Sampling policies in long-term care facilities are currently subject to change. Since the degree of restrictive sampling influences the magnitude of overestimation of resistance percentages, this may result in spurious time trends. Therefore, time trends were not calculated for isolates from long-term care facilities.

We considered two-sided p-values for trend <0.05 to be statistically significant. When the absolute difference in predicted resistance from the logistic regression model between 2018 and 2022 was larger than the square root of the predicted resistance in 2018, we considered the trend to be microbiologically relevant. Statistically significant increasing trends that were considered to be microbiologically relevant are indicated in a red font, together with an up arrow, whereas decreasing trends that meet the same criteria are indicated in green, together with a down arrow. In addition, for each pathogen-agent combination for which the resistance levels were higher than 0.5% for at least one year and lower than 30% for at least three years, the resistance levels from 2018 to 2022 are shown in bar charts. For co-amoxiclav resistance levels are also shown when resistance levels were lower than 30% for less than three years. In those cases exact resistance levels are mentioned in a footnote. Trends that meet the criteria for significant and microbiologically relevant are indicated with an asterisk.

[1] Rijksinstituut voor volksgezondheid en milieu (RIVM) 2019, *Regionale aanpak*, accessed 16 March 2022, https://www.rivm.nl/antibioticaresistentie/nationale-aanpak-antibioticaresistentie/zorgnetwerken.

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[6] Werkgroep Infectiepreventie 2017, *Bijzonder resistente micro-organismen (BRMO)*, Rijksinstituut voor volksgezondheid en milieu (RIVM), accessed 16 March 2022, https://www.rivm.nl/wip-richtlijn-brmo-bijzonder-resistente-micro-organismen-zkh.

4.1.2 Description of the ISIS-AR data

In this section, several descriptive characteristics of the data from the ISIS-AR antimicrobial resistance surveillance system are presented. In figure 4.1.2.1, the smoothed distribution of isolates over the country, based on the percentage of inhabitants for whom at least one isolate was included in the analyses in sections 4.2 through 4.6, is shown by 4-digit postal code area. Furthermore, in the same figure the geographical distribution of laboratories is presented by status of connection to ISIS-AR and inclusion in the analyses in sections 4.2 through 4.6 (see section 4.1.1 for inclusion criteria). In table 4.1.2.1, characteristics of included isolates are listed by pathogen.

Each year all laboratories are included that send data on at least the last year to ISIS-AR. This results in variation in the mixture of included laboratories through the years, and data from this chapter can not be compared to data from Nethmap 2022.

Figure 4.1.2.1 Geographical distribution of laboratories, by status of connection to ISIS-AR and inclusion in the analyses in sections 4.2 to 4.6, together with smoothed geographical distribution of isolates, based on the percentage of inhabitants for whom at least one isolate was included in those analyses, by 4-digit postal code area and with regional cooperative network borders, ISIS-AR 2022

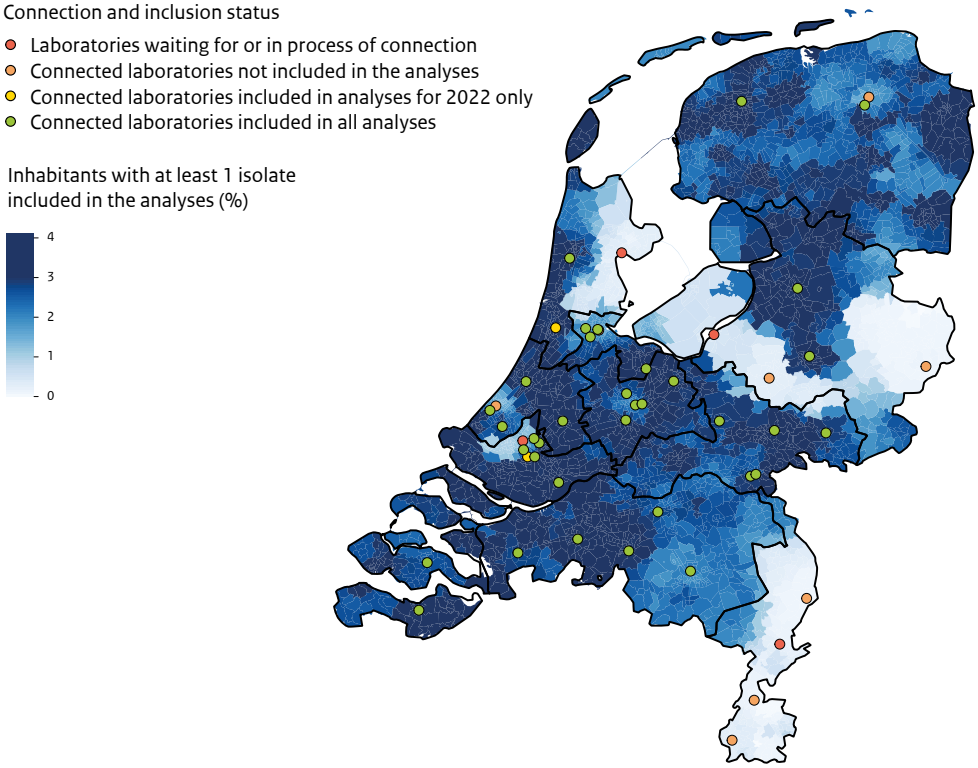


Table 4.1.2.1 Characteristics of 431,455 isolates, sampled in 2022, that were included in the analyses in sections 4.2 through 4.6, by pathogen

	E. coli	*K. pneumoniae*	*P. mirabilis*	*E. cloacae* complex	*P. aeruginosa*	*Acinetobacter* spp.	*E. faecalis*	*E. faecium*	*S. aureus*
Total number of isolates	187,232	31,887	22,214	12,195	24,914	4,152	31,591	6,515	58,478
Sex of patient (%)									
Male	27	34	43	55	54	53	55	52	54
Female	73	66	57	45	46	47	45	48	46
Setting of care (%)									
General practices	60	49	47	33	32	43	42	8	27
Outpatient departments	14	18	18	25	30	26	22	13	38
Inpatient departments (excl. Intensive Care Units)	19	23	21	33	28	24	28	58	28
Intensive Care Units	1	2	1	4	3	4	2	16	3
Long-term care facilities	6	8	13	5	7	3	6	4	4
Age category of patient in years (%)									
0-4	3	1	3	3	2	5	4	1	5
5-18	5	2	2	2	5	4	2	1	7
19-64	33	26	21	29	29	32	26	32	43
>65	59	70	75	65	64	59	68	67	45
Isolate source (%)									
Blood	3	3	2	4	2	5	3	13	5
Respiratory tract	1	3	2	8	16	8	0	1	15
Urine	90	86	82	59	44	58	85	52	14
Wound or Pus	3	5	11	23	32	23	9	27	54
Genital	1	0	1	0	1	0	0	0	3
Other	2	2	3	6	5	6	2	6	9
Type of hospital (hospital isolates only, %)									
General	38	36	41	34	33	32	36	26	34
Top clinical	48	48	47	47	45	47	51	52	47
University hospital	13	16	13	19	21	21	13	21	18

The first isolate per patient, per pathogen, per setting of care was selected.

Table 4.1.2.1 (continued) Characteristics of 431,455 isolates, sampled in 2022, that were included in the analyses in sections 4.2 through 4.6, by pathogen

	β-haemolytic *Streptococcus* spp. group A	β-haemolytic *Streptococcus* spp. group B	β-haemolytic *Streptococcus* spp. group C and G	*S. anginosus*	*S. mitis*/*S. oralis*	*B. fragilis* complex	*C. perfringens*	*S. pneumoniae*	*H. influenzae*	*M. catarrhalis*	*H. pylori*
Total number of isolates	7,182	20,715	2,299	2,093	1,364	1,567	435	4,592	9,320	2,352	358
Sex of patient (%)											
Male	43	23	55	52	58	57	54	54	51	49	37
Female	57	77	45	48	42	43	46	46	49	51	63
Setting of care (%)											
General practices	40	50	30	8	14	3	3	6	10	10	2
Outpatient departments	28	26	31	29	23	23	21	27	43	39	69
Inpatient departments (excl. Intensive Care Units)	29	21	36	56	59	68	71	61	41	42	27
Intensive Care Units	3	1	1	5	3	4	4	6	5	7	1
Long-term care facilities	1	3	2	2	1	2	1	0	1	2	0
Age category of patient in years (%)											
0-4	19	1	1	1	4	2	0	8	11	13	0
5-18	19	3	4	4	5	6	1	3	4	3	6
19-64	46	65	51	54	43	37	37	37	35	28	80
>65	16	31	44	41	48	55	61	52	49	55	14
Isolate source (%)											
Blood	9	2	10	11	34	24	29	31	2	1	0
Respiratory tract	11	1	3	3	3	0	0	55	84	88	0
Urine	8	54	14	19	22	1	3	1	0	0	0
Wound or Pus	49	11	53	60	37	66	55	12	11	10	14
Genital	17	28	12	3	1	1	4	0	2	0	0
Other	7	4	9	4	4	8	9	2	0	0	86
Type of hospital (hospital isolates only, %)											
General	36	37	40	37	37	35	34	37	32	31	36
Top clinical	49	50	46	52	46	43	48	50	50	54	55
University hospital	16	12	14	11	17	22	18	13	18	14	9

The first isolate per patient, per pathogen, per setting of care was selected.

Key results

- For the 2022 analyses, data of 37 laboratories could be used, resulting in inclusion of data on 431,455 isolates.
- Included laboratories were well distributed throughout the Netherlands although the proportion of laboratories from which data could be included in the analyses was relatively low in the regions 'Noord-Holland West', 'Noord-Holland Oost/ Flevoland', and 'Euregio-Zwolle', and 'Limburgs infectiepreventie en antibioticaresistentie netwerk (LINK)'. The distribution of included laboratories was reflected in the geographical distribution of isolates.

4.2 Primary care

The distribution of pathogens in diagnostic urine, wound or pus, respiratory, and genital samples from general practitioners' (GP) patients in 2022 is presented in table 4.2.1. The resistance levels in 2022 for *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* isolates from urine samples are presented in table 4.2.2. In accordance with age categories used in the guidelines of the Dutch College of General Practitioners (NHG) for urinary tract infections, resistance levels and five-year trends for urine isolates are calculated separately for patients aged ≤12 years and patients aged >12 years. For *S. aureus* isolates from wound or pus samples resistance levels in 2022 are presented in table 4.2.3, and for ß-haemolytic *Streptococcus* spp. group A isolates from wound/pus, respiratory, or genital samples as well as for ß-haemolytic *Streptococcus* spp. group B isolates from urine or genital samples in table 4.2.4. Five-year trends in resistance are shown in figure 4.2.1 (*E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa*), figure 4.2.4 (*S. aureus*) and figure 4.2.6 (ß-haemolytic *Streptococcus* spp. group A and group B). Finally, the smoothed geographical distribution of diagnostic isolates, and resistance levels for a selection of antibiotics in *E. coli*, *K. pneumoniae*, and *S. aureus* are shown by regional cooperative network in figures 4.2.2a and 4.2.2b (*E. coli*), 4.2.3a and 4.2.3b (*K. pneumoniae*), and 4.2.5a and 4.2.5b (*S. aureus*).

in accordance with the NHG guidelines, GPs usually send urine, wound, or pus samples for culture and susceptibility testing in case of antimicrobial therapy failure or (with regard to urine samples) complicated urinary tract infection. As a result, the presented resistance levels are likely to be higher than those for all patients with urinary tract infections caused by Enterobacterales or *P. aeruginosa* or wound infections or pus caused by *S. aureus* or β-haemolytic *Streptococcus* spp. group A presenting at the GP. Bias due to selective sampling of patients is expected to be limited for β-haemolytic *Streptococcus* spp. group B, because initial therapy of urinary tract infections is not expected to affect resistance to most antibiotics presented for *Streptococcus* spp. in this report and genital samples are taken as part of routine diagnostics.

Because of the potential bias in results for Enterobacterales, *P. aeruginosa*, *S. aureus* and β-haemolytic *Streptococcus* spp. group A, the patients from whom samples were taken are hereafter referred to as 'selected general practitioners' patients'.

Table 4.2.1 Distribution of isolated pathogens in diagnostic urine samples (by patient age category) and diagnostic wound or pus, respiratory, and genital samples from selected general practitioners' patients, ISIS-AR 2022

	Urine		Wound or pus	Respiratory tract	Genital
Pathogen	Age≤12 N (%)	Age>12 N (%)	N (%)	N (%)	N (%)
E. coli	9,536 (69)	107,431 (55)	773 (3)	82 (3)	433 (7)
K. pneumoniae	328 (2)	15,759 (8)	245 (1)	48 (2)	53 (1)
P. mirabilis	643 (5)	9,788 (5)	572 (3)	31 (1)	58 (1)
Other Enterobacterales[1]	708 (5)	21,378 (11)	2,054 (9)	241 (8)	126 (2)
P. aeruginosa	234 (2)	4,716 (2)	3,255 (14)	210 (7)	76 (1)
Other non-fermenters[2]	185 (1)	2,499 (1)	742 (3)	272 (9)	15 (0)
Other Gram-negatives[3]	8 (0)	25 (0)	396 (2)	615 (20)	94 (2)
S. aureus	139 (1)	3,590 (2)	10,495 (47)	1,170 (38)	963 (16)
β-haemolytic *Streptococcus* spp. group A	302 (2)	178 (0)	1,515 (7)	209 (7)	1,104 (18)
β-haemolytic *Streptococcus* spp. group B	140 (1)	7,358 (4)	546 (2)	32 (1)	2,647 (44)
Other Gram-positives[4]	1,575 (11)	24,016 (12)	1,863 (8)	196 (6)	460 (8)

[1] *In order of frequency: Klebsiella spp. (non-pneumoniae), Citrobacter spp., Enterobacter spp., Morganella spp., Serratia spp., Proteus spp. (non-mirabilis), Raoultella spp., Providencia spp., Pantoea spp., Escherichia spp. (non-coli), Hafnia spp., Salmonella spp., Cronobacter spp., Mixta spp.*

[2] *In order of frequency: Acinetobacter spp., Pseudomonas spp. (non-aeruginosa), S. maltophilia, M. catarrhalis, B. cepacia.*

[3] *In order of frequency: H. parainfluenzae, H. influenzae, B. fragilis complex, N. meningitidis, H. pylori.*

[4] *In order of frequency: Enterococcus spp., Staphylococcus spp. (non-aureus), A. urinae, S. anginosus, S. dysgalactiae n.n.g., S. pneumoniae, β-haemolytic Streptococcus spp. groups C and G, S. mitis/S. oralis, S. dysgalactiae subsp. equisimilis, C. perfringens, L. monocytogenes.*

Table 4.2.2 Resistance levels (%) among diagnostic urine isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* from selected general practitioners' patients, by age category, ISIS-AR 2022

	E. coli		*K. pneumoniae*		*P. mirabilis*		*P. aeruginosa*	
	age≤12	age>12	age≤12	age>12	age≤12	age>12	age≤12	age>12
median age	5	68	4	74	3	75	3	79
Antibiotic								
amoxicillin/ampicillin	32	34	-	-	19	19	-	-
co-amoxiclav[a]	24	25 ↓	30	18	4	5	-	-
piperacillin-tazobactam	-	-	-	-	-	-	2	4
cefuroxime	4	7	6	10 ↓	1	1	-	-
cefotaxime/ceftriaxone[b]	3	4	4	3	1	1	-	-
ceftazidime	2	3	4	3	0	0	1	1
meropenem[b]	-	-	-	-	-	-	0	1
imipenem	-	-	-	-	-	-	4	4
ciprofloxacin	5	9	3	10	6	10	1	9
gentamicin	3	4	1	1	3	5	-	-
tobramycin	3	4	1	2	4	4	1	1
fosfomycin[1]	1	2	-	-	-	-	-	-
trimethoprim	19	20	6 ↓	16 ↓	26	29	-	-
co-trimoxazole	17	18	4 ↓	7 ↓	21	22	-	-
nitrofurantoin	0	2	-	-	-	-	-	-
Multidrug resistance								
MDOT[a]	1	3	0 ↓	2	0	1	-	-

10 ↑	Significant and clinically relevant increasing trend since 2018.
10 ↓	Significant and clinically relevant decreasing trend since 2018.
10°	Trend not calculated because data from the years before 2022 did not meet the criteria for trend analysis.
10	No significant and clinically relevant time trend.

(For the criteria for trend analysis and the definition of a clinically relevant trend see section 4.1.1).

- = Resistance not calculated.

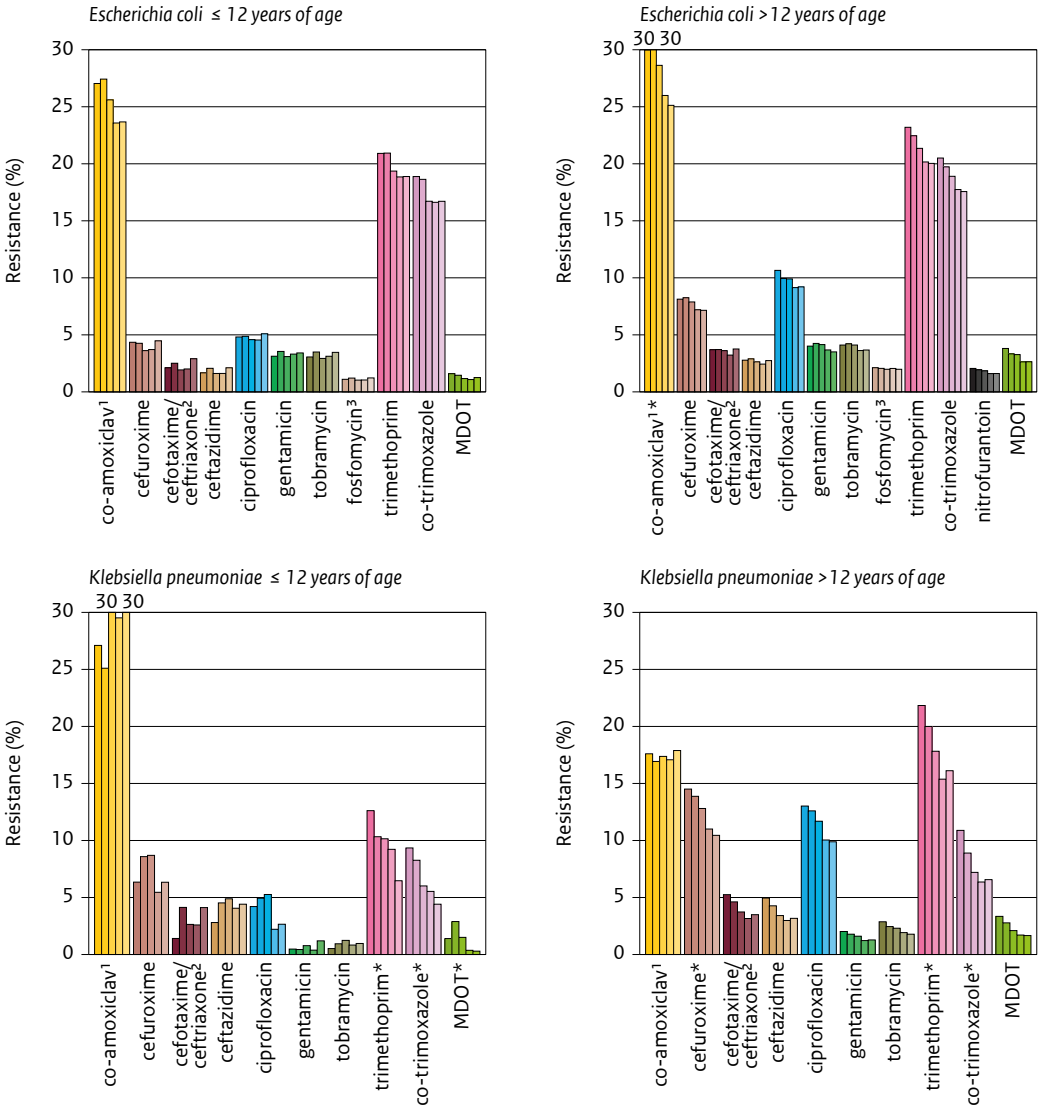
MDOT = multidrug resistance to oral therapy, defined as resistance to all of the following oral agents: co-amoxiclav (according to breakpoint for indications other than uncomplicated urinary tract infection), ciprofloxacin, and co-trimoxazole.

[1] *Resistance percentage calculated using an mic cut-off of 16 mg/L and a diameter cut-off of 24 mm. For more details see section 4.1.1.*

[a] *According to breakpoint for indications other than uncomplicated urinary tract infections. For more details see section 4.1.1.*

[b] *According to breakpoint for indications other than meningitis. For more details see section 4.1.1.*

Figure 4.2.1 Trends in antibiotic resistance (from left to right 2018 to 2022) among diagnostic urine isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* from selected general practitioners' patients in ISIS-AR, by age category



Only pathogen-agent combinations are shown for which the resistance levels were higher than 0.5% for at least one year and lower than 30% for at least three years, except for co-amoxiclav in Enterobacterales, for which resistance levels are always shown.
MDOT = Multidrug resistance for oral therapy, defined as resistance to all following oral agents: co-amoxiclav (according to breakpoint for indications other than uncomplicated urinary tract infection), ciprofloxacin, and co-trimoxazole.

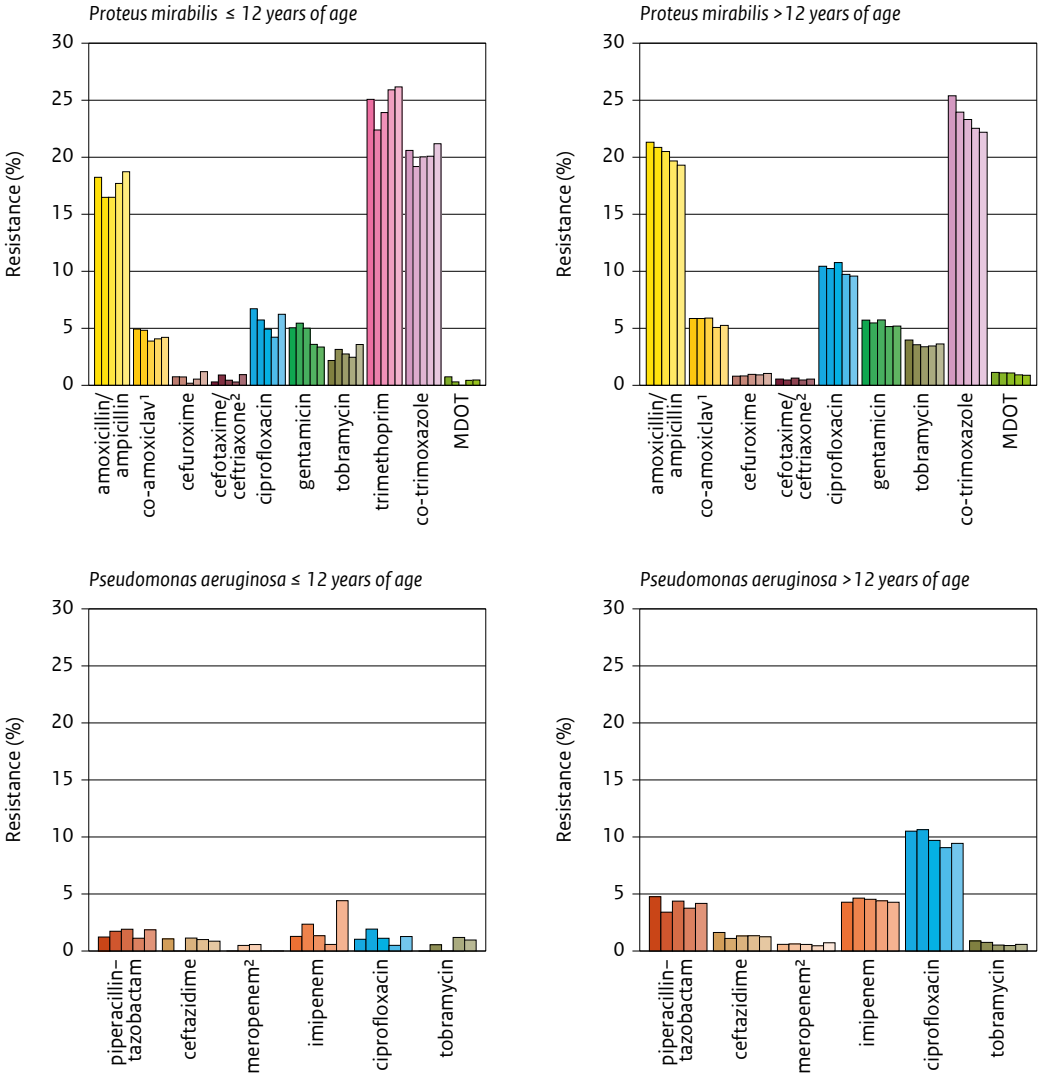
* *Trend is significant and microbiologically relevant (for details see section 4.1.1).*

1 *According to breakpoint for indications other than uncomplicated urinary tract infection.*

2 *According to breakpoint for indications other than meningitis.*

3 *Resistance percentage calculated using an mic cut-off of 16 mg/L and a diameter cut-off of 24 mm. For more details see section 4.1.1.*

Figure 4.2.1 (continued) Trends in antibiotic resistance (from left to right 2018 to 2022) among diagnostic urine isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* from selected general practitioners' patients in ISIS-AR, by age category



Only pathogen-agent combinations are shown for which the resistance levels were higher than 0.5% for at least one year and lower than 30% for at least three years, except for co-amoxiclav in Enterobacterales, for which resistance levels are always shown.
MDOT = Multidrug resistance for oral therapy, defined as resistance to all following oral agents: co-amoxiclav (according to breakpoint for indications other than uncomplicated urinary tract infection), ciprofloxacin, and co-trimoxazole.

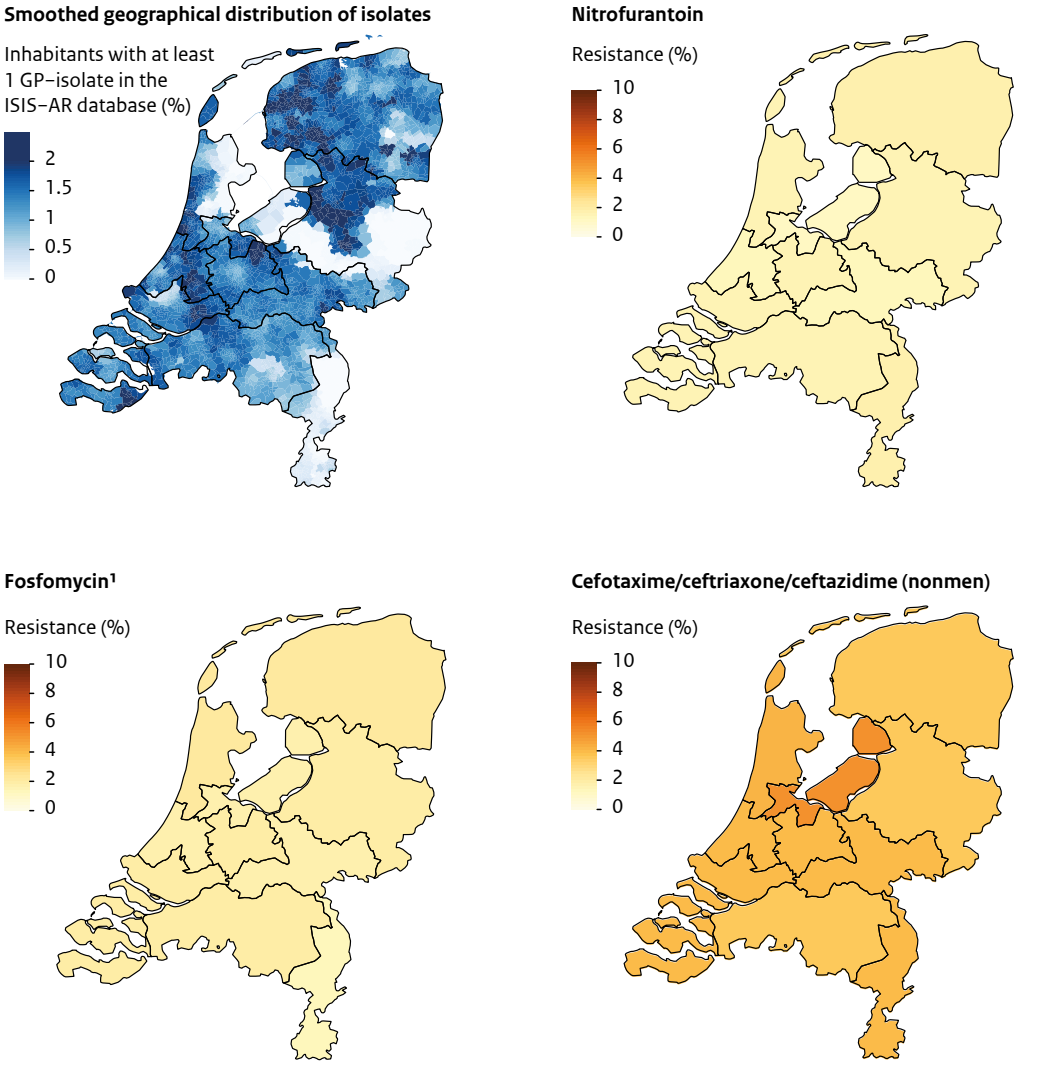
* *Trend is significant and microbiologically relevant (for details see section 4.1.1).*

[1] *According to breakpoint for indications other than uncomplicated urinary tract infection.*

[2] *According to breakpoint for indications other than meningitis.*

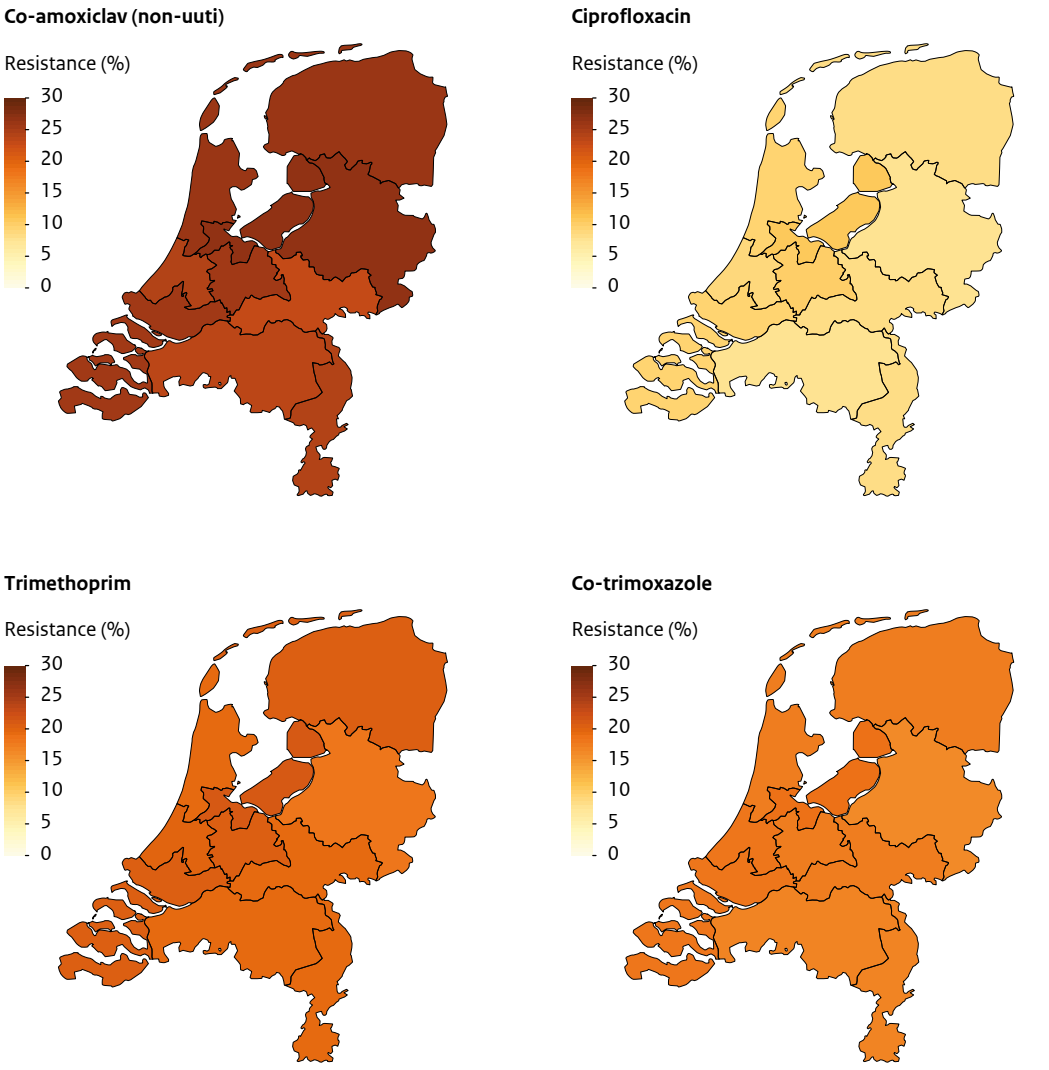
[3] *Resistance percentage calculated using an mic cut-off of 16 mg/L and a diameter cut-off of 24 mm. For more details see section 4.1.1.*

Figure 4.2.2a Smoothed geographical distribution of isolates from selected general practitioners' patients, based on percentage of inhabitants for whom at least one isolate was included in the analyses, and the resistance levels in diagnostic urinary *E. coli* isolates on a gradient scale between 0 and 10% for nitrofurantoin, fosfomycin[1], and cefotaxime/ceftriaxone/ceftazidime by regional cooperative network, ISIS-AR 2022



nonmen = according to breakpoint for indications other than meningitis.
Note: No statistically significant and microbiologically relevant differences of regional resistance levels were found for the selected antibiotics in comparison to all regions combined (for details see section 4.1.1).
[1] *Resistance percentage calculated using an mic cut-off of 16 mg/L and a diameter cut-off of 24 mm. For more details see section 4.1.1.*

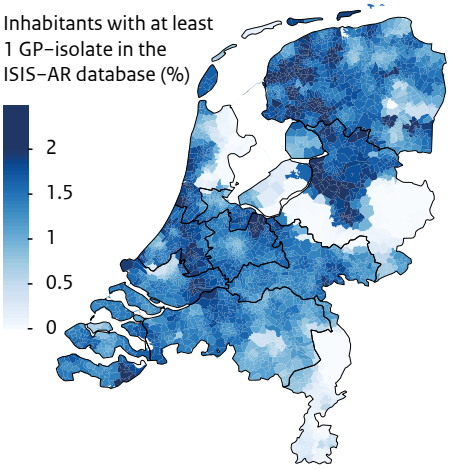
Figure 4.2.2b Resistance levels in diagnostic urinary *E. coli* isolates on a gradient scale between 0 and 30% for co-amoxiclav, ciprofloxacin, trimethoprim, and co-trimoxazole from selected general practitioners' patients, by regional cooperative network, ISIS-AR 2022



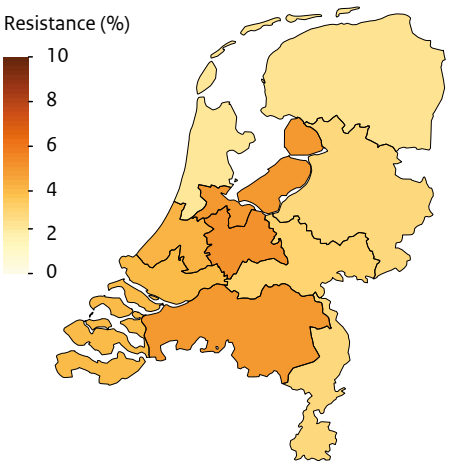
non-uuti = according to breakpoint for indications other than uncomplicated urinary tract infection.
Note: No statistically significant and microbiologically relevant differences of regional resistance levels were found for the selected antibiotics in comparison to all regions combined (for details see section 4.1.1).

Figure 4.2.3a Smoothed geographical distribution of isolates from selected general practitioners' patients, based on percentage of inhabitants for whom at least one isolate was included in the analyses, and the resistance levels in diagnostic urinary *K. pneumoniae* isolates on a gradient scale between 0 and 10% for cefotaxime/ceftriaxone/ceftazidime by regional cooperative network, ISIS-AR 2022

Smoothed geographical distribution of isolates

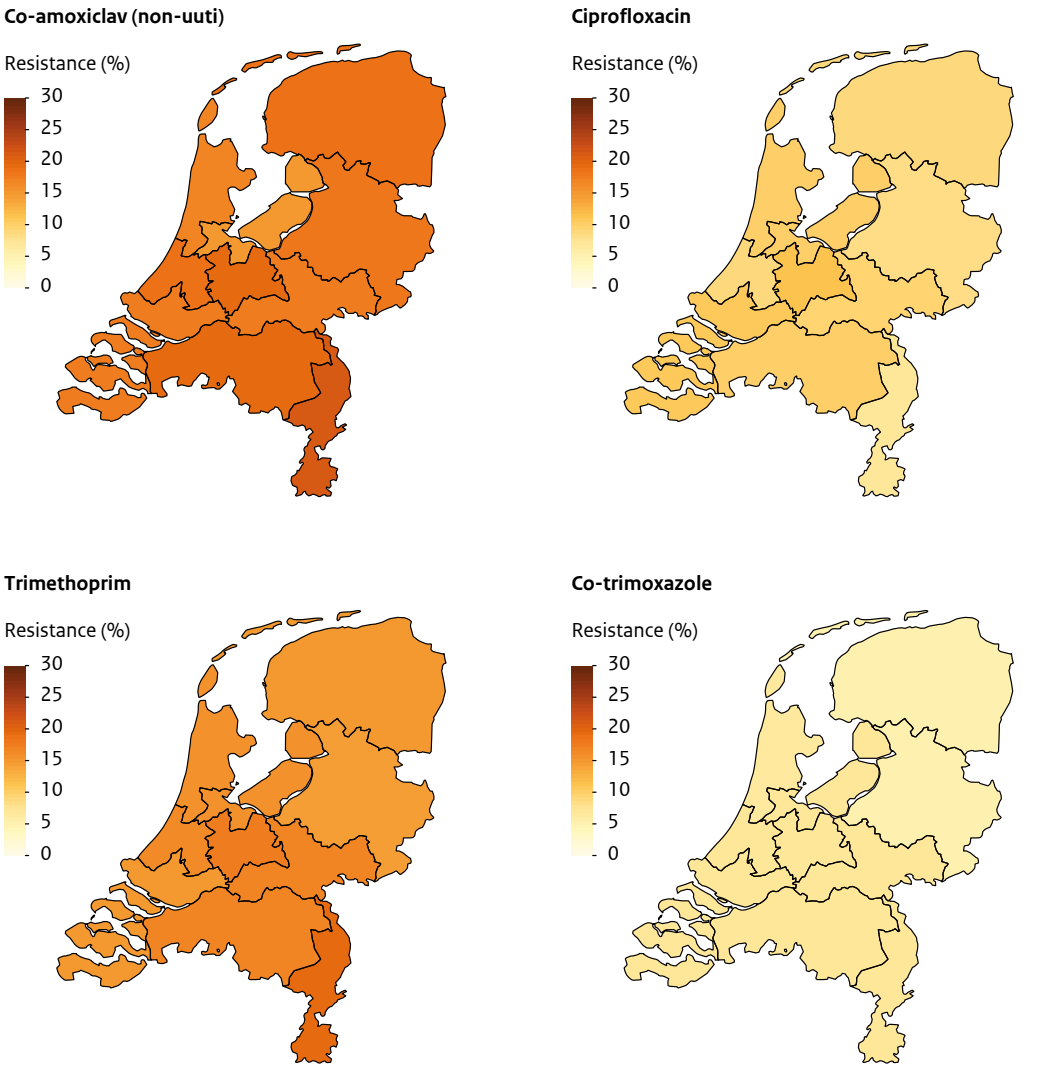


Cefotaxime/ceftriaxone/ceftazidime (nonmen)



nonmen = according to breakpoint for indications other than meningitis.
Note: No statistically significant and microbiologically relevant differences of regional resistance levels were found for the selected antibiotics in comparison to all regions combined (for details see section 4.1.1).

Figure 4.2.3b Resistance levels in diagnostic urinary *K. pneumoniae* isolates on a gradient scale between 0 and 30% for co-amoxiclav, ciprofloxacin, trimethoprim, and co-trimoxazole from selected general practitioners' patients, by regional cooperative network, ISIS-AR 2022



non-uuti=according to breakpoint for indications other than uncomplicated urinary tract infection.
Note: No statistically significant and microbiologically relevant differences of regional resistance levels were found for the selected antibiotics in comparison to all regions combined (for details see section 4.1.1).

Table 4.2.3 Resistance levels (%) among diagnostic wound or pus isolates of *S. aureus* from selected general practitioners' patients, ISIS-AR 2022

	S. aureus
Antibiotic	
MRSA	3
ciprofloxacin[1]	3
erythromycin[c]	14
clindamycin (including inducible resistance)[2]	13
doxycycline/tetracycline[c]	4
fusidic acid	19
co-trimoxazole	2
mupirocine[a]	1
mupirocine[b]	1

10 ↑	Significant and clinically relevant increasing trend since 2018.
10 ↓	Significant and clinically relevant decreasing trend since 2018.
10°	Trend not calculated because data from the years before 2022 did not meet the criteria for trend analysis.
10	No significant and clinically relevant time trend.

(For the criteria for trend analysis and the definition of a clinically relevant trend see section 4.1.1).

MRSA = Methicillin resistant Staphylococcus aureus. For the estimation method of MRSA see section 4.1.1.

[1] *Resistance to ciprofloxacin is intended to be a class indicator for resistance to fluoroquinolones.*

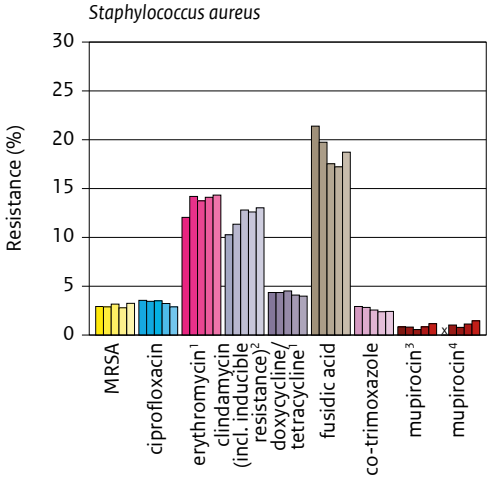
[2] *For the method used to estimate clindamycin resistance (including inducible resistance) see section 4.1.1.*

[a] *According to breakpoint for nasal decontamination. For more details see section 4.1.1.*

[b] *According to breakpoint for topical use. For more details see section 4.1.1.*

[c] *According to breakpoint for screening. For more details see section 4.1.1.*

Figure 4.2.4 Trends in antibiotic resistance (from left to right 2018 to 2022) among diagnostic wound or pus isolates of *S. aureus* from selected general practitioners' patients in ISIS-AR



Only pathogen-agent combinations are shown for which the resistance levels were higher than 0.5% for at least one year and lower than 30% for at least three years.
MRSA = Methicillin resistant Staphylococcus aureus.
For the estimation method of MRSA see section 4.1.1.
Note: None of the trends were statistically significant and microbiologically relevant (for details see section 4.1.1).

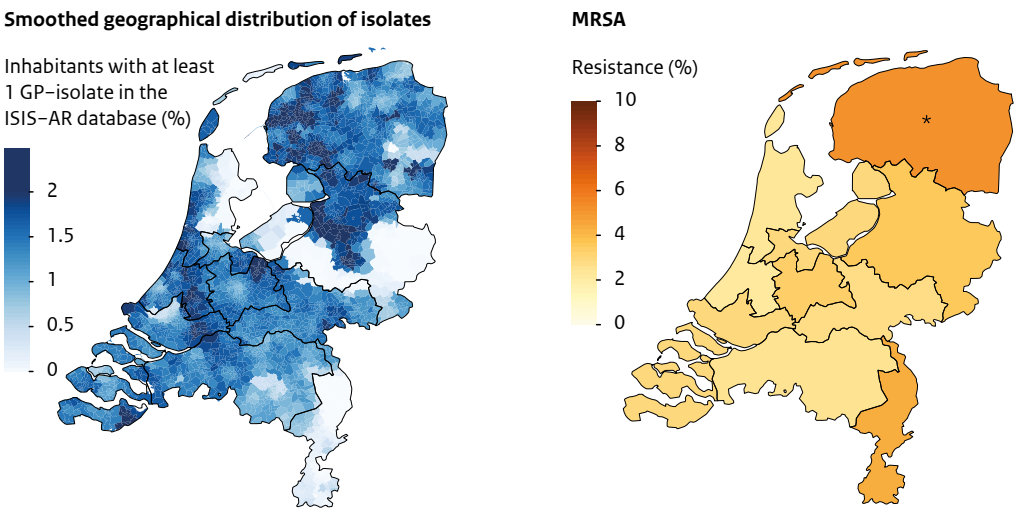
[1] *According to breakpoint for screening.*

[2] *For the method used to estimate clindamycin resistance (including inducible resistance) see section 4.1.1.*

[3] *According to breakpoint for nasal decontamination.*

[4] *According to breakpoint for topical use.*

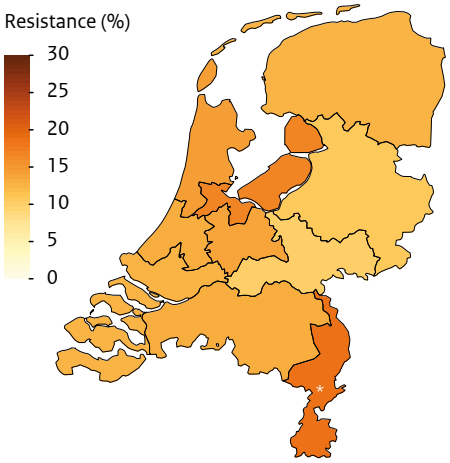
Figure 4.2.5a Smoothed geographical distribution of isolates from selected general practitioners' patients, based on percentage of inhabitants for whom at least one isolate was included in the analyses, and the resistance levels in diagnostic wound or pus *S. aureus* isolates on a gradient scale between 0 and 10% for MRSA by regional cooperative network, ISIS-AR 2022



MRSA = Methicillin resistant Staphylococcus aureus. For the estimation method of MRSA see section 4.1.1.

** Statistically significant and microbiologically relevant difference of resistance in the regional cooperative network compared with all regions combined (for details see section 4.1.1).*

Figure 4.2.5b Resistance levels in diagnostic wound or pus *S. aureus* isolates on a gradient scale between 0 and 30% for clindamycin including inducible resistance by regional cooperative network, ISIS-AR 2022



* *Statistically significant and microbiologically relevant difference of resistance in the regional cooperative network compared with all regions combined (for details see section 4.1.1).*

[1] *For the method used to estimate clindamycin resistance (including inducible resistance) see section 4.1.1.*

Table 4.2.4 Resistance levels (%) among diagnostic wound/pus, respiratory or genital isolates of β-haemolytic *Streptococcus* spp. group A and diagnostic urine or genital isolates of β-haemolytic *Streptococcus* spp. group B from selected general practitioners' patients, ISIS-AR 2022

	β-haemolytic *Streptococcus* spp. group A	β-haemolytic *Streptococcus* spp. group B
Antibiotic		
erythromycin	9	21°
clindamycin (including inducible resistance)[1]	8 ↑	17°
doxycycline/tetracycline[a]	29 ↑	78
co-trimoxazole	4 ↑	1

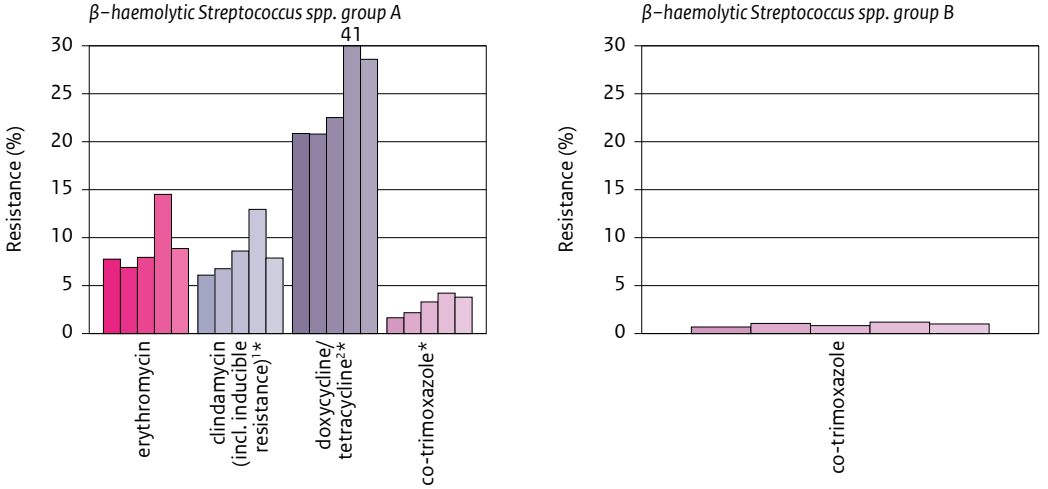
10 ↑	Significant and clinically relevant increasing trend since 2018.
10 ↓	Significant and clinically relevant decreasing trend since 2018.
10°	Trend not calculated because data from the years before 2022 did not meet the criteria for trend analysis.
10	No significant and clinically relevant time trend.

(For the criteria for trend analysis and the definition of a clinically relevant trend see section 4.1.1).

[1] *For the method used to estimate clindamycin resistance (including inducible resistance) see section 4.1.1.*

[a] *According to breakpoint for screening. For more details see section 4.1.1.*

Figure 4.2.6 Trends in antibiotic resistance (from left to right 2018 to 2022) among diagnostic wound/pus, respiratory or genital isolates of β-haemolytic *Streptococcus* spp. group A and diagnostic urine or genital isolates of β-haemolytic *Streptococcus* spp. group B from selected general practitioners' patients in ISIS-AR



Only pathogen-agent combinations are shown for which the resistance levels were higher than 0.5% for at least one year and lower than 30% for at least three years.

* *Trend is significant and microbiologically relevant (for details see section 4.1.1).*

[1] *For the method used to estimate clindamycin resistance (including inducible resistance) see section 4.1.1.*

[2] *According to breakpoint for screening.*

Key results and conclusions

Urine: Enterobacterales and P. aeruginosa

Uncomplicated urinary tract infections (UTI)

- In *E. coli*, resistance levels for **nitrofurantoin** and **fosfomycin**, first and second choice antibiotics for the treatment of uncomplicatied UTI in adults in primary care, were stable and low (2%). For **trimethoprim**, third choice antibiotic for the treatment of uncomplicated UTI in adults, resistance levels decreased slightly over the last 5 years to 20%. For other Enterobacterales, both **nitrofurantoin** and **fosfomycin** are not appropriate for empirical treatment of urinary tract infections.

Complicated UTI

- Resistance levels for **ciprofloxacin**, first choice antibiotic for the treatment of complicated UTI in adults in primary care, was stable at 10% or lower for all Enterobacterales and *P. aeruginosa*. Resistance levels for **co-amoxiclav**, second choice antibiotic for the treatment of complicated UTI in primary care, decreased to 25% in *E. coli* and was stable at 18% in *K. pneumoniae*. Resistance levels for **co-trimoxazole**, third choice antibiotic for this indication, was 18% in *E. coli* and 7% in *K. pneumoniae*, with in *K. pneumoniae* the resistance being remarkably decreased from 11% in 2018.
- Combined resistance for **co-amoxiclav**, **ciprofloxacin**, and **co-trimoxazole** in all Enterobacterales was low (≤3%).

Wound or pus: S. aureus

- Antibiotic resistance levels in *S. aureus* were relatively low except for **erythromycin** (14%), **clindamycin** (including inducible resistance, 13%) and **fusidic acid** (19%). Resistance to **clindamycin** (including inducible resistance) and **macrolides** in *S. aureus* was 13% in 2022, which limits its usefulness in empiric therapy for those infections possibly caused by *S. aureus*, such as skin and soft tissue infections.
- **MRSA** was found in 3% of isolates of primary care patients which was stable over the previous 5 years.
- Resistance to **mupirocine** for nasal decontamination in *S. aureus* was low: 1%.

Wound/pus, respiratory or genital: β-haemolytic Streptococcus spp. groups A and B

- The rise in resistance to **doxycycline/tetracycline** (29%), **clindamycin** (including inducible resistance, 8%), and **co-trimoxazole** (4%) in ß-haemolytic *Streptococcus* spp. group A over the last five years is worrisome. It complicates empirical treatment of skin and soft tissue infections, pharyngitis, and pneumonia, for which these agents are recommended in case of β-lactam allergy.
- Resistance levels for **doxycycline/tetracycline** (78%), **clindamycin** (including inducible resistance, 17%) and **erythromycin** (21%) in ß-haemolytic *Streptococcus* spp. group B were high. However, a time trend could not be calculated due to the low amount of isolates.

4.3 Hospital departments

In this section, resistance levels among isolates from patients in outpatient departments (section 4.3.1), inpatient departments (excluding intensive care units, section 4.3.2), and intensive care units (section 4.3.3) are presented. Additionally, resistance levels are shown separately for blood isolates from patients admitted to inpatient hospital departments (including intensive care units) in section 4.3.4 and for urine isolates from patients in urology departments (outpatient and inpatient departments) in section 4.3.5.

4.3.1 Outpatient departments

The distribution of pathogens isolated from diagnostic samples (lower respiratory tract, urine, and wound or pus) from patients attending outpatient departments in 2022 is presented in table 4.3.1.1. The resistance levels for a selection of pathogens isolated from these patients in 2022 are presented in tables 4.3.1.2 (*E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa*) and 4.3.1.3 (*S. aureus*). Five-year trends in resistance are shown in figures 4.3.1.1 (*E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa*) and 4.3.1.2 (*S. aureus*).

In outpatient departments in the Netherlands, a sample is taken from the majority of patients presenting with infections and susceptibility testing is performed as part of routine diagnostics. Therefore, bias due to selective sampling will be lower than in GP patients and resistance percentages in this section are considered representative of resistance in outpatient departments.

Table 4.3.1.1 Distribution of isolated pathogens in diagnostic samples from patients attending outpatient departments, ISIS-AR 2022

	Lower respiratory tract	Urine	Wound or pus
Pathogen	N (%)	N (%)	N (%)
E. coli	384 (4)	21,633 (41)	2,224 (6)
K. pneumoniae	168 (2)	4,474 (8)	502 (1)
P. mirabilis	95 (1)	2,358 (4)	1,189 (3)
Other Enterobacterales[1]	794 (8)	7,767 (15)	4,007 (11)
P. aeruginosa	1,519 (16)	1,960 (4)	3,302 (9)
Other non-fermenters[2]	1,139 (12)	757 (1)	976 (3)
Other Gram-negatives[3]	3,136 (32)	25 (0)	1,011 (3)
S. aureus	1,613 (16)	1,813 (3)	14,988 (41)
Other Gram-positives[4]	942 (10)	12,227 (23)	8,719 (24)

[1] *In order of frequency: Klebsiella spp. (non-pneumoniae), Enterobacter spp., Citrobacter spp., Serratia spp., Morganella spp., Proteus spp. (non-mirabilis), Providencia spp., Raoultella spp., Pantoea spp., Hafnia spp., Escherichia spp. (non-coli), Salmonella spp., Cronobacter spp., Mixta spp., Yersinia spp.*

[2] *In order of frequency: Acinetobacter spp., M. catarrhalis, S. maltophilia, Pseudomonas spp. (non-aeruginosa), B. cepacia.*

[3] *In order of frequency: H. parainfluenzae, H. influenzae, B. fragilis complex, N. meningitidis, H. pylori.*

[4] *In order of frequency: ß-haemolytic Streptococcus spp. group B, S. pneumoniae, S. anginosus, S. dysgalactiae n.n.g., S. mitis/S. oralis, ß-haemolytic Streptococcus spp. group A, ß-haemolytic Streptococcus spp. groups C and G, S. dysgalactiae subsp. equisimilis, Enterococcus spp., Staphylococcus spp. (non-aureus), A. urinae, C. perfringens, L. monocytogenes.*

Table 4.3.1.2 Resistance levels (%) among diagnostic isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* from patients attending outpatient departments, ISIS-AR 2022

	E. coli	*K. pneumoniae*	*P. mirabilis*	*P. aeruginosa*
Antibiotic				
amoxicillin/ampicillin	39	-	21	-
co-amoxiclav[a]	29 ↓	20	5	-
piperacillin-tazobactam	4	15	0	5
cefuroxime	11	13	1	-
cefotaxime/ceftriaxone[b]	6	7 ↓	1	-
ceftazidime	4	6	0	3
meropenem/imipenem[b]	0	0	-	-
meropenem[b]	-	-	0	1
imipenem	-	-	-	5
ciprofloxacin	15	13	12	13
gentamicin	5	3	6	-
tobramycin	5	4	4	3
fosfomycin[1]	3	-	-	-
trimethoprim	25	19 ↓	31	-
co-trimoxazole	22	11 ↓	24	-
nitrofurantoin	3	-	-	-
Empiric therapy combinations				
co-amoxiclav + gentamicin[a]	4	2	1	-
cefuroxime + gentamicin	2	2	0	-
cefotaxime/ceftriaxone + gentamicin[b]	1	2	0	-
co-amoxiclav + ciprofloxacin[a]	8	6 ↓	1	-
cefuroxime + ciprofloxacin	6	8	0	-
cefotaxime/ceftriaxone + ciprofloxacin[b]	4	5	0	-
Multidrug resistance				
MDOT[a]	5	4 ↓	1	-

10 ↑	Significant and clinically relevant increasing trend since 2018.
10 ↓	Significant and clinically relevant decreasing trend since 2018.
10°	Trend not calculated because data from the years before 2022 did not meet the criteria for trend analysis.
10	No significant and clinically relevant time trend.

(For the criteria for trend analysis and the definition of a clinically relevant trend see section 4.1.1).

- = Resistance not calculated.

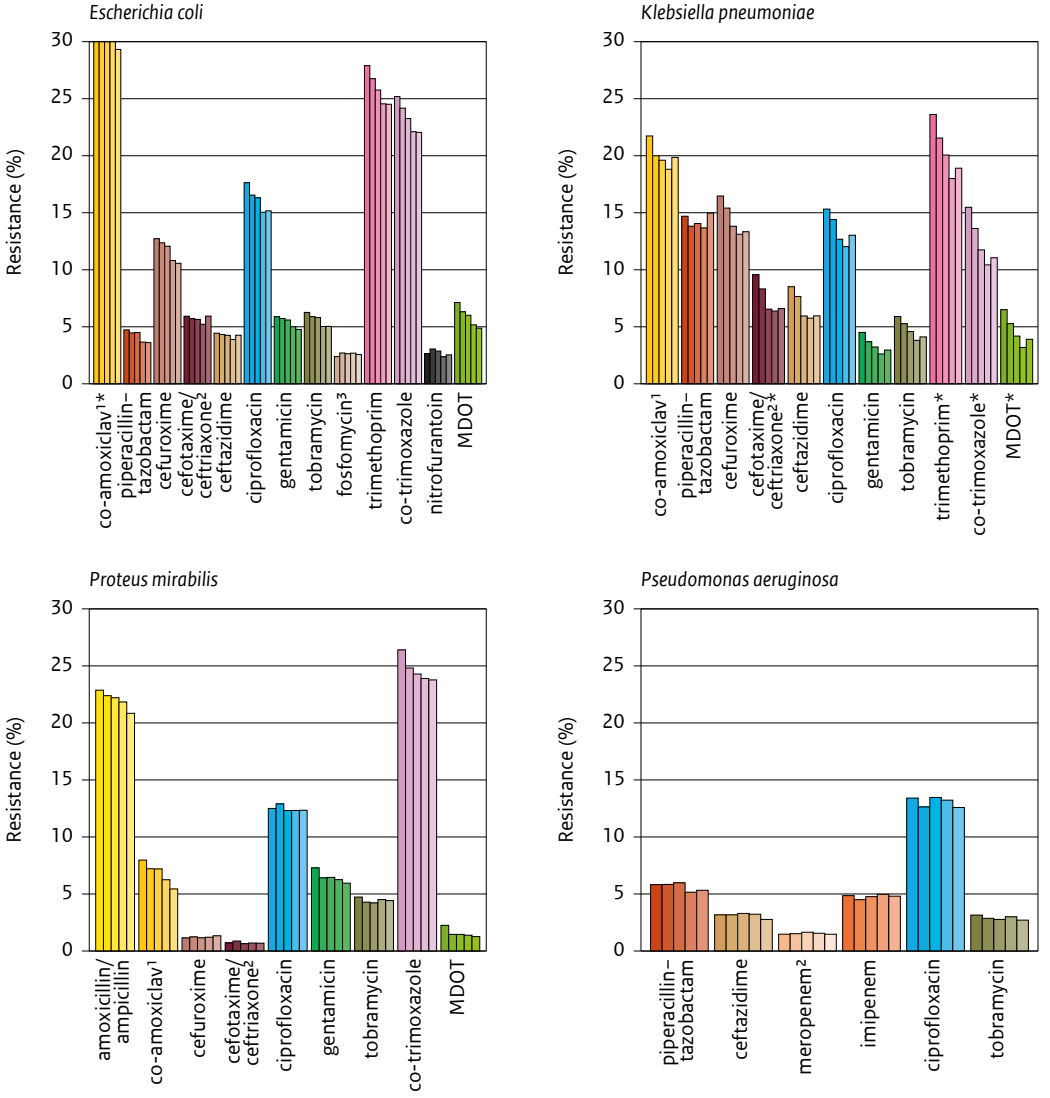
MDOT = multidrug resistance to oral therapy, defined as resistance to all of the following oral agents: co-amoxiclav (according to breakpoint for indications other than uncomplicated urinary tract infection), ciprofloxacin, and co-trimoxazole.

[1] *Resistance percentage calculated using an mic cut-off of 16 mg/L and a diameter cut-off of 24 mm. For more details see section 4.1.1.*

[a] *According to breakpoint for indications other than uncomplicated urinary tract infections. For more details see section 4.1.1.*

[b] *According to breakpoint for indications other than meningitis. For more details see section 4.1.1.*

Figure 4.3.1.1 Trends in antibiotic resistance (from left to right 2018 to 2022) among diagnostic isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* from patients attending outpatient departments in ISIS-AR



Only pathogen-agent combinations are shown for which the resistance levels were higher than 0.5% for at least one year and lower than 30% for at least three years, except for co-amoxiclav in Enterobacterales, for which resistance levels are always shown.
MDOT = Multidrug resistance for oral therapy, defined as resistance to all following oral agents: co-amoxiclav (according to breakpoint for indications other than uncomplicated urinary tract infection), ciprofloxacin, and co-trimoxazole.

* *Trend is significant and microbiologically relevant (for details see section 4.1.1).*

[1] *According to breakpoint for indications other than uncomplicated urinary tract infection. E. coli: Resistance percentages for co-amoxiclav are from left to right (2018 to 2022) 36%, 35%, 34%, 31%, and 29%.*

[2] *According to breakpoint for indications other than meningitis.*

[3] *Resistance percentage calculated using an mic cut-off of 16 mg/L and a diameter cut-off of 24 mm. For more details see section 4.1.1.*

Table 4.3.1.3 Resistance levels (%) among diagnostic isolates of *S. aureus* from patients attending outpatient departments, ISIS-AR 2022

	S. aureus
Antibiotic	
MRSA	2
ciprofloxacin[1]	4
gentamicin	1
erythromycin[c]	17
clindamycin (including inducible resistance)[2]	16
doxycycline/tetracycline[c]	4
fusidic acid	8
linezolid	0
co-trimoxazole	2
rifampicin	0
mupirocine[a]	0
mupirocine[b]	1

10 ↑	Significant and clinically relevant increasing trend since 2018.
10 ↓	Significant and clinically relevant decreasing trend since 2018.
10°	Trend not calculated because data from the years before 2022 did not meet the criteria for trend analysis.
10	No significant and clinically relevant time trend.
	(For the criteria for trend analysis and the definition of a clinically relevant trend see section 4.1.1).

MRSA = Methicillin resistant Staphylococcus aureus. For the estimation method of MRSA see section 4.1.1.

[1] *Resistance to ciprofloxacin is intended to be a class indicator for resistance to fluoroquinolones.*

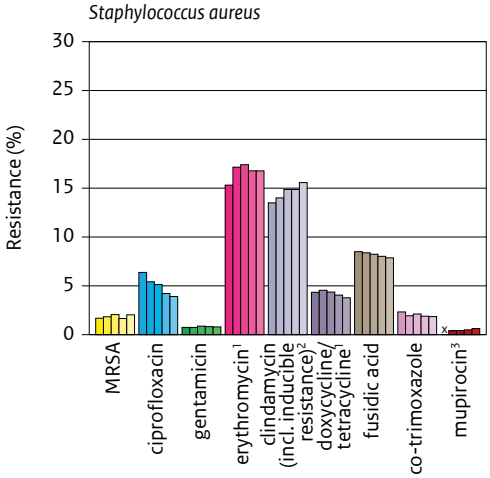
[2] *For the method used to estimate clindamycin resistance (including inducible resistance) see section 4.1.1.*

[a] *According to breakpoint for nasal decontamination. For more details see section 4.1.1.*

[b] *According to breakpoint for topical use. For more details see section 4.1.1.*

[c] *According to breakpoint for screening. For more details see section 4.1.1.*

Figure 4.3.1.2 Trends in antibiotic resistance (from left to right 2018 to 2022) among diagnostic isolates of *S. aureus* from patients attending outpatient departments in ISIS-AR



Only pathogen-agent combinations are shown for which the resistance levels were higher than 0.5% for at least one year and lower than 30% for at least three years.

MRSA = Methicillin resistant Staphylococcus aureus. For the estimation method of MRSA see section 4.1.1.

Note: None of the trends were statistically significant and microbiologically relevant (for details see section 4.1.1).

[1] *According to breakpoint for screening.*

[2] *For the method used to estimate clindamycin resistance (including inducible resistance) see section 4.1.1.*

[3] *According to the breakpoint for topical use.*

Key results and conclusions

Urine, wound/pus and respiratory: E. coli, K. pneumoniae, and P. aeruginosa

- For both Enterobacterales and *P. aeruginosa*, resistance levels for all tested antibiotics were higher or equal in isolates of OPD patients compared to resistance levels in isolates from primary care patients.
- For all Enterobacterales, resistance to **second and third generation cephalosporins** seemed to plateau or slightly decrease over the past five years. Moreover, in *K. pneumoniae*, a relevant decrease was observed for **cefotaxime/ceftriaxone**, probably reflecting a decline in ESBL-producing isolates.
- For the three most important oral antibiotics used in OPD setting, **co-amoxiclav**, **co-trimoxazole** and **ciprofloxacin**, a similar decreasing or stable trend was found as observed in primary care. In *E. coli*, resistance to **co-amoxiclav** decreased to 29% and was stable at 20% in *K. pneumoniae*. Resistance to **co-trimoxazole** decreased to 11% in *K. pneumoniae* and was 22% in *E. coli*. Resistance levels for **ciprofloxacin** were 15% in *E. coli*, 13% in *K. pneumoniae* and 13% in *P. aeruginosa*. However, because resistance levels for each of the three individual antibiotics were more than 10% the a priori chance of successful empirical oral treatment is limited. Therefore, culture and antibiotic susceptibility testing are necessary for successful treatment. Fortunately, most often at least one of

the three antibiotics can be used for successful treatment, given the relatively low (≤5%) combined resistance rates for these oral agents.

Urine, wound/pus and respiratory: S. aureus

- In *S. aureus*, no significant and microbiologically relevant trends in resistance levels were found over the last five years.
- Resistance was generally low (≤4%), with the exception of resistance to **fusidic acid** (8%), **clindamycin** (including inducible resistance, 16%) and **erythromycin** (17%).
- Resistance to **clindamycin** (including inducible resistance), which was 16%, seems to have slightly increased over the last five years, which limited its usefulness in empiric therapy for those infections possibly caused by *S. aureus*, such as skin and soft tissue infections.
- Resistance to **fusidic acid** was much lower in *S. aureus* isolates of OPD patients (8%) than in *S. aureus* isolates of GP patients (19%).
- **MRSA** was found in 2% of isolates of OPD patients which was stable over the previous 5 years.
- Resistance to **mupirocine** for nasal decontamination in *S. aureus* was less than 1%.

4.3.2 Inpatient hospital departments (excl. ICU)

The distribution of pathogens from diagnostic samples (blood or cerebrospinal fluid, lower respiratory tract, urine, and wound or pus) from patients admitted to inpatient hospital departments (excl. ICU) in 2022 is presented in table 4.3.2.1. The resistance levels for a selection of pathogens isolated from these patients in 2022 are presented in tables 4.3.2.2 (*E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, *P. aeruginosa*, and *Acinetobacter* spp.), 4.3.2.3 (*E. faecalis* and *E. faecium*), 4.3.2.4 (*S. aureus*), 4.3.2.5 (β-haemolytic *Streptococcus* spp. groups A, B, C and G, *S. anginosus*, and *S. mitis/S. oralis*), and 4.3.2.6 (*B. fragilis* complex and *C. perfringens*). Five-year trends in resistance are shown in figures 4.3.2.1 (*E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, *P. aeruginosa*, and *Acinetobacter* spp.), 4.3.2.2 (*E. faecalis* and *E. faecium*), 4.3.2.3 (*S. aureus*), 4.3.2.4 (β-haemolytic *Streptococcus* spp. groups A, B, C and G, and *S. anginosus*), and 4.3.2.5 (*B. fragilis* complex and *C. perfringens*).

In inpatient hospital departments in the Netherlands, a sample is taken from the majority of patients presenting with infections and susceptibility testing is performed as part of routine diagnostics. Therefore, bias due to selective sampling of patients is expected to be limited.

Table 4.3.2.1 Distribution of isolated pathogens in diagnostic samples from patients admitted to inpatient departments (excl. intensive care units), ISIS-AR 2022

Pathogen	Blood or cerebrospinal fluid N (%)	Lower respiratory tract N (%)	Urine N (%)	Wound or pus N (%)
E. coli	5,308 (18)	1,198 (7)	22,537 (42)	3,756 (11)
K. pneumoniae	954 (3)	544 (3)	4,188 (8)	778 (2)
P. mirabilis	335 (1)	224 (1)	3,070 (6)	919 (3)
E. cloacae complex	496 (2)	608 (4)	1,425 (3)	1,442 (4)
Other Enterobacterales[1]	1,443 (5)	1,987 (12)	5,687 (11)	3,200 (9)
P. aeruginosa	578 (2)	1,714 (11)	2,760 (5)	1,944 (6)
Acinetobacter spp.	193 (1)	173 (1)	299 (1)	354 (1)
Other non-fermenters[2]	151 (1)	1,670 (10)	279 (1)	480 (1)
B. fragilis complex	336 (1)	2 (0)	6 (0)	632 (2)
Other Gram-negatives[3]	315 (1)	3,804 (23)	8 (0)	250 (1)
E. faecalis	947 (3)	43 (0)	5,845 (11)	2,015 (6)
E. faecium	734 (2)	53 (0)	1,872 (3)	1,337 (4)
S. aureus	2,818 (9)	2,626 (16)	1,599 (3)	8,870 (25)
ß-haemolytic *Streptococcus* spp. group A	584 (2)	101 (1)	68 (0)	1,063 (3)
ß-haemolytic *Streptococcus* spp. group B	385 (1)	122 (1)	1,444 (3)	728 (2)
ß-haemolytic *Streptococcus* spp. groups C and G	225 (1)	17 (0)	58 (0)	421 (1)
S. anginosus	220 (1)	24 (0)	96 (0)	827 (2)
S. mitis/S. oralis	457 (2)	13 (0)	41 (0)	285 (1)
C. perfringens	120 (0)	2 (0)	7 (0)	154 (0)
Other Gram-positives[4]	13,365 (45)	1,393 (9)	2,672 (5)	5,355 (15)

[1] *In order of frequency: Klebsiella spp. (non-pneumoniae), Citrobacter spp., Serratia spp., Morganella spp., Proteus spp. (non-mirabilis), Raoultella spp., Providencia spp., Hafnia spp., Pantoea spp., Salmonella spp., Enterobacter spp. (non-cloacae complex), Escherichia spp. (non-coli), Cronobacter spp., Yersinia spp., Mixta spp.*

[2] *In order of frequency: M. catarrhalis, S. maltophilia, Pseudomonas spp. (non-aeruginosa), B. cepacia.*

[3] *In order of frequency: H. parainfluenzae, H. influenzae, N. meningitidis, C. coli, C. lari, C. jejuni, H. pylori.*

[4] *In order of frequency: Staphylococcus spp. (non-aureus), S. dysgalactiae subsp. equisimilis, S. pneumoniae, S. dysgalactiae n.n.g., A. urinae, Enterococcus spp. (non-faecalis, non-faecium), L. monocytogenes.*

Table 4.3.2.2 Resistance levels (%) among diagnostic isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, *P. aeruginosa*, and *Acinetobacter* spp. from patients admitted to inpatient departments (excl. intensive care units), ISIS-AR 2022

	E. coli	*K. pneumoniae*	*P. mirabilis*	*E. cloacae* complex	*P. aeruginosa*	*Acinetobacter* spp.
Antibiotic						
amoxicillin/ampicillin	39	-	21	-	-	-
co-amoxiclav[a]	29 ↓	20	6	-	-	-
piperacillin-tazobactam	4	15	0	-	6	-
cefuroxime	11	13	1	-	-	-
cefotaxime/ceftriaxone[b]	6	7	1	-	-	-
ceftazidime	4	6	0	-	3	-
meropenem/imipenem[b]	0	0	-	0	-	2
meropenem[b]	-	-	0	-	1	-
imipenem	-	-	-	-	5	-
ciprofloxacin	13	11	11	4	10	4
gentamicin	5	3	5	3	-	4
tobramycin	5	4	4	3	2	3
fosfomycin[1]	2	-	-	-	-	-
trimethoprim	23	16	30	7	-	-
co-trimoxazole	20	10 ↓	24	6	-	4
nitrofurantoin	2	-	-	-	-	-
Empiric therapy combinations						
co-amoxiclav + gentamicin[a]	3	3	1	-	-	-
cefuroxime + gentamicin	2	3	0	-	-	-
cefotaxime/ceftriaxone + gentamicin[b]	1	3	0	-	-	-
ceftazidime + tobramycin	-	-	-	-	0	-
ciprofloxacin + tobramycin	-	-	-	-	1	-
co-amoxiclav + ciprofloxacin[a]	7	5	1	-	-	-
cefuroxime + ciprofloxacin	5	7	1	-	-	-
cefotaxime/ceftriaxone + ciprofloxacin[b]	4	5	0	-	-	-
Multidrug resistance						
MDOT[a]	4	4 ↓	1	-	-	-

10 ↑	Significant and clinically relevant increasing trend since 2018.
10 ↓	Significant and clinically relevant decreasing trend since 2018.
10°	Trend not calculated because data from the years before 2022 did not meet the criteria for trend analysis.
10	No significant and clinically relevant time trend.
	(For the criteria for trend analysis and the definition of a clinically relevant trend see section 4.1.1).

- = Resistance not calculated.

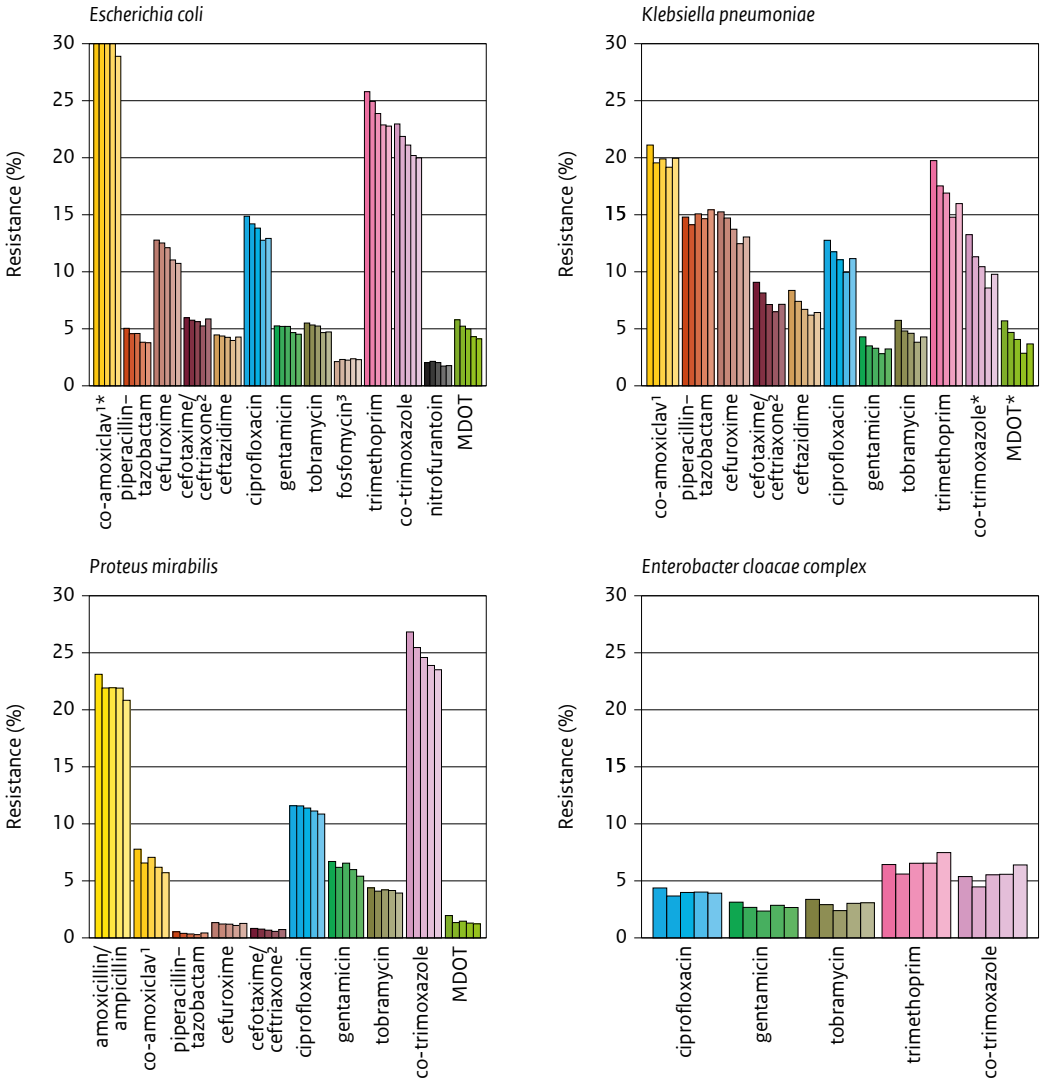
MDOT = multidrug resistance to oral therapy, defined as resistance to all of the following oral agents: co-amoxiclav (according to breakpoint for indications other than uncomplicated urinary tract infection), ciprofloxacin, and co-trimoxazole.

[1] *Resistance percentage calculated using an mic cut-off of 16 mg/L and a diameter cut-off of 24 mm. For more details see section 4.1.1.*

[a] *According to breakpoint for indications other than uncomplicated urinary tract infections. For more details see section 4.1.1.*

[b] *According to breakpoint for indications other than meningitis. For more details see section 4.1.1.*

Figure 4.3.2.1 Trends in antibiotic resistance (from left to right 2018 to 2022) among diagnostic isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, *P. aeruginosa*, and *Acinetobacter* spp. from patients admitted to inpatient departments (excl. intensive care units) in ISIS-AR



Only pathogen-agent combinations are shown for which the resistance levels were higher than 0.5% for at least one year and lower than 30% for at least three years, except for co-amoxiclav in Enterobacterales, for which resistance levels are always shown.
MDOT = Multidrug resistance for oral therapy, defined as resistance to all following oral agents: co-amoxiclav (according to breakpoint for indications other than uncomplicated urinary tract infection), ciprofloxacin, and co-trimoxazole.

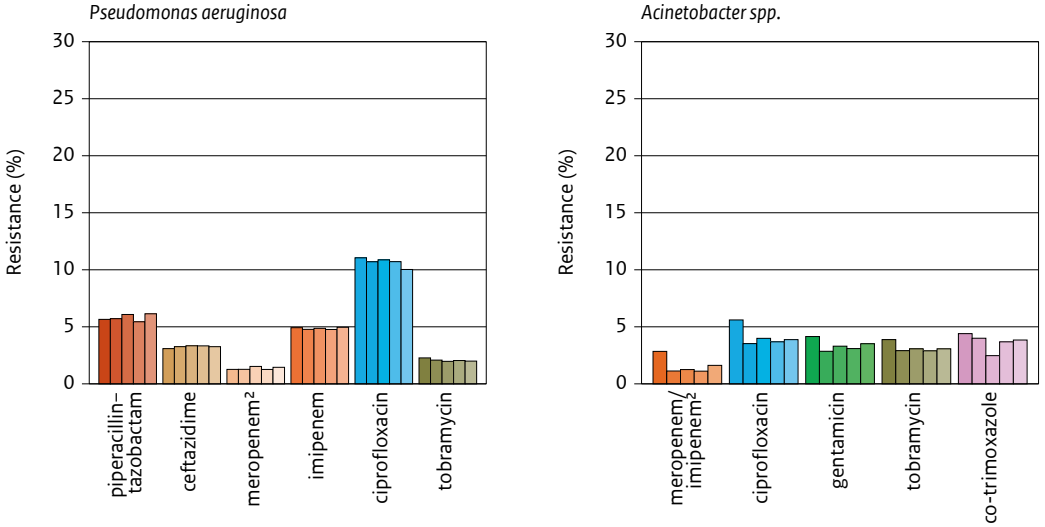
* *Trend is significant and microbiologically relevant (for details see section 4.1.1).*

[1] *According to breakpoint for indications other than uncomplicated urinary tract infection. E. coli: Resistance percentages for co-amoxiclav are from left to right 35%, 35%, 33%, 30%, and 29%.*

[2] *According to breakpoint for indications other than meningitis.*

[3] *Resistance percentage calculated using an mic cut-off of 16 mg/L and a diameter cut-off of 24 mm. For more details see section 4.1.1.*

Figure 4.3.2.1 (continued) Trends in antibiotic resistance (from left to right 2018 to 2022) among diagnostic isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, *P. aeruginosa*, and *Acinetobacter* spp. from patients admitted to inpatient departments (excl. intensive care units) in ISIS-AR



Only pathogen-agent combinations are shown for which the resistance levels were higher than 0.5% for at least one year and lower than 30% for at least three years, except for co-amoxiclav in Enterobacterales, for which resistance levels are always shown.
MDOT = Multidrug resistance for oral therapy, defined as resistance to all following oral agents: co-amoxiclav (according to breakpoint for indications other than uncomplicated urinary tract infection), ciprofloxacin, and co-trimoxazole.

* *Trend is significant and microbiologically relevant (for details see section 4.1.1).*

[1] *According to breakpoint for indications other than uncomplicated urinary tract infection. E. coli: Resistance percentages for co-amoxiclav are from left to right 35%, 35%, 33%, 30%, and 29%.*

[2] *According to breakpoint for indications other than meningitis.*

[3] *Resistance percentage calculated using an mic cut-off of 16 mg/L and a diameter cut-off of 24 mm. For more details see section 4.1.1.*

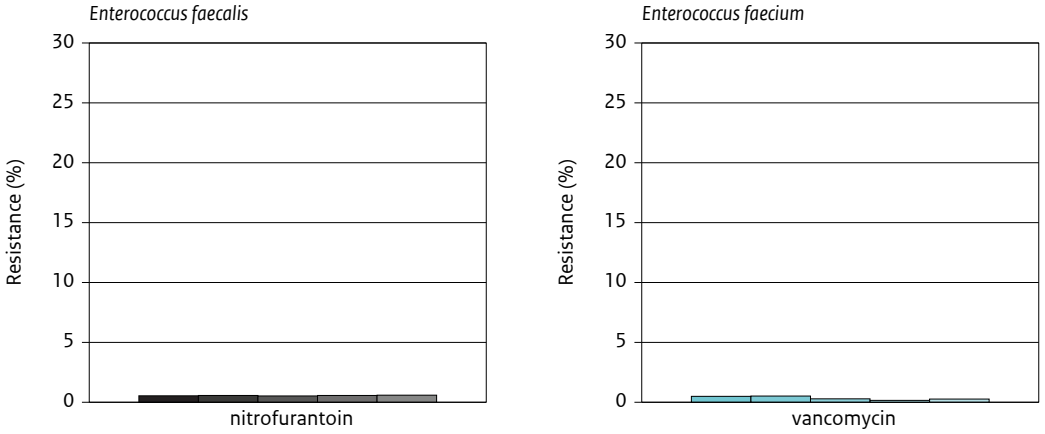
Table 4.3.2.3 Resistance levels (%) among diagnostic isolates of *E. faecalis* and *E. faecium* from patients admitted to inpatient departments (excl. intensive care units), ISIS-AR 2022

	E. faecalis	*E. faecium*
Antibiotic		
amoxicillin/ampicillin	-	85
vancomycin	0	0
linezolid	-	0
nitrofurantoin	1	-

10 ↑	Significant and clinically relevant increasing trend since 2018.
10 ↓	Significant and clinically relevant decreasing trend since 2018.
10°	Trend not calculated because data from the years before 2022 did not meet the criteria for trend analysis.
10	No significant and clinically relevant time trend.
	(For the criteria for trend analysis and the definition of a clinically relevant trend see section 4.1.1).

- = Resistance not calculated.

Figure 4.3.2.2 Trends in antibiotic resistance (from left to right 2018 to 2022) among diagnostic isolates of *E. faecalis* and *E. faecium* from patients admitted to inpatient departments (excl. intensive care units) in ISIS-AR



Only pathogen-agent combinations are shown for which the resistance levels were higher than 0.5% for at least one year and lower than 30% for at least three years.
Note: None of the trends were statistically significant and microbiologically relevant (for details see section 4.1.1).

Table 4.3.2.4 Resistance levels (%) among diagnostic isolates of *S. aureus* from patients admitted to inpatient departments (excl. intensive care units), ISIS-AR 2022

	S. aureus
Antibiotic	
MRSA	2
ciprofloxacin[1]	4
gentamicin	1
erythromycin[c] ╞	17
clindamycin (including inducible resistance)[2]	15
doxycycline/tetracycline[c]	4
fusidic acid	7
linezolid	0
co-trimoxazole	2
rifampicin	0
mupirocine[a]	0
mupirocine[b]	1

10 ↑	Significant and clinically relevant increasing trend since 2018.
10 ↓	Significant and clinically relevant decreasing trend since 2018.
10°	Trend not calculated because data from the years before 2022 did not meet the criteria for trend analysis.
10	No significant and clinically relevant time trend.
	(For the criteria for trend analysis and the definition of a clinically relevant trend see section 4.1.1).

MRSA = Methicillin resistant Staphylococcus aureus. For the estimation method of MRSA see section 4.1.1.

[1] *Resistance to ciprofloxacin is intended to be a class indicator for resistance to fluoroquinolones.*

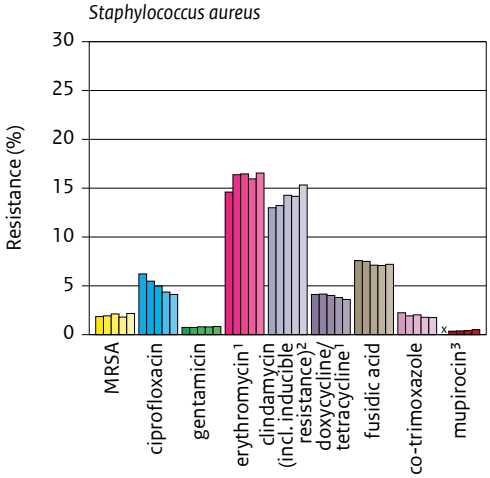
[2] *For the method used to estimate clindamycin resistance (including inducible resistance) see section 4.1.1.*

[a] *According to breakpoint for nasal decontamination. For more details see section 4.1.1.*

[b] *According to breakpoint for topical use. For more details see section 4.1.1.*

[c] *According to breakpoint for screening. For more details see section 4.1.1.*

Figure 4.3.2.3 Trends in antibiotic resistance (from left to right 2018 to 2022) among diagnostic isolates of *S. aureus* from patients admitted to inpatient departments (excl. intensive care units) in ISIS-AR



Only pathogen-agent combinations are shown for which the resistance levels were higher than 0.5% for at least one year and lower than 30% for at least three years.
Note: None of the trends were statistically significant and microbiologically relevant (for details see section 4.1.1).
MRSA = Methicillin resistant Staphylococcus aureus.
For the estimation method of MRSA see section 4.1.1.

1 *According to breakpoint for screening.*
2 *For the method used to estimate clindamycin resistance (including inducible resistance) see section 4.1.1.*
3 *According to the breakpoint for topical use.*

Table 4.3.2.5 Resistance levels (%) among diagnostic isolates of β-haemolytic *Streptococcus* spp. groups A,B,C and G, *S. anginosus*, and *S. mitis/S. oralis* from patients admitted to inpatient departments (excl. intensive care units), ISIS-AR 2022

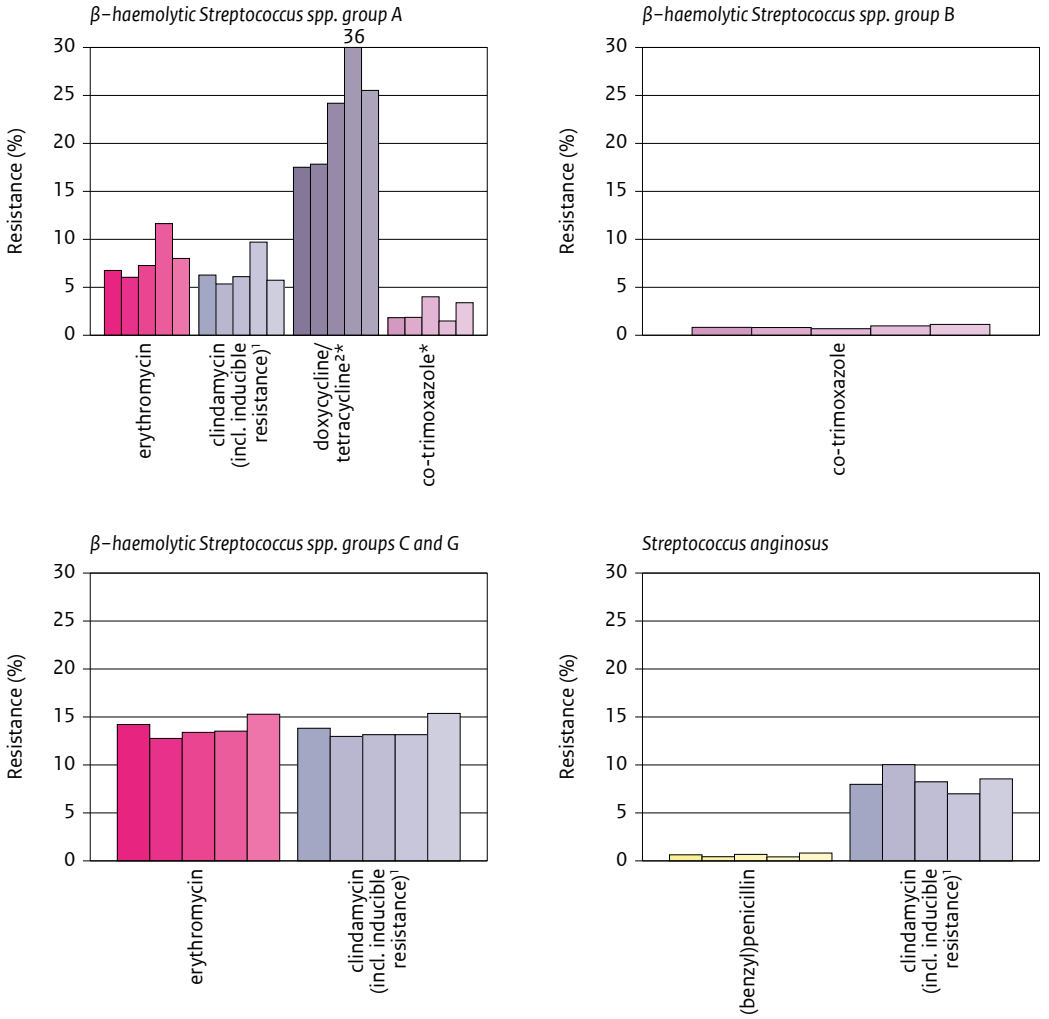
	β-haemolytic *Streptococcus* spp. group A	β-haemolytic *Streptococcus* spp. group B	β-haemolytic *Streptococcus* spp. groups C and G	*S. anginosus*	*S. mitis/ S. oralis*
Antibiotic					
(benzyl)penicillin	-	-	-	0	4°
amoxicillin/ampicillin	-	-	-	0°	6°
erythromycin	8	20°	15	-	-
clindamycin (including inducible resistance)[1]	6	17°	15	9	9°
doxycycline/tetracycline[a]	26 ↑	-	-	-	-
co-trimoxazole	3 ↑	1	0°	-	-

10 ↑	Significant and clinically relevant increasing trend since 2018.
10 ↓	Significant and clinically relevant decreasing trend since 2018.
10°	Trend not calculated because data from the years before 2022 did not meet the criteria for trend analysis.
10	No significant and clinically relevant time trend.

(For the criteria for trend analysis and the definition of a clinically relevant trend see section 4.1.1).

- = *Resistance not calculated.*
1 *For the method used to estimate clindamycin resistance (including inducible resistance) see section 4.1.1.*
a *According to breakpoint for screening. For more details see section 4.1.1.*

Figure 4.3.2.4 Trends in antibiotic resistance (from left to right 2018 to 2022) among diagnostic isolates of β-haemolytic *Streptococcus* spp. groups A,B,C and G, and *S. anginosus* from patients admitted to inpatient departments (excl. intensive care units) in ISIS-AR



Only pathogen-agent combinations are shown for which the resistance levels were higher than 0.5% for at least one year and lower than 30% for at least three years.

* *Trend is significant and microbiologically relevant (for details see section 4.1.1).*

1 *For the method used to estimate clindamycin resistance (including inducible resistance) see section 4.1.1.*

2 *According to breakpoint for screening.*

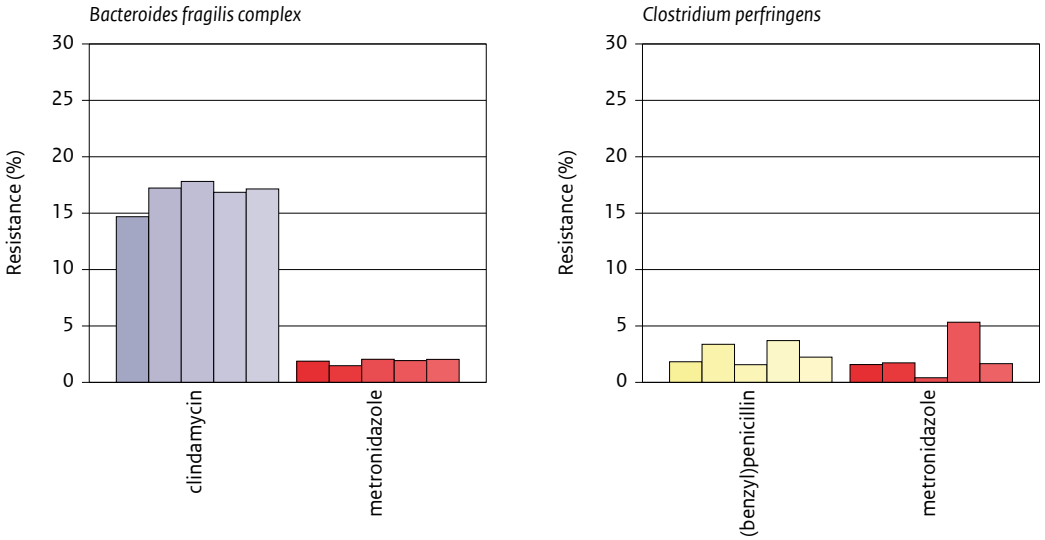
Table 4.3.2.6 Resistance levels (%) among diagnostic isolates of *B. fragilis* complex and *C. perfringens* from patients admitted to inpatient departments (excl. intensive care units), ISIS-AR 2022

	B. fragilis complex	*C. perfringens*
Antibiotic		
(benzyl)penicillin	-	2
clindamycin	17	64
metronidazole	2	2

10 ↑	Significant and clinically relevant increasing trend since 2018.
10 ↓	Significant and clinically relevant decreasing trend since 2018.
10°	Trend not calculated because data from the years before 2022 did not meet the criteria for trend analysis.
10	No significant and clinically relevant time trend.
	(For the criteria for trend analysis and the definition of a clinically relevant trend see section 4.1.1).

- = Resistance not calculated.

Figure 4.3.2.5 Trends in antibiotic resistance (from left to right 2018 to 2022) among diagnostic isolates of *B. fragilis* complex and *C. perfringens* from patients admitted to inpatient departments (excl. intensive care units) in ISIS-AR



Only pathogen-agent combinations are shown for which the resistance levels were higher than 0.5% for at least one year and lower than 30% for at least three years.
Note: None of the trends were statistically significant and microbiologically relevant (for details see section 4.1.1).

Key results and conclusions

Blood, urine, wound/pus and respiratory: E. coli, K. pneumoniae, P. aeruginosa, and Acinetobacter spp.

- For both Enterobacterales and *P. aeruginosa*, resistance levels for all tested antibiotics were comparable to resistance levels in isolates of OPD patients. Except for **ciprofloxacin** and **co-trimoxazole**, for which resistance levels were lower in isolates from hospital patients.
- For all Enterobacterales, resistance to **second and third generation cephalosporins** seemed to have plateaued or slightly decreased over the past five years. In 2022, resistance to **cefuroxime** was 11% in *E. coli* and 13% in *K. pneumoniae*. Resistance to **cefotaxime/ceftriaxone** was 6% in *E. coli* and 7% in *K. pneumoniae*. This is encouraging but nevertheless, patients that are infected with *K. pneumoniae* or *E. coli* have a considerable risk of non-adequate empiric treatment with a **second or** (to a lesser extent) **third generation cephalosporin**. In case of severe infection, empiric combination therapy with **aminoglycosides**, reducing likelihood of resistance to 3% or less, might be a suitable option.
- For the three most important oral antibiotics, **co-amoxiclav**, **co-trimoxazole** and **ciprofloxacin**, a similar trend was found as observed in isolates from primary care and OPD patients. In *E. coli*, resistance to **co-amoxiclav** decreased to 29% and was stable at 20% in *K. pneumoniae*. Resistance to **co-trimoxazole** decreased to 10% in *K. pneumoniae* and was 20% in *E. coli*. Resistance levels for **ciprofloxacin** was 13% in *E. coli*, 11% in *K. pneumoniae* and 10% in *P. aeruginosa*.
- Resistance to **co-amoxiclav** in Enterobacterales is high. In 2022, the resistance percentage in *E. coli* was 29% and in *K. pneumoniae* 20%. This renders the drug unsuitable for empiric therapy for any infection potentially caused by gram-negative bacteria, unless it is combined with a second drug, preferably an **aminoglycoside**.
- For *P. aeruginosa*, resistance is relatively low and stable for all antibiotics over the last five years. Empirical treatment with **ceftazidime** when infections are potentially caused by *P. aeruginosa* remains therefore adequate.
- Resistance in *Acinetobacter* spp. for all tested antibiotics remained low at 4% or less.

Blood, urine, wound/pus and respiratory: S. aureus

- In *S. aureus* resistance was high for **clindamycin** (including inducible resistance, 15%), **erythromycin** (17%) and **fusidic acid** (7%). Resistance percentages in *S. aureus* were comparable to isolates from OPD patients.
- The 15% resistance for **clindamycin** (including inducible resistance) indicates that culture and susceptibility testing are mandatory before starting treatment with this drug.
- **MRSA** was found in 2% of *S. aureus* isolates of hospital patients which remained stable over the previous 5 years.

Blood, urine, blood, wound/pus, and respiratory: β-haemolytic Streptococcus spp. groups A, B and C/G

- Resistance to **clindamycin** (including inducible resistance) and **erythromycin** in β-haemolytic *Streptococcus* spp. group A remained stable at 6% for **clindamycin** (including inducible resistance) and 8% for **erythromycin**. Resistance to **doxycycline/tetracycline** increased over the last five years and was 26% in 2022 (36% in 2021).

- In β-haemolytic *Streptococcus* spp. groups B and C/G, resistance levels for **clindamycin** (including inducible resistance, 17% group B, 15% group C/G), and **erythromycin** (20% group B, 15% group C/G) were higher than for group A. For β-haemolytic *Streptococcus* spp. group C/G, these levels remained stable over the last five years. However, for β-haemolytic *Streptococcus* spp. group B, a time trend could not be calculated due to the low number of isolates tested for these antibiotics in earlier years.

Blood, urine, blood, wound/pus, and respiratory: Anaerobes

- The European Committee on Antimicrobial Susceptibility Testing (EUCAST) publishes clinical minimum inhibitory concentration (MIC) breakpoints for anaerobes. Prior to 2022, these breakpoints were not species-specific. In EUCAST breakpoints version 12.0 (2022), no breakpoints were available for **benzylpenicillin** or **co-amoxiclav** for *B. fragilis*. In addition, the breakpoint for **clindamycin** for *C. perfringens* was lowered from 4 mg/L (not species-specific) to 0.25 mg/L (species-specific). This resulted in a much higher resistance level for **clindamycin** (64%) in *C. perfringens* using the new 2022 breakpoint version compared to the resistance percentage reported for 2021 in Nethmap 2022 (11%). However, when all test values were reinterpreted, according to EUCAST breakpoints version 12.0, including those from previous years, resistance to **clindamycin** in *C. perfringens* remained stable over the last five years.
- Resistance to **metronidazole** in both *B. fragilis* and *C. perfringens* remained low at 2% and was not influenced by the new species-specific breakpoints.

4.3.3 Intensive Care Units

The distribution of pathogens from diagnostic samples (blood or cerebrospinal fluid, lower respiratory tract, urine, and wound infections or pus) from patients admitted to intensive care units in 2022 is presented in table 4.3.3.1. The resistance levels for a selection of pathogens isolated from these patients in 2022 are presented in tables 4.3.3.2 (*E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, *P. aeruginosa*, and *Acinetobacter* spp.), 4.3.3.3 (*E. faecalis* and *E. faecium*), 4.3.3.4 (*S. aureus*) and 4.3.3.5 (β-haemolytic *Streptococcus* spp. group A and group B). Five-year trends in resistance are shown in figures 4.3.3.1 (*E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, *P. aeruginosa*, and *Acinetobacter* spp.), 4.3.3.2 (*S. aureus*) and 4.3.3.3 (β-haemolytic *Streptococcus* spp. group A and group B). For *E. faecium* and *E. faecalis* trends, and for β-haemolytic *Streptococcus* spp. groups C and G, *S. anginosus*, *S. mitis/S. oralis*, *B. fragilis* complex, and *C. perfringens*, resistance levels and trends were not calculated because in 2022 results for the majority of antibiotics were available for less than 100 isolates.

In intensive care units in the Netherlands, a sample is taken from almost all patients presenting with infections and susceptibility testing is performed as part of routine diagnostics. Bias due to selective sampling of patients is therefore unlikely.

Table 4.3.3.1 Distribution of isolated pathogens in diagnostic samples from patients admitted to intensive care units, ISIS-AR 2022

Pathogen	Blood or cerebrospinal fluid N (%)	Lower respiratory tract N (%)	Urine N (%)	Wound or pus N (%)
E. coli	164 (5)	407 (9)	556 (40)	333 (13)
K. pneumoniae	42 (1)	157 (4)	102 (7)	70 (3)
P. mirabilis	6 (0)	63 (1)	70 (5)	44 (2)
E. cloacae complex	36 (1)	228 (5)	36 (3)	117 (5)
Other Enterobacterales[1]	83 (3)	738 (17)	142 (10)	217 (8)
P. aeruginosa	46 (1)	332 (8)	75 (5)	165 (6)
Acinetobacter spp.	16 (0)	69 (2)	7 (0)	28 (1)
Other non-fermenters[2]	7 (0)	397 (9)	8 (1)	51 (2)
Other Gram-negatives[3]	28 (1)	454 (10)	1 (0)	50 (2)
E. faecalis	138 (4)	23 (1)	164 (12)	247 (10)
E. faecium	283 (9)	49 (1)	120 (9)	345 (13)
S. aureus	240 (7)	1,036 (24)	51 (4)	284 (11)
ß-haemolytic *Streptococcus* spp. group A	22 (1)	48 (1)	0 (0)	118 (5)
ß-haemolytic *Streptococcus* spp. group B	10 (0)	56 (1)	11 (1)	25 (1)
Other Gram-positives[4]	2,118 (65)	278 (6)	58 (4)	490 (19)

[1] *In order of frequency: Klebsiella spp. (non-pneumoniae), Serratia spp., Citrobacter spp., Morganella spp., Proteus spp. (non-mirabilis), Hafnia spp., Raoultella spp., Enterobacter spp. (non-cloacae complex), Pantoea spp., Providencia spp., Escherichia spp. (non-coli), Salmonella spp., Cronobacter spp.*

[2] *In order of frequency: S. maltophilia, M. catarrhalis, Pseudomonas spp. (non-aeruginosa).*

[3] *In order of frequency: H. influenzae, H. parainfluenzae, B. fragilis complex, N. meningitidis, C. jejuni.*

[4] *In order of frequency: Staphylococcus spp. (non-aureus), S. anginosus, S. mitis/S. oralis, S. dysgalactiae n.n.g., S. pneumoniae, ß-haemolytic Streptococcus spp. groups C and G, S. dysgalactiae subsp. equisimilis, Enterococcus spp. (non-faecalis, non-faecium), A. urinae, C. perfringens, L. monocytogenes.*

Table 4.3.3.2 Resistance levels (%) among diagnostic isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, *P. aeruginosa*, and *Acinetobacter* spp. from patients admitted to intensive care units, ISIS-AR 2022

	E. coli	*K. pneumoniae*	*P. mirabilis*	*E. cloacae* complex	*P. aeruginosa*	*Acinetobacter* spp.
Antibiotic						
amoxicillin/ampicillin	40	-	23	-	-	-
co-amoxiclav[a]	28 ↓	25	8	-	-	-
piperacillin-tazobactam	5	18	1	-	16 ↑	-
cefuroxime	14	18	2	-	-	-
cefotaxime/ceftriaxone[b]	8	14	1	-	-	-
ceftazidime	6	12	0	-	10 ↑	-
meropenem/imipenem[b]	0	1	-	1	-	6
meropenem[b]	-	-	0	-	4	-
imipenem	-	-	-	-	9	-
ciprofloxacin	11	14	10	5	11	6
gentamicin	4	7	4	6	-	7
tobramycin	4	8	3	6	4	5
co-trimoxazole	18 ↓	14	27	7	-	7
Empiric therapy combinations						
co-amoxiclav + gentamicin[a]	3	6	1	-	-	-
cefuroxime + gentamicin	2	7	1	-	-	-
cefotaxime/ceftriaxone + gentamicin[b]	2	7	1	-	-	-
ceftazidime + tobramycin	-	-	-	-	2	-
ciprofloxacin + tobramycin	-	-	-	-	3	-
co-amoxiclav + ciprofloxacin[a]	6 ↓	10	2	-	-	-
cefuroxime + ciprofloxacin	6	11	1	-	-	-
cefotaxime/ceftriaxone + ciprofloxacin[b]	5	10	1	-	-	-
Multidrug resistance						
MDOT[a]	4	8	2	-	-	-

10 ↑	Significant and clinically relevant increasing trend since 2018.
10 ↓	Significant and clinically relevant decreasing trend since 2018.
10°	Trend not calculated because data from the years before 2022 did not meet the criteria for trend analysis.
10	No significant and clinically relevant time trend.

(For the criteria for trend analysis and the definition of a clinically relevant trend see section 4.1.1).

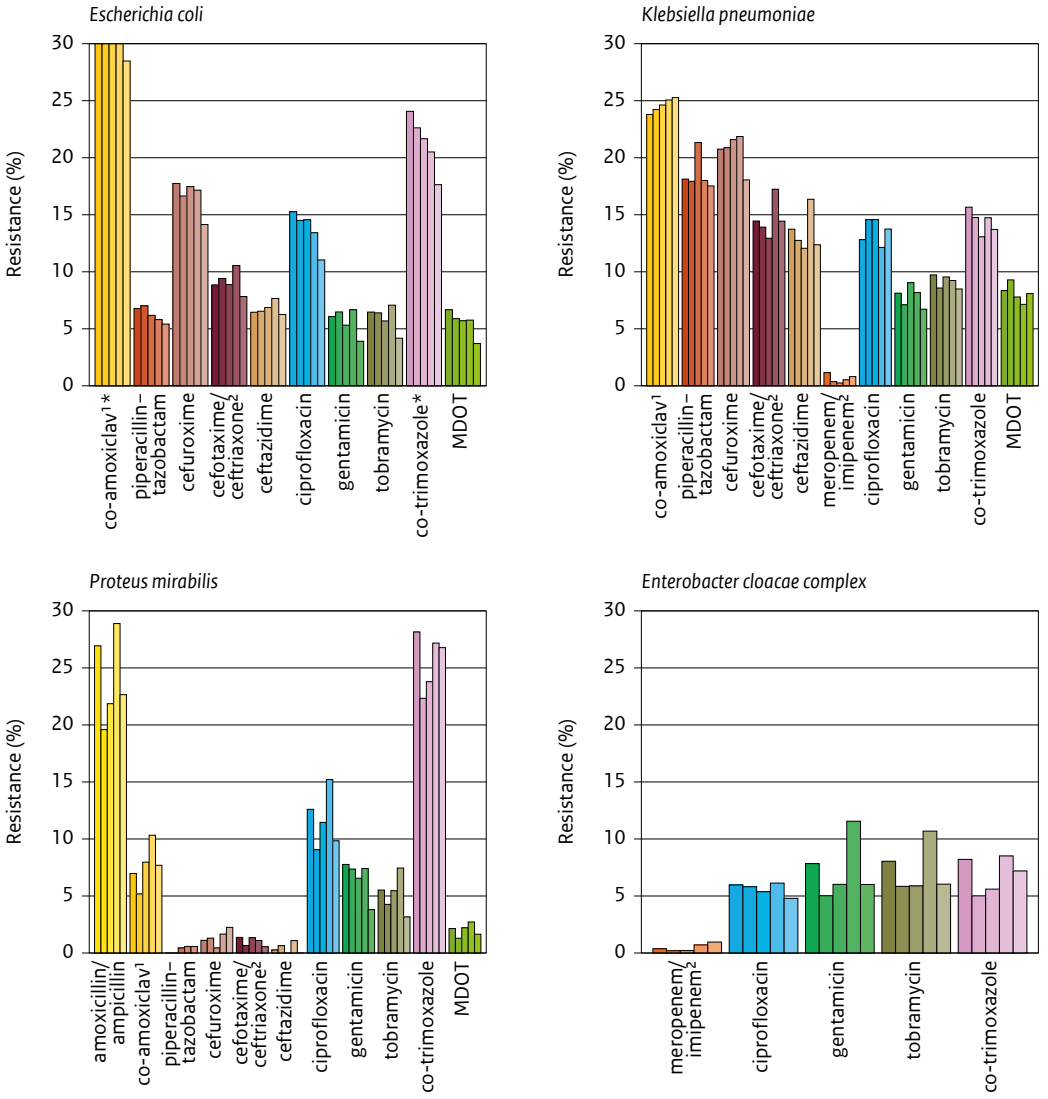
- = Resistance not calculated.

MDOT = multidrug resistance to oral therapy, defined as resistance to all of the following oral agents: co-amoxiclav (according to breakpoint for indications other than uncomplicated urinary tract infection), ciprofloxacin, and co-trimoxazole.

[a] *According to breakpoint for indications other than uncomplicated urinary tract infections. For more details see section 4.1.1.*

[b] *According to breakpoint for indications other than meningitis. For more details see section 4.1.1.*

Figure 4.3.3.1 Trends in antibiotic resistance (from left to right 2018 to 2022) among diagnostic isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, *P. aeruginosa*, and *Acinetobacter* spp. from patients admitted to intensive care units in ISIS-AR



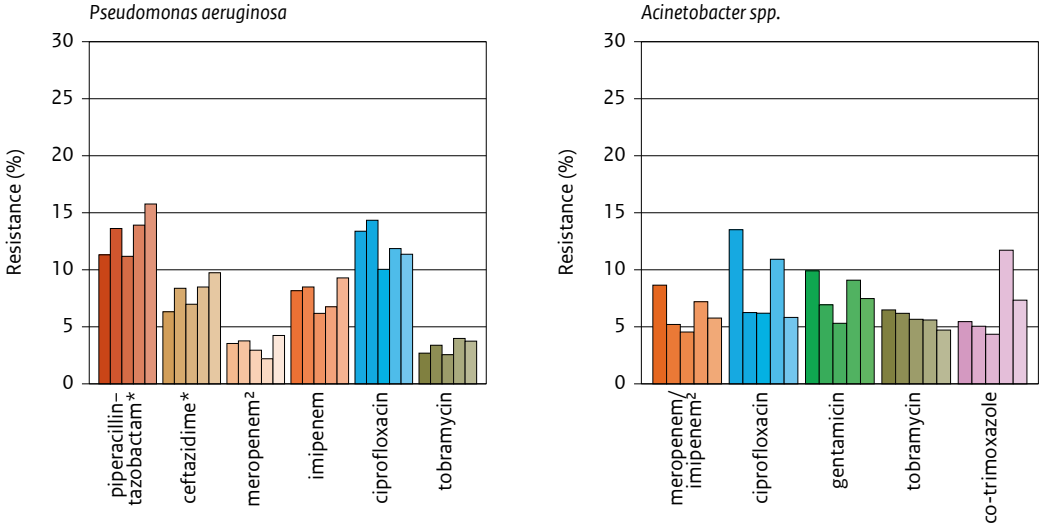
Only pathogen-agent combinations are shown for which the resistance levels were higher than 0.5% for at least one year and lower than 30% for at least three years, except for co-amoxiclav in Enterobacterales, for which resistance levels are always shown.
MDOT = Multidrug resistance for oral therapy, defined as resistance to all following oral agents: co-amoxiclav (according to breakpoint for according to the breakpoint for indications other than uncomplicated urinary tract infection), ciprofloxacin, and co-trimoxazole.

* *Trend is significant and microbiologically relevant (for details see section 4.1.1).*

[1] *According to breakpoint for indications other than uncomplicated urinary tract infection. E. coli: Resistance percentages for co-amoxiclav are from left to right 37%, 39%, 37%, 34% and 28%.*

[2] *According to breakpoint for indications other than meningitis.*

Figure 4.3.3.1 (continued) Trends in antibiotic resistance (from left to right 2018 to 2022) among diagnostic isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, *P. aeruginosa*, and *Acinetobacter* spp. from patients admitted to intensive care units in ISIS-AR



Only pathogen-agent combinations are shown for which the resistance levels were higher than 0.5% for at least one year and lower than 30% for at least three years, except for co-amoxiclav in Enterobacterales, for which resistance levels are always shown.
MDOT = Multidrug resistance for oral therapy, defined as resistance to all following oral agents: co-amoxiclav (according to breakpoint for according to the breakpoint for indications other than uncomplicated urinary tract infection), ciprofloxacin, and co-trimoxazole.

* *Trend is significant and microbiologically relevant (for details see section 4.1.1).*

[1] *According to breakpoint for indications other than uncomplicated urinary tract infection. E. coli: Resistance percentages for co-amoxiclav are from left to right 37%, 39%, 37%, 34% and 28%.*

[2] *According to breakpoint for indications other than meningitis.*

Table 4.3.3.3 Resistance levels (%) among diagnostic isolates of *E. faecalis* and *E. faecium* from patients admitted to intensive care units, ISIS-AR 2022

	E. faecalis	*E. faecium*
Antibiotic		
amoxicillin/ampicillin	-	87
vancomycin	0	0
linezolid	-	0

10 ↑	Significant and clinically relevant increasing trend since 2018.
10 ↓	Significant and clinically relevant decreasing trend since 2018.
10°	Trend not calculated because data from the years before 2022 did not meet the criteria for trend analysis.
10	No significant and clinically relevant time trend.

(For the criteria for trend analysis and the definition of a clinically relevant trend see section 4.1.1).

- = Resistance not calculated.

Table 4.3.3.4 Resistance levels (%) among diagnostic isolates of *S. aureus* from patients admitted to intensive care units, ISIS-AR 2022

	S. aureus
Antibiotic	
MRSA	4 ↑
ciprofloxacin[1]	3
gentamicin	2 ↑
erythromycin[c]	16
clindamycin (including inducible resistance)[2]	15
doxycycline/tetracycline[c]	4
fusidic acid	4
linezolid	0
co-trimoxazole	2
rifampicin	0
mupirocine[a]	0
mupirocine[b]	1

10 ↑	Significant and clinically relevant increasing trend since 2018.
10 ↓	Significant and clinically relevant decreasing trend since 2018.
10°	Trend not calculated because data from the years before 2022 did not meet the criteria for trend analysis.
10	No significant and clinically relevant time trend.
	(For the criteria for trend analysis and the definition of a clinically relevant trend see section 4.1.1).

- = Resistance not calculated.

MRSA = Methicillin resistant Staphylococcus aureus. For the estimation method of MRSA see section 4.1.1.

[1] *Resistance to ciprofloxacin is intended to be a class indicator for resistance to fluoroquinolones.*

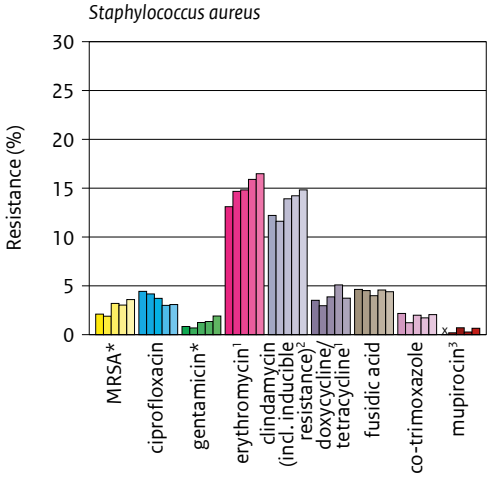
[2] *For the method used to estimate clindamycin resistance (including inducible resistance) see section 4.1.1.*

[a] *According to breakpoint for nasal decontamination. For more details see section 4.1.1.*

[b] *According to breakpoint for topical use. For more details see section 4.1.1.*

[c] *According to breakpoint for screening. For more details see section 4.1.1.*

Figure 4.3.3.2 Trends in antibiotic resistance (from left to right 2018 to 2022) among diagnostic isolates of *S. aureus* from patients admitted to intensive care units in ISIS-AR



Only pathogen-agent combinations are shown for which the resistance levels were higher than 0.5% for at least one year and lower than 30% for at least three years.
** Trend is significant and microbiologically relevant (for details see section 4.1.1).*
MRSA = Methicillin resistant Staphylococcus aureus.
For the estimation method of MRSA see section 4.1.1.
[1] *According to breakpoint for screening.*
[2] *For the method used to estimate clindamycin resistance (including inducible resistance) see section 4.1.1.*
[3] *According to the breakpoint for topical use.*

Table 4.3.3.5 Resistance levels (%) among diagnostic isolates of β-haemolytic *Streptococcus* spp. group A and group B from patients admitted to intensive care units, ISIS-AR 2022

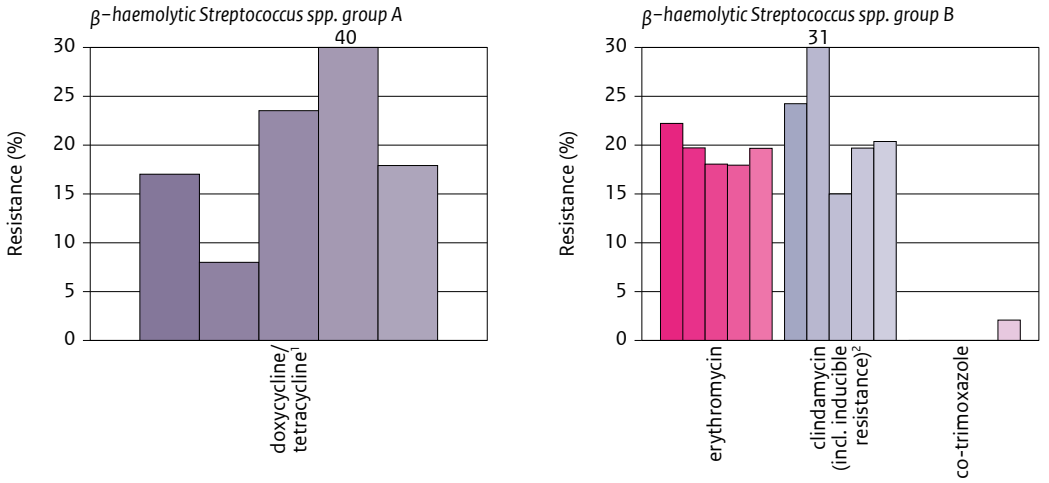
	ß-haemolytic *Streptococcus* spp. group A	ß-haemolytic *Streptococcus* spp. group B
Antibiotic		
erythromycin	2°	20
clindamycin (including inducible resistance)[1]	2°	20
doxycycline/tetracycline[a]	18°	-
co-trimoxazole	3°	2

10 ↑	Significant and clinically relevant increasing trend since 2018.
10 ↓	Significant and clinically relevant decreasing trend since 2018.
10°	Trend not calculated because data from the years before 2022 did not meet the criteria for trend analysis.
10	No significant and clinically relevant time trend.

(For the criteria for trend analysis and the definition of a clinically relevant trend see section 4.1.1).

- = Resistance not calculated.
[1] *For the method used to estimate clindamycin resistance (including inducible resistance) see section 4.1.1.*
[a] *According to breakpoint for screening. For more details see section 4.1.1.*

Figure 4.3.3.3 Trends in antibiotic resistance (from left to right 2018 to 2022) among diagnostic isolates of β-haemolytic *Streptococcus* spp. group A and group B from patients admitted to intensive care units in ISIS-AR



Only pathogen-agent combinations are shown for which the resistance levels were higher than 0.5% for at least one year and lower than 30% for at least three years.

Note: None of the trends were statistically significant and microbiologically relevant (for details see section 4.1.1).

[1] *According to breakpoint for screening.*

[2] *For the method used to estimate clindamycin resistance (including inducible resistance) see section 4.1.1.*

Key results and conclusions

Blood, urine, blood, wound/pus, and respiratory: Enterobacterales, P. aeruginosa and Acinetobacter spp.

- For *E. coli*, resistance levels for all tested antibiotics in ICU patients were comparable to resistance levels in *E. coli* from non-ICU patients.
- For *K. pneumoniae* and *P. aeruginosa*, resistance levels were much higher in ICU patients than in isolates from non-ICU patients.
- In *K. pneumoniae*, resistance to **piperacillin-tazobactam** and **cefuroxime** was almost 20% and remained stable over the last five years. Resistance to **third generation cephalosporins** was 14% for **cefotaxime/ceftriaxone** and 12% for **ceftazidime**. This means that ICU patients with infections due to *K. pneumoniae* had considerable risk of non-adequate empiric treatment with a **second or a third generation cephalosporin**. In case of severe infection, empiric combination therapy with **aminoglycosides**, reducing likelihood of resistance to 7% or less, might be a suitable option.
- In *P. aeruginosa* isolates from ICU patients, resistance to **piperacillin-tazobactam** and **ceftazidime**, the two first choice agents for the treatment of severe *P. aeruginosa* infections, increased to 16% for **piperacillin-tazobactam** and 10% for **ceftazidime** over the last five years. This might complicate empirical treatment of severe infections due to *P. aeruginosa*. However, in many Dutch ICUs, routine surveillance cultures are taken for monitoring resistance in gram-negative aerobic enteric bacteria.

This means that empirical treatment can often be guided by these culture results in case of infection. Based on the resistance levels in gram-negative bacteria of ICU patients in 2022, routine culturing with susceptibility testing remains mandatory to tailor therapy to the individual patient.
- Resistance in *Acinetobacter* spp. in ICU patients was higher than for non-ICU patients but still remained low for all suitable antibiotics at 7% or less.

Blood, urine, blood, wound/pus, and respiratory: S. aureus
- **MRSA** percentage in ICU patients increased to 4% over the last five years. The **MRSA** level was higher than in non-ICU patients.
- Also in the ICU setting, *S. aureus* showed an ongoing rise of **clindamycin** resistance (including inducible resistance, 15%).

4.3.4 Blood isolates from inpatient departments (incl. intensive care units)

The distribution of pathogens isolated from blood of patients admitted to non-intensive care inpatient departments (non-ICU) and intensive care units (ICU) in 2022 is presented in table 4.3.4.1. Resistance levels for a selection of pathogens isolated from these patients in 2022 are presented in tables 4.3.4.2 (*E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, *P. aeruginosa* and *Acinetobacter* spp.), 4.3.4.3 (*E. faecalis* and *E. faecium*), 4.3.4.4 (*S. aureus*), 4.3.4.5 (β-haemolytic *Streptococcus* spp. groups A, B, C and G, *S. anginosus*, and *S. mitis/S. oralis*), and 4.3.4.6 (*B. fragilis* complex). Five-year trends in resistance are presented in figures 4.3.4.1 (*E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, *P. aeruginosa*, and *Acinetobacter* spp.), 4.3.4.2 (*E. faecium*), 4.3.4.3 (*S. aureus*), 4.3.4.4 (β-haemolytic *Streptococcus* spp. groups A, B, C and G, *S. anginosus*, and *S. mitis/S. oralis*), and 4.3.4.5 (*B. fragilis* complex). For *C. perfringens* both resistance levels and trends were not calculated because in 2022 less than 100 isolates were available for analysis.

In most hospitals, blood samples are taken from all patients suspected of having sepsis and susceptibility testing is performed as part of routine diagnostics. Bias due to selective sampling of patients is therefore unlikely. However, a substantial part of isolates is likely to be contamination rather than cause of infection.

Table 4.3.4.1 Distribution of pathogens in diagnostic blood samples from patients admitted to non-intensive care inpatient departments (non-ICU) and intensive care units (ICU), ISIS-AR 2022

Pathogen	Non-ICU N (%)	ICU N (%)
E. coli	6,366 (22)	165 (5)
K. pneumoniae	1,129 (4)	48 (1)
P. mirabilis	419 (1)	5 (0)
E. cloacae complex	531 (2)	46 (1)
Other Enterobacterales[1]	1,567 (5)	102 (3)
P. aeruginosa	648 (2)	63 (2)
Acinetobacter spp.	174 (1)	15 (0)
Other non-fermenters[2]	148 (1)	12 (0)
B. fragilis complex	333 (1)	11 (0)
Other Gram-negatives[3]	295 (1)	14 (0)
E. faecalis	947 (3)	140 (4)
E. faecium	554 (2)	311 (10)
S. aureus	3,077 (10)	193 (6)
ß-haemolytic *Streptococcus* spp. group A	610 (2)	25 (1)
ß-haemolytic *Streptococcus* spp. group B	394 (1)	10 (0)
ß-haemolytic *Streptococcus* spp. groups C and G	226 (1)	3 (0)
S. anginosus	224 (1)	11 (0)
S. mitis/S. oralis	436 (1)	18 (1)
Other Gram-positives[4]	11,413 (39)	2,024 (63)

[1] *In order of frequency: Klebsiella spp. (non-pneumoniae), Serratia spp., Citrobacter spp., Salmonella spp., Morganella spp., Pantoea spp., Raoultella spp., Proteus spp. (non-mirabilis), Providencia spp., Hafnia spp., Enterobacter spp. (non-cloacae complex), Yersinia spp., Escherichia spp. (non-coli), Mixta spp., Cronobacter spp.*

[2] *In order of frequency: Pseudomonas spp. (non-aeruginosa), S. maltophilia, M. catarrhalis, B. cepacia.*

[3] *In order of frequency: H. parainfluenzae, H. influenzae, N. meningitidis, C. lari, C. coli, C. jejuni.*

[4] *In order of frequency: Staphylococcus spp. (non-aureus), S. dysgalactiae n.n.g., S. dysgalactiae subsp. equisimilis, S. pneumoniae, C. perfringens, Enterococcus spp. (non-faecalis, non-faecium), A. urinae, L. monocytogenes.*

Table 4.3.4.2 Resistance levels (%) among diagnostic blood isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, *P. aeruginosa* and *Acinetobacter* spp. from patients admitted to inpatient departments (incl. intensive care units), ISIS-AR 2022

	E. coli	*K. pneumoniae*	*P. mirabilis*	*E. cloacae* complex	*P. aeruginosa*	*Acinetobacter* spp.
Antibiotic						
amoxicillin/ampicillin	40	-	21	-	-	-
co-amoxiclav[a]	29 ↓	18	7	-	-	-
piperacillin-tazobactam	4	13	0	-	7	-
cefuroxime	11	12	1	-	-	-
cefotaxime/ceftriaxone[b]	7	8	1	-	-	-
ceftazidime	6	7	1	-	5	-
meropenem/imipenem[b]	0	0	-	0	-	1
meropenem[b]	-	-	0	-	2	-
imipenem	-	-	-	-	6	-
ciprofloxacin	13	12	11	5	7	3 ↓
gentamicin	5	4	5	4	-	1
tobramycin	6	5	3	4	0	2
co-trimoxazole	21	13	22	8	-	4
Empiric therapy combinations						
co-amoxiclav + gentamicin[a]	4	4	2	-	-	-
cefuroxime + gentamicin	2	3	0	-	-	-
cefotaxime/ceftriaxone + gentamicin[b]	2	3	0	-	-	-
ceftazidime + tobramycin	-	-	-	-	0	-
ciprofloxacin + tobramycin	-	-	-	-	0	-
co-amoxiclav + ciprofloxacin[a]	7 ↓	6	2	-	-	-
cefuroxime + ciprofloxacin	6	7	1	-	-	-
cefotaxime/ceftriaxone + ciprofloxacin[b]	5	5	1	-	-	-
Multidrug resistance						
MDOT[a]	4	5	1	-	-	-

10 ↑	Significant and clinically relevant increasing trend since 2018.
10 ↓	Significant and clinically relevant decreasing trend since 2018.
10°	Trend not calculated because data from the years before 2022 did not meet the criteria for trend analysis.
10	No significant and clinically relevant time trend.

(For the criteria for trend analysis and the definition of a clinically relevant trend see section 4.1.1).

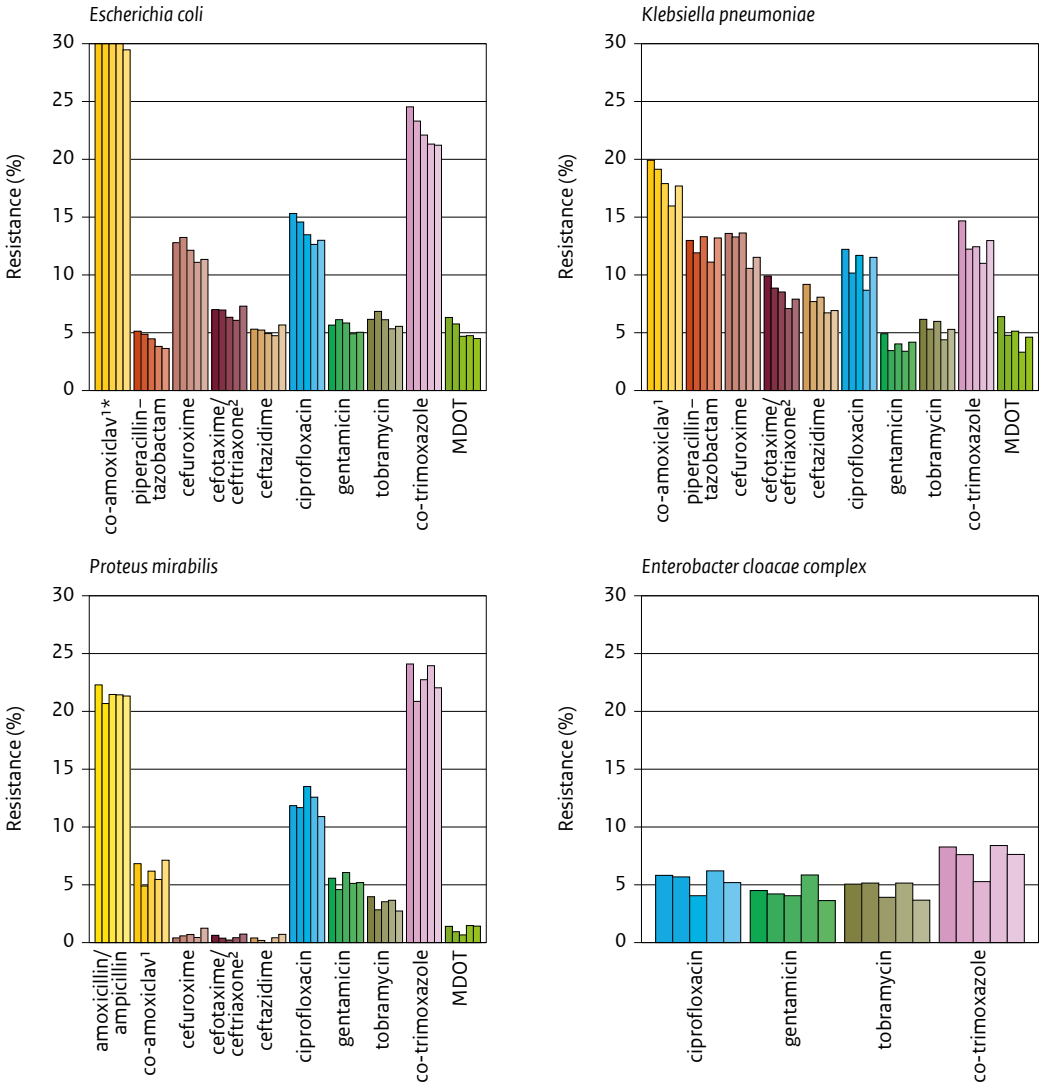
- = Resistance not calculated.

MDOT = multidrug resistance to oral therapy, defined as resistance to all of the following oral agents: co-amoxiclav (according to breakpoint for indications other than uncomplicated urinary tract infection), ciprofloxacin, and co-trimoxazole.

[a] *According to breakpoint for indications other than uncomplicated urinary tract infections. For more details see section 4.1.1.*

[b] *According to breakpoint for indications other than meningitis. For more details see section 4.1.1.*

Figure 4.3.4.1 Trends in antibiotic resistance (from left to right 2018 to 2022) among diagnostic blood isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, *P. aeruginosa* and *Acinetobacter* spp. from patients admitted to inpatient departments (incl. intensive care units) in ISIS-AR



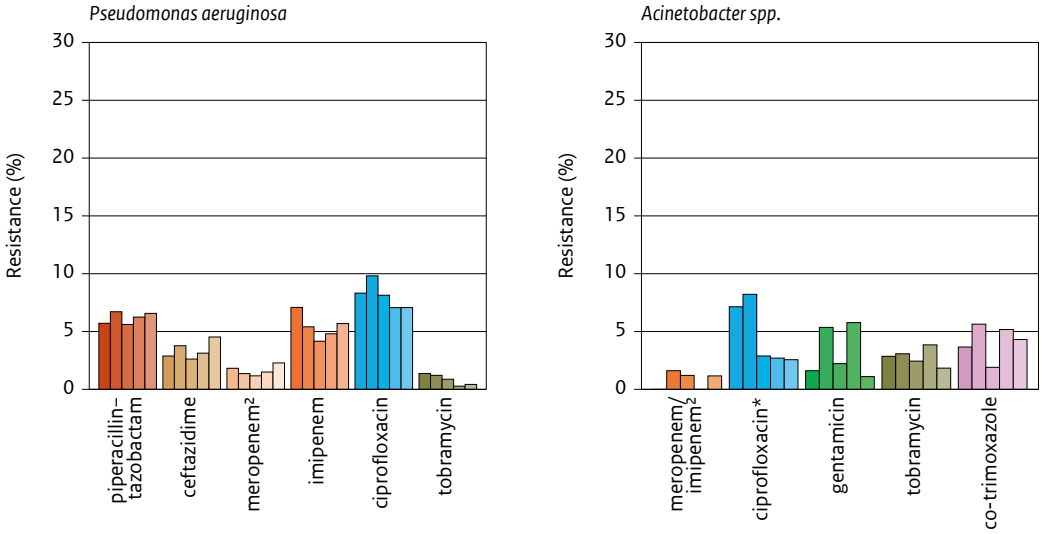
Only pathogen-agent combinations are shown for which the resistance levels were higher than 0.5% for at least one year and lower than 30% for at least three years, except for co-amoxiclav in Enterobacterales, for which resistance levels are always shown.
MDOT = Multidrug resistance for oral therapy, defined as resistance to all following oral agents: co-amoxiclav (according to breakpoint for indications other than uncomplicated urinary tract infection), ciprofloxacin, and co-trimoxazole.

* *Trend is significant and microbiologically relevant (for details see section 4.1.1).*

[1] *According to breakpoint for indications other than uncomplicated urinary tract infection. E. coli: Resistance percentages for co-amoxiclav are from left to right 37%, 37%, 34%, 31% and 29%.*

[2] *According to breakpoint for indications other than meningitis.*

Figure 4.3.4.1 (continued) Trends in antibiotic resistance (from left to right 2018 to 2022) among diagnostic blood isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, *P. aeruginosa* and *Acinetobacter* spp. from patients admitted to inpatient departments (incl. intensive care units) in ISIS-AR



Only pathogen-agent combinations are shown for which the resistance levels were higher than 0.5% for at least one year and lower than 30% for at least three years, except for co-amoxiclav in Enterobacterales, for which resistance levels are always shown.
MDOT = Multidrug resistance for oral therapy, defined as resistance to all following oral agents: co-amoxiclav (according to breakpoint for indications other than uncomplicated urinary tract infection), ciprofloxacin, and co-trimoxazole.

* *Trend is significant and microbiologically relevant (for details see section 4.1.1).*

[1] *According to breakpoint for indications other than uncomplicated urinary tract infection. E. coli: Resistance percentages for co-amoxiclav are from left to right 37%, 37%, 34%, 31% and 29%.*

[2] *According to breakpoint for indications other than meningitis.*

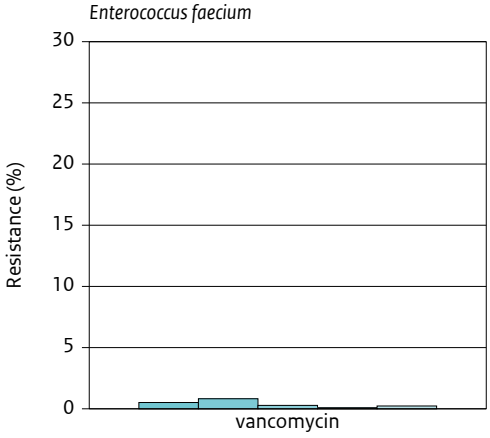
Table 4.3.4.3 Resistance levels (%) among diagnostic blood isolates of *E. faecalis* and *E. faecium* from patients admitted to inpatient departments (incl. intensive care units), ISIS-AR 2022

	E. faecalis	*E. faecium*
Antibiotic		
amoxicillin/ampicillin	-	88
vancomycin	0	0

10 ↑	Significant and clinically relevant increasing trend since 2018.
10 ↓	Significant and clinically relevant decreasing trend since 2018.
10°	Trend not calculated because data from the years before 2022 did not meet the criteria for trend analysis.
10	No significant and clinically relevant time trend.
	(For the criteria for trend analysis and the definition of a clinically relevant trend see section 4.1.1).

- = Resistance not calculated.

Figure 4.3.4.2 Trends in antibiotic resistance (from left to right 2018 to 2022) among diagnostic blood isolates of *E. faecium* from patients admitted to inpatient departments (incl. intensive care units) in ISIS-AR



Only pathogen-agent combinations are shown for which the resistance levels were higher than 0.5% for at least one year and lower than 30% for at least three years.
Note: None of the trends were statistically significant and microbiologically relevant (for details see section 4.1.1).

Table 4.3.4.4 Resistance levels (%) among diagnostic blood isolates of *S. aureus* from patients admitted to inpatient departments (incl. intensive care units), ISIS-AR 2022

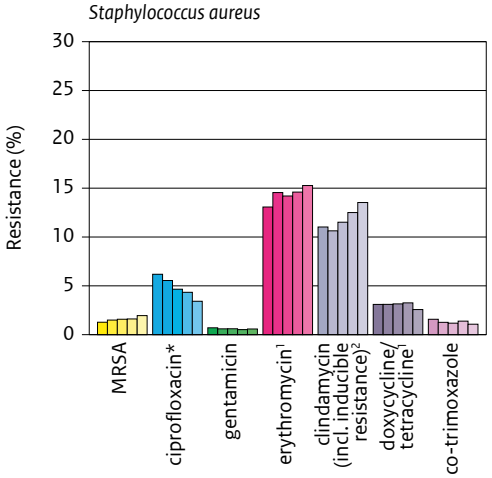
	S. aureus
Antibiotic	
MRSA	2
ciprofloxacin[1]	3 ↓
gentamicin	1
erythromycin[a]	15
clindamycin (including inducible resistance)[2]	14
doxycycline/tetracycline[a]	3
linezolid	0
co-trimoxazole	1
rifampicin	0

10 ↑	Significant and clinically relevant increasing trend since 2018.
10 ↓	Significant and clinically relevant decreasing trend since 2018.
10°	Trend not calculated because data from the years before 2022 did not meet the criteria for trend analysis.
10	No significant and clinically relevant time trend.

(For the criteria for trend analysis and the definition of a clinically relevant trend see section 4.1.1).

- = Resistance not calculated.
MRSA = Methicillin resistant Staphylococcus aureus. For the estimation method of MRSA see section 4.1.1.
[1] *Resistance to ciprofloxacin is intended to be a class indicator for resistance to fluoroquinolones.*
[2] *For the method used to estimate clindamycin resistance (including inducible resistance) see section 4.1.1.*
[a] *According to breakpoint for screening. For more details see section 4.1.1.*

Figure 4.3.4.3 Trends in antibiotic resistance (from left to right 2018 to 2022) among diagnostic blood isolates of *S. aureus* from patients admitted to inpatient departments (incl. intensive care units) in ISIS-AR



Only pathogen-agent combinations are shown for which the resistance levels were higher than 0.5% for at least one year and lower than 30% for at least three years.
MRSA = Methicillin resistant Staphylococcus aureus.
For the estimation method of MRSA see section 4.1.1.

* *Trend is significant and microbiologically relevant (for details see section 4.1.1).*

[1] *According to breakpoint for screening.*

[2] *For the method used to estimate clindamycin resistance (including inducible resistance) see section 4.1.1.*

Table 4.3.4.5 Resistance levels (%) among diagnostic blood isolates of β-haemolytic *Streptococcus* spp. groups A,B,C and G, *S. anginosus*, and *S. mitis/S. oralis* from patients admitted to inpatient departments (incl. intensive care units), ISIS-AR 2022

	β-haemolytic *Streptococcus* spp. group A	β-haemolytic *Streptococcus* spp. group B	β-haemolytic *Streptococcus* spp. groups C and G	*S. anginosus*	*S. mitis/ S. oralis*
Antibiotic					
(benzyl)penicillin	-	-	-	0	5
amoxicillin/ampicillin	-	-	-	1	8°
erythromycin	6	20	9	-	-
clindamycin (including inducible resistance)[1]	4	18 ↓	11	5	9°
doxycycline/tetracycline[a] ⊨	19 ↑	76	34	-	-
co-trimoxazole	3°	1	1°	-	-

10 ↑	Significant and clinically relevant increasing trend since 2018.
10 ↓	Significant and clinically relevant decreasing trend since 2018.
10°	Trend not calculated because data from the years before 2022 did not meet the criteria for trend analysis.
10	No significant and clinically relevant time trend.

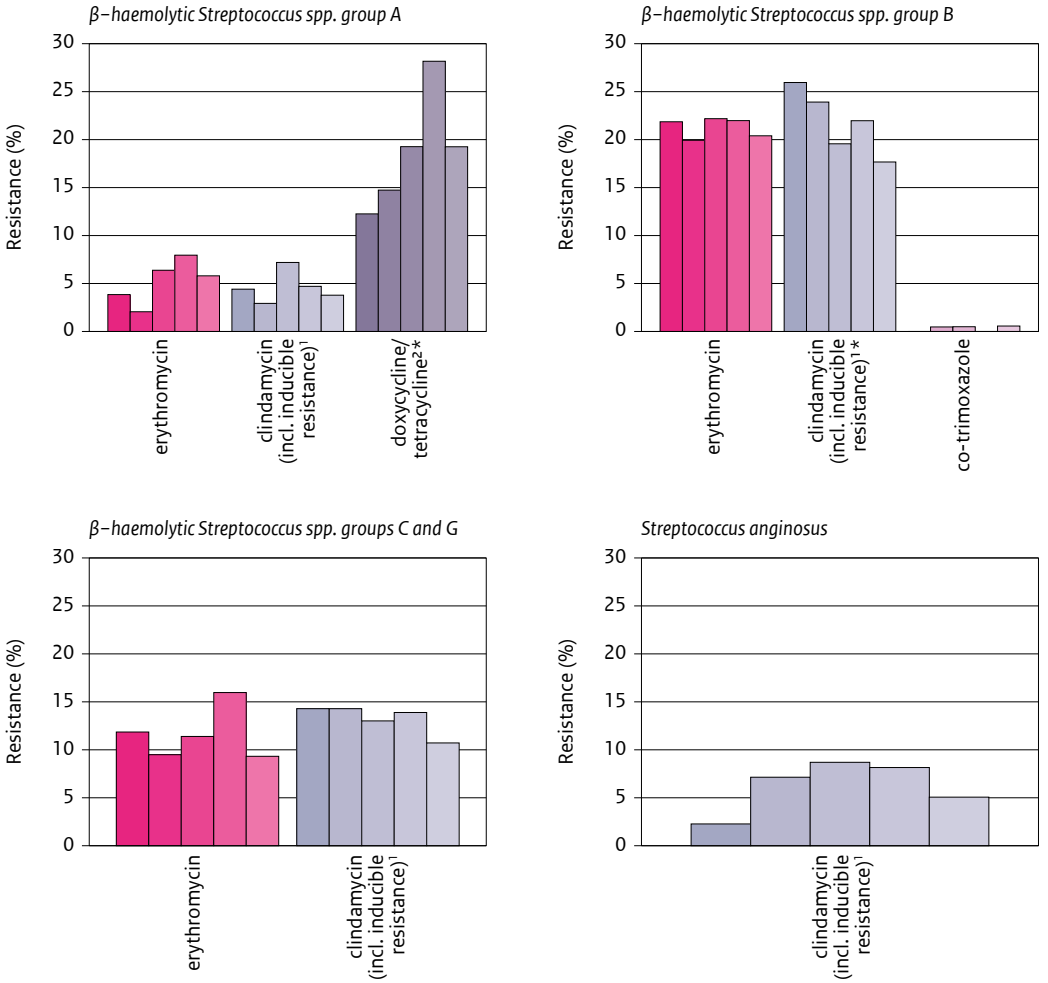
(For the criteria for trend analysis and the definition of a clinically relevant trend see section 4.1.1).

- = Resistance not calculated.

[1] *For the method used to estimate clindamycin resistance (including inducible resistance) see section 4.1.1.*

[a] *According to breakpoint for screening. For more details see section 4.1.1.*

Figure 4.3.4.4 Trends in antibiotic resistance (from left to right 2018 to 2022) among diagnostic blood isolates of β-haemolytic *Streptococcus* spp. groups A,B,C and G, *S. anginosus*, and *S. mitis/S. oralis* from patients admitted to inpatient departments (incl. intensive care units) in ISIS-AR



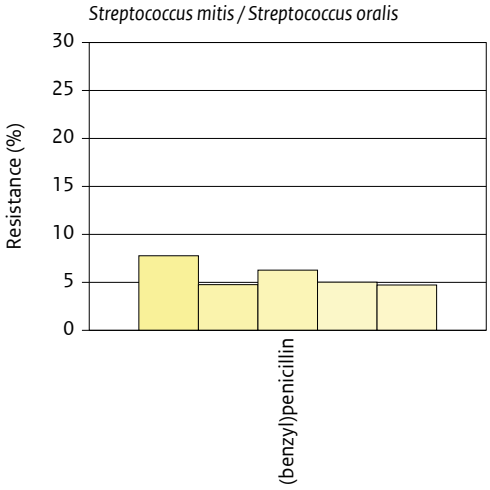
Only pathogen-agent combinations are shown for which the resistance levels were higher than 0.5% for at least one year and lower than 30% for at least three years.

* *Trend is significant and microbiologically relevant (for details see section 4.1.1).*

1 *For the method used to estimate clindamycin resistance (including inducible resistance) see section 4.1.1.*

2 *According to breakpoint for screening.*

Figure 4.3.4.4 (continued) Trends in antibiotic resistance (from left to right 2018 to 2022) among diagnostic blood isolates of β-haemolytic *Streptococcus* spp. groups A,B,C and G, *S. anginosus*, and *S. mitis/S. oralis* from patients admitted to inpatient departments (incl. intensive care units) in ISIS-AR



Only pathogen-agent combinations are shown for which the resistance levels were higher than 0.5% for at least one year and lower than 30% for at least three years.

* *Trend is significant and microbiologically relevant (for details see section 4.1.1).*

[1] *For the method used to estimate clindamycin resistance (including inducible resistance) see section 4.1.1.*

[2] *According to breakpoint for screening.*

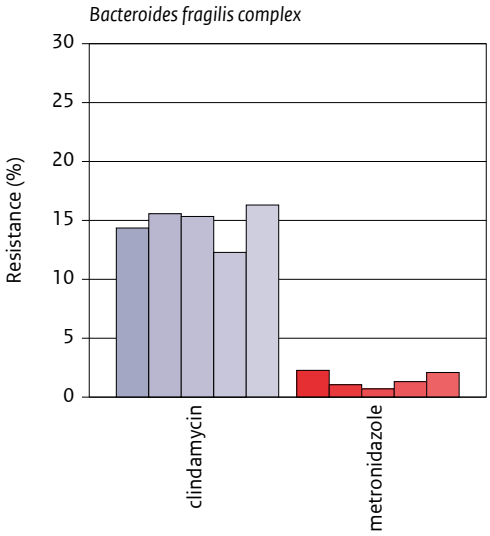
Table 4.3.4.6 Resistance levels (%) among diagnostic blood isolates of *B. fragilis* complex from patients admitted to inpatient departments (incl. intensive care units), ISIS-AR 2022

	***B. fragilis* complex**
Antibiotic	
clindamycin	16
metronidazole	2

10 ↑	Significant and clinically relevant increasing trend since 2018.
10 ↓	Significant and clinically relevant decreasing trend since 2018.
10°	Trend not calculated because data from the years before 2022 did not meet the criteria for trend analysis.
10	No significant and clinically relevant time trend.
	(For the criteria for trend analysis and the definition of a clinically relevant trend see section 4.1.1).

- = Resistance not calculated.

Figure 4.3.4.5 Trends in antibiotic resistance (from left to right 2018 to 2022) among diagnostic blood isolates of *B. fragilis* complex from patients admitted to inpatient departments (incl. intensive care units) in ISIS-AR



Only pathogen-agent combinations are shown for which the resistance levels were higher than 0.5% for at least one year and lower than 30% for at least three years.
Note: None of the trends were statistically significant and microbiologically relevant (for details see section 4.1.1).

Key results and conclusions

Enterobacterales and P. aeruginosa

- For *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *Acinetobacter* spp. in blood isolates no significant and microbiologically relevant trends were found, except for a decrease in resistance to **co-amoxiclav** in *E. coli* (from 37% in 2018 to 29% in 2022) and **ciprofloxacin** in *Acinetobacter* spp. (from 7% to 3%).
- Resistance levels to **second and third generation cephalosporins** in 2022 were comparable in *E. coli* and *K. pneumoniae* and remained stable over the last five years. This is encouraging but nevertheless, patients with a bloodstream infection with *K. pneumoniae* or *E. coli* have a considerable risk of non-adequate empiric treatment with a **second or** (to a lesser extent) **third generation cephalosporin**. In case of severe infection, empiric combination therapy with **aminoglycosides**, reducing likelihood of resistance to 3% or less, might be a suitable option.
- After initial iv treatment, a switch to either **ciprofloxacin**, **co-trimoxazole**, or **co-amoxiclav** was most often possible given the relatively low (≤5%) combined resistance rates for these oral agents.
- Compared to *P. aeruginosa* isolates from ICU patients, resistance to **ceftazidime**, **piperacillin-tazobactam** and **ciprofloxacin** in *P. aeruginosa* isolates from blood cultures was much lower (≤7%).

S. aureus

- **MRSA** was found in 2% of *S. aureus* isolates in blood cultures which remained stable over the previous 5 years.

4.3.5 Urology services

The distribution of pathogens in urine samples from patients attending urology outpatient departments (OPD) and patients admitted to urology inpatient departments (IPD) in 2022 is presented in table 4.3.5.1. Resistance levels for a selection of pathogens isolated from these patients in 2022 are presented by type of department in tables 4.3.5.2 (*E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa*) and 4.3.5.3 (*E. faecalis* and *E. faecium*). Five-year trends in resistance are shown in figure 4.3.5.1 (*E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa*) and 4.3.5.2 (*E. faecalis* and *E. faecium*).

In urology departments of Dutch hospitals, a urine sample is routinely taken from patients presenting with complicated urinary tract infections and susceptibility testing is performed as part of routine diagnostics. However, guidelines do not indicate sampling in case of uncomplicated urinary tract infections. As a result, for those infections often only a sample is taken after therapy failure, and the presented resistance levels are likely to be higher than those for all patients with urinary tract infections at urology departments.

Table 4.3.5.1 Distribution of isolated pathogens in diagnostic urine samples from patients attending urology outpatient departments (OPD) and patients admitted to urology inpatient departments (IPD), ISIS-AR 2022

	OPD	IPD
Pathogen	N (%)	N (%)
E. coli	11,739 (37)	1,871 (32)
K. pneumoniae	2,721 (9)	424 (7)
P. mirabilis	1,408 (4)	289 (5)
Other Enterobacterales[1]	5,379 (17)	1,115 (19)
P. aeruginosa	1,207 (4)	376 (6)
Other non-fermenters[2]	529 (2)	145 (2)
Other Gram-negatives[3]	11 (0)	1 (0)
E. faecalis	3,724 (12)	819 (14)
E. faecium	262 (1)	151 (3)
Other Gram-positives[4]	4,930 (15)	732 (12)

1 *In order of frequency: Klebsiella spp. (non-pneumoniae), Enterobacter spp., Citrobacter spp., Serratia spp., Morganella spp., Proteus spp. (non-mirabilis), Providencia spp., Raoultella spp., Pantoea spp., Hafnia spp., Escherichia spp. (non-coli), Salmonella spp., Cronobacter spp.*

2 *In order of frequency: Acinetobacter spp., S. maltophilia, Pseudomonas spp. (non-aeruginosa), B. cepacia.*

3 *In order of frequency: H. parainfluenzae, H. influenzae, B. fragilis complex.*

4 *In order of frequency: Staphylococcus spp., A. urinae, ß-haemolytic Streptococcus spp. groups C and G, S. dysgalactiae n.n.g., S. dysgalactiae subsp. equisimilis, ß-haemolytic Streptococcus spp. group A, S. anginosus, ß-haemolytic Streptococcus spp. group B, S. pneumoniae, S. mitis/S. oralis, Enterococcus spp. (non-faecalis, non-faecium), C. perfringens.*

Table 4.3.5.2 Resistance levels (%) among diagnostic urine isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* from patients attending urology outpatient departments (OPD) and patients admitted to urology inpatient departments (IPD), ISIS-AR 2022

	E. coli		*K. pneumoniae*		*P. mirabilis*		*P. aeruginosa*	
	OPD	IPD	OPD	IPD	OPD	IPD	OPD	IPD
Antibiotic								
amoxicillin/ampicillin	40	43 ↓	-	-	22	23	-	-
co-amoxiclav[a]	30 ↓	32 ↓	20	26	6	7	-	-
piperacillin-tazobactam	4	4	15	19	0	1	4	6
cefuroxime	12	15	14	14 ↓	2	2	-	-
cefotaxime/ceftriaxone[b]	7	11	7 ↓	10	1	1	-	-
ceftazidime	5	8	6 ↓	8 ↓	0	1	1	3
meropenem/imipenem[b]	0	0	0	0	-	-	-	-
meropenem[b]	-	-	-	-	0	0	1	1
imipenem	-	-	-	-	-	-	6	5
ciprofloxacin	19	23 ↓	15	15	14	20	15	9
gentamicin	5	5 ↓	3	8	7	6	-	-
tobramycin	5	6 ↓	4	9	5	4	1	2
fosfomycin[1]	3	3	-	-	-	-	-	-
trimethoprim	27	26	21 ↓	22	33	36	-	-
co-trimoxazole	24	24	12 ↓	18	26	25	-	-
nitrofurantoin	3	4	-	-	-	-	-	-
Empiric therapy combinations								
co-amoxiclav + gentamicin[a]	4	4 ↓	2	6	1	1	-	-
cefuroxime + gentamicin	2	2 ↓	2	6	1	0	-	-
cefotaxime/ceftriaxone + gentamicin[b]	1	2	2	6	1	0	-	-
ceftazidime + tobramycin	-	-	-	-	-	-	0	0
ciprofloxacin + tobramycin	-	-	-	-	-	-	1	1
co-amoxiclav + ciprofloxacin[a]	10	12 ↓	6 ↓	9	2	2	-	-
cefuroxime + ciprofloxacin	7	10	9 ↓	8 ↓	1	1	-	-
cefotaxime/ceftriaxone + ciprofloxacin[b]	5	8	5	7	0	1	-	-
Multidrug resistance								
MDOT[a]	6	8 ↓	4 ↓	6	1	3	-	-

10 ↑	Significant and clinically relevant increasing trend since 2018.
10 ↓	Significant and clinically relevant decreasing trend since 2018.
10°	Trend not calculated because data from the years before 2022 did not meet the criteria for trend analysis.
10	No significant and clinically relevant time trend.

(For the criteria for trend analysis and the definition of a clinically relevant trend see section 4.1.1).

- = Resistance not calculated.

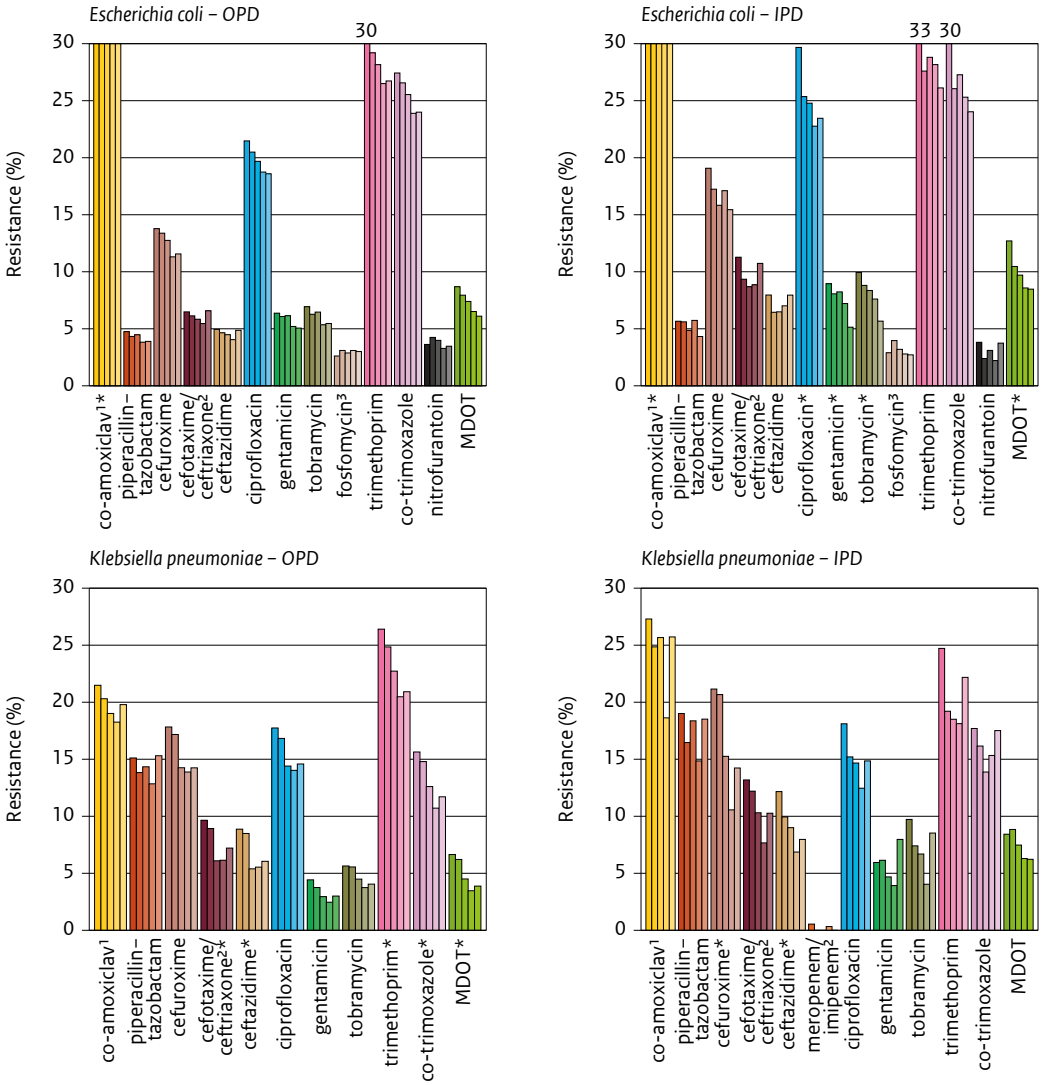
MDOT = multidrug resistance to oral therapy, defined as resistance to all of the following oral agents: co-amoxiclav (according to breakpoint for indications other than uncomplicated urinary tract infection), ciprofloxacin, and co-trimoxazole.

[1] *Resistance percentage calculated using an mic cut-off of 16 mg/L and a diameter cut-off of 24 mm. For more details see section 4.1.1.*

[a] *According to breakpoint for indications other than uncomplicated urinary tract infections. For more details see section 4.1.1.*

[b] *According to breakpoint for indications other than meningitis. For more details see section 4.1.1.*

Figure 4.3.5.1 Trends in antibiotic resistance (from left to right 2018 to 2022) among diagnostic urine isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* from patients attending urology outpatient departments (OPD) and patients admitted to urology inpatient departments (IPD) in ISIS-AR



Only pathogen-agent combinations are shown for which the resistance levels were higher than 0.5% for at least one year and lower than 30% for at least three years, except for co-amoxiclav in Enterobacterales, for which resistance levels are always shown.
MDOT = Multidrug resistance for oral therapy, defined as resistance to all following oral agents: co-amoxiclav (according to breakpoint for indications other than uncomplicated urinary tract infection), ciprofloxacin, and co-trimoxazole.

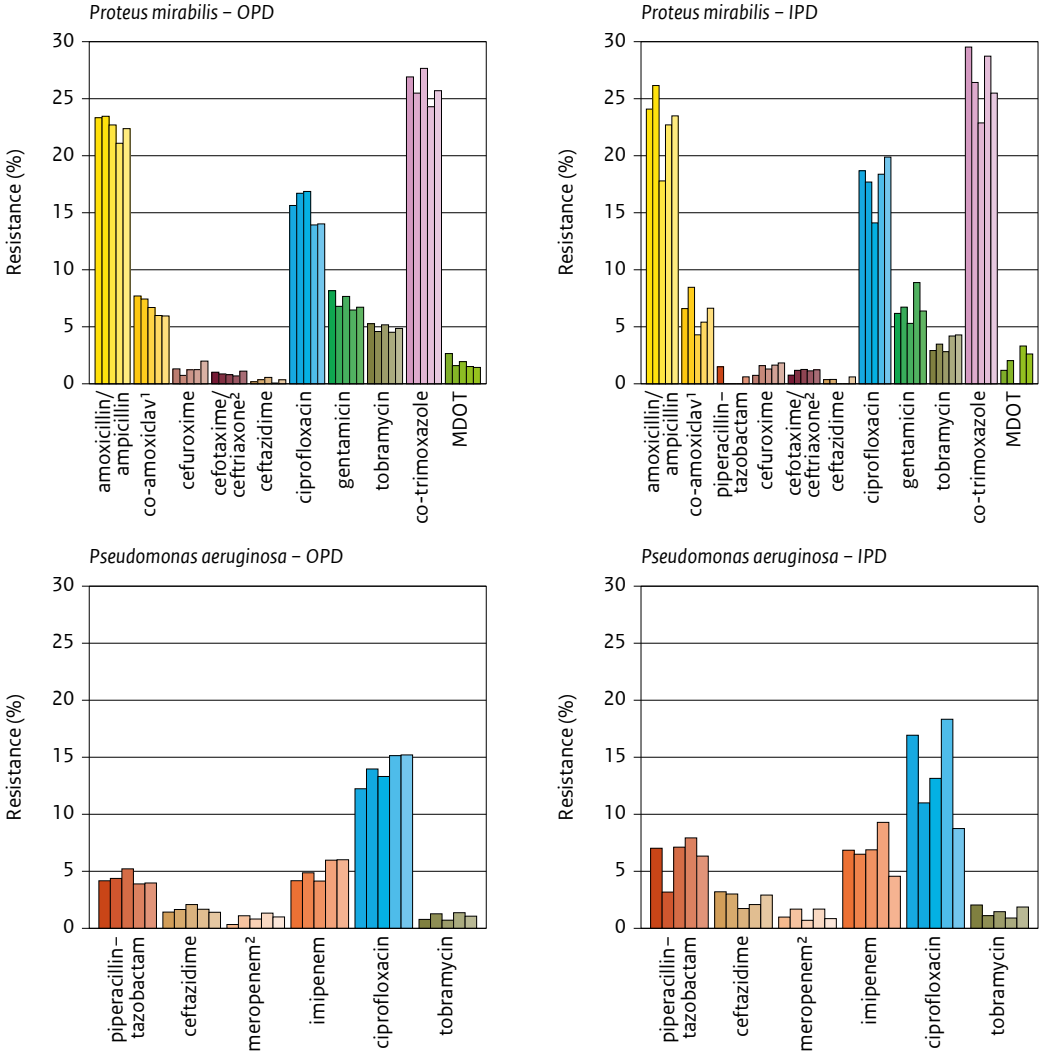
* *Trend is significant and microbiologically relevant (for details see section 4.1.1).*

1 *According to breakpoint for indications other than uncomplicated urinary tract infection. Resistance percentages for co-amoxiclav are from left to right 37%, 36%, 35%, 32%, and 30% for E. coli in OPD, and 41%, 40%, 40%, 35%, and 32% for E. coli in IPD.*

2 *According to breakpoint for indications other than meningitis.*

3 *Resistance percentage calculated using an mic cut-off of 16 mg/L and a diameter cut-off of 24 mm. For more details see section 4.1.1.*

Figure 4.3.5.1 (continued) Trends in antibiotic resistance (from left to right 2018 to 2022) among diagnostic urine isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* from patients attending urology outpatient departments (OPD) and patients admitted to urology inpatient departments (IPD) in ISIS-AR



Only pathogen-agent combinations are shown for which the resistance levels were higher than 0.5% for at least one year and lower than 30% for at least three years, except for co-amoxiclav in Enterobacterales, for which resistance levels are always shown.
MDOT = Multidrug resistance for oral therapy, defined as resistance to all following oral agents: co-amoxiclav (according to breakpoint for indications other than uncomplicated urinary tract infection), ciprofloxacin, and co-trimoxazole.

* *Trend is significant and microbiologically relevant (for details see section 4.1.1).*

1 *According to breakpoint for indications other than uncomplicated urinary tract infection. Resistance percentages for co-amoxiclav are from left to right 37%, 36%, 35%, 32%, and 30% for E. coli in OPD, and 41%, 40%, 40%, 35%, and 32% for E. coli in IPD.*

2 *According to breakpoint for indications other than meningitis.*

3 *Resistance percentage calculated using an mic cut-off of 16 mg/L and a diameter cut-off of 24 mm. For more details see section 4.1.1.*

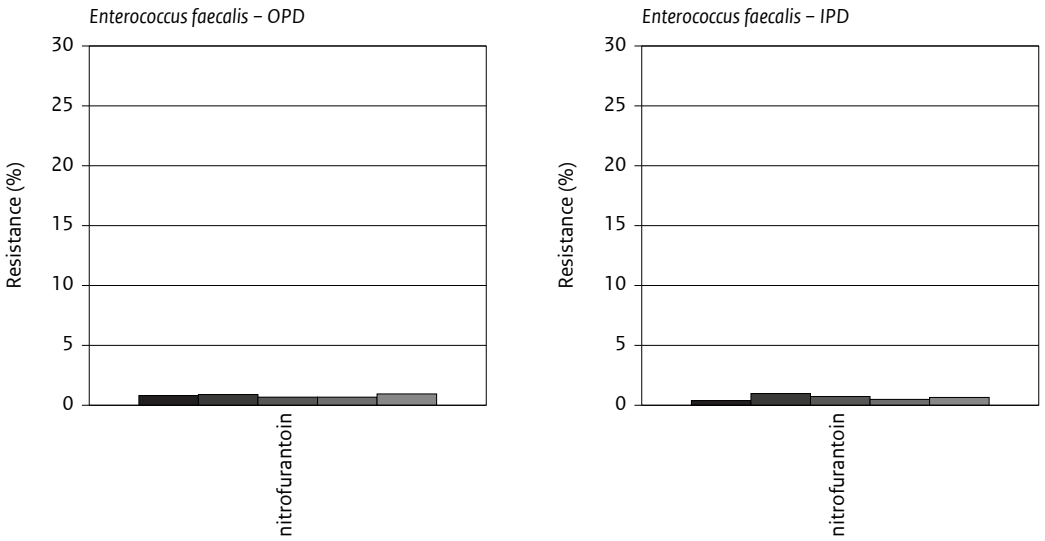
Table 4.3.5.3 Resistance levels (%) among diagnostic urine isolates of *E. faecalis* and *E. faecium* from patients attending urology outpatient departments (OPD) and patients admitted to urology inpatient departments (IPD), ISIS-AR 2022

	E. faecalis		*E. faecium*	
	OPD	IPD	OPD	IPD
Antibiotic				
amoxicillin/ampicillin	-	-	82	93
vancomycin	0	0	0	0
nitrofurantoin	1	1	-	-

10 ↑	Significant and clinically relevant increasing trend since 2018.
10 ↓	Significant and clinically relevant decreasing trend since 2018.
10°	Trend not calculated because data from the years before 2022 did not meet the criteria for trend analysis.
10	No significant and clinically relevant time trend.
	(For the criteria for trend analysis and the definition of a clinically relevant trend see section 4.1.1).

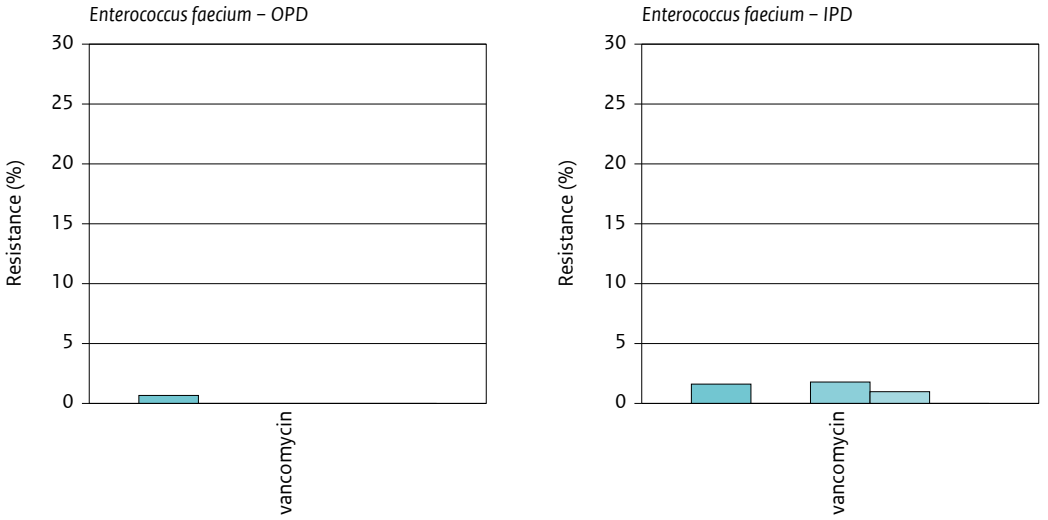
- = Resistance not calculated.

Figure 4.3.5.2 Trends in antibiotic resistance (from left to right 2018 to 2022) among diagnostic urine isolates of *E. faecalis* and *E. faecium* from patients attending urology outpatient departments (OPD) and patients admitted to urology inpatient departments (IPD) in ISIS-AR



Only pathogen-agent combinations are shown for which the resistance levels were higher than 0.5% for at least one year and lower than 30% for at least three years.
Note: None of the trends were statistically significant and microbiologically relevant (for details see section 4.1.1).

Figure 4.3.5.2 (continued) Trends in antibiotic resistance (from left to right 2018 to 2022) among diagnostic urine isolates of *E. faecalis* and *E. faecium* from patients attending urology outpatient departments (OPD) and patients admitted to urology inpatient departments (IPD) in ISIS-AR



Only pathogen-agent combinations are shown for which the resistance levels were higher than 0.5% for at least one year and lower than 30% for at least three years.
Note: None of the trends were statistically significant and microbiologically relevant (for details see section 4.1.1).

Key results and conclusions

Urine: Enterobacterales and P. aeruginosa

- Resistance levels in Enterobacterales from patients in urology services traditionally have been higher than in Enterobacterales from non-urology patients.
- Within urology services, resistance levels were higher in isolates from patients that were admitted compared to patients seen in OPD.
- Resistance to **ciprofloxacin** (23%) and **co-trimoxazole** (24%) in *E. coli* from admitted patients remains a problem.
- However, resistance in *E. coli* and *K. pneumoniae* to antibiotics that are used to treat complicated urinary tract infections such as **cefuroxime**, **cefotaxime/ceftriaxone**, **ciprofloxacin**, **co-trimoxazole**, and **co-amoxiclav** showed a decreasing trend or remained stable over the last five years.

4.4 Long-term care facilities

The distribution of pathogens in diagnostic urine and wound or pus samples from residents of long-term care facilities (LTCF) in 2022 is presented in table 4.4.1. The resistance levels in 2022 for *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* isolates from urine samples are presented in table 4.4.2 and for *S. aureus* isolates from wound or pus samples in table 4.4.3.

LTCFs usually send urine, wound, or pus samples for culture and susceptibility testing in case of antimicrobial therapy failure or (with regard to urine samples) complicated urinary tract infection. As a result, the presented resistance levels are likely to be higher than those for all residents with urinary tract infections caused by Enterobacterales or *P. aeruginosa*, or wound infections or pus caused by *S. aureus* presenting in LTCFs. Therefore, residents from whom samples were taken are hereafter referred to as 'selected residents of long-term care facilities'.

In 2018 a new sampling guideline for LTCFs was implemented, leading to changes in sampling policies. Since the degree of restrictive sampling influences the magnitude of overestimation of resistance percentages, this may result in spurious time trends. Therefore, time trends were not calculated for this section.

Table 4.4.1 Distribution of isolated pathogens in diagnostic urine and wound or pus samples from selected residents of long-term care facilities, ISIS-AR 2022

	Urine	Wound or pus
Pathogen	**N (%)**	**N (%)**
E. coli	11,361 (41)	195 (8)
K. pneumoniae	2,746 (10)	66 (3)
P. mirabilis	2,861 (10)	203 (8)
Other Enterobacterales[1]	2,964 (11)	246 (10)
P. aeruginosa	1,423 (5)	309 (12)
Other non-fermenters[2]	213 (1)	46 (2)
Other Gram-negatives[3]	0 (0)	32 (1)
S. aureus	1,090 (4)	1,055 (42)
Other Gram-positives[4]	4,823 (18)	334 (13)

[1] *In order of frequency: Klebsiella spp. (non-pneumoniae), Citrobacter spp., Enterobacter spp., Morganella spp., Serratia spp., Proteus spp. (non-mirabilis), Providencia spp., Raoultella spp., Hafnia spp., Pantoea spp., Escherichia spp. (non-coli), Salmonella spp., Mixta spp., Cronobacter spp.*

[2] *In order of frequency: Acinetobacter spp., Pseudomonas spp. (non-aeruginosa), S. maltophilia, M. catarrhalis.*

[3] *In order of frequency: B. fragilis complex, H. parainfluenzae, H. influenzae.*

[4] *In order of frequency: Enterococcus spp., A. urinae, β-haemolytic Streptococcus spp. groups C and G, S. dysgalactiae n.n.g., S. dysgalactiae subsp. equisimilis, β-haemolytic Streptococcus spp. group A, S. anginosus, β-haemolytic Streptococcus spp. group B, S. pneumoniae, S. mitis/S. oralis, Staphylococcus spp. (non-aureus), C. perfringens.*

Table 4.4.2 Resistance levels (%) among diagnostic urine isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* from selected residents of long-term care facilities, ISIS-AR 2022

	E. coli	*K. pneumoniae*	*P. mirabilis*	*P. aeruginosa*
Antibiotic				
amoxicillin/ampicillin	40	-	20	-
co-amoxiclav[a]	31	21	6	-
piperacillin-tazobactam	5	17	0	8
cefuroxime	11	11	1	-
cefotaxime/ceftriaxone[b]	5	6	0	-
ceftazidime	4	6	0	3
meropenem/imipenem[b]	0	0	-	-
meropenem[b]	-	-	0	1
imipenem	-	-	-	5
ciprofloxacin	15	12	14	10
gentamicin	5	2	5	-
tobramycin	5	4	3	1
fosfomycin[1]	3	-	-	-
trimethoprim	21	17	33	-
co-trimoxazole	18	9	25	-
nitrofurantoin	3	-	-	-
Multidrug resistance				
MDOT[a]	4	3	1	-

- = Resistance not calculated.

MDOT = multidrug resistance to oral therapy, defined as resistance to all of the following oral agents: co-amoxiclav (according to breakpoint for indications other than uncomplicated urinary tract infection), ciprofloxacin, and co-trimoxazole.

[1] *Resistance percentage calculated using an mic cut-off of 16 mg/L and a diameter cut-off of 24 mm. For more details see section 4.1.1.*

[a] *According to breakpoint for indications other than uncomplicated urinary tract infections. For more details see section 4.1.1.*

[b] *According to breakpoint for indications other than meningitis. For more details see section 4.1.1.*

Table 4.4.3 Resistance levels (%) among diagnostic wound or pus isolates of *S. aureus* from selected residents of long-term care facilities, ISIS-AR 2022

	S. aureus
Antibiotic	
MRSA	2
ciprofloxacin[1]	16
erythromycin[c]	16
clindamycin (including inducible resistance)[2]	16
doxycycline/tetracycline[c]	3
fusidic acid	7
co-trimoxazole	2
mupirocine[a]	0
mupirocine[b]	0

- = Resistance not calculated.
MRSA = Methicillin resistant Staphylococcus aureus. For the estimation method of MRSA see section 4.1.1.
[1] *Resistance to ciprofloxacin is intended to be a class indicator for resistance to fluoroquinolones.*
[2] *For the method used to estimate clindamycin resistance (including inducible resistance) see section 4.1.1.*
[a] *According to breakpoint for nasal decontamination. For more details see section 4.1.1.*
[b] *According to breakpoint for topical use. For more details see section 4.1.1.*
[c] *According to breakpoint for screening. For more details see section 4.1.1.*

Key results and conclusions

Urine: Enterobacterales and P. aeruginosa

- Resistance levels in *E. coli*, *K. pneumoniae* and *P. aeruginosa* urine isolates from LTCF patients were higher than resistance levels in GP patients and comparable to resistance levels in OPD and hospital patients.
- Resistance levels for **nitrofurantoin** and **fosfomycin** in *E. coli*, first and second choice antibiotics for the treatment of uncomplicated UTI in adults, were low (3%).
- Resistance levels for **ciprofloxacin**, first choice antibiotic for the treatment of complicated UTI in adults, was 15% in *E. coli* and 12% in *K. pneumoniae*. Resistance levels for **co-amoxiclav**, second choice antibiotic for the treatment of complicated UTI was 31% in *E. coli* and 21% in *K. pneumoniae*. Resistance levels for **co-trimoxazole**, third choice antibiotic for this indication, was 18% in *E. coli* and 9% in *K. pneumoniae*. Combined resistance for **co-amoxiclav**, **ciprofloxacin**, and **co-trimoxazole** in all Enterobacterales was low (≤4%).

Wound/pus: S. aureus

- Resistance levels in *S. aureus* isolates from LTCF patients were higher than resistance levels in GP patients and comparable to resistance levels in OPD and hospital patients, with the exception of resistance to **ciprofloxacin** (16%), which was much higher in *S. aureus* from LTCF patients than in *S. aureus* from OPD (4%), hospital (4%) and ICU patients (3%).

4.5 Respiratory pathogens

The distribution of pathogens isolated from diagnostic lower and upper respiratory tract samples from general practitioners' (GP) patients and hospital patients (outpatients and inpatients, including intensive care patients) in 2022 is presented in table 4.5.1. Resistance levels for respiratory pathogens (*S. pneumoniae*, *H. influenzae*, and *M. catarrhalis*) in 2022 are presented by patient group in table 4.5.2. Five-year trends in resistance are shown in figure 4.5.1.

Although patients from general practitioners are assumed to be representative of the community with respect to resistance levels of pathogens, in accordance with the NHG guidelines, general practitioners do not routinely take a sample when respiratory tract infection is suspected. Therefore, the results may be biased towards higher resistance levels due to overrepresentation of more severe or recurrent cases of respiratory tract infections.

In hospitals in the Netherlands, according to the guidelines a sample should be taken for routine diagnostic purposes when lower respiratory tract infection is suspected. Although often it is not possible to take a sample because a patient does not produce sputum, it is not expected that this is correlated to resistance, and selective sampling bias is expected to be small. Nevertheless, resistance levels in hospital patients may be higher than in the community, as hospital patients are likely to be more severely ill and patients with previous treatment failure, chronic obstructive pulmonary diseases (COPD), and cystic fibrosis (CF) may be overrepresented.

Table 4.5.1 Distribution of isolated pathogens in diagnostic respiratory samples from general practitioners' patients (GP) and in diagnostic blood or cerebrospinal fluid and respiratory samples from hospital patients (outpatient and inpatient departments, incl. intensive care units), ISIS-AR 2022

	GP		Hospital departments		
	Lower respiratory tract	Upper respiratory tract	Blood or cerebrospinal fluid	Lower respiratory tract	Upper respiratory tract
Pathogen	N (%)	N (%)	N (%)	N (%)	N (%)
S. pneumoniae	127 (8)	3 (0)	1,378 (4)	2,064 (8)	111 (2)
Other Gram-positives[1]	203 (13)	1,282 (86)	19,383 (59)	4,713 (18)	3,549 (63)
H. influenzae	558 (34)	32 (2)	224 (1)	6,175 (24)	430 (8)
M. catarrhalis	152 (9)	21 (1)	32 (0)	1,651 (6)	119 (2)
Other non-fermenters[2]	274 (17)	35 (2)	1,010 (3)	4,326 (17)	384 (7)
Enterobacterales[3]	287 (18)	115 (8)	10,318 (31)	6,002 (24)	944 (17)
Other Gram-negatives[4]	23 (1)	2 (0)	437 (1)	584 (2)	65 (1)

[1] *In order of frequency: Staphylococcus spp., ß-haemolytic Streptococcus spp. groups C and G, ß-haemolytic Streptococcus spp. group A, S. dysgalactiae n.n.g., S. mitis/S. oralis, ß-haemolytic Streptococcus spp. group B, S. anginosus, S. dysgalactiae subsp. equisimilis, Enterococcus spp., C. perfringens, A. urinae, L. monocytogenes.*

[2] *In order of frequency: Pseudomonas spp., S. maltophilia, Acinetobacter spp., B. cepacia.*

[3] *In order of frequency: Escherichia spp., Klebsiella spp., Enterobacter spp., Serratia spp., Proteus spp., Citrobacter spp., Morganella spp., Raoultella spp., Salmonella spp., Pantoea spp., Hafnia spp., Providencia spp., Yersinia spp., Mixta spp., Cronobacter spp.*

[4] *In order of frequency: H. parainfluenzae, B. fragilis complex, N. meningitidis, C. jejuni, C. coli, C. lari.*

Table 4.5.2 Resistance levels (%) among diagnostic isolates of *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* from general practitioners' patients and hospital patients (outpatient and inpatient departments, incl. intensive care units), ISIS-AR 2022

	S. pneumoniae		*H. influenzae*		*M. catarrhalis*	
	GP	Hospital	GP	Hospital	GP	Hospital
Antibiotic						
(benzyl)penicillin (R)[1] ⊧	0	0	-	-	-	-
(benzyl)penicillin (I)[1] ⊧	7	8	-	-	-	-
amoxicillin/ampicillin[2]	-	-	27	27	-	-
co-amoxiclav[2]	-	-	11	10	1	2 ↑
ciprofloxacin	-	-	4	4	5	6°
erythromycin	13	9	-	-	2	2
doxycycline/tetracycline[3]	15	10	2	3	1	1
co-trimoxazole	5	9	16	22	6	4

10 ↑	Significant and clinically relevant increasing trend since 2018.
10 ↓	Significant and clinically relevant decreasing trend since 2018.
10°	Trend not calculated because data from the years before 2022 did not meet the criteria for trend analysis.
10	No significant and clinically relevant time trend.
	(For the criteria for trend analysis and the definition of a clinically relevant trend see section 4.1.1).

- = Resistance not calculated.

In 2020 and 2021, the number of S. pneumoniae isolates from GP patients for which data were available were below 100. Reliability of resistance percentages in these years may be lower.

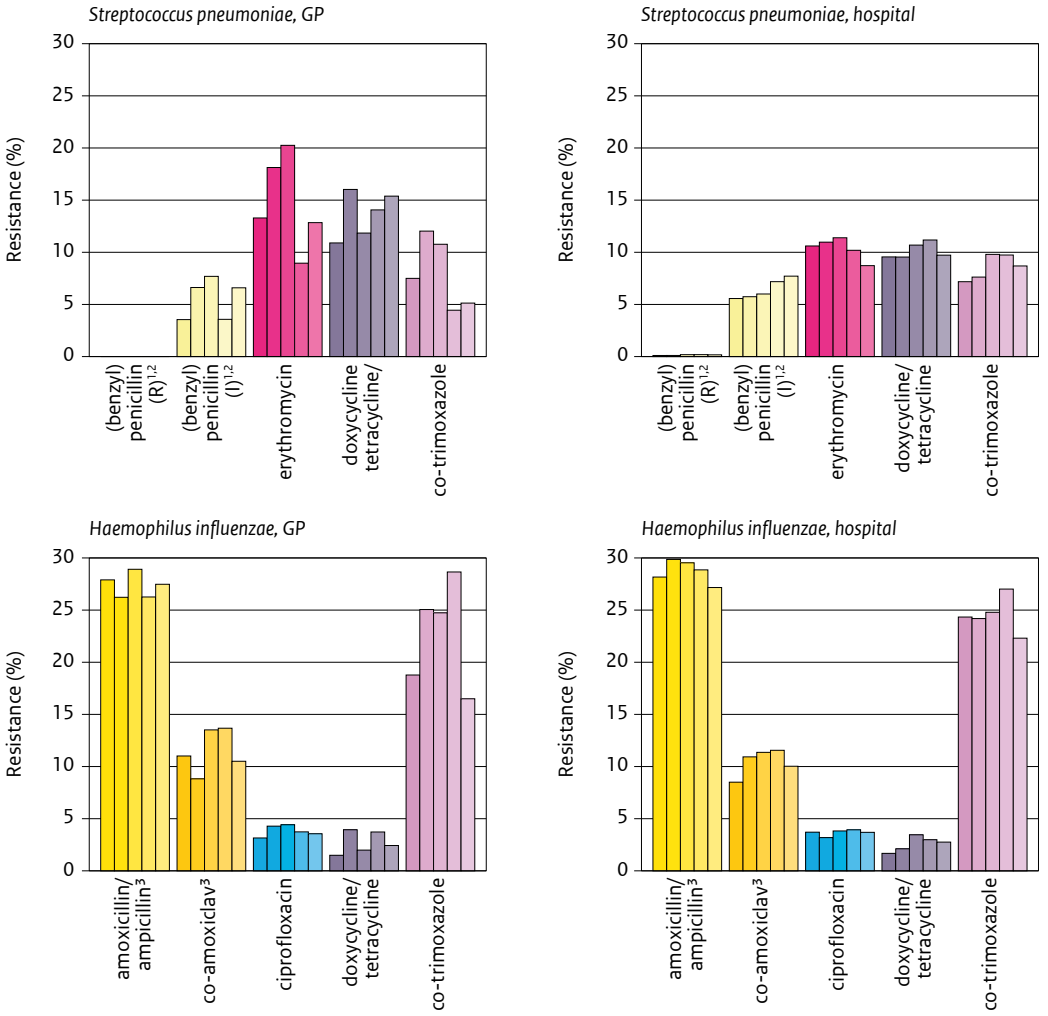
[1] *Resistance to (benzyl)penicillin was estimated based on reinterpretation of oxacillin test values, or, if the result for oxacillin was I or R, on reinterpreatation of testvalues for (benzyl)penicillin. Available gradient strip tests (Etest™ and MTS™) systematically underestimate (benzyl)penicillin MIC values in S. pneumoniae (for details see section 4.1.1). Resistance percentages may therefore be biased toward a lower level.*

[2] *Resistance to amoxicillin/ampicillin and co-amoxiclav in H. influenzae was calculated according to directions for intravenous use in the flow-diagram from EUCAST guidelines 2022. For details see section 4.1.1.*

[3] *For S. pneumoniae according to breakpoint for screening.*

[a] *According to breakpoint for indications other than meningitis. For more details see section 4.1.1.*

Figure 4.5.1 Trends in antibiotic resistance (from left to right 2018 to 2022) among diagnostic isolates of *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* from general practitioners' patients and hospital patients (outpatient and inpatient departments, incl. intensive care units) in ISIS-AR



Only pathogen-agent combinations are shown for which the resistance levels were higher than 0.5% for at least one year and lower than 30% for at least three years.

In 2020 and 2021 the number of S. pneumoniae isolates from GP patients for which data were available were below 100. Reliability of resistance percentages in these years may be lower.

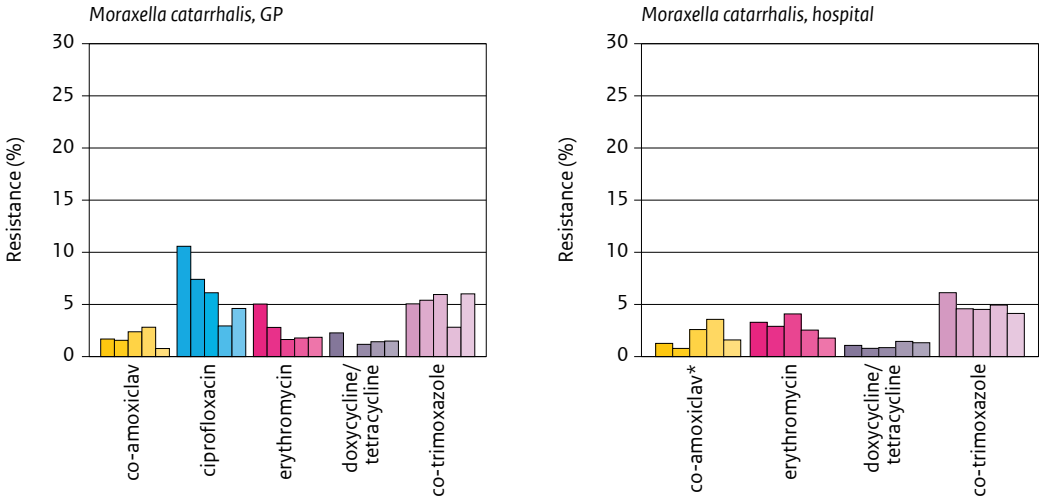
* *Trend is significant and microbiologically relevant (for details see section 4.1.1).*

[1] *According to breakpoint for non-meningitis.*

[2] *Available gradient strip tests (EtestTM and MTSTM) systematically underestimate (benzyl)penicillin MIC values in S. pneumoniae (for details see section 4.1.1). Resistance percentages may therefore be biased toward a lower level.*

[3] *Resistance to amoxicillin/ampicillin and co-amoxiclav in H. influenzae was calculated according to directions for intravenous use in the flow-diagram from EUCAST guidelines 2022. For details see section 4.1.1.*

Figure 4.5.1 (continued) Trends in antibiotic resistance (from left to right 2018 to 2022) among diagnostic isolates of *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* from general practitioners' patients and hospital patients (outpatient and inpatient departments, incl. intensive care units) in ISIS-AR



Only pathogen-agent combinations are shown for which the resistance levels were higher than 0.5% for at least one year and lower than 30% for at least three years.
In 2020 and 2021 the number of S. pneumoniae isolates from GP patients for which data were available were below 100. Reliability of resistance percentages in these years may be lower.

* *Trend is significant and microbiologically relevant (for details see section 4.1.1).*

[1] *According to breakpoint for non-meningitis.*

[2] *Available gradient strip tests (EtestTM and MTSTM) systematically underestimate (benzyl)penicillin MIC values in S. pneumoniae (for details see section 4.1.1). Resistance percentages may therefore be biased toward a lower level.*

[3] *Resistance to amoxicillin/ampicillin and co-amoxiclav in H. influenzae was calculated according to directions for intravenous use in the flow-diagram from EUCAST guidelines 2022. For details see section 4.1.1.*

Key results and conclusions

S. pneumoniae

- In *S. pneumoniae*, the percentages I+R results for **(benzyl)penicillin** are low (≤8%) in GP patients and hospital patients.
- Resistance to **doxycycline/tetracycline** in *S. pneumoniae* was higher in GP patients (15%) than in hospital patients (10%).

H. influenzae

- In *H. influenzae* isolates, resistance to **amoxicillin/ampicillin** was 27% in both GP and hospital patients. Resistance to **co-amoxiclav** was 11% in GP patients and 10% in hospital patients. These resistance levels remained stable over the last five years.

4.6 *Helicobacter pylori*

Resistance levels for *Helicobacter pylori* from diagnostic isolates in 2022 are presented in table 4.6.1. Five-year trends in resistance are shown in figure 4.6.1.

For the culture of *H. pylori* and subsequent phenotypical antimicrobial susceptibility testing, a biopsy from the gastric epithelium is required. However, usually an *H. pylori* infection is primarily diagnosed using non-invasive methods such as a stool antigen test or a urea breath test. Only when empirical treatment was unsuccessful, a biopsy is likely to be performed. Therefore, the results may be biased towards higher resistance levels compared to the resistance levels in the total population with an *H. pylori* infection. Nonetheless, because the degree of bias is assumed to be constant over the years, we expect the calculated time trends to be a valid estimate of the changes through the years.

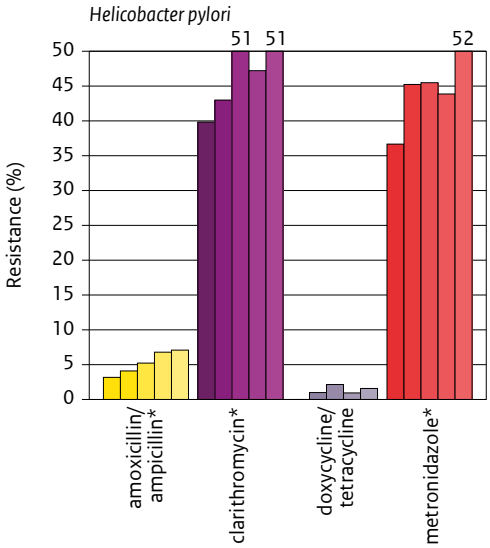
Table 4.6.1 Resistance levels (%) among 304 diagnostic isolates of *H. pylori*, ISIS-AR 2022

	Helicobacter pylori
Antibiotic	
amoxicillin/ampicillin	7 ↑
levofloxacin	32°
clarithromycin	51 ↑
doxycycline/tetracycline	2
metronidazole	52 ↑
clarithromycin + metronidazole	38°

10 ↑	Significant and clinically relevant increasing trend since 2018.
10 ↓	Significant and clinically relevant decreasing trend since 2018.
10°	Trend not calculated because data from the years before 2022 did not meet the criteria for trend analysis.
10	No significant and clinically relevant time trend.

(For the criteria for trend analysis and the definition of a clinically relevant trend see section 4.1.1).

Figure 4.6.1 Trends in antibiotic resistance (from left to right 2018 to 2022) among diagnostic isolates of *H. pylori* in ISIS-AR



Warning: The Y-axis of this figure differs from the standard format. The Y-axis is scaled up to 50%.
* *Trend is significant and microbiologically relevant (for details see section 4.1.1).*

Key results and conclusions

H. pylori

- Since 2020, we have added data for *H. pylori* to NethMap. The results may be biased towards higher resistance levels due to sampling policies. However, resistance to **amoxicillin/ampicillin** (7%) and **doxycycline/tetracycline** (2%) was low. Over the last years, an increasing trend in resistance is seen for most antimicrobial agents. This means that treatment failures are expected to be more common. Therapy after treatment failure therefore should be guided by culture and susceptibility testing.

4.7 Highly resistant microorganisms

4.7.1 Carbapenem-resistant and carbapenemase-producing Enterobacterales

Introduction

Carbapenem-resistant Enterobacterales (CRE) and carbapenemase-producing Enterobacterales (CPE) have been reported all over the world. Because carbapenems represent a group of antibiotics of last resort for treatment of many bacterial infections, resistance poses a significant challenge to clinicians and negatively impacts patient care.[1] In Europe, CRE were first described in the early 2000's and their prevalence has since increased.[2] The current epidemiology in Europe varies from sporadic imported cases, to sporadic hospital outbreaks, to (inter-) regional spread between hospitals, to CRE being endemic in healthcare settings.[3] In the Netherlands, CRE are mainly a problem in hospitals so far, but community-spread has been described. CRE are therefore considered a growing public health threat.[4] Measured prevalence of CRE is influenced by test procedures and methods. Up to 2021, the Dutch national guideline suggested a gradient strip test as the first step in further investigation of isolates with an elevated MIC based on automated tests.[5] However, the guideline has been adapted in 2021 and now suggests to directly perform tests for carbapenemase production (phenotypic) or carbapenemase genes (genotypic) when further investigation is necessary.[6] This chapter describes the prevalence and confirmatory testing of CRE/CPE, (molecular) epidemiology and outbreaks of CPE in the Netherlands.

Methods

Data on CRE/CPE were obtained from the ISIS-AR and the Type-Ned databases, mandatory notifications in OSIRIS, and outbreaks reported to the Early warning and response meeting of Healthcare associated Infections and AntiMicrobial Resistance (SO-ZI/AMR).[7]

Prevalence and confirmatory testing of CRE/CPE

These analyses focus on all Enterobacterales, divided into 4 categories: *E. coli*, *K. pneumoniae*, *E. cloacae* complex and all other Enterobacterales species. The category *E. cloacae* complex contains the following species: *Enterobacter cloacae*, *E. homaechei*, *E. asburiae*, *E. cancerogenus*, *E. kobei*, *E. bugandensis*, *E. roggenkampii*, and *E. cloacae* complex not further defined. We searched the ISIS-AR database (years 2018-2022) for diagnostic (infection-related) isolates that were tested for meropenem and/or imipenem by an automated system. For *Proteus* spp., *Providencia* spp., *Serratia* spp. and *Morganella morganii*, only meropenem test results were included and analysed because of an intrinsic imipenem resistance.[6] Several breakpoints are used in this chapter: i) the screening breakpoint as defined by the Dutch national guideline[6] (which is 0.25 mg/L for meropenem and 1 mg/L for imipenem), and ii) the clinical breakpoints according to EUCAST, namely the clinical S (which is 2 mg/L for both imipenem and meropenem) and clinical R breakpoint (which is 8 mg/L for meropenem and 4 mg/L for imipenem). Based on the crude automated test values, we categorized them as having either an:

i) MIC ≤ the screening breakpoint,
ii) MIC > the screening breakpoint and ≤ the EUCAST clinical S breakpoint,
iii) MIC > the clinical S breakpoint and ≤ the clinical R breakpoint, or
iv) MIC > the clinical R breakpoint.

Categories ii, iii and iv are together referred to as elevated MIC. Subsequently, we searched the ISIS-AR and Type-Ned database for data on confirmatory tests (i.e., gradient strip tests and tests for carbapenemase production (phenotypic) or carbapenemase genes (genotypic)). We included only one isolate per patient

per organism category per year: an isolate with data on confirmatory tests (further referred to as CRE/CPE confirmed) was prioritized over an isolate with an automated test result only. If, subsequently, multiple isolates were eligible for inclusion, we prioritized the most resistant isolate. Based on data of isolates from 39 laboratories, we calculated numbers of isolates with automated MIC in the respective categories in 2022, the number of isolates with data on confirmatory tests and the number of isolates that were CRE/CPE confirmed per organism category. Based on data from 37 laboratories that continuously submitted data to ISIS-AR from 2018 to 2022, we assessed the percentage of isolates i) with an elevated MIC based on automated testing, ii) with elevated automated MIC that underwent confirmatory testing, and iii) that are CRE/CPE confirmed, by year.

Molecular characteristics of CPE isolates

For the enhanced surveillance of CPE via Type-Ned, all -except one- Dutch medical microbiology laboratories participate (n=50). Dutch laboratories are requested to submit screening or diagnostic Enterobacterales isolates to the RIVM with an MIC for meropenem of >0.25 mg/L and/or an MIC for imipenem >1 mg/L (until 2016) and/or carbapenemase production and/or a detected carbapenemase encoding gene. A restriction is that the laboratory can only send the first isolate/carbapenemase gene combination per person per year. The RIVM allows consecutive isolates from the same person if these are Enterobacterales species with other carbapenemase-encoding gene combinations when compared to the first isolate. The RIVM confirms the species by MALDI-ToF, determines the MIC for meropenem by Etest, and detects carbapenemase production by the carbapenem inactivation method (CIM).[8] The presence of carbapenemase-encoding genes are assessed by PCR (carba-PCR on bla_{NDM}, bla_{KPC}, bla_{IMP}, bla_{VIM}, and bla_{OXA}), and next-generation sequencing (NGS) and Nanopore long-read sequencing is performed for all isolates that are CIM positive.[9] The data described in this chapter are based on the first unique CIM-positive Enterobacterales species/carbapenemase-encoding allele combination per person for the period 2018-2022. This includes all isolates belonging to genetic clusters. A genetic cluster is defined per bacterial species and includes ≥2 highly related isolates of one species that differ typically ≤20 wgMLST alleles (25 for *E. coli*). In 2022, due to the Russia-Ukraine war, samples without a person ID (BSN, n=61) were included for further analysis if it represented a unique person, based on sex, age and postal code and when country of origin was Ukraine. Whole-genome multi-locus sequence typing (wgMLST) was used to detect genetic clusters consisting of genetically highly related *E. coli*, *K. pneumoniae*, *E. cloacae* complex and *C. freundii* complex isolates and are systematically assigned consecutive cluster numbers. Assigning genetic clusters started in 2018 and all sequenced isolates available from the national surveillance since 2014 were included in wgMLST analysis. Except for the first isolate, clusters solely consisting of multiple isolates from the same patient, including over different years and/or submitted by different laboratories, were not counted. Since the end of 2019, genetic cluster numbers for CPE are reported in Type-Ned.

Clinical/epidemiological characteristics of persons with CPE

From 1 July 2019 onwards, CPE, either phenotypically or genotypically confirmed, is mandatorily notifiable on person level (not on isolate level). Since then epidemiological patient data are collected by Municipal Health Services (MHS) and entered into the national web-based system for notifiable diseases (OSIRIS). Only notifications with a sampling date between 1 January and 31 December 2022 with status 'definite' are included in this chapter. Notifications are reported to the RIVM and stratified into persons with diagnostic and screening isolates.

Outbreaks
The SO-ZI/AMR database was interrogated for CPE outbreaks that were reported in 2022.

Results

Prevalence and confirmatory testing of CRE/CPE
Absolute numbers of isolates and categorization according to automated MICs in 2022 are presented in Table 4.7.1.1. Of a total number of 319,652 isolates with an automated test value for meropenem or imipenem (205,325 *E. coli*, 35,150 *K. pneumoniae*, 12,899 *E. cloacae* complex, and 66,278 other Enterobacterales species), an elevated MIC on automated testing was found in 4.7% of isolates (14,958). CRE/CPE confirmed isolates were mostly found in the organism category *E. cloacae* complex (0.81%) followed by *K. pneumoniae* (0.29%), other Enterobacterales (0.25%), and *E. coli* (0.03%). Most CRE/CPE confirmation tests were performed for species in the category other Enterobacterales (8.0%), followed by *E. cloacae* complex (4.2%), *K. pneumoniae* (1.1%), and *E. coli* (0.39%).

Figure 4.7.1.1 shows automated and confirmatory carbapenem susceptibility testing results of the past 5 years. The overall prevalence of *E. coli* strains with CRE/CPE confirmation has been fluctuating around an average of 0.04% (Figure 4.7.1.1a). For *K. pneumoniae*, the prevalence of strains with CRE/CPE confirmation has decreased between 2018 and 2020 from 0.40% to 0.25% and increased in 2021 to 0.29% and in 2022 to 0.32% (Figure 4.7.1.1a). The use of phenotypical and genotypical carbapenemase tests as well as gradient strip tests to confirm elevated automated carbapenem MIC values decreased between 2018 and 2022 for both species.

As for *E. cloacae* complex, the overall prevalence of strains with CRE/CPE confirmation decreased between 2018 and 2021 from 1.2% to 0.75% and increased in 2022 to 0.95% (Figure 4.7.1.1b). For the other Enterobacterales species, the prevalence of strains with CRE/CPE confirmation increased between 2018 and 2020 from 0.23% to 0.25%, decreased in 2021 to 0.24% and increased again in 2022 to 0.28% (Figure 4.7.1.1b). The use of gradient strip tests are fluctuating around an average 65.9% for *E. cloacae* complex species (not depicted in Figure 4.7.1.1b) and 37.1% for the other Enterobacterales species. The use of phenotypical carbapenemase confirmation tests also fluctuates around an average of 16.4% for *E. cloacae* complex species and 6.3% for the other Enterobacterales species. The use of genotypical carbapenemase confirmation tests decreased for both organism categories between 2018 and 2022 (Figure 4.7.1.1b).

Table 4.7.1.1 Results of automated and confirmatory carbapenem susceptibility testing among diagnostic (infection-related) Enterobacterales isolates in 2022, in 39 laboratories participating in ISIS-AR

	Automated MIC				
	MIC ≤ screening breakpoint *	MIC > screening* and ≤ clinical S breakpoint **	MIC > clinical S and ≤ clinical R breakpoint **	MIC > clinical R breakpoint **	Total
E. coli					
Total (N)	204,049	1,012	183	81	205,325
CRE/CPE confirmatory test performed (N (%))	149 (0.07)	506 (50.0)	87 (47.5)	68 (84.0)	810 (0.39)
CRE/CPE confirmed (N (% of total))	3 (0.00)	36 (3.5)	8 (4.4)	22(27.2)	69 (0.03)
K. pneumoniae					
Total (N)	34,543	434	83	90	35,150
CRE/CPE confirmatory test performed (N (%))	30 (0.09)	259 (59.7)	44 (53.0)	67 (74.4)	400 (1.1)
CRE/CPE confirmed (N (% of total))	0 (0.00)	38 (8.8)	8 (9.6)	56 (62.2)	102 (0.29)
***E. cloacae* complex**					
Total (N)	12,170	616	81	32	12,899
CRE/CPE confirmatory test performed (N (%))	49 (0.40)	397 (64.4)	65(80.2)	28 (87.5)	539 (4.2)
CRE/CPE confirmed (N (% of total))	2 (0.02)	75 (12.2)	11 (13.5)	17 (53)	105 (0.81)
Other Enterobacterales* **					
Total (N)	53,932	12,122	162	62	66,278
CRE/CPE confirmatory test performed (N (%))	465 (0.86)	4,705 (38.8)	105(64.8)	51 (82.3)	5,326 (8.0)
CRE/CPE confirmed (N (% of total))	8 (0.01)	133 (1.1)	13 (8.0)	12 (19.4)	166 (0.25)
All isolates					
Total (N)	304,694	14,184	509	265	319,652

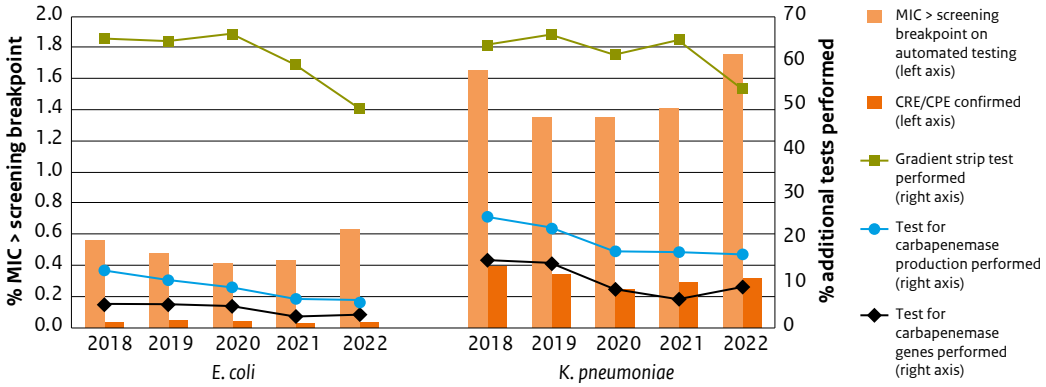
CRE/CPE confirmed = confirmation through gradient strip test (>screening breakpoint) and/or phenotypical carbapenemase production test and/or genotypical carbapenemase gene test.

** screening breakpoint according to NVMM guideline laboratory detection of highly resistant microorganisms (published November 2021).*

*** Clinical breakpoint according to EUCAST guideline v.12.0.*

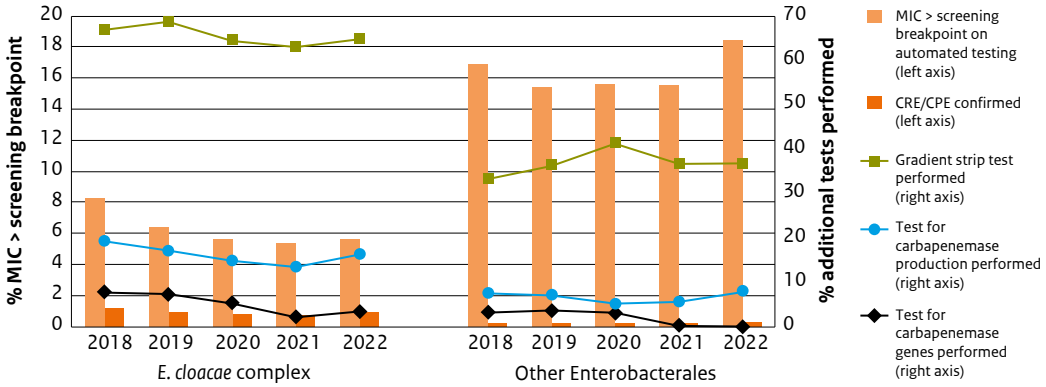
**** All other Enterobacterales species present in the ISIS-AR database. For species within the Proteus spp., Serratia spp., Providencia spp. and Morganella morganii results of imipenem were excluded. Top 5 species are: Proteus mirabilis, Klebsiella oxytoca, Citrobacter koseri, Serratia marcescens, Morganella morganii.*

Figure 4.7.1.1a Additional testing of elevated carbapenem MIC (%) in *E. coli* and *K. pneumoniae* by year, in 37 laboratories, ISIS-AR 2018-2022



Screening breakpoint: meropenem 0.25 mg/L, imipenem 1 mg/L.
The percentages of gradient tests and tests for carbapenemase production and carbapenemase genes performed were calculated for isolates with MIC > screening breakpoint on automated testing. The percentages of elevated automated MIC and CRE/CPE confirmed were calculated for all isolates with an automated test result.
One isolate per patient per organism group was selected: the most completely tested and most resistant isolate was included (see Methods section).

Figure 4.7.1.1b Additional testing of elevated carbapenem MIC (%) in *E. cloacae* complex and other Enterobacterales by year, in 37 laboratories, ISIS-AR 2018-2022



Screening breakpoint: meropenem 0.25 mg/L, imipenem 1 mg/L.
The percentages of gradient tests and tests for carbapenemase production and carbapenemase genes performed were calculated for isolates with MIC > screening breakpoint on automated testing. The percentages of elevated automated MIC and CRE/CPE confirmed were calculated for all isolates with an automated test result.
One isolate per patient per organism group was selected: the most completely tested and most resistant isolate was included (see Methods section).
Other Enterobacterales = all other Enterobacterales species present in the ISIS-AR database. For species within the Proteus spp., Serratia spp., Providencia spp. and Morganella morganii results of imipenem were excluded. Top 5 species are: Proteus mirabilis, Klebsiella oxytoca, Citrobacter koseri, Serratia marcescens, Morganella morganii.

Molecular characteristics of CPE isolates obtained through Type-Ned

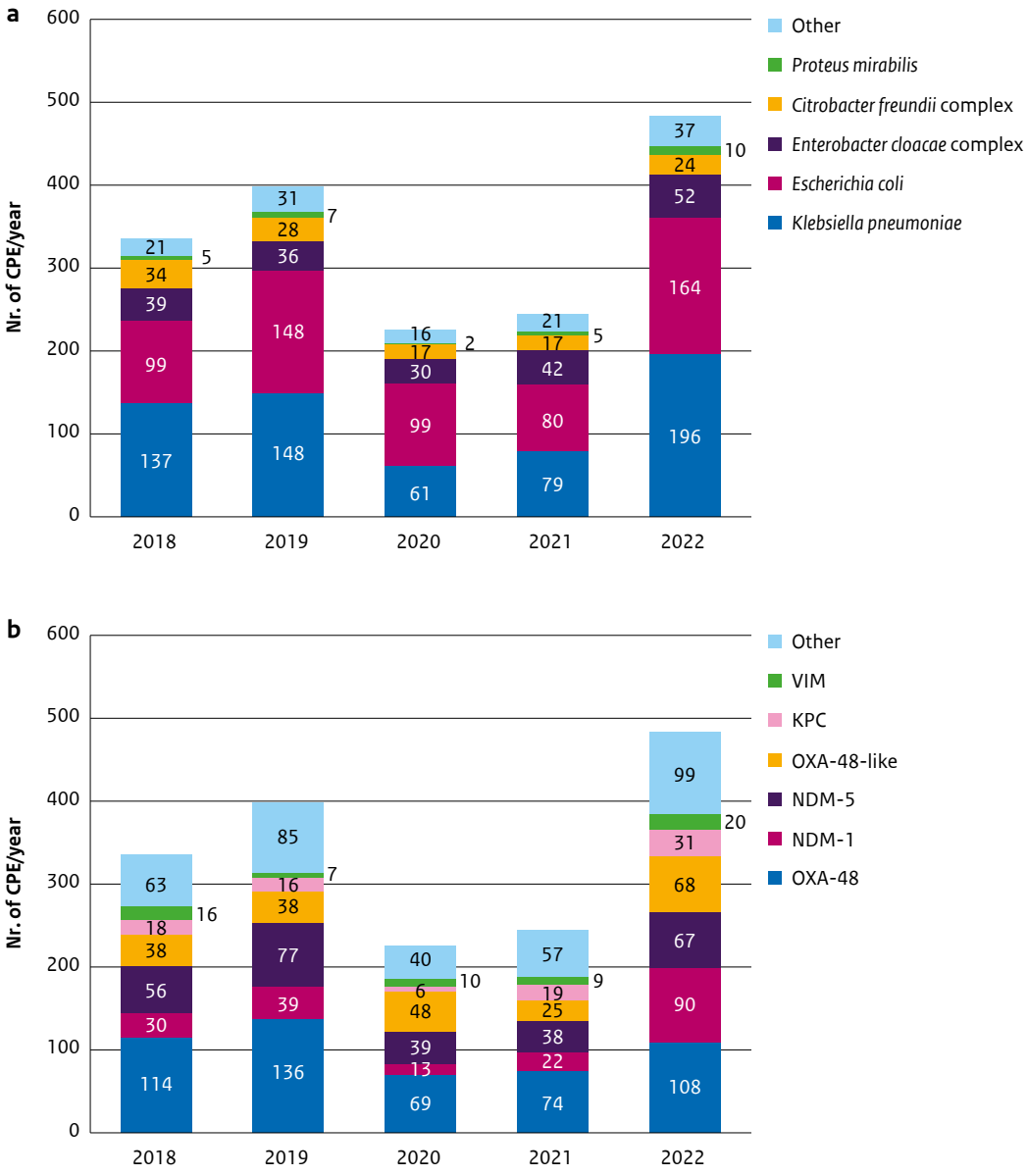
Carbapenemase-production was confirmed in 483 Enterobacterales isolates (unique species/carbapenemase allele combinations per person) obtained in 2022 from 356 patients with a person ID, 61 patients without BSN from the Ukraine (17%) and 39 patients without BSN with unknown origin (11%) at time of analyses. The screening and diagnostic isolates were submitted to the RIVM by 49 of the 50 Dutch medical microbiology laboratories. The number of unique CPE isolates submitted to the RIVM increased from 335 in 2018, to 398 in 2019, decreased to 225 and 244 in 2020 and 2021, respectively, followed by an increase to 483 in 2022 (Figure 4.7.1.2a).
Of the 483 CPE isolates, 196 (41%) were *K. pneumoniae* complex, 164 (34%) *E. coli*, 52 (11%) *E. cloacae* complex, and 24 (5%) *C. freundii* complex and the remaining 47 (10%) isolates belonged to other species. When the EUCAST clinical breakpoints were applied, 198/483 (41%) had an MIC for meropenem above the cut-off of 8 mg/L.

As in previous years, the bla_{OXA-48} gene was the most frequently (22%, 108/483) identified carbapenemase-encoding gene in CPE isolates cultured and submitted in 2022 (Figure 4.7.1.2b). In addition, of the CPE analysed in 2022, 44% (212/483) carried a bla_{OXA-48} or bla_{OXA-48}-like gene ($bla_{OXA-181}$, $bla_{OXA-232}$, and $bla_{OXA-244}$) either alone or in combination with another carbapenemase allele. The bla_{OXA-48} allele, either alone or in combination with another carbapenemase-encoding gene, was present in 29%, 21% and 40% of the *K. pneumoniae*, *E. coli*, *E. cloacae* complex and 42% *C. freundii* complex isolates, respectively. The bla_{OXA-48}-like alleles were found in 14% and 29% of the *K. pneumoniae* and *E. coli* isolates, respectively. In *E. coli*, 30% (50/164) of the isolates carried bla_{NDM-5} and the gene was found in 8% (15/196) of the *K. pneumoniae* isolates. Conversely, bla_{NDM-1} was found predominantly in *K. pneumoniae* isolates (35%, 69/196) and only in 6% (10/164) of the *E. coli* isolates. In 2022, there was a marked increase in NDM- and OXA-48-like-carrying CPE when compared to previous years (Figure 4.7.1.2b). This increase of CPE and NDM-carrying CPE was observed among persons in the Netherlands with a BSN, but a proportion of this increase can also be attributed to the Ukraine/Russia war with the resulting increased Ukrainian patient population in hospitals since March 2022.[10]

Twenty-six of the thirty-two (81%) $bla_{OXA-244}$ *E. coli* isolates submitted in 2022 had MICs for meropenem ≤2 mg/L. Eight percent (39/483) of the CPE carried two carbapenemase-encoding genes. In 13/483 (3%) no carbapenemase-encoding gene was detected. Of these isolates, 9 (69%) were *Enterobacter* spp. and 4 (31%) from other species. The nature of the apparent carbapenemase production in *Enterobacter* spp. is still under investigation in the RIVM, but carbapenemase activity of AmpC-type of enzymes seems to be the likely explanation.

There was a strong correlation between the MIC for meropenem and the presence of particular species/carbapenemase-encoding allele combinations. In general, a larger proportion of the *K. pneumoniae* isolates (68%, 134/196) was meropenem resistant compared to the *E. coli* isolates (27%, 45/164), irrespective of the carbapenemase-encoding genes present. Only one *E. coli* isolate carrying bla_{OXA-48} had an MIC above the clinical breakpoint for meropenem resistance (MIC >8 mg/L), while 46% percent of the *K. pneumoniae* carrying bla_{OXA-48} were meropenem resistant.

Figure 4.7.1.2 Numbers of carbapenemase-producing Enterobacterales (CPE), species (a) and carbapenemase allele (b), based on Type-Ned, 2018-2022



Genetic clusters
Between 2018 and 2022, 605 of the 1530 (40%) isolates of *E. coli*, *K. pneumoniae*, *E. cloacae* complex and *C. freundii* complex fell in one of 190 clusters, including clusters partially containing older isolates. The four largest clusters containing isolates from 2022 concerned *C. freundii* complex NDM-5 with 54 isolates, *E. cloacae* complex OXA-48 with 34 isolates, *E. coli* OXA-244 with 20 isolates, and *E. coli* OXA-48 with 18 isolates. These four clusters spanned multiple years, between 5 and 8. The two *E. coli* clusters were multi-institutional, whereas the other two clusters were predominantly from one institute each.[11] MMLs are notified by email that isolates they submitted within a period of one year are part of a genetic cluster. Forty-five new clusters were created in 2022, 22 involving *K. pneumoniae*, 18 *E. coli*, three *C. freundii* complex and two *E. cloacae* complex. The majority (34) of the new clusters contained only two isolates, the largest contained 10 *K. pneumoniae* and 30 clusters concerned multi-institutional genetic clusters. Thirty two clusters involved isolates from 2022 only, including the 10-isolate cluster. Twelve of the new clusters, of which 10 *K. pneumoniae*, involved persons from Ukraine.

Clinical/epidemiological characteristics of persons with CPE
Additional epidemiological questionnaire data was available in OSIRIS for 368 CPE positive persons with a sampling date in between 1 January 2022 and 31 December 2022 (Table 4.7.1.2). This is much higher than the previous two years (n= 180 in 2019 (July-December), n=170 in 2020, n=201 in 2021). The median age of the 368 persons was 58 (range 0 - 100) years and 222 (60%) were male.

A sample taken for diagnostic purposes was the reason for sampling in 22% (82/368) of the notified persons in 2022 (Table 4.7.1.2), compared to 31% (55/180) in 2019 (July-December), 28% in 2020 (48/170), and 25% (50/201) in 2021. Most patients with a diagnostic isolate had no known risk factor identified (57%) (Figure 4.7.1.3). Hospitalization abroad for at least 24 hours within the previous two months was higher than previous years (21% in 2022 compared to 15% in 2019 (July-December), 10% in 2020, and 8% in 2021) (Table 4.7.1.2, Table 4.7.1.3, and Figure 4.7.1.3). Ukraine (n=6), followed by Turkey, Morocco and Egypt (all n=2) were most often reported as countries of hospitalization. The most common reported infection among patients with a diagnostic isolate was urinary tract infection (47%, 39/83), followed by sepsis (14%, 12/83) and wound infection (13%, 11/83).

Screening as part of routine screening (e.g., on admission, because of prolonged hospital stay or as part of selective decontamination regimens) or targeted screening because of suspected CPE carriage was the reason for sampling in 75% (276/368) of the persons in 2022, compared to 67% (120/180) in 2019 (July-December), 69% (118/170) in 2020, and 73% (146/201) in 2021. Hospitalization abroad for at least 24 hours within the previous two months was the most common reported risk factor for the presence of CPE among persons with a screening isolate and this was higher than previous years (61% in 2022 compared to 54% in 2019 (July-December), 42% in 2020, and 49% in 2021) (Table 4.7.1.2, Table 4.7.1.3, and Figure 4.7.1.3). Turkey (n=40), Ukraine (n=30) and Morocco (n=20) were most often reported as countries where hospitalization had occurred. No risk factor could be identified in 18%.

For the 10 (3%) remaining persons the reason for sampling was different from diagnostic or screening or was unknown.

Table 4.7.1.2 Epidemiological data of notifications of persons carrying CPE, OSIRIS, 2022

Characteristic	Total[a] n (%)	Diagnostic n (%)	Screening n (%)
n	368	82	276
Location of sampling			
Outpatient/emergency departments or by a general practitioner	146 (39.7)	44 (53.7)	99 (35.9)
Inpatient departments (excl. Intensive care units)	180 (48.9)	29 (35.4)	145 (52.5)
Intensive care units	41 (11.1)	9 (11.0)	31 (11.2)
Other/unknown	1 (0.3)	0 (0.0)	1 (0.4)
Residence			
Living independently	255 (69.3)	62 (75.6)	186 (67.4)
Rehabilitation centre	13 (3.5)	1 (1.2)	12 (4.3)
Nursing or elderly home/facilities for small-scale housing for elderly	13 (3.5)	5 (6.1)	8 (2.9)
Asylum seekers centre	31 (8.4)	4 (4.9)	25 (9.1)
Other/unknown	56 (15.2)	10 (12.2)	45 (16.3)
Invasive medical procedure/diagnostics			
No	189 (51.4)	41 (50.0)	141 (51.1)
Surgery	100 (27.2)	16 (19.5)	83 (30.1)
Other (incl. invasive procedure like endoscopy, cystoscopy, urinary catheter, renal dialysis)	72 (19.6)	25 (30.5)	45 (16.3)
Unknown	7 (1.9)	0 (0.0)	7 (2.5)
Identified risk factors			
No risk factor known/unknown	104 (28.3)	47 (57.3)	50 (18.1)
Hospitalization abroad >24 hours during the previous two months	188 (51.1)	18 (22.0)	169 (61.2)
Contact with a hospital abroad in the past twelve months in a different way than >24 hours during the previous two months	34 (9.2)	8 (9.8)	25 (9.1)
Travelling abroad in the past twelve months without hospitalization or visiting a hospital	35 (9.5)	8 (9.8)	26 (9.4)
Already known carrier of CPE	5 (1.4)	1 (1.2)	4 (1.4)
Received care in a department of a healthcare facility with an ongoing outbreak of CPE in the previous two months	2 (0.5)	0 (0.0)	2 (0.7)

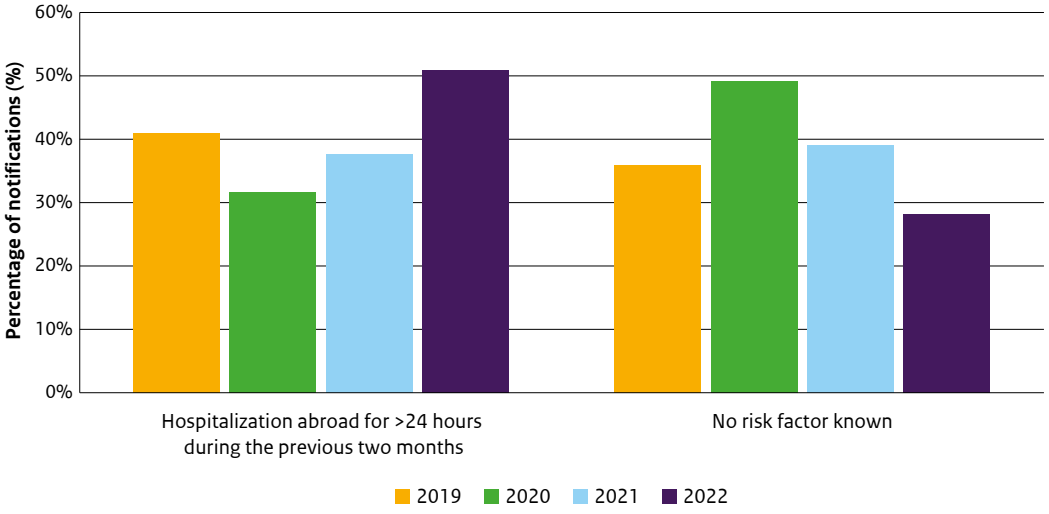
[a] *Including persons for whom the reason for sampling was unknown.*

Table 4.7.1.3 Top 5 parts of the world where persons with hospitalization abroad for at least 24 hours during the previous two months were admitted, OSIRIS, 2022

Characteristic	Total[a] n (%)	Diagnostic n (%)	Screening n (%)
n	188	18	169
Hospitalized in a country in			
Western Asia	49 (26.1)	3 (16.7)	46 (27.2)
Eastern Europe	46 (24.5)	7 (38.9)	38 (22.5)
Northern Africa	29 (15.4)	4 (22.2)	25 (14.8)
Southern Europe	22 (11.7)	1 (5.6)	21 (12.4)
Western Europe	12 (6.4)	0 (0.0)	12 (7.1)
Other region of the world/unknown	30 (16.0)	3 (16.7)	27 (16.0)

[a] *Including persons for whom the reason for sampling was unknown.*

Figure 4.7.1.3 Hospitalization abroad for at least 24 hours during the previous two months and no risk factor known among CPE positive persons (diagnostic and screening combined), OSIRIS, 2019-2022[a]



[a] *Data from 2019 only includes notifications from 1 July-31 December 2019 since mandatory notification started on 1 July 2019.*

Outbreaks

In 2022, three new outbreaks with carbapenemase-producing Enterobacterales in hospitals were reported to SO-ZI/AMR (Table 4.7.1.4). See chapter 4.7.6 for more details about SO-ZI/AMR.

Table 4.7.1.4 Outbreaks of carbapenemase-producing Enterobacterales reported in 2022 to the Early warning and response meeting for Healthcare-associated Infections and Antimicrobial Resistance (SO-ZI/AMR)

Region	Main organism	Gene	No. of patients
Zuidwest NL	*E. cloacae*	OXA-48	7
GAIN	*K. pneumoniae*	NDM-1	2
Euregio Zwolle	*K. pneumoniae*	NDM-1	2

Discussion

In ISIS-AR, the prevalence of confirmed carbapenem resistance of *E. coli* was 0.03% in 2022 which was comparable to the previous years. Carbapenem resistance in *K. pneumoniae* is significantly higher compared to *E. coli*, but has also been stable in the previous years, with a resistance percentage around 0.3% in the last few years. Other Enterobacterales show similar patterns. Since 2021, the revised Dutch national guideline suggests to perform tests for carbapenemase production (phenotypic) or carbapenemase genes (genotypic) of isolates with an elevated MIC based on automated tests, and gradient strip tests are not warranted anymore. The percentage of isolates with elevated automated MIC with a gradient strip test performed has already been decreasing since 2017, especially for *E. coli* and *K. pneumoniae*. On the other hand, the percentages of confirmatory carbapenemase tests among Enterobacterales isolates with elevated automated MIC has been stable or increasing since 2021, in accordance with the new guideline. The percentage of confirmed CRE/CPE isolates differed between the various species categories within Enterobacterales. Even among the isolates with automated MIC > the clinical R breakpoint, the confirmation percentage was below 50% for both *E. coli* and 'Other Enterobacterales'. This was partly a result of lab-specific algorithms for additional testing after automated antimicrobial susceptibility testing, which was not always in accordance with the national guideline. Furthermore, the data showed that there could be substantial discrepancies between automated MIC values and MICs determined through gradient strip test, regularly leading to an overestimation of phenotypical resistance measured by automated MIC testing.

In 2022, the number of carbapenemase-producing Enterobacterales isolates that was submitted to the RIVM was considerably higher than in previous years and was succeeding pre-COVID totals. In 2020-2021 the decrease of the number of CPE was presumably the result of the COVID-19 pandemic associated measures, such as travel restrictions, social isolation, and a reduction in regular healthcare. The increase in 2022 is partially attributable to the transfer of Ukrainian patients to the Netherlands due to the Ukraine/Russia war. We indeed noted an increase in patients from Ukraine.[10] This resulted in an increase of the percentage of patients with a reported hospitalization abroad for more than 24 hours during the preceding two months, and Ukraine being the second country of all reported countries where hospitalization occurred. A large number (45) of new genetic clusters were found in 2022, compared to previous years (five in 2021, 14 in 2020). Most of the clusters, especially with *E. coli*, were small clusters of 2 isolates only, whereas a number of the *K. pneumoniae* clusters involved a larger number of isolates, up to 10. The introduction of

next-generation sequencing and Nanopore long-read-generation sequencing on all carbapenemase-producing isolates allows the identification of genetic clusters that may indicate transmission within and between healthcare centres. Genetic clustering does not prove direct transmission or an outbreak. Isolates that cluster together based on wgMLST may still be different in plasmid content and/or resistome and may lack an epidemiological link in time and place. For some genetic clusters, sampling dates are several years apart. To identify direct transmission, information on epidemiological links would be needed.
The absolute number of persons with a sample taken for diagnostic purposes as well as for routine or targeted screening increased in 2022 compared to previous years, while the percentage of persons with a diagnostic sample decreased over time and the percentage with a screening isolate increased over time. The increase of screening samples is potentially related to the increased travel after releasing the COVID-19 measures and to the transfer of patients from Ukraine. The reason why more diagnostic isolates were seen are unclear.

Conclusions

- The overall percentage of Enterobacterales isolates with elevated automated test value for meropenem or imipenem (i.e., > the screening breakpoint) was 4.7% in 2022. The prevalence of CRE/CPE confirmed isolates among *E. coli* was 0.03%, among *K. pneumoniae* 0.29%, *E. cloacae* complex 0.81% and other Enterobacterales 0.25%.
- In 2022, the number of carbapenemase-producing Enterobacterales isolates submitted to the RIVM was considerably higher than in previous years and was succeeding pre-COVID totals. The increase is partially attributable to the transfer of Ukrainian patients to the Netherlands.
- The predominant carbapenemase-producing Enterobacterales species in 2022 were *K. pneumoniae*, *E. coli*, *E. cloacae* complex, and *C. freundii* complex.
- The most frequently identified carbapenemase encoding genes in Enterobacterales were $bla_{\text{OXA-48}}$, $bla_{\text{OXA-48}}$-like, $bla_{\text{NDM-1}}$ and $bla_{\text{NDM-5}}$.
- The MIC for meropenem was generally higher for *K. pneumoniae* than for *E. coli* isolates harbouring $bla_{\text{OXA-048}}$ or $bla_{\text{OXA-48}}$-like genes. Still, these isolates were more sensitive for meropenem than isolates carrying other carbapenemase-encoding genes.
- Of the *K. pneumoniae* complex, *E. coli*, *E. cloacae* complex and *C. freundii* complex isolates found between 2018 and 2022, 40% could be categorized into one of 190 genetic clusters.
- Forty-five new genetic clusters arose in 2022, thirty of which concerned multi-institutional genetic clusters. Thirty-four of the 45 new genetic clusters comprise two isolates only.
- Twenty-two percent of CPE cases were identified in diagnostic samples, 75% were identified upon routine screening or targeted screening because of suspected CPE carriage, and for 3% a different/unknown reason was reported.
- In 51% of patients, there is a relation with hospitalization abroad for more than 24 hours during the preceding two months (22% and 61% among persons with a diagnostic and screening isolate, respectively), which was higher than previous years. Turkey, Ukraine, and Morocco are the countries that are most often reported.
- In 28% of the CPE positive persons no known CPE risk factor was identified (57% and 18% among persons with a diagnostic and screening isolate, respectively), which is lower than previous years.

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4.7.2 Vancomycin-resistant Enterococci

Introduction

In the last few years, a considerable number of Dutch hospitals have been confronted with outbreaks of vancomycin-resistant *Enterococcus faecium* (VRE_{fm}). There is no national surveillance program with centrally organised characterisation of VRE-strains in The Netherlands. Here we give an overview of available data that describe the epidemiology of VRE in The Netherlands.

Methods

VRE_{fm} outbreaks are reported through the Early warning and response meeting for Healthcare associated Infections and Antimicrobial Resistance (SO-ZI/AMR, see section 4.7.6). In addition, based on the national surveillance system of antimicrobial resistance, ISIS-AR, the proportion of vancomycin resistance in *E. faecium* isolates among patients in various healthcare settings in the Netherlands was determined. The prevalence of VRE_{fm} isolates was based on positivity of confirmation tests (molecular detection of VanA/B), or, if these tests were lacking, on re-interpretation of test-values for amoxicillin/ampicillin and vancomycin according to EUCAST 2022, with VRE_{fm} being defined as resistant to amoxicillin/ampicillin and vancomycin. Both diagnostic isolates (isolates cultured from clinical material) and screening isolates (predominantly rectal swabs) were included. Numbers are based on data from 37 laboratories in the Netherlands that continuously reported to the ISIS-AR database in the past five years. The first diagnostic or screening *E. faecium* isolate per patient was selected.

Results

In 2022, 9 outbreaks with VRE_{fm} in the Netherlands were reported to the SO-ZI/AMR (see section 4.7.6), all were in hospitals, with a median reported number of 9 patients involved (range, 3 – 33 patients). This number of outbreaks is comparable to the 8 outbreaks that were reported in 2021, but much lower than the 19 outbreaks reported in 2019. In the years before 2019, 10 to 15 outbreaks per year were reported. In total, since the start of SO-ZI/AMR in April 2012, 128 outbreaks with VRE_{fm} have been reported in the Netherlands. The contribution of VRE_{fm} outbreaks to the total number of reported outbreaks in hospitals was substantial in the previous years, with a proportion varying between 20 and 32% of all reported outbreaks in SO-ZI/AMR yearly.

The percentage of diagnostic VRE_{fm} isolates of the total number of *E. faecium* isolates from general practitioners and (outpatient and inpatient) hospital departments in 2022 in the Netherlands based on ISIS-AR is shown in table 4.7.2.1. Figure 4.7.2.1 shows the trends in vancomycin resistance in diagnostic *E. faecium* isolates over the years. The proportion of diagnostic isolates with VRE_{fm} was persistently low, although a slight increase is seen in ICU departments.

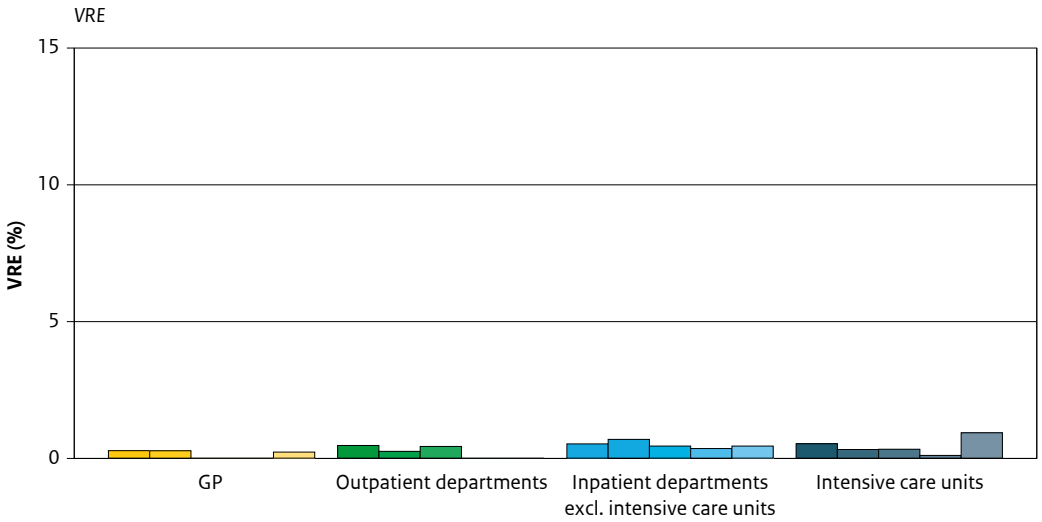
The absolute numbers of VRE_{fm} isolates from screening samples of inpatient hospital departments (including intensive care units), from 37 laboratories continuously reporting to ISIS-AR show a range of 86-158 positive isolates per year, with the lowest number in 2020 (Table 4.7.2.2).

Table 4.7.2.1 Vancomycin-resistant *E. faecium* (VRE_{fm}) in diagnostic isolates in the Netherlands in 2022, based on ISIS-AR data

Type of setting	Tested isolates, N	VRE, N (%)
General practitioner	450	1 (0)
Outpatient departments	546	0 (0)
Inpatient departments excl. intensive care units	2,711	12 (0)
Intensive care units	645	6 (1)
Total	**4,352**	**19 (0)**

Numbers are based on a selection of 37 laboratories that continuously reported to the ISIS-AR database in the past five years.
The first diagnostic E. faecium isolate per patient was selected.
The prevalence of VRE_{fm} isolates was based on positivity of confirmation tests, or, if these tests were lacking, on re-interpretation of test-values for amoxicillin/ampicillin and vancomycin according to EUCAST 2022, with VRE_{fm} being defined as resistant to amoxicillin/ampicillin and vancomycin.

Figure 4.7.2.1 Trends in vancomycin-resistant *E. faecium* (VRE_{fm}) in diagnostic isolates in the Netherlands (from left to right 2018 to 2022), based on ISIS-AR data



Numbers are based on a selection of 37 laboratories that continuously reported to the ISIS-AR database in the past five years.
The first diagnostic E. faecium isolate per patient per year was selected.
The prevalence of VRE_{fm} isolates was based on positivity of confirmation tests, or, if these tests were lacking, on re-interpretation of test-values for amoxicillin/ampicillin and vancomycin according to EUCAST 2022, with VRE_{fm} being defined as resistant to amoxicillin/ampicillin and vancomycin.

Table 4.7.2.2 Absolute numbers of vancomycin-resistant *E. faecium* (VRE_{fm}) isolates in the Netherlands, 2018-2022, based on ISIS-AR data

	General practitioner and outpatient departments			Inpatient departments including intensive care units			Total		
Year	Diagnostic	Screening	Total	Diagnostic	Screening	Total	Diagnostic	Screening	Total
2018	3	79	82	17	141	158	20	220	240
2019	2	54	56	20	105	125	22	159	181
2020	2	35	37	15	71	86	17	106	123
2021	0	40	40	11	89	100	11	129	140
2022	1	41	42	18	111	129	19	152	171

Numbers are based on a selection of 37 laboratories that continuously reported to the ISIS-AR database in the past five years.
The first screening E. faecium isolate per patient per year was selected with a preference for the first diagnostic isolate if both diagnostic and screening samples were taken.
The prevalence of VRE_{fm} isolates was based on positivity of confirmation tests, or, if these tests were lacking, on re-interpretation of test-values for amoxicillin/ampicillin and vancomycin according to EUCAST 2022, with VRE_{fm} being defined as resistant to amoxicillin/ampicillin and vancomycin.

Discussion

The number of reported VRE_{fm} outbreaks in 2022 was comparable to that of 2021 and higher than the low number in 2020, but still lower compared to the previous years. This could be related to the COVID-19 pandemic, which led to downscaling non-urgent healthcare in hospitals and a change in infection prevention measures. Although the number of screening samples that were tested is unknown, the absolute number of positive screening isolates remains the same in the Netherlands over the years. This seems in contrast to the majority of European countries, where the number of VRE_{fm} isolates is considerably increasing.[1]
Currently, there are no centrally collected data on molecular typing of VRE_{fm} isolates in the Netherlands, even though the WHO marked VRE_{fm} as a "high priority antibiotic resistant organism". Thus, there are no reliable data available on the molecular epidemiology of VRE_{fm} in Dutch hospitals.
In the coming period, the need for a national VRE_{fm} surveillance system will be discussed.[2]

Conclusions

- The number of reported hospital outbreaks with VRE_{fm} in 2022 was comparable to 2021, but still lower compared to 2019, which was probably due to the COVID-19 pandemic.
- The proportion of VRE_{fm} in infection-related isolates with *E. faecium* in various healthcare settings varies marginally below 1% and has not changed in the previous five years, although an increase of the proportion of VRE_{fm} was seen in intensive care units after several years of decrease.
- The absolute number of positive screening VRE_{fm} isolates is substantially higher than 2020 and 2021 (COVID-19 period) and comparable to the pre-COVID-19-period.
- There are no national data available on the molecular epidemiology of VRE_{fm} in Dutch hospitals.

References

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4.7.3 Methicillin-resistant *Staphylococcus aureus* (MRSA)

Introduction

The Netherlands remains a country with a low MRSA prevalence. This is most probably explained by the strict infection prevention rules ("search and destroy" MRSA policy) and the limited use of antibiotics. The ISIS-AR database contains, among others, information regarding *S. aureus* culture and susceptibility testing results from routine practices in medical microbiology laboratories (MML). In addition, in 1989, a more enhanced molecular MRSA surveillance at a national level was started at the RIVM which includes the submission of MRSA isolates. For the enhanced surveillance, the Type-Ned system is used to monitor the occurrence of MRSA, clinical/epidemiological characteristics of persons with MRSA and the molecular characteristics of circulating MRSA types.

Methods

Objectives

The objectives were to assess the (trends in) MRSA prevalence, molecular isolate characteristics and the clinical/epidemiological person characteristics during the five previous years (2018 to 2022) in the Netherlands.

MRSA prevalence

From the ISIS-AR database, *S. aureus* isolates, including MRSA, that were cultured in the period of 2018 to 2022 were identified. Data were originating from 37 laboratories that continuously reported complete data to the ISIS-AR database during the selected period. The first diagnostic *S. aureus* isolate per person per year from blood, cerebrospinal fluid, urine, lower respiratory tract, or wound/pus was selected. Prevalence of MRSA was calculated as the percentage of *S. aureus* isolates for which the MRSA confirmation test (presence of *mecA* gene or *pbp2*) was positive, or, if these tests were lacking, laboratory S/R interpretation for cefoxitin was R, or, if no data on a cefoxitin test was available, the S/R laboratory interpretation for flucloxacillin/oxacillin was R. An additional prevalence assessment was conducted for *S. aureus* isolates from blood only.

Molecular characteristics of MRSA isolates

For the enhanced MRSA surveillance, Dutch laboratories are requested to submit MRSA isolates that are identified in routine practices using the Type-Ned system for molecular typing by multiple-locus variable number of tandem repeat analysis (MLVA), in which detection of the *mecA*, *mecC* and *lukS-PV-lukF-PV*-genes (encoding *pbp2* and Panton-Valentine leucocidin, respectively) are additionally incorporated. MLVA types that differ on 1 locus in MLVA profile were combined into MLVA complexes. Since 2020, one isolate per person within a three-year period is eligible to be submitted. Isolates in the database were categorised as either diagnostic (isolated from samples of infection-related materials, i.e. blood, cerebrospinal fluid, sputum, pus, urine or wound) or screening (isolated from human MRSA-screening materials, i.e. swabs from throat, nose, skin, ear, perineum and/or rectum). Livestock-associated MRSA (LA-MRSA) are defined as isolates from the MLVA-complex MC0398. To investigate trends in molecular results, the first MRSA isolate per person sampled in the period 2018 to 2022 was included, with the exception that only the first diagnostic isolate is included when both a screening and a diagnostic sample are submitted from the same person. Samples from non-human origin, isolates lacking a *mecA* or *mecC* gene, samples that could not be typed by MLVA, and isolates without a person ID were also excluded from molecular analysis described herein. Since 2019, a semi-random selection of MRSA isolates is analysed through whole-

genome sequencing (150 bp paired-end reads, Illumina NovaSeq 6000). It concerns a random selection of 40 isolates per month that meet the following criteria: one isolate per person, per MLVA type, per laboratory. All liquor and blood-derived isolates that are not part of the initial selection are additionally included. Sequencing data were used for multi-locus sequence typing (MLST) using SeqSphere software and antimicrobial resistance genes were identified using the ResFinder database.

Clinical/epidemiological characteristics of persons with MRSA
Since 2017, as part of the enhanced surveillance, a clinical/epidemiological questionnaire on person characteristics is requested to be completed for each submitted MRSA isolate, except for persons who are part of a contact tracing investigation. Questionnaires related to isolates from employees in a healthcare facility that were screened as part of a local screening programme were excluded. Clinical/epidemiological data from the included isolates are described for 2022 and compared with previous years, for all isolates combined and after stratification into diagnostic and screening isolates.

Results

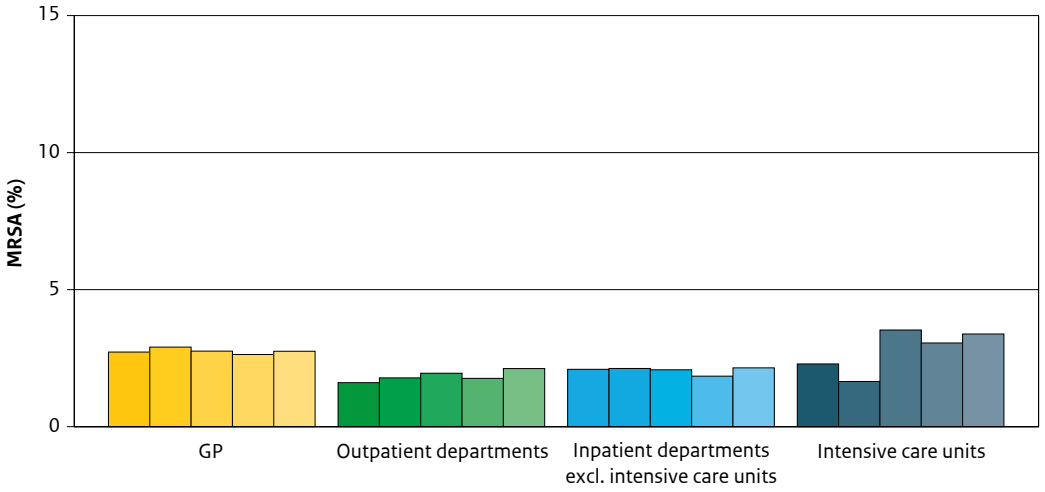
MRSA prevalence
In ISIS-AR, the overall proportion of diagnostic *S. aureus* isolates in 2022 that was identified as MRSA was 2% (n=738/31,518). A higher proportion of 3% was seen for isolates cultures requested by general practitioners (GPs) and for intensive care units (Table 4.7.3.1). In blood isolates only, the prevalence of MRSA in 2022 was 2% (n=60/3,198). Figure 4.7.3.1 shows the trends in MRSA from 2018 to 2022 in all diagnostic isolates. Percentages MRSA were quite stable, except in intensive care units in which the prevalence increased from ~2% in the first two years (35/1,527 in 2018, and 22/1,333 in 2019) to ~3% in the last three years (n=50/1,415 in 2020, 54/1,765 in 2021, and 44/1,299 in 2022).

Table 4.7.3.1 Methicillin-resistant *S. aureus* (MRSA) in the Netherlands in 2022, based on ISIS-AR data

Type of department	Tested isolates, N	MRSA, N(%)
General practitioners	7,839	216 (3)
Outpatient departments	11,259	239 (2)
Inpatient departments excl. intensive care units	11,121	239 (2)
Intensive care units	1,299	44 (3)
Total	**31,518**	**738 (2)**

Numbers are based on a selection of 37 laboratories that continuously reported to the ISIS-AR database in the past five years.
The first diagnostic S. aureus isolate per patient was selected.
The prevalence of MRSA isolates was based on positivity of MRSA confirmation tests (presence of mecA or mecC gene or pbp2). If these tests were lacking, prevalence was based on laboratory S/R interpretation for cefoxitin or, if no data on a cefoxitin test was available, for flucloxacillin/oxacillin.

Figure 4.7.3.1 Trends in methicillin-resistant *S. aureus* (MRSA) in the Netherlands (from left to right 2018 to 2022), based on ISIS-AR data



GP: general practitioner
Numbers are based on a selection of 37 laboratories that continuously reported to the ISIS-AR database in the past five years.
The first diagnostic S. aureus isolate per patient per year was selected.
The prevalence of MRSA isolates was based on positivity of MRSA confirmation tests (presence of mecA or mecC gene or pbp2). If these tests were lacking, prevalence was based on laboratory S/R interpretation for cefoxitin or, if no data on a cefoxitin test was available, for flucloxacillin/oxacillin.

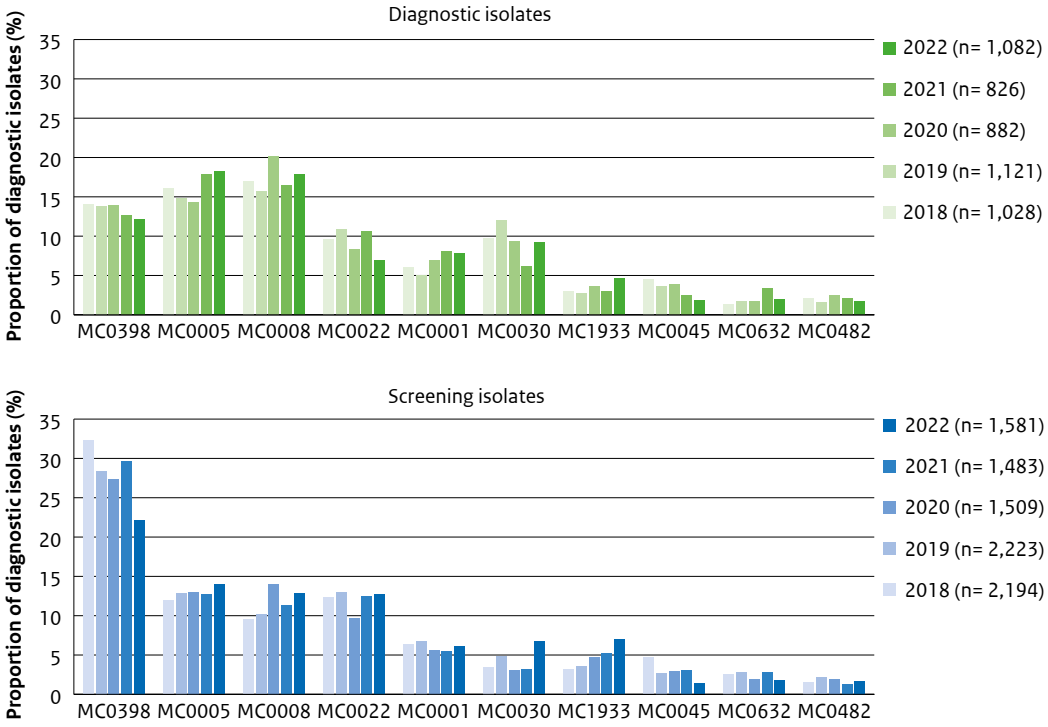
Molecular characteristics of MRSA isolates
In 2022, the RIVM received 3,015 *S. aureus* isolates that were *mecA* or *mecC* gene positive, from human origin and from persons with a known personal identification number. As only the first isolate per person for the period 2018-2022 was used, 2,672 isolates from 2,672 persons were included for analyses of the year 2022. The absolute number of MRSA isolates included in the analysis in 2022 is higher compared to 2020 (2,396 isolates) and 2021 (2,322 isolates), but lower than in the years 2018 (3,239 isolates) and 2019 (3,350 isolates), most likely attributable to the COVID-19 pandemic.

The majority of the isolates in 2022 were cultured from samples submitted to the MML from hospitals (n= 1,682; 63%), followed by GPs (n= 782; 29%) and long-term care facilities (n= 90; 3%). Based on origin of the material, 59% (n= 1,581) of the isolates were isolated from screening samples. A total of 1,082 isolates (40%) were categorised as diagnostic isolates, of which the majority were cultured from wound material or pus (n= 801; 74%) and 48 diagnostic isolates were cultured from blood (4%). The above-mentioned numbers are comparable to previous years.

For 2022, the MRSA population could be divided into 715 MLVA-types. The majority (632 MLVA-types, 2,515 isolates) could be grouped into 24 MLVA-complexes (MCs). For 83 MLVA-types (157 isolates) no MC could be assigned. The most frequently identified MC in 2022 was MC0398 (n= 486; 18%), also known as LA-MRSA, followed by MC0005 (n= 418; 16%) and MC0008 (n= 399; 15%). The proportion of MC0398 was higher in screening isolates (22%) than in diagnostic isolates (12%), like in previous

years. During the 2018-2022 surveillance period, there has been a trend of increasing proportions of screening isolates belonging to MC0008 and MC1933 and of diagnostic isolates belonging to MC0005 and MC0001 (Figure 4.7.3.2). In both, screening and diagnostic isolates, there has been a trend of decreasing proportions of isolates belonging to MC0398 and MC0045 (Figure 4.7.3.2).

Figure 4.7.3.2 Trends in the ten most frequently identified MLVA complexes of MRSA in the Netherlands (2018 to 2022) among diagnostic and screening isolates, based on enhanced MRSA surveillance data

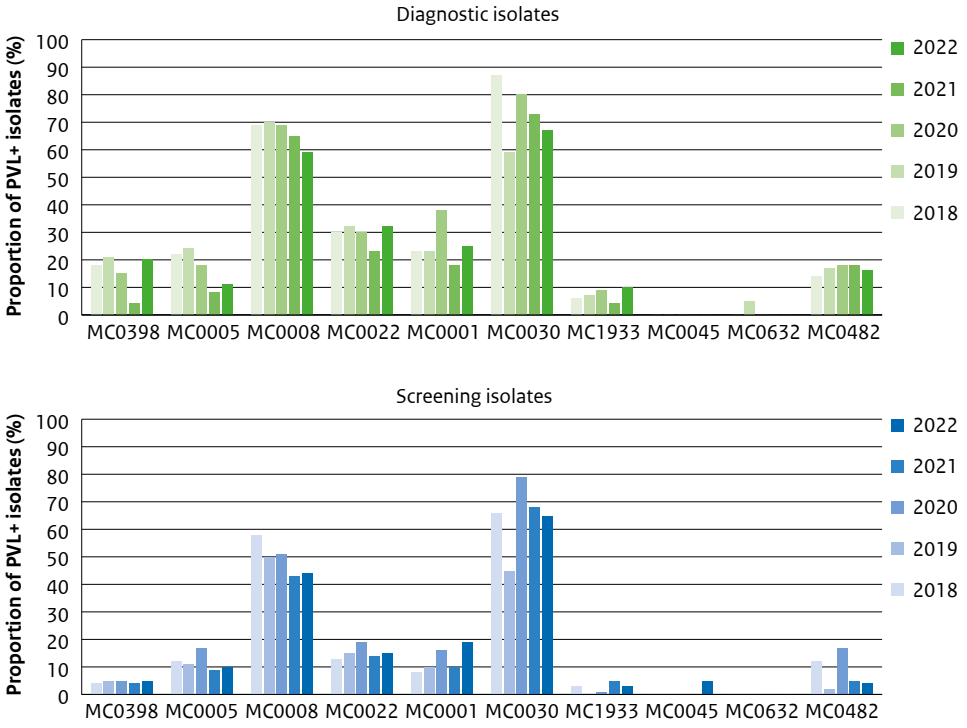


The graph displays the proportion of MLVA-complexes per sampling year.
The first MRSA isolate per person sampled in the period 2018 to 2022 was selected, with the exception that only the first diagnostic isolate is included when both a screening and a diagnostic sample are submitted from the same person.

In 2022, six non-MLVA-typeable isolates, submitted as MRSA, were shown to be *Staphylococcus argenteus*. Of these, five (83%) were screening isolates and one was a diagnostic isolate (17%).

Of the 2,672 MRSA isolates submitted in 2022, 14 isolates (0.5%) contained the *mecC* gene instead of the *mecA* gene, of which ten were diagnostic isolates. Seven hundred twenty-eight isolates (27%) were Panton-Valentine Leukocidin (PVL) positive, which is a higher fraction than in 2021 (21%), but comparable to the years 2018-2020 (respectively 25%, 25% and 28%). Of the 728 PVL positive isolates, 332 (46%) were screening isolates and 391 (54%) were diagnostic isolates. The proportion of PVL positivity was higher in diagnostic isolates (396/1,082; 37%) than in screening isolates (332/1,581; 21%). MC0030 and MC0008 had the highest proportion of PVL positive isolates during the surveillance period 2018-2022 in, both, diagnostic and screening samples (Figure 4.7.3.3). However, during 2019-2022, there has been a trend of decreasing proportion of PVL positive isolates in MC0008.

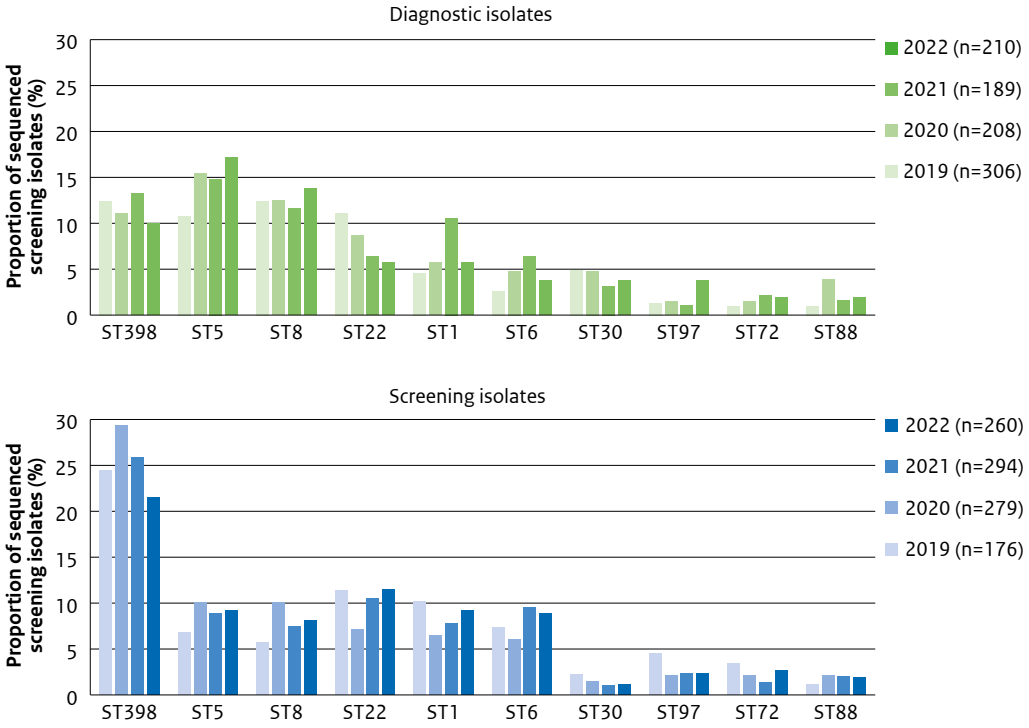
Figure 4.7.3.3 Trends in PVL positivity among the ten most frequently identified MLVA complexes of MRSA in the Netherlands (2018 to 2022), based on enhanced MRSA surveillance data



The graph displays the proportion of PVL-positive isolates per MLVA-complex per sampling year.
The first MRSA isolate per person sampled in the period 2018 to 2022 was selected, with the exception that only the first diagnostic isolate is included when both a screening and a diagnostic sample are submitted from the same person.

In 2022, 474 of the MRSA isolates included for molecular analyses were also analysed through whole-genome sequencing (14-21% of the total number of isolates per year included in the analysis from 2019-2022). In 2022, the isolates belonged to 40 different multi-locus sequence types (STs) of which ST398 was most frequently identified (n= 77; 16%), followed by ST5 (n= 60; 13%) and ST8 (n= 50; 11%). These MLST results are in line with the MLVA-based data (*vide supra*)[1]. The proportion of isolates belonging to ST398 was particularly high in screening isolates (22%, Figure 4.7.3.4). However, during 2020-2022, there has been a trend of decreasing proportion of screening isolates of ST398. Furthermore, a trend of increasing proportion of diagnostic isolates belonging to ST5 and of decreasing proportion of ST22 isolates could be observed between 2019 and 2022 (Figure 4.7.3.4).

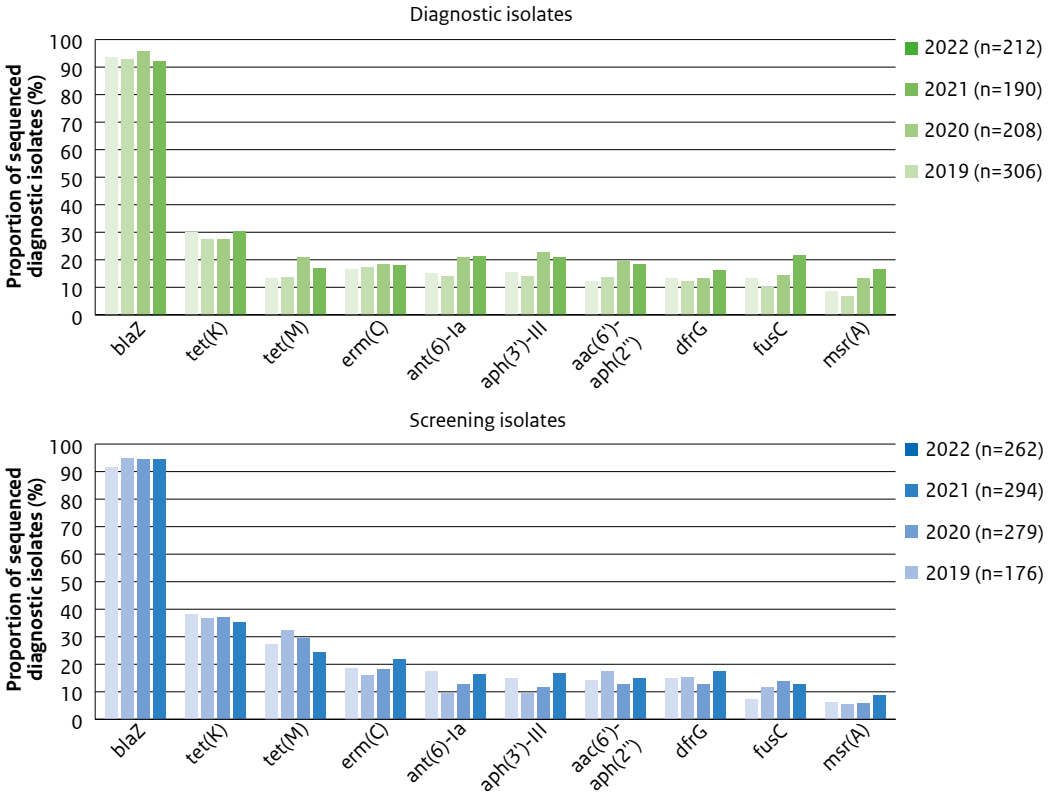
Figure 4.7.3.4 Trends in the ten most frequently identified multi-locus sequence types of MRSA in the Netherlands (2019 to 2022), based on enhanced MRSA surveillance data



The graph displays the proportion of MLST types per sampling year.
MRSA isolates that were sequenced and fulfilled the inclusion criteria for molecular analysis are included.

Besides *mecA*, the antimicrobial resistance genes *blaZ* (encoding β-lactamase; 94%) and *tet(K)* (encoding tetracycline resistance ribosomal protection protein; 33%) were most frequently identified in the genomes of MRSA isolates obtained between 2019 and 2022. The other eight most frequently identified antimicrobial resistance genes in MRSA among diagnostic and screening isolates putatively encode for tetracyclin resistance (*tet(M)*), aminoglycosides (*ant(6)-Ia*, *aph(3')-III*, *aac(6')-aph(2'')*), fusidic acid (*fusC*) and erythromycin and streptogramin B (*msr(A)*), (Figure 4.7.3.5).

Figure 4.7.3.5 The ten most frequently identified antimicrobial resistance genes in MRSA in the Netherlands (2019 to 2022), based on enhanced MRSA surveillance data



The graph displays the proportion of isolates in which the depicted antimicrobial resistance genes were identified per sampling year.
The mecA and mecC genes are not included since their presence was part of the isolate inclusion criteria.
MRSA isolates that were sequenced and fulfilled the inclusion criteria for molecular analysis are included.

Clinical/epidemiological characteristics of persons with MRSA

In 2022, the persons from whom MRSA was cultured had a median age of 42 (range 0 - 101) years and 1,505 (56%) were male. Diagnostic purposes were the reason for sampling in 50% of the persons (1,339/2,672), which is much higher than in the previous four years (increase from 30% in 2018 to 37% in 2021). Screening was the reason for sampling in 45% (1,200/2,672): in 35% (948/2,672) screening was performed because of presumed increased risk for MRSA carriage including active surveillance, and in 9% (252/2,672) because the person was part of a contact tracing investigation. For 5% (n=133) the reason of sampling was unknown. For 34 (1%) persons it was recorded in the questionnaire that they were employees in a healthcare facility that were tested as part of a local screening programme, and for 840, including contacts in a contact tracing investigation, no additional data were available. Therefore, additional epidemiological questionnaire data for 2022, was available for 67% (n=1,798) of the persons.

In Table 4.7.3.2 a selection of the clinical/epidemiological data of included persons is summarised. Seventy percent (1,258/1,798) were sampled in the hospital, of which 45% in outpatient departments, 31% in inpatient departments and 5% during their stay in the Intensive Care Unit (ICU). In the group of persons that were sampled for screening/active surveillance, the large majority met the WIP risk category 1, 2, or 3[2] (94%), whereas in diagnostic isolates this proportion was - as expected - much lower (45%) but increased compared to 39% in 2020, and 43% in 2021. Work-related exposure to livestock animals was reported for 4% of persons with diagnostic samples and 28% of persons with samples that were taken for screening/ active surveillance. The main group of livestock animals to which this group was exposed were pigs (74%), and from 95% of persons with a livestock-related profession a LA-MRSA was sampled. Out of all persons with LA-MRSA, 21% (n=22/103) of persons with diagnostic samples, and 77% (n=134/173) of persons that were sampled for screening/active surveillance, were persons with work related exposure to livestock animals. The number of persons for whom hospitalisation abroad for at least 24 hours during the previous two months was recorded was comparable to 2019 after a drop in 2020-2021 due to travel restrictions during the COVID-19 pandemic (156/1,728 (9%) in 2019, 46/1,058 (4%) in 2020, 64/1,056 (6%) in 2021, and 93/1,112 (8%) in 2022). The main geographic regions of previous hospitalisations in 2022 were Western Asia (including Turkey) with 19%, and Southern and Western Europe both with 18%. Turkey was the country most frequently reported (n=14/93, 15%). In 370/1,071 (35%) persons an underlying illness was reported (underlying illness unknown in 727 persons), with diabetes being the most frequent illness in 106/370 (29%) persons.

Table 4.7.3.2. Epidemiological data of 1,798 MRSA-positive persons (excluding employees of healthcare facilities) with a genotyped isolate in the enhanced MRSA surveillance system, with a sampling date in 2022

Characteristic	Total[a]		Diagnostic		Screening/active surveillance	
	Data available (N)	n (%)	Data available (N)	n (%)	Data available (N)	n (%)
Sample location (hospital only)						
Outpatient departments	1,258	563 (45)	725	322 (44)	517	232 (45)
Inpatient departments (excluding Intensive Care Units)	1,258	388 (31)	725	232 (32)	517	153 (30)
Intensive Care Units	1,258	57 (5)	725	28 (4)	517	29 (6)
Other/unknown	1,258	250 (20)	725	143 (20)	517	103 (20)
Risk factors						
Meeting WIP risk category 1,2, or 3[b]	1,526	1,023 (67)	833	373 (45)	664	627 (94)
Work-related exposure to livestock animals	1,230	170 (14)	710	27 (4)	498	138 (28)
Pigs	170	126 (74)	27	16 (59)	138	106 (77)
Cattle	170	36 (21)	27	10 (37)	138	25 (18)
Other/unknown	170	8 (5)	27	1 (4)	138	7 (5)
Hospitalization abroad >24 hours during the previous two months	1,112	93 (8)	630	18 (3)	471	74 (16)
Western Asia (including Türkiye)	93	18 (19)	18	2 (11)	74	16 (22)
Southern Europe	93	17 (18)	18	3 (17)	74	14 (19)
Western Europe	93	17 (18)	18	1 (6)	74	15 (20)
Other/unknown country	93	41 (44)	18	12 (67)	74	29 (39)
Living in asylum centre	1,645	141 (9)	917	21 (2)	688	117 (17)

WIP: Working Party in Infection Control.

[a] *Including persons for whom the reason for sampling was unknown.*

[b] *WIP risk category 1: the person is known to be MRSA positive; risk category 2: person at high-risk for MRSA carriage; risk category 3: person at low-risk for MRSA carriage; risk category 4: person not suspected of MRSA carriage.*

Discussion

Within the ISIS-AR database, routine culture results from MMLs are collected. However, this can introduce overestimation of resistance percentages due to selective sampling by general practitioners which occurs to a lesser extent in hospital departments. Blood cultures isolates are taken routinely in case of suspected bloodstream infection or meningitis, and, therefore, isolates from blood cultures are considered to be the least biased to calculate resistance percentages. MRSA screening isolates originate from selective cultures for MRSA that do not detect sensitive isolates and cannot be used to calculate the percentage of MRSA among all *S. aureus*. Therefore, we only included diagnostic isolates to assess MRSA prevalence. An increase was found in the proportion of MRSA in ICUs from 2020 onwards. The explanation of this finding is currently unclear. Increased transmission is not a likely explanation since no large clusters were found

based on the molecular data of the enhanced MRSA surveillance nor were large outbreaks in hospitals, including ICUs, reported to the Early warning and response meeting of Healthcare associated Infections and AntiMicrobial Resistance (SO-ZI/AMR) (see also Chapter 4.7.6).
Misclassification of screening and diagnostic isolates might have occurred in the molecular results since distinction between screening and diagnostic isolates is solely based on the material of origin. The most common MLVA-complex found in the enhanced surveillance still is MC0398 (LA-MRSA), which is probably attributable to active screening of MRSA carriage in persons with professional exposure to livestock.

Conclusions

- The overall proportion of routinely collected diagnostic *S. aureus* isolates that were MRSA positive in 2018-2022 was 2%. A proportion of 3% was seen for *S. aureus* isolates that were obtained from material collected by GPs and intensive care units, which is still low compared to other countries[3].
- Gradual shifts occurred in the proportion of isolates belonging to the ten most frequently identified MLVA-complexes and sequence types. Trends varied between screening and diagnostic samples.
- LA-MRSA MC0398 remains the predominant MRSA clade constituting 22% of all screening isolates and 12% of all diagnostic isolates.
- In 2022, 37% of the diagnostic MRSA-isolates carried the PVL-encoding genes, whereas 21% of the screening isolates were PVL-positive. MC0008 and MC0030 isolates had the highest proportion of PVL-positive isolates, but the proportion is decreasing over time in MC0008 isolates.
- The top 10 most occurring antimicrobial resistance genes among diagnostic and screening samples encode for tetracycline, aminoglycoside, fusidic acid and erythromycin and streptogramin B. In 50% of MRSA-positive persons, the samples were obtained for diagnostic reasons and this proportion is increasing over the years.
- The majority of persons with samples that were taken for screening/active surveillance, met WIP-category 1,2, or 3[2] (94%), with the main risk factor being work-related exposure to livestock animals (28%).
- Hospitalisation abroad for at least 24 hours during the previous two months was recorded in 8% of the MRSA positive persons. The main geographic regions of recent hospitalisations abroad of MRSA positive persons were Western Asia (19%), and Southern and Western Europe (both with 18%) in 2022.

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4.7.4 Carbapenem-resistant and carbapenemase-producing *Pseudomonas aeruginosa*

Introduction

Pseudomonas aeruginosa is a common nosocomial pathogen, intrinsically resistant to various broad-spectrum antibiotics. The emergence of multidrug-resistant (MDR) *P. aeruginosa* by acquired resistance mechanisms is a problem of global concern, and in 2017 the World Health Organization classified carbapenem-resistant *P. aeruginosa* as 'priority 1: critical'.[1]

Methods

Data on carbapenem-resistant and carbapenemase-producing *P. aeruginosa* (CPPA) were obtained from two different surveillance systems of the RIVM: ISIS-AR and the national enhanced CPPA surveillance via Type-Ned.

Prevalence of carbapenem-resistant and MDR P. aeruginosa based on ISIS-AR

From the ISIS-AR database, the first *P. aeruginosa* isolate per patient per year was extracted for the period 2018-2022. To avoid overestimation of the percentage carbapenem-resistant *P. aeruginosa* caused by active screening for highly resistant isolates, only data on diagnostic cultures (as categorized by the reporting laboratory) from blood, cerebrospinal fluid, urine, lower respiratory tract, and wound or pus were included in the analysis. Carbapenem resistance was defined as (1) a positive test for carbapenemase (production) and/or (2) phenotypic resistance to meropenem and/or imipenem. The phenotypical tests were reinterpreted according to the 2022 EUCAST breakpoints for meropenem (applying the cut-off of 8 mg/L or 14 mm) and/or imipenem (cut-off 4 mg/L or 20 mm). In addition, the percentage *P. aeruginosa* that was MDR was calculated. Multidrug resistance was defined as resistance to ≥3 antimicrobials or antimicrobial groups among fluoroquinolones, aminoglycosides, carbapenems, ceftazidime, and piperacillin-tazobactam, based on re-interpretation of test-values according to the EUCAST 2022 breakpoints. Only isolates which were tested for all five (groups of) antimicrobials were included in the latter analysis. CPPA strains were also classified as carbapenem-resistant. The numbers were based on a selection of 37 laboratories (out of a total of 51 laboratories in the Netherlands), which provided complete data on the last five years (2018 to 2022).

Molecular characteristics of carbapenemase-producing P. aeruginosa and patient related characteristics based on Type-Ned

Since 2020 the enhanced CPPA surveillance via Type-Ned is implemented. All but one Dutch medical microbiology laboratory (MML) participate (n=50), however, participation is voluntary, and not all laboratories submit all eligible isolates. MMLs are requested to submit *P. aeruginosa* isolates to the RIVM with an MIC for meropenem of >2 mg/L and/or an MIC for imipenem >4 mg/L and/or carbapenemase production and/or a detected carbapenemase-encoding gene. A restriction is that the laboratory can only send the first isolate per person per year. The RIVM allows consecutive isolates from the same person if these are *P. aeruginosa* with other carbapenemase-encoding gene combinations when compared to the first isolate. The RIVM confirms the species by MALDI-ToF, determines the MIC for meropenem by Etest, and detects carbapenemase production by the carbapenem inactivation method (CIM). The presence of carbapenemase-encoding genes are assessed by PCR (carba-PCR on bla_{NDM}, bla_{KPC}, bla_{IMP}, bla_{VIM}, and bla_{OXA}), and next-generation sequencing (NGS) and Nanopore sequencing is performed for all isolates that are CIM positive. The data described in this chapter are based on the first unique CIM-positive *P. aeruginosa* species/carbapenemase-encoding allele combination per person with a person ID for the period 2018-2022, and

all of these isolates collected in 2022 were included. In contrast to the analyses from previous years, samples without a person ID from persons whom had country of origin Ukraine were included for further analysis if it was confirmed that it represented a unique person, based on sex, age and postal code. Other samples without a person ID were excluded. Due to the Ukraine/Russia war, Ukrainian patients migrated to multiple European countries and carried highly resistant microorganisms.[2] Based on whole-genome multi-locus sequence typing (wgMLST), genetically closely related CPPA isolates are grouped in genetic clusters and assigned consecutive cluster numbers. A genetic cluster is defined per bacterial species and includes ≥2 isolates that differ ≤15 wgMLST alleles. Assigning genetic cluster numbers in the surveillance started in 2020, but the genetic cluster numbers in the results of this report include all sequenced *P. aeruginosa* isolates available from (pilot) surveillance studies in the RIVM. Except the first isolate, clusters of multiple isolates from the same patient, including over multiple years and/or submitted by different laboratories, were not counted.
In addition to submitting an isolate, Dutch laboratories are also requested to fill in a clinical/epidemiological questionnaire on characteristics of the patient from whom the CPPA isolate was obtained.

Results

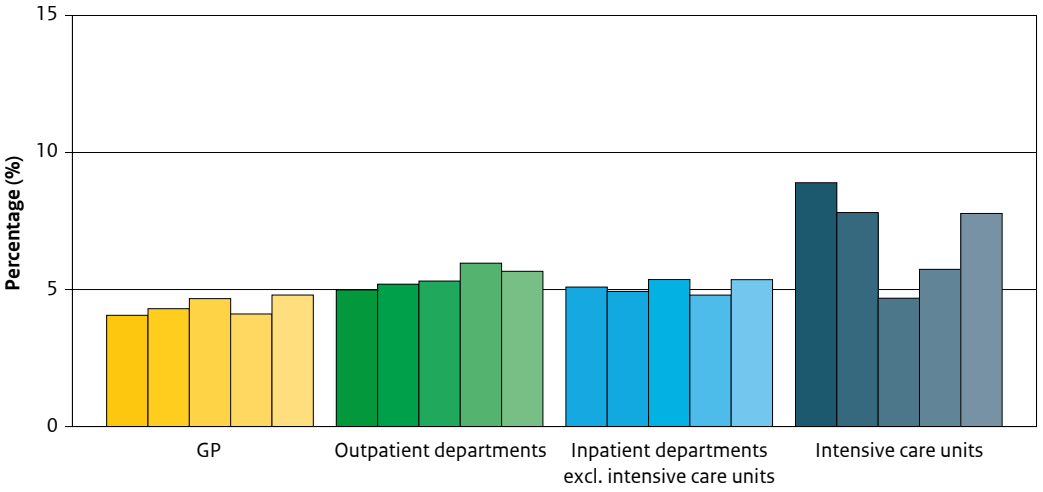
Prevalence of carbapenem-resistant and MDR P. aeruginosa
Based on the ISIS-AR database, 5% (830/15,570) of the diagnostic *P. aeruginosa* isolates were carbapenem-resistant in 2022 (Table 4.7.4.1). The percentage of carbapenem-resistant *P. aeruginosa* isolates was higher among diagnostic samples from intensive care units (ICUs) compared to other departments. The observed proportion of resistance appears to be relatively stable over the 2018-2022 time period, except for a decrease in the proportion of carbapenem resistance in *P. aeruginosa* isolates from ICUs during the COVID-years 2020 and 2021 (Figure 4.7.4.1). Of the total number of 830 carbapenem-resistant *P. aeruginosa* isolates, for 118 (14%) isolates, data on tests for carbapenemase production were available, of which 16 (14%) showed a positive result.

Table 4.7.4.1 Carbapenem-resistant *P. aeruginosa* in the Netherlands in 2022, based on ISIS-AR data

Type of department	Tested isolates, N	Carbapenem-resistant *P. aeruginosa*, N (%)
General practitioners	5,469	263 (5)
Outpatient departments	4,333	246 (6)
Inpatient departments excl. intensive care units	5,306	285 (5)
Intensive care units	462	36 (8)
Total	**15,570**	**830 (5)**

Numbers are based on a selection of 37 laboratories that continuously reported to the ISIS-AR database in the past five years.
The first diagnostic P. aeruginosa isolate per patient was selected.
Carbapenem resistance was defined as (1) a positive test for carbapenemase production and/or (2) phenotypic resistance to meropenem and/or imipenem. The phenotypical tests were reinterpreted according to the 2022 EUCAST breakpoints for meropenem (applying the cut-off of 8 mg/L or 14 mm) and/or imipenem (cut-off 4 mg/L or 20 mm).

Figure 4.7.4.1 Percentages of carbapenem-resistant *P. aeruginosa* in the Netherlands (from left to right 2018 to 2022), based on ISIS-AR data



Numbers are based on a selection of 37 laboratories that continuously reported to the ISIS-AR database in the past five years.
The first diagnostic P. aeruginosa isolate per patient per year was selected.
Carbapenem resistance was defined as (1) a positive test for carbapenemase production and/or (2) phenotypic resistance to meropenem and/or imipenem. The phenotypical tests were reinterpreted according to the 2022 EUCAST breakpoints for meropenem (applying the cut-off of 8 mg/L or 14 mm) and/or imipenem (cut-off 4 mg/L or 20 mm).

Additional analyses in the 2022 ISIS-AR database showed that 2% (n=243) of 15,052 diagnostic *P. aeruginosa* isolates tested for all five (groups of) antimicrobials included in the MDR definition, were MDR (Table 4.7.4.2) and 63% (152/243) of the MDR isolates were carbapenem-resistant.

Table 4.7.4.2 Multidrug resistant (MDR) *P. aeruginosa* in the Netherlands in 2022, based on ISIS-AR data

Type of department	Tested isolates, N	MDR *P. aeruginosa*, N (%)	Carbapenem-resistant MDR *P. aeruginosa*, N (%)
General practitioners	5,381	34 (1)	20 (59)
Outpatient departments	4,146	94 (2)	57 (61)
Inpatient departments excl. intensive care units	5,092	97 (2)	63 (65)
Intensive care units	433	18 (4)	12 (67)
Total	**15,052**	**243 (2)**	**152 (63)**

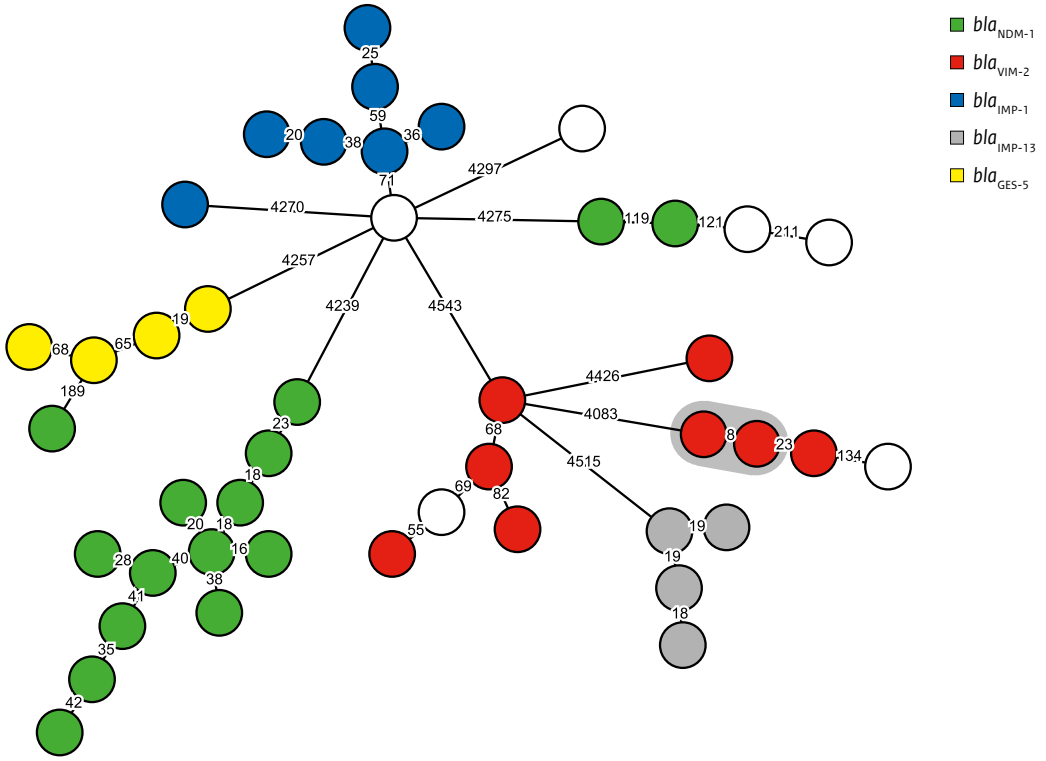
Numbers are based on a selection of 37 laboratories that continuously reported to the ISIS-AR database in the past five years.
The first diagnostic P. aeruginosa isolate per patient was selected.
Multidrug resistance was defined as resistant to ≥3 antimicrobial groups among fluoroquinolones, aminoglycosides, carbapenems, ceftazidime, and piperacillin-tazobactam, based on re-interpretation of test-values according to EUCAST 2022; Carbapenem resistance was defined as (1) a positive test for carbapenemase production and/or (2) phenotypic resistance to meropenem and/or imipenem.
The phenotypical tests were reinterpreted according to the 2022 EUCAST breakpoints for meropenem (applying the cut-off of 8 mg/L or 14 mm) and/or imipenem (cut-off 4 mg/L or 20 mm).

Molecular characteristics of carbapenemase-producing P. aeruginosa

In the enhanced surveillance, the RIVM received 50 *P. aeruginosa* isolates from samples (20 screening and 30 infection-related samples) collected in 2022 through Type-Ned, all of which produced carbapenemase according to the CIM. The CPPA were from 50 patients and these isolates were submitted by 21 MMLs. Twenty of the 50 CPPA isolates were obtained from Ukrainian patients. From 45 of the 50 isolates whole-genome sequencing data was available upon analysis. Analysis of whole-genome sequencing data of 45/50 CPPA isolates revealed that 15 of the 45 carbapenemase-producing isolates (33%) carried a $bla_{\text{NDM-1}}$ allele, eight harboured $bla_{\text{VIM-2}}$ (18%), seven $bla_{\text{IMP-1}}$ (16%), four $bla_{\text{IMP-13}}$ (9%), and four $bla_{\text{GES-5}}$ (9%). Six single isolates carried either $bla_{\text{VIM-1}}$, $bla_{\text{VIM-5}}$, $bla_{\text{IMP-7}}$, $bla_{\text{IMP-10}}$, $bla_{\text{VIM-11}}$, or $bla_{\text{OXA-427}}$ and in one other isolate (2%) no carbapenemase-encoding gene could be identified. Of the twenty CPPA isolates from Ukrainian patients, seven harboured a $bla_{\text{IMP-1}}$ allele, six $bla_{\text{NDM-1}}$, three $bla_{\text{VIM-1}}$ and four isolates carried various carbapenemase alleles.

The genetic relations of the 45 sequenced isolates were assessed by performing wgMLST (Figure 4.7.4.2). This revealed that most of the isolates resided in five distinct groups, according to the carbapenemase allele they harbored. Two Ukrainian patients, whose isolates were submitted by two different MMLs differed in ≤15 wgMLST alleles (8 alleles) and could therefore be regarded as a genetic cluster. Of the CPPA isolates, 92% (46/50) had MICs for meropenem above the clinical EUCAST breakpoint of 8 mg/L (Table 4.7.4.3), whereas one CPPA isolate with a $bla_{\text{VIM-2}}$ allele (2%) had an MIC below the clinical breakpoint. The following sample materials were reported: twenty CPPA were from screening swabs, twelve were from wound/ulcer, eight isolates from pus/punctate/biopsy, four from urine samples and three from sputum/bronchoalveolar lavage fluid. The majority (46/50) of the CPPA were obtained from materials sent in by hospitals.

Figure 4.7.4.2 wgMLST-based minimum spanning tree of 45/50 CPPA isolates from patients sampled in 2022, based on enhanced CPPA surveillance data



Each node represents an isolate, the numbers on the connecting lines indicate allelic distances between isolates. A grey halo indicates ≥2 isolates differing ≤15 wgMLST alleles.

Table 4.7.4.3 Distribution of carbapenemase-encoding genes based on whole-genome sequencing in carbapenemase-producing *P. aeruginosa* isolates received via the enhanced CPPA surveillance in 2022

MIC meropenem (mg/L)	*bla*$_{NDM-1}$	*bla*$_{VIM-2}$	*bla*$_{IMP-1}$	*bla*$_{IMP-13}$	*bla*$_{GES-5}$	*bla*$_{VIM-1}$	*bla*$_{VIM-5}$	*bla*$_{VIM-11}$	*bla*$_{IMP-7}$	*bla*$_{IMP-10}$	*bla*$_{OXA-427}$	Carba gene not found	No WGS	Total
>32	14	5	7	4	4	1	1	1	1	1	1	1	5	46
32	1	1												2
16		1												1
4		1												1
Total	**15**	**8**	**7**	**4**	**4**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**5**	**50**

NB: "No WGS" indicates that whole-genome sequencing sequencing has not been performed.

Clinical/epidemiological characteristics of patients with carbapenemase-producing P. aeruginosa
Clinical/epidemiological questionnaire data in Type-Ned were available for 23 of the 50 CPPA-carrying persons, all of whom were hospital patients. Twenty patients were male and the median age was 45 years (range 2-86 years). Three patients (3/23, 13%) were admitted to the ICU at the moment of sampling, 19 (83%) were admitted to a non-ICU hospital department, and one (4%) sample was taken at the outpatient department.
Twelve questionnaires concerned Ukrainian patients. For 8/12 (67%) of the Ukrainian patients, diagnostics of an infection was mentioned as the reason for taking the sample. Ten of these patients (10/12, 83%) had been admitted >24 hours in a hospital abroad in the previous two months (all in Ukraine and two also in Poland). Two Ukrainian patients were reported to have severe pre-existing underlying internal or neurological medical conditions.
Eleven questionnaires concerned non-Ukrainian patients. In these patients, 4/11 samples (36%) were taken for diagnostic purposes, while for the other seven (64%), the reason for sampling was screening. Five patients (5/11, 45%) had been admitted >24 hours in a hospital abroad in the previous two months, namely two in India, one in Morocco, one in Turkey and one in Kenya and Somalia. Three of the non-Ukrainian patients were admitted to the ICU at the moment of sampling, and six patients (including two of the ICU patients) had (severe) comorbidities, of whom two patients with malignancies.

Discussion

In 2022, in ISIS-AR, 5% of *P. aeruginosa* in diagnostic isolates were resistant to carbapenems. For only 14% of these isolates, data on carbapenemase tests (phenotypically or genotypically) performed by the participating MMLs, were available in the ISIS-AR database. Of the 118 carbapenem-resistant isolates with carbapenemase test results, 16 were positive for carbapenemase production. Not all phenotypically carbapenem-resistant isolates are routinely tested on carbapenemase production or carbapenemase genes in the MMLs (which is in accordance with the Dutch national guideline for detection of highly resistant microorganisms[3]), and such results are also not always routinely included in the data submitted to the surveillance system; therefore, the percentage of carbapenemase-producing *P. aeruginosa* may be an underestimation. The proportion of carbapenem-resistant *P. aeruginosa* in ICUs returned to pre-COVID-19 levels in 2022, after two years in which this proportion was remarkably lower. An important source for acquisition of carbapenemase-carrying *P. aeruginosa* in ICU patients, is contaminated environmental sources or acquisition originating from patient-to-patient transfer.[4] Possibly, intensified hygienic measures in ICUs during the COVID-19-pandemic have decreased the transmission from environment to patients or between patients.
The 2022 results of the enhanced CPPA surveillance submitted via Type-Ned were very different to those of 2021, likely due to migration and medical evacuation of Ukrainian patients to the Netherlands. Forty percent of the CPPA isolates in the enhanced surveillance in 2022 were from samples of Ukrainian patients, thereby introducing $bla_{\text{IMP-1}}$ and $bla_{\text{NDM-1}}$ carrying CPPA leading to a changed molecular epidemiology.
Up until 2021 the most predominant (63%) carbapenemase-encoding allele in CPPA was $bla_{\text{VIM-2}}$, in 2022 this was reduced to 18% and dominated the $bla_{\text{NDM-1}}$ allele in 33% of the isolates. CPPA with $bla_{\text{IMP-1}}$ were only found in Ukrainian patients. CPPA with $bla_{\text{GES-5}}$ were found in 9% of the isolates, however, since this gene is often not included in carbapenemase-PCR gene panels, this gene may be missed. There was a single case in which a carbapenemase allele was not identified; it is likely that the carbapenem resistance was caused by other resistance mechanisms than carbapenemase production.
For more than half of the CPPA positive persons in Type-Ned CPE/CPPA no additional epidemiological data were available. However, based on the available information we can conclude that half of the patients had been hospitalized abroad and one third had severe comorbidities reported.

Unfortunately, it is not yet possible to get a complete overview of carbapenem-resistant and carbapenemase-producing *P. aeruginosa* in the Netherlands, because not all laboratories routinely perform tests for carbapenemase production, and because only a selection of the relevant isolates and data were submitted to one or both of the surveillance systems ISIS-AR and Type-Ned in 2022. Therefore, the data as shown here are most likely an underestimation of the number present in the Netherlands.

Conclusions

- In 2022, in ISIS-AR, 5% of *P. aeruginosa* in diagnostic isolates were resistant to carbapenems. For only 14% of these isolates, information was reported on tests for carbapenemase production; of these, 14% produced carbapenemase. The proportion of carbapenem-resistant *P. aeruginosa* in ICUs returned to pre-COVID-19 levels in 2022, after two years during the COVID-19 pandemic in which this proportion was remarkably lower.
- In 2022, two percent of the total number of *P. aeruginosa* isolates was MDR and 63% of these MDR isolates were carbapenem-resistant.
- Forty percent of the CPPA isolates in the enhanced CPPA surveillance in 2022 were from samples of Ukrainian patients.
- The predominant (33%) carbapenemase-encoding allele in carbapenemase-producing *P. aeruginosa* was bla_{NDM-1} in contrast to 2021 when the dominant carbapenemase encoding allele in CPPA was bla_{VIM-2}.
- Ukrainian patients carried CPPA with bla_{IMP-1} or bla_{NDM-1}, thereby contributing to the changed molecular epidemiology of CPPA in the Netherlands.
- A total of 92% of the carbapenemase-producing *P. aeruginosa* had MICs for meropenem above the EUCAST defined clinical breakpoint (8 mg/L).
- The majority (83%) of the Ukrainian patients with CPPA had been hospitalized in a hospital abroad >24 hours and 67% of the samples were taken for diagnostic purposes, while these proportions were 45% and 36% among non-Ukrainian patients, respectively.
- Data from both ISIS-AR and the enhanced surveillance via Type-Ned could not give a complete overview of carbapenem-resistant and carbapenemase-producing *P. aeruginosa* in the Netherlands, because laboratories did not always routinely perform tests for carbapenemase production (in accordance with the Dutch national guideline), and/or submitted only a selection of the relevant isolates and data to one or both of the surveillance systems.

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4.7.5 Extended spectrum β-lactamases

Introduction

Extended spectrum β-lactamase producing Enterobacterales (ESBL-E) have become a major concern worldwide. The prevalence of ESBL-E carriage has become quite widespread, also in the WHO European Region.[1] Over the last years, the percentage of ESBLs in clinical isolates of Enterobacterales in the Netherlands was estimated using the ISIS-AR database. We here present data from ISIS-AR for *Escherichia coli* and *Klebsiella pneumoniae*.

Methods

Data were extracted from the ISIS-AR database. The percentages of ESBL producing *E. coli* and *K. pneumoniae* were estimated based on positivity of confirmation tests (available >99% of the ESBL positive isolates), or, if data from these tests were lacking, resistance for third generation cephalosporins (cefotaxime/ceftriaxone/ceftazidime) based on EUCAST 2022 clinical breakpoints.

Results

In table 4.7.5.1 and 4.7.5.2 the estimated percentages of ESBL carrying *E. coli* and *K. pneumoniae* are shown by healthcare setting or department, i.e. general practice (GP), outpatient departments, inpatient departments and intensive care units (ICUs), in 2022. In figure 4.7.5.1, trends in ESBL percentages (from left to right 2018 to 2022) among clinical isolates of *E. coli* and *K. pneumoniae* by site are shown. The percentages of ESBL have been stable or have slightly decreased for *E. coli* and *K. pneumoniae* from 2018 to 2022 in most healthcare settings. In contrast, since 2020 the ESBL percentage for *K. pneumoniae* in ICUs increased from around 12 to 15%.

Table 4.7.5.1 Extended spectrum β-lactamase (ESBL) producing *E. coli* in the Netherlands in 2022, based on ISIS-AR data

Type of department	Tested isolates, N	ESBL, number (%)[1]
General practitioners	113,151	3,930 (3)
Outpatient departments	19,278	1,000 (5)
Inpatient departments excl. intensive care units	27,317	1,563 (6)
Intensive care units	1,093	69 (6)
Total	**160,839**	**6,562 (4)**

Numbers are based on a selection of 37 laboratories that continuously reported to the ISIS-AR database in the past five years. The first diagnostic E. coli isolate per patient was selected.

[1] *The percentage of ESBL producing E. coli was estimated based on positivity of ESBL confirmatory tests, or, if no data on confirmatory tests were available, by resistance to cefotaxime/ceftriaxone (according to a cut-off of 1 mg/L for both cefotaxime or ceftriaxone or 20 mm for cefotaxime and 25 mm for ceftriaxone) and/or ceftazidime, based on re-interpretation of testvalues according to EUCAST 2022.*

Table 4.7.5.2 Extended spectrum β-lactamase (ESBL) producing *K. pneumoniae* in the Netherlands in 2022, based on ISIS-AR data

Type of department	Tested isolates, N	ESBL, number (%)[1]
General practitioners	15,632	538 (3)
Outpatient departments	4,312	248 (6)
Inpatient departments excl. intensive care units	5,705	462 (8)
Intensive care units	328	48 (15)
Total	**25,977**	**1,296 (5)**

Numbers are based on a selection of 37 laboratories that continuously reported to the ISIS-AR database in the past five years. The first diagnostic K. pneumoniae isolate per patient was selected.

[1] *The percentage of ESBL producing K. pneumoniae was estimated based on positivity of ESBL confirmatory tests, or, if no data on confirmatory tests were available, by resistance to cefotaxime/ceftriaxone (according to a cut-off of 1 mg/L for both cefotaxime or ceftriaxone or 20 mm for cefotaxime and 25 mm for ceftriaxone) and/or ceftazidime, based on re-interpretation of testvalues according to EUCAST 2022.*

Figure 4.7.5.1 Trends in extended spectrum β-lactamase (ESBL) producing *E. coli* and *K. pneumoniae* in the Netherlands (from left to right 2018 to 2022), based on ISIS-AR data

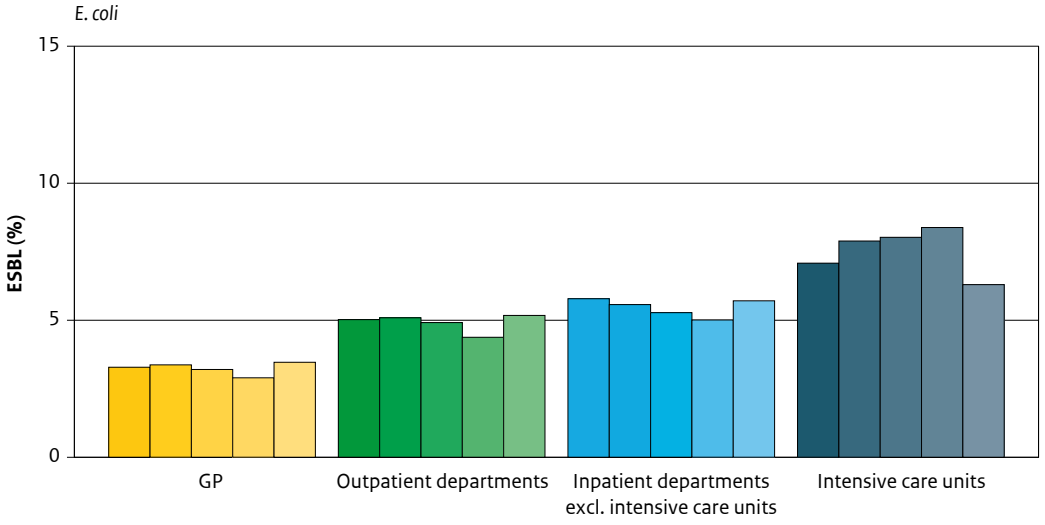
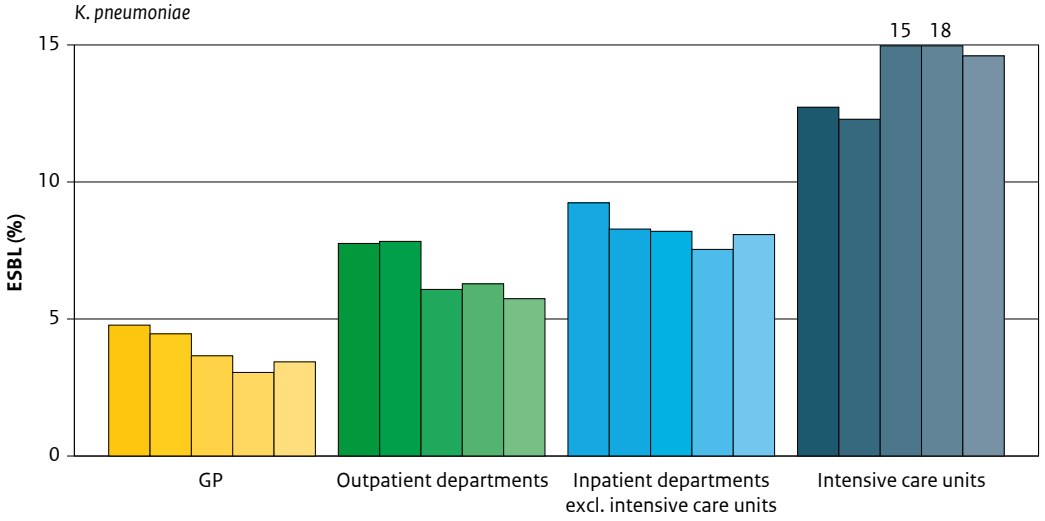


Figure 4.7.5.1 (continued) Trends in extended spectrum β-lactamase (ESBL) producing *E. coli* and *K. pneumoniae* in the Netherlands (from left to right 2018 to 2022), based on ISIS-AR data



Numbers are based on a selection of 37 laboratories that continuously reported to the ISIS-AR database in the past five years. The first diagnostic E. coli and K. pneumoniae isolate per patient per year was selected.

[1] *The percentage of ESBL producing E. coli and K. pneumoniae was estimated based on positivity of ESBL confirmatory tests, or, if no data on confirmatory tests were available, by resistance to cefotaxime/ceftriaxone (according to a cut-off of 1 mg/L for both cefotaxime or ceftriaxone or 20 mm for cefotaxime and 25 mm for ceftriaxone) and/or ceftazidime, based on re-interpretation of testvalues according to EUCAST 2022.*

Discussion

Over the past 5 years, the proportion of ESBL-producing *E. coli* was at a stable level for GP, out- and in-patients. In the ICUs, the ESBL-producing *E. coli* proportion returned to the level of before the COVID-19 pandemic. Among the patients that visited the GP, proportions of ESBL-producing *E. coli* remained low at 3-4%. The average proportion of ESBL-producing *E. coli* carriage in the community in Europe is 6%, whereas globally this average rate is 16.5%.[3]

After 2019, there was an increase of the proportion of ESBL-producing *K. pneumoniae* in ICUs, presumably related to the presence of a higher number of patients receiving antibiotics, with longer length of stays during the COVID-19 pandemic. This proportion reached a top of 18% in 2021, and was slightly lower in 2022. In the outpatient departments and community, the pattern of proportions of ESBL-producing *K. pneumoniae* appeared to be reverse, with a decrease during the COVID-19 years, presumably as a result of social distancing, increased hygiene and hand washing measures, showing the lowest proportion in 2021. This drop in proportion was not clearly noticed in *E. coli*.

The trends in proportions of ESBLs could be influenced by changes in detection methods. In the end of 2021, the revised guideline for detection of highly resistant microorganisms was published.[4] This guideline recommends the use of enrichment broths for detection of ESBL carriage, because this increases the sensitivity of the cultures substantially.[5-7] The true impact of this change in detection methods on the proportions of ESBL in Enterobacterales will become more clear as data are collected over a longer period of time.

Conclusions

- From 2018 to 2022, the proportion of ESBL *E. coli* was stable in general practice (GP), outpatient departments and inpatient departments. In the ICUs the proportion increased until 2021, and dropped in 2022.
- After 2019, there was an increase of the proportion of ESBL-producing *K. pneumoniae* in ICUs, which stabilized in 2021, presumably related to the presence of a higher number of patients using antibiotics, with longer lengths of stay during the COVID-19 pandemic. These trends could also have been influenced by changes in detection methods.

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4.7.6 Early warning and response meeting for Healthcare associated Infections and AntiMicrobial Resistance (SO-ZI/AMR)

Introduction

In 2012, the Early warning and response meeting for Hospital-acquired Infections and AntiMicrobial Resistance (SO-ZI/AMR) was founded. The initial purpose of the SO-ZI/AMR is to mitigate large-scale outbreaks of AMR in hospitals and to prevent spread to other health care facilities through early warning and reporting. Since 2015 long-term care facilities (LTCFs) are also invited to report outbreaks of highly-resistant microorganisms (HRMO). Since then, the name of the early warning and response meeting was changed to Healthcare associated Infections and AntiMicrobial Resistance (SO-ZI/AMR).
The SO-ZI/AMR consists of experts in the field of clinical microbiology, infection prevention, elderly care and public health and meets once a month. The SO-ZI/AMR assesses the risk of the outbreak to public health, monitors the course of the outbreak and facilitates – on request of the hospital or LTCF – in the acquisition of external expertise. An overview of active outbreaks is reported to professionals involved in infection prevention on a monthly basis.
Notifications are voluntary. All hospitals and LTCFs have committed to participate in SO-ZI/AMR. In 2017, a financial compensation rule was introduced to compensate for detection and control of HRMO outbreaks in LTCF, provided that these outbreaks are reported to the SO-ZI/AMR.[1]

Methods

Until April 2022, health care facilities reported outbreaks using a standardized web-based form to RIVM or NVMM (the Dutch Society of Medical Microbiology), where the information was copied into one database at the RIVM. Monthly updates were provided by institutions until the outbreak was considered ended. Since April 2022, notifications and monthly updates of outbreaks were submitted through a newly developed web-based application Osiris. In addition, the criteria for reporting an outbreak were adapted to make them more unambiguous and to facilitate a more objective interpretation. Simultaneously, a new method of transmission risk assessment during the monthly meetings was introduced, according to which each outbreak was categorized in one of four phases with 0 as lowest, III as highest risk. Once an outbreak is contained, it is classified as phase 0. Otherwise, the categories are: Phase I: transmission is under control, all necessary information on the situation is available; phase II: active transmission cannot be ruled out, active contact screening is still ongoing; phase III: transmission is ongoing in spite of infection control measures, which are thus not effective or incomplete. In monthly meetings, the SO-ZI/AMR assesses the phase of each notified outbreak which has not yet been classified as phase 0.

Results

Table 4.7.6.1 provides an overview of the 36 outbreaks reported in 2022. These were reported by 29 different healthcare institutions: 26 outbreaks in hospitals, 10 in LTCFs, including one in a home care organization. Most outbreaks (n=19) ended in 2022 and 17 ended in 2023. The median number of patients involved in outbreaks in hospitals (6) was lower than in 2021 when this number was 12, but comparable to 2020 and 2019 (4 in both years). In LTCFs the median number was 3. The maximum number of involved patients was much higher in hospitals compared to LTCF (61 vs 22).
In hospitals, vancomycin-resistant *Enterococcus faecium* (VRE) outbreaks were most often reported. Furthermore, 5 methicillin-resistant *Staphylococcus aureus* (MRSA) outbreaks were reported in hospitals, and 5 outbreaks with carbapenemase-producing (CP) gram-negatives (3 outbreaks with CP Enterobacterales and 2 with CP non-fermenting gram-negative bacteria).

In LTCFs MRSA was most often reported. The number of 8 MRSA outbreaks in LTCFs was higher than the number in 2021, but still lower than the previous years (11 in 2020, 17 in 2019). Eight of the 36 reported outbreaks included more than 10 patients.
Four outbreaks were classified as phase III. One was an extensive, long-lasting MRSA outbreak in a nursing home which was notified in April 2022. Retrospectively, the outbreak had already started in October 2021. Various closely related MLVA types were involved in this outbreak, and transmission occurred throughout multiple departments within the healthcare institute. In total, 22 clients and nine healthcare workers were found positive with one of the outbreak strains. The outbreak was considered to be contained in June 2023 and had a duration of more than 600 days.
Another outbreak that was classified as phase III was an outbreak of ESBL-producing multi-resistant (fluoroquinolone- and aminoglycoside-resistant) *K. pneumoniae* in a hospital. It was notified in July 2022, but had already started in September 2021 retrospectively, and the outbreak was still ongoing in June 2023. Again, various strains were detected in this outbreak, with at least five different multi-locus sequence types of *K. pneumoniae*. Multiple clinical departments were involved, including the intensive care unit, but most patients were identified in the surgical ward. Since there were clear indications that the sinks were involved in transmission of the pathogen (several environmental samples of the sinks were found to be positive for the outbreak strains), construction works were planned and the effects can only be determined after the improvements are finished. Since the beginning of 2022 until June 2023, a total number of 61 patients were positive with one of the outbreak strains.
Finally, the other two outbreaks which were classified in phase III were VRE outbreaks in hospitals, with 31 and 33 VRE-positive patients involved.

Table 4.7.6.1 Characteristics of outbreaks reported to the SO-ZI/AMR in 2022

	Hospitals (n=26) n (%)	LTCFs[4] (n=10) n (%)	Total 2022 (n=36) n (%)
Microorganism (resistance mechanism)[1]			
Enterococcus faecium (VRE)	9		9
Staphylococcus aureus (MRSA)	5	8	13
Sarcoptes scabiei	1	1	2
Enterobacterales (CPE) (various species)	3		3
Acinetobacter (CPAB)	1		1
Citrobacter freundii (ESBL)		1	1
Klebsiella pneumoniae (ESBL)	2		2
Escherichia coli (ESBL)	1		1
Pseudomonas aeruginosa (CPPA)	1		1
Streptococcus pyogenes	1		1
Parainfluenzavirus type 3	1		1
Norovirus	1		1
Highest level phase[2]			
phase 0		1	1
phase I	3	2	5
phase II	13	6	19
phase III	3	1	4
Median number of patients[3] (range)	6 (2-61)	3 (1-22)	4 (1-61)
Median duration outbreak in days from start or reporting date until end of the outbreak (range)	93 (17-636)	57 (27-620)	90 (17-636)

n: number of outbreaks

1 *VRE=vancomycin-resistant Enterococcus faecium; MRSA=methicillin-resistant Staphylococcus aureus; CPE=carbapenemase-producing Enterobacterales; CPAB=carbapenemase-producing Acinetobacter baumannii complex; ESBL=extended-spectrum β-lactamase producing; CPPA=carbapenemase-producing Pseudomonas aeruginosa.*

2 *Only available for notifications since April 2022 (hospitals n=19, LTCF n=10), when new method of risk assessment was introduced. Outbreaks are categorized in one of four phases with 0 as lowest, III as highest risk. Once an outbreak is contained it is classified as phase 0. Phase I: transmission is under control, all necessary information on the situation is available, phase II: active transmission cannot be ruled out, active contact screening is still ongoing, phase III: transmission is ongoing in spite of infection control measures, which are thus not completely effective.*

3 *In two outbreaks, one patient and multiple health care workers were involved.*

4 *Including one home care organisation.*

Discussion

The total number of 36 outbreaks in 2022 was higher than the number in 2021 (27), more comparable to 2020, but still remarkably lower than in 2017-2019, when around 60 outbreaks were reported each year. The lower number of outbreaks in 2022 might still be related to the COVID-19 pandemic which continued until the beginning of 2022. A lower number of healthcare-associated outbreaks during the COVID-19 pandemic could be attributed to various factors, such as downscaling of provided regular healthcare in hospitals and an intensified infection prevention policy both in hospitals and LTCF. On the other hand, it cannot be ruled out that a higher number of outbreaks did happen in healthcare facilities which have not been reported to SO-ZI/AMR, possibly because of diminished capacity for reporting outbreaks.
In 2022, some changes in the SO-ZI/AMR were implemented, concerning the criteria for reporting an outbreak and a renewed classification method, and a simplified web-based reporting system was introduced.[2] This should be taken into account when analyzing trends, since the newly formulated reporting criteria might lead to a change in outbreak notifications because of altered interpretation by the healthcare institutes. Furthermore, the collected data could differ slightly depending on the reporting system used. Still, the modifications to SO-ZI/AMR are expected to result in improved surveillance and overview of the ongoing outbreaks in healthcare institutes in the future, leading to more timely insight in and control of potential public health threats for the Netherlands.

Conclusions

- On average three outbreaks a month were reported to the SO-ZI/AMR in 2022, which is still lower compared to the pre-COVID-19 era, but higher compared to 2021.
- Most outbreaks were classified maximally as phase II and three hospital outbreaks and one LTCF outbreak as phase III.
- Most outbreaks were due to MRSA, of which the majority were reported by LTCFs. VRE was the most frequent cause of notified HRMO outbreaks in hospitals.
- The median number of patients involved in an outbreak was 4, which was considerably lower compared to a median of 10 patients in 2021.
- Modifications to the SO-ZI/AMR workflow introduced in 2022 will improve the surveillance and overview of healthcare-associated HRMO outbreaks.

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4.8 Resistance in specific pathogens

4.8.1 *Neisseria meningitidis*

Introduction

Neisseria meningitidis isolates cultured from cerebrospinal fluid (CSF) and/or blood in microbiological laboratories in the Netherlands are submitted for serogrouping and susceptibility testing to the Netherlands Reference Laboratory for Bacterial Meningitis (NRLBM) at the Amsterdam UMC. For *N. meningitidis*, the EUCAST criteria for phenotypic penicillin susceptibility testing have been altered as of 1-1-2021 with a single breakpoint defining susceptible (≤ 0.25 mg/L) or resistant strains (> 0.25 mg/L).

Methods

From 2013-2022, 1,183 invasive meningococcal disease cases were registered, including culture-negative PCR-positive cases. In 29 cases, a positive blood culture was available for patients with a culture-negative but PCR-positive CSF-sample, which could be used for assessment of penicillin resistance. In total, 1,037 isolates were available for the study period; 317 isolates from patients with culture-positive CSF-sample or a positive blood culture in combination with a PCR-positive CSF-sample, and 720 isolates from blood culture. The number of meningococcal isolates per year in this period ranged between 25 isolates (2021) and 186 isolates (2018). The MIC for penicillin was determined by Etest using Mueller-Hinton Fastidious Agar (MHF) plates and incubation at 37°C under 5% CO_2 for 18-24 h. EUCAST criteria for resistance were applied. In case of resistance to penicillin, susceptibility to ceftriaxone was also assessed by Etest using MHF plates and incubation at 37°C under 5% CO_2 for 18-24 h. All isolates were tested for rifampicin resistance by plating on MH chocolate agar plates containing rifampicin (0.25 µg/ml).

Results

In 2022, all received meningococcal isolates (n=56), whether isolated from CSF or blood, were susceptible to penicillin, which is similar to data from 2021 (tables 4.8.1.1 and 4.8.1.2). Overall, only 7 out of 1,037 (0.7%) isolates displayed phenotypic penicillin resistance in the previous 10 years. None of the 2022 isolates was resistant to rifampicin.

Discussion

The breakpoint for phenotypic penicillin susceptibility was altered by EUCAST in 2021, whereby only 2 categories remain; isolates are either susceptible or resistant to penicillin. It is clear that penicillin resistance in *N. meningitidis* isolates in the Netherlands is rare, whether applying the former or current EUCAST criteria for penicillin susceptibility.

Table 4.8.1.1 Susceptibility of *N. meningitidis* isolated from CSF or CSF and blood to penicillin, 2013-2022

	Penicillin				
	MIC* ≤ 0.25		MIC* > 0.25		Total
	n	%	n	%	
2013	41	**98**	1	**2**	42
2014	33	**100**	0	**0**	33
2015	32	**100**	0	**0**	32
2016	36	**100**	0	**0**	36
2017	46	**100**	0	**0**	46
2018	54	**98**	1	**2**	55
2019	33	**100**	0	**0**	33
2020	14	**93**	1	**7**	15
2021	7	**100**	0	**0**	7
2022	18	**100**	0	**0**	18

* *MIC values in mg/L.*

Table 4.8.1.2 Susceptibility of *N. meningitidis* isolated from blood only to penicillin, 2013-2022

	Penicillin				
	MIC* ≤ 0.25		MIC* > 0.25		Total
	n	%	n	%	
2013	69	**99**	1	**1**	70
2014	40	**100**	0	**0**	40
2015	52	**100**	0	**0**	52
2016	101	**100**	0	**0**	101
2017	128	**99**	1	**1**	129
2018	129	**99**	2	**1**	131
2019	102	**100**	0	**0**	102
2020	39	**100**	0	**0**	39
2021	18	**100**	0	**0**	18
2022	38	**100**	0	**0**	38

* *MIC values in mg/L.*

Conclusions

- Number of invasive meningococcal disease isolates decreased by 60% in 2022 compared to 2019 (pre-COVID-19) but more than doubled compared to 2021.
- Penicillin and rifampicin resistance in *N. meningitidis* isolates is rare in the Netherlands.

4.8.2 *Neisseria gonorrhoeae*

Introduction

Neisseria gonorrhoeae is a species of Gram-negative bacteria which can cause gonorrhoea after sexual transmission. Gonorrhoea is the second most common bacterial sexually transmitted infection (STI) in the Netherlands. It can result in severe reproductive complications and can increase the transmission of HIV. Third generation cephalosporins, such as ceftriaxone and cefixime, are the current first-line treatment for gonorrhoea in most countries. In the Netherlands, cefotaxime was the first-line therapy for gonorrhoea from 2003-2006, and ceftriaxone from 2006 onwards. In the past, *N. gonorrhoeae* has developed antimicrobial resistance to all drugs used for treatment of gonorrhoea. While resistance to ceftriaxone has been reported in Europe only incidentally, resistance levels in the Asian-Pacific region surpass 5% in several countries.[1]

Methods

The national Gonococcal Resistance to Antimicrobials Surveillance (GRAS) programme started in 2006, collecting epidemiological data on gonorrhoea and resistance patterns of isolated strains from Sexual Health Centres (SHC) across the Netherlands. In 2022, 16 out of the 24 SHC participated in GRAS, which together accounted for 86% of SHC gonorrhoea diagnoses. Diagnosis of gonorrhoea is made by PCR on patients' materials. For GRAS, additional culture and susceptibility testing using Etest, are performed. The aim is to perform culture and susceptibility testing for all gonorrhoea patients in these SHC, but due to logistical and financial restrictions in practice there is a culture performed for around 75% of patients. Isolates are tested for susceptibility to ciprofloxacin, cefotaxime, ceftriaxone, and azithromycin. Resistance levels are calculated using the EUCAST breakpoints for resistance.[2]

Results

The number of gonorrhoea diagnoses reported by SHC participating in GRAS ranged between 5,000 and 6,000 in the years 2015 to 2020, and increased since then to 9,131 diagnoses in 2022. The percentage of diagnoses including a susceptibility test remained stable around 39% since 2016 (39.3% in 2022, Figure 4.8.2.1).

Gonococcal resistance to ciprofloxacin fluctuated around 30% between 2012 and 2018 but increased in the past few years and was 61.5% in 2022. Resistance to cefotaxime has been slowly decreasing since 2012 and was 0.1% in 2022. For azithromycin, resistance increased from 2.1% in 2012 to 26.6% in 2022. No resistance was reported to ceftriaxone (Figure 4.8.2.2). In the MIC distribution of ceftriaxone a shift was observed in 2019 where the proportion of isolates with an MIC ≤ 0.002 mg/L decreased and the proportion of isolates with slightly higher MIC values (MIC 0.008-0.016 mg/L) increased (Figure 4.8.2.3a). This continued until 2022. For azithromycin a shift towards higher MICs is observed over time (Figure 4.8.2.3b).

Figure 4.8.2.1 Number of reported gonorrhoea diagnoses and number and percentage of diagnoses including an antimicrobial susceptibility test at Sexual Health Centres participating in GRAS, 2013-2022

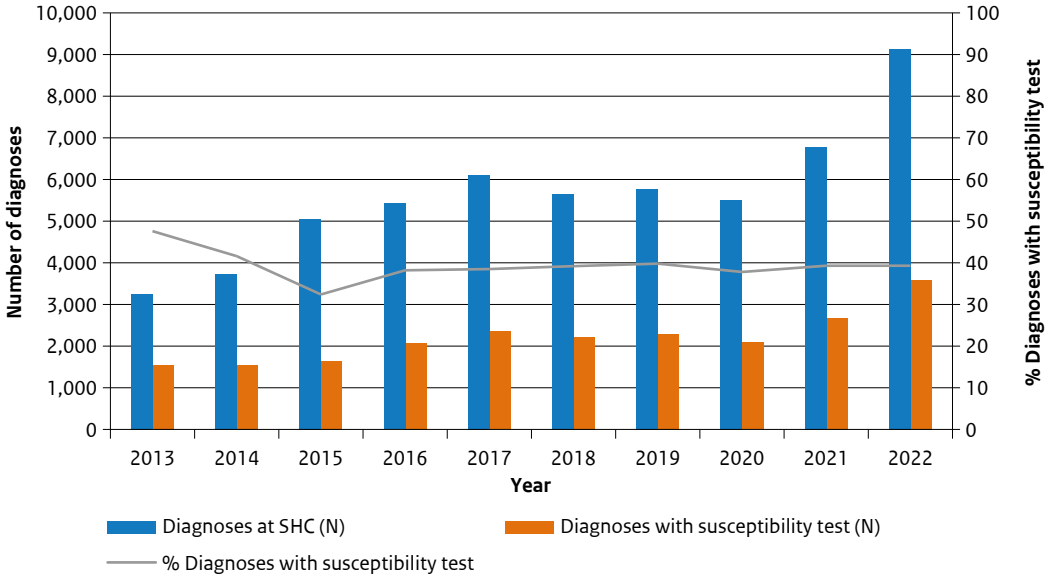
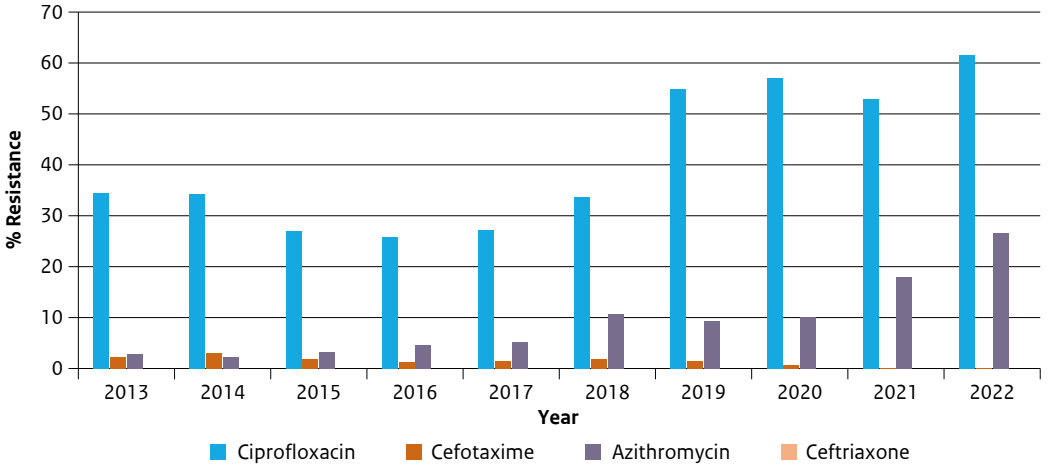


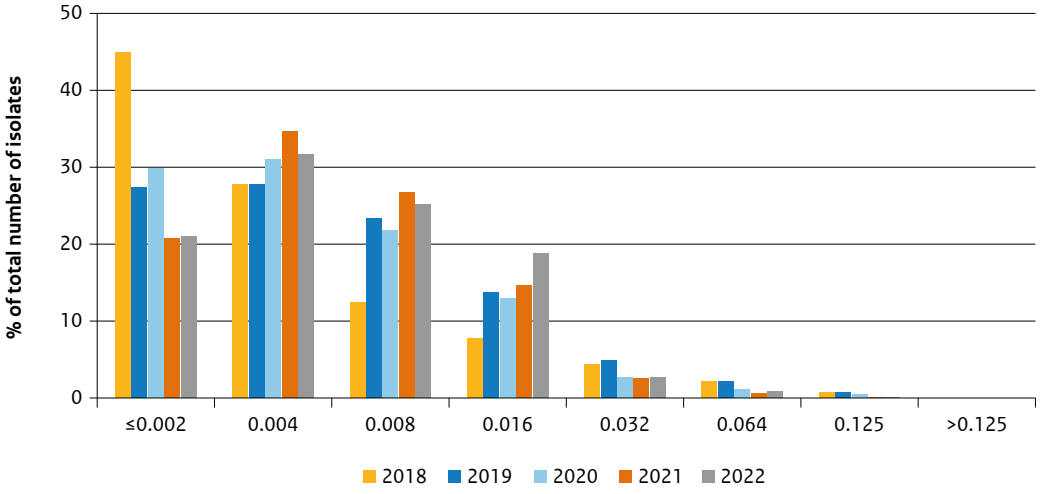
Figure 4.8.2.2 Trends in antimicrobial resistance among *Neisseria gonorrhoeae* (following EUCAST breakpoints) in the Netherlands, 2013-2022



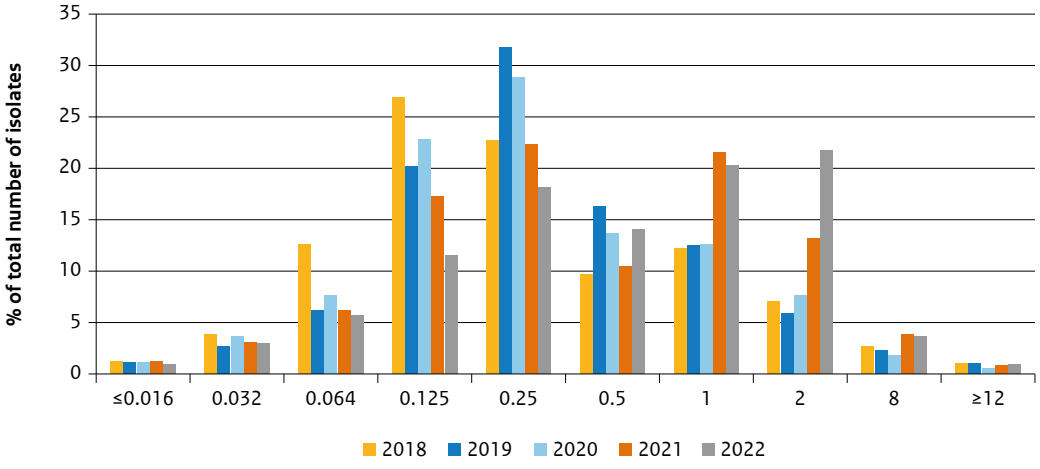
No resistance to ceftriaxone has been reported.

Figure 4.8.2.3 MIC distributions of ceftriaxone and azithromycin for *Neisseria gonorrhoeae*, 2018-2022

a. MIC distribution for ceftriaxone. Following EUCAST breakpoints, an MIC of >0.125 mg/L is considered resistant



b. MIC distribution for azithromycin. Following EUCAST breakpoints, an MIC of >1 mg/L is considered the epidemiological cut-off value for resistance



Discussion

In 2022 in less than half (39.3%) of all gonorrhoea diagnoses at the SHCs participating in GRAS susceptibility testing was performed. This low number can partially be explained by a large proportion of diagnoses being culture negative and/or only based on PCR, making susceptibility testing impossible. The number of gonorrhoea diagnoses increased greatly in 2022 compared with 2021; from 6,784 to 9,131 within SHC participating in GRAS. This increase in diagnoses was mainly due to increased gonorrhoea positivity rates among heterosexuals, and especially among women.[3] This is also reflected in the distribution of isolates between sexes: in 2021, 85% of isolates were from men who have sex with men (MSM), 7% from heterosexual men and 7% from women. In 2022, 78% of isolates were from MSM, 9% from heterosexual men and 12% from women. In the Netherlands, the recommended treatment for gonorrhoea is a single injection with ceftriaxone (500mg). Thus far, no ceftriaxone resistance has been reported. Yet, trends of decreasing susceptibility have been observed for multiple antimicrobial agents monitored in GRAS. This calls for a continued effort to monitor trends and emergence of antimicrobial resistance in gonococci.

Conclusions

- The execution of GRAS is very stable over time, with around 39% of diagnoses including susceptibility testing results within SHCs participating in GRAS per year.
- No resistance to ceftriaxone, the current first-line treatment for gonorrhoea, has been reported. However, the MIC distribution has shifted towards higher MICs since 2019.
- Resistance to ciprofloxacin yearly increases and more than doubled since 2016, to 61.5% in 2022, despite the fact that ciprofloxacin is not prescribed for gonorrhoea, according to guidelines.
- MIC values for azithromycin continue to increase after a stable period between 2018-2020.

References

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4.8.3 *Mycobacterium tuberculosis*

Introduction

Of all infectious diseases, tuberculosis (TB) remains one of the deadliest infectious diseases worldwide. Although the incidence is slowly declining globally, it has been estimated that about one third of the global population is still latently infected by its main causative agent; *Mycobacterium tuberculosis*. In the Netherlands we have reached the elimination phase in the native population; more than 75% of the TB cases currently diagnosed are in foreign-born persons. Compared to 2021 the total number of notified TB cases in 2022 declined by 6%, to 635 cases. This follows an increase of 10% to 673 cases in 2021 vs 2020. These dynamics are most likely related to the COVID-19 pandemic and reflect a combination of reduced immigration, less transmission and delayed diagnosis.

Worldwide, there is a concern about the development of resistance, which hampers adequate treatment of tuberculosis. After the initial diagnosis at peripheral and regional laboratories, resistance testing, species identification and the typing of *M. tuberculosis* isolates in the Netherlands is performed at the RIVM and the results are used to guide the therapy of individual patients, as well as investigations of transmission and surveillance. The RIVM participates in the proficiency studies of the WHO for international TB reference laboratories to monitor the quality of the resistance testing.

Methods

Around 30 laboratories in the Netherlands are involved in the diagnosis of TB and send all *M. tuberculosis* isolates to the RIVM for epidemiological typing to support TB transmission investigations performed by Municipal Health Services. Before 2020, the resistance testing was based on the phenotypic approach using the Mycobacteria Growth Indicator Tubes (MGIT). From 2020 on, all isolates are initially screened by WGS (Whole Genome Sequencing) for the presence of resistance mutations in the nine major resistance genes in *M. tuberculosis*. In absence of such mutations, isolates are determined to be susceptible for standard first line therapy and no further testing is performed. If resistance mutations are detected, phenotypic resistance testing is performed.[1,2,3,4] As injectables are no longer part of the TB treatment regimen, since 2020 we no longer predict resistance against streptomycin. From 2020 onwards, we also monitor resistance to pyrazinamide, for which the combination of the results of WGS and phenotypic testing yield more reliable predictions than phenotypic testing alone. Comparisons of molecular and phenotypic resistance testing have been described by Jajou *et al*[1] and Walker *et al*[2]. These studies form the basis of the current testing algorithm.[4]

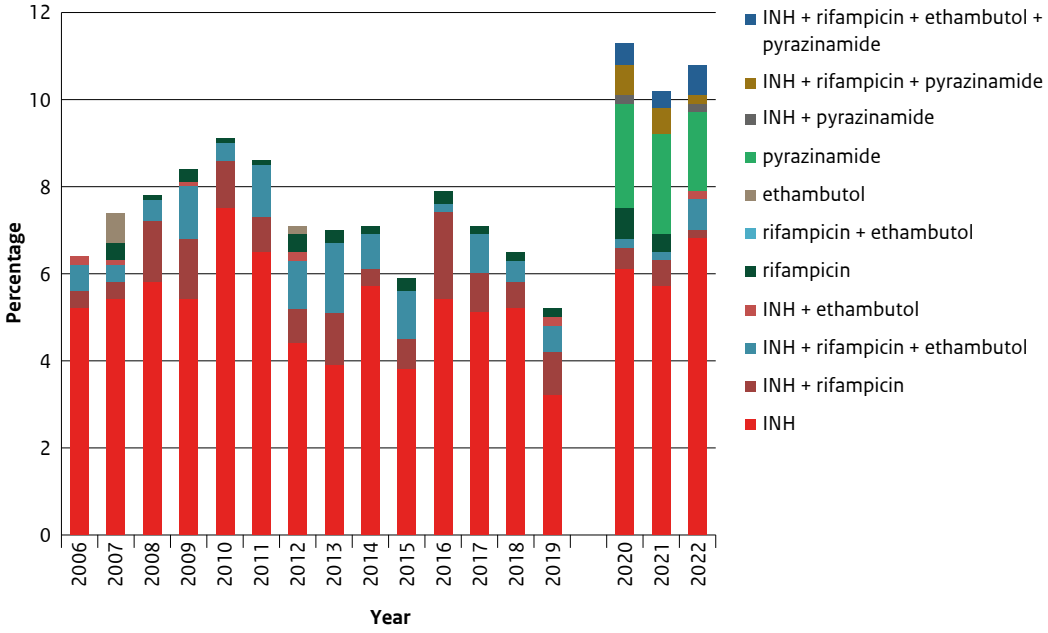
Results

In 2022, of the 635 notified cases, 441 (69%) represented bacteriologically confirmed cases. Isolates from these confirmed cases were received at the RIVM.

Any form of resistance was detected in 10.9% (48/441) of the isolates tested. In total 8 multidrug-resistant (MDR)-TB cases (defined as resistant to at least isoniazid (INH) and rifampicin) were detected. These observations were initially based on the detection of resistance mutations in WGS data and confirmed by phenotypic resistance testing.

MDR cases represented 1.8% (8/441) of the cases in 2022, which is a small decrease compared to the combined MDR and RR (rifampicin resistant) resistance in 2021 (2.3%).

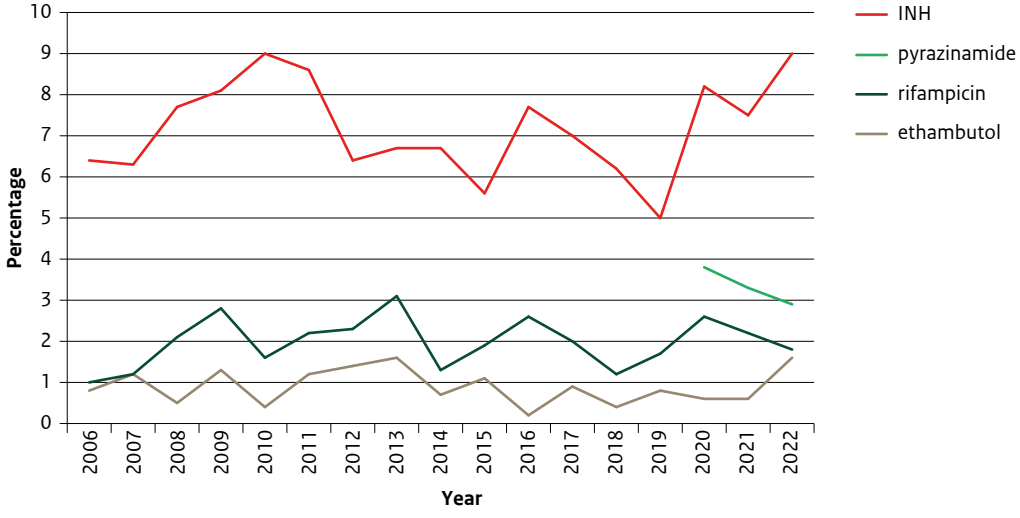
Figure 4.8.3.1 Percentage combined antibiotic resistance for *M. tuberculosis* complex isolates 2006-2022*



INH = Isoniazid

** From 2020 the primary screen for resistance was based on WGS rather than phenotypic testing.[4] Prior to 2020 resistance to pyrazinamide was not monitored as the reliability is much higher for this drug when WGS data is used to support the determination.*

Figure 4.8.3.2 Percentage antibiotic resistance for *M. tuberculosis* complex isolates 2006-2022



INH = Isoniazid

Discussion

In 2022, 10.9% (48/441) of the isolates tested in the Netherlands revealed some form of resistance, compared to 10.3% (49/472) in 2021.

The number of MDR isolates remained low in 2022 with a total of only 8 MDR-TB cases detected in the Netherlands. Nonetheless the extended hospitalization, complicated treatment of MDR-TB patients, and infectious nature of this disease continues to justify special attention for these infections.

Worldwide, resistance is an important aspect in TB control. As the majority of TB cases in the Netherlands are diagnosed in patients originating from high prevalence areas, it remains important to continue the structural surveillance for local transmission and of resistance.

Conclusions

- Resistance to the antibiotics to treat tuberculosis remained almost stable over the last years, although there may be somewhat larger fluctuations in INH resistance.
- The number of MDR-TB cases remained stable in the recent years (average of 10 cases per year).
- There was a sharp decline in TB notifications in 2020 (621 cases; 17% less than the previous year), presumably related to the COVID-19 pandemic. In 2021 there was an increase of TB cases of 10% compared to 2020, although also in this year the COVID-19 pandemic influenced travel and work. In 2022 a decrease of 6% of TB cases re-establishes the trend of decreasing TB notifications of the last years.

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4.8.4 Antiviral resistance

The current antimicrobial resistance (AMR) awareness and monitoring activities along with the preventive/mitigation measures still focus largely on antibacterial resistance, notorious for the death of thousands worldwide, each year. Importantly, resistance to drugs and therapies occurs in a vast array of other pathogens as well, from viruses and parasites to fungi and others.

In the Netherlands, antiviral resistance monitoring is carried out by several clinical virology laboratories and working groups, mainly in case of treatment failure in different viral induced infections, namely Human Immunodeficiency virus (HIV), Hepatitis B virus (HBV) and Hepatitis C virus (HCV).

Since 2009, NethMap has been entailing data on the systematic monitoring of antiviral resistance for Influenza viruses treatments. Herein, the update on Influenza antiviral resistance data is accompanied with a resumed chapter on antiviral resistance in HIV, extracted from the SHM annual report.[1]

This overview on antiviral resistance considers that antiviral compounds are pharmaceutical agents that inhibit viral replication either by interfering with viral or host factors. Drug resistance is caused by viral genetic changes/mutations which in turn confer fitness advantage over the wildtype virus. The risk of resistance acquisition varies among different viruses as it is related to virus-specific mutation frequency, replication effectiveness, viral load, exposure to antiviral pressure, etc. Antiviral resistance characterisation relies on **phenotypic assays** measuring reduced drug susceptibility in cell culture or reduced functional activities of antiviral drug targeted proteins. Phenotypic methods are the gold standard but often much more laborious since they require viral isolates. Resistance to antiviral drugs can additionally be detected by **genotypic methods** identifying amino acid substitutions that are associated with reduced susceptibility. These substitutions can either be directly or indirectly involved in the mechanism of action of antiviral agents. This is only possible if these amino acid substitutions have been characterized fully by phenotypic methods. Noteworthy, this report considers resistance to antiviral drugs and not vaccine escape phenomena nor specific immunoglobulin treatments. Different active substances, mostly targeting the viral pathogen or essential host pathways/structures for viral replication are used as therapeutical antiviral drugs. Besides the reported antiviral resistance data (Influenza (chapter 4.8.4.1) and HIV (chapter 4.8.4.2)), there are ongoing studies on antiviral susceptibility monitoring accounting other viral infections, as HBV, HCV, Herpes virus, Varicella Zoster virus (VZV), Cytomegalovirus (CMV), Polio/Enterovirus, Mumps virus, Measles virus, Rubella virus, Respiratory Syncytial virus (RSV) and SARS-CoV-2.

There have been few documented instances of antiviral resistance observed among the aforementioned viral pathogens. Consequently, these cases have not been the focus of attention within this report. Nonetheless, the continuous development and increased use of direct-acting antiviral agents as therapeutic options used in clinical practice is, simultaneously, associated to the growing possibility of antiviral resistance occurrence.

Like other resistance phenomena, antiviral drug pressure confers survival advantage to subpopulations of viral isolates relatively less susceptible to the drug effect, driving the emergence of resistant viral isolates. Spontaneous mutations due to drug exposure can also occur and alter the virus genetic stability, pathogenicity and transmissibility. High viral replicative loads, elevated viral mutation rates and prolonged

drug exposure (specifically inadequate antiviral therapy or inappropriate dosages) also favour the selection of resistance viral isolates, rendering antiviral drugs ineffective.

On the clinical aspect, the concern around antiviral resistance is to limit its significance and manifestations, along with reducing the possibility of mutants' development and transmission, particularly to immunocompromised and vulnerable populations.

Noteworthy, efforts to analyse antiviral treatment failure should rely on systematic antiviral resistance monitoring activities for all relevant treatable viral infections, along with enhanced characterisation of both antiviral susceptibility and underlying drivers for the emergence of resistance events.

Respiratory viral pathogens

Antiviral resistance among influenza strains (see chapter 4.8.4.1) is a major public health concern because of the continuous emergence and spread of influenza viruses with enhanced potential of resistant strains upsurge which can rapidly spread among the communities with a considerable burden of disease, especially during the winter season. Currently, the monitoring of **influenza** antiviral susceptibility is embedded in the surveillance of influenza performed by general practitioners (GP), which is coordinated by the Nivel Netherlands Institute for Health Services Research and the RIVM location of the National Influenza Centre (NIC), and the surveillance of influenza viruses received from mainly hospital laboratories by the Erasmus Medical Centre or the RIVM locations of the NIC.[2] The GP network offers an opportunity to study other respiratory viruses that potentially have an impact on the public health, such as **SARS-CoV-2**, and **respiratory syncytial virus (RSV)** for which antiviral agents are available or will become available.

Remdesivir is the antiviral agent (viral RNA-dependent RNA polymerase inhibitor) used for COVID-19 treatment since November 2020, following a repurposing strategy of herpesvirus treatment. Still, susceptibility tests are performed on a small scale. Several antiviral agents are under development, varying from preclinical to approval testing.

RSV is a common respiratory virus mainly associated with mild, cold-like symptoms, but also the origin of severe lower respiratory tract infections and hospitalization in children less than 1 year old. It is estimated to have a similar disease burden as influenza in elderly and patients with comorbidities, with fatalities among younger children. Presently, only one antiviral is under approval process and treatments are mainly based on supportive measures and the use of immunoglobulins (in severe cases). Still, many other drugs are being studied for future use.[3]

Herpes simplex virus (HSV-1 and HSV-2) infections are associated with significant morbidity, mostly induced by recurrent infections of the oral and genital regions. Severe infections can also occur with the involvement of the central nervous system (encephalitis), ocular compromise (keratitis), and severe neonatal infections. HSV infections are not subject to mandatory notification in the Netherlands, and may also be sexually transmitted. Although an acyclovir resistant HSV isolate was first reported in 1982, it is still the worldwide antiviral drug of choice to treat HSV infections, as it is converted by virus-encoded thymidine kinase (TK) to monophosphate derivatives in a considered low toxic therapy. The resistance of acyclovir, valacyclovir, famciclovir and foscarnet are mainly associated to mutations of HSV TK gene or the viral DNA polymerase gene. Further characterisation of these mutations are crucial. So far, information on cidofovir resistance events is missing.

Varicella-zoster virus (VZV/HSV-3), part of the Herpesvirus group is commonly associated to paediatric exanthema diseases (chickenpox), but also to a few severe outcomes such as the involvement of the central nervous system. As all herpesvirus, its infection has latent stages in nervous termini which can induce a resurgence/reactivation, later in life. Worldwide vaccination programs have significantly reduced the disease burden. Acyclovir is also the predominant therapeutic drug used to treat VZV disease. Similarly to HSV, TK gene mutations are reported in cases of antiviral resistance and therapy failure.

Cytomegalovirus (CMV) infections remain a concern for immunocompromised patients (both primary and reactivation stage) mainly presenting systemic symptoms. Congenital and perinatal infections occur during pregnancy or by exposure shortly after birth. The frequency of antiviral resistance to ganciclovir, foscarnet (inhibiting CMV DNA polymerase) and cidofovir varies between 0% and 10% between different patient populations based on Dutch data. Letermovir resistance has also been described, along with multidrug resistance associated with amino acid substitutions. No consensus is available on when antiviral resistance to CMV should be suspected or tested.[4]

HCV is a bloodborne virus which causes acute and chronic hepatitis. Estimations describe that 1.5 million new infections occur yearly, with around 70% of all infections becoming chronic. From these, around 5% incur high probability of developing hepatocellular carcinoma (primary liver cancer). It is a mandatory reporting disease. Even though no effective vaccine is available, treatment options have increased with combined drugs, which are more successful in preventing genetic mutations and leading to effective cure in many cases. The HCV therapy goal is complete resolution of infection (cure). Currently, direct-acting antivirals as NS5B, NS5A and NS3/4A inhibitors are used in standard treatment (e.g. sofosbuvir, voxilaprevir and velpatasvir, respectively). Antiviral resistance events are monitored in the Netherlands, mainly when treatment failure occurs.

HBV infections are mostly transmitted by blood and body fluids, with a considerable mother to child transmission rate. In the Netherlands, prevalence has been decreasing due to the vaccination programme. As HBV infections in adults have a 5% probability to become chronic hepatitis, infections in childhood are estimated to become chronic hepatitis in about 95% of cases. HBV testing and treatment guidelines along with the alternative drugs to use in case of treatment failure can be found on '*HBV richtsnoer*'.[5] Several nucleoside/nucleotide analogues (viral transcription inhibitors) are approved antiviral drugs to treat HBV: lamivudine, adefovir, entecavir, telbivudine, and tenofovir. Genotypic studies have characterised HBV polymerase mutations which confer viral resistance to specific or multiple drugs.

HIV infections have been decreasing in the Netherlands mostly due to efficient antiretroviral (ART) drugs which suppress viral loads, reducing the risk of transmission. Newly HIV diagnosed patients are recommended to initiate ART as soon as possible, regardless of CD4 count, and without the need to characterise the virus susceptibility to the available drugs. The following viral replication inhibitors are: nucleoside analogue inhibitors of the viral reverse transcriptase (RT) enzyme, non-nucleoside inhibitors of the viral RT enzyme, and inhibitors of the viral protease. Also, the non-nucleoside RT inhibitors (NNRTIs) bind to the HIV-1 RT while protease inhibitors (PIs) interfere with the cleavage of viral polypeptides (release of immature virions). HIV resistant mutants have been characterised for all classes of antiviral drugs which led to the combined therapy strategy, reducing treatment failure. See chapter 4.8.4.2.

Table 4.8.4.1 Overview of surveillance of viral pathogens and antiviral resistance in the Netherlands

Virus	Estimated burden of disease	Antiviral treatment	National surveillance in the Netherlands	National monitoring of antiviral resistance
Influenza	High	Amantadine, rimantadine, oseltamivir, zanamivir, baloxavir marboxil	Yes	Yes
SARS-CoV-2	Very high	Remdesivir	Yes	No
Respiratory syncytial virus	High	In development	No	No
Herpes simplex virus 1 and 2	High	Acyclovir and its derivates, foscarnet, cidofovir	No	No
Varicella-zoster virus	Low	Acyclovir	Yes (Chickenpox)	No
Cytomegalovirus	High in immunocompromised patients, low in neonates	Acylcovir, ganciclovir, foscarnet, letermovir	No	No
Hepatitis B	High in specific populations, for which vaccination is recommended	See https://www.hbvrichtsnoer.nl/	No	No
Hepatitis C	High in specific subpopulations	See https://hcvrichtsnoer.nl/	No	AMC, Erasmus MC and UMCU.
Mumps, measles, rubella and poliovirus/enterovirus	Low	In development	Part of vaccination programme monotoring	No
Human Immunodeficiency virus	High	20 agents belonging to 5 different classes	Yes, by “HIV monitoring”, See: https://www.hiv-monitoring.nl/en/resources/monitoring-report-2022	Yes

Rubella-, measles-, mumps-virus, and poliovirus/enterovirus infections are nationally surveyed as part of monitoring of the vaccination programme. There are currently no treatment options for rubella, measles and mumps, except for the human hyperimmune globulins treatment against measles. Apart from vaccination against polio (and Enterovirus (EV)-A71 related hand-foot and mouth disease in Asia), there are also no treatment options available for poliovirus and enteroviruses, such as EV-D68, which has recently been associated with paralytic disease (acute flaccid myelitis) in higher incidence when compared to polio associated paralysis.

Currently, new antiviral drugs are being developed and studied, from laboratory experiments up to clinical trials assessment with patients. Also, repurposing experiments and off-label antiviral drugs use contribute to expand therapeutic possibilities to treat these and many other viral infections[6,7]. Understanding that viruses mutation rates and selective pressure may contribute to the emergence of resistant mutants, urges for improved surveillance on antiviral resistance events, and the development of appropriate assays.

Conclusions

- Current antimicrobial resistance (AMR) awareness and monitoring activities primarily emphasize antibacterial resistance, with less attention given to antiviral resistance.
- Antiviral resistance monitoring in the Netherlands primarily focuses on specific viral infections like HIV, Hepatitis B, and Hepatitis C. The systematic monitoring of antiviral resistance in influenza viruses has been ongoing since 2009, and additional research on other viral infections, such as Herpes-, Varicella-Zoster-, and Cytomegalovirus, is also in progress.
- Reliable techniques to detect resistant clinical isolates should be developed to improve antiviral resistance understanding and rapidly adjust therapeutic options (minimising mutant transmission).
- Limited documented antiviral resistance exists among specific viruses, but the continuous use of antiviral drugs raises concerns. Vigilant monitoring and understanding the drivers of resistance are crucial, especially for immunocompromised individuals.

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4.8.4.1 Influenza virus

Introduction

When vaccination against influenza is not available or fails due to antigenic mismatch with circulating viruses, influenza antiviral drugs can be used for (post exposure) prophylaxis as well as for treatment of influenza cases with (expected) severe course of disease. In the Netherlands the M2 ion channel blockers (M2B) amantadine and rimantadine acting against type A viruses only, the neuraminidase enzyme inhibitors (NAI) oseltamivir and zanamivir and the acidic endonuclease inhibitor baloxavir marboxil (Xofluza®) (BXM), acting against both type A and B viruses, are approved. The M2B prevent uncoating of the virus in the cell and BXM inhibits replication of the virus genome, both thereby inhibiting virus replication. In contrast, NAI prevent release of progeny virus from the cell and thereby limiting the spread to and infection of other cells. Seasonal influenza type A viruses have become fully resistant against M2B by 2010. Monitoring of NAI susceptibility of seasonal human influenza viruses is performed since the 2005/2006 winter season.[1]

Methods

Monitoring of influenza antiviral susceptibility is embedded in the integrated clinical and virological surveillance of influenza using general practitioner (GP) sentinels, that is carried out by the Nivel Netherlands Institute for Health Services Research and the National Institute for Public Health and the Environment (RIVM) location of the National Influenza Centre (NIC). A subset of viruses detected in hospital and peripheral laboratories are submitted to, and analysed at, either the Erasmus Medical Centre or the RIVM locations of the NIC. For both virus sources, patient information on antiviral treatment, travel history and immune competence status in the 14 days preceding the time of specimen collection are collected. The footnotes to Table 4.8.4.2 list whether these characteristics for patients with antiviral reduced susceptible virus are known. In addition, influenza viruses retrieved from municipal health service COVID-19 testing streets in 2021/2022 and obtained via the community based surveillance system on respiratory infections (Infectieradar, see: https://www.infectieradar.nl/welcome) in 2022/2023 are included in the assessment. Techniques to monitor antiviral susceptibility include whole genome Nanopore sequencing and site-specific polymerase chain reaction (PCR) assays for known reduced antiviral susceptibility markers for both NAIs and BXM. For a subset of influenza viruses, the susceptibility to NAIs is determined using an enzyme inhibition assay, which generates a 50% inhibitory concentration of the drug (IC50) to confirm the impact of known markers for reduced antiviral susceptibility and to discover new markers. The use of antiviral drugs is monitored using data available from the Foundation for Pharmaceutical Statistics (SFK) collecting data from more than 97% of the community pharmacies in the Netherlands serving 15.8 million people (93%) of the Dutch population.

Results

Findings for the influenza seasons 2005/2006 through 2009/2010 are presented in NethMap 2016 and for M2Bs up to 2018/2019 in NethMap 2019.[1,2] Table 4.8.4.2 displays an overview of the antiviral susceptibility of influenza viruses since the 2010/2011 influenza season. Each season, none or only very few viruses with reduced antiviral susceptibility to NAI and recently BXM have been detected, and, if status reported, frequently associated with antiviral drug use. However, information on preceding antiviral treatment is often lacking. Figure 4.8.4.1 shows the utilization of oseltamivir and zanamivir since 2010, as reported by the SFK. Oseltamivir prescriptions peak when the circulation of influenza viruses peaks. Zanamivir has not been prescribed since 2020 and BXM has still not been prescribed since its EU authorization early 2021. In the 2022/2023 season so far, one A(H1N1)pdm09 with phenotypically confirmed reduced inhibition by oseltamivir has been detected.

Discussion

In the Netherlands (Table 4.8.4.2), and globally, the proportion of NAI reduced susceptible influenza viruses remains very low.[3] Except for the emergence and sustained worldwide circulation of oseltamivir reduced susceptible former seasonal A(H1N1) in 2007/2008 and some small clusters of oseltamivir reduced susceptible A(H1N1)pdm09 since 2009, most of the NAI reduced susceptible viruses for which treatment status of the patient is known come from antiviral treated patients and do not spread. This highlights that NAIs are still appropriate for prophylaxis and treatment and that it is important to continue monitoring the susceptibility of influenza viruses for NAIs. Very few natural BXM reduced susceptible viruses were detected in the Netherlands, similar to the very low prevalence globally.[3]

Table 4.8.4.2 (Highly) reduced inhibition/susceptibility of influenza viruses by NAIs and BXM in the Netherlands, 2010/2011 - 2022/2023[1]

	A(H1N1)pdm09		A(H3N2)		B	
Season	NAI	BXM	NAI	BXM	NAI	BXM
2010/2011	0/58	ND	0/2	ND	0/64	ND
2011/2012	2/7 (29%)[2]	ND	0/257	ND	0/10	ND
2012/2013	3/125 (2.4%)[3]	ND	0/156	ND	0/8	ND
2013/2014	1/150 (<1%)[4]	ND	2/220 (<1%)[5]	ND	0/4	ND
2014/2015	1/130 (<1%)[6]	ND	0/727	ND	0/42	ND
2015/2016	1/1191(<1%)[7]	ND	0/44	ND	1/69 (1%)[8]	ND
2016/2017	2/11 (18%)[9]	ND	0/911	ND	0/14	ND
2017/2018	1/233(<1%)[10]	ND	0/355	ND	0/156	ND
2018/2019	3/331(<1%)[11]	ND	0/421	ND	0/4	ND
2019/2020[12]	0/206	0/58	0/303	0/139	0/16	0/1
2020/2021[13]	ND	ND	0/20	ND	0/1	ND
2021/2022	1/432 (<1%)[14]	0/285	3/1772 (<1%)[15]	2/1158 (<1%)[16]	0/62	0/41
2022/2023[17]	1/555 (<1%)[18]	0/443	0/339	0/292	0/415[19]	0/406

1 *Combined results obtained with phenotypic (virus isolates) and genotypic (clinical specimens) assays. Season defined as week 40 of the first year to week 39 of the following year. Abbreviations: NAI = neuraminidase inhibitor; BXM = baloxavir marboxil; ND = not done.*

2 *Two viruses with highly reduced inhibition by oseltamivir due to the NA-H275Y amino acid substitution, isolated from two epidemiological unlinked not antiviral treated patients returning from holiday at the Spanish coast.*

3 *Three viruses with highly reduced inhibition by oseltamivir due to the NA-H275Y amino acid substitution. Two isolated from epidemiological unlinked immunocompromised hospitalised patients treated with oseltamivir. No details available for the third patient.*

4 *One virus with highly reduced inhibition by oseltamivir due to the NA-H275Y amino acid substitution. No patient characteristics or antiviral exposure data available.*

5 *Two clinical specimens from two patients with mixture of NA-292R and NA-292K amino acid composition; NA-R292K is associated with highly reduced inhibition for oseltamivir and zanamivir. No patient characteristics or antiviral exposure data available.*

6 *One virus with highly reduced inhibition by oseltamivir due to mixture NA-275H/Y amino acid substitution. The patient was treated with oseltamivir prior to specimen collection.*

7 *One virus with highly reduced inhibition by oseltamivir due to mixture NA-275H/Y amino acid substitution. No patient characteristics or antiviral exposure data available.*

[8] *One virus with highly reduced inhibition by zanamivir and reduced inhibition by oseltamivir due to an NA-E105K amino acid substitution. However, highly likely induced by virus isolation as in the clinical specimen this amino acid substitution was not detectable. The patient was not treated with antivirals prior to specimen collection.*

[9] *Two viruses from one patient taken 10 days apart with both highly reduced inhibition by oseltamivir due to a NA-H275Y amino acid substitution. The patient was treated with oseltamivir prior to specimen collection.*

[10] *One virus with highly reduced inhibition by oseltamivir due to mixture NA-275H/Y amino acid substitution. No patient characteristics or antiviral exposure data available.*

[11] *Three viruses with highly reduced inhibition by oseltamivir due to NA-H275Y (n=1) or mixture NA-275H/Y (n=2) amino acid substitution. Two patients were admitted to ICU of which one was treated with oseltamivir prior to specimen collection and the other had an unknown treatment status. One community patient had no prior treatment with oseltamivir*

[12] *Late in the season additionally a case of swine influenza A(H1N1)v was detected that showed highly reduced inhibition by oseltamivir due to NA-H275Y amino acid substitution following oseltamivir treatment.*

[13] *During the winter period 2020/2021 no influenza viruses were detected. Only very late in the season after COVID-19 measures were partly lifted in summer 2021 few influenza viruses were detected and anlysed for antiviral susceptibility.*

[14] *One virus with NA-H275Y associated with highly reduced inhibition by oseltamivir was detected; additional data on treatment status of the patient unknown.*

[15] *Three viruses with NA-N329R associated with reduced inibition by zanamivir; by phenotypic testing two were indeed RI by zanamvir and with fold-change around the RI threshold and one was NI by both oseltamivir and zanamivir. All three viruses came from the same submitter. Influenza antiviral treatment history of all three patients was unknown.*

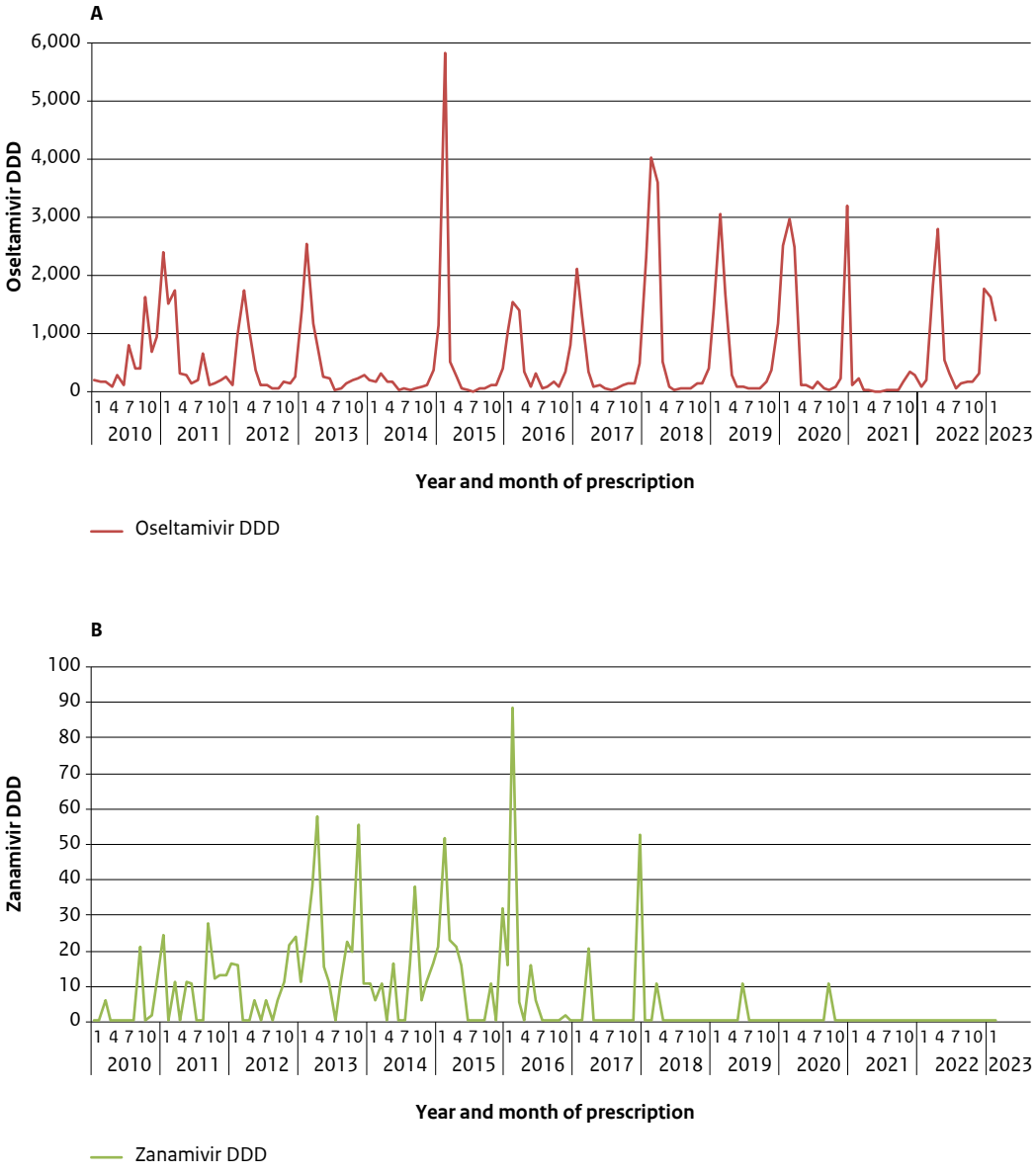
[16] *Two viruses showed the amino acid substitution PA-E23G, previously associated with mild reduced susceptibility to baloxavir marboxil. By phenotypic testing at the WHO CCs for influenza in Tokyo and Atlanta of one virus, the virus was clearly reduced susceptible for baloxavir marboxil. One patient was hospitalized. The status of the other patient was unknown. For both patients no antiviral exposure data available.*

[17] *Preliminary data up to week 13/2023.*

[18] *One virus with NA-D199G previously associated with reduced inhibition by oseltamivir that appeared highly reduced inhibited by oseltamivir and normal inhibited by zanamivir by phenotypic testing. The patient did not receive influenza antiviral treatment prior to specimen collection.*

[19] *A cluster of B/Victoria viruses emerged almost exclusively in The Netherlands with NA-K360E previously associated with highly reduced inhibition by peramivir. However, by phenotypic testing of 9 of these virus isolates they appeared normal inhibited by peramivir, likely due to compensating additional amino acid substitutions A395V and L396F/S in the close vicinity of the 360 position in the 3D structure of the neuraminidase.*

Figure 4.8.4.1 Prescriptions of oseltamivir (A) and zanamivir (B) in the Netherlands, 2010/2011 - 2022/2023. Shown are the Defined Daily Doses (ddd) cumulated by month. Data kindly provided by Foundation for Pharmaceutical Statistics (SFK), the Netherlands



Conclusions

- Over the last 13 seasons type A and type B influenza viruses remained susceptible to the neuraminidase inhibitors oseltamivir and zanamivir, and since approval also to baloxavir marboxil.
- Sporadically, a neuraminidase inhibitor or baloxavir marboxil reduced susceptible virus has been detected; mostly associated with the use of antivirals prior to specimen collection if antiviral treatment status was known.
- Prescriptions of oseltamivir remain low with sharp increases every influenza epidemic, even during the 2020/2021 season when hardly any influenza viruses were detected due to the COVID-19 pandemic measures.
- Zanamivir has not been prescribed since late 2020 and baloxavir marboxil has not been prescribed since its registration in 2021.

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4.8.4.2 Human Immunodeficiency virus

Antiviral Drug Resistance data - HIV Summary as part of the national repository with reference to HIV Monitoring Rapport 2022 | Stichting HIV Monitoring (hiv-monitoring.nl)

Introduction

Human Immunodeficiency virus (HIV) is characterised as HIV-1 and HIV-2 and subdivided in groups (M, N, O, P for HIV-1; A to H for HIV-2), subtypes (A-D, F-H, J-K for HIV-1 group M), and sub-subtypes (A1-A7 for subtype A, F1 and F2 for subtype F in HIV-1 group M). Circulating recombinant forms (CRF), and unique recombinant forms have also been characterised in a small number of infections. From its first isolation in 1983, virological diagnostic and monitoring techniques have greatly improved.
Innovative treatment developments lowered the morbidity and mortality associated with HIV. In the Netherlands effective antiretroviral therapy (ART) is available since 1996. Currently 96% of people on antiretroviral treatment (ART) have a suppressed HIV-1 RNA. In countries where viral load and resistance testing is not standard of care, HIV drug resistance strains have increase throughout the years (WHO, 2022). This antiretroviral drugs resistance phenomena is caused by alterations in the virus genetic structure which in turn affect drug's efficacy. A full characterisation and adequacy of ART is crucial to prevent HIV transmission and HIV-associated morbidity and mortality.
HIV resistance can be categorized as pre-treatment drug resistance resulting from the transmission of resistant strains and acquired resistance which occurs during treatment resulting from decreased exposure to ART and subsequently the selection of resistant strains and treatment failure.
In the Netherlands, as in other countries, viral resistance tests are recommended at the start of the treatment (or establish baseline viral loads) since 2003, though not performed in all HIV centres.
These tests help determine the most appropriate therapeutic scheme while attempting to minimize the emergence/spread of HIV drug resistance. Still, in a global context, little is known on antiviral resistance events in underdeveloped countries (mostly due to costs limitation).

Methods

HIV genotypic resistance analyses are performed in the Netherlands at the treatment centres, recommended at entry into care and later on if treatment fails and obtaining a sequence is necessary. Sequencing analysis is part of baseline information collection (viral mutations characterization), intending to optimize individual patient treatment and improve therapeutical guidelines. Surveillance of HIV drug resistance is performed before antiretroviral therapy (ART) is implemented, both for adults and naïve infants, as recommended by WHO in order to select first-line regimens. These antiviral resistance (AVR) studies are annually published in the HIV Monitoring report (SHM). Genotypic resistance test assess Reverse transcriptase (RT) resistance-associated mutations (RAM), protease or integrase resistance-associated mutations. These data are then analysed using HIVdb which infers antiretroviral drug susceptibility and resistance scores.

Results

By the end of December 2021, 20,804 people with HIV were in care in the Netherlands. HIV-1 subtype B (74.7%), followed by non-B subtypes (25.3%), including circulating recombinant forms (i.e. CRF_02AG (6.7%), subtype C (5.0%), and CRF_01AE (3.7%)) were the main identified infection subtypes. Antiviral resistance is mainly studied when pre-treatment sequences are available, as after the therapy is started, suppressed viral loads hamper the resistance characterisation effort, and not considered useful for clinical follow-up. Antiviral resistance is usually understood as transmitted or acquired, detailed below.

Transmitted drug resistance

Transmitted drug resistance is considered when presence of at least one resistance-associated mutation can be detected before initiation of ART. HIV drug resistance mutation list is used to score major RAM. From 2003-2021, 8,637 HIV-1 sequences were obtained from 8,327 ART-naïve people (prior to ART). Simultaneously, 8,627 reverse transcriptase sequences were available (from 8,320 individuals) and 8,133 protease sequences were available from 7,835 individuals along with 202 integrase sequences from 201 individuals. Overall, 247 (3.0%) individuals screened for transmitted drug resistance harboured high-level resistance to at least one antiretroviral drug, 45 (0.5%) to at least one nucleotide/nucleoside reverse transcriptase inhibitor (NRTI); 182 (2.2%) to at least one non-nucleoside reverse transcriptase inhibitor (NNRTI); and 34 (0.4%) to at least one protease inhibitor (PI), as depicted in Figure 4.8.4.2. Noteworthy, from the 201 people that had an integrase sequence available prior the time of entry into care (ART), all but three exceptions were ARV-naïve. Integrase inhibitor resistance associated mutations could not be detected in any of all individual sequences, probably due to the fact that integrase inhibitor resistances is only assessed when previous NRTI characterisation reveal mutations.

Overall, more than 97% of individual infection isolates were fully susceptible to all antiretroviral drugs while 2.6% (n=212) harboured high-level resistance in one drug class; 0.3% (n=24) in two drug classes; and less than 0.1% (n=5) to three drug classes (i.e. NRTIs, NNRTIs and PIs).

Figure 4.8.4.2 Annual percentage of patients with evidence of transmitted HIV drug resistance over time
Note: RAS, resistance associate substitutions. ***In*** HIV Monitoring report 2022 (p. 136)

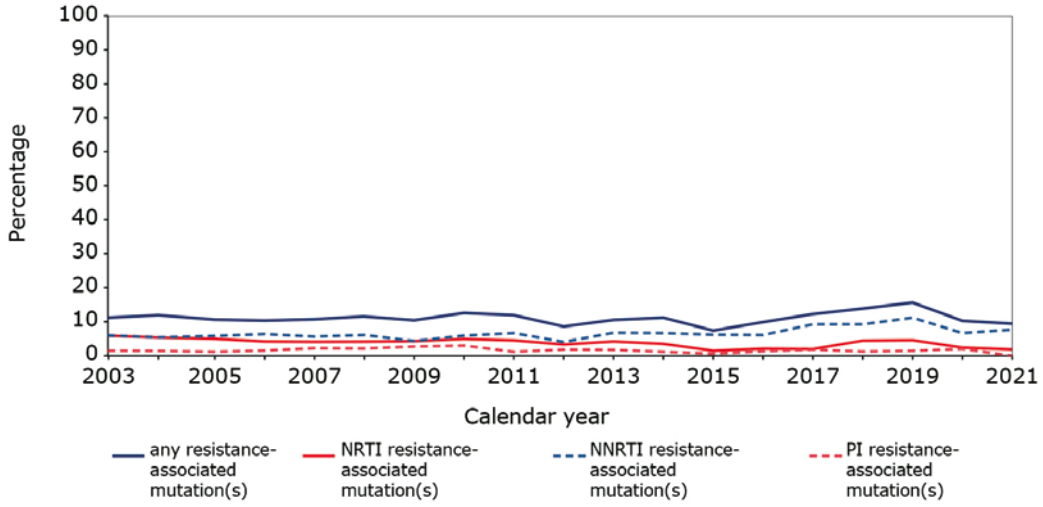

Figure retrieved from the original SHM report

PrEP related drug resistance

Contrastingly, pre-treatment drug-resistance data is related mostly to MSM (67.4%). Genotypic resistance test results were performed for 46 (61.3%) of the 75 men who reported having used PrEP prior to first entering HIV care, with 14 (30.4%) showing Reverse transcriptase (RT) RAM. Detailed findings can be found in Table 4.8.4.3. Further resistance tests of 37 other patients exclusively yielded wild-type RT or naturally occurring polymorphisms most probably unrelated to PrEP intake, and no major protease or integrase resistance-associated mutations were observed.

Table 4.8.4.3 HIV mutations and antiviral resistance features (2018-2021) adapted from SHM (p.92, p.156)

	2018-01 to 2019-09	2019-10 & later
Resistance test performed after testing HIV-positive	16 (53.3%)	30 (66.7%)
M184V/I [a]	4 (25.0%)	5 (16.7%)
K65R [b]		1 (3.3%)
E138A [c]	1 (6.3%)	3 (10.0%)
V74I [d]		2 (14.3%)
V103R [e]		2 (6.7%)
V108I [f]		2 (6.7%)

[a] *decreases susceptibility to lamivudine and emtricitabine*
[b] *decreases susceptibility to tenofovir, abacavir, lamivudine and emtricitabine*
[c] *natural polymorphism, especially in non-B HIV-1 subtypes (also selected for by the use of rilpivirine)*
[d] *decreases susceptibility to abacavir and didanosine*
[e] *possibly a naturally occurring polymorphism*
[f] *a non-polymorphic accessory mutation conferring decreased susceptibility to nevirapine and efavirenz*
NOTE: The two individuals with HIV-1 subtype B and an M184V/I RT RAM may have acquired HIV from a person on a failing rilpivirine containing regimen

Acquired drug resistance

From 2000 to 2021, 4,587 HIV-1 sequences from 2,757 people who received ART for at least four months were analysed. From these, 736 (2,757-2,021) were treated with mono or dual therapy, representing (27%, 736/2757) of the people with a sequence, while constituting 7% of all people in care in 2021. In summary, analysis was performed on 3,225 sequences from 2,021 people who had been ART-naïve before initiating ART; 4,511 reverse transcriptase sequences from 2,731 individuals; 4,343 protease sequences from 2,616 individuals, and 371 integrase sequences were available from 295 individuals.
Sequencing of HIV-1 integrase gene was performed seldom (in cases of virological failure on ART) as was the case for 295 individuals who had received ART for at least four months (most of them had many years of ART). In total, 97.6% (n=288) had followed an Integrase strand transfer inhibitors (INSTI) containing regimen revealing a median time between initial ART intake and integrase inhibitor resistance testing of 10.4 years (IQR 4.6-15.7). From these 295 individuals, 46 displayed at least one acquired major mutation associated with integrase inhibitor resistance. Also, additional RAM could be detected as follows: N155H/N, R263R/K, E92E/Q, Y143R and Y143Y/C, T66T/I, Q148H/R, S147S/G (major INSTI resistance mutations); T97 (T97A), T66T/A, T66T/K, T66K, L74I/L/M, G140S, G140G/S, E138K (minor mutations). Four of these 46 individuals developed INSTI-associated resistance mutations during monotherapy with dolutegravir (*n*=3) or raltegravir (*n*=1).

Figure 4.8.4.3 The percentage of sequences with evidence of high-level resistance by drug class per year, obtained at the time of virological failure when receiving combination ART, among previously antiretroviral drug-naïve people. Graphs depicting high level resistance to antiretroviral drug (class): A) at least one drug within class, B) nucleoside reverse transcriptase inhibitors, C) non-nucleoside reverse transcriptase inhibitors, D) protease inhibitors. Note: additional information on high-level resistance to nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and protease inhibitors, available in original report. ***In*** HIV Monitoring report 2022 (p. 141)

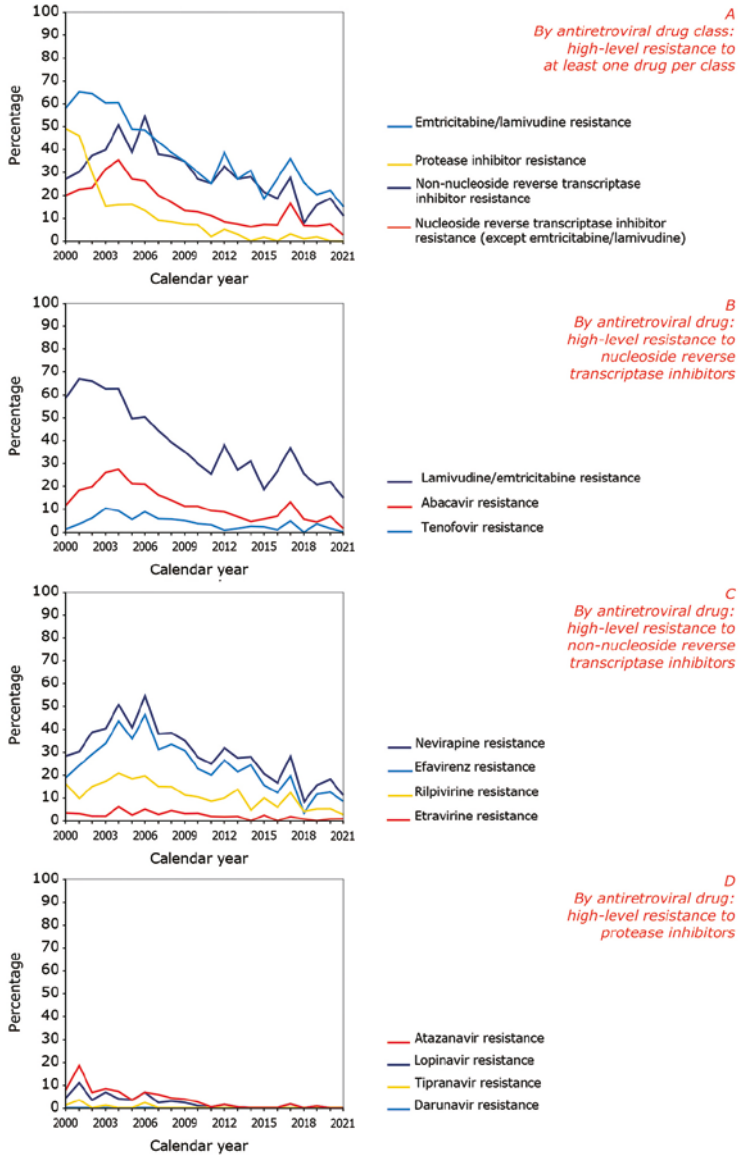

Figure retrieved from the original SHM report and adapted presentation

Discussion

In the Netherlands, the proportion of antiretroviral resistance shows decreasing trends (Figure 4.8.4.2). While transmitted HIV drug resistance results from the infection with an HIV strain containing drug-resistant mutations, acquired resistance mostly results from non-compliance to the prescribed treatment (also considering a susceptible virus to ART). In extraordinary cases acquired resistance may be the result of undetected transmitted drug resistance, either because pre-treatment sequence was unavailable or because the resistant variant was already overgrown by wild-type virus (possibility of resistant variant still present in resting CD4 cells). Data shows high rate of NRTI resistance among MSM that had used PrEP. Noteworthy, antiretroviral therapy incomplete suppression of viral replication, may enable HIV mutations, henceforth a possible ART failure since all antiretroviral drugs, including newer classes, have an associated risk of ineffectiveness due to drug-resistant HIV virus emergence. Continuous monitoring in both newly diagnosed infections and in case of ART failure is crucial will enabling appropriate response to HIV drug resistance with comprehensive and effective HIV care, contributing to suppress viral loads, and minimise the risk of HIV transmission.

The presented data may not be representative of the entire population in HIV care in the Netherlands as some HIV treatment centres and laboratories did not share data for the SHM.

Conclusions – directly extracted from SHM

- Globally, viral suppression rates in patients with HIV receiving ART is high and has been improving. Among those with ART failure, the annual percentage with acquired antiviral resistance remained low; similarly to other high-income settings.
- Transmitted drug resistance was a rare occurrence and prevalence remains low, with equal reported rates from other European countries.
- Data on Integrase inhibitor resistance remain limited with no detected cases among the 201 people tested during 2021. Genotypic testes show low rates of acquired integrase inhibitor resistance, with a few cases of significant resistance to dolutegravir.

References

1 Stichting HIV Monitoring (hiv-monitoring.nl)

2 Fact Sheet: HIV Drug Resistance (who.int) (data from 17.November.2022; accessed on July, 2023)

3 Global action plan on HIV drug resistance 2017–2021. Geneva: World Health Organization; 2017. Licence: CC BY-NC-SA 3.0 IGO.

4 HIV Drug Resistance Database (stanford.edu)

4.8.5 The antimicrobial susceptibility profile of clinically relevant anaerobic bacteria in the Netherlands

Introduction

The large variation in antimicrobial resistance (AMR) of clinically relevant anaerobic bacteria between countries and regions makes it important to regularly perform surveillance studies (1). Within this report we describe the routinely performed antimicrobial susceptibility testing (AST) of anaerobic bacteria isolated at the University Medical Center Groningen (UMCG) in 2022, and the results of AST of Bacteroidales isolates derived from 8 Dutch laboratories, which were tested for 9 different antibiotics.

Methods

At the UMCG, anaerobic isolates from clinical infection-related samples are routinely tested for antimicrobial susceptibility using Etest (BioMérieux), according to the manufacturer's recommendations. Resistance of gram-negative isolates was determined for amoxicillin (except β-lactamase producers), amoxicillin-clavulanic acid, clindamycin, metronidazole and meropenem (only *Bacteroides* and *Prevotella*). Gram-positive isolates were tested for amoxicillin, clindamycin, and metronidazole (except genera with intrinsic resistance; e.g. *Actinomyces* and *Cutibacterium*).
In addition, for a national surveillance study, 8 different Dutch laboratories (7 university medical centers, 1 peripheral laboratory) collected 20-25 *Bacteroides* (including *Parabacteroides*) and 10-15 *Prevotella* isolates in March - June 2021, which were sent to the UMCG for antimicrobial susceptibility testing (AST). Resistance was determined for amoxicillin, amoxicillin-clavulanic acid, piperacillin-tazobactam, meropenem, imipenem, clindamycin, metronidazole, tetracycline, and moxifloxacin; using agar dilution. Whole genome sequencing (WGS) was performed of metronidazole resistant isolates.
MIC50 and MIC90 were calculated for all genera/species of which at least 10 strains were available. Antimicrobial resistance (AMR) was determined using EUCAST breakpoints v11.0 (2021) and v12.0 (2022) (only for isolates which were sampled in 2022). The v11.0 breakpoints were included for both the isolates sampled in 2021 and 2022 in order to compare current resistance rates of with the ones from previous years. For all anaerobic bacteria, from both studies, one isolate of each species per patient was included and they were all identified using MALDI-TOF MS (Bruker Daltonics, Bremen, Germany) at the UMCG.

Results

Anaerobic isolates at UMCG in 2022

Variations in AMR were observed between the different genera (Table 4.8.5.1). The resistance percentage for the different antibiotics is similar as in previous years (data not shown). Resistance to metronidazole among the gram-negative anaerobic bacteria was only observed in one *Prevotella* isolate in 2022. This *P. bivia* isolate was not only resistant to metronidazole, but also to clindamycin and produced β-lactamase. Remarkable is the high rate of resistance of *Parabacteroides* species for amoxicillin-clavulanic acid, 26.3%. Of the *Bacteroides* isolates, 3.3% (n=5) were resistant to meropenem. Four of these resistant isolates were identified as *B. fragilis* and one as *B. ovatus*. One *Prevotella oris* isolate was resistant to meropenem, but susceptible to amoxicillin-clavulanic acid.
Applying the new breakpoints resulted in an increase of the resistance percentage for most of the antibiotics (if applicable) (Table 4.8.5.1).

Differences in AMR were also observed for the gram-positive anaerobic bacteria. Among the gram-positive anaerobic cocci (GPAC) genera, resistance for amoxicillin was only observed among *Peptostreptococcus* isolates. In contrast, none of these *Peptostreptococcus* isolates was resistant to clindamycin, while this varied among the other GPAC genera. One *Peptoniphilus harei* isolate was resistant to metronidazole, but susceptible to amoxicillin and clindamycin.

National surveillance study
Of 165 *Bacteroides* and 96 *Prevotella* isolates resistance was determined using the EUCAST v11.0 breakpoints and differed between species (Table 4.8.5.2).

Even though *B. fragilis* is considered to be the most virulent *Bacteroides* species, the most resistant *Bacteroides* species in this study was *B. thetaiotaomicron/faecis*, with notably high resistance to piperacillin-tazobactam (42%) and amoxicillin-clavulanic acid (29%). This stands out when compared to the other *Bacteroides* species, where resistance against these antimicrobial agents was between 0% and 16%. Clindamycin resistance was mostly found in *B. thetaiotaomicron/faecis* and *B. ovatus/xylanisolvens*, 42% and 36% respectively. Resistance in all other *Bacteroides* species was notably lower ranging from 5% to 18%. Resistance to meropenem and imipenem were similar, and was found in the most prevalent *Bacteroides* species in this study (i.e. *B. fragilis*, *B. thetaiotaomicron/faecis*, *B. ovatus/xylanisolvens*, *B. vulgatus*), although still in low numbers. Metronidazole resistance was found in one *B. thetaiotaomicron/faecis*, which was susceptible to meropenem and imipenem.
When comparing with *Bacteroides* species, resistance among *Parabacteroides* isolates was higher to amoxicillin-clavulanic acid and tetracycline, but lower to piperacillin-tazobactam, clindamycin and moxifloxacin. None of the *Parabacteroides* isolates were resistant to metronidazole or carbapenems.
Of all *Prevotella* isolates included, two isolates were resistant to amoxicillin-clavulanic acid, identified as *P. buccae* and *P. intermedia*. Amoxicillin and clindamycin resistance were highest in *P. bivia*, 77% and 33%, respectively. Two *Prevotella* isolates were resistant to metronidazole, a *P. melaninogenica* and a *P. nanceiensis*. All *Prevotella* isolates were susceptible to piperacillin-tazobactam and carbapenems.

Discussion

As in previous years, metronidazole resistance in *Bacteroides* and *Prevotella* remained low. Among the Bacteroidales isolates from the UMCG, this year only one resistant *P. bivia* isolate was observed. One *Peptoniphilus* isolate was reported to be metronidazole resistant. This isolate will be subjected to WGS to determine which AMR mechanism is present. Also, meropenem resistance remained low, even though, for the first time, one *Prevotella* isolate was resistant.
Three metronidazole resistant *Bacteroides/Prevotella* isolates were encountered, which were all subjected to WGS. Analysis of the genomes showed that two isolates harboured a *nim* gene in their genome, while one did not (*P. nanceiensis*). The *B. thetaiotaomicron/faecis* isolate harbored a *nimE* gene on a plasmid, while *P. melaninogenica* had a *nimA* gene in its chromosome. The fact that a *nimE* gene was located on a plasmid increases the chance of exchange of this gene between different bacterial strains. An important additional finding was that mobile genetic elements (MGEs) harboring an antimicrobial resistance gene previously described by Boiten *et al*. (2) were also present in the *B. thetaiotaomicron/faecis* and *P. melaninogenica* isolate. More studies are needed to assess the prevalence of these MGEs in bacterial isolates.

Table 4.8.5.1 MIC50, MIC90 and percentage resistance of clinically anaerobic isolates at UMCG for the different antibiotics, 2022

	amoxicillin			amoxicillin/clavulanic acid			clindamycin				metronidazole				meropenem			
Genus	MIC50	MIC90	%R v11	MIC50	MIC90	%R v11	MIC50	MIC90	%R v11	%R v12	MIC50	MIC90	%R v11	%R v12	MIC50	MIC90	%R v11	%R v12
Gram-negative anaerobic bacteria																		
Bacteroides spp. (151-152)[a]	nd [b]	nd	nd	0.25	2	4	3	>256	32	32	0.38	0.75	0	0	0.19	0.75	3	7
Prevotella spp. (127-129)[a]	nd	nd	nd	0.016	0.094	0	0.016	>256	17	19	0.125	0.75	1	1	0.064	0.094	1	2
Fusobacterium spp. (33)	nd	nd	nd	<0.016	0.023	3	0.047	0.38	6	na	<0.016	0.19	0	na	nd	nd	nd	
Fusobacterium spp. (32)	na [c]	na	na	na	na	na	0.047	0.38	na	13	na	na	na	0	nd	nd	nd	
Parabacteroides spp. (19)	nd	nd	nd	3	96	26	3	8	21	90	0.38	0.75	0	0	nd	nd	nd	
Porphyromonas spp. (13)	nd	nd	nd	<0.016	0.047	0	<0.016	0.016	0	0	0.016	0.38	0	0	nd	nd	nd	
Bilophila spp. (9)	128	>256	100	0.75	1.5	0	0.38	1	0	22	0.047	0.125	0	0	nd	nd	nd	
Dialister spp. (10)	0.125	0.5	0	<0.016	0.016	0	0.19	0.5	0	10	2	24	20	20	nd	nd	nd	
Veillonella spp. (29-32)[a]	0.25	3	13	0.064	1.5	0	0.19	0.38	3	6	1.5	2	3	3	nd	nd	nd	
Gram-positive anaerobic bacteria																		
Actinomyces spp. (104)	0.125	0.38	1	nd	nd	nd	0.19	12	11	17	nd	nd	nd	nd	nd	nd	nd	
Cutibacterium spp. (191)	0.094	0.25	1	nd	nd	nd	0.047	0.125	3	na	nd	nd	nd	nd	nd	nd	nd	
C. acnes (161)	na	na	na	nd	nd	nd	0.047	0.125	na	3	nd	nd	nd	nd	nd	nd	nd	
Cutibacterium spp. (30)	na	na	na	nd	nd	nd	0.032	0.25	na	10	nd	nd	nd	nd	nd	nd	nd	
F. magna (40)	0.19	0.38	0	nd	nd	nd	1	>256	25	65	0.19	0.5	0	0	nd	nd	nd	
P. micra (41-42)	0.023	0.064	0	nd	nd	nd	0.19	0.75	5	17	0.064	0.25	0	0	nd	nd	nd	
Peptoniphilus spp. (43)	0.023	0.19	0	nd	nd	nd	0.5	>256	23	49	0.38	1.5	2	2	nd	nd	nd	
Peptostreptococcus spp. (13)	0.25	4	8	nd	nd	nd	0.19	0.5	0	0	0.094	0.38	0	0	nd	nd	nd	
Anaerococcus spp. (41)	0.023	0.125	0	nd	nd	nd	0.064	>256	17	22	0.19	0.75	0	0	nd	nd	nd	
Clostridium spp. (43)	0.38	1	0	nd	nd	nd	2	128	33	na	0.38	1	0	na	nd	nd	nd	
Clostridium spp. (34)	na	na	na	nd	nd	nd	1	128	na	68	na	na	na	0	nd	nd	nd	
C. perfringens (9)	na	na	na	nd	nd	nd	3	24	na	89	na	na	na	0	nd	nd	nd	
Atopobium spp. (8-9)	0.125	0.19	0	nd	nd	nd	0.064	2	0	33	0.19	0.38	0	0	nd	nd	nd	
Bifidobacterium spp. (12-13)	0.125	>256	15	nd	nd	nd	0.047	0.19	0	8	nd	nd	nd	nd	nd	nd	nd	

[a] *Not all antibiotics were tested for all isolates.*
[b] *Not determined.*
[c] *Not applicable.*

Table 4.8.5.2 MIC50, MIC90 and the percentage resistance for all tested antibiotics of all isolates from 8 Dutch laboratories of the national surveillance study, March - June 2021

	amoxicillin			amoxicillin-clavulanic acid (fixed ratio)			piperacillin/tazobactam		
Isolates (n)	**MIC50**	**MIC90**	**%R**	**MIC50**	**MIC90**	**%R**	**MIC50**	**MIC90**	**%R**
***Bacteroides* spp.**									
B. fragilis (62)	32	256	97	1	4	7	2	8	2
B. thetaiotaomicron/faecis (31)	64	256	100	2	32	29	16	32	42
B. ovatus/xylanisolvens (25)	64	>256	100	2	16	16	8	32	16
B. vulgatus (24-25)	128	>256	100	2	16	16	8	32	12
Bacteroides spp. (21-22)	32	>256	96	2	4	0	4	8	0
Bacteroides total (163-165)	32	>256	98	2	16	13	4	32	13
***Parabacteroides* spp.**									
Parabacteroides total (19)	>256	>256	68	8	32	37	4	16	5
***Prevotella* spp.**									
P. bivia (12-13)	16	64	77	2	4	0	1	4	0
P. buccae (14-15)	0.125	256	40	0.25	8	7	2	8	0
Prevotella spp. (60-68)	4	128	53	0.5	4	2	2	4	0
Prevotella total (87-96)	4	128	54	0.5	4	2	2	4	0

	meropenem			imipenem			metronidazole		
Isolates (n)	**MIC50**	**MIC90**	**%R**	**MIC50**	**MIC90**	**%R**	**MIC50**	**MIC90**	**%R**
***Bacteroides* spp.**									
B. fragilis (62)	0.25	1	3	0.25	2	7	0.5	1	0
B. thetaiotaomicron/faecis (31)	0.5	4	3	1	4	3	0.5	1	3
B. ovatus/xylanisolvens (25)	0.5	4	4	1	4	8	0.5	1	0
B. vulgatus (24-25)	0.5	2	4	1	4	8	0.5	1	0
Bacteroides spp. (21-22)	0.25	1	0	1	1	0	0.5	0.5	0
Bacteroides total (163-165)	0.5	2	3	1	4	6	0.5	1	1
***Parabacteroides* spp.**									
Parabacteroides total (19)	1	2	0	2	2	0	1	1	0
***Prevotella* spp.**									
P. bivia (12-13)	0.125	0.125	0	0.125	0.125	0	1	1	0
P. buccae (14-15)	0.125	0.5	0	0.25	0.5	0	0.5	1	0
Prevotella spp. (60-68)	0.0625	0.25	0	0.125	0.25	0	0.25	1	3
Prevotella total (87-96)	0.125	0.25	0	0.125	0.5	0	0.5	1	2

Table 4.8.5.2 (continued) MIC50, MIC90 and the percentage resistance for all tested antibiotics of all isolates from 8 Dutch laboratories of the national surveillance study, March - June 2021

	clindamycin			tetracyclin			moxifloxacin		
Isolates (n)	**MIC50**	**MIC90**	**%R**	**MIC50**	**MIC90**	**%R**	**MIC50**	**MIC90**	**%R**
***Bacteroides* spp.**									
B. fragilis (62)	0.25	>256	18	64	128	60	0.5	16	13
B. thetaiotaomicron/faecis (31)	4	>256	42	32	128	81	2	16	16
B. ovatus/xylanisolvens (25)	3	>256	36	64	128	72	2	16	20
B. vulgatus (24-25)	0.023	0.75	8	32	64	72	1	128	44
Bacteroides spp. (21-22)	0.75	2	5	32	64	68	2	4	9
Bacteroides total (163-165)	0.75	>256	22	32	128	69	1	16	19
***Parabacteroides* spp.**									
Parabacteroides total (19)	1	3	5	64	64	90	0.5	1	5
***Prevotella* spp.**									
P. bivia (12-13)	0.023	>256	33	1	64	39	2	4	8
P. buccae (14-15)	0.016	>256	13	0.5	32	27	0.5	1	7
Prevotella spp. (60-68)	<0.016	>256	22	1	64	34	0.5	2	9
Prevotella total (87-96)	0.016	>256	22	1	64	33	1	4	8

Conclusions

- Significant differences in AMR in anaerobic bacteria were observed among clinically relevant anaerobic bacteria, with notable variations between genera and species.
- *Bacteroides thetaiotaomicron/faecis* is the most resistant *Bacteroides* species in the Netherlands.
- *Parabacteroides* species demonstrated significant resistance (26%) to amoxicillin-clavulanic acid, while *Bacteroides* isolates showed 3% resistance to meropenem, including *B. fragilis*, highlighting the emergence of resistance in important clinical strains.

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2 Boiten KE, Kuijper EJ, Schuele L, van Prehn J, Bode LGM, Maat I, van Asten SAV, Notermans DW, Rossen JWA, Veloo ACM. Characterization of mobile genetic elements in multidrug-resistant Bacteroides fragilis isolates from different hospitals in the Netherlands. Anaerobe. 2023;81:102722.

4.8.6 *Clostridioides difficile*

Introduction

Clostridioides difficile is an anaerobic, spore-forming bacterium which can colonise the intestine of humans and animals. Toxin-producing *C. difficile* strains can cause mild diarrhoea, severe colitis or a life-threatening toxic megacolon depending on host susceptibility and the virulence of the infecting strain. The Centre for Infectious Disease Control (CIb) of the National Institute for Public Health and the Environment (RIVM) started a National Reference Laboratory for *C. difficile* at the Leiden University Medical Centre (LUMC) soon after recognition of *C. difficile* ribotype (RT) 027 outbreaks in 2005. A sentinel surveillance program started in May 2009 to monitor the incidence and characteristics of *C. difficile* infections (CDI). In 2022, as part of a restructuring effort, the number of participating hospitals was reduced from 22 to five.

Methods

In 2022, the sentinel surveillance was continued in 5 centres (3 academic, 2 general hospitals) geographically spread over the Netherlands. Inclusion criteria for surveillance were: hospitalized patients with a clinical picture compatible with CDI and a positive CDI diagnostic test (toxin enzyme-immunoassay, or toxigenic *C. difficile* polymerase chain reaction (PCR)), or presence of pseudomembranous colitis. Of the included cases, *C. difficile* isolates or fecal samples were submitted to the Expertise Centre for *C. difficile* culture and typing. The web-based system OSIRIS was used to capture epidemiological data such as location of onset, disease severity, disease course and CDI-attributable mortality. Severe CDI was defined as meeting one or more of the following criteria: (i) bloody diarrhoea, (ii) pseudomembranous colitis, (iii) diarrhoea with dehydration, (iv) diarrhoea with hypoalbuminemia <20g/L, (v) temperature >38.0°C with leukocytosis >15 x 10^9/L. To calculate CDI incidence, the number of included CDI cases was divided by the number of clinical patient days in the centers participating in the sentinel surveillance program.
In addition to the sentinel surveillance, the *ad hoc* typing service continued to be offered for all microbiology laboratories in the Netherlands for typing *C. difficile* isolates of patients with severe disease, or isolates from a suspected outbreak. After comparison of Whole-Genome Sequence (WGS)-based typing to PCR ribotyping using a large *C. difficile* reference strain collection, core genome MLST is currently being implemented for routine typing of strains.[1] We here present PCR ribotyping results.

Results

In 2022, 417 fecal samples/isolates were sent to the Expertise Centre for the sentinel surveillance and *ad hoc* typing. In the CDI sentinel surveillance, 291 cases (median age 69, IQR 57-78) met inclusion criteria, of which 246 *C. difficile* isolates could be cultured and typed. We received 42 samples for *ad hoc* typing.

CDI incidence remained stable with 3.1/10,000 patient days (95%CI 2.8-3.4) in 2018-2019 (pre-COVID19) compared to 3.2/10,000 (2.9-3.5, n=291 cases) in 2022. CDI-attributable mortality was also stable: 1.1% (0.4-1.8) in 2018-2019 and 1.0% (0.0-2.2) in 2022. The proportion of patients with severe disease increased from 16.1% (13.7-18.5) in 2018-2019 to 30.3% (25.0-35.6) in 2022. The age of severe CDI cases remained stable with a median age of 69 years in 2018-2019 and 2022. A sensitivity analysis was performed limiting the 2018-2019 analysis to the five currently participating centers: in this analysis the proportion severe CDI also increased, from 15.0% (11.1-18.9) to 30.3%. Key epidemiological characteristics are shown in Table 4.8.6.1.

The prevalence of the so-called hypervirulent ribotype (RT) 027 was 0.8% in 2022 (0.6% in 2018-2019). RT014/020 continued to be the most prevalent: 16.7% (19.5% in 2018-2019). Additionally, an increase in RT106 was observed from 1.5% (2018-2019) to 5.3% (2022). See Figure 4.8.6.1 for the proportions of the 5 most common ribotypes over the years in *C. difficile* sentinel surveillance samples (with a larger proportion RT106 compared to RT001 and RT015). No outbreaks were reported in 2022.

Discussion

The trend of more severe CDI cases during the second COVID-19 wave in the Netherlands has continued, and warrants further investigation.[2] It must be noted that the surveillance was continued in 2022 with five participating centers (previously 22), yet a sensitivity analysis seems to support the aforementioned conclusion. Ribotype distribution and CDI-incidence mirrored previous (pre-COVID-19 and COVID-19) years. The prevalence of RT027 remained below 1%. The sentinel surveillance program continues to be of importance to monitor circulating ribotypes and *ad hoc* typing remains relevant for early recognition of potential outbreaks. Surveillance will be especially important in the light of the current European infection control guideline (by European Society for Clinical Microbiology and Infectious Diseases) in which in low endemic settings it is acceptable to forgo contact precautions providing surveillance is in place.[3] With the 2021 update of the ESCMID CDI treatment guidance document[4] and the concept of the Dutch Working Party on Antibiotic Policy (SWAB) guideline for acute infectious diarrhoea, it is anticipated that fidaxomicin will be increasingly used to treat CDI. Fidaxomicin resistance has recently been observed in Europe in recurrent CDI after treatment with fidaxomicin.[5] Thus, monitoring of fidaxomicin resistance becomes increasingly important. Whole genome sequencing is currently being implemented for typing, this will allow for typing with higher resolution and in-depth genomic analyses.

Figure 4.8.6.1 Proportions of the 7 most common ribotypes in time in *C. difficile* sentinel surveillance samples

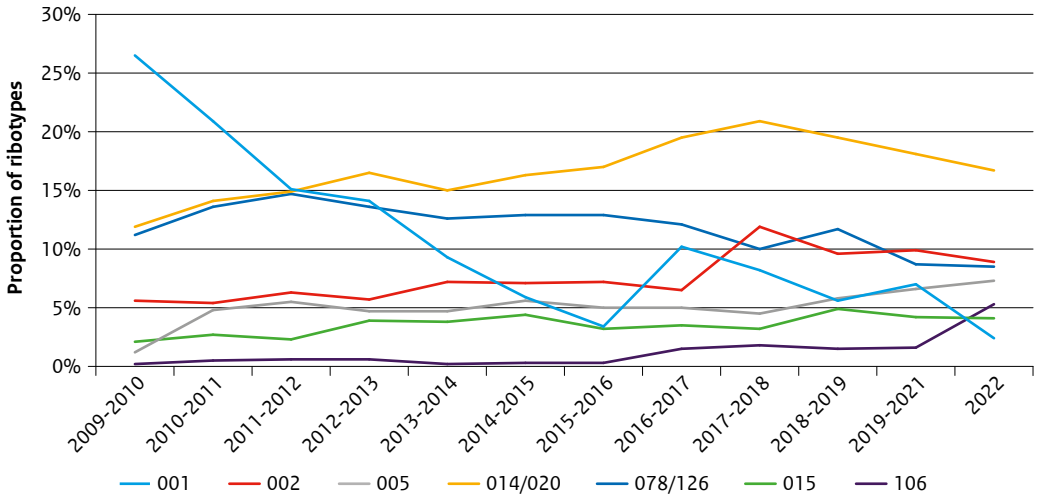

Table 4.8.6.1 Key epidemiological characteristics of *C. difficile* surveillance over the years 2009-2022

Surveillance period (May-May)	2009-2010	2010-2011	2011-2012	2012-2013	2013-2014	2014-2015	2015-2016	2016-2017	2017-2018	2018-2019	2019-2021[4]	2022[5]
Incidence												
Per 10,000 patient-days	2.7	2.8	2.9	2.9	2.9	3.0	3.1	3.0	2.9	3.1	3.2	3.2
Location of onset												
Within healthcare facility	63%	73%	69%	63%	64%	59%	58%	59%	55%	54%	55%	53%
At home	37%	27%	31%	37%	36%	41%	42%	41%	45%	46%	45%	47%
Course and outcome[1]												
Severe CDI[2]	28%	20%	27%	25%	21%	24%	21%	17%	20%	16%	21%	30%
Uncomplicated course[3]	66%	86%	87%	88%	87%	86%	89%	87%	87%	90%	89%	83%
Deaths contributable to CDI	4%	3%	4%	2%	3%	4%	2%	2%	3%	1%	2%	1%
PCR ribotype 027												
Prevalence	4.2%	2.4%	2.3%	3.4%	3.2%	0.7%	1.2%	0.6%	1.2%	0.6%	0.2%	0.9%
N reported 027 outbreaks-sentinel surveillance	1	1	0	1	0	0	0	0	0	0	0	0
N reported 027 outbreaks-ad hoc typing	2	2	1	2	5	1	0	1	0	0	0	0

[1] *Data on complicated course and mortality from between the 2nd of November 2020 until the 10th of January 2021 were excluded due to technical issues with absence of some answer possibilities, indicating missingness at random.*

[2] *Severe CDI is defined as bloody diarrhoea and/or diarrhoea with hypovolaemia or hypoalbuminaemia (<20 g/L) and/or with fever (T >38.0°C) and leucocytosis (WBC count >15x10⁹/l), and/or with pseudomembranous colitis.*

[3] *Uncomplicated course is defined as not admitted to the intensive care unit as a consequence of the C. difficile infection, no need for surgery as a consequence of the C. difficile infection and no death within 30 days after sample date.*

[4] *Sentinel surveillance period May 2019 - January 2021.*

[5] *2022 data is based on 5 laboratories.*

Conclusions

- The National Reference Laboratory for *C. difficile* with 22 participating centres transitioned to the Expertise Centre for CDI with five participating centres.
- The current surveillance set-up seems to have an adequate sample size.
- No CDI outbreaks occurred in 2022.
- There is a continued increase in severe CDI cases.
- Core genome MLST is currently being implemented for routine typing of strains.
- Antibiotic resistance monitoring in CDI surveillance has not yet been implemented but becomes increasingly important.

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4.8.7 *Aspergillus fumigatus*

Introduction

Aspergillus fumigatus is a saprobic mold that thrives on decaying plant material and may cause opportunistic fungal diseases in humans, including invasive aspergillosis. Specific host groups that are susceptible to develop invasive aspergillosis include patients with neutropenia, leukemia and (solid organ) transplant recipients. Viral associated pulmonary aspergillosis has emerged in patients with severe influenza and COVID-19, and although less patients required mechanical ventilation for COVID-19 since widespread implementation of vaccination, those that did require mechanical ventilation were more likely to present an EORTC/MSGERC host factor for invasive mold disease.[1] Mold-active triazoles (voriconazole, posaconazole and isavuconazole) are first choice antifungals to treat *Aspergillus* diseases, but response rates and survival is affected by acquired triazole resistance. In *A. fumigatus*, resistance is mainly due to isolates harboring TR_{34}/L98H or TR_{46}/Y121F/T289A mutations in the *Cyp51A* gene, which are generally pan-azole resistant. TR-mutations are associated with environmental resistance selection through exposure to triazole fungicides with activity against *A. fumigatus*. Due to high azole resistance rates, the national SWAB guideline recommends combination therapy for the treatment of invasive aspergillosis, at least in those cases where resistance cannot be demonstrated or excluded rapidly.

Methods

In five University Medical Centers and five teaching hospitals all clinical *A. fumigatus* isolates were screened for triazole resistance using a four-well agar plate (VIPcheck™, MediaProducts, Groningen, the Netherlands). Three agars contain medical triazoles, itraconazole, voriconazole and posaconazole, and one well acts as growth control. Growth on one of the triazole containing wells is highly indicative for resistance and these isolates were sent to the reference laboratory for MIC-testing and sequence-analysis of the *Cyp51A* gene. MIC testing was performed using the EUCAST microbroth dilution method and using recommended clinical breakpoints. Underlying disease information was collected for patients harboring a triazole-resistant isolate. The resistance frequency based on the number of patients screened was determined for all participating centers and compared with previous years.

Results

In 2022, *A. fumigatus* isolates from 1,542 culture-positive patients were screened for triazole resistance, including 756 (range 81 to 206 per center) patients from UMCs and 786 (range 98 to 237 per center) patients from teaching hospitals. Overall 143 azole-resistant *A. fumigatus* isolates were recovered from 118 patients. The overall resistance frequency was 7.7%, with a resistance frequency of 10.6% (80 of 756 patients) in UMCs and 4.8% (38 of 786 patients) in teaching hospitals (Table 4.8.7.1). The resistance frequency in three UMCs was above 10%, which is the recommended threshold to consider changing empirical antifungal treatment regimen to combination therapy. In all teaching hospitals the resistance frequency was below 10%, ranging from 2.0% to 7.1%.

Overall, 143 *A. fumigatus* isolates were analyzed for resistance mutations in the *Cyp51A* gene. A tandem repeat (TR_{34} or TR_{46}) was present in 118 isolates (118 of 143; 82.5%), of which 78 TR_{34} and 40 TR_{46}.

The presence of TR_{34} or TR_{46} is indicative of environmental resistance selection rather than in-host resistance development during antifungal therapy. Isolates harboring TR_{34} included 74 with TR_{34}/L98H only, while four isolates (4 of 78; 5.1%) harbored additional short nucleotide polymorphisms (SNPs). These four TR_{34}-variants were recovered from a single hospital (UMCG). Additional SNPs were found in 30 of 40 isolates (75%) with a TR_{46}, recovered from patients from seven different hospitals.

Of the 118 patients with triazole-resistant *A. fumigatus*, data regarding underlying condition was available for 107 patients. Of these, 57 (53.3%) suffered from a chronic lung disease, of which 10 patients with cystic fibrosis. Only 8 (7.5%) patients were reported to have COVID-19 pneumonia as underlying condition.

Discussion

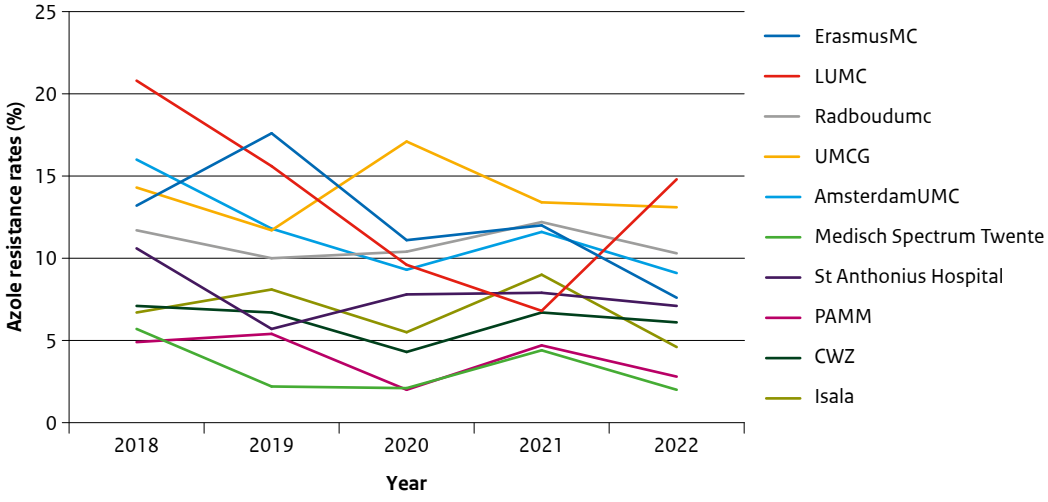
The azole resistance rates have shown overall a declining trend over the past years, although the frequency in individual centers may vary (Figure 4.8.7.1). In 2022 seven of the 10 surveillance centers had an azole resistance rate below 10%. An expert panel previously recommended that a resistance rate above 10% should prompt reconsideration of azole monotherapy as first line treatment option.[2] In the Netherlands the 2017 SWAB guideline recommends to start with combination antifungal therapy in order to cover azole resistance empirically, but declining resistance rates may require reconsideration of this recommendation. Furthermore, PCR-based tests may allow for rapid detection of resistance. A recent prospective multicenter study evaluated the performance of *Aspergillus* PCR and resistance PCR in bronchoalveolar lavage (BAL)-fluid of 323 patients with hematological malignancy.[3] *A. fumigatus* DNA was detected in 89/293 (30%) patients with sufficient DNA in the BAL fluid for PCR testing. The resistance PCR was conclusive in 58/89 (65%) and resistance detected in 8/58 (14%). Although in 35% of patients the resistance PCR was not conclusive, 6 of 8 patients with azole-resistant invasive aspergillosis were culture positive. Improving diagnostic sensitivity of resistance PCR remains an important goal, while resistance detection is also challenged by the emergence of TR-variants. TR-variants are TR_{34}/L98H or TR_{46}/Y121F/T289A isolates that contain additional SNPs or variations in the number of TR's. Due to the use of predefined PCR targets, such variations might not be detected with current resistance PCR-tests, while TR-variations may alter the azole phenotype. In 2022, variations were frequent in isolates harboring TR_{46}, where 75% of mutations included variations in the TR_{46}/Y121F/T289A background.

Table 4.8.7.1 Triazole resistance proportion in unselected clinical *A. fumigatus* isolates in 5 University Medical Centers and 5 teaching hospitals, 2018-2022

	2018		2019		2020		2021		2022	
	Screened	Azole R (%)	Screened	Azole R (%)	Screened	Azole R (%)	Screened	Azole R (%)	Screened	Azole R (%)
UMCs										
ErasmusMC	129	17 (13.2)	102	18 (17.6)	108	12 (11.1)	142	17 (12)	119	7 (7.6)
LUMC	120	25 (20.8)	90	14 (15.6)	83	8 (9.6)	103	7 (6.8)	81	12 (14.8)
Radboudumc	196	23 (11.7)	230	23 (10)	193	20 (10.4)	205	25 (12.2)	175	18 (10.3)
UMCG	238	34 (14.3)	230	27 (11.7)	181	31 (17.1)	209	28 (13.4)	206	27 (13.1)
AmsterdamUMC	81	13 (16)	51	6 (11.8)	172[a]	16 (9.3)	173	20 (11.6)	175	16 (9.1)
Total UMCs	**764**	**112 (14.7)**	**703**	**88 (12.5)**	**737**	**87 (11.8)**	**832**	**97 (11.7)**	**756**	**80 (10.6)**
Teaching hospitals										
Medisch Spectrum Twente	88	5 (5.7)	90	2 (2.2)	95	2 (2.1)	182	8 (4.4)	98	2 (2.0)
St Antonius Hospital	265	28 (10.6)	177	10 (5.7)	193	15 (7.8)	151	12 (7.9)	211	15 (7.1)
PAMM	81	4 (4.9)	147	8 (5.4)	150	3 (2)	129	6 (4.7)	141	4 (2.8)
CWZ	155	11 (7.1)	90	6 (6.7)	163	7 (4.3)	120	8 (6.7)	99	6 (6.1)
Isala	195	13 (6.7)	222	18 (8.1)	183	10 (5.5)	222	20 (9)	237	11 (4.6)
Total teaching hospitals	**784**	**50 (7.8)**	**726**	**42 (6.1)**	**784**	**37 (4.7)**	**804**	**54 (6.7)**	**786**	**38 (4.8)**

[a] *Includes both VUmc and AMC, since 2020 AmsterdamUMC.*

Figure 4.8.7.1 Trends in azole resistance rates (from left to right 2018 to 2022) in 10 Dutch surveillance centers



Conclusions

- Triazole resistance frequency in 2022 was 10.6% in UMCs and 4.8% in teaching hospitals, which represents a resistance level similar to 2020 but a declining trend over the last 5 years.
- In two of five UMCs and all teaching hospitals the azole resistance frequency was below the 10% threshold.
- Overall, 82.5% of azole-resistant isolates harbored a TR-mediated resistance mechanism, with TR-variants especially frequent in TR_{46} isolates.

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5 Antimicrobial stewardship monitor in hospitals

5.1 Antimicrobial stewardship teams in the Netherlands

Methods and results

In 2022, a web-based survey was sent to all 72 acute care hospitals in the Netherlands to assess the composition and financial support of the antimicrobial stewardship teams (A-teams) in the Netherlands and the monitoring performed by them. The results of the hospitals that completed the questionnaire (39; 54%) are presented as percentages of the responding hospitals in Table 5.1.1. The A-team characteristics are described comparing the data with the previous four years.

Table 5.1.1 Trends in A-team characteristics and monitoring between 2018 and 2022

	2018	2019	2020	2021	2022
Survey response rate, N (%)*	35 (45%)	39 (51%)	37 (51%)	60 (85%)	39 (54%)
A-team characteristics					
Presence of an A-team in responding hospitals	100%	97%	100%	100%	100%
A-team consists of at least:					
≥1 clinical microbiologist	100%	100%	97%	95%	100%
≥1 hospital pharmacist	100%	97%	100%	95%	100%
≥1 infectious disease specialist	86%	71%	76%	68%	77%
≥1 nurse	23%	21%	32%	18%	33%
≥1 infection prevention specialist	14%	16%	14%	15%	13%
Time spent on stewardship per team, median [hours per week], (range)	34.0 (4-134)	21.0 (2-144)	not available	15.0 (0-98)	16.0 (3-73)
Budget provided by hospital board of directors	79%	55%	54%	67%	77%
Financial support, median [FTE], (range)	0.7 (0.1-3.1)	0.6 (0.05-3.30)	0.9 (0.1-2.6)	not available	0.9 (0.1-2.9)
*Occasional and continuous monitoring of***					
Restricted antimicrobials****	92%	95%	not available	not available	100%
Guideline adherence empirical antimicrobial use	51%	39%	not available	not available	49%
IV-oral switch	80%	58%	not available	not available	77%
De-escalation	40%	37%	not available	not available	49%
Bedside consultation *S. aureus* bacteremia	72%	77%	not available	not available	94%
Therapeutic drug monitoring	69%	44%	not available	not available	72%
Correct diagnostics	34%	13%	not available	not available	18%
Surgical prophylaxis	14%	3%	not available	not available	15%

* *Total number of hospitals in the Netherlands has changed. Total number of hospitals in 2018: 78, in 2019: 76, in 2020: 73, in 2021: 71, in 2022: 72*

** *Meaning postprescription review for all objectives except bedside consultation and restricted antimicrobials*

*** *Includes all types of interventions to improve the use of restricted antimicrobials*

5.2 Quality of antimicrobial use

Methods

Participating hospitals
The antimicrobial stewardship monitor supports hospitals in obtaining information on the quality of antibiotic use.

Data acquisition
Data reported here were extracted from the interactive dashboard of the antimicrobial stewardship monitor. This dashboard provides benchmarked feedback information to A-teams and uses structured data already recorded in the electronic medical records (EMR). Participating hospitals were asked to provide antimicrobial prescriptions (both clinical and those started at discharge with ATC code starting with J01, J02, or J04) for all patients admitted to the hospital and had antimicrobials prescribed during admission. Participating hospitals update their data annually. The 'basic set' further consisted of date of admission, date of discharge, surgery date(s) (if applicable) and if possible indications for the prescriptions. Hospitals could also provide more data in addition to the basic set ('extensive set'). This included data on when antimicrobial drug concentrations were determined (therapeutic drug monitoring) and, if recorded as structured data in the EMR, the judgment by the A-team on whether the indications of the prescriptions were according to the local antibiotic guidelines. Here, data are shown from hospitals that provided complete data (i.e., data that contained antimicrobial prescriptions from all hospitalized patients) for 2021. At the time the data was retrieved from the dashboard, comprehensive data has been submitted by all 19 participating hospitals since 2020. Therefore, complete data from 2020 is available for comparison across all hospitals.

Indicators
We derived so-called 'proxy indicators' from the volume data. These metrics are in between pure quantity metrics and quality indicators and can suggest on the appropriateness of different aspects of antibiotic use. We included 'proxy indicators' on empiric treatment, IV-oral switch, streamlining, therapeutic drug monitoring, and surgical prophylaxis. In addition, for the hospitals that provided data on the A-team's judgement, the performance of quality indicators on the appropriateness of the indication of reserve antibiotics were calculated.

Definitions
Individual antimicrobial prescriptions included all individual oral and IV prescriptions of antimicrobial therapy. An antimicrobial course was defined as a consecutive prescription of antimicrobials with the same ATC code irrespective of route of administration and with < 24 hours between stop and start of individual prescriptions. A prescription was considered as surgical prophylaxis if it was started on the day of surgery, regardless of route of administration. Empiric treatment was defined as an antibiotic course/combination of courses started on the day of admission, which was not considered as surgical prophylaxis. Course(s) active on the day at admission were used as a proxy for empiric treatment.

Results

5.2.1 Participating hospitals

Nineteen hospitals participated (3 academic and 16 non-academic) and provided data for the basic set, including five hospitals that provided data for the extensive data set (1 academic, 4 non-academic hospitals). Only data from the basic set are shown.

5.2.2 Reserve antimicrobials

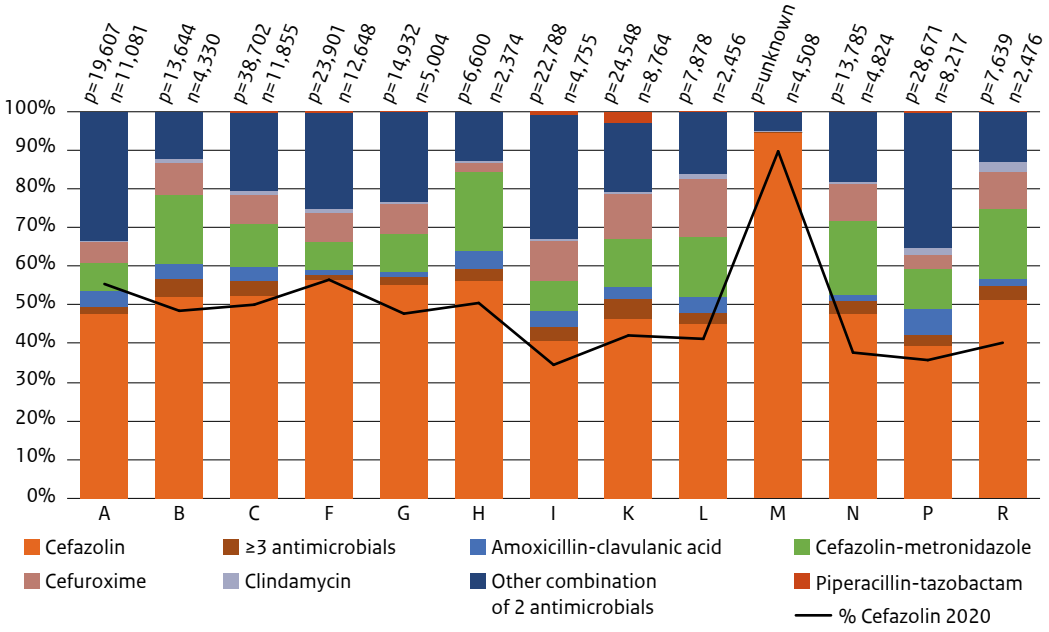
The percentage of carbapenem courses as part of total prescribed courses varied from 0% (0/6,600) to 2.9% (657/22,778) for non-academic hospitals and from 1.5% (372/24,458) to 3.6% (1,391/38,702) for academic hospitals. The percentage of quinolone courses varied from 2.3% (545/23,901) to 13.8% (2,946/21,414) for non-academic hospitals and from 5.1% (1,245/24,548) to 7.2% (1,990/27,777) for academic hospitals. Glycopeptide courses varied from 0.3% (58/16,321) to 2.5% (144/5,691) for non-academic hospitals and from 2.5% (606/24,548) to 3.5% (1,342/38,702) for academic hospitals. The percentage of amoxicillin/clavulanic acid courses as part of total prescribed courses varied from 0.35% (20/5,691) to 18.7% (3,058/16,321) for non-academic hospitals and from 6.7% (2,579/38,702) to 8.6% (2,119/24,548) for academic hospitals. The percentage of piperacillin/tazobactam varied from 0% (0/13,785) to 5.0% (1,068/21,414) for non-academic hospitals and from 1.9% (522/27,777) to 5.9% (1,439/24,548) for academic hospitals.

5.2.3 Surgical prophylaxis

The most commonly prescribed agents as preoperative prophylaxis, according to our proxy definition, are summarized in Figure 5.2.1. To compare the cefazolin use in 2021 with 2020, the percentage of cefazolin use of 2020 is depicted in the figure as well. Some of the preoperative prescriptions were missing because they were documented in a separate system. These hospitals were excluded in this analysis. Data for the remaining 13 hospitals is shown. Cefazolin was used as backbone of surgical antimicrobial prophylaxis in all hospitals.

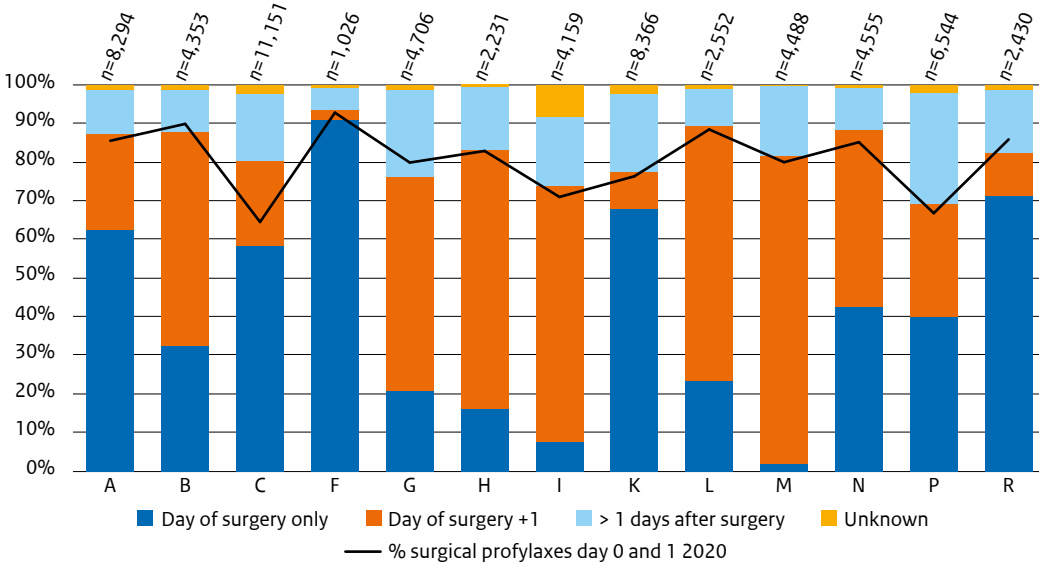
Figure 5.2.2 shows the duration of antimicrobial prophylaxis after surgery. Perioperative antimicrobial prophylaxis should generally be discontinued within 24 hours after surgery. On average, 82.3% (range 76-94%) of surgical antimicrobial prophylaxis courses were discontinued at the day of surgery or the day after. In most hospitals, this proportion in 2021 was comparable to 2020.

Figure 5.2.1 Antibiotics used for surgical antimicrobial prophylaxis in thirteen hospitals in 2021



Total number of courses used for surgical prophylaxis (n) and total number of prescribed antimicrobial courses (p) are displayed above the columns.
The black line depicts the percentage of cefazolin use in each hospital in 2020.

Figure 5.2.2 Distribution of the duration of surgical antimicrobial prophylaxis in thirteen hospitals (A-R) in 2021



Total number of courses used for surgical prophylaxis is displayed above the columns.
The black line depicts the percentage of surgical prophylaxis discontinued on day of surgery or the day after in each hospital in 2020.

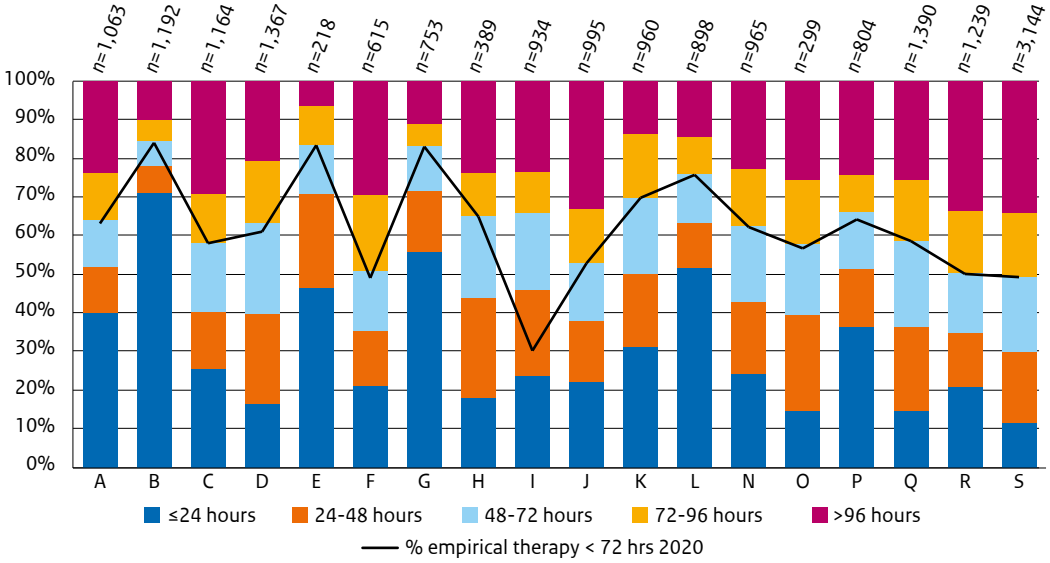
5.2.4 Intravenous to oral switch and escalation

Most hospitals in the Netherlands use either cefuroxime or ceftriaxone as empirical treatment of most infections, including sepsis of unknown origin. Figure 5.2.3 shows the duration of these antibiotic courses, and amoxicillin-clavulanic acid that was the backbone in one hospital. Courses started on the day of admission, as proxy of empiric therapy, most frequently had a duration of less than 24 hours, but there was clear variation in duration between the hospitals. Both ceftriaxone and cefuroxime as empirical therapy had a duration of less than 24 hours most frequently (36% versus 29% respectively).

Fifty-nine percent (mean, range 48-72%) of these cefuroxime/ceftriaxone/amoxicillin-clavulanic acid courses were discontinued without starting another course, while 26% (mean, range 14-41%) were switched to oral treatment and 14% (mean, range 8-22%) to other intravenous antibiotics between 24 hours before or after stop of cefuroxime/ceftriaxone (Figure 5.2.4). For the cefuroxime/ceftriaxone courses that had a duration of less than 24 hours, antibiotic courses were switched to other iv antibiotics more often compared to the cefuroxime/ceftriaxone courses that had a duration of 48-96 hours (32% versus 5%) (data available for 18 hospitals). For the cefuroxime/ceftriaxone courses that had a duration of 48-96 hours, antibiotic courses were switched to oral treatment more frequently compared to the courses that had a duration of <24 hours (39% versus 16%) (Figure 5.2.5).

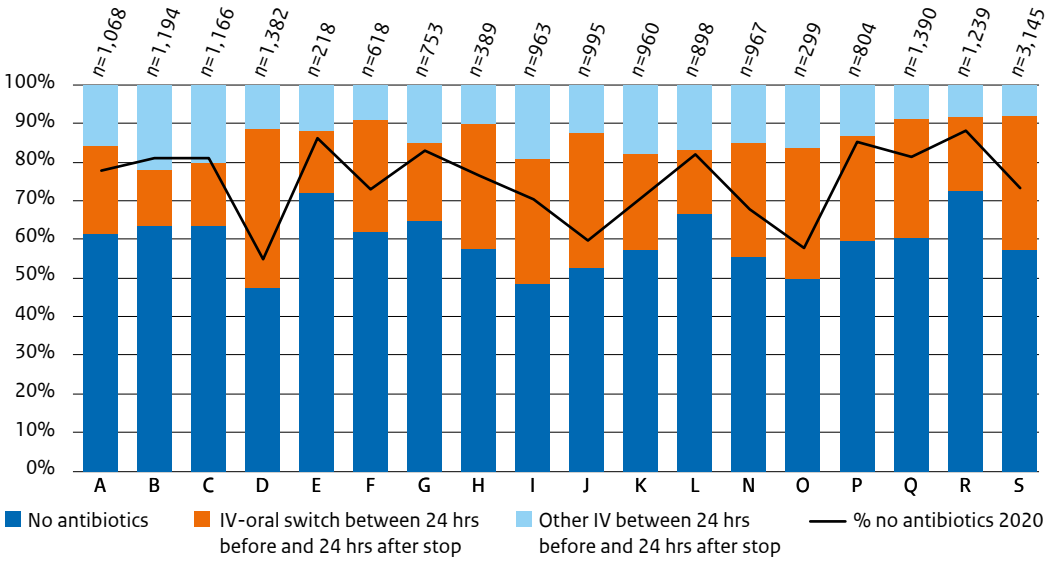
Empiric treatment of cefuroxime or ceftriaxone was only in a very small fraction of the patients escalated to an aminoglycoside-containing regimen, piperacillin/tazobactam or carbapenem, with little variation between the hospitals (Figure 5.2.6).

Figure 5.2.3 Duration of cefuroxime, ceftriaxone or amoxicillin-clavulanic acid* courses started on the day of admission ('empiric treatment') in eighteen hospitals (A-S in 2021)



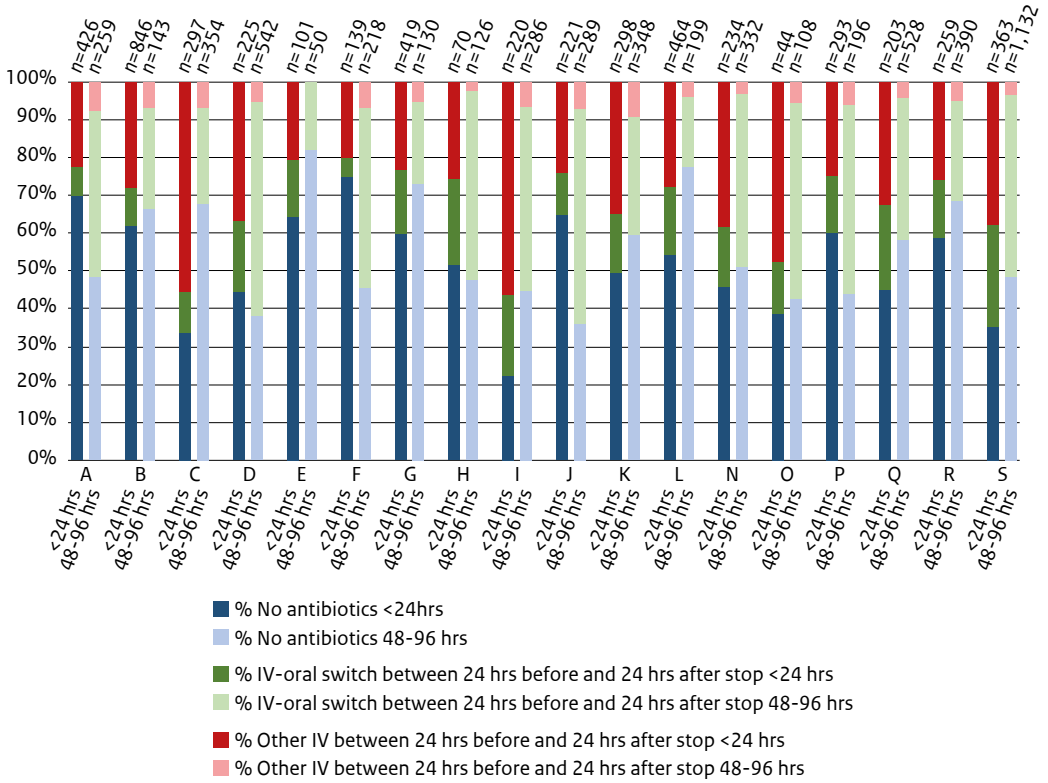
** cefuroxime, ceftriaxone or amoxicillin-clavulanic acid, depending on the preferred empiric treatment for sepsis of unknown origin.*
Total number of courses used as empirical treatment is displayed above the columns.
M: Data is lacking because of its hospital type.
The black line depicts the percentage of empiric treatment courses with a duration of < 72 hrs for each hospital in 2020.

Figure 5.2.4 Discontinuation or change to oral or other intravenous antibiotic treatment of all cefuroxime, ceftriaxone or amoxicillin-clavulanic acid* courses started on the day of admission ('empiric treatment') in eighteen hospitals (A-S) in 2021



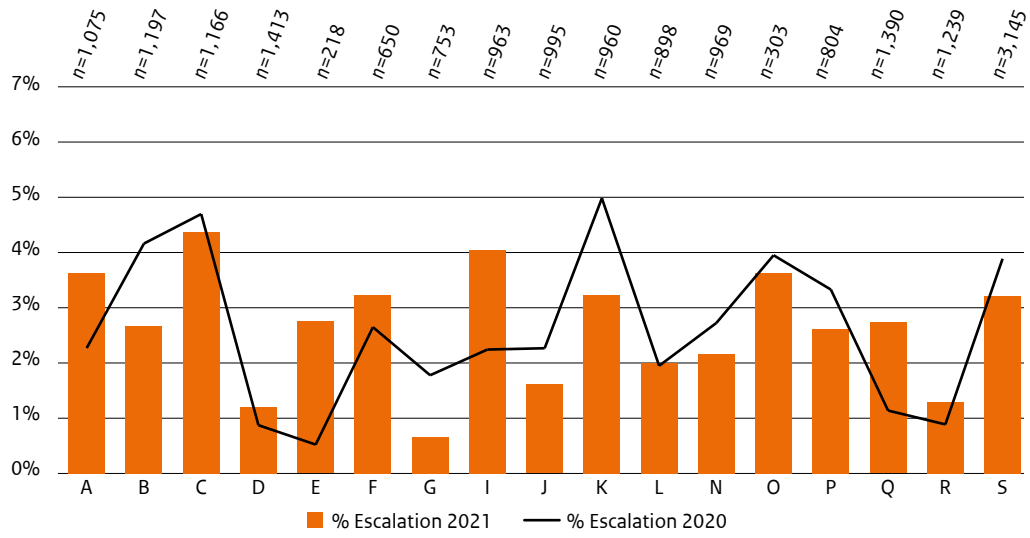
** cefuroxime, ceftriaxone or amoxicillin-clavulanic acid, depending on the preferred empiric treatment for sepsis of unknown origin.*
Total number of courses used as empirical treatment is displayed above the columns.
M: Data is lacking because of its hospital type.
The black line depicts the percentage of discontinuation of empiric treatment in each hospital in 2020.

Figure 5.2.5 Discontinuation or change to oral or other intravenous antibiotic treatment of all cefuroxime, ceftriaxone or amoxicillin-clavulanic acid* courses started on the day of admission ('empiric treatment') in eighteen hospitals (A-S) in 2021 depending on duration of initial IV therapy



** cefuroxime, ceftriaxone or amoxicillin-clavulanic acid, depending on the preferred empiric treatment for sepsis of unknown origin.*
M: Data is lacking because of its hospital type.
Total number of courses used as empirical treatment is displayed above the columns.

Figure 5.2.6 Percentage of empiric antibiotic cefuroxim or ceftriaxone courses*, started on the day of admission ('empiric treatment'), where a new course with aminoglycosides, piperacillin/tazobactam or meropenem was started between 24 and 96 hours after initiation of cefuroxim/ceftriaxon in seventeen hospitals (A-S) in 2021



** cefuroxime or ceftriaxone, depending on what the preferred empiric treatment for sepsis of unknown origin.*
Total number of courses is displayed above the columns.
H: Data is lacking since amoxicillin/clavulanic acid is used as empiric treatment for sepsis of unknown origin.
M: Data is lacking because of its hospital type.
The black line depicts the percentage of empiric treatment courses which were 'escalated' between 24 and 96 hours after initiation of cefuroxim/ceftriaxone in each hospital in 2020.

Discussion

For several years now, all hospitals have actually an A-team. The A-team's composition is more or less unchanged compared to previous years. In 2017 and 2021 response rates were > 80% and showed similar results. In addition to a clinical microbiologist and a hospital pharmacist, approximately 77% of the A-teams have an infectious disease specialist and approximately 33% a nurse. There is no further increase in the number of A-teams with a nurse. Equal to previous years, financial support remained on average less than the national staffing standard.

This is the second year that we have extracted data from the interactive dashboard of the SWAB antimicrobial stewardship monitor. This monitor provides benchmarked feedback to A-teams and uses structured data already recorded in the EMR. For 2021 the dashboard contained data from 19 hospitals compared to 7 hospitals in 2020. The results of this year's data are in line with those of the previous year.

We have derived so-called 'proxy indicators' from the volume data. For certain of these metrics, it is immediately clear that they reflect the appropriateness of different aspects of antibiotic use. For example, we used an indicator to reflect the extent to which surgical prophylaxis was given after the operation, whereas postoperative continuation is never indicated. Eighty-two percent of surgical antimicrobial prophylaxis courses were discontinued at the day of surgery or the day after, ranging from 76% to 93%. We chose the day of surgery and the day after as adequate surgical prophylaxis because in some hospitals prophylaxis prescription is continued until 0.00 AM and therefore it counts for the day after. There was variation between hospitals in duration of surgical prophylaxis and the data suggest that some hospitals may choose this a topic for an improvement intervention.

The percentage of cefazoline as surgical prophylaxis is 53% (39-95%), which is relatively low. Because we used a proxy indicator to calculate the percentage of cefazolin as surgical prophylaxis, we might have included some therapeutic prescriptions instead of prophylaxis. Data validation is important to overcome this uncertainty.

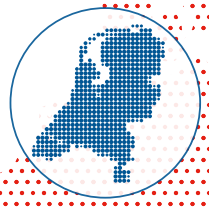
We used the antibiotic course(s) started on the day of admission and not considered as surgical prophylaxis as a proxy for empiric treatment. Focus on the cornerstone of local antibiotic policy, we described how this therapy was used. Future validation studies and linking indications to antibiotic use will provide more insight into how to interpret these data, but the variation in IV-oral switch suggests that some A-teams should perform more in-depth audits on this topic.

Conclusions

- All hospitals participating in the stewardship monitor have a formal A-team, but in 20% of hospitals the financial support for their A-team is than the standard staffing recommendation.
- Nineteen (~25%) acute care hospitals extracted structured data from the electronic medical records and provided these to the interactive dashboard of the antimicrobial stewardship monitor.
- Cefazolin was used as backbone of surgical antimicrobial prophylaxis in all hospitals.
- Based on prescriptions started on the day of surgery as a proxy for surgical prophylaxis, on average 82% (range 76-93%) of surgical antimicrobial prophylaxis courses were discontinued at the day of surgery or the day after.
- In 59% (range 48-72%) of the patients that received cefuroxime/ceftriaxone as empiric treatment upon admission, antibiotics were discontinued without starting another course.

MARAN 2023

Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2022



National Institute for Public Health and the Environment
Ministry of Health, Welfare and Sport

Netherlands Food and Consumer Product Safety Authority
Ministry of Agriculture, Nature and Food Quality

WAGENINGEN UNIVERSITY & RESEARCH

Universiteit Utrecht

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MARAN 2023

Monitoring of Antimicrobial Resistance
and Antibiotic Usage in Animals in the Netherlands
in 2022

November 2023

Contents

Colophon

This report is published under the acronym MARAN-2023 by Wageningen Bioveterinary Research (WBVR) in collaboration with the Food and Consumer Product Safety Authority (NVWA), Wageningen Food Safety Research (WFSR), the National Institute for Public Health and the Environment (RIVM), the Netherlands Veterinary Medicines Institute (SDa) and the Faculty of Veterinary Medicine, Utrecht University (FD).
The information presented in MARAN-2023 is based on total sales data and animal specific usage of antimicrobial agents in livestock and the occurrence of antimicrobial resistance and specific resistance genes in bacteria of animal origin and of relevance to public health in 2022.

MARAN-2023 is published in a combined back-to-back report with NETHMAP-2023. The combined report is available on the website of WBVR at www.wur.nl/maran. More detailed information on the usage of antibiotics per animal species is available on the website of the Netherlands Veterinary Medicines Institute (www.autoriteitdiergeneesmiddelen.nl).

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1 Summary

Antibiotic Usage

In 2022, a total of 112 tonnes of Antimicrobial Veterinary Medicinal Products (AVMPs) were sold, which is a decrease of 22.9% compared to 2021. A decrease in sales by 77.5 % over the years 2009-2022 is attained (with 2009 considered a reference year by the Dutch Government). The decreased sales of AVMPs in the Netherlands in 2022 is supported by an overall decrease in Antimicrobial use (AMU) as observed in the use monitoring data. The calculation of consumption is based on national conversion factors (DDDAs) of authorized veterinary medicinal products. The use of antibiotics of critical importance to human health care (especially cephalosporins of 3rd and 4th generation) is low, even in sectors which are not monitored for use. Use and sales of polymyxins decreased in 2022, for which the overall decrease since 2011 is 82.6% in sales. Of the fluoroquinolones, 52% is applied in sectors currently not yet monitored; an overall decrease of 93.1% since 2011 is observed.

Antimicrobial resistance

In 2022, over all *Salmonella* isolates, the highest resistance proportions were observed for nalidixic acid (27%), ciprofloxacin (26%), ampicillin (23%), sulfamethoxazole (23%), tetracycline (22%), trimethoprim (10%) and chloramphenicol (7%). Over all sources, the highest levels of resistance were observed for *S.* Heidelberg, *S.* Infantis, *S.* Paratyphi B var. Java, monophasic *S.* Typhimurium and *S.* Typhimurium. Among S. Typhimurium, no substantial changes in resistance were observed compared to previous years. Among human clinical *S.* Enteritidis isolates, an increase in the resistance against ampicillin (7% in 2021, 18% in 2022), ciprofloxacin and nalidixic acid (both 21% in 2021, 38% in 2022) was observed. In total, 14 (1.8%) ESBL-producing human clinical isolates were detected, which is more than previous years. The prevalence of ESBL-producing *Salmonella* from domestic meat is considered low. The highest frequency of ESBL-producing *Salmonella* isolates were detected in imported meat from outside the EU. No carbapenemase-producing *Salmonella* were found in 2022.

In 2022, resistance proportions in *Campylobacter jejuni* (*C. jejuni*) isolates from caecal samples of broilers and meat thereof remained at a high level for quinolones and tetracycline. Resistance to erythromycin was not detected in *C. jejuni* isolates from broilers and poultry meat, but was present at low levels in *C. jejuni*

in veal calves and *C. coli* in broilers and poultry meat. A notably higher level of erythromycin resistance was observed in *C. coli* from veal calves. In humans, resistance proportions were higher among *C. coli* than in *C. jejuni* isolates. Resistance levels of human *Campylobacter* isolates increased in 2022 compared to 2020 and 2021, when resistance most likely dropped due to a substantial reduction of travel-related campylobacteriosis as a result of the COVID-19 travel restrictions. However, resistance levels were generally still lower than before the COVID-19 pandemic. Ciprofloxacin resistance in *Campylobacter* isolates from humans was high again in 2022, which is a concern for public health. Resistance to erythromycin, first choice antibiotic in human medicine for campylobacteriosis, remained low.

In human Shiga toxin-producing *E. coli* (STEC) O157 isolates, a tendency of increasing proportions of resistance against ampicillin and trimethoprim and decreasing levels of resistance against sulfamethoxazole and tetracycline compared to 2021 was observed, which was in range with the fluctuating levels of 2011-2017 after a decrease in 2018-2020. The proportion of resistance to ciprofloxacin was higher in human STEC/EPEC non-O157 *E. coli* than in human STEC O157 in 2022. No ESBL-producing isolates were detected in STEC O157, but an O111 STEC isolate was confirmed as ESBL-producer carrying $bla_{\text{CTX-M-15}}$.

Amongst indicator *E. coli* from animals, resistance levels to ampicillin, tetracycline, sulfamethoxazole and trimethoprim were still relatively high in broilers, pigs, and (white) veal calves. In broilers, pigs and veal calves, levels of resistance stabilised for most antibiotics, whereas resistance in dairy cattle remained traditionally low. Resistance to third generation cephalosporins was low or absent amongst (randomly isolated) indicator *E. coli* from caecal samples of all animal species. Resistance to fluoroquinolones was still commonly present in indicator *E. coli* from caecal samples of broilers, in contrast to the low prevalence observed in pigs and white veal calves and the complete absence in rosé veal calves and dairy cattle. For most antibiotics tested, levels of resistance in *E. coli* from caecal samples of rosé veal calves were substantially lower than those from white veal calves. In fresh retail meat from bovine and chicken, resistance proportions in *E. coli* were similar to isolates from caecal content. In imported poultry meat, resistance proportions were substantially higher compared to fresh domestic retail meat. For the first time, resistance monitoring was performed in rabbits, showing high levels of resistance to tetracycline, sulfamethoxazole and trimethoprim, while resistance to other antibiotics was low or absent.

The prevalence of extended-spectrum cephalosporin (ESC) resistance in randomly selected *E. coli* has been steadily low for several years in all livestock species. While the prevalence of selectively isolated ESC-resistant *E. coli* remained stable or decreased in most livestock sectors, in dairy cattle, an increase was measured compared to previous years. The inclusion of imported meat to the monitoring has shown that the prevalence of ESC-resistant *E. coli* on imported chicken and turkey meat is substantially higher than for domestic meat products.
As in 2021, Whole Genome Sequencing of ESC-resistant *E. coli* shows that over 20% of isolates are clonally related. In 2022, clones were also detected that are shared between livestock sectors. In 2022, no carbapenemase-producing Enterobacterales (CPE) were detected in livestock, but on one occasion an OXA-48-producing *E. coli* was identified in a faecal sample of a dog. As in former years, the prevalence of *mcr* genes, encoding for colistin resistance, in *E. coli* was low in livestock and meat. In 2022, over 25% of the investigated veal calf farms were tested positive for MRSA. The incidence of MRSA on farms rearing white veal calves was significantly higher than that of those rearing rosé veal calves.

It can be concluded that antibiotic reduction policies in the Netherlands has resulted in more than 77% reduction of sales of Antimicrobial Veterinary Medicinal Products for veterinary use since 2009. Antimicrobial resistance has decreased simultaneously in isolates from most livestock species. In spite of the AMU reduction continuous high levels of resistance are observed for fluoroquinolones and tetracycline in *Campylobacter* isolates from humans and poultry. ESBL and colistin-resistance remain present at low levels, while no CPE was detected in samples from livestock or meat.

2
Usage of antibiotics in animal husbandry in the Netherlands

Sales and use of antimicrobial veterinary medicinal product (AVMPs) are monitored by the Netherlands Veterinary Medicines Institute (SDa, Diergeneesmiddelenautoriteit). The information described in this part of MARAN is presented in more detail in the annual reports of the SDa (https://www.autoriteitdiergeneesmiddelen.nl/en/publications/general-reports).

2.1 Total sales of veterinary antibiotics in the Netherlands 2022

2.1.1 Analysis of sales data

FIDIN, the federation of the Dutch veterinary pharmaceutical industry, provided sales data for all Antimicrobial Veterinary Medicinal Products (AVMPs) on package level sold in 2022 in the Netherlands, as extracted from the Vetindex and supplemented with AVMPs data of non-FIDIN members. These data are estimated to cover approximately 98% of all sales in the Netherlands, according to FIDIN. 3.6% (in mass) of the sold AVMPs (including all administration forms like tablets and injectables) is exclusively authorized for companion animals or horses. AVMPs that are marketed in accordance with legal exemptions such as products that are imported from other EU member states in accordance with cascade legislation, are not included. Actual use in animal husbandries can be somewhat different from the quantities sold due to stock piling and cross border use. Monitored mass used in the major livestock farming sectors (pigs, broilers, turkey, other poultry, veal calves, dairy- and other cattle, meat rabbits) covered 96.8% of total sales in 2022. This coverage fluctuates over the years, due to not yet monitored sectors (e.g. sheep, horses, companion animals) and stockage differences between the years.

AVMPs are reported as active base substance mass (excluding mass of salts and esters), including oral products, injectables, intramammary injectors and topical applications like ointments, eye drops and sprays. The sales data in this report involves total sales for all animals, not stratified by animal species. Detailed information about antibiotic usage by animal species in the Netherlands is reported in paragraph 2.2.

2.1.2 Trends in total sales

Table 1 shows the trends in the total sales of antibiotics licenced for therapeutic use in animals in the Netherlands. In 2022 in total 112 tonnes of AVMPs were sold, which is a decrease of 22.9% compared to 2021. A decrease in sales by 77.5 % over the years 2009-2022 is attained (with 2009 considered the reference year by the Dutch Government).

Figure 1 shows the trends in sales (mass, black line) in relation to the dynamics of liveweight of Dutch livestock (dashed line) and the total use on farms (mass, bars) in the livestock sectors monitored, from 2009 to 2022. Antimicrobial use (in kg) in livestock sectors is presented as bars in which the use in different animal species can be distinguished. Figure 1 shows a slightly decreasing trend in liveweight of Dutch livestock. Compared to 2009 the liveweight of Dutch livestock has decreased by around 10%. The decrease in antimicrobial use is much greater, demonstrating that trends in total mass sold and used cannot be explained by a drop in the liveweight of Dutch livestock. Veal calves (light blue) and pigs (green) used almost 80% of the total mass of all antibiotics used for therapy. Animals treated in these two sectors are large and therefore need more antibiotics per administration than small animals like broiler chickens. This illustrates that sales data provide limited information about exposure of animals at risk. Use data based on mass may result in the suggestion that exposure of broiler chickens to antibiotics is limited based on the small proportion of total mass used in these animals.

The discrepancy in mass in 2022 between sales and usage in monitored sectors was 3.2% as illustrated in Figure 1. The difference between sales and use data fluctuates as described by the difference between the solid black line (mass sold) and bars (mass used in monitored sectors).

As demonstrated in Figure 2, antimicrobial sales by antibiotic class show a fluctuating pattern over the years, with an overall decreasing tendency in most antibiotic classes, and some variation from year to year.

Penicillins
First place in mass for the first time, although sales of penicillins (including aminopenicillins) are still decreasing in 2022 compared to 2021, with 14.7%. The distribution of broad and narrow spectrum penicillins (in mass sold) is comparable to previous years with 70% aminopenicillins.

Tetracyclines
Second place when expressed in mass are the tetracyclines, the sales in 2022 have sharply decreased with 33.6% compared to 2021. The fraction of doxycycline (not specified in Figure 2) was in 2022 55.9% of the total sales of tetracyclines (67.8% in 2021, fluctuations between 31% and 69% in the years 2011-2020).

(Fluoro)quinolones

The sales of fluoroquinolones decreased again with 16kg (13.9%) in 2022. An overall reduction of 93.2% was realized since 2011. In 2022, 48% of the sold AVMP's were applied in the monitored sectors. Extending monitoring to other animal species (as is regulated with EU 2019/6) is warranted. The sales of quinolones (flumequine) increased with 17.4% in 2022 when compared to 2021; these AVMPs are exclusively applied in food producing animals, and partly substitute the use of colistin. Although the EMA Antimicrobial Advice ad hoc Expert Group (AMEG) decided not to differentiate between quinolones and fluoroquinolones (both category B), in the Netherlands quinolones are still classified at a level of lower importance (2nd line, comparable with category C) than fluoroquinolones (3rd line, comparable with category B). This discrepancy will be evaluated in the near future for the Dutch situation.

Cephalosporins 3rd/4th generation

Sales of these AVMPs were relatively stable at a low level since 2016, fluctuating in kg range. In 2021 a marked increase was observed in sales data, to 5.3kg, which was halved to 2.6kg again on 2022. A reduction of 99.7% of cephalosporins 3rd/4th generation sales has been achieved since 2011.

Polymyxins

Colistin sales decreased in 2022 again, with 26.8%. The reduction since 2011 is 82.6%. Based on the classification of polymyxins as *Highest Priority Critically Important Antimicrobials* (CIAs) in the 6th revision of the WHO CIA list (2019), the *Expert Panel of the Netherlands Veterinary Medicines Institute* considers polymyxins as third choice antibiotics, and this antibiotic class is reported as such. This implies that similar as for fluoroquinolones and 3rd/4th generation cephalosporins the Dutch target for use since 2020 is 0 DDDAF. The ESVAC group introduced in 2016 the colistin desirable-level-benchmark for EU member states. This benchmark is below 1 mg/PCU for sales data, irrespective of the sectors in which colistin is used. Netherlands is below that unified benchmark, but for some sectors (laying hens) specific use data show differently. Moreover, many farms have zero colistin usage, this proportion of zero-use is increasing over years.

2.2 Usage in pigs, veal calves, cattle, broilers, turkeys and rabbits in the Netherlands

In Figure 3, antimicrobial use (AMU) based on annual prescription data is presented for each livestock sector. Important reductions in AMU have been achieved in all sectors (Figure 3) since monitoring of AMU was established.

Figure 4 shows that in most sectors first choice antimicrobials (green and blue bars) are dominant. In most sectors, except for pigs, broilers and turkeys, this proportion of first choice AVMP's has attained a stable level, at over 80%. Figure 4 also illustrates that use of fluoroquinolones (red bar) is the highest in turkeys, although a reduction of 87% has been observed since 2013. In veal calves, a large sector using 86% first choice AVMP's, the steady decrease in total use was halted in 2020, and in 2022 the use increased with 5.6%. In rabbits, the use of colistin was abandoned in 2020, at the cost of introducing flumequine. Total AMU in this sector has attained a huge reduction in 2022, the use almost halved since 2018.
Expressing antibiotic use in number of Defined-Daily Dosage Animal like in Figure 3 and 4 shows that AMU in broilers and in pigs is comparable in number of DDDA, although the distinct differences in applied antibiotic classes is notable.

For more details about all animal sectors, annual reports of the SDa should be consulted (https://www.autoriteitdiergeneesmiddelen.nl/en/publications/general-reports).

EU regulation 2019/6 (VMP-reg)
EU Regulations about, amongst others, monitoring of veterinary antimicrobial use, starting from 2023 (reporting in 2024) will be implemented in national legislation for all EU member states, coming into effect since January 28th 2022. Sales data will have to be reported to EMA, as is already in place for most EU member states in the ESVAC project. Additionally, use data will have to be reported, starting with pigs, cattle and broilers in 2024 (with regard to 2023 use data). Monitoring of sales and use data may be expanded from anti*bacterial* substances to anti*microbial* substances including antimycotic, antifungal, antiviral and anticoccidial substances. Cascade use of products imported from other EU countries will have to be incorporated in sales (and use) data.
In 2026, monitoring of use of indicated products will be extended to rabbits, sheep, goats, ducks, geese, finfish and horses. Most of these sectors are already preparing the implementation of a monitoring system, rabbits are already included in the Dutch AMU monitoring. In 2029 the use of these products will also be monitored in cats and dogs. For horses and companion animals cascade use of antimicrobial medicinal products for human use will have to be included as well in the use monitoring.

Conclusion
Maximal transparency has been created since 2011 through monitoring antibiotics use by veterinarians and farmers. The decreased sales of AVMPs in the Netherlands in 2022 is supported by an overall decrease in AMU as observed in the use monitoring data. The calculation of consumption is based on national conversion factors (DDDAs) of authorized veterinary medicinal products.
The use of antibiotics of critical importance to human health care (especially cephalosporins of 3rd and 4th generation) is low, even in the unmonitored sectors. Use and sales of polymyxins decreased in 2022, overall decrease since 2011 is 82.6% in sales. Of the fluoroquinolones, 52% is applied is sectors not yet monitored; an overall decrease 93.1% since 2011 is observed.

Table 1 Antimicrobial veterinary medicinal product sales from 1999-2022 in kg (thousands) (FIDIN, 2022)

Year	'99	'00	'01	'02	'03	'04	'05	'06	'07	'08	'09	'10	'11	'12	'13	'14	'15	'16	'17	'18	'19	'20	'21	'22
Betalactam antibiotics	35	36	38	38	36	43	51	57	61	70	73	71	66	54	45	48	45	39	42	43	36	40	34	29
Tetracyclines	162	194	200	214	216	256	292	301	321	257	251	217	157	102	80	69	82	62	68	65	51	49	47	31
Macrolides & lincosamides	10	15	17	19	17	23	28	42	55	52	46	39	34	26	25	28	23	23	25	25	23	24	21	19
Aminoglycosides	13	12	11	10	9	9	11	11	12	11	10	8.6	7.3	5.8	3.4	1.8	2.7	2.1	1.9	2.0	1.8	1.7	1.8	1.5
(Fluoro) quinolones	7	7	6	6	5	7	8	7	9	8	8	6.6	5.1	3.1	2.8	3.8	4.2	3.4	3.4	3.9	2.7	2.6	2.1	2.4
Trimethoprim/ sulfonamides	72	80	92	92	88	91	91	93	99	100	92	78	58	48	53	49	42	39	34	33	29	30	32	22
Other antibacterials	11	12	11	11	7	6	6	8	8	7	15	13	10	10	8.1	7.8	7.5	7.4	7.2	7.5	7.4	7.2	6.9	6.3
Total sales	310	356	376	390	378	434	487	519	565	506	495	433	338	249	217	207	206	176	181	179	150	154	145	112

Figure 1 Mass balance of AVMPs sales data (black line, left y-axis) and use data (colored bars, left x-axis, kg), combined with total live weight of the food animal population (dotted line, right y-axis, 1,000 kg) from 2009-2022

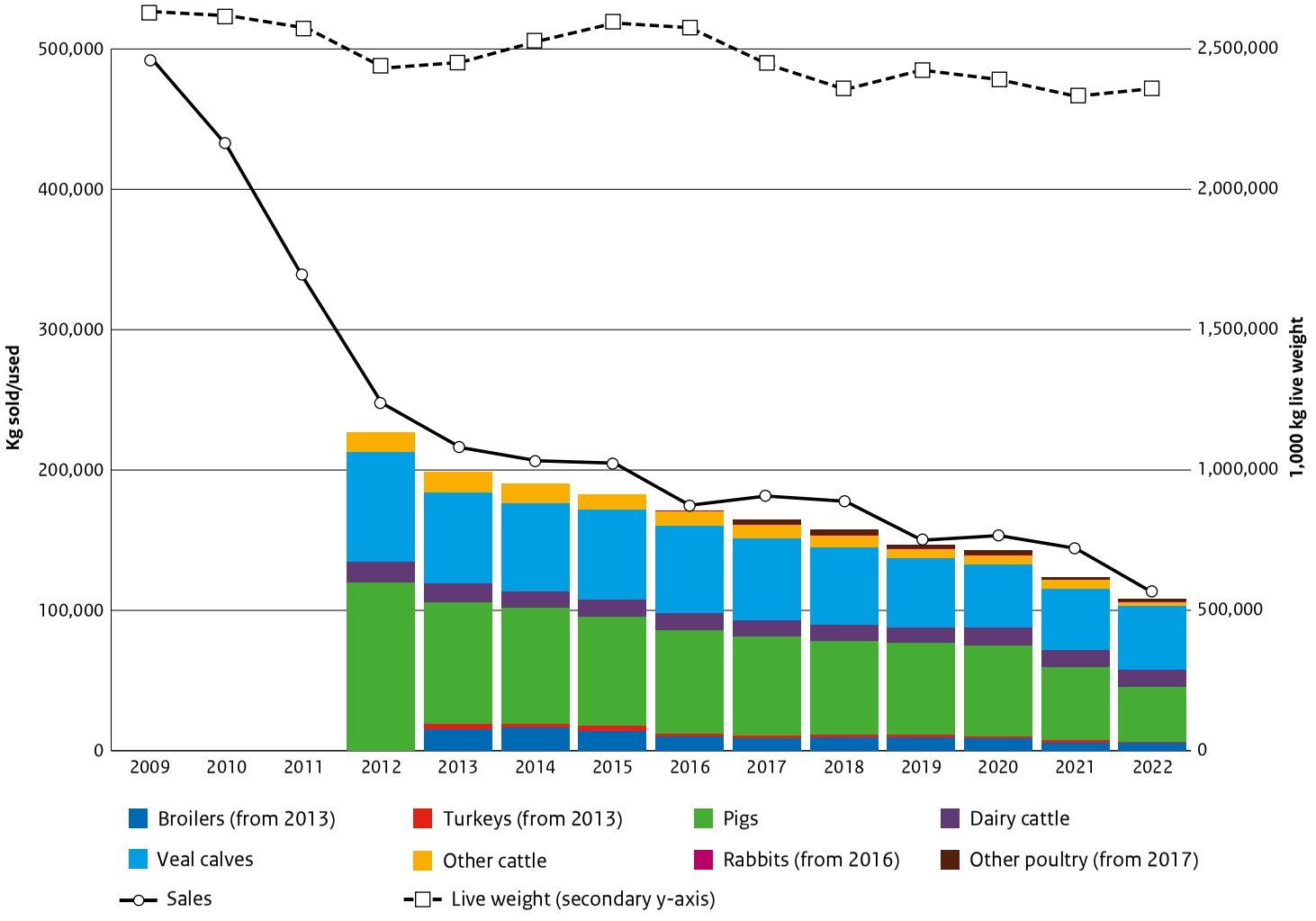


Figure 2 Antimicrobial Veterinary Medicinal Product sales by antibiotic class from 2011-2022 in kg (thousands)

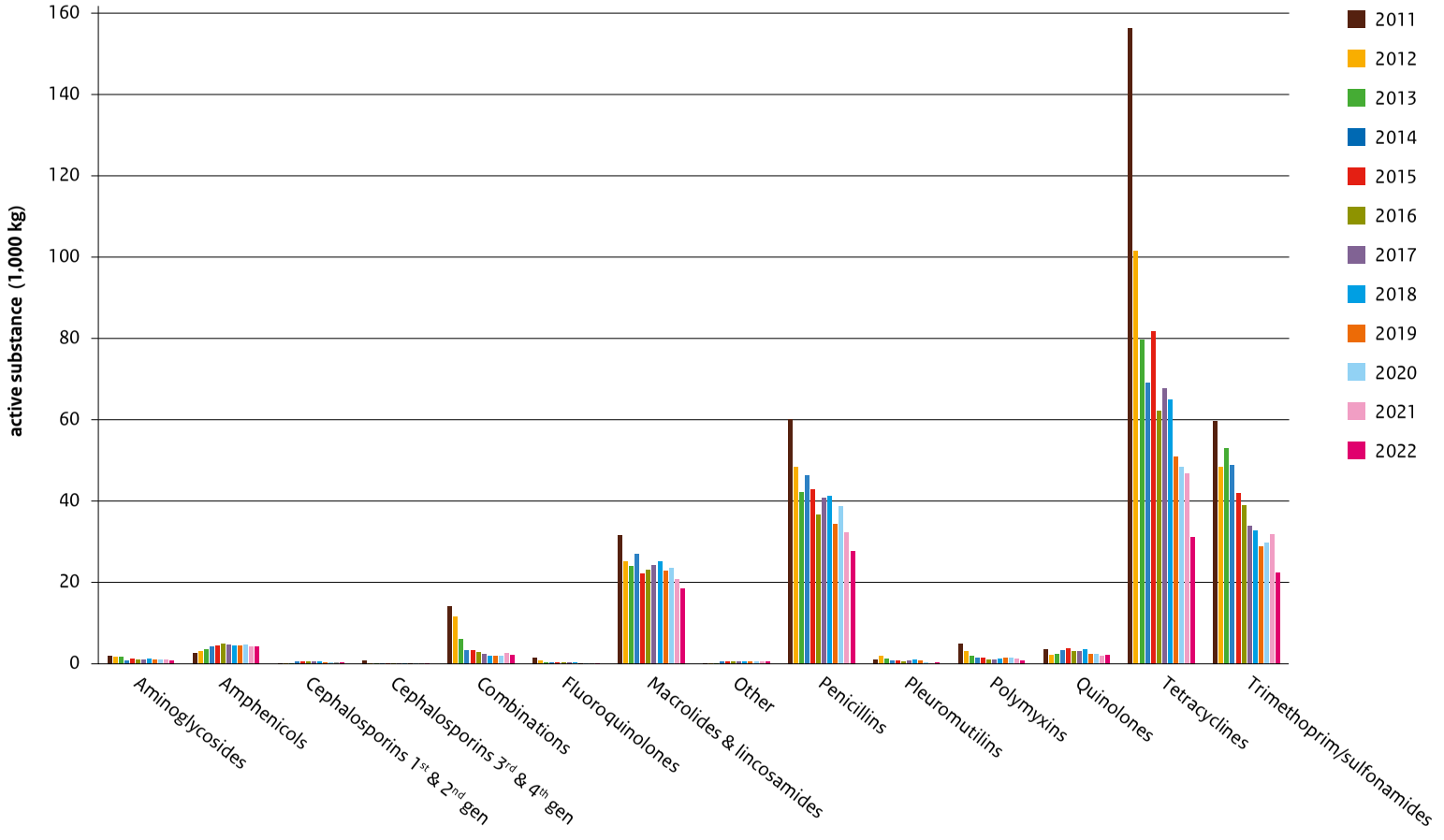


Figure 3 Number of animal-defined daily dosages per animal-year for rabbits (grey), turkeys (purple), veal calves (blue), broilers (orange), pigs (light green) and dairy cattle (dark green) farms as reported by LEI WUR-MARAN (years 2007-2010 as DD/AY) and by SDa (years 2011-2022 as $DDDA_{NAT}$) depicting point estimates (dots), 95% confidence limits (error bars), smoothed trend line (penalized spline) and 95% confidence limits for the spline (shaded area)

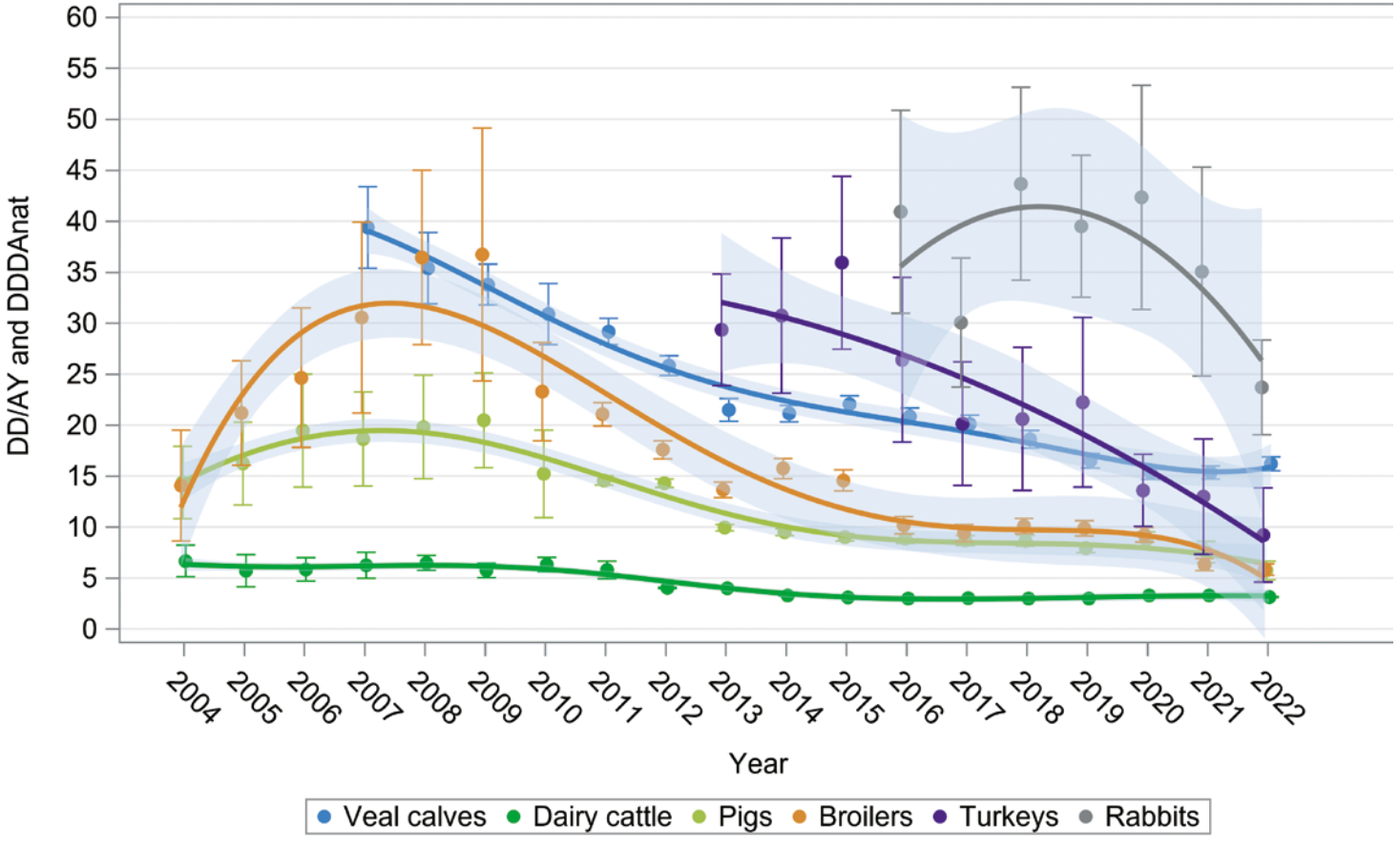
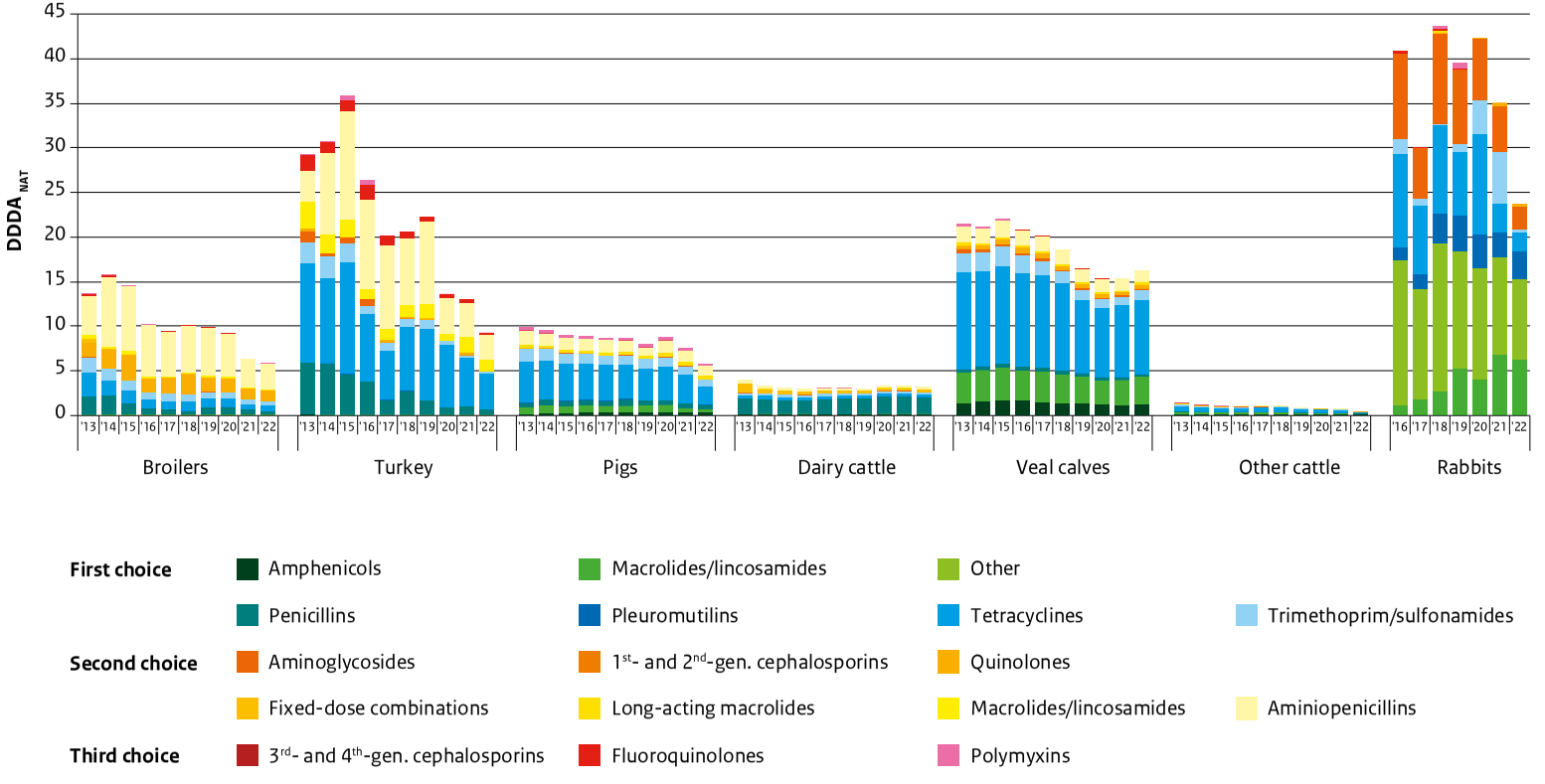


Figure 4 Number of $DDDA_{NAT}$ per animal-year of antimicrobial veterinary medicinal products specified by antibiotic class per animal sector over the years 2013-2022



3
Resistance data

This chapter describes susceptibility test results as determined in 2022 for the food-borne pathogens *Salmonella enterica* subsp. *enterica*, *Campylobacter* spp., *Escherichia coli* O157 and the commensal organism *E. coli*. Epidemiological cut-off values (www.eucast.org) were used for the interpretation of minimum inhibitory concentrations (MIC). Epidemiological cut-off (ECOFF) values are in most cases lower than clinical breakpoints; therefore, depending on the antibiotic in question, non-wild-type susceptible isolates (i.e. isolates displaying MICs above the ECOFFs) cannot automatically be classified as clinically resistant. For the purpose of this report, we designated all non-wild-type susceptible isolates as "resistant", and specified this per antibiotic if necessary.

3.1 Food-borne pathogens

3.1.1 *Salmonella*

This chapter presents resistance percentages of *Salmonella* isolates. These isolates were obtained from human salmonellosis patients, food-producing animals, food products of animal origin and other food products as potential sources of infection for humans via the food chain, and animal feed as potential source of infection for food-producing animals.

Highlights

1. Over all isolates, the highest resistance proportions were observed for nalidixic acid (27%), ciprofloxacin (26%), ampicillin (23%), sulfamethoxazole (23%), tetracycline (22%), trimethoprim (10%) and chloramphenicol (7%).
2. Over all sources, the highest levels of resistance were observed for *S.* Heidelberg, *S.* Infantis, *S.* Paratyphi B var. Java, monophasic *S.* Typhimurium and *S.* Typhimurium.
3. Over all *Salmonella* isolates tested in 2022 (n=1156), resistance against quinolones (ciprofloxacin and nalidixic acid) was 10% higher than previous years.
4. Among *S.* Typhimurium, no substantial changes in resistance were observed compared to previous years.
5. Among human clinical *S.* Enteritidis isolates, an increase in the resistance against ampicillin (7% in 2021, 18% in 2022), ciprofloxacin and nalidixic acid (both 21% in 2021, 38% in 2022) was observed.
6. In total, 14 (1.8%) ESBL-producing human clinical isolates were detected, which is more than previous years.
7. In 2022, no carbapenemase-producing *Salmonella* were found.

Resistance proportions overall

A selection of all human *Salmonella* isolates received by the RIVM from regional public health and other clinical laboratories (n = 762) was sent to WBVR for susceptibility testing. Moreover, 394 isolates from non-human sources were tested. These included isolates from broilers (n = 211), cattle (n = 36), pigs (n = 24), and layers (n = 42), as well as isolates from a diversity of other sources, including animal feed (n = 8), vegetables and other animals (e.g. sheep, goats, rabbits, ducks, etc., n = 73). The non-human isolates included also 128 isolates from food products (e.g. meat and products thereof) analysed for antibiotic susceptibility by WFSR, the official food safety laboratory of the NVWA. Non-human isolates were mainly sent to the RIVM by the Animal Health Services in Deventer from a diversity of surveillance programs and diagnostic activities for clinical infections in animals, or they were obtained from WFSR (mainly non-clinical isolates) through its routine *Salmonella*-control activities on farms, slaughterhouses (e.g. EC/2073.2005 verification projects broiler neck skin) and food products sampled at retail. *Salmonella* isolates from primary poultry farms (n=157) and from imported fresh poultry meat gathered at border control points (n=41) were tested by WBVR and WFSR, respectively, in line with Decision (EU) 2020/1729).

MIC distributions and resistance percentages of 1156 *Salmonella* isolates from different sources (human and non-humane) tested for susceptibility in 2022 are presented in Table S01. The highest resistance proportions were observed for nalidixic acid (27%), ciprofloxacin (26%), ampicillin (23%), sulfamethoxazole (23%), tetracycline (22%), trimethoprim (10%), and chloramphenicol (7%). No resistance was detected to the carbapenem antibiotic meropenem and low proportions of resistance (<5%) were found for tigecycline, azithromycin, cefotaxime, ceftazidime, and gentamicin.

The class of fluoroquinolones is regarded as the treatment of choice for severe salmonellosis in human adults. Currently, EUCAST recommends a clinical breakpoint of 0.06 mg/L for *Salmonella enterica*, based on clinical evidence that there is a poor therapeutic response in systemic infections caused by *Salmonella* spp. with low-level ciprofloxacin resistance (MIC >0.06 mg/L) (www.eucast.org). Using the EUCAST recommended epidemiological cut off value of 0.06 mg/L as breakpoint, 26% of *Salmonella* isolates from 2022 demonstrated an acquired resistance phenotype for ciprofloxacin (Table S01), which is 10% higher than previous years. Overall very high levels of ciprofloxacin resistance were observed for *S.* Heidelberg (96%) and *S.* Chester (86%) (Table S02). High levels were also detected for *S.* Infantis (58%) and *S.* Paratyphi B var. Java (39%), and *S.* Enteritidis (34%). Medium to low levels were observed for monophasic *S.* Typhimurium (21%) and *S.* Typhimurium (7%). Similar patterns were observed for the quinolone nalidixic acid. Table S05 shows that the resistance proportions against (fluoro)quinolones among isolates from broiler meat increased from just below 60% in 2021 to 76% in 2022 when combining Dutch/EU and imported broiler meat. However, this increase is partly due to differences in relative abundance of isolated serovars, with a high number of *S.* Heidelberg isolates (n=22, of which 18/22 were isolated from imported meat, and 4 from human cases) in 2022 (n=22).

Table S01 MIC distribution (in %) and resistance percentages (R%) for all *Salmonella* isolates (N=1,156) tested for antibiotic susceptibility during 2022

Salmonella N = 1,156	MIC (%) distribution mg/L																		R%	95% CI
	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048		
Ampicillin							30.9	41.0	4.7	0.4	0.3	0.2	22.6						23.4	21.0 - 25.9
Cefotaxime					92.5	3.4	0.2	0.0	0.0	4.0									4.2	3.0 - 5.3
Ceftazidime					45.7	43.3	7.1	0.0	0.2	0.4	3.4								3.9	2.8 - 5.0
Gentamicin						89.5	7.1	0.7	0.2	0.3	0.4	1.8							2.7	1.7 - 3.6
Tetracycline								73.8	4.2	0.1	0.3	0.1	21.5						21.9	19.5 - 24.3
Sulfamethoxazole										38.2	21.5	10.7	5.9	0.4	0.1	0.0	23.3		23.3	20.8 - 25.7
Trimethoprim					68.1	20.7	1.2	0.1	0.2	0.0	0.0	9.8							10.0	8.2 - 11.7
Ciprofloxacin	17.7	53.1	2.9	2.5	11.7	9.1	1.2	0.4	0.6	0.3	0.5								26.2	23.7 - 28.8
Nalidixic acid									67.7	5.7	3.3	4.2	0.6	18.5					26.6	24.0 - 29.1
Chloramphenicol										83.6	9.8	0.7	0.2	5.8					6.7	5.2 - 8.1
Azithromycin*								0.6	19.9	70.8	7.2	0.8	0.4	0.4					1.6	0.8 - 2.3
Colistin**							70.7	13.8	9.0	6.5	0.0								-	-
Meropenem		70.7	29.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0								0.0	0.0 - 0.3§
Tigecycline***					55.5	34.1	7.8	2.2	0.5	0.0									2.7	1.7 - 3.6
Amikacin					0.0	0.0	0.0	0.0	99.7	0.3									0.3	0.0 - 0.6

The white areas indicate the dilution range tested for each antimicrobial agent. Values above this range indicate MIC values > the highest concentration in the range. Values at the lowest concentration tested indicate MIC-values ≤ the lowest concentration in the range. Vertical bars indicate the epidemiological cut-off values (ECOFF), used as breakpoints. If available, dashed bars indicate the clinical breakpoints. For ampicillin, ciprofloxacin and chloramphenicol the ECOFF and clinical breakpoints are identical.

* *tentative set ECOFF during the EURL AMR WP meeting on 25 April 2015 in Lyngby (DK).*

** *Because of differences in natural susceptibility for colistin between serovars there is no general Salmonella ECOFF available for colistin. For this reason the percentage of resistance is not depicted*

*** *Since 2019 the ECOFF is no longer available for Salmonella. The former defined ECOFF of EUCAST for tigecycline was used for monitoring purposes in 2018.*

§ *One-sided, 97.5% confidence interval*

Table S02 Resistance (%) of the most prevalent *Salmonella* serovars (>20 isolates) in the Netherlands in 2022 (N tested)

	Enteritidis (271)	Typhimurium monophasic (98)	Typhimurium (89)	Infantis (88)	Typhi (72)	Dublin (44)	Virchow (27)	Paratyphi B vr. Java (33)	Agona (22)	Heidelberg (22)	Chester (21)
Ampicillin	15.5	88.8	44.9	10.2	4.2	4.5	0.0	30.3	18.2	95.5	14.3
Cefotaxime	0.0	3.1	1.1	4.5	2.8	0.0	0.0	0.0	0.0	81.8	0.0
Ceftazidime	0.0	1.0	0.0	4.5	2.8	0.0	0.0	0.0	0.0	81.8	0.0
Gentamicin	0.0	11.2	4.5	3.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Tetracycline	7.4	81.6	33.7	53.4	0.0	4.5	0.0	3.0	22.7	95.5	9.5
Sulfamethoxazole	6.6	80.6	32.6	58.0	2.8	0.0	3.7	60.6	4.5	86.4	9.5
Trimethoprim	1.1	16.3	5.6	39.8	2.8	0.0	0.0	75.8	13.6	13.6	14.3
Ciprofloxacin	33.9	21.4	6.7	58.0	11.1	2.3	14.8	39.4	27.3	95.5	85.7
Nalidixic acid	34.3	21.4	5.6	59.1	11.1	2.3	14.8	45.5	22.7	95.5	85.7
Chloramphenicol	1.1	22.4	15.7	4.5	2.8	0.0	0.0	0.0	18.2	9.1	14.3
Azithromycin	0.7	6.1	1.1	1.1	0.0	0.0	0.0	0.0	4.5	0.0	4.8
Meropenem	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Tigecycline	0.0	10.2	1.1	8.0	0.0	0.0	0.0	0.0	0.0	13.6	4.8
Amikacin	0.0	0.0	1.1	0.0	0.0	0.0	3.7	0.0	0.0	0.0	4.8

Resistance proportions of the most prevalent serovars

The resistance patterns of *S.* Typhimurium and monophasic *S.* Typhimurium, which are both primarily associated with pigs and cattle as a reservoir, among human and non-human isolates are shown in Table S03. Resistance remained high for ampicillin, sulfamethoxazole, and tetracycline, especially among monophasic *S.* Typhimurium. No obvious changes in resistance in human *S.* Typhimurium isolates were observed compared to previous years (Figure S01). Resistance to the clinically important drug cefotaxime was only detected among human isolates.

In the Netherlands, *S.* Enteritidis is mainly associated with layers and their produced eggs. Fractions of resistance in *S.* Enteritidis are generally lower compared to *S.* Typhimurium. However, an increase in resistance against ampicillin (7% in 2021, 18% in 2022), ciprofloxacin and nalidixic acid (both 21% in 2021, 38% in 2022) was observed among human isolates (Table S04 and Figure S02). Interestingly, in contrast to 2021, resistance against ciprofloxacin and nalidixic was not observed among isolates from layers while the level of resistance increased considerably among isolates from broilers (4% in 2021, 44% in 2022). However, the numbers of non-human isolates are relatively small and changes over time in the fraction of resistance should be interpreted with caution.

Table S03 Resistance percentages of *S.* Typhimurium (N tested), including its monophasic variant, isolated from humans, cattle, pigs and other known sources in 2022

	S. Typhimurium (89)[a]	
	Humans (71)	Non-human sources (18)[b]
Ampicillin	45.1	44.4
Cefotaxime	1.4	0.0
Ceftazidime	0.0	0.0
Gentamicin	0.0	22.2
Tetracycline	31.0	44.4
Sulfamethoxazole	29.6	44.4
Trimethoprim	2.8	16.7
Ciprofloxacin	5.6	11.1
Nalidixic acid	5.6	5.6
Chloramphenicol	12.7	27.8
Azithromycin	1.4	0.0
Meropenem	0.0	0.0
Tigecycline	1.4	0.0
Amikacin	0.0	5.6

a Monophasic variant is excluded.

b Non-human sources include broilers (5), layers (4), pigs (2), goats (2), feed (2), cattle (1), ducks (1) and unspecified animals (1).

Figure S01 Trends in resistance (%) of *S.* Typhimurium isolated from humans in 2000-2022

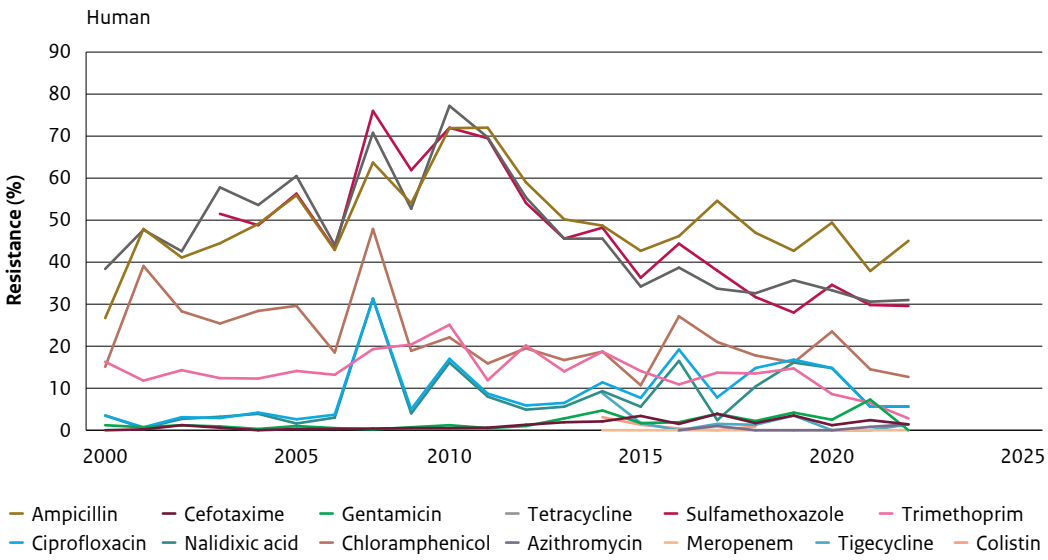
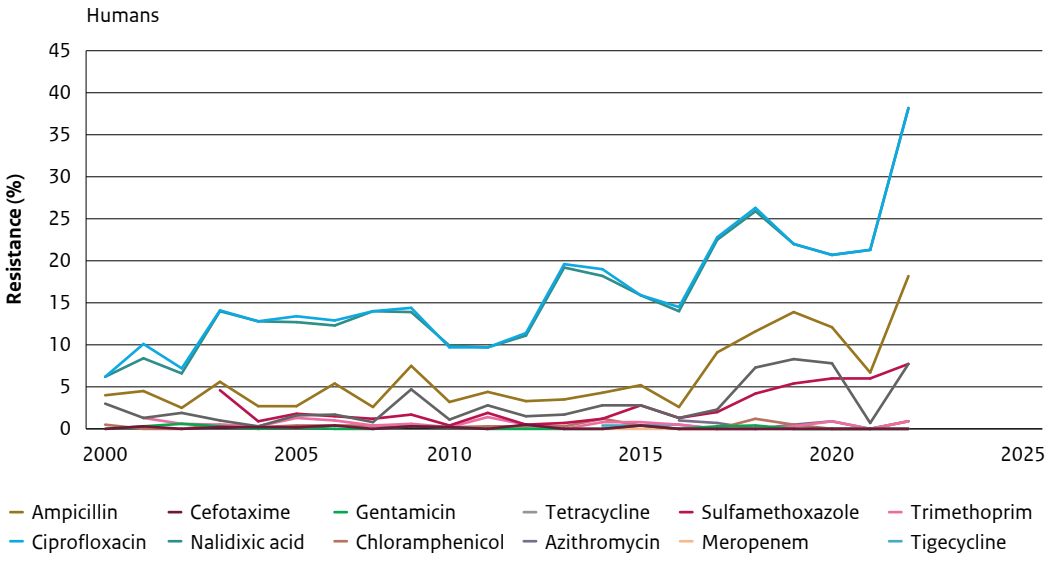


Table S04 Resistance percentages of *S.* Enteritidis (N tested) isolated from humans and broilers in 2022

	S. Enteritidis (271)			
	Humans (220)	Layers (24)	Broilers (16)	Other non-human sources (11)[a]
Ampicillin	18.2	4.2	0.0	9.1
Cefotaxime	0.0	0.0	0.0	0.0
Ceftazidime	0.0	0.0	0.0	0.0
Gentamicin	0.0	0.0	0.0	0.0
Tetracycline	7.7	4.2	6.3	9.1
Sulfamethoxazole	7.7	0.0	6.3	0.0
Trimethoprim	0.9	0.0	6.3	0.0
Ciprofloxacin	38.2	0.0	43.8	9.1
Nalidixic acid	38.2	0.0	43.8	18.2
Chloramphenicol	0.9	0.0	0.0	9.1
Azithromycin	0.9	0.0	0.0	0.0
Meropenem	0.0	0.0	0.0	0.0
Tigecycline	0.0	0.0	0.0	0.0
Amikacin	0.0	0.0	0.0	0.0

a Other sources include cattle (2), goats (2), and unspecified animals (7).

Fig S02 Trends in resistance (%) of *S.* Enteritidis isolated from humans from 2000-2022



ESBLs in *Salmonella*

The emergence of multidrug resistant *Salmonella* strains with resistance to fluoroquinolones and extended-spectrum cephalosporins is a serious development, which results in severe limitations for effective treatment of human infections. In 2022, the total number of genotypic confirmed ESBL *Salmonella* isolates was 14/1156 (1.2%) (compared to 10/1264 (0.8%) in 2021, 6/1170 (0.5%) in 2020, and 24/1880 (1.3%) in 2019). These included, *S.* Infantis (n=4, all human), *S.* Kentucky (n=1, human), *S.* Typhi (n=2, both human), monophasic *S.* Typhimurium (n=2, both human), *S.* Heidelberg (n=1, human), *S.* Anatum (n=2, both human) and *S.* Schwarzengrund (n=1, human), and *S.* Oslo (n=1, non-human).
In addition 79.5% (31/39) of *Salmonella* isolates obtained from imported poultry meat (outside EU) were ESBL-producers: *S.* Heidelberg (n=15), *S.* Minnesota (n=15) and *S.* Infantis.

Table S05 Resistance (%) of *Salmonella enterica* isolated from different types of raw meat in the Netherlands in 2022

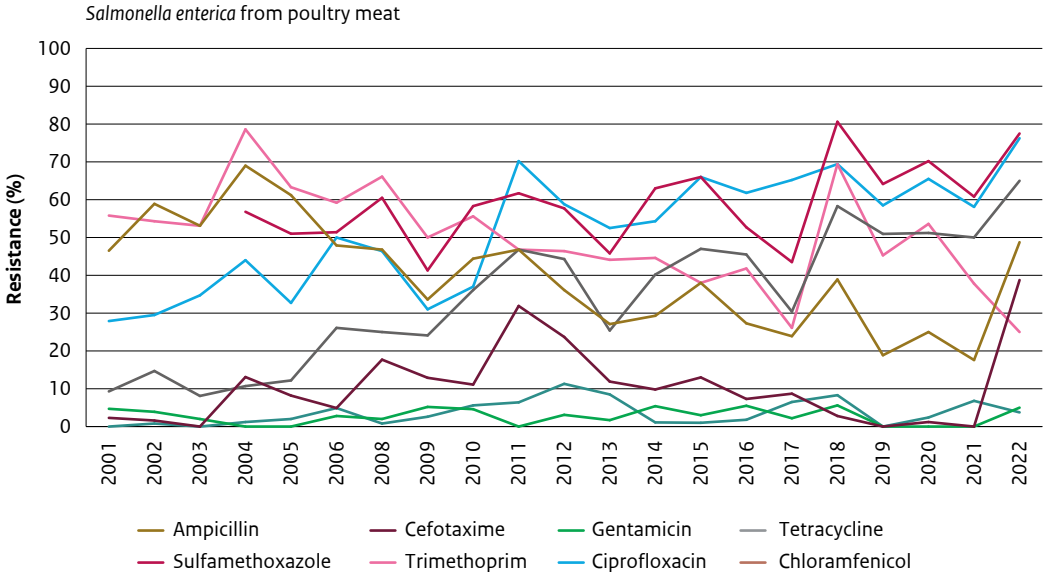
	Broiler meat[a]	Imported broiler meat	Pork	Other meat[b]
	N = 41	N=39	N = 23	N = 26
Ampicillin	14.6	84.6	26.1	26.9
Cefotaxime	0.0	79.5	0.0	7.7
Ceftazidime	0.0	79.5	0.0	7.7
Gentamicin	0.0	10.3	0.0	3.8
Tetracycline	46.3	84.6	30.4	30.8
Sulfamethoxazole	65.9	89.7	39.1	23.1
Trimethoprim	41.5	7.7	43.5	7.7
Ciprofloxacin	65.9	87.2	8.7	11.5
Nalidixic acid	65.9	87.2	4.3	7.7
Chloramphenicol	2.4	5.1	4.3	11.5
Azithromycin	2.4	0.0	0.0	0.0
Meropenem	0.0	0.0	0.0	0.0
Tigecycline	12.2	23.1	4.3	0.0
Amikacin	0.0	0.0	4.3	0.0

a Fresh chicken meat sampled at retail and chicken neck skin from verification projects.
b Other meat includes beef (10), sheep (7), turkeys (2), ducks (2), goats (2), rabbit (1), cangaroo (1), and unspecified meat (1).

Salmonella from chicken meat and other meat sources

Table S05 shows resistance data of *Salmonella* isolates from broiler meat, pork and other meat sources. Of notice here is the considerable consistent higher levels of resistance with meat imported from outside the EU (n=39) compared to Dutch/EU meat (n=41). Also of notice is the continuation of the long year upward trend of resistance against tetracycline (reaching 65% in 2022), and the stable high levels of resistance against ciprofloxacin (76%) and sulfamethoxazole (78%) as shown in Figure S03.

Figure S03 Trends in resistance (%) of *Salmonella enterica* isolated from poultry meat in the Netherlands from 2001-2022



3.1.2 Campylobacter

In this chapter, the occurrence and trends in antimicrobial resistance in *Campylobacter jejuni* and *C. coli* are described. Isolates were obtained from samples collected from food animals, meat and humans. As a result of prioritization and changes in legislation, from 2014 until 2020 the surveillance of antimicrobial resistance in *Campylobacter* focused mainly on broiler chickens (and meat thereof). From 2021 onwards, the mandatory monitoring of antimicrobial resistance in *Campylobacter* has been extended to *C. jejuni* and *C. coli* from veal calves (< 1 year) as well as *C. coli* obtained from slaughter pigs due to a new legislation (2020/1729/EU). It was decided to include isolates from both bovines and swine beside broiler isolates, for their potential role in human *Campylobacter* cases. In particular, *C. coli* from poultry, fattening pigs and veal calves were included, because of its potential reservoir of antimicrobial resistance genes.
As a result of the implementation of the new legislation the mandatory panel of antimicrobials for susceptibility testing of *Campylobacters* was updated by removing nalidixic acid and streptomycin and adding chloramphenicol and ertapenem. Chloramphenicol was included to screen for presumptive strains with an altered sequence of the CmeABC efflux pump and its regulating region exhibiting resistance to chloramphenicol and florfenicol. Since 2018[1*], carbapenem-non-susceptible strains have been reported from several countries (France and Japan). Therefore ertapenem was included in the panel as indicator for carbapenem resistance. Due to an ongoing discussion within Europe about the interpretation of the surprisingly high ertapenem resistance in *C. coli* reported in 2021 in several European countries, together with the lack of approved ECOFFs for both *C. jejuni* and *C. coli* for ertapenem, it was decided to stop reporting ertapenem resistance in MARAN until further notice.
From 2019 onwards, data on human isolates were obtained from ISIS-AR (see chapter 4 in NethMAP), whereas these data were previously obtained from a different laboratory surveillance system (with partly overlapping laboratories). Comparability of resistance proportions between these surveillance systems were assessed in 2019 which revealed negligible differences in resistance proportions.

Highlights

1. In 2022, resistance proportions in *C. jejuni* isolates from caecal samples of broilers and meat thereof remained at a high level for fluoroquinolones and tetracycline.
2. Resistance to erythromycin was not detected in *C. jejuni* isolates from broilers and poultry meat, and was observed at low levels in *C. jejuni* in veal calves and *C. coli* in broilers and poultry meat. A notably higher level of erythromycin resistance was observed in *C. coli* from veal calves.
3. In humans, resistance proportions were higher among *C. coli* than in *C. jejuni* isolates. Resistance levels increased in 2022 compared to 2020 and 2021, when resistance most likely dropped due to a substantial reduction of travel-related campylobacteriosis as a result of the COVID-19 travel restrictions, which is associated with higher resistance proportions than domestically acquired campylobacteriosis. However, resistance levels were generally still lower than before the COVID-19 pandemic.
4. Ciprofloxacin resistance in *Campylobacter* isolates from humans was again high in 2022, which is a concern for public health. It was, however, lower compared to 2019, before the COVID-19 pandemic.
5. Resistance to erythromycin, first choice antibiotic in human medicine for campylobacteriosis, remained low.

[1*] European Food Safety A, Aerts M, Battisti A, et al. Technical specifications on harmonised monitoring of antimicrobial resistance in zoonotic and indicator bacteria from food-producing animals and food. EFSA J. Jun 2019;17(6):e05709.

Table C01 presents the MIC distributions and resistance percentages for all *Campylobacter jejuni* and *C. coli* strains isolated in 2022 from caecal samples of broilers, veal calves and pigs. Resistance percentages of *C. jejuni* isolated from caecal samples of broilers and veal calves, neck skin samples of broilers (originating from hygiene control verifications at slaughter) as well as chicken meat are presented in Table C02. This table also contains resistance percentages for *C. coli* from caecal samples of broilers, veal calves and pigs, neck skin from broilers and chicken meat. (Trends in resistance of *C. jejuni* and *C. coli* from broilers and poultry meat products are presented in Figures C01 and C02. National surveillance data for *Campylobacter* spp. isolated from humans are shown in Figure C03 (from 2002 onwards) and in Table C03 (from 2009 onwards).

Resistance proportions

As in former years, resistance proportions were high for ciprofloxacin and tetracycline in both *C. jejuni* and *C. coli* isolates (Table C01 and C02). In contrast, resistance against chloramphenicol, erythromycin and gentamicin was low or absent in *C. jejuni* from different types of samples. Resistance against erythromycin was more frequently observed in *C. coli*, especially in veal calves.
Figure C01 presents the resistance levels of *C. jejuni* from poultry meat and broilers over the last 19 and 23 years, respectively. This figure demonstrates a high similarity in resistance trends between *C. jejuni* obtained from caecal samples at slaughter and those obtained from retail meat. It can be seen in Figure C02 that until 2018, similar parallel resistance trends were observed for *C. coli* for both antibiotics. However, ciprofloxacin resistance seems to increase since 2018 and the opposite is observed for tetracycline resistance, resulting in growing differences in resistance levels between these two antimicrobials over time.

Fluoroquinolones (ciprofloxacin)

The continuously high proportion of *Campylobacter* spp. isolates from animal origin resistant to fluoroquinolones (Figures C01 and C02) and especially from human patients (Figure C03) is a serious public health concern. The proportion of *C. jejuni* isolates from broilers resistant to ciprofloxacin remained at a high level over the last 10 years, with 58.8% in 2021 and 61.8% in 2022. The proportion of fluoroquinolone resistance in *C. jejuni* from poultry meat increased from 63.3 in 2021 to 68.3% in 2022. This high level of fluoroquinolone resistance was confirmed in *C. jejuni* isolates from chicken neck skin samples with 80.0% (Table C02). In 2022, the level of ciprofloxacin resistance in *C. coli* isolates from broilers, chicken meat and neck skin was even higher with 87.5%, 95.0% and 85.3%, respectively. In veal calves, high proportions of resistance to fluoroquinolones were observed in both *C. jejuni* (49.4%) and *C. coli* (80.5%) for the second year in row. Traditionally, a relatively low level of resistance is observed in *C. coli* obtained from slaughter pigs reflecting the long-term low use of fluoroquinolones in the pig sector.

Macrolides (erythromycin)

Erythromycin, or other macrolides (e.g. clarithromycin), are the first-choice drugs for the treatment of campylobacteriosis in humans. As in former years, resistance proportions to macrolides in isolates from animals and humans were low with exception of *C. coli* from veal calves. Table C02 shows that no macrolide resistance was detected in *C. jejuni* from caecal samples of broilers, chicken meat and neck skin, but a low proportion of resistance (3.0%) was detected in *C. jejuni* isolates from caecal samples of veal calves. Table C03 shows that between 1.6% and 3.4% (average: 2.3%) of human *C. jejuni* isolates were resistant for erythromycin in the period 2015-2022. It should be noted that for human isolates a lower breakpoint has been applied for erythromycin (≥ 1.5-2.0 mg/L); for animal and meat isolates the EUCAST epidemiological cut-off values were used (> 4 mg/L for *C. jejuni*, and > 8 mg/L for *C. coli*).
Different from *C. jejuni*, erythromycin resistance was detected at low levels in *C. coli* from caecal samples of broilers (4.2%) and pigs (5.4%), chicken meat (5.0%), but not in isolates from neck skin. (Table C02). Notably high levels of macrolide resistance were observed in *C. coli* isolates from caecal samples of veal calves (31.7%) for the second year in row.

Broiler chickens and chicken meat

In *Campylobacter* from poultry, resistance profiles were determined for isolates recovered from caecal samples of broilers, as well as from samples of chicken meat and neck skin.
Figure C01 demonstrates a high similarity in resistance trends between *C. jejuni* obtained from caecal samples at slaughter and those obtained from retail meat suggesting an overlap in the bacterial population examined from the different matrixes. Resistance percentages for ciprofloxacin and tetracycline have been high with considerable fluctuations over the years, while an increase of ciprofloxacin resistance in broilers and chicken meat was observed in 2022. The resistance levels for chloramphenicol, erythromycin and gentamicin were very low to zero as in former years.
Trends in resistance of *C. coli* isolates from broilers and poultry meat are presented in Figure C02. These levels show more fluctuation over the years than in *C. jejuni*, which is most likely caused by the lower number of isolates in the survey. As in former years, gentamicin resistance in *C. coli* from broilers and poultry meat was completely absent. In *C. coli*, proportions of macrolide resistance have been fluctuating substantially over the years with resistance proportions below 10% from 2019 onwards. Resistance percentages for ciprofloxacin in broilers and poultry meat have been fluctuating on a high level since 2001 and increased even further in 2022 to proportions of resistance above 85%. Because of the relatively low number of *C. coli* isolates tested (especially from meat), these results might not be very representative.
Table C02 shows that the proportions of resistance for tetracycline and ciprofloxacin in *C. jejuni* isolates were at high levels for isolates from chicken meat, as well as for isolates from caecal samples of broilers. Resistance levels for *C. coli* isolates from broilers and chicken meat for ciprofloxacin were even higher.
No resistance to gentamicin and chloramphenicol was detected in both *C. jejuni* and *C. coli* isolates from broilers and broiler meat. Resistance to erythromycin was not detected in *C. jejuni* isolates from broilers, chicken meat and neck skin, but was present at low levels in *C. coli* from broilers and meat thereof.
Higher resistance proportions were observed for almost all antimicrobials in *C. coli* isolates from broilers and chicken meat, compared to *C. jejuni* isolates from the same sources.

Table C01 MIC distributions (in %) for *Campylobacter jejuni* (N = 197) and *C. coli* (N = 451) isolated from caecal samples of broilers, veal calves and pigs in 2022

***C. jejuni*, broilers and veal calves**	**MIC (%) distribution mg/L**															**R%**	**95% CI**
(N = 197)	**0.06**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**1024**		
Chloramphenicol						42.1	47.7	6.6	3.6	0.0	0.0					0.0	0.0 - 0.2
Ciprofloxacin		44.7	3.0	0.5	0.0	0.0	1.0	24.4	19.8	5.6	1.0					51.8	44.6 - 58.9
Erythromycin					65.0	28.4	4.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.5	2.5	0.8 - 5.8
Ertapenem		71.6	17.3	7.1	2.0	1.5	0.5									-	-
Gentamicin			91.4	7.6	1.0	0.0	0.0	0.0	0.0							0.0	0.0 - 0.2
Tetracycline				18.8	1.0	0.0	1.0	0.5	1.0	0.5	18.3	58.9				80.2	74.0 - 85.5

***C. coli*, broilers, veal calves and pigs**	**MIC (%) distribution mg/L**															**R%**	**95% CI**
(N = 451)	**0.06**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**1024**		
Chloramphenicol						3.1	43.9	36.4	16.6	0.0	0.0					0.0	0.0 - 0.1
Ciprofloxacin		43.9	12.2	1.6	0.0	0.0	4.0	11.8	17.7	8.6	0.2					42.4	37.7 - 47.1
Erythromycin					28.6	31.5	23.3	4.2	0.2	0.0	0.0	0.0	0.2	0.7	11.3	12.4	9.5 - 15.8
Ertapenem		33.5	25.1	17.1	16.0	5.3	2.0	1.1								-	-
Gentamicin			47.5	49.7	1.1	0.0	0.0	0.0	0.0	1.3						1.3	0.5 - 2.9
Tetracycline				16.2	2.9	1.8	0.7	0.9	1.6	1.8	7.8	66.5				79.2	75.1 - 82.8

Figure C01 Trends in resistance (%) of *Campylobacter jejuni* isolated from broilers and chicken meat in the Netherlands

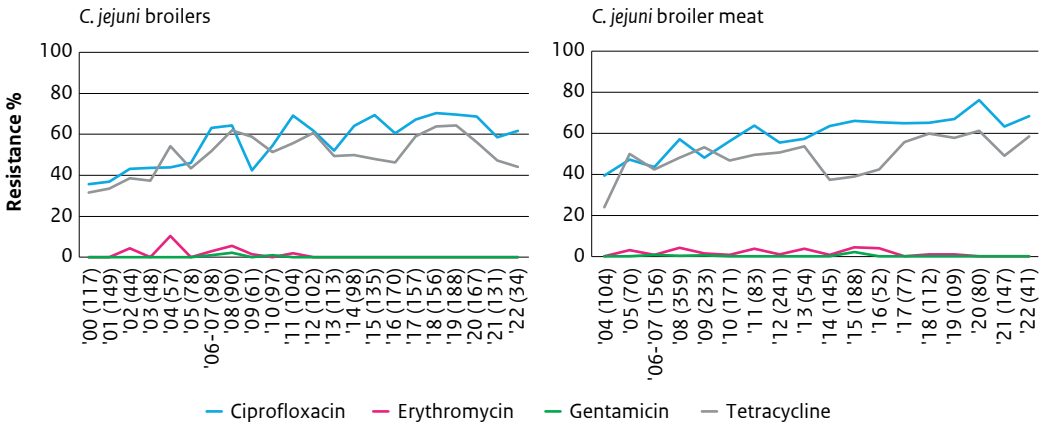


Figure C02 Trends in resistance of *Campylobacter coli* isolated from broilers and chicken meat in the Netherlands

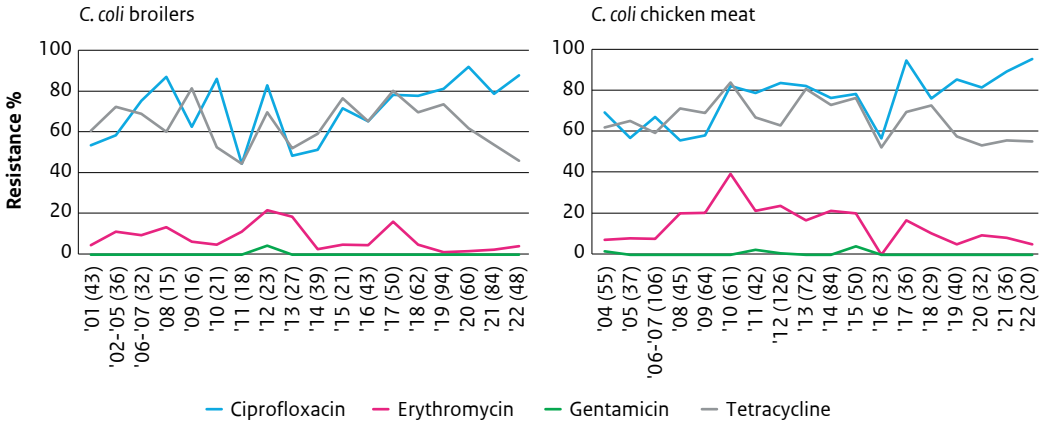


Table C02 Resistance percentages of *C. jejuni* and *C. coli* isolated from faecal samples of livestock and meat in 2022

	C. jejuni (R%)				*C. coli* (R%)				
	Broilers	Chicken meat	Chicken nek skin	Veal calves	Broilers	Chicken meat	Chicken nek skin	Veal calves	Pigs
	N = 34	N = 41	N = 45	N = 164	N = 48	N = 20	N = 34	N = 123	N = 280
Chloramphenicol	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ciprofloxacin	61.8	68.3	80.0	49.4	87.5	95.0	85.3	80.5	17.9
Erythromycin	0.0	0.0	0.0	3.0	4.2	5.0	0.0	31.7	5.4
Gentamicin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.9	0.0
Tetracycline	44.1	58.5	60.0	87.8	45.8	55.0	55.9	95.1	77.9

Veal calves and fattening pigs

From 2021 onwards, the mandatory monitoring of antimicrobial resistance in *Campylobacter* has been extended to *C. jejuni* and *C. coli* from veal calves (< 1 year) as well as *C. coli* obtained from slaughter pigs due to a new legislation (2020/1729/EU). As a result, susceptibility testing was performed on 164 *C. jejuni* and 123 *C. coli* isolates collected from veal calves and 280 *C. coli* isolates from pigs (Table C02). Similarly as in 2021, very high proportions of tetracycline resistance were measured for *C. jejuni* and *C. coli* from veal calves as well as for *C. coli* from pigs. Resistance levels were also high in veal calves for ciprofloxacin in both *C. jejuni* and *C. coli*. Clearly lower levels of ciprofloxacin resistance were detected in *C. coli* from pigs confirming the relatively low levels observed in former years. In *C. jejuni* from veal calves resistance was undetected for chloramphenicol and gentamicin, but resistance to erythromycin was detected for the first time at a low level in 2022. For the second year in a row erythromycin resistance was frequently observed in *C. coli* from veal calves, whereas gentamicin resistance was rare and chloramphenicol resistance completely absent. In *C. coli* from pigs, resistance to erythromycin was rare and resistance against chloramphenicol and gentamicin was not detected.

Campylobacter in humans

In 2022, an estimated 4857 campylobacteriosis cases occurred in The Netherlands (based on 3030 notifications with a national coverage of 62%). Although the number of cases were higher than during the COVID-19 pandemic, they are still lowed compared to the average of 6244 cases during 2015-2019 (range: 5558 – 7269 cases). Resistance levels in isolates from human patients were determined for ciprofloxacin, tetracycline and erythromycin, and are shown in Table C03 and Figure C03. Figure C03 shows a continuously increasing trend of ciprofloxacin and tetracycline resistance. In 2020 and 2021, however, resistance levels for all measured antibiotics dropped, most likely due to a substantial reduction in travel-related campylobacteriosis as a result of the COVID-19 lockdown, which is associated with higher resistance levels than domestically acquired campylobacteriosis. Because data on travel history it not available, this cannot be confirmed. In 2022, the resistance level increased compared to 2020 and 2021, but are still lower than pre-pandemic levels.

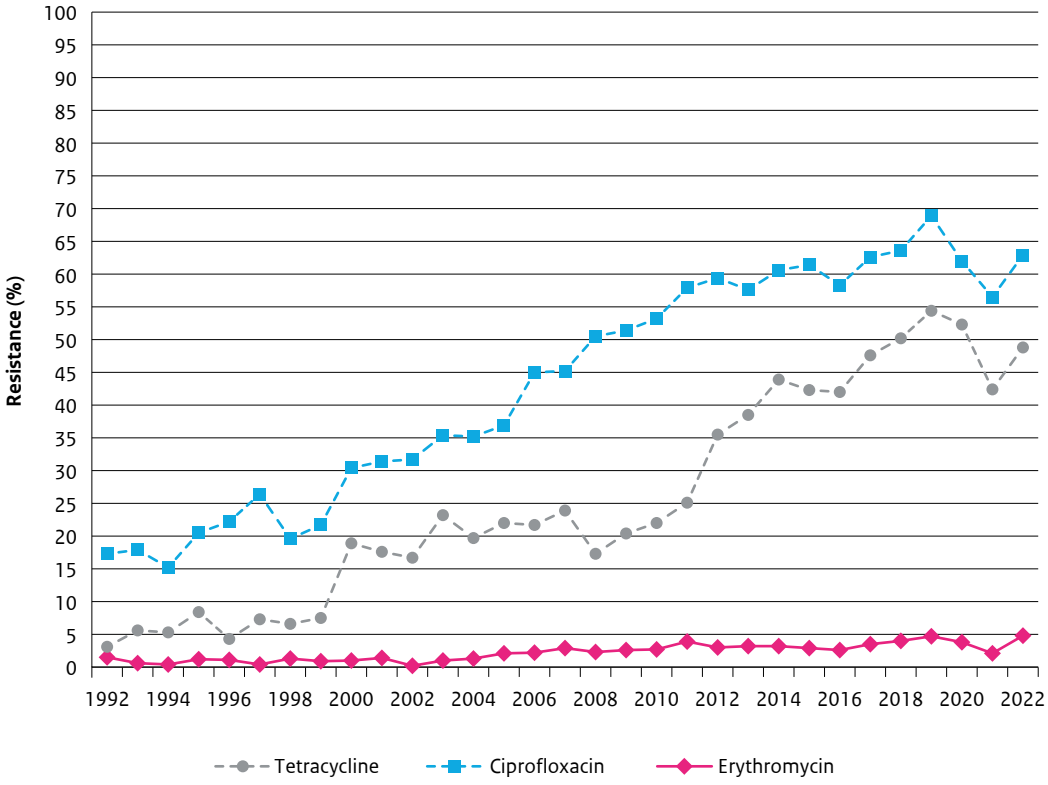
In 2022, the resistance level for ciprofloxacin in human *Campylobacter* spp. isolates was 62.9%, which is a public health concern. Although not back at the level of before the COVID-19 pandemic, it is higher than during 2020 and 2021. Tetracycline resistance had a similar trend and increased to 48.8% in 2022. Erythromycin resistance was 4.8% in 2022. Although still low, it is higher than before the COVID-19 pandemic.

Table C03 shows the resistance levels for human *C. jejuni* and *C. coli* isolates since 2015. Overall, the resistance levels in human *Campylobacter* spp. isolates for all three antimicrobials show an increasing trend until 2019, a reduction in resistance levels in 2020 and 2021, followed by an increase in 2022. Resistance proportions were higher for *C. coli* isolates than *C. jejuni* isolates.

Table C03 Resistance in *C. jejuni* and *C. coli* isolated from humans from 2015 - 2022

	C. jejuni						*C. coli*					
	Fluoroquinolone		Tetracycline		Erythromycin		Fluoroquinolone		Tetracycline		Erythromycin	
	N	R%	N	R%	N	R%	N	R%	N	R%	N	R%
2015	1,970	61.7	1,831	42.1	1,949	1.6	120	66.7	104	65.4	116	19.0
2016	1,834	61.3	1,658	45.1	1,819	2.0	142	66.2	121	67.8	140	14.3
2017	1,649	63.9	1,453	49.0	1,629	2.5	142	79.6	126	77.0	138	19.6
2018	1,753	62.7	1,575	54.6	1,730	2.3	153	80.4	138	73.2	150	35.3
2019	1,673	67.7	1,517	52.7	1,646	2.5	178	80.9	157	75.8	172	25.6
2020	1,147	60.9	1,009	49.9	1,133	2.1	104	68.3	98	74.5	103	20.4
2021	1,303	55.3	1,161	40.7	1,295	1.9	93	72.0	84	65.5	93	5.4
2022	1,435	60.6	1,256	46.7	1,423	3.4	133	67.7	118	70.3	131	20.6

Figure C03 Trends in resistance (%) of *Campylobacter* spp. isolated from humans between 1992 and 2022



3.1.3 Shiga-toxin producing *E. coli* (STEC) and enteropathogenic *E. coli* (EPEC)

Highlights

1. In human STEC O157 isolates, a tendency of increasing proportions of resistance against ampicillin and trimethoprim and decreasing levels of resistance against sulfamethoxazole and tetracycline compared to 2021 was observed.
2. The proportion of resistance of ciprofloxacin was higher in human STEC/EPEC non-O157 *E. coli* than in human STEC O157.
3. No ESBL-producing isolates were detected in STEC O157, but an O111 STEC isolate was confirmed as ESBL-producer carrying $bla_{CTX\text{-}M\text{-}15}$.

Shiga-toxin producing *E. coli* (STEC) is a bacterial zoonotic agent associated with human disease with varying clinical manifestations, including diarrhea, hemorrhagic colitis and hemolytic uremic syndrome (HUS), a leading cause of acute renal failure among children. The natural reservoir of STEC is the gastrointestinal tract of ruminants, especially cattle and small ruminants[2]. Although, therapeutic treatment of STEC infections with antimicrobials is not regularly advised, monitoring AMR in STEC from symptomatic human cases is useful to assess the risk of transmission of resistant bacteria, and resistance genes from ruminants to humans.

In contrast to the years before 2020, in 2022 not only STEC O157 but a larger collection of isolates from human clinical cases (N = 271) consisting of multiple STEC/aEPEC/tEPEC[2*] O157 and non-O157 serotypes were tested for susceptibility. The set consisted of 90 STEC/EPEC O157 isolates, 181 STEC/EPEC non-O157 isolates: O26 (n=33), O103 (n=21), O145 (n=17), O146 (n=17), O63 (n=10), and other O-types (n=83). All isolates were obtained from the RIVM national laboratory surveillance of STEC. Table STEC01 shows the MIC results for *E. coli* O157 isolates from humans; Table STEC02 shows resistance proportions of *E. coli* O157 and STEC/EPEC non-O157 isolates; Figure STEC01 presents the trends over time for STEC O157; Figure STEC02 presents the trends in resistance over time for STEC/EPEC non-O157.

Compared to 2021, an increase in resistance proportions among STEC O157 was observed for ampicillin and trimethoprim in 2022, which was in range with the fluctuating levels of 2011-2017 after a decrease in 2018-2020 (Figure STEC01). After a sharp increase in 2021 following a decrease in 2018-2020, resistance proportions for tetracycline and sulfamethoxazole decreased again to the fluctuating trend of 2008-2017. Levels of resistance for gentamicin, chloramphenicol and ciprofloxacin remain similar to the fluctuating trend that is observed since 2017-2018. No ESBL-producing isolates were detected in 2022 among STEC O157.

[2*] aEPEC = atypical enteropathogenic *E. coli*, which share the LEE-pathogenicity island with STEC but lack shiga-toxin genes a well as the EPEC adherence factor plasmid. tEPEC = typical enteropathogenic *E. coli*, which possesses the LEE-pathogenicity island as well as the EPEC adherence factor plasmid, but lack shiga-toxin genes.

Figure STEC01 Trends in resistance (in %) of *E. coli* STEC O157 isolated from humans in the Netherlands from 1999-2022

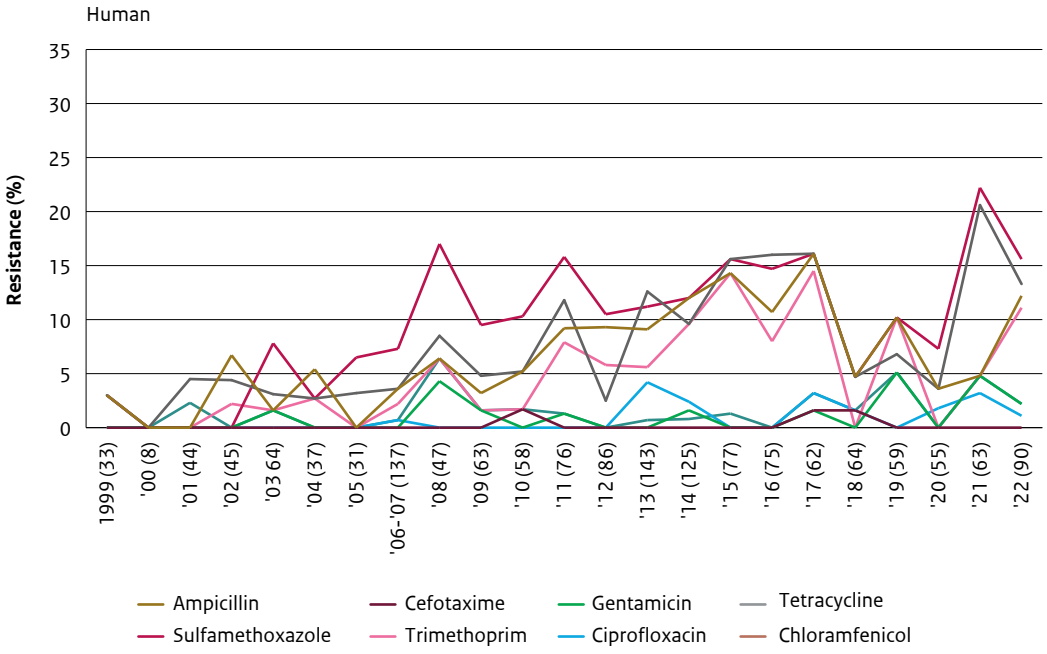


Figure STEC02 Figure STEC02 Trends in resistance (in %) of *E. coli* STEC/EPEC non-O157 isolated in the Netherlands from 2020-2022

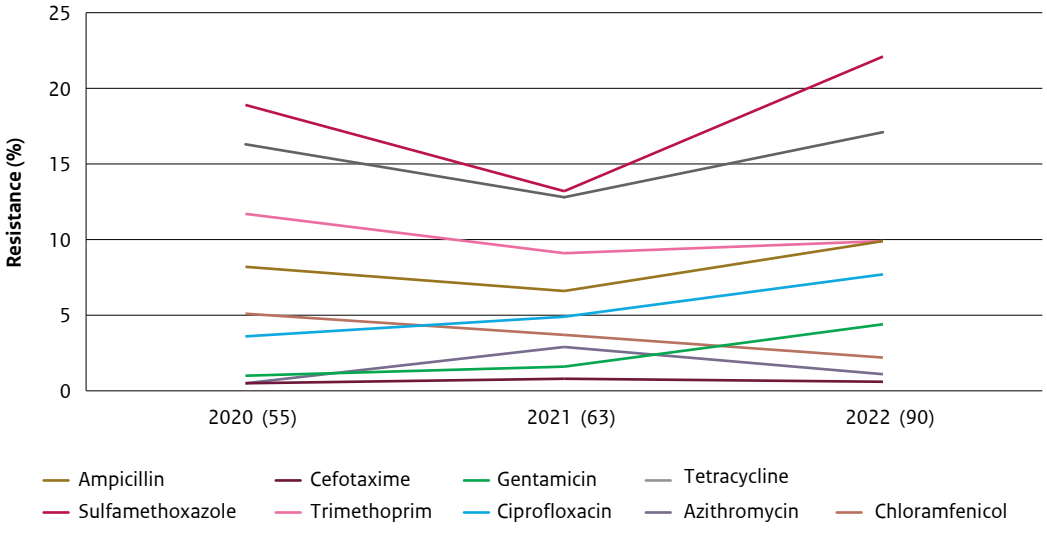


Table STEC01 MIC distribution (in %) and resistance percentages (R%) for *E. coli* STEC O157 (N=90) isolated from humans the Netherlands in 2022

E. coli N = 90	MIC (%) distribution mg/L																		R%	95% CI
	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048		
Amikacin							0.0	0.0	98.9	1.1	0.0	0.0	0.0						0.0	0.0 - 4.0
Ampicillin							0.0	3.3	78.9	5.6	0.0	1.1	11.1						12.2	6.3 - 20.8
Cefotaxime					100.0	0.0	0.0	0.0	0.0										0.0	0.0 - 4.0
Ceftazidime					97.8	2.2	0.0	0.0	0.0	0.0									0.0	0.0 - 4.0
Gentamicin						93.3	3.3	1.1	0.0	0.0	2.2	0.0							2.2	0.3 - 7.8
Tetracycline								85.6	1.1	0.0	0.0	0.0	13.3						13.3	7.1 - 22.1
Sulfamethoxazole										80.0	4.4	0.0	0.0	0.0	0.0	0.0	15.6		15.6	8.8 - 24.7
Trimethoprim					86.7	2.2	0.0	0.0	0.0	0.0	0.0	11.1							11.1	5.5 - 19.5
Ciprofloxacin	84.4	12.2	2.2	0.0	0.0	0.0	1.1	0.0	0.0	0.0	0.0								1.1	0.0 - 6.0
Nalidixic acid									96.7	2.2	0.0	0.0	0.0	1.1					1.1	0.0 - 6.0
Chloramphenicol										97.8	0.0	0.0	0.0	2.2					2.2	0.3 - 7.8
Azithromycin								27.8	68.9	1.1	0.0	0.0	1.1	1.1					2.2	0.3 - 7.8
Colistin							100.0	0.0	0.0	0.0	0.0								0.0	0.0 - 4.0
Meropenem		100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0								0.0	0.0 - 4.0
Tigecycline					100.0	0.0	0.0	0.0	0.0	0.0									0.0	0.0 - 4.0

The white areas indicate the dilution range tested for each antimicrobial agent. Values above this range indicate MIC values > the highest concentration in the range. Values at the lowest concentration tested indicate MIC-values ≤ the lowest concentration in the range. Vertical bars indicate the epidemiological cut-off values, used as breakpoints. Dashed bars indicate the clinical breakpoints.

Table STEC02 shows differences in proportion of resistance between STEC O157 and STEC/EPEC non-O157 isolates. The only statistically significant difference in proportion is observed for ciprofloxacin, with a higher resistance level for non-O157 isolates (8%) compared to O157 isolates (1%, χ^2, p =0.012). The proportion of multidrug resistance[3*] was 12% in both STEC O157 and non-O157 isolates, with 11 out of 90 and 22 out of 181 isolates, respectively. Resistance to 3rd generation cephalosporins (cefotaxime and ceftazidime) was detected in one O111: H8, ST16 isolate (*stx1* and *eae* positive). Resistance marker detection in the genome sequence confirmed the presence of the ESBL gene $bla_{\text{CTX-M-15}}$. Azithromycin resistance was detected in two O157:H7 isolates and two O71:H2 isolates from the same patient at different sample dates.

Table STEC02 Resistance percentages (R%) of pathogenic *E. coli* in the Netherlands in 2022

E. coli	O157	Other serotypes
	N = 90	N = 181
Amikacin	0.0	0.6
Ampicillin	12.2	9.9
Cefotaxime	0.0	0.6
Ceftazidime	0.0	0.6
Gentamicin	2.2	4.4
Tetracycline	13.3	17.1
Sulfamethoxazole	15.6	22.1
Trimethoprim	11.1	9.9
Ciprofloxacin	1.1	7.7
Nalidixic acid	1.1	5.0
Chloramphenicol	2.2	2.2
Azithromycin	2.2	1.1
Colistin	0.0	0.0
Meropenem	0.0	0.0
Tigecycline	0.0	0.0

Reference

L. Mughini-Gras, W. van Pelt, M. van der Voort, M. Heck, I. Friesema E. Franz, Attribution of human infections with Shiga toxin-producing Escherichia coli (STEC) to livestock sources and identification of source-specific risk factors, The Netherlands (2010–2014), Zoonosis and Public Health, Volume65, Issue1, February 2018. https://doi.org/10.1111/zph.12403

[3*] Multidrug resistant defined here as resistant against ≥2 classes of antimicrobials.

3.2 Commensal indicator organisms

This chapter describes the susceptibility profiles of commensal bacteria from the gastro-intestinal tract of food-producing animals, meat and vegetables. The level of antimicrobial resistance in bacteria inhabiting the intestinal tract directly reflects the selection pressure as a result of the use of antibiotics in animals, especially over time. *E. coli* is therefore included as indicator organism for the Gram-negative flora. As a result of less priority for including enterococci representing the Gram-positive flora in the surveillance, no enterococci are reported since 2017.

EFSA[1,3] prescribes the sampling strategy and isolation methodology of bacteria from caeca of randomly selected food-producing animals at slaughter with the aim to detect the occurrence and trends in resistance at the bacterial population level in food animals. In the Netherlands, this monitoring is conducted in slaughter pigs and broilers since 1998. From 2005 onwards, resistance in isolates from both dairy cattle, veal calves and meat samples have been included. In the years 2010 and 2011, samples of individual dairy cattle were collected at slaughter houses; in all other years pooled or individual faecal samples were collected at dairy farms. Until 2012, pooled veal calf samples were collected at farms. Monitoring programs in veal calves at farms stopped in 2012. From then onwards, the monitoring program for veal calves was carried out similar as for pigs and poultry by collecting samples from caeca of individual veal calves at slaughterhouses, and resistance levels were reported separately for white and rosé veal calves.

It should be noted that the sampling strategies used are inherently insensitive to detect resistance at the population level, as only one randomly selected isolate from a single sample collected from one animal per epidemiological unit (herd or flock) is tested for susceptibility. The total number of isolates is intended to represent the *E. coli* population of each animal species of the entire country. One percent resistance in e.g. *E. coli* indicates that in all animals of that animal species 1% of the *E. coli* bacteria are resistant. This means that the absence of resistance in these datasets does not exclude the possibility that resistance is present in individual animals.

3.2.1 *Escherichia coli*

In this chapter, information is presented on resistance in *E. coli*, as indicator organism for the occurrence and trends in resistance in Gram-negative bacteria in the gastro-intestinal tract of food-producing animals, meat and other products in the Netherlands.
A new EU legislation on monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria (2020/1729/EU) was implemented in 2021. Indicator commensal *E. coli* isolates obtained from samples of caecal content taken at slaughter, and from samples of fresh meat taken at the border control posts are to be gathered and examined. This includes susceptibility testing by broth microdilution according to ISO 20776-1:2019 with updated mandatory panels of antimicrobials. The former panel for testing Gram-negative bacteria was amended by shortening the ranges of several antibiotics on the upper side of the concentration range thereby generating space for an extra antibiotic: amikacin. Amikacin is one of the most commonly used aminoglycosides in hospitals for the treatment of infections by Gram-negative bacteria in a number of European countries. It's presence in the new panel improves the detection of the 16S rRNA methyltransferases associated with carbapenemases, AmpC or ESBLs and fluoroquinolone resistance in Gram-negative Enterobacterales.

Results are interpreted with epidemiological cut-off values (ECOFF's) according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST). In this report non-wild type susceptible isolates are classified as resistant. These isolates all harbour an acquired resistance mechanism, but may not be clinically resistant for some antibiotics.

Highlights 2022

1. Amongst indicator *E. coli* from animals, resistance levels to ampicillin, tetracycline, sulfamethoxazole and trimethoprim were still relatively high in broilers, pigs, and (white) veal calves.
2. In broilers, pigs and veal calves, levels of resistance stabilised for most antibiotics, whereas resistance in dairy cattle remained traditionally low.
3. Resistance to third generation cephalosporins was low or absent amongst (randomly isolated) indicator *E. coli* from caecal samples of all animal species.
4. Resistance to fluoroquinolones was still commonly present in indicator *E. coli* from caecal samples of broilers in contrast to the low prevalence observed in pigs and white veal calves and the complete absence in rosé veal calves and dairy cattle.
5. For most antibiotics tested, levels of resistance in *E. coli* from caecal samples of rosé veal calves were substantially lower than those from white veal calves.
6. In fresh retail meat from bovine and chicken, resistance proportions in *E. coli* were similar to isolates from caecal content.
7. In imported poultry meat, resistance proportions were substantially higher compared to fresh domestic retail meat.
8. In contrast to fresh retail meat, the presence of ESBL/AmpC-producing *E. coli* was frequently observed in imported broiler and turkey meat.
9. For the first time, resistance monitoring was carried out in rabbits, showing high levels of resistance to tetracycline, sulfamethoxazole and trimethoprim, while resistance to other antibiotics was low or absent.

Resistance levels

Table Eco01 shows resistance levels, presented as MIC-distributions, of 1201 *E. coli* isolates obtained from caecal samples from broilers, pigs, veal calves collected at slaughter and faecal samples of dairy cows collected at farms in 2022. Table Eco02 presents resistance percentages per animal species and includes for the first time resistance data of *E. coli* obtained from faecal samples of rabbits collected at farms. Trends in resistance levels from 1998 to 2022 are shown in Figure Eco01 and information on trends in multidrug resistance in the different animals sectors (except for rabbits) is shown in Figure Eco02.
Table Eco03 presents resistance percentages of 448 *E. coli* isolates collected from raw meat and shell fish at retail and import in the Netherlands in 2022. Figure Eco03 shows trends in resistance of *E. coli* in the Netherlands from 2002 to 2022 isolated from fresh meat at retail of chicken and bovine.

Table Eco01 MIC distribution (in %) and resistance percentages (R%) for all *E. coli* (N=1,201) isolated as indicator organism from intestines of food producing animals in the Netherlands in 2022

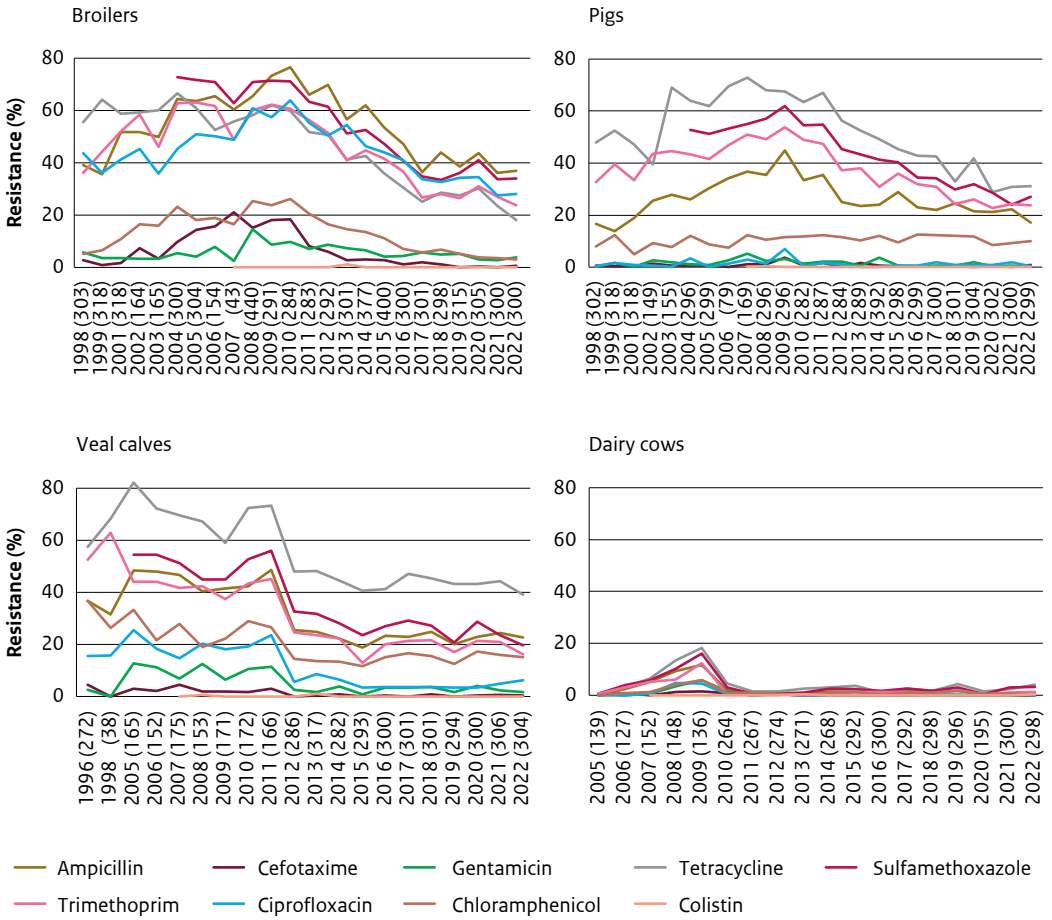
E. coli N = 1,201	MIC (%) distribution mg/L																		R%	95% CI
	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048		
Ampicillin							1.7	19.5	50.5	9.0	0.0	0.2	19.2						19.4	17.2 - 21.8
Cefotaxime					99.6	0.0	0.1	0.2	0.1	0.1									0.4	0.1 - 1.0
Ceftazidime					91.8	7.6	0.2	0.0	0.2	0.1									0.3	0.1 - 0.9
Gentamicin						70.2	26.1	2.3	0.0	0.1	0.4	0.8							1.3	0.8 - 2.2
Tetracycline								67.4	8.9	0.5	0.3	0.3	22.5						23.1	20.8 - 25.6
Sulfamethoxazole										77.9	1.0	0.0	0.0	0.0	0.0	0.0	21.1		21.1	18.8 - 23.5
Trimethoprim					45.1	35.7	2.7	0.2	0.1	0.0	0.0	16.2							16.2	14.2 - 18.5
Ciprofloxacin	73.9	17.2	0.2	0.8	4.9	1.2	0.5	0.1	0.2	0.6	0.3								8.7	7.1 - 10.4
Nalidixic acid									90.4	2.0	0.7	0.1	1.3	5.4					7.6	6.1 - 9.2
Chloramphenicol										79.7	13.2	0.7	1.1	5.4					7.2	5.8 - 8.8
Azithromycin*								3.0	26.2	61.7	8.2	0.2	0.2	0.4					0.8	0.4 - 1.5
Colistin							99.3	0.6	0.1	0.0	0.0								0.1	0.0 - 0.5
Meropenem		99.3	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0								0.0	0.0 - 0.3
Tigecycline					96.3	3.7	0.0	0.0	0.0	0.0									0.0	0.0 - 0.3
Amikacin									98.0	2.0	0.0	0.0	0.0	0.0					0.0	0.0 - 0.3

The white areas indicate the dilution range tested for each antimicrobial agent. Values above this range indicate MIC values > the highest concentration in the range. Values at the lowest concentration tested indicate MIC-values ≤ the lowest concentration in the range. Vertical bars indicate the epidemiological cut-off values (ECOFF), used as breakpoints. If available, dashed bars indicate the clinical breakpoints. For ampicillin, chloramphenicol and colistin the ECOFF and clinical breakpoint are identical.

** tentative ECOFF set by EURL established by EFSA data.*

For most drugs or drug classes, resistance levels varied substantially between the different animal species (Table Eco02). As in previous years, highest resistance levels were found in broilers, slaughter pigs and white veal calves, lower levels in rosé veal calves, and the lowest levels of resistance were observed in isolates from dairy cattle. Overall, the highest resistance levels were detected for ampicillin, tetracycline, sulfamethoxazole and trimethoprim. These drug classes are the most frequently used classes in veterinary medicine in The Netherlands. In addition, high levels of resistance were also observed for (fluoro) quinolones in broilers and for chloramphenicol in white veal calves. The use of chloramphenicol has been banned for many years from the veterinary sector, but resistance to chloramphenicol can be selected by the use of florfenicol. Low resistance was noticed for azithromycin, cefotaxime, ceftazidime, colistin, gentamicin and tigecycline. Resistance for amikacin and meropenem was completely absent.

Figure Eco01 Trends in proportion of resistance (%) of *E. coli* isolated from broilers, slaughter pigs, veal calves and dairy cattle in the Netherlands from 1998-2022



Fluoroquinolone resistance

Highest resistance levels for fluoroquinolones (FQ) were found in *E. coli* from broilers with 28.0% resistance to ciprofloxacin and 26.7% resistance to nalidixic acid (Table Eco02). In samples from other animal sectors, FQ resistance was low or completely absent: 9.3% in white veal calves, 0.3% in pigs, and undetected in isolates from rosé veal calves and dairy cattle. In meat rabbits, the level of resistance was also low with 4.0% resistance against ciprofloxacin.

Resistance to fluoroquinolones in *E. coli* from meat was tested in 2022 for chicken meat, bovine meat (beef), exotic meat and shell fish from retail as well as from imported chicken and turkey meat (Table Eco03). As a result, resistance levels of indicator *E. coli* collected from fresh retail meat and imported poultry meat is separately reported in Table Eco03 showing higher fluoroquinolone resistances in imported poultry meat (chicken and turkey) compared to fresh chicken meat at retail (Table Eco03). Levels of FQ resistance were low in bovine meat (1.7%) and exotic meat (4.8%), but not detected in *E. coli* isolates obtained from shell fish.

Resistance against extended-spectrum cephalosporins (cefotaxime and ceftazidime) in animals

Passive screening (by non-selective isolation)

The prevalence of resistance against extended-spectrum cephalosporins (ESC-R) has declined over time in randomly selected indicator *E. coli* to levels close to the detection limit. As a result, ESC-R *E. coli* is only incidentally observed since 2019. However, in 2022 nine randomly selected indicator *E. coli* isolates showed resistance to third generation cephalosporins (ESC-resistant *E. coli*). These isolates were obtained from caecal samples of rabbits (n=4), pigs (n=3), broilers (n=1) and white veal calves (n=1). Susceptibility testing of these *E. coli* isolates resulted in low proportions of resistance to cefotaxime in most animals species (Table Eco02). It should be noted that all four ESC-R *E. coli* isolates from rabbits were borderline resistant against cefotaxime which an MIC-value (0.5 mg/L) just above the ECOFF and susceptible for ceftazidime. No specific ESBL/AmpC resistance mechanism was detected in these isolates. No ESC-resistant indicator *E. coli* were observed in randomly selected *E. coli* isolates from caecal samples of rosé veal calves and dairy cattle (Figure Eco01). Amongst indicator *E. coli* obtained from meat samples, ESC-R was almost exclusively found in imported poultry meat (Table Eco03).

Active screening (by selective isolation)

In contrast to the low levels ESC-R amongst indicator *E. coli* described above, ESC-R *E. coli* are frequently detected in caecal samples by using selective isolation according to the EURL protocol. The results of selective isolation as well as the molecular typing of the ESC-resistant *E. coli* are discussed in Chapter 4.

Broiler chickens

In 2022, resistance proportions of commensal *E. coli* isolated obtained from caecal samples of broiler chickens were similar to 2021 and stayed below 40% for all antibiotic classes for the second year. Still, relatively high levels of resistance were observed for ampicillin, (fluoro)quinolones, sulfamethoxazole, trimethoprim and tetracycline. Noteworthy, resistance against trimethoprim and tetracycline decreased to the lowest degree since the beginning of the monitoring with 23.7% and 18.0%, respectively. Resistance to cefotaxime, ceftazidime, gentamicin and azithromycin remained low (less than 5%), where resistance to amikacin, colistin, meropenem and tigecycline was not detected amongst indicator *E. coli*.

Table Eco02 Resistance percentages (%) of *E. coli* isolated from faecal samples of broilers, pigs, dairy cows, veal calves and rabbits in the Netherlands in 2022

Faecal samples	Broilers	Pigs	Dairy	Veal calves		Rabbits
	N = 300	N = 299	N = 298	White, N = 205	Rosé, N = 99	N = 75
Ampicillin	37.0	17.1	0.7	29.8	8.1	17.3
Cefotaxime	0.3	1.0	0.0	0.5	0.0	5.3
Ceftazidime	0.3	0.7	0.0	0.5	0.0	0.0
Gentamicin	3.7	0.0	0.0	2.4	0.0	0.0
Tetracycline	18.0	31.1	4.0	50.7	15.2	56.0
Sulfamethoxazole	34.0	27.1	3.4	25.4	8.1	52.0
Trimethoprim	23.7	23.7	1.3	22.9	2.0	54.7
Ciprofloxacin	28.0	0.3	0.0	9.3	0.0	4.0
Nalidixic acid	26.7	0.7	0.0	4.4	0.0	2.7
Chloramphenicol	3.0	10.0	0.3	19.5	6.1	5.3
Azithromycin	0.7	1.3	0.0	2.0	0.0	0.0
Colistin	0.0	0.0	0.3	0.0	0.0	6.7
Meropenem	0.0	0.0	0.0	0.0	0.0	0.0
Tigecycline	0.0	0.0	0.0	0.0	0.0	0.0
Amikacin	0.0	0.0	0.0	0.0	0.0	0.0

Slaughter pigs

The overall resistance proportion stabilised in slaughter pigs (Figure Eco01) with some fluctuation in resistance between the different antibiotic classes. For sulfamethoxazole, tetracycline and trimethoprim resistance levels stabilised between 20% and just above 30% whereas resistance to ampicillin was measured below 20% for the first time since 2001. Chloramphenicol resistance remained stable around 10%. Low levels of resistance were observed for azithromycin, cefotaxime, and (fluoro)quinolones, where resistance to amikacin, colistin, gentamicin, meropenem and tigecycline was not detected.

Veal calves

Resistance data on white and rosé veal calves are reported separately, because of the difference in production systems. As seen in previous years, substantially higher resistance levels were measured in isolates from white veal calves, compared to those from rosé veal calves (Table Eco02). Figure Eco01 illustrates the trends in resistance in *E. coli* isolated from both types of veal calves combined. Resistance levels were relatively stable over time, with a clear decrease in 2012, which was the year in which the sampling strategy changed from sampling at farm at variable ages to sampling at slaughterhouse. This has influenced the results from 2012 onwards, because most antibiotic usage is in the younger calves and less in the period before slaughter.

The ratio of sampled white veal calves versus rosé veal calves changed from 50/50% to 60/40% in 2016, and to 70/30% in 2017 onwards, which better reflects the proportions of slaughtered white and rosé calves in The Netherlands. After 2017, resistance levels in veal calves stabilised (Figure Eco01) with large differences between the two husbandry types (Table Eco02). In 2022, resistance levels in veal calves slightly decreased compared to 2021 for most antibiotic classes including ampicillin, sulfamethoxazole, trimethoprim and tetracycline. As a result, resistance levels stayed below 40% for all antibiotic classes for the first time since the beginning of the monitoring in 1998. Resistance against azithromycin, cefotaxime, ceftazidime, gentamicin and (fluoro)quinolones was only detected in white veal calves. In addition, no resistance was observed for amikacin, colistin, meropenem and tigecycline in both white and rosé veal calves. (TableEco02).

Dairy cattle

Resistance in *E. coli* isolated from dairy cattle was slightly fluctuating but traditionally low compared to pigs, broilers and veal calves (Table Eco02), reflecting the low use of antibiotics in dairy farming. As in previous years resistance to the 3rd generation cephalosporins was not detected.

Rabbits

In 2022, resistance of indicator *E. coli* in rabbits was measured for the first time as part of a policy supporting project exploring the level of resistance in other animal sectors. A total of 75 faecal samples were collected at 15 different meat rabbit farms. The proportion of resistance of 75 indicator *E. coli* are shown in Table Eco02. Resistance levels were the highest compared to other animals sectors involved in the AMR monitoring for sulfamethoxazole (52.0%), trimethoprim (54.7%) and tetracycline (56.0%). However, resistance levels were low for most other antibiotic classes. Borderline resistance against cefotaxime was detected in four isolates from three different farms (5.7%). Analysis of Whole Genome Sequence (WGS) data did not reveal any ESBL/AmpC resistance mechanism. Finally, colistin resistance was identified in 6.7% of the isolates (n=5) with confirmed colistin MIC-values between varying 4 – 16 mg/L. No known colistin resistance mechanisms were identified based on WGS data.

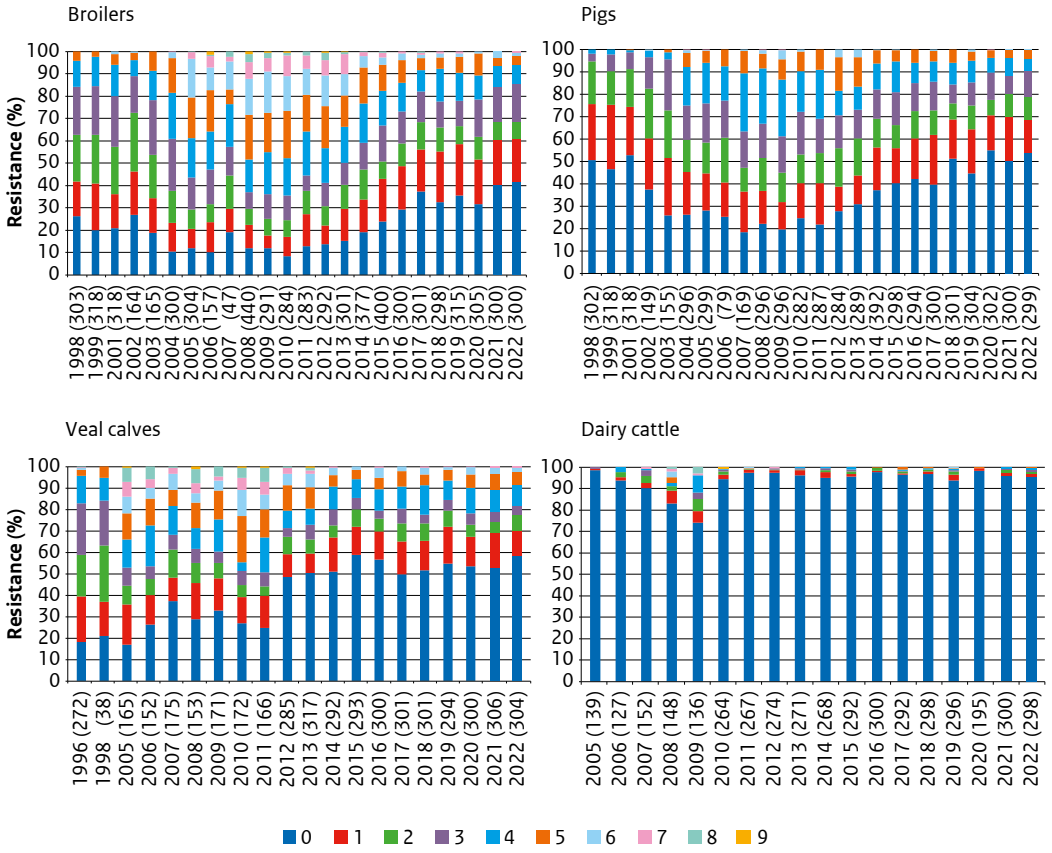
Multidrug resistance

Data to determine multidrug resistance is based on resistance against the following antimicrobial classes: aminopenicillins (ampicillin), 3rd gen. cephalosporins (cefotaxime), carbapenems (meropenem), aminoglycosides (gentamicin), tetracyclines (tetracycline), sulfonamides (sulfamethoxazole), folate pathway inhibitors (trimethoprim), fluoroquinolones (ciprofloxacin), phenicols (chloramphenicol), macrolides (azithromycin) and polymyxins (colistin). The data with the determined level of multidrug resistance over the years are shown in Figure Eco02.

In general, the level of multidrug resistance (showing resistance to three or more classes of antimicrobials) stabilised in the last five years. Compared to 2021, the level of multidrug resistance amongst *E. coli* was identical in broilers (31.3%) and only minor changes were observed for pigs (21.1%) and veal calves (22.0%). As in former years, multidrug resistant *E. coli* in dairy cattle were extremely rare (1.7%) compared to the other animals species.

During the last decade, proportions of complete susceptibility have increased considerably in all animals species. In broilers and in veal calves, the proportion of complete susceptible isolates was the highest since the beginning of the monitoring with 42.3% and 58.6% respectively. Also in pigs, more than half of the *E. coli* (54.2%) isolates was complete susceptible to all antibiotics tested. The proportion of complete susceptible isolates slightly decreased in dairy cattle, but was still very high in 2022 with 95.6%.

Figure Eco02 Proportions of isolates resistant (%) to 0 - 9 antimicrobial classes among *E. coli* isolated from broilers, slaughter pigs, veal calves and dairy cattle in the Netherlands from 1998-2022



E. coli in raw-meat and shell fish

Due to the new legislation, fresh meat products imported from outside the EU were included in the monitoring. Samples of imported chicken meat and turkey meat were collected at border control posts following the annual recommended frequency rates from 159 consignments imported from South America, United Kingdom and South East Asia, (77%, 21% and 2% resp.). Table Eco03 presents resistance percentages of *E. coli* isolated from fresh chicken, bovine meat and exotic fresh meat (duck, goose, guinea fowl, mallard, partridge, pheasant, wood pigeon, dear, hare and wild boar) sampled at retail as well as imported frozen poultry meat collected at border control posts by the Dutch Food and Consumer Product Safety Authority (NVWA). Meat from retail comprises meat produced in the Netherlands, but also in other EU countries. Overall, resistance levels were low in fresh bovine meat, exotic meat and shell fish. Higher proportions of resistance were observed in poultry meat with the highest rates in imported chicken meat and turkey meat. In addition, resistance to third generation cephalosporins was almost exclusively found amongst indicator *E. coli* obtained from imported poultry meat.

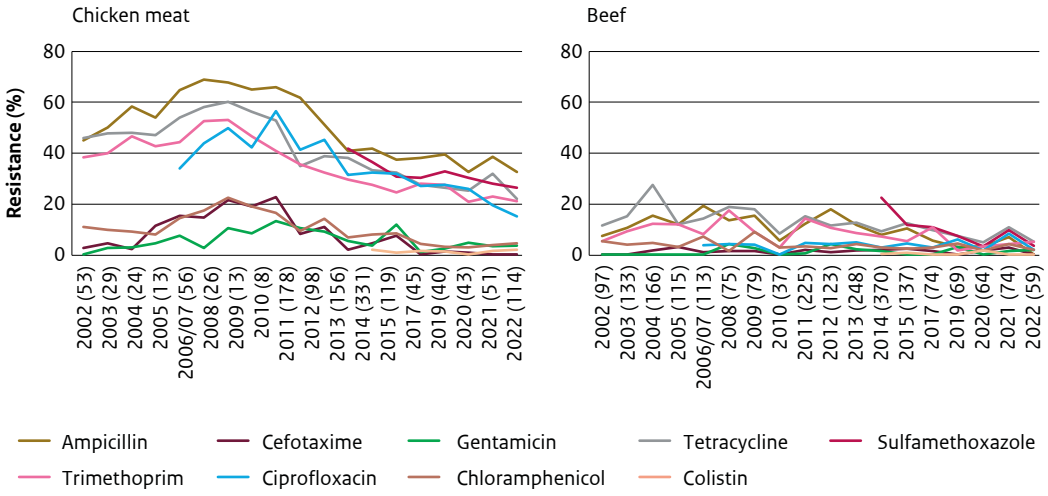
Table Eco03 Resistance percentages (R%) of *E. coli* isolated from raw meat and shellfish in the Netherlands in 2022

Products	Chicken Fresh, retail N = 114	Chicken Imported N = 111	Turkey Imported N = 44	Bovine Fresh, retail N = 59	Exotic meat Fresh, retail N = 63	Shellfish Fresh, retail N = 57
Ampicillin	32.5	51.4	54.5	0.0	12.7	5.3
Cefotaxime	0.0	22.5	11.4	1.7	0.0	0.0
Ceftazidime	0.0	19.8	6.8	0.0	0.0	0.0
Gentamicin	3.5	11.7	11.4	1.7	0.0	0.0
Tetracycline	21.9	37.8	75.0	5.1	25.4	3.5
Sulfamethoxazole	26.3	38.7	61.4	3.4	11.1	1.8
Trimethoprim	21.1	32.4	20.5	5.1	7.9	1.8
Ciprofloxacin	14.0	41.4	40.9	1.7	4.8	0.0
Nalidixic acid	13.2	39.6	34.1	1.7	4.8	0.0
Chloramphenicol	4.4	9.0	20.5	1.7	1.6	1.8
Azithromycin	1.8	0.9	0.0	0.0	0.0	0.0
Colistin	1.8	0.9	0.0	0.0	0.0	0.0
Meropenem	0.0	0.0	0.0	0.0	0.0	0.0
Tigecycline	1.8	4.5	0.0	1.7	4.8	0.0
Amikacin	0.0	0.0	0.0	1.7	0.0	0.0

Samples of exotic meat consisted of: duck, goose, guinea fowl, mallard, partridge, pheasant, wood pigeon, dear, hare and wild boar.

Fig Eco03 shows resistance rates of indicator *E. coli* from fresh chicken meat and bovine meat collected at retail. In general, *E. coli* from chicken meat show similar levels of resistance and trends in time compared to *E. coli* from caecal samples suggesting an overlap in the bacterial population examined from the different matrixes. In bovine meat, levels of resistance are traditionally low with fluctuating percentages below 5% for most antimicrobials tested.

Figure Eco03 Trends in resistance (%) of *E. coli* isolated from chicken meat and beef sampled at retail in the Netherlands from 2002-2022



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4
Screening for ESBL, AmpC, carbapenemase-producing and colistin-resistant Enterobacteriaceae and MRSA in food-producing animals and meat in the Netherlands in 2022

In this chapter, monitoring of organisms of specific interest is described, either due to their resistance mechanism or their potential to spread between the Dutch livestock and human populations. These include resistance against antimicrobials which are classified by the World Health Organisation (WHO) as critically important antimicrobials for human medicine: Gram-negative bacteria which are resistant to 3rd and 4th generation cephalosporins, carbapenems or colistin. Furthermore, the monitoring of the prevalence of Methicillin-resistant Staphylococcus aureus (MRSA) in livestock and food is described here.

Highlights

1. The prevalence of resistance against extended-spectrum cephalosporins (ESC resistance) in randomly selected *E. coli* has been steadily low for several years in all livestock species.
2. While the prevalence of selectively isolated ESC-resistant *E. coli* remained stable or decreased in most livestock sectors, in dairy cattle, an increase was measured compared to previous years.
3. The addition of imported meat to the monitoring has shown that the prevalence of ESC-resistant *E. coli* on imported chicken and turkey meat is much higher than for domestic products.
4. As in 2021, Whole Genome Sequencing of ESC-resistant *E. coli* shows that over 20% of isolates are clonally related. In 2022, clones were also detected that are shared between livestock sectors.
5. The prevalence of ESBL-producing *Salmonella* isolated from human, livestock and domestic meat is considered low. Higher frequency of ESBL-suspected *Salmonella* isolates were detected in imported meat from outside EU.
6. In 2022, no carbapenemase-producing Enterobacterales were detected in livestock, but on one occasion an OXA-48-producing *E. coli* was identified in a faecal sample of a dog.
7. As in former years, the prevalence of *mcr*-genes, encoding for colistin resistance, in *E. coli* was low in livestock and meat.
8. In 2022, 25.4% of the investigated veal calf farms was tested positive for MRSA. The incindence of farms rearing white veal calves (38.3%) were significantly higher than that of those rearing rosé veal calves (14.1%).

4.1 Extended-spectrum cephalosporin (ESC) resistant Enterobacteriaceae

This chapter describes the data for the screening of organisms which are resistant to critically important antimicrobials as defined by the World Health Organisation (Critically important antimicrobials for human medicine, 6th revision, 2019) in the Netherlands. Results include the non-selective and selective screening for ESBL/AmpC producing *E. coli* and Salmonella in livestock and meat, carbapenemase producing Enterobacteriaceae in livestock, companion animals and seafood, colistin resistance in *E. coli* in livestock and meat, and MRSA surveillance in livestock and meat.

New EU legislation on monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria (2020/1729/EU) was implemented in 2021. This allows for the reporting of ESBL, AmpC or carbapenemase producing *E. coli* through whole genome sequencing (WGS) rather than the phenotypic screening through broth microdilution. As WGS allows for an increased level of molecular analysis than was previously carried out, the method was adopted here for all non-selectively and selectively isolated ESBL and AmpC producing *E. coli*, colistin resistant *E. coli* and carbapenemase producing Enterobacteriaceae, and data was compared over the years. All WGS data reported in these sections were analysed using Illumina sequencing technology.

4.1.1 Randomly isolated ESC-resistant *E. coli* from livestock

Random isolation of commensal *E. coli* from caecal samples of broilers, slaughter pigs, veal calves and dairy cows is described in Chapter 3. The prevalence of extended-spectrum cephalosporin resistance (ESC-resistance) in these *E. coli* provides data on the prevalence of the total population of *E. coli* that are present in the livestock sector in the Netherlands. The phenotype of these bacteria was determined by measuring the minimum inhibitory concentration (MIC) and comparing these to the epidemiological cut-off values described by EUCAST. *E. coli* are considered suspected ESBL/pAmpC producers or AmpC promotor mutants when a reduced susceptibility of the isolate is measured against the ESC cefotaxime and/or ceftazidime. After confirmation of the phenotype, WGS is performed. A standardised analysis pipeline was used to asses quality control and perform assembly of the WGS data. Analysis of the resistance mechanisms was determined using Resfinder 4.0 including Pointfinder[1].

Figure ESBL01 shows the trends of randomly isolated ESC-resistant *E. coli* from 1998 until 2022. Over the past 15 years, ESC resistance has reduced to a level where in 2019, no randomly isolated ESC-resistant *E. coli* had been detected. Table ESBL01 includes the number of isolates for each group of animals, and the results of WGS analysis. In 2022, one randomly selected *E. coli* from broilers was isolated which contained the TEM-52B gene. One randomly selected *E. coli* from veal calves contained the CTX-M-1 gene. In pigs, three *E. coli* were isolated, of which two contained ampC promotor mutations that are responsible for resistance to ESC antibiotics.

Figure ESBL01 Trends in cefotaxime resistance (%) of *E. coli* randomly isolated from faeces of broilers, slaughter pigs, veal calves and dairy cows

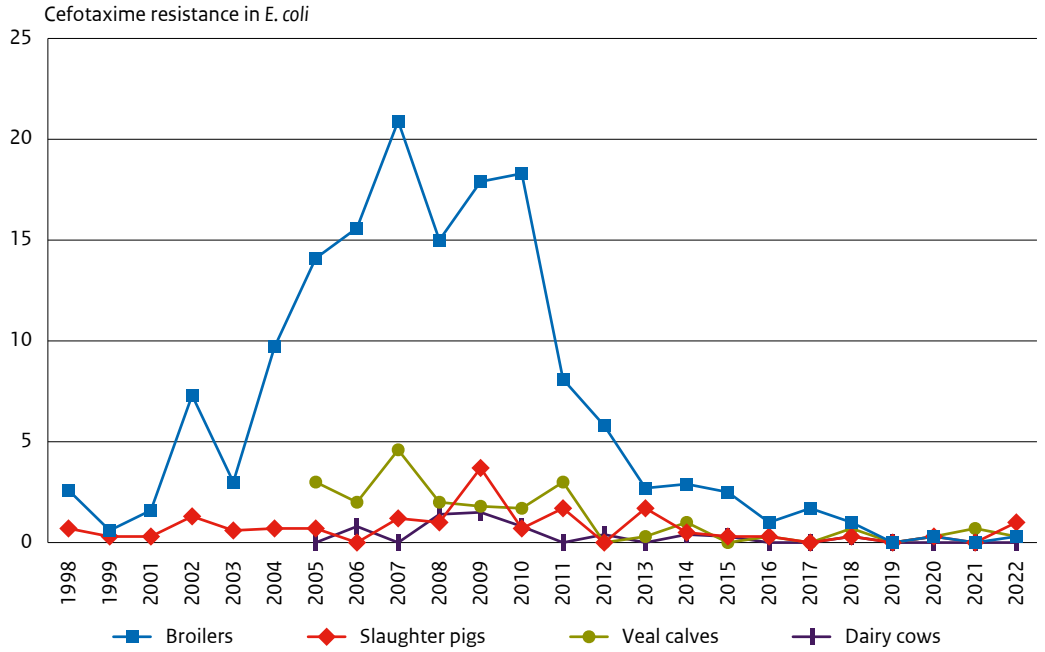


Table ESBL01 ESBL-genes found in *E. coli* isolates with reduced susceptibility to cefotaxime derived from broilers, veal calves, slaughter pigs, dairy cows and turkey (only 2011 and 2012) during 2007-2022

ESBLs isolated from							ESBL-genes detected											
Year	[c]Broilers	Veal calves	Slaughter pigs	[d]Dairy cows	Turkeys	Total ESBL suspected (n)	[a]CTX-M-1-group	CTX-M-2	CTX-M-9-group	TEM-52c	TEM-20	[b]SHV-12	SHV-2	CMY-2	chromosomal *ampC*	no gene found	Total *E. coli* (n)	% ESBL of total *E. coli*
2007	9	6	2	0	*n.t.*	17	3	1		3				1	2	7	539	3.2
2008	66	4	3	2	*n.t.*	75	38	5	1	9			2	12	3	5	1,026	7.3
2009	53	2	11	2	*n.t.*	68	34	7		2	1	8	1	12	3		894	7.6
2010	52	3	2	2	*n.t.*	59	21	6		5	1	9	4	5	3	5	1,002	5.9
2011	23	5	5	0	6	39	9			8		9	2	3	3	5	1,096	3.6
2012	26	2	0	1	0	29	8			4		8		5		4	1,328	2.2
2013	13	1	4	0	*n.t.*	18	7			4		3		3	1		1,371	1.3
2014	11	3	2	0	*n.t.*	16	8			1		4			1	2	1,519	1.1
2015	10	0	1	1	*n.t.*	12	3		2	1		1		2	3		1,283	0.9
2016	3	1	1	0	*n.t.*	5	2			1				1	1		1,492	0.3
2017	5	0	0	0	*n.t.*	5	2			1			2				1,194	0.4
2018	3	2	0	0	*n.t.*	7	2					3			2		1,198	0.6
2019	0	0	0	0	*n.t.*	0											1,209	0.0
2020	1	1	1	0	*n.t.*	3	1						1			1	1,103	0.3
2021	0	2	0	0	*n.t.*	2	1									1	1,206	0.2
2022	1	1	3	0	*n.t.*	5	1			1					2	1	1,276	0.4
Total	**276**	**33**	**35**	**8**	**6**	**350**	**140**	**19**	**3**	**40**	**2**	**45**	**12**	**44**	**24**	**31**	18,736	**1.9**

a All were bla$_{CTX-M-1}$, only in 2011 one bla$_{CTX-M-3}$ gene was found in an isolate from a veal calf.
b One combination of bla$_{SHV-12}$ together with bla$_{TEM-52}$ occured in 2012 in one broiler isolate.
c In broilers, three combinations were found: in 2008: bla$_{CTX-M-1}$ with bla$_{CTX-M-2}$; in 2009: bla$_{CTX-M-1}$ with bla$_{SHV-12}$ and bla$_{CTX-M-1}$ with bla$_{SHV-12}$ and bla$_{CMY-2}$.
d In dairy cows, one combination of bla$_{CMY-42}$ with bla$_{TEM-190}$.
n.t. : not tested

4.1.2 Selectively isolated ESC-resistant *E. coli* from livestock and food products

While the randomly isolated *E. coli* provide an insight into the total prevalence of ESC-resistance in *E. coli* in the livestock population, the selectively isolated *E. coli* provide an insight of prevalence at the level of individual animals.

Selection is performed according to protocols provided by the European Reference Laboratory for Antimicrobial Resistance. Isolation from faeces and caecal content occurs by incubating 1 gram of material in 9 ml of buffered peptone water overnight at 37 °C. While samples from pigs, veal calves and dairy cows represent individual animals per flock, for broiler chickens the caecal contents of 10 animals from a single flock are pooled since 2022 due to a change in EU legislation (2020/1729/EU). Selective isolation is performed on MacConkey agar plates supplemented with 1 mg/L of cefotaxime (EURL AR, Laboratory Protocol; Isolation of ESBL, AmpC and carbapenemase-producing *E. coli* from caecal samples, version 7, December 2019: https://www.eurl-ar.eu/protocols.aspx).

In 2022, 75 faecal samples of meat rabbits collected on 15 rabbit farms were also screened for the presence of ESC-R *E. coli* as part of a policy supporting project, extending AMR monitoring to other animals sectors.

The isolation from food products is performed by adding 25 grams of product to 225 ml of buffered peptone water and incubating overnight at 37 °C. Selective screening is performed on plates of MacConkey agar plates supplemented with 1 mg/L of cefotaxime (EURL AR, Laboratory Protocol; Isolation of ESBL, AmpC and carbapenemase-producing *E. coli* from meat samples, version 7, December 2019: https://www.eurl-ar.eu/protocols.aspx).

Putative resistant *E. coli* colonies are subcultured and species identification is performed using MALDI-TOF (Bruker Biotyper). The MIC of isolates is determined as described in Chapter 3 using a panel of antibiotics specifically aimed at β-lactamase producing *Enterobacteriaceae*. The genotype of all ESC-resistant *E. coli* was confirmed using WGS.

Results of selective isolation and molecular typing of ESC-resistant *E. coli* from livestock

The selective isolation of ESC-resistant *E. coli* has an increased sensitivity and is expected to result in a higher prevalence than the randomly selected ESC-resistant *E. coli*. In contrast to previous years, typing of ESC-resistant *E. coli* now includes the additional category of chromosomal AmpC promotor mutants. This includes *E. coli* with a similar phenotype as pAmpC-producing *E. coli*, with the difference that pAmpC-producing *E. coli* contain a resistance mechanism that can be transmitted between bacteria, while AmpC promotor mutants contain a chromosomal mutation that cannot be transmitted between bacteria and are therefore considered to be less relevant for human health.

Table ESBL02 shows the number of ESC-resistant *E. coli* that were isolated in 2022 and the number of isolates that were confirmed via WGS as ESBL/pAmpC producing *E. coli* or AmpC promotor mutants. The trends over time of confirmed ESBL/pAmpC producing *E. coli* or AmpC promotor mutants are depicted in Figure ESBL02. The results of the molecular typing per animal species are presented in Table ESBL03. The results of this molecular typing of ESC-resistance have previously been used to assess the relative attribution of livestock species and the environment to ESC-resistance in the human population[2].

The prevalence of ESC-resistant *E. coli* in **broilers**, measured by selective isolation, has decreased from 65.8% in 2014 to 9.8% in 2020, while in 2021 a minimal increase to 11.3% was measured. In 2022, the prevalence was further increased to 15.0%, which is a significant increase based on a logistic regression model of the data from 2014 to 2021. However, due to changes in EU legislation (2020/1729/EU), 2022 was the first year in which a pool of the caecal contents of 10 animals was tested per flock, rather than samples from individual animals per flock. According to hypergeometric distribution, the chance of detecting a positive sample increases when multiple samples are pooled, which is expected to have contributed to this increase in prevalence.

The results of analysing the genotype of ESC-resistant *E. coli* in broilers has shown some fluctuations over the years. While the ESBL-gene CTX-M-1 and the plasmid-encoded AmpC-gene CMY-2 were the most prevalent genes in 2014 (respectively 43.25 and 28.6%), over time these decreased in favour of the ESBL-gene SHV-12, which was the most prevalent gene in 2021 (41.2%). In 2022, all three of these prevalent genes decreased and the ESBL-gene CTX-M-15, reached the same prevalence as CMY-2, at 12.8% of the ESC-resistant *E. coli* population.

The prevalence of ESC-resistant *E. coli* in **slaughter pigs** has been stable over time, although a shift in the dominant genotypes has occurred. Previously, chromosomal AmpC promotor mutants were characterised and included in Table ESBL03, but not included as confirmed ESBL in Table ESBL02 and Figure ESBL02, as the resistance mechanism cannot be transferred between bacteria. Due to the shift in genotypic prevalence, these *E. coli* have become more significant and have been added to all relevant tables and figures as an additional category. In 2014, the ESBL-gene CTX-M-1 was the most prevalent resistance mechanism, representing 40.8% of ESC-resistant *E. coli* in slaughter pigs, while AmpC promotor mutants represented 30.6% of isolates. In 2022, CTX-M-1 is detected in 23.9% of isolates while AmpC promotor mutants have increased to 47.8%.

In 2016, the prevalence of ESC-resistant *E. coli* in both **rosé and white veal calves** increased significantly due to unknown cause. In both types of animals, chromosomal AmpC promotor mutants were detected at consistently low levels between 0 to 3.3%. In white veal calves, the prevalence of confirmed ESBL/pAmpC producing *E. coli* increased from 17.9% in 2014 to 33.9% in 2016 and 47.6% in 2018. Since then, the prevalence has seen a slow but consistent decrease to 31.9% in 2022. The ESBL-genes CTX-M-1 and CTX-M-15 have alternated through the years as the most prevalent resistance mechanisms, where both were detected in 27.3% of the isolates in 2022. In rosé veal calves, the prevalence of ESBL/pAmpC producing *E. coli* increased from 12% in 2014 to 28.7% in 2016 and has since fluctuated, reaching 24.8% in 2021 and 11.0% in 2022. Similar to white veal calves, CTX-M-1 and CTX-M-15 are the most prevalent ESBL-genes through the years, and the only genes detected in 2022. However, here the AmpC chromosomal promotor mutants have increased from 3.8% of the population of ESC-resistant *E. coli* in 2021 to 26.7% of the population in 2022.

The prevalence of ESC-resistant *E. coli* in **dairy cows** has seen little fluctuation over time. While the average prevalence of *E. coli* with ESBL/pAmpC genes was 11.8% between 2014 and 2021, the prevalence in 2022 was 18.3%. Analysis to determine werther this increase is statistically significant is ongoing. Similar to veal calves, CTX-M-1, CTX-M-15 and AmpC chromosomal promotor mutants are the most prevalent resistance mechanisms in dairy cows through the years. While CTX-M-1 was the most prevalent resistance mechanism in 2016, which was detected in 41.3% of resistant isolates, in 2022 this has decreased to 12.7%.

Since 2014, the AmpC chromosomal promotor mutants have fluctuated between 13% and 30%, and in 2022 this mechanism was detected in 14.5% of resistant isolates. The gene CTX-M-15 was first detected in 2015 and has since risen to 45.5% of the resistant isolates from dairy cows in 2022.

The differences over time for the various ESBL/pAmpC genes in each of the livestock species described above indicate that selective conditions possibly change over time, affecting the dynamics of ESBL/AmpC *E. coli*. These changing conditions likely provide advantages for either specific *E. coli* lineages that contain this gene, either chromosomally or on a plasmid, to be selected for within the population, or a successful plasmid to be dispersed throughout the microbial communities.

In addition, no ESC-R *E. coli* were detected in faecal samples of meat **rabbits**.

Table ESBL02 Proportion of *E. coli* isolates showing resistance to cefotaxime derived from selective culturing of faecal samples from broilers, slaughter pigs, veal calves and dairy cows collected in 2022

	N samples	N cefotaxime resistant *E. coli*	% cefotaxime resistant *E. coli*	N ESBL/pAmpC carrying *E. coli*	% ESBL/pAmpC carrying *E. coli*	% ESBL/pAmpC carrying *E. coli*	% AmpC promotor mutants
Broilers	300	47	15.7	45	15.0	2	0.7
[a] Pigs	300	44	14.7	24	8.0	22	7.3
Veal calves white	207	70	33.8	66	31.9	4	1.9
Veal calves rosé	100	15	15.0	11	11.0	4	4.0
Dairy cows	300	55	18.3	47	15.7	8	2.7
Total	**1,207**	**231**	**19.1**	**193**	**16.0**	**40**	**3.3**

a In pigs two E. coli isolates carried a CTX-M gene in combination with a mutation in the promotor region of chromosomally located ampC gene.

Figure ESBL02 Trends in prevalence of ESBL/pAmpC-producing *E. coli* in faecal samples of broilers, pigs, white and rosé veal calves and dairy cows from 2014-2022 determined by using selective isolation

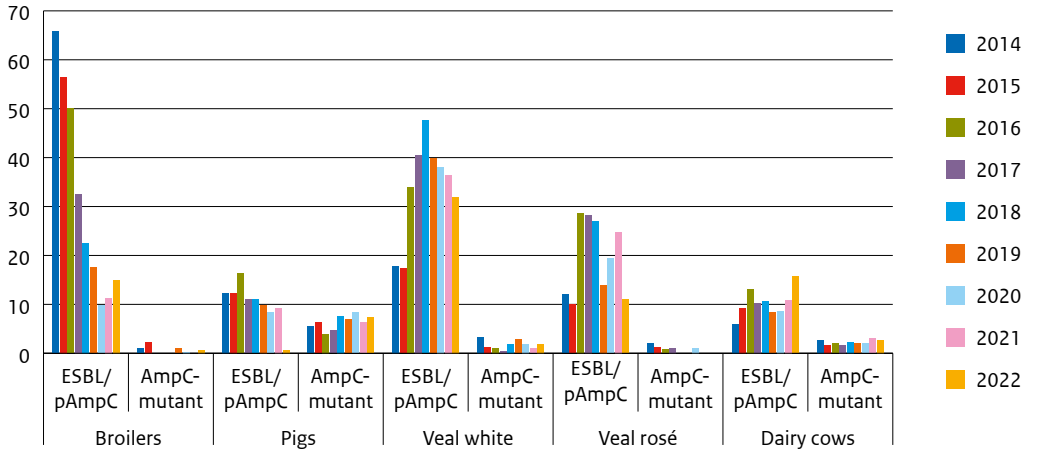


Table ESBL03 β-lactamases identified in *E. coli* derived from selective culturing of faecal samples of broilers, slaughter pigs, veal calves, and dairy cows in 2022

		Broilers	Slaughter pigs	Veal calves		Dairy cows	Total
				White	Rosé		
CTX-M-1 group	CTX-M-1	12	11	18	4	7	52
	CTX-M-15	6	3	18	7	25	59
	CTX-M-32	1	1	9		4	15
	CTX-M-55	5	2	4		4	15
CTX-M-2 group	CTX-M-2			1		1	2
CTX-M-3 group	CTX-M-3	1				2	3
CTX-M-8/25 group	CTX-M-8						0
CTX-M-9 group	CTX-M-9	1					1
	CTX-M-14		1	3			4
	CTX-M-27			1			1
	CTX-M-65		1	1			2
TEM	TEM-52b	1	1				2
	TEM-52c	3	1	8		1	13
SHV	SHV-12	9	1	2		2	14
CMY	CMY-2	6	2			1	9
DHA-1				1			1
Chromosomal *ampC*	[1] *ampC*-type-3	2	20	4	4	8	38
Total		**47**	**44**	**70**	**15**	**55**	**231**

[1] *In pigs two E. coli isolates carried a CTX-M gene in combination with a mutation in the promotor region of chromosomally located ampC gene.*

Results of selective isolation and molecular typing of ESC-resistant E. coli in fresh meat and vegetables

Selective isolation of ESC-resistant *E. coli* was performed from food for human consumption, including samples from fresh meat, poultry, fish, fruit and vegetables from domestic produce and imported goods from outside the EU. Similar to the selective isolation of ESC-resistant *E. coli* from livestock, described above, the selective isolation is more sensitive than the non-selective isolation method described in chapter 3.

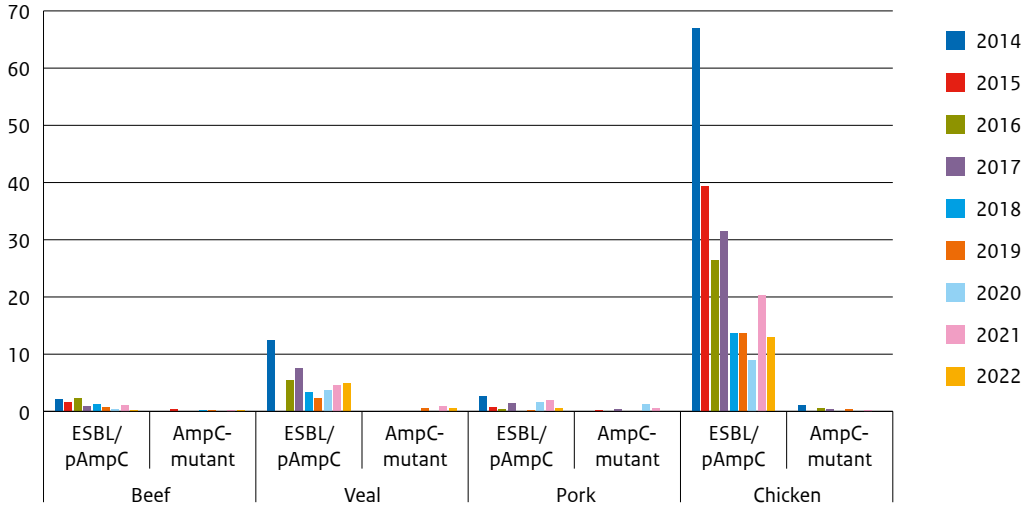
The number of detected ESC-resistant *E. coli*, the number of confirmed ESBL/pAmpC-producing *E. coli* and the number of *E. coli* containing a chromosomal AmpC promotor mutation are included in Table ESBL04. A comparison of the prevalence of these *E. coli* in comparison to previous years is shown in Figure ESBL03 for the categories of food samples of which sufficient numbers were tested through the years. Similarly as described for the samples from livestock above, for these samples from meat, AmpC-promoter mutants were indicated separately in the figure this year. These results indicate that AmpC-promoter mutants are detected in much lower frequency from meat samples than from livestock samples.

Table ESBL04 Prevalence of ESBL/AmpC-positive *E. coli* isolates from raw meat, vegetables, fruits, herbs, mushrooms and aquaculture in the Netherlands in 2022

Animal source	N screened	N ESBL/AmpC suspected	N ESBL/AmpC confirmed	% ESBL/AmpC positive	N AmpC promotor mutants	% AmpC promotor mutants
Beef	350	4	1	0.3	1	0.3
Veal	162	9	8	4.9	1	0.6
Pork	177	2	1	0.6	0	0.0
Chicken	186	32	25	13.4	0	0.0
Turkey	9	1	1	11.1	0	0.0
[1] Exotic meat	89	4	3	3.4	0	0.0
Vegetables/fruit	276	1	0	0.0	0	0.0
Herbs	44	1	1	2.3	0	0.0
Mushrooms	90	0	0	0.0	0	0.0
Imported aquaculture	136	8	7	5.1	0	0.0
Chicken (import, fresh)	106	57	57	53.8	0	0.0
Chicken (import, preparation)	53	36	36	69.9	0	0.0
Turkey (import)	39	29	29	74.4	0	0.0
Total	**1,717**	**184**	**169**	**9.8**	**2**	**0.1**

[1] *Exotic meat consisted duck, goose, guinea fowl, mallard, partridge, pheasant, wood pigeon, dear, hare and wild boar.*

Figure ESBL03 Trends in prevalence of ESBL/AmpC-producing *E. coli* in fresh meat of broilers, pigs, veal calves and dairy cows from 2014-2022 determined by using selective isolation



The results of the WGS performed to confirm the genotype of the isolates are described in Table ESBL05. Further samples are included in this table for ESC-resistant *E. coli* isolated from smaller numbers of samples, ESC-resistant *E. coli* isolated through non-selective culturing, and products imported from outside of the EU.

In **pork** and **beef**, only one and two confirmed ESC-resistant *E. coli* isolates were detected in 2022 respectively, both resulting in a 0.6% prevalence. In beef these included one $bla_{\text{CTX-M-14}}$ containing isolate and an AmpC chromosomal promotor-mutant, and in pork one $bla_{\text{CTX-M-15}}$ containing isolate, all of which were previously detected in these types of meat.

In the past decade, the prevalence for **meat from veal calves** has fluctuated between 3.4-12.5% for ESBL producing *E. coli* and 0-1% for chromosomal AmpC promotor mutants. In 2022, these prevalences were 4.6% and 0.6%, within the expected range. All detected ESBL genes, $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-15}}$, $bla_{\text{CTX-M-32}}$, $bla_{\text{CTX-M-55}}$ and $bla_{\text{TEM-52B}}$, were also detected in previous years.

The prevalence of ESBL/pAmpC producing *E. coli* in **(retail) chicken meat** has reduced considerably, from 67% in 2014 to 12.9% in 2022. This decrease is similar to the decrease in prevalence in caecal samples from broilers. Both in chicken caecal samples and in chicken meat, the pAmpC-gene $bla_{\text{CMY-2}}$ has been replaced by the ESBL-genes $bla_{\text{SHV-12}}$ and $bla_{\text{TEM-52B}}$ as the dominant resistance mechanism.

Similar to chicken, the prevalence of ESC-resistant *E. coli* in **(retail) turkey meat** has also reduced from 50.9% in 2014 to 0% in following years. However, the numbers of samples that are tested is much lower due to its lower consumption in the Netherlands. In 2022, one $bla_{\text{CTX-M-15}}$ producing *E. coli* isolate was detected.

Meat from other animals are collectively reported as **exotic meat** and in 2022 included samples from duck, deer, dove, kangaroo, guinea fowl, partridge and pheasant. One bla_{CMY-2} producing *E. coli* was detected in a sample from partridge, one $bla_{CTX-M-1}$ producing *E. coli* was detected in a sample from deer and one $bla_{CTX-M-15}$ producing *E. coli* was detected in a sample from guinea fowl.

In samples from **fruit and vegetables**, a single ESC-resistant suspected isolate was detected from salad, but both the phenotype and genotype of the isolate did not confirm this. In 2022, just as in 2020 and 2021, no ESC-resistant *E. coli* were detected from **mushrooms**. In samples of fresh imported **herbs**, one $bla_{CTX-M-1}$ producing *E. coli* was detected from a single batch of mint.

Batches of **imported aquaculture** are tested each year and in 2022 these included tilapia, pangasius, shrimp, salmon and tuna. The prevalence of ESC-resistant *E. coli* was 3.7% but due to the variation of countries from which products were imported, this is difficult to compare to previous years. One isolate was detected that produces two ESBLs, $bla_{CTX-M-15}$ and bla_{DHA-1}, three isolates produce $bla_{CTX-M-15}$, and the last isolate produces the pAmpC bla_{VEB-1}.

Imported chicken meat and **imported turkey meat** contain the highest prevalence of ESC-resistant *E. coli.* These food categories are now included into the monitoring due to changes in EU legislation (2020/1729/EU). Imported fresh chicken from Argentina, Brazil, Thailand and the UK was analysed and had a prevalence of 53.8%, whereas samples from preparations of chicken from Thailand and Brazil had a prevalence of 69.9%. Imported turkey meat was included from Brazil and Chile and had a prevalence of ESC-resistant *E. coli* of 74.4%. The resistance mechanisms from imported chicken and turkey were diverse, but $bla_{CTX-M-2}$, $bla_{CTX-M-8}$ and $bla_{CTX-M-55}$ were the three most common genes, which are not commonly found in domestic meat. The high prevalence of ESC-resistant *E. coli* on imported meat is a concern, but it should be noted that imported chicken and turkey meat is almost exclusively used in processed products and not used as fresh meat by end-consumers.

Table ESBL05 β-lactamases identified in *E. coli* from raw meat products, herbs and aquaculture in the Netherlands in 2022

	ESBL gene	Chicken	Pork	Beef	Veal	Turkey	Exotic meat	Herbs	Imported chicken	Imported turkey	Imported aquaculture	Total
CTX-M-1 group	CTX-M-1	3			1		1		2			7
	CTX-M-15		1		1	1	1	1	4	3	3	15
	CTX-M-15; DHA-1										1	1
	CTX-M-32	1			4							5
	CTX-M-55	2			1				36	9		48
	CTX-M-55; CTX-M-2								1			1
	CTX-M-55; CTX-M-8								1			1
CTX-M-2 group	CTX-M-2								18	1		19
CTX-M-8 group	CTX-M-8								22	11		33
CTX-M-9 group	CTX-M-14			1					1			2
	CTX-M-65	1										1
TEM	TEM-52B	7										7
	TEM-52C	1			1							2
	TEM-20	1										1
SHV	SHV-12	7							3	2		12
CMY	CMY-2	2					1		9	2		14
CMH	CMH-3*										1	1
VEB	VEB-1										1	1
ACT	ACT-14*										1	1
Chromosomal *ampC*	*ampC*-type-3			1	1							2
Total		**25**	**1**	**2**	**9**	**1**	**3**	**1**	**97**	**28**	**7**	**174**

** These genes do not show 100% identity to previously described genes.*

Results of genomic comparisons of ESC-resistant E. coli from food and livestock

Since 2021, all ESC-resistant *E. coli* isolated from food and livestock are analysed using whole-genome sequencing (WGS)[28]. WGS-data of the isolates is compared to identify highly related clusters, for which the number of single-nucleotide polymorphisms (SNPs) is determined in pair-wise comparisons, where any isolates with less than 40 SNPs are considered a single clone[3].

In 2021, 16 clusters were identified, consisting of two or more clones of ESC-resistant *E. coli.* Comparing the data of both 2021 and 2022, 32 clusters were identified over the course of these years, of which 14 clusters included isolates of both 2021 and 2022, while 9 clusters included isolates only from 2022.

In 2021 all detected clusters contained clones from within a single production sector, while in 2022, 7 mixed clusters were detected, including caecal samples from broilers, veal calves, dairy cattle or pigs, or meat from pigs. These 7 clusters each contain 2 to 15 isolates and include 6 different resistance mechanisms, indicating this is not one specific mobile genetic element that is transferred across multiple lineages of *E. coli*.

The other 16 clusters contained isolates from chicken meat (n=1, 2022), from pig caecal samples (n=1, 2021-2022), or caecal samples from a combination of caecal samples of veal calves, dairy cattle or meat samples of veal (n=15, 2021-2022).

ESC-resistant Salmonella

Each year, *Salmonella* isolates are typed by the public health institute RIVM and phenotypically analysed for antimicrobial resistance at WBVR. These include bacteria from various sources, but mainly from human patients, see paragraph 3.1.1 for a full description.

A total of fifteen *Salmonella* isolates from human origin (Anatum (n=2), Heidelberg (n=1), Infantis (n=4), Kentucky (n=1), Schwarzengrund (n=1), Typhi (n=2), Typhimurium (n=1), monophasic Typhimurium (n=3) and two non-human isolates (one Infantis and one Oslo) were suspected ESBL-producers based on the phenotypic analysis. Of these, 16 isolates (all except Infantis) were analysed by WGS to confirm the genotype. For 2 human Typhimurium isolates, the phenotype was just one step above the epidemiological cut-off for cefotaxime and WGS showed no presence of known ESBL/pAmpC genes. For the other 14 isolates, 8 different ESBL/pAmpC genes were detected in 8 different serovars, see table ESBL06.

Based on phenotypic analysis at WFSR, 33 suspected ESBL-producing *Salmonella* were isolated from fresh or processed meat imported from outside the EU which belonged to serovars Minnesota, Infantis and Heidelberg. WGS analysis confirmed the presence of ESBL/pAmpC genes in all 33 isolates, see table ESBL06.

In table ESBL07, the results of the ESBL/pAmpC genes detected in *Salmonella* are compared to the results in previous years, showing that the genes of the CTX-M-9 group of ESBLs was the most prevalent resistance mechanism detected in 2022, similar to previous years.

Table ESBL06 β-lactamases identified in *Salmonella* in 2022 (13 human isolates, 1 non-human isolates of unknow origin and 33 isolates from imported poultry meat)

Serovar	CTX-M-1	CTX-M-15	CTX-M-2	CTX-M-55	CTX-M-8	CTX-M-65	CTX-M-14b	SHV-12	CMY-2	Total
Anatum								2		2
Heidelberg									18	18
Infantis						5				5
Kentucky							1			1
Minnesota			2						13	15
Oslo					1					1
Schwarzengrund				1						1
Typhi		2								2
Typhimurium	1								1	2
Total	**1**	**2**	**2**	**1**	**1**	**5**	**1**	**2**	**32**	**47**

Table ESBL07 β-lactamases identified in *Salmonella* isolates collected in 2007-2022

Year	[a] CTX-M-1-group	[b] CTX-M-2	CTX-M-3	CTX-M-8	[c] CTX-M-9-group	TEM-52	TEM-20	[d] SHV-12	[e] CMY-2	ACC-1	DHA-1	Total ESBL	Total *Salmonella* tested	% ESBL of total *Salmonella*
2007	9	13				17	2	4	2			47	1,514	3.1
2008	25	12		1	1	13	1		6	2		61	2,149	2.8
2009	12	4			2	3		1	9			31	2,232	1.4
2010	8	3			1	2		3	4			21	1,715	1.2
2011	5	3			1	1		2	13			25	1,444	1.7
2012	14	5			2	2			10	1		34	1,795	1.9
2013	1	3		5	4	5	1		36			55	1,369	4.0
2014	6			2	3	1			21			33	1,688	2.0
2015	13	2			6	1			12			34	1,761	1.9
[f] 2016	7				15	2			10		1	36	2,117	1.7
[g] 2017	3				23			1	3		1	31	1,697	1.8
[g] 2018	2		1	1	8				2			14	1,718	0.8
2019	4				11			1	3			19	1,880	1.0
2020					4				2			6	1,310	0.5
2021	2				5			1	2			10	1,264	0.8
[h] 2022	4	2		1	6			2	32			47	1,503	3.1
Total	**115**	**47**	**1**	**10**	**92**	**47**	**4**	**15**	**167**	**3**	**2**	**504**	**27,156**	**1.9**

a Contains $bla_{CTX\text{-}M\text{-}1}$, $bla_{CTX\text{-}M\text{-}55}$, $bla_{CTX\text{-}M\text{-}15}$, $bla_{CTX\text{-}M\text{-}3}$ and a combination with $bla_{CMY\text{-}2}$ (n=2, 2014, 2015).

b In 2008 one combination of $bla_{CTX\text{-}M\text{-}2}$ with $bla_{TEM\text{-}52}$ was found in S. Paratyphi B var Java.

c contains $bla_{CTX\text{-}M\text{-}9}$, $bla_{CTX\text{-}M\text{-}14}$ and $bla_{CTX\text{-}M\text{-}65}$.

d In 2007 three S. Concord were found containing both $bla_{SHV\text{-}12}$ and $bla_{CTX\text{-}M\text{-}15}$.

e In 2015 a combination of $bla_{CMY\text{-}2}$ and $bla_{TEM\text{-}52}$ was found in S. Oranienburg and a combination of $bla_{CMY\text{-}2}$ with $bla_{CTX\text{-}M\text{-}1}$ in S. Molade.

f In 2016, one S. Minnesota isolate obtained from poultry meat at NVWA was not included in the molecular analysis.

g In 2017 and 2018 only human isolates were molecularly characterised.

h In 2022 a total of 33 Salmonella isolates obtained from imported fresh or processed meat were included which results in an increase of the % ESBL-positive isolates compared to former years.

4.2 Carbapenemase producing Enterobacteriaceae

4.2.1 Monitoring in livestock

Passive screening
Based on the outcomes of the susceptibility testing, all randomly isolated indicator *E. coli, Salmonella* as well as selectively cultured ESC-resistant *E. coli* isolates are screened for resistance to meropenem as indicator for the presence of carbapenem resistance genes. No meropenem resistant isolates were detected amongst these bacteria in 2022.

Active screening
To screen for the presence of Carbapenemase producing *Enterobacteriaceae* (CPE), faecal samples of livestock are cultured overnight in BPW (1 gram sample in 9 ml BPW) and cultured the next day on two chromogenic agar plates (ChromID CARBA and ChromID OXA, Biomerieux). After incubation, plates are inspected visually for growth of CPE suspected colonies and identified by MALDITOF. In 2022, no CPE were identified using this culture method.

To enhance the sensitivity of the screening all samples are screened in parallel for specific carbapenem resistance genes with an in-house Real-Time PCR. This is important in an environment with a very low anticipated prevalence of carbapenem resistance. Samples were grown overnight in Buffered Peptone Water (BPW) with 0.25 mg/L ertapenem and 50 mg/L vancomycin. After incubation, five individual samples were pooled in lysis buffer, next DNA was isolated with a bead method (NucliSENS easyMag, BioMérieux). A multiplex Real-Time PCR (In house) that can detect the most prevalent carbapenemase gene families (KPC, NDM, VIM, IMP and OXA-48) was used. PCR screening was extended with two additional carbapenem resistance genes (IMI and FRI) which are more frequently found in *Enterobacter cloacae* complex isolates obtained from imported seafood[4,5]. Recently, an Enterobacter isolate carrying an IMI gene was found in a feed mill in Sweden[6]. If Real-Time PCR gave suspected or positive results, a step-wise analysis was performed to confirm the results:

1. Singleplex Real-Time PCR-tests were performed on purified DNA of the 5 individual samples of the pool;
2. If the PCR was positive, the original faecal sample and corresponding broth culture of suspected positive samples were inoculated for bacterial isolation on commercial selective plates (ChromID CARBA and ChromID OXA, Biomerieux, for *Enterobacteriaceae*) and on HIS plates with 0.125 mg/L ertapenem (for *Shewanella* spp);
3. DNA was isolated from bacterial isolates grown on selective agar plates, Real-Time PCR was repeated and genes were confirmed with Sanger sequencing.

The screening of 1207 faecal samples (collected from 300 broilers, 300 slaughter pigs, 307 veal calves and 300 dairy cattle) resulted in eleven OXA-48-like positive faecal samples (0.9%) which is similar to 2021 with 0.8% OXA-48-like positive samples. Positive samples included: veal calves (n=2), dairy cattle (n=8) and broilers (n=1). In seven samples the presence of OXA-carrying *Shewanella* was confirmed by bacterial culturing followed by PCR and sequencing: OXA-48b (n=5), OXA-416-like (n=1) and OXA-547 (n=1). In all four remaining samples OXA-48b (n=4) was detected in the enrichment broth with PCR and confirmed by Sanger sequencing, but culturing of *Shewanella* was negative. These results confirm the findings of the previous years where OXA-48-like genes have also been found in *Shewanella* obtained in faecal samples from livestock. Given the role of *Shewanella* spp. as natural progenitor of this carbapenemase

family[7], these genes were considered of environmental origin and not a public health risk. PCR screening for the remaining six carbapenem resistance genes was negative for all samples. Most importantly, no carbapenemase-producing *Enterobacteriaceae* were detected in faecal samples from livestock in the Netherlands in 2022. Screening for carbapenemase-producing isolates in faecal samples of food-producing animals will continue in 2023.

4.2.2 Monitoring in companion animals

In 2022, 109 faecal samples from dogs and 104 faecal samples from cats were examined. Samples were obtained through the Veterinary Microbiological Diagnostic Centre (VMDC). Because the expected prevalence of CPE in companion animals remains low and reported CPE are frequently multi-resistant, the inclusion criterion for dog faecal samples was recent antimicrobial treatment of the animal. This strategy is not feasible for cats, since cats are less frequently treated with antimicrobials. Therefore, in cats a randomized stratified subset of faecal samples from cats submitted to VMDC were included.
From each sample, 0.5 gram faeces was suspended in 4.5 ml TSB broth, supplemented with 50 mg/L vancomycin for enrichment. The suspension was directly inoculated on ChromID Carba-Smart agar plates (BioMerieux). Both the Smart Agar and the enrichment broth were cultured overnight at 37 °C. After enrichment, the broth was inoculated again and cultured on ChromID Carba-Smart agar (BioMerieux). In addition, total DNA of the enrichment broth was isolated for molecular screening by PCR for the targets NDM[8], KPC[9], IMP[10], VIM[10], OXA-group-23, -24, -51, -58[11] and OXA-group-48[12].

One faecal sample of a dog was positive after selective culture and PCR screening. This was a sample from a 5-month-old dog with gastrointestinal complaints (no appetite, diarrhoea) that was previously treated with metronidazole. Two different bacterial species were found in the sample after selective culture: *Escherichia coli* and *Enterobacter cloacae*. Both strains were PCR positive for OXA-48. No CPE-suspected isolates were found in the other 108 faecal dog samples nor in 104 faecal cat samples. These outcomes indicate the importance of a continuous screening program. Screening for carbapenemase-producing isolates in companion animals will continue in 2023.

4.2.3 Monitoring in imported seafood, seaweed and herbs

In 2022, 242 batches of frozen fish originating from fish farms in South-East Asia as well as 48 batches of shrimps, mainly from farms in South America and Asia, were screened for the presence of carbapenemase producing *Enterobacteriaceae* (CPE) by WFSR through selective culturing. In addition, 44 batches of imported herbs; 49 batches of fruits; and 76 batches of exotic meat were screened for the presence of CPE. In none of these analyzed samples CPE were detected. The monitoring of imported food will be continued in 2023 and extended with PCR screening in order to increase the sensitivity of the method.

4.3 Colistin resistance

In 2022, active screening for the presence of *mcr*-genes in caecal samples was continued using selective culturing (in BPW + 2 mg/L colistin) and PCR. For this purpose, purified DNA of pooled BPW cultures (five samples per pool) from a total of 1207 faecal samples of Dutch livestock were tested for the presence of *mcr*-1, *mcr*-2, *mcr*-3, *mcr*-4 and *mcr*-5 using an in house designed multiplex Real-Time PCR based on the updated EURL-AR protocol (https://www.eurl-ar.eu/CustomerData/Files/Folders/21-protocols/396_mcr-multiplex-pcr-protocol-v3-feb18.pdf). From 2022 onwards, the PCR screening of the samples was extended with *mcr*-6, *mcr*-7, *mcr*-8, *mcr*-9 and *mcr*-10 using an extra in inhouse designed multiplex RT-PCR. In case of a PCR positive pool, individual samples were tested, followed by direct culturing of the original BPW broth on MacConkey agar with 2 mg/L colistin.

In 2022, *mcr*-1 positive *E. coli* were identified in seven samples (0.7%) consisting of caecal samples from four flocks of broilers (1.3%), one white veal calf (0.5%) and two faecal samples from dairy cattle (0.7%). In addition, a *Hafnia alvei* isolate carrying *mcr*-4.6 was detected for the first time in a white veal calf[13]. Notably, *mcr*-4.3 was detected in *E. coli* from veal calves in 2018 and 2019 and in *Hafnia alvei* in 2021 also originating from a veal calf. Finally, for the first time since 2013, a colistin resistant carrying *mcr*-1 isolate was identified amongst the randomly selected indicator *E. coli.* This *mcr*-1 carrying isolate was obtained from a faecal sample of a dairy cow (**Table Eco02**). No other colistin resistant indicator *E. coli* were identified in livestock. Colistin resistance was also detected at low levels in *E. coli* in fresh and imported chicken meat (1.8% and 0.9%, respectively), but not in fresh beef, exotic meat, shell fish and imported turkey meat (**Table Eco03**).

4.4 MRSA surveillance in livestock and humans

MRSA surveillance in livestock and humans

MRSA causes healthcare- and community-associated infections and asymptomatic carriage in humans. During the last two decades, MLST clonal complex (CC) 398 has emerged in livestock and persons in contact with livestock in many countries, including The Netherlands. This type of MRSA is referred to as livestock-associated MRSA (LA-MRSA). The most important risk factor for human carriage of LA-MRSA is professional contact with livestock, especially pigs, poultry and veal calves[14]. During the last decade, however, the number of persons colonized or infected with LA-MRSA in The Netherlands who did not have direct contact with livestock, seemed to be increasing[15-17]. In 2018, a project on surveillance of MRSA in humans, livestock, and meat products was started. This project is a collaboration between NVWA, RIVM, WBVR and WFSR. MRSA isolates obtained from animals, dust from livestock farms, farmers and their family members and meat are compared with isolates collected in the Dutch national MRSA surveillance in humans. For the Dutch national MRSA surveillance, medical microbiology laboratories send MRSA isolates from carriers and from infected persons to the RIVM. The objective of this project is to assess possible changes in the rate or nature of MRSA transmission between animals and humans. Below are the findings obtained within this surveillance project.

Prevalence of MRSA on livestock farms, in caecal samples and in isolates from meat

For the MRSA surveillance in livestock, each year one animal sector is monitored. For the year 2022 veal calf farms and persons working and/or living on these farms were sampled. In addition rabbit farms (only the animals) were investigated as well.

Veal calf farms

In 2022, a total of 173 veal calf farms were investigated. Nasal samples were taken from three animals per barn aged between 5 and 7 months in a maximum of 2 barns. On most farms, calves from two barns were sampled, resulting in a total of 258 barns. A total of 772 nose swabs were examined. One or more samples from 44 farms were MRSA-positive, resulting in a farm prevalence of 25.4% (95%CI: 19.5-32.4%) (Table MRSA01). The sample prevalence was 15.2%, while 22.5% of the barns were tested MRSA-positive. The farm prevalence of farms rearing white veal calves (38.3%) was significantly higher than that of those rearing rosé veal calves (14.1%). The reason for this difference is unknown, but could be due to different management systems. The prevalence seems lower than that found in a study in 2007/2008 by Graveland *et al.*. In that study nasal swabs from 2151 calves on 102 farms were investigated and on 88% (95%CI 81-93%) of the farms one or more samples were MRSA positive[18]. A higher number of calves per farm were sampled, which enhanced the chance of finding MRSA on a farm and may in part explain the difference.

Persons working/living on dairy farms

Persons living and/or working on the veal calf farms were requested to take a nasal swab on a voluntary basis. In total 55 persons living and/or working on 36 farms volunteered to send in a nasal swab. Seven persons (12.7%; 95%CI 6.3-24.0%) were MRSA-positive and all were farmers and had daily contact with veal calves. The results of veal calf farms are summarized in Table MRSA01 together with data from 2018/2019 (broiler farms), 2020 (pig farms), 2021 (dairy farms) and 2022 (rabbit farms).

Table MRSA01 Number of MRSA found on farms and in persons working/living on these farms from 2018-2022

Year	Animal	MRSA positive farms (n)	Total (n)	Prevalence (%)	95%CI	Humans (n)	Total (n)	Prevalence (%)	95%CI
2018/2019	Broilers	0	195	0.0	0.0-2.0	4	133	3.0	3.4-10.3
2020	Pigs	133	149	89.3	83.3-93.3	ns	ns	-	-
2020/2021	Dairy cows	11	181	6.2	3.4-10.6	1	107	0.9	0.2-5.1
2022	Rabbits	0	75	0.0	0–4.9	ns	ns	-	-
2022	Veal calves	44	173	25.4	19.5-32.4	7	55	12.7	6.3-24.0

ns: not sampled

The prevalence of persons working/living on veal calf farms was higher than on broiler- and dairy farms. On pig farms, no samples from humans were taken. The prevalence of 12.7% is comparable to the results found in the same study mentioned above by Graveland *et al.* which investigated nasal carriage of MRSA in 390 persons living or working on 102 veal calf farms. They found a prevalence of 16% and the prevalence was highest in farmers and employees (33% and 26% respectively) compared to 8% in family members[18]. In the Netherlands, the prevalence of MRSA (all MRSA types including LA-MRSA) carriage in individuals in the population at large is low and has varied between 0.1% and 0.8% during the last decade depending on the methods used and the population studied[19-21]. In a Dutch study among persons living in a livestock-dense area, but without professional livestock contact, 0.6% carried MRSA and the prevalence of LA-MRSA was 0.4%[22]. Together with our and previous findings this confirms that persons in close contact with veal calves are at greater risk of MRSA nasal carriage.

Rabbit farms
In addition, in 2022, 75 faecal samples, 75 dust samples and 75 nasal swabs were collected from meat rabbits originating from 15 different farms and were cultured for MRSA. However, no MRSA was found.

MRSA from caecal samples from pigs, veal calves and dairy cattle

In 2022, caecal/faecal samples from the national surveillance taken at slaughter for the monitoring of antimicrobial resistance in zoonotic and commensal bacteria (EU decision (EU) 2020/1729) were investigated. MRSA was found in caecal samples from 48/300 pigs, 60/307 caecal samples from veal calves and 1/300 faecal samples from dairy cattle (Table MRSA02).

Table MRSA02 Number of MRSA found in caecal samples collected in 2022

Animal	Positive samples (n)	Total (n)	Prevalence (%)	95%CI
Pigs	48	300	16.0	12.0-20.6
White veal calves	58	207	28.0	22.0-34.7
Rosé veal calves	2	100	2.0	0.2-7.0
Dairy Cattle	1	300	1.0	0.1-1.8

The data should be interpreted with care, as caecal or faecal samples are not the preferred sample for MRSA. The samples, however, are available from the routine surveillance and as they are collected in the same way each year, trends in time can be analysed. The prevalence found in pigs in 2022 was comparable to the prevalence of 15% found in 2021. The low prevalence found in dairy cows is also consistent with the findings in 2021. Remarkably, MRSA was cultured from 28.0% of the white veal calf samples compared to only 2% from the samples of rosé calves. This difference was also reflected in the farm prevalence found on the veal calf farms. As mentioned before, the reason for this difference is unknown, but could be due to different management systems.

MRSA on meat

MRSA was also found on meat. From 2018 to 2022, 3166 retail meat samples were analysed and 309 (9.8%) were MRSA-positive (Table MRSA03). The prevalence on poultry meat was 14.4% (166/1149), on pork 7.2% (48/668), on beef 4.0% (23/573), on veal 7.3% (35/447), and on lamb meat 12.4% (37/299). The prevalence was highest on turkey meat; 45.3 % (24/53), followed by broiler meat 13.0% (142/1096) and lamb (12.4%). Remarkably, broiler meat was often contaminated with MRSA, although in 2018/2019 MRSA was not found on 190 Dutch broiler farms. The MRSA prevalence on broiler farms might have been underestimated due to the fact that only dust samples were investigated. A Belgian study on broiler farms did also not find MRSA in dust from the broiler houses, although 7.2% of the samples from the broilers themselves were MRSA-positive[23]. Dust samples from broiler farms might contain residues of coccidiostats in concentrations active against Gram-positive bacteria (personal communication M. Rapallini). This would explain the discordance between the MRSA prevalence in dust and meat samples. In addition, poultry meat sold in the Netherlands might originate from other countries with a higher MRSA prevalence. Contamination by humans during handling of meat might also play a role. This is confirmed by the fact that not all MRSA from poultry meat were ST398, but some belonged to human associated MRSA types. Generally it is thought that meat consumption is not an important transmission route for humans, especially if the meat is heated before consumption. In some studies performed abroad, however, food handling has been implicated as a transmission route [24-27].

Table MRSA03 Number of MRSA found on meat (products) from 2018-2022

	2018			2019			2020			2021			2022		
	Positive (n)	Total (n)	Prevalence (%)	Positive (n)	Total (n)	Prevalence (%)	Positive (n)	Total (n)	Prevalence (%)	Positive (n)	Total (n)	Prevalence (%)	Positive (n)	Total (n)	Prevalence (%)
Pork	8	135	5.9	25	296	8.4	2	57	3.5	ns	ns	-	13	180	7.2
Beef	3	140	2.1	11	286	3.8	ns	ns	-	ns	ns	-	9	147	6.1
Veal	ns	ns	-	ns	ns	-	2	52	3.8	18	261	6.9	15	164	9.1
Poultry meat total	29	132	22	50	251	19.9	41	248	16.5	28	324	8.6	18	194	9.3
Chicken	26	129	20.2	41	237	17.3	36	234	15.4	25	310	8.1	14	186	7.5
Turkey	3	3	100	9	14	64.3	5	14	35.7	3	14	21.4	4	8	50.0
Lamb	ns	ns	-	ns	ns	-	ns	ns	-	37	299	12.4	ns	ns	ns

ns: not sampled

Table MRSA04 Resistance percentages (R%) of MRSA isolated from veal calves (nasal swabs, caeca and meat) and pigs (caeca) in 2022

	Veal calves nasal swabs (N=117)	Veal calves caeca (N=60)	Veal calves meat (N=12)	Pigs caeca (N=48)
Cefoxitin	100.0	100.0	100.0	100.0
Chloramphenicol	14.5	3.3	0.0	20.8
Ciprofloxacin	12.0	5.0	0.0	6.3
Clindamycin	88.9	86.7	50.0	45.8
Erythromycin	87.2	85.0	66.7	39.6
Fusidic acid	0.0	6.7	0.0	6.3
Gentamicin	65.8	93.3	50.0	33.3
Kanamycin	68.4	93.3	50.0	20.8
Linezolid	0.0	0.0	0.0	0.0
Mupirocin	0.0	0.0	0.0	0.0
Penicillin	100.0	100.0	100.0	100.0
Quinupristin/dalfopristin	26.5	16.7	8.3	20.8
Rifampicin	0.0	0.0	0.0	0.0
Streptomycine	14.5	8.3	16.7	8.3
Sulfamethoxazole	0.0	1.7	0.0	4.2
Tetracycline	100.0	100.0	75.0	100.0
Tiamulin	19.7	6.7	8.3	20.8
Trimethoprim	89.7	98.3	91.7	58.3
Vancomycin	0.0	0.0	0.0	0.0

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