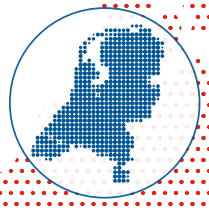
NethMap 2022

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

25 JAAR SWAB Stichting Werkgroep Antibioticabeleid

NethMap 2022

Consumption of antimicrobial agents and
antimicrobial resistance
among medically important bacteria
in the Netherlands
in 2021

June 2022

In memoriam

Kees Verduin

Op 7 december 2021 overleed onze geliefde collega Kees Verduin, hij was een echte all-round arts-microbioloog en werkzaam bij de PAMM. In de afgelopen paar jaren was hij eveneens de eindredacteur van NethMap, de belangrijke overzichtsrapportage van de antimicrobiële consumptie en resistentieontwikkeling in Nederland, waarvan de 2022-editie nu voor u ligt. Met veel enthousiasme en inzet zorgde hij ervoor dat dit werk zorgvuldig en op tijd werd uitgevoerd.
Daarnaast was hij vanaf 2014 namens de NVMM lid van de deelnemersraad en lid van de SWAB-werkgroep Surveillance Antibioticaresistentie, waar hij in december 2018 ook de voorzitter van werd. Daarnaast had hij zitting in diverse richtlijncommissies en was altijd zeer betrokken bij de missie en doelen van de Stichting Werkgroep Antibioticabeleid.
Goed samenwerken met elkaar stond bij hem hoog in het vaandel. Bovenal kenden wij hem allen als een zeer vriendelijke, maar ook als uiterst bekwame arts-microbioloog, die altijd voor je klaar stond als er een vraag was, of er een probleem moest worden opgelost. Wij missen zijn professionele kennis en zijn warme persoonlijkheid zeer.

Synopsis

NethMap/MARAN report

The coronavirus pandemic still impacted heavily on the healthcare system in the Netherlands in 2021. More people ended up in intensive care units and fewer people were able to get appointments in regular healthcare. Despite these changes the number of antibiotic-resistant bacteria has remained the same over the past couple of years. Resistance has even decreased in some strains of bacteria compared to previous years. Furthermore, the number of bacteria that are resistant to various types of antibiotics simultaneously, which makes treatment more difficult, has not changed.

In recent years, there has been an increase in antibiotic resistance in some strains of bacteria that usually cause mild infections such as of the skin. Hospitals and care homes for the elderly have reported fewer outbreaks caused by resistant bacteria since the start of the pandemic in 2020. It is unclear what the effects of the pandemic will be on antibiotic resistance on the long-term.

The overall quantity of antibiotics prescribed by GPs and hospitals fell during the pandemic. That said, more antibiotics per patient were prescribed. This is due to the fact that many patients with Covid-19 had to be treated for longer and more intensively in hospital.

Infections caused by antibiotic-resistant bacteria are becoming increasingly common throughout the world. This problem is not quite so acute in the Netherlands as it is in many other countries as antibiotics are only prescribed if it is absolutely necessary to do so. Still, we do need to remain vigilant in the Netherlands. That entails keeping a close eye on antibiotic use and antibiotic resistance. Monitoring these will enable us to implement measures in a timely fashion to prevent the problem of antibiotic resistance from getting worse.

The measures currently in place in the Netherlands to combat antibiotic resistance extend beyond the healthcare sphere. After all, resistant bacteria are also found in animals, in food and in the environment (One Health approach).

Over the past decade there has been a fall in resistance in gut bacteria in pigs, cows and chickens kept for food production (farmed animals). A lower quantity of antibiotics was sold and used for farmed animals in 2021 than in 2020. Compared to 2009, the reference year, the drop in sales is over 70%. Since 2015, the antibiotics that are crucial to treat infections in people have only been used on farmed animals in highly exceptional circumstances.

This is shown in the annual report NethMap/MARAN 2022, in which various organisations collectively present the data on antibiotic use and antibiotic resistance in the Netherlands, for humans and animals.

Keywords: antibiotic resistance, bacteria, antibiotic use, infection

Publiekssamenvatting

NethMap/MARAN-rapport

Ook in 2021 heeft de uitbraak van het coronavirus de gezondheidszorg in Nederland nog erg belast. Er hebben meer mensen op de IC gelegen en minder mensen konden terecht in de reguliere zorg. Toch is het aantal bacteriën dat resistent is tegen antibiotica afgelopen twee jaar gelijk gebleven. Bij sommige bacteriesoorten is de resistentie zelfs afgenomen ten opzichte van de jaren ervoor. Ook is het aantal bacteriën dat resistent is tegen verschillende antibiotica tegelijk, waardoor ze moeilijker te behandelen zijn, gelijk gebleven.

Wel is de laatste jaren de resistentie toegenomen bij sommige soorten bacteriën die veelal milde infecties van onder andere de huid veroorzaken. Sinds het begin van de coronapandemie in 2020 hebben ziekenhuizen en verpleeghuizen minder uitbraken door resistente bacteriën gemeld. Het is niet duidelijk wat de effecten van de coronapandemie op de antibioticaresistentie op de langere termijn zijn.

Tijdens de coronapandemie hebben huisartsen en ziekenhuizen in totaal minder antibiotica voorgeschreven. Wel is er gemiddeld per patiënt meer antibiotica gegeven. Dit komt doordat veel patiënten met COVID-19 langer en intensiever moesten worden behandeld in het ziekenhuis.

Wereldwijd komt het steeds vaker voor dat infecties worden veroorzaakt door bacteriën die resistent zijn tegen antibiotica. In Nederland is dit probleem minder groot dan in veel andere landen omdat antibiotica alleen wordt voorgeschreven als het echt nodig is. Toch is het belangrijk dat Nederland waakzaam blijft. Dat gebeurt onder andere door antibioticaresistentie en antibioticagebruik in de gaten te houden. Dan kunnen op tijd maatregelen worden genomen om te voorkomen dat het resistentieprobleem groter wordt.

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

De laatste tien jaar zijn darmbacteriën in varkens, koeien en kippen die voor de voedselproductie worden gehouden (landbouwhuisdieren) steeds minder resistent geworden. In 2021 zijn minder antibiotica verkocht en gebruikt voor landbouwhuisdieren dan in 2020. Ten opzichte van 2009, het referentiejaar, is de verkoop met ruim 70 procent gedaald. Sinds 2015 worden de antibiotica die cruciaal zijn om infecties bij de mens te behandelen, alleen nog bij hoge uitzondering gebruikt voor landbouwhuisdieren.

Dit blijkt uit de jaarlijkse rapportage NethMap/MARAN 2022. Hierin presenteren diverse organisaties samen de gegevens over het antibioticagebruik en -resistentie in Nederland, voor mensen en dieren.

Kernwoorden: antibioticaresistentie, bacteriën, antibioticagebruik, 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 2022 and earlier versions are also available from the website of the SWAB: www.swab.nl and www.rivm.nl.

Members of SWAB's working group on Surveillance of Antimicrobial Resistance/ Committee Guidelines Susceptibility Testing (CRG)

Dr NJ Verkaik (chair working group on Surveillance of Antimicrobial Resistance)
Dr AE Muller (chair subcommittee CRG/NAC)
Dr L van Balen
Drs MA Berends
Dr MB Ekkelenkamp
Dr ir SC de Greeff
Dr E Kolwijck
Dr DC Melles
Dr CM Verduin †
Dr AF Schoffelen
Prof Dr C Schultsz

Members of CIb working on Surveillance of Antimicrobial Resistance

Dr Ir W Altorf-van der Kuil
Dr Ir BHB van Benthem
Drs SM Bierman
Dr Ir SC de Greeff
Mrs SR Groenendijk
Ing APJ Haenen
Dr APA Hendrickx
Drs R Hertroys
Dr NA Kampstra
Mrs M Kamst-van Agterveld
Ir TII van der Kooi
Prof Dr EJ Kuijper
Drs N Marchal
Dr A Meijer
Drs JCM Monen
Dr DW Notermans
Mr JEM Polman
Drs WJ van den Reek
Dr C Schneeberger
Dr AF Schoffelen
Prof Dr D van Soolingen
Drs M Visser
Dr CCH Wielders
Drs BJ de Wit
Ir SHS Woudt
Drs RE Zoetigheid

Members of SWAB's working group on Surveillance of Antimicrobial Use

Dr S Natsch (chair)
Drs BJC Hendriks
Dr NGL Jager
Drs PAG de Klaver
Dr TBY Liem
Dr PD van der Linden
Drs M Lourens
Drs AE Nieman
Drs MMB Roukens
Drs M Sijbom
Dr AW van der Velden

Members of SWAB's working group on Antimicrobial Stewardship

Dr J ten Oever (chair)
S Balder
I Groothuis
Drs MMC Hendriks
Dr RM van Hest
Prof Dr MEJL Hulscher
Dr PD van der Linden
Prof Dr JM Prins
Dr J Schouten
Dr KCE Sigaloff
Prof Dr BNM Sinha
D. Struik
Prof Dr A Verbon
Dr CM Verduin †
Dr JEM de Steenwinkel
Prof Dr HFL Wertheim
Dr TFW Wolfs

Acknowledgements

We thank the Foundation for Pharmaceutical Statistics SFK, The Hague, for providing data on community usage of antimicrobial agents and all hospital pharmacists of the centres mentioned below for providing data on hospital usage.

We thank all participants of ISIS-AR, Dr JA Severin, Dr DC Melles, Dr MS Arcilla, the Netherlands Reference Laboratory for Bacterial Meningitis in Amsterdam, GRAS, anaerobic pathogen surveillance, *C. difficile* surveillance, azole resistance surveillance, and the NIVEL for their important contributions; and the staff of the Publishing Department RIVM for preparing this report for printing.

Centres contributing to the surveillance of antibiotic consumption
Alkmaar & Den Helder, NoordWest ziekenhuisgroep; Almelo & Hengelo, ziekenhuisgroep Twente; Almere, Flevoziekenhuis; Amersfoort, Meander MC ; Amsterdam, AMC; Amsterdam, BovenIJ ziekenhuis; Amsterdam, OLVG Oost; Amsterdam, OLVG west; Amsterdam, VUMC; Apeldoorn & Zutphen, Gelre ziekenhuizen; Arnhem, Rijnstate; Assen, Wilhelmina ziekenhuis; Bergen op Zoom & Roosendaal, Bravis ziekenhuis; Boxmeer, Maasziekenhuis Pantein; Breda, Amphia ziekenhuis; Delft, Reinier de Graaf Groep; Den Bosch, Jeroen Bosch ziekenhuis; Den Haag, HMC; Den Haag, HAGA ziekenhuizen; Deventer, Deventer ziekenhuis; Dirksland, van Weel Bethesda ziekenhuis; Doetinchem, Slingerland ziekenhuis; Drachten, Nij Smellinghe; Ede, Gelderse vallei; Eindhoven, Maxima MC; Enschede, Medisch spectrum Twente; Geldrop, St Anna ziekenhuis; Gorinchem, Rivas Zorggroep; Gouda, Groene hart ziekenhuis; Groningen, Martini ziekenhuis; Groningen, Ommelander ziekenhuis; Groningen, UMCG; Haarlem, Spaarne Gasthuis; Hardenberg, Ropcke Zweers; Harderwijk, St.Jansdal; Heerenveen, De Tjongerschans; Hilversum, Tergooiziekenhuizen; Hoogeveen & Emmen, Treant Zorggroep; Hoorn, Westfries gasthuis; Leeuwarden, Medisch centrum Leeuwarden; Leiden, LUMC; Maastricht, MUMC; Nieuwegein, St.Antonius ziekenhuis; Nijmegen, CWZ; Nijmegen, Radboudumc; Roermond, Laurentius ziekenhuis; Rotterdam, Erasmus MC; Rotterdam, Ikazia ziekenhuis; Rotterdam, Maasstad ziekenhuis; Rotterdam, St.Franciscus gasthuis; Schiedam, Vlietland ziekenhuis; Spijkenisse, Spijkenisse medisch centrum; Terneuzen, ZorgSaam; Tiel, Ziekenhuis Rivierenland; Tilburg, ETZ; Uden, Ziekenhuis Bernhoven; Utrecht, Diakonessenhuis; Utrecht, UMCU; Venlo, Vie Curi MC; Winterswijk, Streekziekenhuis Koningin Beatrix; Zeeland, ADRZ; Zoetermeer, Lange Land ziekenhuis; Zwolle & Meppel, Isala klinieken.

Centres contributing to the surveillance of resistance to antimicrobial agents (ISIS-AR)
Alkmaar, Noordwest Ziekenhuisgroep; Amersfoort, Meander MC; Amsterdam, Amsterdam UMC; Amsterdam, Atalmedial; Amsterdam, OLVG / Almere; Amsterdam, Streeklaboratorium Amsterdam – GGD; Apeldoorn, Gelre Ziekenhuizen; Breda, Microvida locatie Amphia; Capelle aan den IJssel, IJssellandziekenhuis; Delft, Microbiologisch laboratorium Reinier de Graaf Groep; Deventer, Deventer Ziekenhuis; Doetinchem, Slingeland Ziekenhuis; Dordrecht, Regionaal Laboratorium Medische Microbiologie; Ede, Ziekenhuis Gelderse Vallei; Etten-Leur, SHL-Groep; Goes, Admiraal De Ruyter Ziekenhuis; Gouda, Groene Hart Ziekenhuis; Groningen, Certe; Groningen, UMC Groningen; Haarlem, Streeklaboratorium voor de Volksgezondheid; Hengelo, Laboratorium voor Medische Microbiologie Twente/Achterhoek; Hilversum, Centraal Bacteriologisch en Serologisch Laboratorium; Leeuwarden, Izore, Centrum Infectieziekten Friesland; Leiden, LUMC; Leiden, Eurofins Clinical Diagnostics; Maastricht, Maastricht UMC+; Nieuwegein, St. Antonius Ziekenhuis; Nijmegen, Canisius Wilhelmina Ziekenhuis; Nijmegen, RadboudUMC; Roosendaal, Bravis ziekenhuis, locatie Roosendaal; Rotterdam, Erasmus MC;

Rotterdam, Ikazia Ziekenhuis; Rotterdam, Maasstad Ziekenhuis; 's-Gravenhage, HagaZiekenhuis; 's-Gravenhage, MC Haaglanden Westeinde; 's-Hertogenbosch, Jeroen Bosch Ziekenhuis; Sittard-Geleen, Zuyderland MC; Terneuzen, Microvida locatie ZorgSaam; Tilburg, Streeklab v.d. Volksgezondheid; Utrecht, Diakonessenhuis; Utrecht, Saltro; Utrecht, UMC Utrecht; Veldhoven, Stichting PAMM; Velp, Ziekenhuis Rijnstate, loc. Velp; Venlo, VieCuri; Zwolle, Isala.

Contents

1 Introduction

This is NethMap 2022, the SWAB/RIVM report on the use of antibiotics, trends in antimicrobial resistance and antimicrobial stewardship programmes in the Netherlands in 2021 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 resistance surveillance data. 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 health care 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 2022 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 appearance of 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 and is described for the seventh consecutive year.

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.

The editors:
Dr Ir SC de Greeff
Dr E Kolwijck
Dr AF Schoffelen

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 2022) and the veterinary sector (MARAN 2022). Without any doubt, the COVID-19 epidemic still has had an enormous impact on the Dutch healthcare system. As a consequence, any of the data presented in this edition of NethMap will be influenced by this epidemic.

2.1 Most important trends in antimicrobial use

In outpatients

- In 2021, total outpatient use of systemic antibiotics remained very stable with 7.61 DDD/1,000 inhabitant days (DID). As in 2020, in 2021 COVID-19 has had a major impact on antibiotic use in outpatients.
- Slight decreases in antibiotic use were seen in tetracyclines and macrolides.
- Two third of the antibiotics used in outpatients were prescribed by GPs.

In hospitals

- In 2020 COVID-19 has had a major impact on the inpatient antibiotic use.
- The inpatient use of antibiotics in 2020 increased by 8.2% to 85.79 DDD/100 patient-days and increased by 4.6% to 333.1 when expressed in DDD/100 admissions.
- Several antibiotics showed remarkable increases: Flucloxacillin (+12.5% to 11.97 DDD/100 patient-days), second- and third-generation cephalosporins (8.48 and 9.93 DDD/100 patient-days (+6.1 and +28.5% resp.)). Macrolides, especially azithromycin (+16.1% to 2.02 DDD/100 patient-days), vancomycin (+21.4% to 2.21 DDD/100 patient-days).
- Although still rarely used, antibiotics such as fosfomycin and linezolid showed a remarkable increase in use by +27.3 and +50% respectively.
- A large variation in systemic antibiotic drug use was seen between Dutch hospitals, with highest use in large teaching hospitals.

- The increase in use of third-generation cephalosporins was mainly seen in general and large-teaching hospitals. This is due to higher use of ceftriaxone. Increase in meropenem use was only seen in university hospitals.
- Antimycotics were mainly used in academic hospitals.

In long-term care facilities

- Compared to 2019, the mean antibiotic use in long-term care facilities remained stable with 50.4 DDD/1,000 residents/day. The use varied highly between the different long-term care facilities.
- In the point prevalence study in long-term care facilities of the SNIV network of RIVM in 2021, antimycotics are the most frequently used antimicrobials for prophylaxis as well as treatment.

2.2 Most important trends in antimicrobial resistance

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 2022.

Table 2.2.1 Overview of antimicrobial resistance surveillance programs included in NethMap 2022

Surveillance program	Origin of isolates	Availability	Sources 2021	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-	46 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-	15 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)	Sanger sequencing, whole genome NGS, or site-specific PCR; Neuraminidase enzyme inhibition assay
Resistance among anaerobic pathogens	Hospital	2010-	8 laboratories	Central testing	Gradient testing
Clostridioides difficile	Hospital, LTCF	2005-	22 hospitals	(De)central testing	Agar dilution testing and PCR
Azole resistance in *Aspergillus fumigatus*	Hospital	2011-	5 university hospitals + 5 teaching hospitals	Central testing	EUCAST microbroth dilution methodology
MRSA	GP, hospital, LTCF	2008-	Nationwide	Central testing	MLVA, NGS
CPE	GP, hospital, LTCF	2011-	Nationwide	Central testing	Gradient testing, Carba-PCR, NGS
CPPA	GP, hospital, LTCF	2016-	Nationwide	Central testing	Gradient testing, multiplex PCR, NGS

ISIS-AR: Infectious disease Surveillance Information System on Antibiotic Resistance; LTCF: long-term care facility; SHC: Sexual Health Centres; MGIT: Mycobacteria growth indicator tube; NIVEL: Netherlands Institute for health services research; GP: General practitioner; SNIV: National sentinel surveillance network for infectious diseases in nursing homes; WHO-CC: World Health Organisation Collaborating Centre; NGS: Next Generation Sequencing; PCR: Polymerase Chain Reaction; MRSA: methicillin-resistant Staphylococcus aureus; MLVA: Multiple-Locus Variable number of tandem repeat Analysis; CPE: carbapenemase-producing Enterobacterales; CPPA: carbapenemase-producing Pseudomonas aeruginosa

In GPs

Urine: *E. coli*, *K. pneumoniae*, and *P. aeruginosa*

- For isolates from urine cultures a distinction is made for patients aged below and above 12 years of age in accordance with age categories used in the urinary tract infection guidelines of the Dutch College of General Practitioners (NHG).
- In general, resistance percentages in the older age group were higher than in the younger age group, except for the resistance of *K. pneumoniae* for co-amoxiclav which was higher in the age group below 12 years.
- In *E. coli*, resistance levels for fosfomycin and nitrofurantoin were low (≤2%) in both age groups. Resistance levels for ciprofloxacin were 5% in patients below 12 years of age and 9% in patients above 12 years of age. Resistance levels for amoxicillin, co-amoxiclav, trimethoprim, and co-trimoxazole varied between 16% and 34% for both age groups. Combined resistance for co-amoxiclav, ciprofloxacin, and co-trimoxazole was low (≤3%). For all antibiotics, resistance percentages in *E. coli* remained stable compared to previous five years.
- In *K. pneumoniae*, resistance levels for ciprofloxacin were 1% in patients below 12 years of age and 10% in patients above 12 years of age. In both groups, this was a significant and clinically relevant decrease compared to previous years. As for trimethoprim and co-trimoxazole in patients above 12 years, resistance levels decreased significantly and to a clinically relevant extent. There was an increase in resistance to co-amoxiclav in *K. pneumoniae* in patients aged below 12 years. Combined resistance for co-amoxiclav, ciprofloxacin, and co-trimoxazole was low (≤2%).
- HRMO and ESBL percentages were stable and low: ≤4% in both *E. coli* and *K. pneumoniae*.
- In patients above 12 years of age, resistance of *P. aeruginosa* to ciprofloxacin is stable and around 10%.
- Resistance percentages per region for *E. coli* and *K. pneumoniae* indicate that there are only minor differences in susceptibility between regions in the Netherlands.

Wound or pus: *S. aureus*

- In *S. aureus* resistance is generally low (≤4%), with the exception of resistance to fusidic acid (17%), (inducible) resistance to clindamycin (12%), and erythromycin (14%).
- Resistance percentages per region for *S. aureus* indicate that a higher resistance percentage was found for clindamycin (inducible) resistance in the regional cooperative network 'Noord-Holland Oost/Flevoland' (18% in the region versus 12% in all regions combined). However, because coverage in this region was low, this percentage might not be representative for the region.

Wound, pus, respiratory, genital or urine: β-haemolytic Streptococci

- For β-haemolytic *Streptococcus* spp. group A there was a statistically significant and clinically relevant increase in resistance to doxycycline (from 17% in 2017 to 40% in 2021), to clindamycin (from 5% in 2017 to 11% in 2021), and to erythromycin (from 7% in 2017 to 13% in 2021).
- Resistance for doxycycline, clindamycin, and erythromycin in β-haemolytic *Streptococcus* spp. group B already was high (75%, 15%, and 18% respectively).

Respiratory: *S. pneumoniae*

- The number of *S. pneumoniae* isolates from GPs in 2020 and 2021 was lower than the years before, and too low to fulfill the criteria for inclusion of resistance calculation.

In outpatient departments

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

- In *E. coli*, resistance levels for fosfomycin and nitrofurantoin were low (≤3%). Resistance levels for ciprofloxacin were 15%, resistance levels for amoxicillin, co-amoxiclav, and co-trimoxazole were 41%, 31%, and 23%, respectively. Combined resistance for co-amoxiclav, ciprofloxacin, and co-trimoxazole was low (5%). HRMO percentage was 7%. For none of the antibiotics, a significant and clinically relevant trend was found for resistance percentages in *E. coli*.
- In *K. pneumoniae*, resistance percentages seem to plateau for now. Moreover, a significant and clinically relevant decrease was observed for cefotaxime/ceftriaxone (from 9% to 7%), trimethoprim (from 25% to 19%), and co-trimoxazole (from 16% to 11%). No significant and clinically relevant trend was found for resistance levels of ciprofloxacin and co-amoxiclav. A significant and clinically relevant decrease was observed in the percentage of HRMO (10% to 8%) and multidrug resistance (6%-3%).
- For all antibiotics, resistance percentages in *P. aeruginosa* remained stable compared to previous five years. Ciprofloxacin resistance percentage was 14%.

Urine, wound/pus and respiratory: *S. aureus*

- In *S. aureus* resistance is generally low (≤4%), with the exception of resistance to fusidic acid (8%), (inducible) resistance to clindamycin (14%) and erythromycin (16%). Resistance percentages in *S. aureus* have remained stable over the last five years.

In hospital departments

Urine, wound/pus and respiratory, non-ICU: *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *B. fragilis* complex

- In *E. coli*, resistance levels for cefuroxime, cefotaxime/ceftriaxone, and ciprofloxacin were 12%, 6%, and 12%, respectively. Resistance levels for amoxicillin, co-amoxiclav, and co-trimoxazole were 40%, 31%, and 20%, respectively. Combined resistance for co-amoxiclav, ciprofloxacin, and co-trimoxazole was 4% and HRMO percentage was 7%. For all antibiotics, resistance percentages in *E. coli* remained stable compared to the previous five years and were comparable to resistance percentages in *E. coli* from outpatient departments.
- In *K. pneumoniae*, for none of the antibiotics, except for tobramycin and co-trimoxazole, a significant and clinically relevant trend was found for resistance percentages. Cefuroxime, cefotaxime/ceftriaxone, and ciprofloxacin resistance in *K. pneumoniae* was 13%, 8%, and 10%, respectively. Combined resistance for co-amoxiclav, ciprofloxacin, and co-trimoxazole was 4% and HRMO percentage was 9%. Resistance percentages were comparable to resistance percentages in *K. pneumoniae* from outpatient departments.
- For all antibiotics, resistance percentages in *P. aeruginosa* remained stable compared to the previous five years. Ciprofloxacin resistance percentage was 10%, which was lower than ciprofloxacin resistance in *P. aeruginosa* isolates from outpatient departments.
- Resistance in *B. fragilis* complex was less than 3% for metronidazole and co-amoxiclav. Resistance to clindamycin was 15%.

Urine, blood, wound/pus, and respiratory, non-ICU: *S. aureus*

- In *S. aureus* resistance is generally low (≤5%), with the exception of (inducible) resistance to clindamycin (13%) and erythromycin (15%). Resistance percentages in *S. aureus* were comparable to isolates from outpatient departments and no significant and clinically relevant trends were found.

Urine, blood, wound/pus, and respiratory, non-ICU: β-haemolytic Streptococci

- Resistance to clindamycin and erythromycin in β-haemolytic *Streptococcus* spp. group A increased significantly and to a clinically relevant extent from 4% in 2017 to 9% in 2021 and from 6% to 11% in 2021, respectively.
- In β-haemolytic *Streptococcus* spp. group B, resistance for doxycycline, clindamycin, and erythromycin in β-haemolytic *Streptococcus* spp. group B was high (75%, 17%, and 20% respectively). A statistically significant and clinically relevant decrease was found for clindamycin (inducible) resistance in β-haemolytic *Streptococcus* spp. group B (from 21% to 17%).

Urine, wound/pus, and respiratory, ICU only: *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *Acinetobacter*

- Resistance percentages in *E. coli* and *K. pneumoniae* from ICU patients were comparable to non-ICU patients except for second and third generation cephalosporines and aminoglycosides, which were higher in ICU patients.
- In *E. coli* from ICU patients, resistance to cefuroxime, cefotaxime/ceftriaxone, ceftazidime, gentamicin, and tobramycin was 18%, 11%, 8%, 6%, and 7% respectively. HRMO percentages were 11%, which is higher than the 7% in non-ICU patients.
- In *K. pneumoniae* from ICU patients, resistance to cefuroxime, cefotaxime/ceftriaxone, ceftazidime, gentamicin, and tobramycin was 20%, 16%, 15%, 8%, and 9% respectively. HRMO percentages were 17%, which is much higher than the 9% in non-ICU patients.
- Especially in *K. pneumoniae*, resistance to second and third generation cephalosporins increased compared to previous years.
- HRMO percentages in *P. aeruginosa* and *Acinetobacter* from ICU patients were much higher than HRMO percentages in *P. aeruginosa* and *Acinetobacter* from non-ICU patients (4% vs. 2% in *Pseudomonas* and 8% vs. 2% in *Acinetobacter*).

Blood, including ICU: *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *Acinetobacter*

- For *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *Acinetobacter* in blood isolates no significant and clinically relevant trends were found, except for resistance for tobramycin in *P. aeruginosa*, which decreased significantly and to a clinically relevant extent compared to previous years.
- In *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *Acinetobacter* HRMO percentages were 8%, 10%, 1%, and 2%, respectively. For *P. aeruginosa*, HRMO percentage decreased significantly and to a clinically relevant extent compared to previous years.
- Resistance to empiric therapy combinations was ≤10% for both *E. coli* and *K. pneumoniae*. In *E. coli*, resistance for empiric therapy combinations was 4% for co-amoxiclav plus gentamicin, 2% for cefuroxime plus gentamicin, and 6% for cefuroxime plus ciprofloxacin. In *K. pneumoniae*, resistance for empiric therapy combinations was 4% for co-amoxiclav plus gentamicin, 4% for cefuroxime plus gentamicin, and 7% for cefuroxime plus ciprofloxacin.

Respiratory and blood, including ICU: *S. pneumoniae* and *H. influenzae*

- Resistance to doxycycline/tetracycline in *S. pneumoniae* was 10% and stable over the last five years. Resistance to co-trimoxazole increased from 7% to 9%.
- Resistance to co-amoxiclav in *H. influenzae* increased from 8% to 15%. Resistance to co-trimoxazole and doxycycline/tetracycline remained stable around 26% and 1% respectively.

Specific pathogens and situations

Helicobacter pylori

- In 2021, resistance in *H. pylori* was high for levofloxacin (21%), clarithromycin (48%), metronidazole (44%), and the combination of clarithromycin/metronidazole (30%), and low for amoxicillin (7%) and tetracycline (1%). Resistance for levofloxacin, clarithromycin, and metronidazole was stable over the last three years.

Carbapenem-resistant and carbapenemase-producing *Enterobacterales*

- The overall percentage of gradient strip test-confirmed carbapenem non-susceptible *E. coli* and *K. pneumoniae* in diagnostic and non-diagnostic isolates was low (0.04% in *E. coli* and 0.36% in *K. pneumoniae*).
- In 2021, 242 unique carbapenemase-producing *Enterobacterales* (CPE) isolates were obtained from 209 patients. This is comparable to the 225 isolates found in 2020 but lower than the 397 isolates found in 2019, which is most likely the result of reduced travel and a reduction in regular healthcare during the COVID-19 pandemic.
- Thirty-seven percent of the CPE had an MIC for meropenem above the clinical resistant breakpoint of 8 mg/L.
- In 2021, bla_{OXA-48} gene or bla_{OXA-48}-like genes were the most frequently identified carbapenemase-encoding genes in CPE isolates (42% of isolates).

Vancomycin-resistant *E. faecium*

- Vancomycin resistant *E. faecium* (VRE) in diagnostic isolates remains very low, around 0.3%.
- The number of outbreaks with VRE reported to SO-ZI/AMR was eight, compared to five in 2020 and 19 in 2019.

Methicillin-resistant *Staphylococcus aureus*

- MRSA prevalence in diagnostic isolates of *S. aureus* was 2% and remained stable over the past 5 years. The MRSA prevalence in blood culture isolates also remained low, at 2%. The percentages were similar among the various types of settings, except for intensive care units in which the prevalence was 3%.
- Most frequently identified MLVA complex of MRSA was MC0398 (23%), also known as LA-MRSA.
- In 2021, 21% of submitted MRSA isolates were Panton-Valentine Leukocidin (PVL) positive, which is lower than in the years 2017-2020 (respectively 22%, 25%, 25%, and 28%). The proportion of PVL positivity was higher in diagnostic isolates (30%) than in screening isolates (16%).

Carbapenem-resistant and carbapenemase-producing *Pseudomonas aeruginosa*

- In 2021, 5% of diagnostic *P. aeruginosa* isolates were phenotypically resistant to carbapenems. One percent of the *P. aeruginosa* isolates was MDR (resistant to ≥3 antimicrobial groups) and 64% of these MDR isolates were carbapenem-resistant.
- The proportion of phenotypically carbapenem-resistant *P. aeruginosa* in ICUs was remarkably lower in 2020 and 2021 (~5%) compared to the preceding three years (~8%), while these proportions in the other types of departments did not change.
- The national surveillance on CPPA via Type-Ned CPE/CPPA revealed that 38% of submitted *P. aeruginosa* isolates in 2021 produced (a) carbapenemase(s). This was much higher than the 20% carbapenemase positivity in 2020 via Type-Ned CPE/CPPA. This might be due to altered submission criteria for CPPA surveillance in 2021. From May 2021 on, only isolates showing presence of carbapenemase production

and/or presence of genes coding for carbapenemase production could be submitted. Before 2021, also *P. aeruginosa* isolates with increased MICs for meropenem or imipenem were allowed for submission.
- The predominant (73%) carbapenemase-encoding gene in carbapenemase-producing *P. aeruginosa* was bla_{VIM-2}.

Extended spectrum beta-lactamases

- The proportion of ESBL-production in *E. coli* from diagnostic isolates of GP, outpatient departments, inpatient departments, and ICUs was 3%, 4%, 5%, and 9% respectively. For *K. pneumoniae*, this was 3%, 6%, 8%, and 15%, respectively.
- From 2019 to 2021, the percentages of ESBL-producing *E. coli* and *K. pneumoniae* slightly decreased in GP, outpatient departments and inpatient departments, while there was an increase in the percentage of ESBL-producing *K. pneumoniae* in ICUs to 15%.

Neisseria gonorrhoeae

- In *N. gonorrhoeae*, no resistance was reported to ceftriaxone, the current first-line treatment. However, MIC values of ceftriaxone, when compared to previous years, are higher since 2019. Resistance to ciprofloxacin more than doubled since 2016, to 52.9% in 2021. Resistance to azithromycin increased from 2.1% in 2012 to 10.8% in 2018, was stable around 10% in 2019 and 2020, but increased to 18.0% in 2021.

Neisseria meningitidis

- The number of invasive meningococcal disease cases decreased by 82% in 2021 compared to 2019 (pre-COVID-19).
- In 2021, all cultured *N. meningitidis* isolates were susceptible to penicillin, ceftriaxone or rifampicin.

Mycobacterium tuberculosis

- In 2021, resistance to rifampicin, ethambutol and pyrazinamide was less than 4% and remained almost stable over the last years. Resistance to INH resistance was 7.5% and fluctuated over the years (5%-9%). MDR-TB cases remained stable (average of 10 per year).

Clostridioides difficile

- Since 2019, submitted *C. difficile* strains are tested for the presence of plasmid-mediated metronidazole resistance. *S*ince then, 0.18% of the strains tested positive.

Aspergillus fumigatus

- Triazole resistance frequency in 2021 was 11.7% in UMCs and 6.7% in teaching hospitals, which represents a resistance level similar to 2019.

SO-ZI/AMR

- In 2021, 27 outbreaks were reported to the Early warning and response meeting of Healthcare-associated Infections and AntiMicrobial Resistance (SO-ZI/AMR). The number was lower than the number in 2020 (34), and remarkably lower than in 2017-2019, when 59 or 60 outbreaks were reported each year.

2.3 Antibiotic use and resistance in animals

Antimicrobial use

- In 2021 in total 145 tonnes of Antimicrobial Veterinary Medicinal Products (AVMPs) were sold, which is a decrease of 5.8% compared to 2020. A decrease in sales by 70.8% over the years 2009-2021 is attained (with 2009 considered a reference year by the Dutch Government).
- The decreased sales of AVMPs in the Netherlands in 2021 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 the unmonitored sectors. Use and sales of polymyxins decreased in 2021, overall decrease since 2011 is 77% in sales.

Antimicrobial resistance

Salmonella from livestock, meat and humans

- In 2021, *Salmonella* Enteritidis (25%) followed by *S.* Typhimurium (19%) and monophasic *S.* Typhimurium (19%) were most frequently isolated from humans suffering from clinical salmonellosis. In pigs, the monophasic variant of *S.* Typhimurium (32%) and *S.* Derby (27%) dominated. In cattle, the most frequently identified serovars were *S.* Dublin (42%) and *S.* Typhimurium (27%). In broilers, *S.* Infantis (35%) and *S.* Paratyphi B var. Java (19%) dominated, while in layers *S.* Enteritidis (59%) and monophasic *S.* Typhimurium (15%) were the most common serovars.
- Over all serovars, the highest resistance proportions were observed for sulfamethoxazole (29.6%), tetracycline (26.6%) and ampicillin (24.5%), with approximately similar levels as in 2020. Serovars showing the highest levels of resistance were *S.* Infantis, *S.* Paratyphi B var. Java, monophasic *S.* Typhimurium variants, and *S.* Typhimurium, with resistance to ampicillin, tetracycline, sulfamethoxazole, trimethoprim, ciprofloxacin, and nalidixic acid reaching maximum levels of between 64% and 92%.
- Among *S.* Typhimurium, resistance to fluoroquinolones decreased considerably among human isolates, while it increased sharply among cattle isolates.
- Among *S.* Enteritidis, the fraction of resistance to ciprofloxacin and nalidixic acid among human isolates remained relatively stable but resistance to ampicillin and tetracycline decreased.
- In total, 10 (0.8%) ESBL-producing (human clinical) *Salmonella* isolates were detected.
- In 2021, no carbapenemase-producing *Salmonella* were found.

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

- Due to a new legislation *Campylobacter jejuni* and *C. coli* isolates obtained from veal calves as well as *C. coli* from fattening pigs are included in the mandatory AMR monitoring program in livestock from 2021 onwards.
- In 2021, resistance proportions in *C. jejuni* isolates from caecal samples of broilers and meat thereof decreased but remained at a high level for quinolones and tetracycline.
- Resistance to macrolides was not detected in *C. jejuni* isolates from broilers and poultry meat, and was present at low levels in *C. coli* isolates from broilers and poultry meat. A notably higher level of macrolide resistance was observed in *C. coli* from veal calves.
- In human isolates, resistance proportions were higher in *C. coli* than in *C. jejuni*, but similar to 2020, these were overall lower in 2021 compared to previous years. This is most likely 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.
- Ciprofloxacin resistance in *Campylobacter* isolates from humans was again high in 2021, which is a concern

for public health. It was, however, lower compared to 2017-2020. Resistance to erythromycin, first choice antibiotic in human medicine for campylobacteriosis, remained low.

Pathogenic *E. coli* (STEC/EPEC/aEPEC) from human patients

- In Shiga toxin-producing *E. coli* (STEC) O157, after a decrease in resistance for 2020, a tendency of increasing resistance towards the fluctuating levels of in 2018-2019 was observed. Resistance to the quinolones (ciprofloxacin and nalidixic acid) was very low in both (STEC) O157 and STEC/enteropathogenic *E. coli* (EPEC) non-O157 human isolates in 2021.
- Proportions of resistance were higher in human STEC/EPEC non-O157 *E. coli* than in STEC O157 for all antimicrobials, except gentamicin, tetracycline and sulfamethoxazole.
- No ESBL-producing isolates were detected in STEC O157, but a-typical enteropathogenic *E. coli* (aEPEC) O163 isolates from one case were confirmed as ESBL-producer carrying bla_{CMY-41}. Almost all STEC O146 isolates - associated with human infections linked to consumption of raw milk products from small ruminants - were pan-susceptible.

Indicator *E. coli* from livestock, meat and vegetables

- 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, resistance in indicator *E. coli* from caecal samples further decreased to the lowest levels since 1998.
- In pigs and veal calves levels of resistance stabilised, whereas resistance in dairy cattle remained traditionally low. Resistance to third generation cephalosporins was very 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 with the low prevalence observed in pigs and veal calves.
- For almost all antibiotics tested, levels of resistance in *E. coli* from caecal samples of rosé veal calves were substantially lower than those from white veal calves.
- Resistance proportions in *E. coli* from pig and bovine meat were low compared to isolates from caeca.
- Low levels of resistance were observed in different types of retail meat as well as in imported meat. In vegetables, levels of resistance were very low for all antibiotic classes.

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

- In 2021, only one confirmed ESBL-producing *E. coli* was detected through random isolation. Selective isolation of ESBL/pAmpC producing *E. coli* from broilers showed that after six years of reduction in prevalence, a plateau was reached.
- For the first time, whole-genome sequencing (WGS) was performed for all extended-spectrum cephalosporin resistant *E. coli* isolates from livestock and food products. WGS showed evidence of clonal transmission within livestock sectors and into the meat that these produce.
- The prevalence of ESBL-producing *Salmonella* isolated from human, livestock and food is considered low.
- In 2021, no carbapenemase-producing *Enterobacterales* were detected in livestock and companion animals, but occasionally in imported food products.
- As in former years, the prevalence of *mcr* encoding *E. coli* was low in livestock and meat.

MRSA from livestock and meat

- Within the study period, the MRSA prevalence varied substantially between the animal sectors: 89% on pig farms, 6% on dairy farms and no MRSA on broilers farms.
- On retail meat, the highest prevalence of MRSA was found on turkey meat, followed by lamb, chicken and veal.

2.4 Implications for therapy

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 2021, 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) in 2021. For now, the data on resistance look encouraging but still we have to keep focus on the use of antimicrobial agents.
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 even 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 those that stay longer in the hospital, is often higher than reported here. On the other hand, resistance may be overestimated in GP and LTCF 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, some but not all 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.
In the summary below, the most important implications for therapy are provided, based on the general trends of resistance. As implications differ by category of patient and indication of use, the summary is organized as such. 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

Enterobacterales

- For empirical treatment of uncomplicated UTI, first and second choice antibiotics nitrofurantoin and fosfomycin, are suitable as resistance levels are stable and low (≤2%) in *E. coli*. For other *Enterobacterales*, both nitrofurantoin and fosfomycin are not appropriate for empirical treatment of urinary tract infections.
- The empirical treatment of complicated UTI is challenged by the relatively high resistance levels for co-amoxiclav (second choice), and to a lesser extent for ciprofloxacin (first choice) and co-trimoxazole (third choice) in *Enterobacterales*. Encouragingly, resistance levels for ciprofloxacin and co-trimoxazole are stable (*E. coli*) or have even decreased (*K. pneumoniae*). Nevertheless, urinary culture is often necessary to guide antibiotic therapy, which is most often possible given the relatively low (≤3%) combined resistance levels for the oral agents co-amoxiclav, ciprofloxacin, and co-trimoxazole in both *E. coli* and *K. pneumoniae*.

S. aureus and β-haemolytic Streptococci

- Clindamycin (inducible) resistance and resistance to macrolides in *S. aureus* rises every year, and was 12% in 2021, which limits its usefulness in empiric therapy for those infections possibly caused by *S. aureus*, such as skin and soft tissue infections.
- The rise in resistance to tetracycline (doxycyclin), clindamycin, and erythromycin 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 beta-lactam allergy.
- Studies have shown that beta-lactam allergy labels are associated with increased antibiotic use, including the use of (often unnecessary) second choice antibiotics, and more health care use. In 2022, SWAB published its first guideline on the approach to antibiotic (beta-lactam) allergy. Implementation of this guideline in hospitals, GP practices, and LTCF may hopefully lead to increased safe reintroduction of beta-lactam antibiotics in potentially beta-lactam-allergic patients.

In hospitals

Except for ICUs, resistance levels in gram-negative bacteria (e.g. *Enterobacterales*, *P. aeruginosa*, and *Acinetobacter* spp.) are stable for now and for some antibiotics the levels even seem to decrease slightly over the last three years. It seems that the strict policy for prescription of antimicrobials and antimicrobial stewardship programs in the Netherlands are paying off. Therefore, we should not lose focus and continue to keep investing in optimizing our antibiotic policy.
Of note, local resistance levels in hospitals and even hospital wards varied significantly. Tailored therapy and culture remain the mainstay of therapy.

Outpatient departments

Enterobacterales and *P. aeruginosa*

- Resistance levels are stable and seem to decrease slightly in all *Enterobacterales* and *P. aeruginosa*. The rise in resistance of *K. pneumoniae* to many antimicrobial agents seen in the previous years has plateaued in the last three years and even decreased in 2021 for a few antibiotics. However, resistance levels in *Enterobacterales* for most oral antibiotics are still more than 10% and therefore limit the chance of success of empirical treatment with oral agents for complicated UTI. Culture and tailored therapy will mostly be necessary for successful treatment. Fortunately, this is most often possible given the relatively low (≤5%) combined resistance rates for the oral agents co-amoxiclav, ciprofloxacin, and co-trimoxazole.

S. aureus

- Clindamycin (inducible) resistance and resistance to macrolides in *S. aureus* rises every year, and is now almost 15%, which limits its usefulness in empiric therapy for e.g. skin and soft tissue infections.

Unselected hospital patient departments

Enterobacterales and *P. aeruginosa*

- For all *Enterobacterales* including *K. pneumoniae*, it is encouraging to see that resistance to most antimicrobials is stable or even declining. Nevertheless, patients with an infection with *K. pneumoniae* and (to a lesser extent) *E. coli* have a considerable risk of non-adequate empiric treatment with monotherapy with a second or (to a lesser extent) third generation cephalosporin. In case of severe infection and increased risk of involvement of third generation cephalosporin resistant *Enterobacterales*, empiric combination therapy might be a suitable option (as a carbapenem sparing strategy).

In 2021, combinations of a second or third generation cephalosporin plus gentamicin/tobramycin had resistance levels of 3% or less.

- Resistance to co-amoxiclav in *Enterobacterales* is high. In 2021, the resistance percentage in *E. coli* was 31% and in *K. pneumoniae* 21%. 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. Empirical treatment with ceftazidime when infections are potentially caused by *P. aeruginosa* remains therefore adequate.

S. aureus

- Overall, susceptibility of *S. aureus* is stable, with the exception of the ongoing rise of macrolide resistance and clindamycin (inducible) resistance. The 13% resistance for clindamycin indicates that culture and susceptibility testing are mandatory before starting treatment with this drug.

Anaerobes

- Antimicrobial resistance in *B. fragilis* complex is low, with the exception of clindamycin resistance of 15%, limiting its use as part of empiric therapy in infections of the gastro-intestinal tract.

Intensive care units

Enterobacterales and *P. aeruginosa*

- Compared to hospital departments, resistance levels for second and third generation cephalosporins in *E. coli* and *K. pneumoniae* are worrisome. Resistance for cefuroxime was ~20%, which is much higher than in other settings. Resistance to third generation cephalosporins was 11% in *E. coli* and 16% in *K. pneumoniae*. These percentages increased compared to previous years. This might be due to increased antimicrobial prescribing in COVID-19 patients. As a result, monotherapy with a second or, to a lesser extent, third generation cephalosporin as empirical treatment in ICU patients should be handled with caution and based on individual risk of involvement of third generation cephalosporin resistant *Enterobacterales* and also local epidemiology. Combination treatment of cephalosporins with gentamicin or tobramycin might be suitable.
- For *P. aeruginosa*, resistance to piperacillin-tazobactam is stable around 13%, which is much higher than in other settings. This makes the drug increasingly less suitable for empiric therapy for infections potentially caused by *P. aeruginosa*.
- In many Dutch ICUs, routine 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 in 2021, routine culturing with susceptibility testing remains mandatory to tailor therapy to the individual ICU patient.

S. aureus and β-haemolytic Streptococci group A

- Also in the ICU setting, *S. aureus* showed an ongoing rise of clindamycin (inducible) resistance (14%). This might be especially worrisome when clindamycin is used for toxin inhibition in case of severe *S. aureus* toxin-related infections. Toxin inhibition by clindamycin occurs in clindamycin-susceptible *S. aureus* strains but is abolished in constitutive clindamycin-resistant strains. It seems that clindamycin anti-toxin effect is retained for inducible clindamycin-resistant *S. aureus* isolates.
- Although no resistance levels were calculated for β-haemolytic Streptococci group A (GAS) in ICU patients due to a limited amount of samples, rising clindamycin resistance in GAS was seen in other settings.

Particularly in the ICU, clindamycin is used for toxin inhibition in case of severe GAS toxin-related infections such as necrotizing fasciitis or toxic shock syndrome. It is still unclear whether toxin inhibition occurs in clindamycin-resistant isolates.

Specific pathogens and situations

- Carbapenemase-production in *Enterobacterales* and in *P. aeruginosa* isolates is rare, and risk of infection caused by or carriage of these specific pathogens is closely monitored.
- MRSA prevalence remains stable at 2%. Spread is controlled by an effective search-and-destroy policy in the Netherlands.
- ESBL-producing *Enterobacterales* are of special concern, particularly the increase in ESBL-producing *K. pneumoniae* in ICUs. For ESBL-producing *Enterobacterales*, treatment is often difficult, with few options remaining. A SWAB advice on practical use of carbapenem-sparing antibiotics will be published in 2022.

2.5 Antimicrobial stewardship

Since 2014, following the recommendation of the Dutch Health Care Inspectorate (IGJ) in response to the statement of the SWAB to contain antimicrobial resistance, all hospitals have established antimicrobial stewardship teams (A-teams) that are responsible for the implementation of an antimicrobial stewardship program.

The most important developments concerning stewardship teams are:

- The composition of the A-team remains more or less the same: almost all included expertise of a clinical microbiologist and a hospital pharmacist, two thirds have an infectious disease specialist and one fifth included a nurse.
- There has been no increase in time spent on antimicrobial stewardship in recent years and a third of the A-teams still receives no funding from the hospital board.
- Seven (~10%) acute care hospitals extracted structured data from the electronic medical records and provided these to the interactive dashboard of the antimicrobial stewardship monitor.
- Based on prescriptions started on the day of surgery as a proxy for surgical prophylaxis, on average 85% (range 76-93%) of surgical antimicrobial prophylaxis courses were discontinued at the day of surgery or the day after.
- Seventeen percent (mean, range 11-24%) of the patients that received cefuroxime/ceftriaxone as empiric treatment upon admission were switched to oral treatment.
- Improvements should be made to link the indication to the antimicrobial prescription within hospital registrations. Only then, the quality of antimicrobial use can be better assessed using structured data extracted from the electronic medical record.

2.6 Implications for public health and health policy

In 2021, the COVID-19 pandemic which had started in 2020, was still ongoing. The pandemic has led to a huge amount of hospitalizations and intensive care admissions. The treatment of and care for these patients, and the downscaling of regular care, may have affected trends in antibiotic use and the occurrence of healthcare-associated infections. Moreover, the increased hygiene precautions and control measurements may have affected transmission of micro-organisms in general. Most of the findings in the various AMR surveillance systems in 2021 were comparable to 2020 when the effects of the COVID-19 pandemic could be noticed. The number of reported HRMO outbreaks in healthcare institutes was even lower in 2021 compared to 2020, when it had almost halved already compared to the previous years. Although the numbers of medical microbiological laboratories reporting their data to the national surveillance system of antimicrobial resistance (ISIS-AR) did not change compared to previous years, the absolute number of isolates per month was obviously lower during the COVID-19 waves compared to the period before and in-between the waves, most likely as a result of the alterations in the patient population in hospitals and at the GPs.
The absolute numbers of carbapenem-resistant *Enterobacterales* submitted to the national surveillance system Type-Ned was still decreased in 2021 with almost 40% compared to 2019, which is most likely the result of reduced travel and a downscaling of regular health care. The outpatient use of systemic antibiotics in 2021 was stable compared to 2020, but 10% lower than in 2019. Probably changes in healthcare delivery due to COVID-19 have played a role here as well.

In the meanwhile, antibiotic resistance continues to be a serious threat to public health worldwide and in Europe, leading to increased healthcare costs, prolonged hospital stays, treatment failures and sometimes death. Data from the European Antimicrobial Resistance Surveillance Network (EARS-Net) show that in Europe in 2020 wide variations in the occurrence of antimicrobial resistance across the EU/EEA exist.[1]
Although in many countries in Europe MRSA percentages among *S. aureus* isolates decline, 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 in *E. coli* remained rare (0.2%) in 2020. More concerning, almost a quarter of EU/EEA countries reported carbapenem resistance percentages above 10% for *K. pneumoniae*, and even higher in *Pseudomonas aeruginosa* and *Acinetobacter* species. As a result, in these settings, only a limited number of therapeutic options are available, often leading to inferior treatment, more toxicity and side-effects.
In contrast to the Netherlands, also combined resistance to different antimicrobial groups was high for *K. pneumoniae*, with 30.4% of the clinical isolates reported to EARS-Net for 2020 being resistant to at least two and 22.1% to at least three of the surveyed antimicrobial groups (fluoroquinolones, aminoglycosides, third-generation cephalosporines, carbapenems). In *E. coli*, combined resistance to at least three antibiotic groups was lower with a percentage of 10.8% in 2020, although resistance to third-generation cephalosporins was still high at 14.9% and to fluoroquinolones 23.8%.
In addition, recent outbreaks of carbapenemase-producing and colistin-resistant *K. pneumoniae* in EU/EEA countries have highlighted the concomitant increase in virulence, transmissibility and AMR among

[1] https://www.ecdc.europa.eu/en/publications-data/antimicrobial-resistance-surveillance-europe-2022-2020-data

certain *K. pneumoniae* strains.[2] A close watch on the early detection of cases and clusters of emerging hypervirulent *K. pneumoniae* strains carrying carbapenemase genes is crucial to avoiding spread among the patient population in the EU/EEA and the Netherlands. A pilot study on the prevalence of hypervirulent *K. pneumoniae* in the Netherlands has been initiated in the beginning of 2022 by the RIVM.
In the Netherlands, the prevalence of resistance of most pathogens is stable or even declining. Overall in 2021, resistance percentages among Gram-negative micro-organisms in general practice, outpatient departments and inpatient departments were stable or declining, while resistance percentages on the intensive care units were generally higher and sometimes even still increasing. Carbapenem resistance among *Enterobacterales* remained rare. The overall percentage of confirmed non-susceptible *E. coli* and *K. pneumoniae* in 2021 was low (0.04% and 0.36%) and there was no significant increase in the last years. The current Dutch policy regarding the control of targeted HRMO shows to be effective and the investments seem to be paying off. Still, 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.

In 2015 the Minister of Health initiated a National Program to combat antimicrobial resistance in the Netherlands. The program propagated a One Health-approach with specific measures for all relevant domains, including human health care, the veterinary sector, the food chain, the environment and international involvement.[3] In February 2021, the Minister provided an update on the progress made and decided to continue the current program and policy.[4] Five goals for the coming years were defined: 1) Promoting and improving a high quality of antimicrobial use both for humans and animals, 2) To slow down the emergence of new resistant microorganisms, by investing in dedicated research, 3) To prevent transmission of highly resistant microorganisms between patients within and outside healthcare centres, and the environment and livestock sectors, 4) To decrease the number of healthcare-associated infections caused by HRMO and to decrease the number of outbreaks in healthcare institutes by surveillance and adequate infection prevention, 5) To intensify international cooperation on this subject. Furthermore, the scope of the program was broadened from antibiotic resistance to antimicrobial resistance which includes resistance against antifungal therapy and against antiviral therapy as well.

Conclusions and discussion

The data presented in NethMap/MARAN 2022 demonstrate that ongoing attention is needed to combat antibiotic resistance and optimize antimicrobial use in humans and animals. In 2021, the COVID-19 pandemic was still having a major impact on healthcare systems and its effects could be noticed in the various AMR surveillance systems, comparable to the year before. It is very positive to see that, in spite of the ongoing crisis, all surveillance systems continued to work properly and that data were available for the indicators described in this report, comparable to earlier years. Still, the interpretation of the data is complicated by the wide variety of changes that took place during the pandemic. 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. Extra vigilance and analyses of data are needed in the coming period when the COVID-19 pandemic is declining.

[2] https://www.ecdc.europa.eu/en/publications-data/risk-assessment-emergence-hypervirulent-klebsiella-pneumoniae-eu-eea
[3] https://www.rijksoverheid.nl/documenten/kamerstukken/2015/06/24/kamerbrief-over-aanpak-antibioticaresistentie
[4] https://www.rijksoverheid.nl/documenten/kamerstukken/2021/02/09/kamerbrief-over-voortgang-aanpak-antibioticaresistentie

For now, it is encouraging to see that use of antimicrobials in humans is stable and 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 70% compared to 2009 and this was reflected in the reduction of the level of resistance in of some bacterial species in livestock. This particularly accounts for ESBLs in poultry and chicken meat. Carbapenem 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 rising resistance percentages among other groups of micro-organisms as well. Antimicrobial stewardship programs and A-teams have been implemented universally in Dutch hospitals. With adequate surveillance systems the impact of these measures on 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.
Some surveillance systems and reference laboratory functions may need more attention. For instance, a recent EU inventarisation among 30 European countries highlighted that there is a need for further capacity building activities for carbapenem-resistant and/or colistin-resistant *Acinetobacter baumannii* complex as part of the molecular surveillance under the European Antimicrobial Resistance Genes Surveillance Network (EURGen-Net). Moreover, the usefulness and necessity for an *Enterococcus faecium* reference laboratory needs to be considered.

3
Use of antimicrobials

3.1 Outpatient antibiotic use

Methods

Data on outpatient antibiotic use in the Netherlands over 2021 was obtained from the SFK (Foundation for Pharmaceutical Statistics, the Hague) and is expressed in Defined Daily Doses (DDD) for each ATC-5 code. The SFK collects dispensing data from 90% of the Dutch community pharmacies (serving 93% of the Dutch population) and extrapolates the data to 100%. These data include prescriptions from general practitioners, as well as prescriptions from outpatient clinics and dentists. Data is presented as DDD per 1,000 inhabitants per day (DID). In 2019, two major changes in DDD were implemented by the World Health Organisation (WHO): for penicillins with extended spectrum and penicillins with beta-lactamase inhibitors.[1] From 2019 onwards, the data were processed using these new DDD definitions. To enable comparison of these data with the years before 2019, the data from 2018 are presented as they were in 2018, as well as using the 2019 DDD definitions.
For the first time, we analyzed the type of caregiver who prescribed the antibiotics issued at community pharmacies.

Results

Total outpatient use of systemic antibiotics remained very stable with 7.61 DID in 2021 (Table 3.1.1). Slight decreases in use were particularly seen in tetracyclines (from 1.54 DID in 2020 to 1.43 DID in 2021) and macrolides (from 1.13 DID in 2020 to 1.07 DID in 2021).
68% of the antibiotics used in outpatients were prescribed by GPs, 22% by medical specialists (residents and attending physicians) and 10% by others (mainly dentists and midwives).
In figure 3.1.3. the relative distribution for the 5 most widely used antibiotics is shown (comprising 68% of total outpatient antibiotic use).

Discussion

Antibiotic use in 2021 was still influenced by the ongoing COVID-19 pandemic. Preventive measures such as social distancing, school closure and working from home has decreased transmission of other micro-organisms and altered help seeking behavior for infectious illness at GP practices. Total outpatient antibiotic use in 2021 in the Netherlands was comparable to 2020, which was lower than before the covid-pandemic.

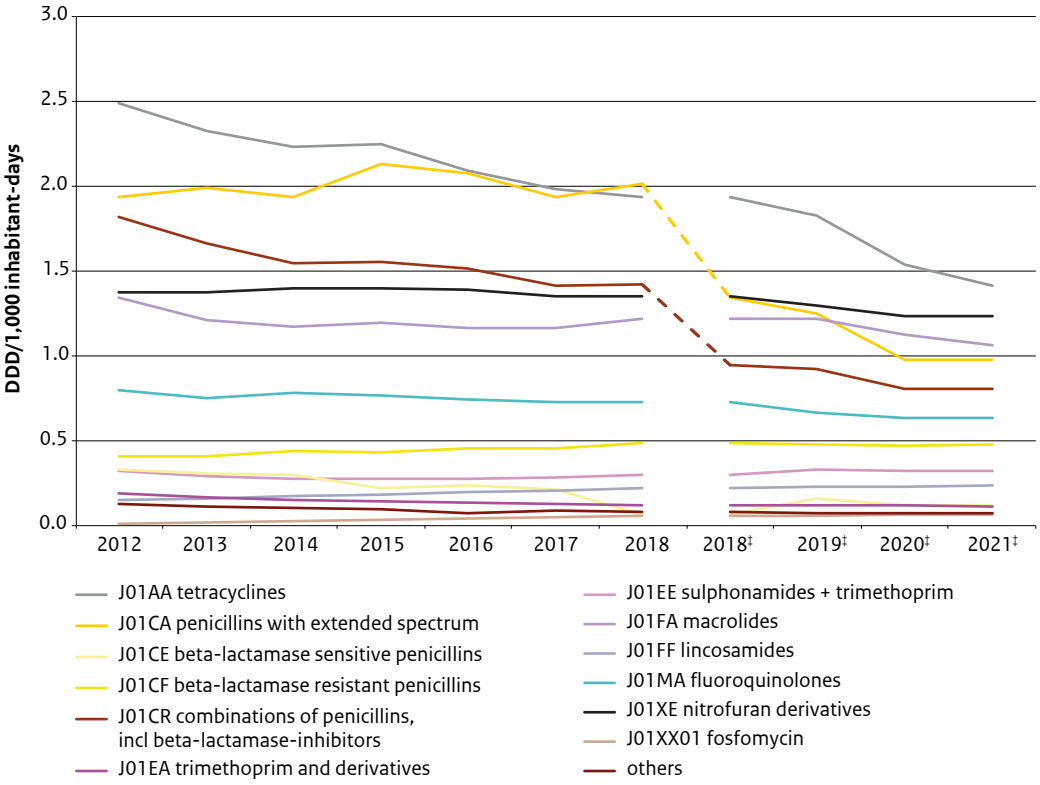
Nitrofurantoin was mainly prescribed by GP's, while a substantial part of amoxicillin and co-amoxiclav was prescribed by the subgroup "others". A possible reason could be that dentists prescribe amoxicillin for (endocarditis-)prophylaxis.
However, it is not possible to identify which type of caregiver initiated the antibiotic treatment. It could be that GP's take over chronic treatments initiated by medical specialists. We previously analyzed the amount and type of consecutive prescriptions[2].

Table 3.1.1 Ten years data on the use of antibiotics for systemic use (J01) in outpatients (DDD/1,000 inhabitant-days), 2012-2021 (source: SFK)

ATC Group*	Therapeutic group	2012	2013	2014	2015	2016	2017	2018	2018‡	2019‡	2020‡	2021‡
J01AA	Tetracyclines	2.49	2.33	2.23	2.25	2.10	1.98	1.94	1.94	1.83	1.54	1.42
J01CA	Penicillins with extended spectrum	1.94	1.99	1.94	2.13	2.08	1.94	2.02	1.35	1.26	0.98	0.98
J01CE	Beta-lactamase sensitive penicillins	0.33	0.31	0.30	0.23	0.24	0.22	0.07	0.07	0.16	0.12	0.13
J01CF	Beta-lactamase resistant penicillins	0.41	0.41	0.44	0.43	0.46	0.46	0.49	0.49	0.48	0.47	0.48
J01CR	Penicillins + beta-lactamase-inhibitors	1.82	1.67	1.55	1.56	1.52	1.42	1.42	0.95	0.93	0.81	0.81
J01D	Cephalosporins & carbapenems	0.04	0.04	0.04	0.04	0.03	0.03	0.03	0.03	0.03	0.03	0.03
J01EA	Trimethoprim and derivatives	0.19	0.17	0.16	0.14	0.14	0.13	0.13	0.13	0.12	0.12	0.12
J01EE	Sulphonamides + trimethoprim	0.33	0.29	0.28	0.28	0.28	0.29	0.30	0.30	0.33	0.33	0.33
J01FA	Macrolides	1.34	1.22	1.18	1.20	1.17	1.17	1.22	1.22	1.22	1.13	1.07
J01FF	Lincosamides	0.16	0.17	0.18	0.19	0.20	0.21	0.23	0.23	0.23	0.23	0.24
J01GB	Aminoglycosides	0.04	0.03	0.03	0.03	0.02	0.02	0.02	0.02	0.02	0.02	0.02
J01MA	Fluoroquinolones	0.80	0.76	0.79	0.77	0.75	0.73	0.73	0.73	0.67	0.64	0.64
J01XE	Nitrofuran derivatives	1.38	1.37	1.40	1.40	1.39	1.36	1.35	1.35	1.30	1.24	1.24
J01XX01	Fosfomycin	0.01	0.02	0.03	0.04	0.05	0.05	0.07	0.06	0.06	0.07	0.07
	others	0.05	0.04	0.04	0.04	0.02	0.05	0.04	0.04	0.03	0.03	0.03
J01	**Antibiotics for systemic use (total)**	**11.34**	**10.83**	**10.58**	**10.72**	**10.44**	**10.06**	**10.06**	**8.90**	**8.68**	**7.77**	**7.61**

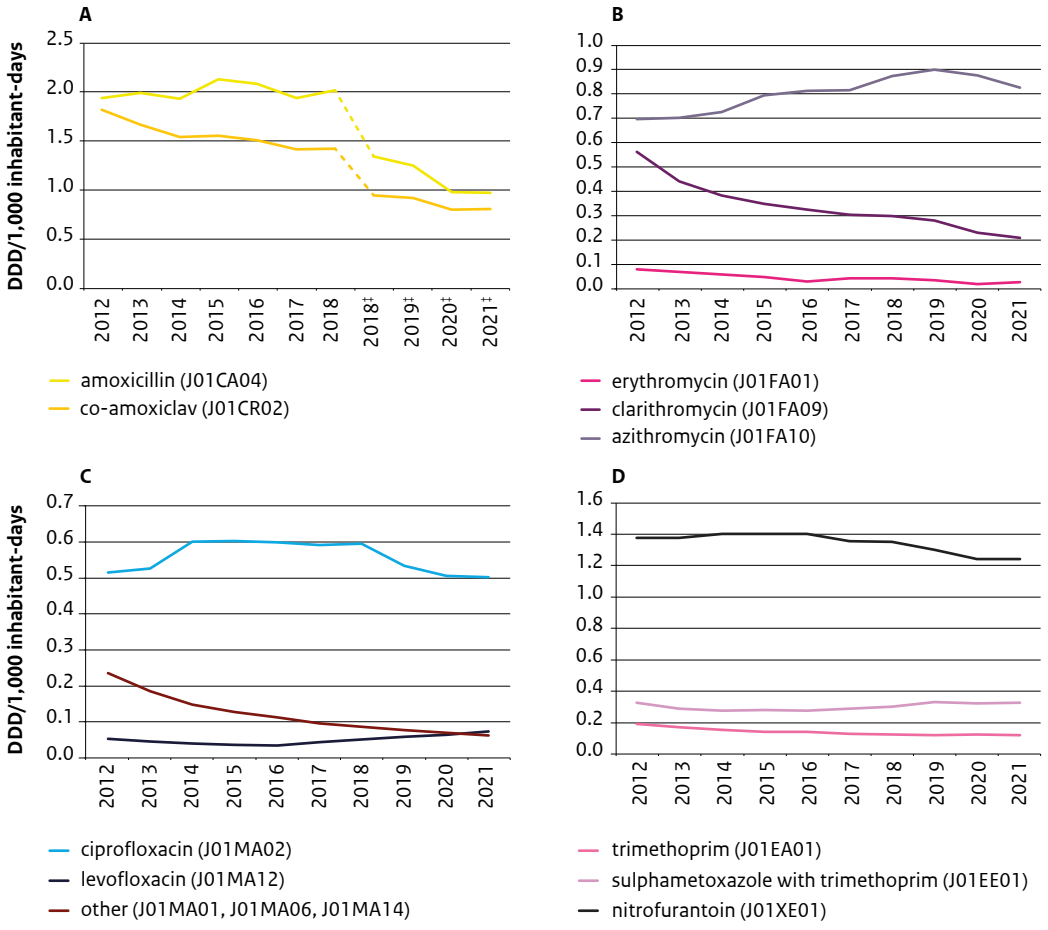
** From the 2021 edition of the Anatomical Therapeutic Chemical (ATC) classification system*
‡ DDD including changes as of 2019 (source: WHO)

Figure 3.1.1 Use of antibiotics for systemic use (J01) in outpatients at ATC-4 level, 2012-2021 (source: SFK)



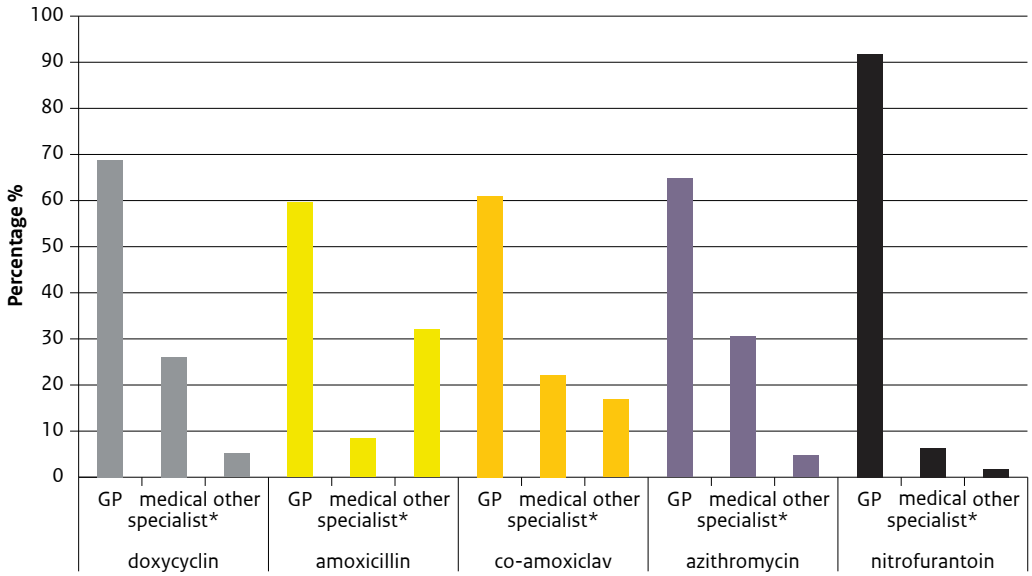
‡ *DDD including changes as of 2019 (source: WHO)*

Figure 3.1.2 A-D Use of antibiotics for systemic use (J01) in outpatients at ATC-5 level, 2012-2021 (source: SFK)



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

Figure 3.1.3 Relative distribution by type of caregiver of the 5 most frequently used antibiotics in outpatients in 2021 (source: SFK)



** residents and attending physicians*

Antibiotic prescribing quality indicators in primary care

To assess the antibiotic prescribing quality at general practitioners (GPs) practice level, quality indicators (QIs) were identified in the SABEL project (*Spiegelinformatie Antibiotica Eerstelijn*)*. These QIs linked antibiotic prescribing to clinical indications (ICPC-codes derived from GP practices' electronic patient information systems). In this pilot the PHARMO database network was used to obtain these SABEL QIs from routine care data of 278 GP practices.

The fourteen SABEL QIs for primary care are:
General QIs:

- Total number of systemic antibiotic prescriptions/1000 registered patients/year
- 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 for:

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

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

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

Data of 278 GP practices from the PHARMO database network were collected from 2018 to 2020. These practices cover a catchment area representing 2.5 million residents. This is the first time Nethmap reports the outcomes of the SABEL QIs. The QI outcomes show high consistency for 2018 and 2019, but also high variability between individual practices with respect to numbers of prescribed antibiotics (QI1) and prescribing quality (other QIs). Notably, the changes due to the COVID-19 pandemic are also reflected in the outcomes for 2020: a marked decrease in overall antibiotic prescribing (QI1 from 269 in 2019 to 216 in 2020), as well as in antibiotic prescribing percentages for URTI and LRTI.

Table 3.1.2 Outcomes of the antibiotic prescribing quality indicators in primary care

	2018		2019		2020	
Quality Indicator	Median	25 - 75 percentile	Median	25 - 75 percentile	Median	25 - 75 percentile
Antibiotics prescriptions/1000 patients/year	284	236 – 333	269	229 – 320	216	165 – 261
% Amoxicillin/clavulanic acid	13	11 – 16	13	11 – 16	13	11 – 16
% Macrolides	7	6 – 9	7	5 – 10	6	5 – 9
% Quinolones	7	6 – 8	7	6 – 8	7	6 – 9
% Amoxicillin/clavulanic acid + Macrolides + Quinolones	29	26 – 33	29	25 – 33	28	25 – 32
Antibiotic prescribing % Otitis media	44	32 – 58	44	33 – 61	42	28 – 59
Antibiotic prescribing % URTI	26	19 – 36	26	18 – 34	18.5	13 – 27
Antibiotic prescribing % LRTI	28	23 – 37	28	22 – 36	16	11 – 23
Antibiotic prescribing % Impetigo	30	21 – 40	30	22 – 39	26	17 – 35
% 1st Choice antibiotic prescribing otitis media	85	75 – 92	85	77 – 93	87	75 – 97
% 1st Choice antibiotic prescribing tonsillitis	25	9 – 43	42	23 – 56	50	19 – 64
% 1st Choice antibiotic prescribing pneumonia	74.5	61 – 82	75	64 – 84	71	60 – 83
% 1st Choice antibiotic prescribing cystitis	85	80 – 89	86	81 – 88	86	82 – 89
% 1st Choice antibiotic prescribing impetigo	50	28 – 68	50	28 – 66	50	25 – 75

References

* The Dutch National Institute for Public Health and the Environment (RIVM).
Aanpak antibioticaresistentie in de eerste lijn | RIVM [Accessed May 18, 2022]

van der Velden AW, van Triest MI, Schoffelen AF, Verheij TJM. Structural Antibiotic Surveillance and Stewardship via Indication-Linked Quality Indicators: Pilot in Dutch Primary Care. Antibiotics (Basel). 2020 Oct 3;9(10):670.

3.2 Hospital care

Methods

Data on the use of antibiotics in Dutch hospitals in 2020 was collected by means of a questionnaire distributed to all Dutch hospital pharmacies. DDDs per ATC-code and route of administration, according to the WHO in 2020[3] were extracted from the Dutch drug database (Z-index) on unit and product level, and used to calculate total antibiotic use. 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) DDDs are depicted for the year 2018 in the tables and figures (as a dashed line), to enable long-term comparison of surveillance data.

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 hospital consumption in the Netherlands. Methods are further described by Kwint *et al.*[4] 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

Data over 2020 were received from 62 hospital locations together with the annual number of bed-days and admissions. The inpatient use of systemic antibiotics increased (+8,2%) to 85.79 DDD/100 patient-days in 2020 (Table 3.2.1). Expressed as DDD/100 admissions, total inpatient use of systemic antibiotics increased to 333.1 in 2020 (+4,6%; Table 3.2.1).
Calculated as DDD/1,000 inhabitant-days (DID) however, total use of antibiotics for systemic use decreased from 0.799 in 2019 to 0.760 in 2020 (-4.9%) (Table 3.2.2).
Several antibiotics show remarkable increases (Figure 3.2.2): flucloxacillin rose by 12.5% to 11.97 DDD/100 patient-days. Second- and third-generation cephalosporins rose to 8.48 and 9.93 DDD/100 patient-days (+6.1 and +28.5% resp.). From the group of the macrolides, especially azithromycin use increased by 16.1% to 2.02 DDD/100 patient-days. Vancomycin use rose by 21.4% to 2.21 DDD/100 patient-days. Although still rarely used, antibiotics like fosfomycin and linezolid show a remarkable increase in use by +27.3 and +50% respectively.
The only group of antibiotics that shows a further decrease in use are the aminoglycosides, and more specifically the parenteral use of gentamicin decreased for the second year in a row to 1.32 DDD/100 patient-days (-13.7%) in 2020.

A large variation in systemic antibiotic drug use is seen between Dutch hospitals (Figure 3.2.3, 3.2.4 and 3.2.5). Considering site of care, in 2020, antibiotic use in university hospitals was higher than in general and large teaching hospitals (median 97.0 DDD/100 patient-days vs 84.8 and 82.0 DDD/100 patient-days resp) as shown in Figure 3.2.4.
The increase in use of third-generation cephalosporins was mainly seen in general and large-teaching hospitals (Figure 3.2.6). This is due to higher use of ceftriaxone (for details see Figure 3.2.7) Increase in meropenem use was only seen in university hospitals, whereas vancomycin use increased in university and in general hospitals and to a lesser extent also in large teaching hospitals (Figure 3.2.6).

Antimycotics were mainly used in university hospitals with a total of 12.64 DDD/100 patient-days, an increase of 11% from 2019. Two-third comprises of triazole derivatives such as fluconazole and voriconazole (Table 3.2.3).

Discussion

Care in hospitals in 2020 was for a great part dominated by the care for COVID-19 patients. Much had to be learned in how to treat these patients. Changes in antibiotic use in 2020 probably reflect in part this journey of the unknown. Overall antibiotic use decreased in hospitals, but use per patient and use per bed-day increased which indicates intensification of treatment of individual patients. There was a remarkable increase in the use of 2nd and 3rd generation cephalosporins, probably due to more frequent treatment of severe pulmonary infections. Use of 1st generation cephalosporins, mainly used for surgical prophylaxis, slightly decreased as surgical procedures were downscaled during COVID-waves.

The use of azithromycin also shows a remarkable increase. In 2020, there was much discussion whether azithromycin would have favorable effects in the treatment of COVID-19.

Use of flucloxacillin increased again, probably indicating use of increasing doses. The same holds true for the increase in use of vancomycin, as higher target trough-levels were advised since 2020. However, the use of antibiotics for gram-positive bacteria in general showed an increase, as also the use of linezolid increased substantially.

The increase in use of antimycotics could be a consequence of the use of high dose dexamethasone in COVID-19 patients with the risk of subsequent fungal superinfections.

The large variation of use between the Dutch hospitals is difficult to explain. There is a large influence of local practices, like guidance through culture-results vs empiric regimens, and differing policies concerning adjustments based on lab-results of CRP, POCT's and radiological results. Also the location of hospitals and cross-border collaboration could influence local practices.

Table 3.2.1 Ten years use of antibiotics for systemic use (J01) in hospitals (DDD/100 patient-days), 2011-2020 (source: SWAB)

ATC group*	Therapeutic group	2011	2012	2013	2014	2015	2016	2017	2018	2018‡	2019‡	2020‡
J01AA	Tetracyclines	1.84	1.74	1.75	1.90	1.89	1.96	1.97	2.05	2.05	2.10	2.00
J01CA	Penicillins with extended spectrum	7.31	7.62	7.95	8.42	9.24	10.88	10.22	11.08	5.26	4.92	5.01
J01CE	Beta-lactamase sensitive penicillins	1.52	1.74	1.86	2.40	2.39	2.55	2.50	2.26	2.26	2.49	2.60
J01CF	Beta-lactamase resistant penicillins	6.73	7.14	8.09	8.67	7.74	8.73	9.59	10.76	10.76	10.64	11.97
J01CR	Combinations of penicillins, incl. beta-lactamase-inhibitors	15.85	14.96	14.84	14.48	14.31	14.62	14.73	14.48	11.98	10.13	10.60
J01DB	First-generation cephalosporins	3.49	3.64	3.71	4.35	4.59	4.63	5.29	6.43	6.43	6.68	6.55
J01DC	Second-generation cephalosporins	3.68	4.09	4.68	4.98	5.33	5.75	5.87	7.99	7.99	7.99	8.48
J01DD	Third-generation cephalosporins	3.90	4.37	5.04	5.67	5.49	5.95	6.39	6.88	6.88	7.73	9.93
J01DH	Carbapenems	1.38	1.48	1.65	1.65	1.74	1.83	1.98	1.93	1.32	1.41	1.53
J01EA	Trimethoprim and derivatives	0.39	0.31	0.30	0.26	0.26	0.25	0.27	0.23	0.23	0.20	0.23
J01EE	Combinations of sulfonamides and trimethoprim, incl. derivatives	1.89	1.77	1.92	1.89	1.76	2.13	2.38	2.15	2.15	2.41	3.00
J01FA	Macrolides	2.86	2.81	2.64	2.88	2.74	2.97	2.82	2.66	2.66	2.75	3.18
J01FF	Lincosamides	2.29	2.21	2.30	2.30	2.35	2.45	2.43	2.54	2.54	2.36	2.34
J01GB	Aminoglycosides	3.95	3.26	3.55	3.57	3.66	3.70	3.62	3.76	3.76	3.34	2.97
J01MA	Fluoroquinolones	9.16	8.90	8.65	9.02	8.39	9.15	8.65	8.45	7.67	6.99	7.39
J01XA	Glycopeptides	1.28	1.36	1.49	1.59	1.60	1.62	1.72	1.73	1.73	1.99	2.39
J01XB	Polymyxins	0.22	0.16	0.23	0.19	0.23	0.23	0.24	0.14	0.11	0.15	0.14
J01XD	Imidazole derivatives	2.16	2.33	2.55	2.60	2.58	2.80	3.00	3.20	3.20	3.21	3.28
J01XE	Nitrofuran derivatives	1.24	1.22	1.30	1.55	1.42	1.67	1.73	1.63	1.63	1.40	1.77
J01XX	Other antibacterials **	0.09	0.10	0.10	0.09	0.12	0.13	0.28	0.24	0.24	0.28	0.31
	Others***	0.07	0.10	0.08	0.07	0.07	0.07	0.08	0.10	0.10	0.13	0.10
J01	**Antibiotics for systemic use (total)**	**71.31**	**71.31**	**74.68**	**78.55**	**77.89**	**84.05**	**85.68**	**90.71**	**80.98**	**79.29**	**85.79**
	expressed in DDD/100 admissions:											
J01	**Antibiotics for systemic use (total)**	**306.4**	**295.7**	**307.8**	**326.0**	**330.1**	**326.1**	**340.2**	**339.7**	**303.2**	**318.5**	**333.1**

* *From the 2020 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 hospital care (DDD/1,000 inhabitant-days), 2011-2020 (source: SWAB)

ATC group*	Therapeutic group	2011	2012	2013	2014	2015	2016	2017	2018	2018‡	2019‡	2020‡
J01AA	Tetracyclines	0.026	0.024	0.022	0.023	0.025	0.022	0.021	0.023	0.023	0.021	0.019
J01CA	Penicillins with extended spectrum	0.103	0.100	0.099	0.101	0.118	0.125	0.117	0.110	0.052	0.063	0.050
J01CE	Beta-lactamase sensitive penicillins	0.020	0.023	0.023	0.028	0.028	0.029	0.029	0.033	0.033	0.024	0.022
J01CF	Beta-lactamase resistant penicillins	0.089	0.093	0.100	0.105	0.097	0.102	0.103	0.105	0.105	0.104	0.103
J01CR	Combinations of penicillins, incl. beta-lactamase-inhibitors	0.223	0.211	0.199	0.187	0.186	0.171	0.159	0.153	0.128	0.109	0.098
J01DB	First-generation cephalosporins	0.045	0.049	0.047	0.052	0.055	0.053	0.065	0.070	0.070	0.066	0.056
J01DC	Second-generation cephalosporins	0.050	0.052	0.055	0.058	0.065	0.066	0.067	0.070	0.070	0.077	0.073
J01DD	Third-generation cephalosporins	0.050	0.057	0.062	0.066	0.067	0.068	0.067	0.072	0.072	0.074	0.085
J01DH	Carbapenems	0.018	0.019	0.020	0.019	0.021	0.020	0.021	0.020	0.014	0.014	0.013
J01EA	Trimethoprim and derivatives	0.006	0.005	0.004	0.003	0.003	0.003	0.003	0.003	0.003	0.002	0.002
J01EE	Combinations of sulfonamides and trimethoprim, incl. derivatives	0.026	0.024	0.024	0.022	0.021	0.024	0.023	0.022	0.022	0.022	0.024
J01FA	Macrolides	0.037	0.038	0.034	0.034	0.034	0.034	0.030	0.030	0.030	0.026	0.027
J01FF	Lincosamides	0.032	0.031	0.032	0.028	0.030	0.028	0.027	0.026	0.026	0.024	0.022
J01GB	Aminoglycosides	0.054	0.044	0.045	0.044	0.046	0.043	0.037	0.037	0.037	0.033	0.027
J01MA	Fluoroquinolones	0.127	0.124	0.116	0.112	0.112	0.106	0.097	0.087	0.079	0.071	0.066
J01XA	Glycopeptides	0.017	0.017	0.018	0.018	0.019	0.019	0.019	0.018	0.018	0.018	0.019
J01XB	Polymyxins	0.003	0.002	0.003	0.002	0.003	0.002	0.001	0.002	0.001	0.001	0.001
J01XD	Imidazole derivatives	0.027	0.029	0.030	0.030	0.032	0.032	0.034	0.033	0.033	0.033	0.031
J01XE	Nitrofuran derivatives	0.015	0.018	0.016	0.018	0.018	0.018	0.019	0.017	0.017	0.015	0.016
J01XX	Other antibacterials**	0.001	0.002	0.002	0.001	0.002	0.002	0.003	0.003	0.003	0.003	0.003
	Others***	0.001	0.002	0.000	0.000	0.001	0.000	0.001	0.001	0.001	0.001	0.001
J01	**Antibiotics for systemic use (total)**	**0.971**	**0.963**	**0.950**	**0.953**	**0.982**	**0.968**	**0.942**	**0.934**	**0.836**	**0.799**	**0.760**

* *From the 2020 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, 2011-2020 (source: SWAB)

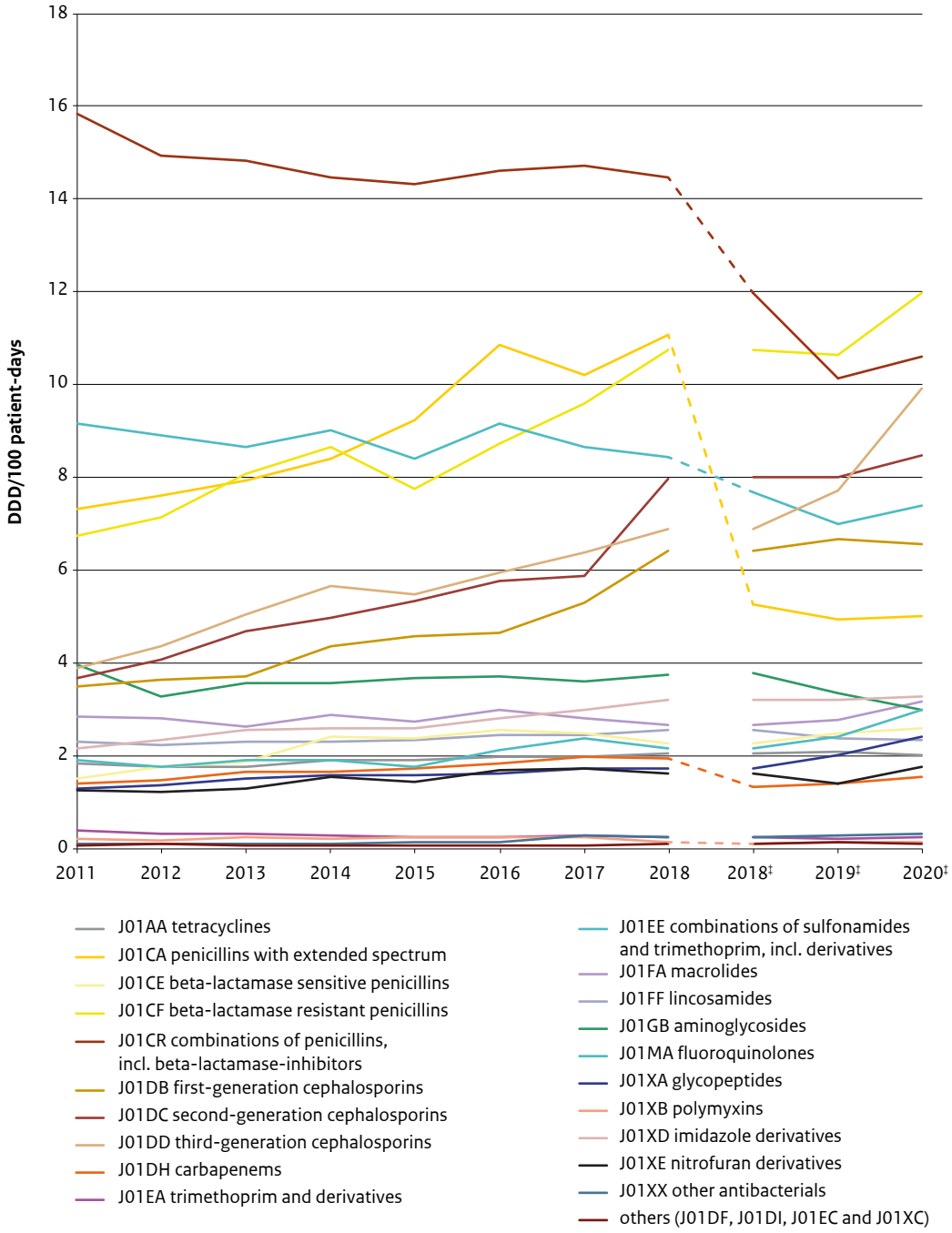
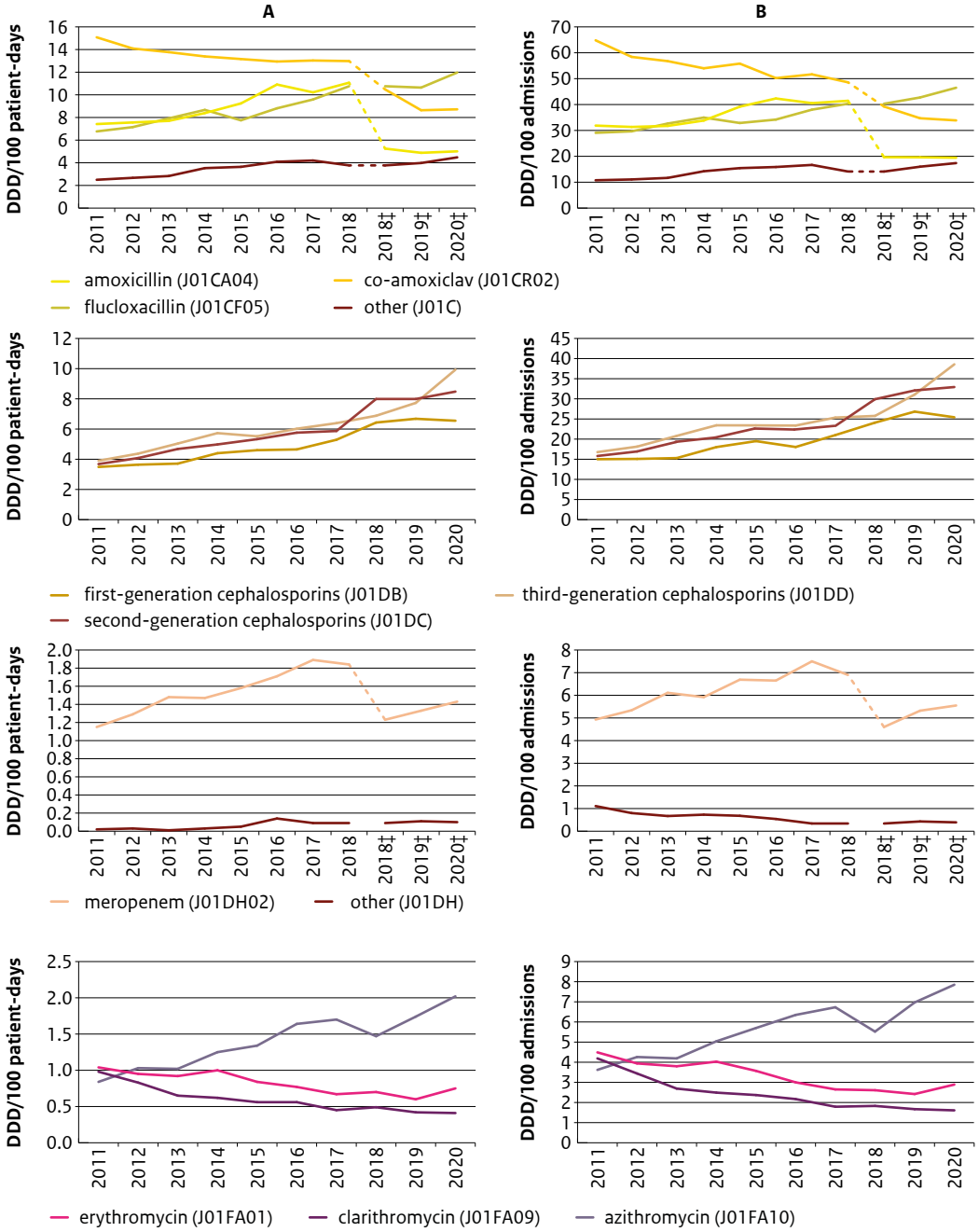
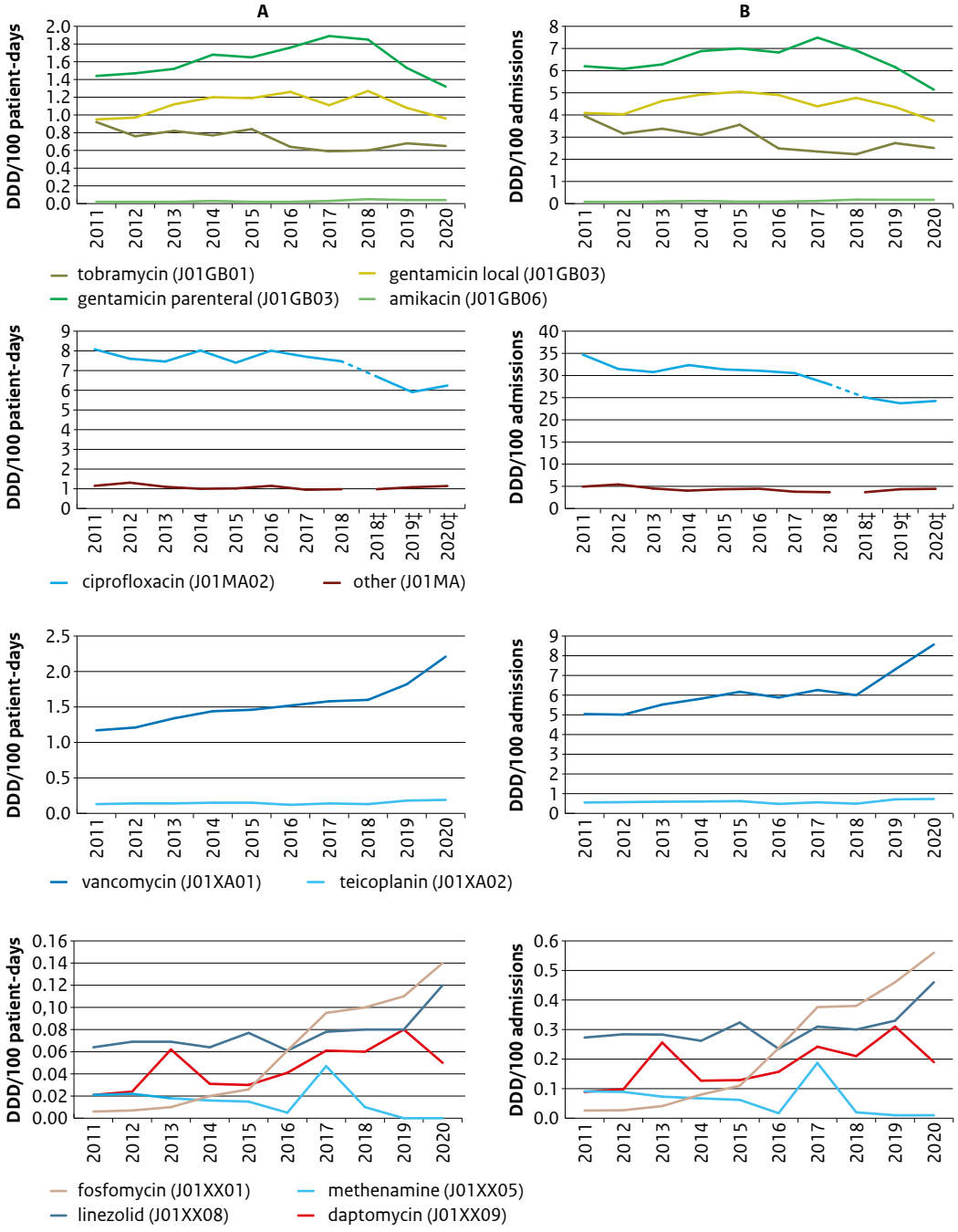


Figure 3.2.2 Use of beta-lactams, macrolides, aminoglycosides, fluoroquinolones, glycopeptides and other antibiotics in hospitals expressed as DDD/100 patient-days (A) and DDD/100 admissions (B) 2011-2020 (source: SWAB)



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 beta-lactams, macrolides, aminoglycosides, fluoroquinolones, glycopeptides and other antibiotics in hospitals expressed as DDD/100 patient-days (A) and DDD/100 admissions (B) 2011-2020 (source: SWAB)



‡ *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 2020 (source: SWAB)

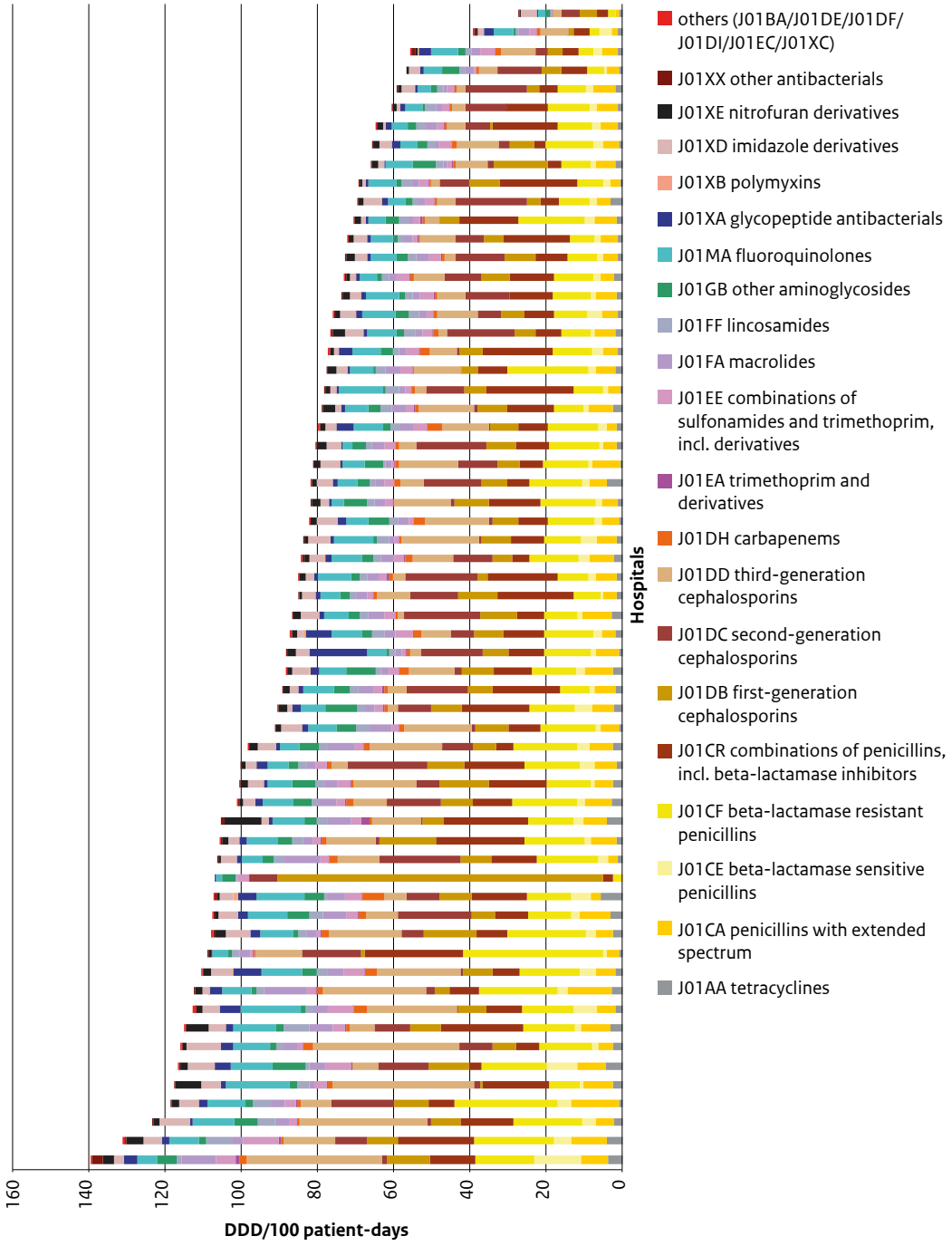
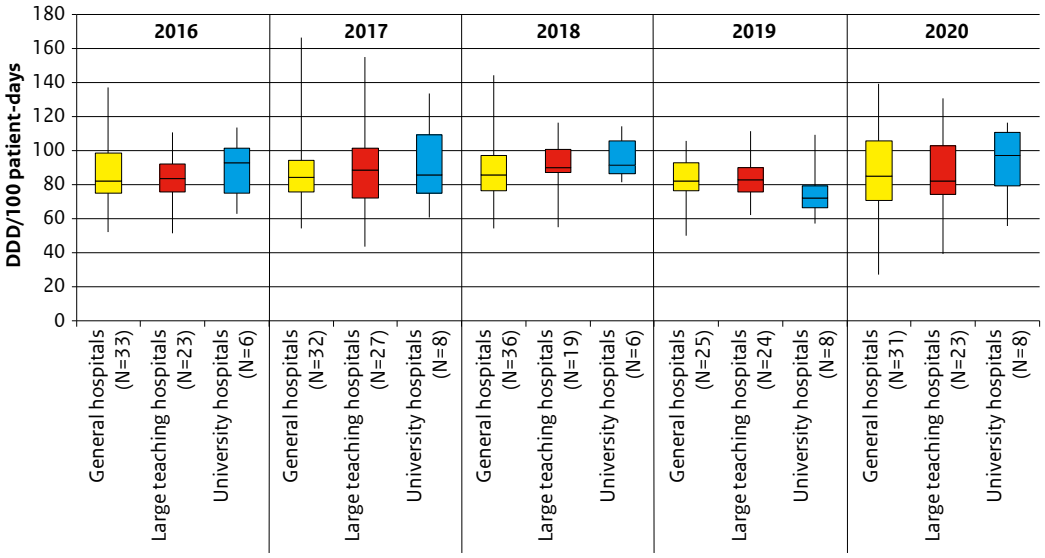


Figure 3.2.4 Five years use of total systemic antibiotic use (J01) and comparison across university, large teaching and general hospitals (source: SWAB)



boxplot shows minimum - P25 - median - P75 - maximum

Figure 3.2.5 Distribution (%) of the use of antibiotics for systemic use (J01) in hospitals, 2020 (source: SWAB)

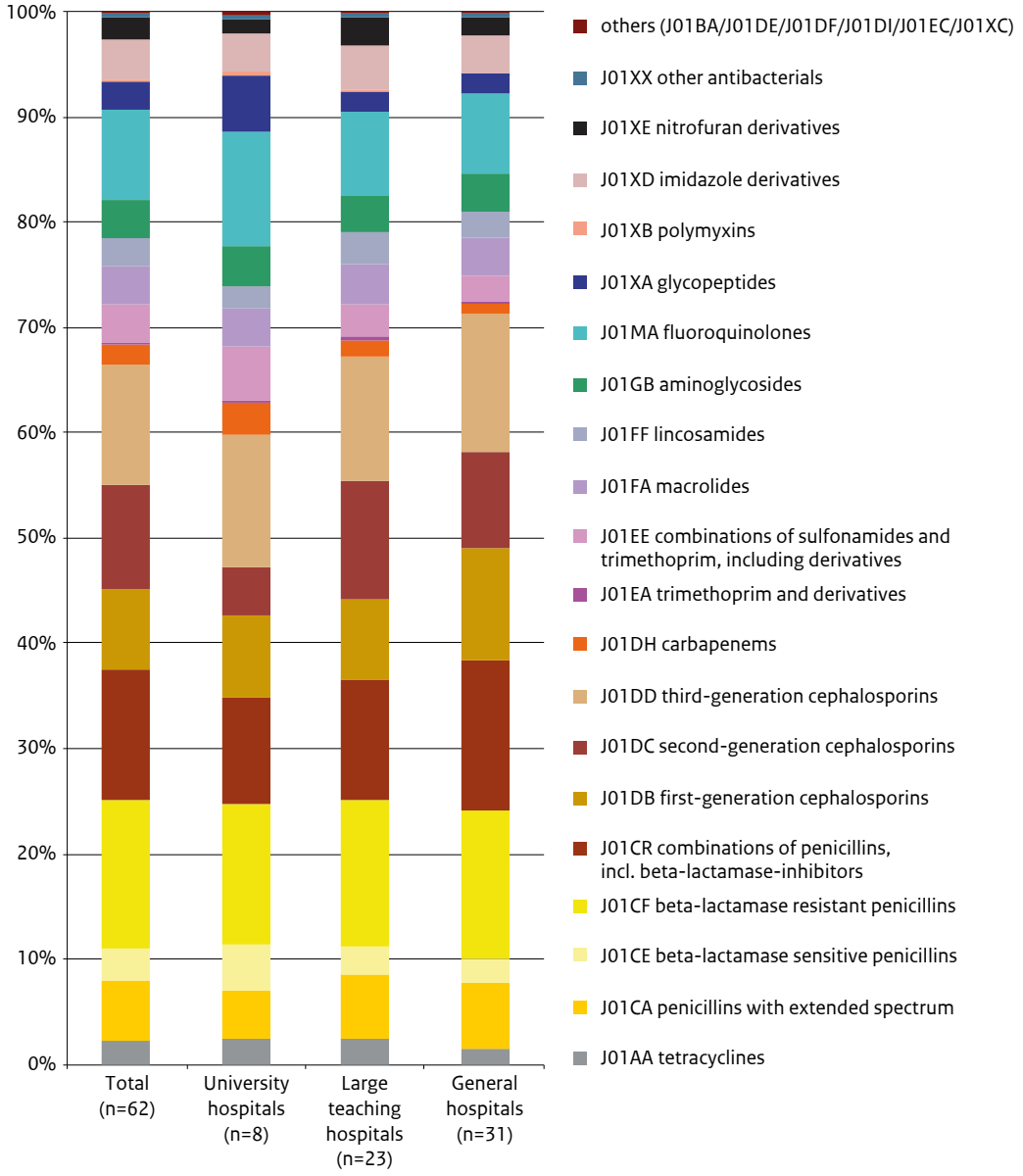
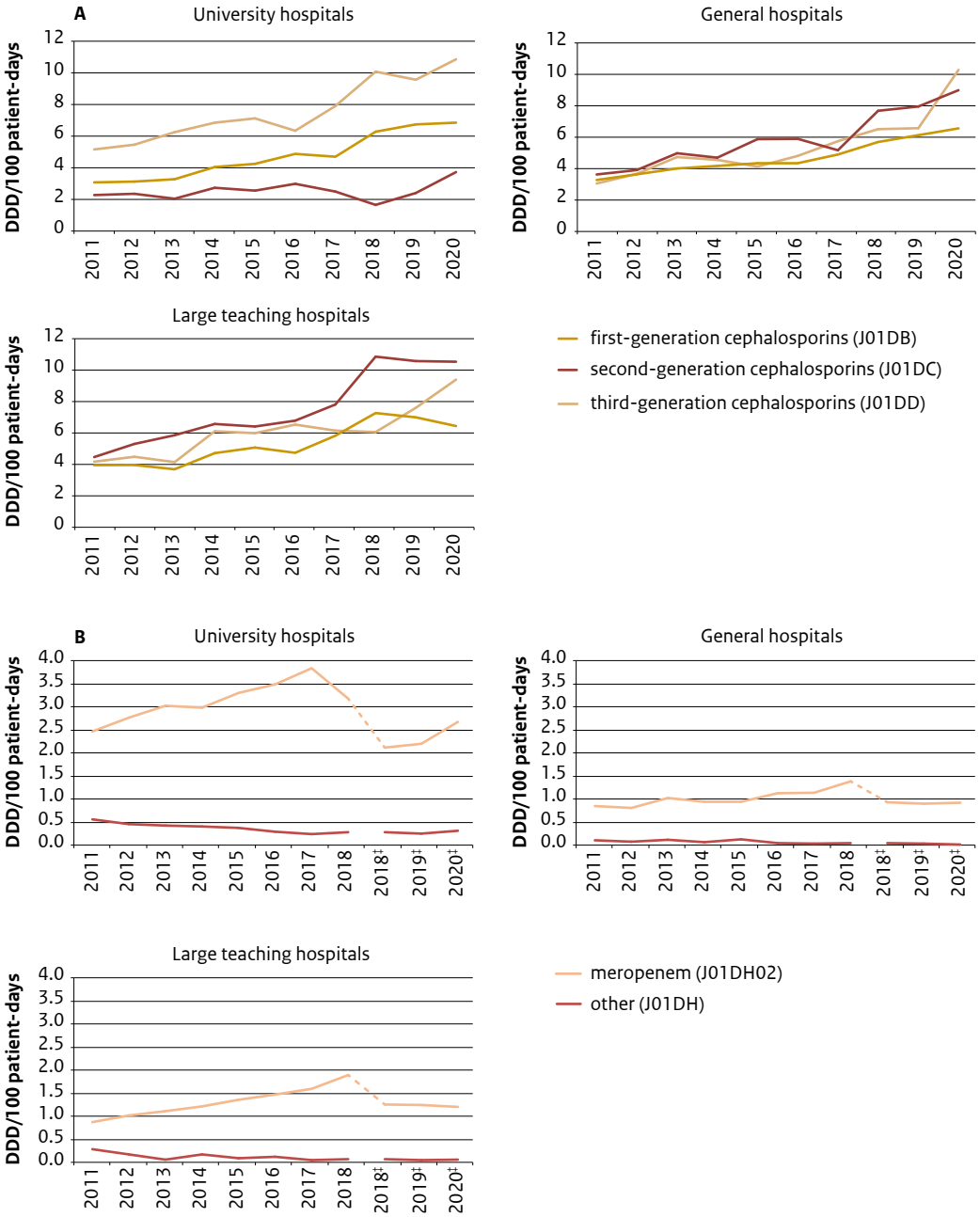


Figure 3.2.6 Use of cephalosporins (A), carbapenems (B), aminoglycosides (C), glycopeptides (D) and fluoroquinolones (E) in hospitals broken down by type of hospital, expressed as DDD/100 patient-days (2011-2020, source: SWAB)



‡ *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 (2011-2020, source: SWAB)

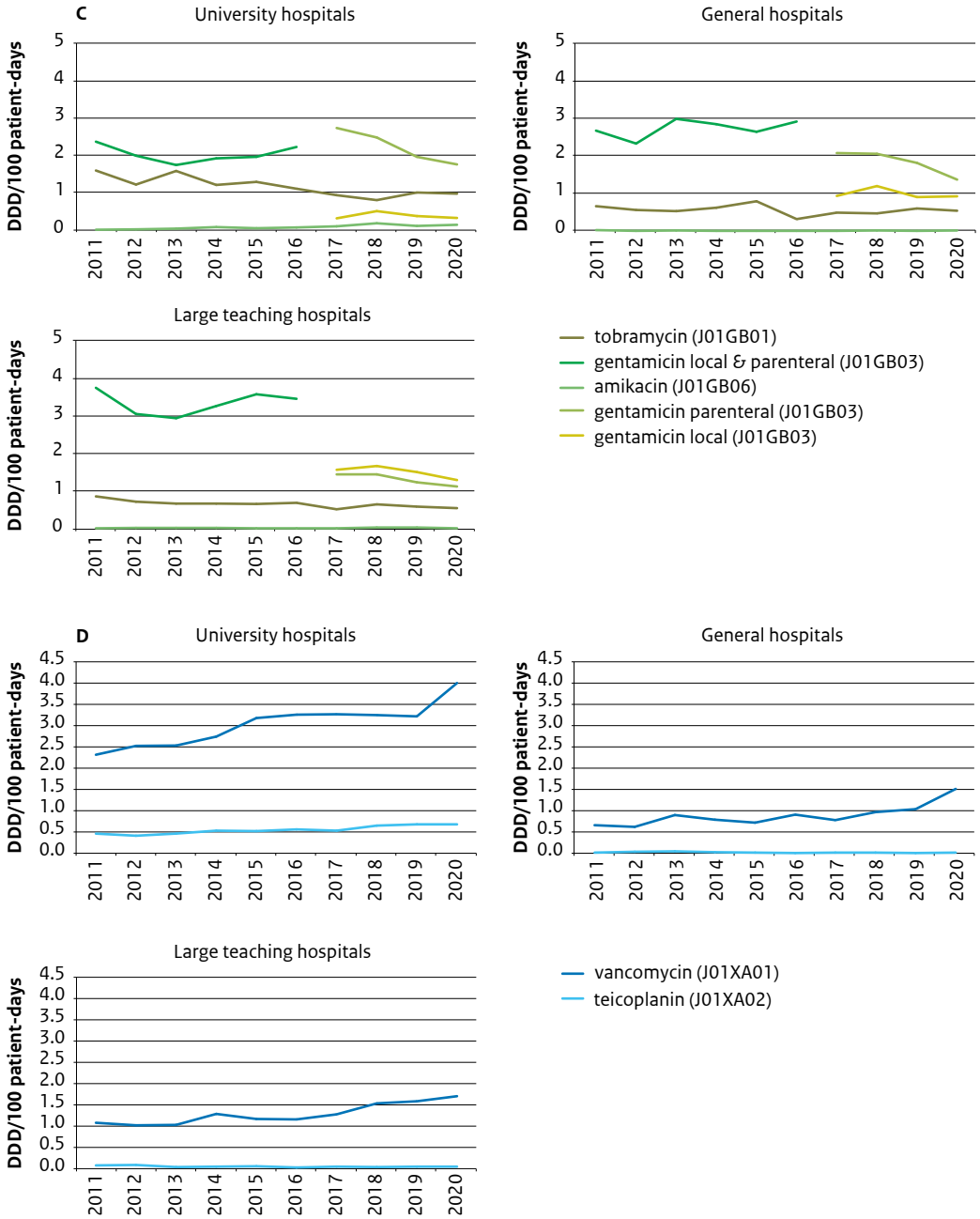
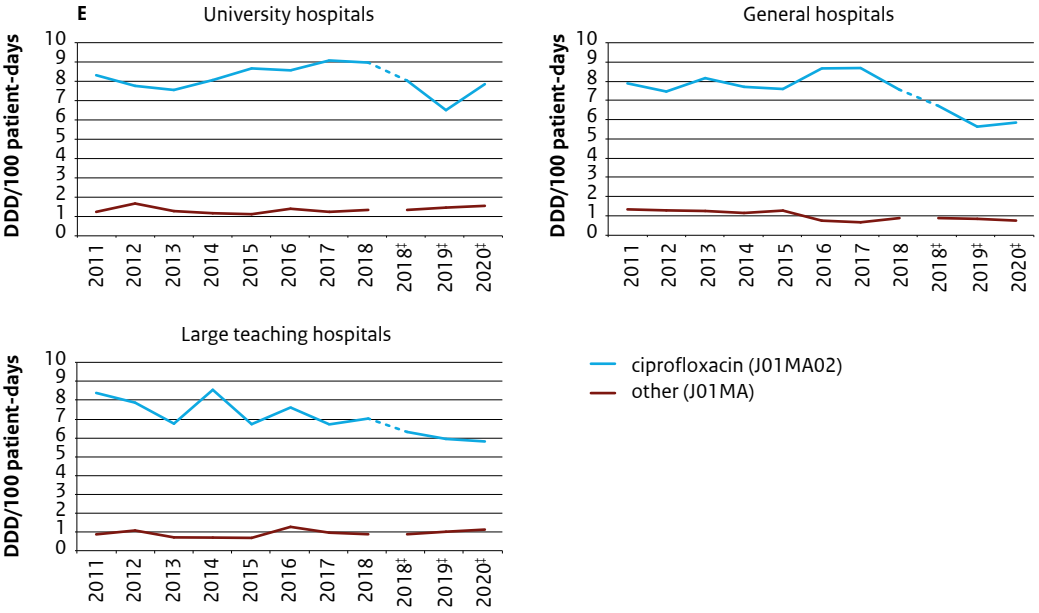


Figure 3.2.6 (continued) Use of cephalosporins (A), carbapenems (B), aminoglycosides (C), glycopeptides (D) and fluoroquinolones (E) in hospitals broken down by type of hospital, expressed as DDD/100 patient-days (2011-2020, source: SWAB)



‡ *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 (2011-2020) (source: SWAB)

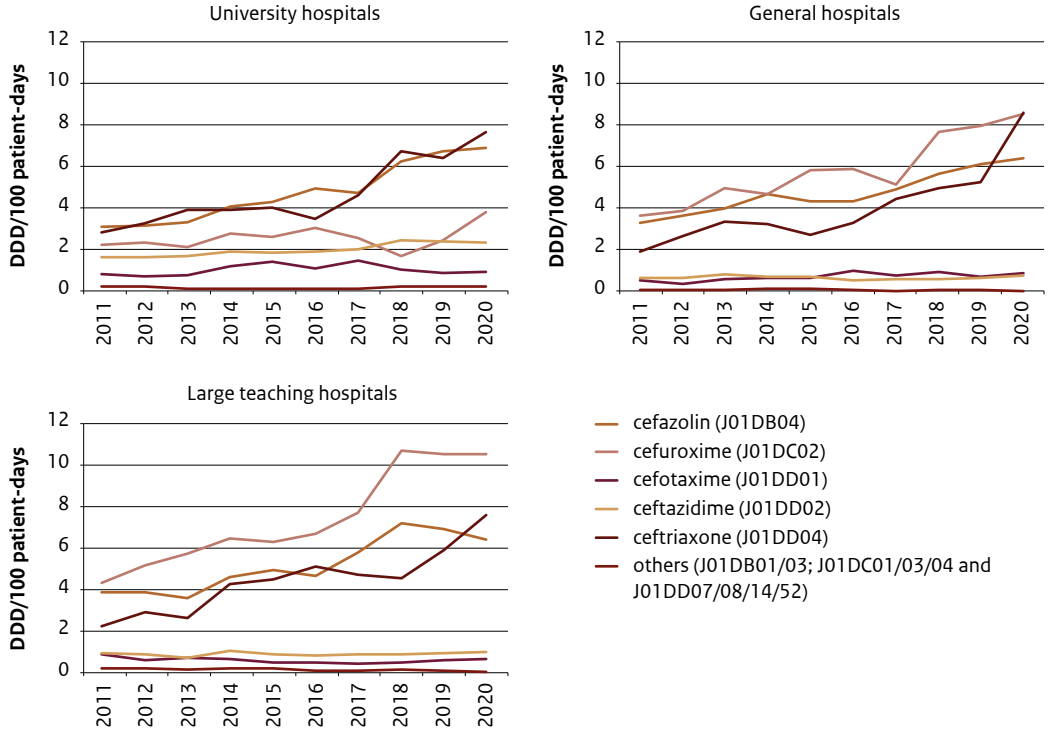


Table 3.2.3 Use of antimycotics (J02) in hospitals (DDD/100 patient-days), 2020 (source: SWAB)

ATC group	Therapeutic group	Total	Academic hospitals	Large teaching hospitals	General hospitals
J02AA01	Antibiotics (amphotericin B)	1.36	3.42	0.95	0.33
J02AB02	Imidazole derivatives (ketoconazole)	0.01	0.03	0.00	0.01
J02AC	Triazole derivatives	3.72	8.16	2.77	1.63
J02AX	Other antimycotics for systemic use (mainly echinocandines)	0.63	1.03	0.54	0.44
J02	**Antimycotics for systemic use (total)**	**5.71**	**12.64**	**4.27**	**2.42**

** From the 2020 edition of the Anatomical Therapeutic Chemical (ATC) classification system*

3.3 Long-term care facilities

Methods

Data on antibiotic use in long-term care facilities originate from two different sources; several hospital pharmacies provided systemic antibiotic consumption data from long-term care facilities that their pharmacy was serving for 2020, collected over 365 days. The second source is the point prevalence study executed by the SNIV network of the RIVM in 2021[5], i.e. prescriptions for systemic and topical antibiotics and antimycotics on an index day.

All hospital pharmacists participating in the SWAB surveillance of antibiotic use in hospitals were asked to provide antibiotic consumption data from long-term care facilities their pharmacy was serving for 2020. For each facility the amount of DDD/1,000 residents/day was calculated, while assuming occupancy of 100%, and their weighed mean, capacity based, was calculated.

In 2021 a point prevalence study was performed in long-term care facilities of the SNIV network of the RIVM. Dutch long-term care facilities participating in SNIV collected detailed data on antibiotic usage on an index day, in addition to data collection on healthcare associated infections. All residents admitted to somatic, psychogeriatric and geriatric revalidation departments 24 hours before the registration date, and present in the long-term care facilities on the registration date, were included. Only systemic and topical antibiotics and antimycotics were included, with a maximum of four concomitant substances per patient.

Results

The antibiotic use of 13380 residents of long-term facilities was included in the data analysis for 2020, originating from 26 long-term care facilities or organizations. The size of long-term facilities varied from 55-1650 residents per home or organization, with a mean of 515 residents.

Compared to 2019, the mean antibiotic use in long-term care facilities remained stable with 50.4 DDD/1,000 residents/day. The use varied highly between the different long-term care facilities with a minimum of 2,1 and a maximum of 288,7 DDD/1,000 residents/day. The use of tetracyclines, beta-lactamase resistant penicillins, combination of penicillins, lincosamides and nitrofurantoin decreased compared to 2019; the use of penicillins with extended spectrum, macrolides and fluoroquinolones increased (Table 3.3.1).

Figure 3.3.1 depicts antimicrobial medication used in the point prevalence study performed in 36 long-term care facilities of the SNIV network of RIVM in 2021. Of the 2541 residents that participated, 283 received antimicrobial medication, with a total of 310 prescriptions, of which 138 were for prophylactic use. Antimycotics are the most frequently used antimicrobials, for prophylaxis (31% ketoconazole, 9% miconazole, 5% clotrimazole) as well as treatment (ketoconazole 21%, miconazole 21%, clotrimazole 5%).

Discussion

Overall antibiotic use in long-term care facilities remained stable. However, we observed a huge variation in total use across different organisations. Although there are slight changes in the pattern of use, amoxicillin with clavulanic acid, fluoroquinolones and nitrofuran derivatives remain the most widely used systemic antibiotics in long-term care facilities. The high use of nitrofurantoin is not surprising, as urinary tract infections are one of the most common infections among elderly patients. With respect to broad spectrum antibiotics, the increasingly high use of fluoroquinolones is especially worrisome.

The results from the point prevalence study show widespread use of topical antimycotics. However, the data stem from a different cohort of long-term care facilities and therefore the two sources of data are not directly comparable.

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, 2011-2020 (source: SWAB)

ATC group*	Therapeutic group	2011	2012	2013	2014	2015	2016	2017	2018	2018‡	2019‡	2020‡
J01AA	Tetracyclines	5.4	6.0	6.2	4.7	3.9	4.9	4.0	5.0	5.0	3.7	2.9
J01CA	Penicillins with extended spectrum	4.5	6.6	4.3	5.1	5.0	5.6	4.6	3.8	2.4	2.6	4.8
J01CE	Beta-lactamase sensitive penicillins	0.3	0.2	0.5	0.5	0.7	0.3	0.6	0.4	0.4	0.5	0.4
J01CF	Beta-lactamase resistant penicillins	2.5	3.7	1.7	1.4	2.3	1.8	2.2	3.3	3.3	3.0	2.5
J01CR	Combinations of penicillins, incl. beta-lactamase-inhibitors	18.8	18.8	19.5	16.3	17.9	16.1	15.5	18.0	12.1	12.0	10.2
J01DB	First-generation cephalosporins	0.0	0.0	0.0	0.1	0.1	0.0	0.2	0.1	0.1	0.0	0.2
J01DC	Second-generation cephalosporins	0.2	0.1	0.2	0.1	0.2	0.1	0.3	0.1	0.1	0.2	1.0
J01DD	Third-generation cephalosporins	0.5	1.0	0.6	0.6	0.8	0.4	0.5	0.4	0.4	0.4	0.5
J01DH	Carbapenems	0.1	0.0	0.0	0.0	0.1	0.0	0.1	0.1	0.1	0.3	0.1
J01EA	Trimethoprim and derivatives	2.2	2.3	2.4	1.9	1.4	1.6	1.6	1.2	1.2	0.8	1.2
J01EE	Combinations of sulfonamides and trimethoprim, including derivatives	3.2	2.5	1.7	1.5	1.6	1.1	1.2	1.9	1.9	3.0	2.6
J01FA	Macrolides	1.8	2.1	1.8	1.8	2.1	2.4	2.8	2.7	2.7	2.7	3.0
J01FF	Lincosamides	3.1	4.0	2.4	2.0	2.6	3.7	2.9	3.0	3.0	2.9	2.2
J01GB	Aminoglycosides	0.1	0.1	0.0	0.2	0.2	0.1	0.3	0.1	0.1	0.0	0.0
J01MA	Fluoroquinolones	10.3	10.7	8.3	8.4	8.9	8.2	6.9	8.7	8.7	7.3	9.1
J01XA	Glycopeptides	0.1	0.1	0.1	0.1	0.2	0.1	0.2	0.2	0.2	0.4	0.1
J01XB	Polymyxins	0.3	0.2	0.0	0.0	0.1	0.2	0.0	0.1	0.1	0.0	0.1
J01XD	Imidazole derivatives	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0
J01XE	Nitrofuran derivatives	9.5	11.0	11.1	10.4	11.4	9.6	8.3	11.3	11.3	9.5	8.2
J01XX	other antibacterials**	0.5	0.6	0.4	0.2	0.5	0.8	0.8	0.7	0.7	0.9	1.4
	others***	0.4	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
J01	**Antibiotics for systemic use (total)**	**63.8**	**70.3**	**61.1**	**55.3**	**60.0**	**57.2**	**52.9**	**61.4**	**53.9**	**50.4**	**50.4**

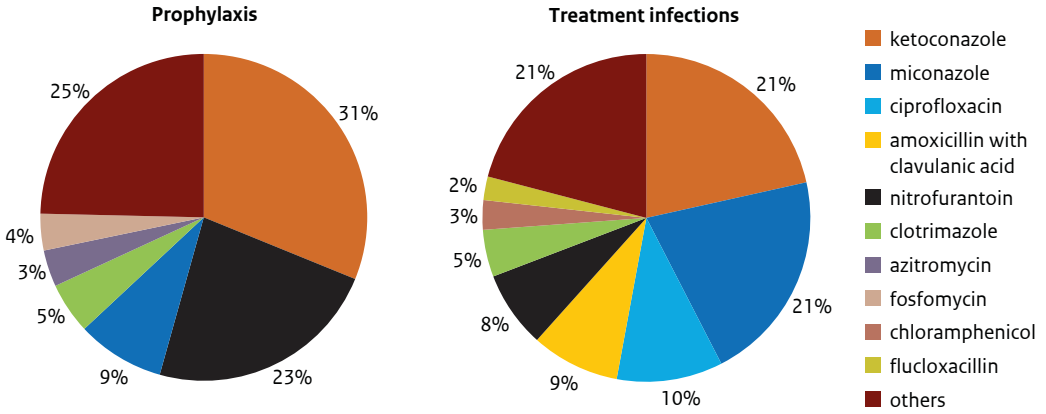
* *From the 2020 edition of the Anatomical Therapeutic Chemical (ATC) classification system*

** *fosfomycin, methenamine, linezolid, daptomycin*

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

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

Figure 3.3.1 Distribution of the use of antibiotics for systemic use (J01); results of the point-prevalence studies 2021 (source: SNIV)



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 de Jong LAW, van der Linden PD, Roukens MMB, van de Garde EMW, van der Velden AW, Natsch S; SWAB's Working Group on Surveillance of Antimicrobial Use. Consecutive antibiotic use in the outpatient setting: an extensive, longitudinal descriptive analysis of antibiotic dispensing data in the Netherlands. BMC Infect Dis. 2019 Jan 24;19(1):84.

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

4 Kwint HM, Van der Linden PD, Roukens MMB et al. Intensification of antibiotic use within acute care hospitals in the Netherlands, J. Antimicrob. Chemother 2012: 2283-2288.

5 The Dutch National Institute for Public Health and the Environment (RIVM). SNIV: Surveillance Netwerk Infectie-ziekten in Verpleeghuizen. Available from: https://www.rivm.nl/sniv/prevalentiemeting. [Accessed May 12, 2022]

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 2021, 46 laboratories were connected to ISIS-AR, all performing antimicrobial susceptibility testing (AST) according to EUCAST guidelines. Out of these 46 laboratories, 34 provided complete data on the last five years (2017 to 2021). Five of these 34 laboratories exclusively served university hospitals; 26 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, and 4.5 we selected only data from these 34 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 32 non-university laboratories for which at least complete data on 2021 were available (29 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 care), 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 *Staphylococcus aureus / Staphylococcus argenteus*, 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. Finally, 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.

Since the number of *S. argenteus* isolates was too small for separate analyses, the data for *S. argenteus* and *S. aureus*, both 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 0.0 to 0.02% of the isolates from this complex. *Staphylococcus schweitzeri*, the third member of the *S. aureus* complex, was not found in the laboratories selected for analysis.

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 of / 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.

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 11.0 (2021). 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 11.0 (2021), 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, reinterpreting 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. In the same year, 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. 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 according to EUCAST breakpoints version 11.0 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 (e.g., meningitis and all indications other than meningitis). 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 R-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 *E. 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 R-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; being 16 mg/L, whereas we reinterpreted diameters according to the EUCAST breakpoint for oral administration (24 mm). In contrast to previous years we did not calculate resistance levels for fosfomycin in *K. pneumoniae*, *P. mirabilis*, and *E. cloacae*

complex, because recent evidence shows that oral fosfomycin is not appropriate for treatment of urinary tract infections with these pathogens[3,4] and breakpoints for those pathogens-agent combinations were omitted in EUCAST breakpoints version 11.0. 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.

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 laboratory S/I/R interpretation, for which we assumed that results of inducible resistance tests were taken into account.

Because not all laboratories used cefoxitin to screen for MRSA, and because part of the laboratories reported flucloxacillin results based on cefoxitin screening methods, we estimated resistance to flucloxacillin in *S. aureus* and coagulase-negative *Staphylococcus* spp. based on laboratory S/R interpretation for cefoxitin, or, if no cefoxitin interpretation was available, for oxacillin/flucloxacillin.

As some laboratories did not report (benzyl)penicillin results for *S. pneumoniae* if the isolate was susceptible to oxacillin, we estimated resistance based on reinterpretation of oxacillin test values, or, if the result for oxacillin was I or R, 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.

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 *P. 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 *E. cloacae* complex and *Acinetobacter* spp., we calculated resistance to specific combinations of agents that are frequently used for empiric therapy (for *Enterobacterales*: gentamicin + co-amoxiclav, gentamicin + cefuroxime, gentamicin + cefotaxime/ceftriaxone, ciprofloxacin + co-amoxiclav, ciprofloxacin + cefuroxime, and ciprofloxacin + cefotaxime/ceftriaxone; for *P. aeruginosa*: tobramycin + ceftazidime and tobramycin + ciprofloxacin). For these combinations, we defined resistance as resistance to both agents.

For *S. aureus* and coagulase-negative *Staphylococcus* spp., 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.

To calculate the percentage of highly resistant microorganisms (HRMO), we used the definitions of the Working Group on Infection Prevention as determined in 2017 (WIP)[6]. We considered *E. coli*, *K. pneumoniae*, and *P. mirabilis* to be an HRMO if they were 1) extended-spectrum β-lactamase (ESBL)-producing, estimated by 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 or 20 mm for cefotaxime and 25 mm for ceftriaxone) and/or

ceftazidime, 2) resistant to both fluoroquinolones and aminoglycosides, or 3) carbapenemase producing (CPE), estimated by confirmatory tests of carbapenemase production (either phenotypical or molecular), or, if no data on confirmatory tests were available, by resistance to meropenem (according to a cut-off of 2 mg/L or 22 mm) or imipenem (for *P. mirabilis*: meropenem only). We considered *E. cloacae* complex to be an HRMO if at least one of the situations 2 and 3, as described for the other *Enterobacterales*, was true. We considered *P. aeruginosa* to be an HRMO if it was resistant to ≥3 antimicrobial groups among fluoroquinolones, aminoglycosides, meropenem (according to a cut-off of 2 mg/L or 24 mm) or imipenem (or, if a confirmatory test for carbapenemase production, either phenotypical or molecular, was available, we prioritized this), ceftazidime, and piperacillin-tazobactam. Finally, for *Acinetobacter* spp., we defined HRMO as at least one of the following: 1) carbapenemase producing, estimated by confirmatory tests of carbapenemase production, or, if no data on confirmatory tests were available, by resistance to meropenem (according to a cut-off of 2 mg/L or 21 mm) or imipenem, or 2) resistant to both fluoroquinolones and aminoglycosides.

In addition, for *Enterobacterales* isolates, we calculated the percentage of isolates that was multidrug resistant, 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*, *K. pneumoniae*, and *S. aureus* isolates from general practitioners' patients, we conducted an extra analysis to calculate resistance to a selection of agents in 2021 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 clinically relevant. In the corresponding figures, differences in resistance percentages that were both statistically significant and clinically relevant are indicated by an asterisk.

Calculation of time trends

In addition to resistance levels in 2021, we calculated for sections 4.2, 4.3, and 4.5 time trends over the last five years (2017 to 2021) using logistic regression models, except when data in one or more years before 2021 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 11.0. 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 2017 and 2021. However, for coagulase-negative *Staphylococcus* spp., breakpoints for cefoxitin were changed in 2017. Therefore, we did not calculate a time trend for flucloxacillin resistance in this pathogen.

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 2017 and 2021 was larger than the square root of the predicted resistance in 2017, we considered the trend to be clinically relevant. Statistically significant increasing trends that are considered to be clinically 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 2017 to 2021 are shown in bar charts.

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

[2] Altorf-van der Kuil W, Schoffelen AF, de Greeff SC, et al. (2017) National laboratory-based surveillance system for antimicrobial resistance: a successful tool to support the control of antimicrobial resistance in the Netherlands. Euro Surveill 22(46).

[3] Abbott IJ, Meletiadis J, Belghanch I, Wijma RA, Kanioura L, Roberts JA, et al. Fosfomycin efficacy and emergence of resistance among *Enterobacteriaceae* in an in vitro dynamic bladder infection model. J Antimicrob Chemother. 2018;73(3):709-19.

[4] Abbott IJ, van Gorp E, Wyres KL, Wallis SC, Roberts JA, Meletiadis J, et al. Oral fosfomycin activity against Klebsiella pneumoniae in a dynamic bladder infection in vitro model. J Antimicrob Chemother. 2022;77(5):1324-33.

[5] EUCAST 2019, *Warning against the use of gradient tests for benzylpenicillin MIC in Streptococcus pneumoniae*, accessed 16 March 2022, http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Warnings/Warnings_docs/Warning_-_gradient_for_benzyl_and_pnc_21nov2019.pdf.

[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 4.5, 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 4.5 (see section 4.1.1 for inclusion criteria). In table 4.1.2.1, characteristics of included isolates are listed by pathogen.

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 4.5, 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 2021

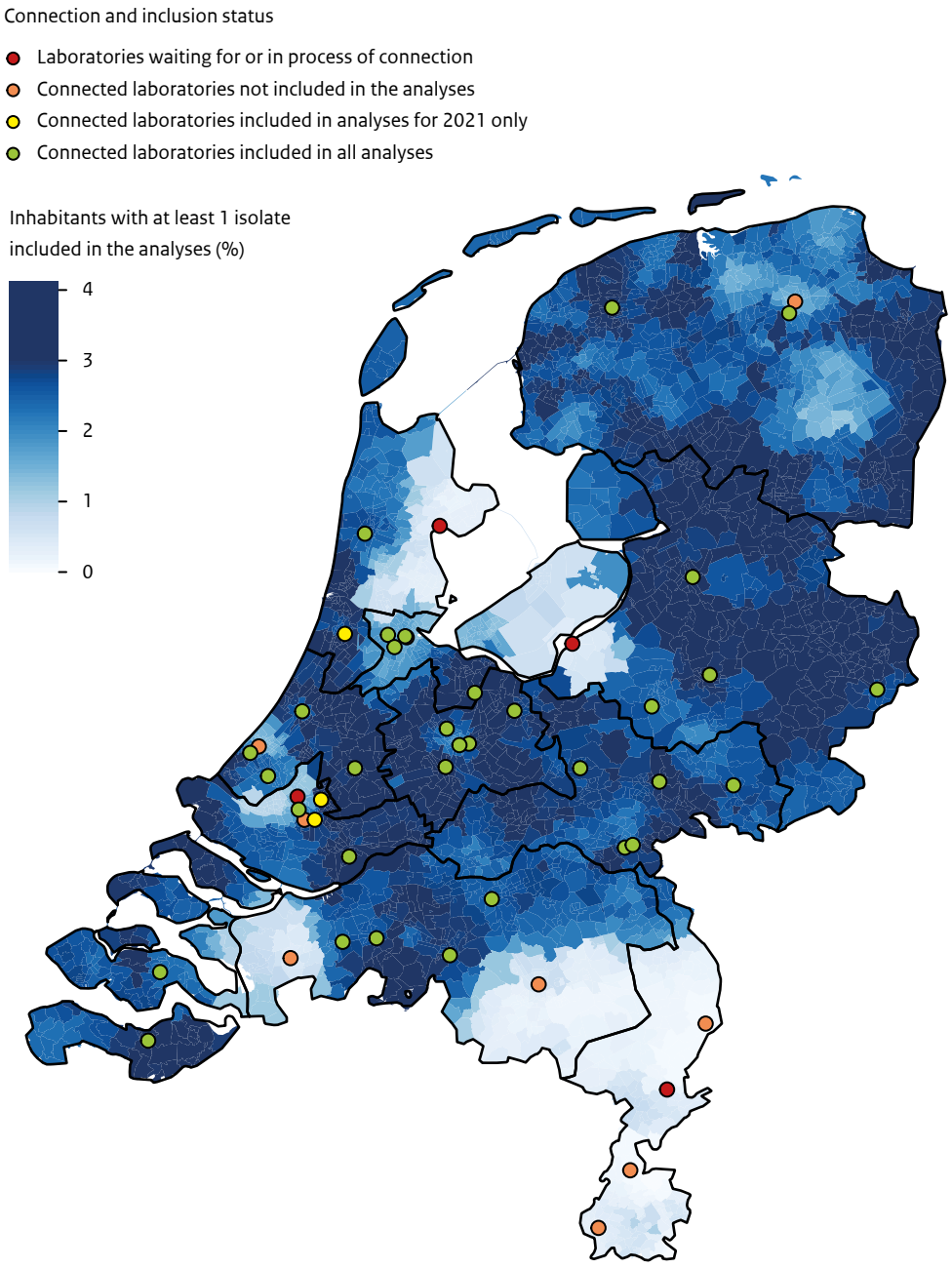


Table 4.1.2.1 Characteristics of 440,870 isolates, sampled in 2021, that were included in the analyses in sections 4.2 through 4.5, by pathogen

	E. coli	*K. pneumoniae*	*P. mirabilis*	*E. cloacae* complex	*P. aeruginosa*	*Acinetobacter* spp.	*E. faecalis*	*E. faecium*	*S. aureus*	CNS
Total number of isolates	185,174	31,203	22,707	11,865	24,481	4,353	31,056	7,041	55,283	25,497
Sex of patient (%)										
Male	27	33	42	55	53	53	54	56	55	60
Female	73	67	58	45	47	47	46	44	45	40
Setting of care (%)										
General practices	62	50	48	36	33	44	43	8	27	9
Outpatient departments	13	18	18	24	30	25	20	11	38	12
Inpatient departments (excl. Intensive Care Units)	18	22	20	32	27	22	27	54	27	59
Intensive Care Units	1	2	1	4	4	5	4	22	4	20
Long-term care facilities	6	9	13	5	7	4	6	4	4	1
Age category of patient in years (%)										
0-4	3	1	3	3	2	5	4	1	4	4
5-18	5	2	2	2	4	4	2	1	7	3
19-64	33	26	21	29	30	33	27	33	44	40
>65	58	71	74	65	64	58	67	65	45	53
Isolate source (%)										
Blood	3	3	2	4	2	4	4	15	5	52
Respiratory tract	1	3	2	8	16	9	0	2	16	0
Urine	90	86	83	59	45	60	83	49	15	13
Wound or Pus	4	5	11	23	32	21	10	26	53	25
Genital	1	0	0	0	1	0	0	0	3	0
Other	2	2	2	6	4	5	3	8	8	10
Type of hospital (hospital isolates only, %)										
General	34	32	36	30	29	28	32	24	30	27
Top clinical	52	51	51	50	49	49	53	54	51	49
University hospital	14	18	13	20	23	23	14	22	20	24

CNS = Coagulase-negative Staphylococcus spp., including S. epidermidis.
The first isolate per patient, per pathogen, per setting of care was selected.

Table 4.1.2.1 (Continued) Characteristics of 440,870 isolates, sampled in 2021, that were included in the analyses in sections 4.2 through 4.5, by pathogen

	β-haemolytic Streptococcus spp. group A	β-haemolytic Streptococcus spp. group B	β-haemolytic Streptococcus spp. group C	β-haemolytic Streptococcus spp. group G	*S. anginosus*	*S. mitis*/*S. oralis*	*B. fragilis* complex	*C. perfringens*	*S. pneumoniae*	*H. influenzae*	*M. catarrhalis*
Total number of isolates	2,123	21,193	1,355	1,842	2,164	991	1,609	341	3,054	5,937	1,601
Sex of patient (%)											
Male	43	22	53	53	50	56	56	57	56	53	51
Female	57	78	47	47	50	44	44	43	44	47	49
Setting of care (%)											
General practices	44	52	33	26	9	15	4	4	6	9	12
Outpatient departments	29	25	29	32	26	25	20	18	30	45	39
Inpatient departments (excl. Intensive Care Units)	25	20	34	38	57	55	69	70	56	40	41
Intensive Care Units	1	1	1	1	6	3	5	7	8	6	7
Long-term care facilities	1	3	3	2	2	2	1	2	0	1	1
Age category of patient in years (%)											
0-4	15	1	1	1	1	7	1	1	8	10	17
5-18	13	3	4	2	5	5	5	0	3	5	3
19-64	57	65	53	50	52	44	37	31	38	36	30
>65	16	30	42	47	42	44	57	68	51	49	50
Isolate source (%)											
Blood	5	2	7	10	11	31	25	28	27	2	1
Respiratory tract	10	1	5	4	2	3	0	0	59	85	87
Urine	9	55	18	19	20	28	1	3	1	0	0
Wound or Pus	48	11	47	50	60	34	65	61	11	10	12
Genital	21	27	14	13	3	0	1	2	0	2	0
Other	7	4	8	4	4	3	7	7	1	1	1
Type of hospital (hospital isolates only, %)											
General	32	33	29	32	34	20	30	27	31	27	25
Top clinical	53	53	56	48	50	53	48	56	54	53	55
University hospital	15	14	16	20	17	27	22	16	15	20	20

CNS = Coagulase-negative Staphylococcus spp., including S. epidermidis.
The first isolate per patient, per pathogen, per setting of care was selected.

Key results

Coverage

- Included laboratories were well distributed throughout most of the country, although the proportion of laboratories from which the data could be included in the analyses was relatively low in the regions 'Noord-Holland West', 'Noord-Holland Oost/ Flevoland', 'Noord-Brabant', and 'Limburgs infectiepreventie en antibioticaresistentie netwerk (LINK)'.
- The distribution of included laboratories was reflected in the geographical distribution of isolates. The coverage was relatively high in most regions except in the regions 'Noord-Holland West', 'Noord-Holland Oost/ Flevoland', 'Noord-Brabant', and 'Limburgs infectiepreventie en antibioticaresistentie netwerk (LINK), where the coverage was lower and less evenly distributed.

Isolate characteristics

- *E. coli* (73%), *K. pneumoniae* (67%), *P. mirabilis* (58%), and β-haemolytic *Streptococcus* spp. groups A (57%) and B (78%) were more often isolated from female patients than from male patients. *E. faecium* (56%), Coagulase-negative *Staphylococcus* spp. (60%), *S. mitis/S. oralis* (56%), *B. fragilis complex* (56%), *C. perfringens* (57%), and *S. pneumoniae* (56%) were more often isolated from males. For the other pathogens, the percentage was similar between male and female patients.
- *E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, *P. aeruginosa*, *Acinetobacter* spp., *E. faecalis*, *S. aureus*, β-haemolytic *Streptococcus* spp. groups A, B, C, G, *H. influenzae*, and *M. catarrhalis* were most often isolated from patients receiving outpatient care (general practices, outpatient hospital departments, and long term care facilities, combined 60%-81%, depending on the pathogen), whereas a large part of *E. faecium*, coagulase-negative *Staphylococcus* spp., *S. anginosus*, *S. mitis/S. oralis*, *B. fragilis complex*, *C. perfringens*, and *S. pneumoniae* was isolated from inpatients (combined 58%-79%, depending on the pathogen).
- For all included *Enterobacterales*, *P. aeruginosa*, *Acinetobacter* spp., *E. faecium*, *E. faecalis*, *B. fragilis complex*, and *C. perfringens*, the majority of isolates originated from patients of 65 years and older (57-74%, depending on the pathogen). For β-haemolytic *Streptococcus* spp. groups A, and B 57-65% of the isolates originated from patients aged 19-64 years.
- *E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, *P. aeruginosa*, *Acinetobacter* spp., *E. faecalis*, *E. faecium*, and β-haemolytic *Streptococcus* spp. group B were mainly isolated from urine (45-90%, depending on the pathogen), whereas *S. aureus*, β-haemolytic *Streptococcus* spp. groups A, C and G, *S. anginosus*, *B. fragilis complex*, and *C. perfringens* were mainly isolated from wound or pus (47-65%, depending on the pathogen). Coagulase-negative *Staphylococcus* spp. were mainly isolated from blood (52%), and *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* from the respiratory tract (59-87%).
- Depending on the pathogen, 13 to 27% of the isolates originated from university hospital patients.

4.2 Primary care

The distribution of pathogens in diagnostic urine, wound or pus, respiratory, and genital samples from general practitioners' (GP) patients in 2021 is presented in table 4.2.1. The resistance levels in 2021 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 2021 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*).

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 does not affect *Streptococcus* spp. 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 2021

	Urine		Wound or pus	Respiratory tract	Genital
Pathogen	Age≤12 N (%)	Age>12 N (%)	N (%)	N (%)	N (%)
E. coli	10,396 (72)	107,867 (54)	730 (4)	87 (3)	385 (7)
K. pneumoniae	279 (2)	15,655 (8)	238 (1)	51 (2)	41 (1)
P. mirabilis	694 (5)	10,076 (5)	595 (3)	31 (1)	43 (1)
Other *Enterobacterales*[1]	779 (5)	22,574 (11)	2,113 (10)	275 (10)	124 (2)
P. aeruginosa	218 (2)	5,076 (3)	3,096 (15)	207 (8)	74 (1)
Other non-fermenters[2]	177 (1)	2,728 (1)	693 (3)	202 (8)	9 (0)
Other Gram-negatives[3]	2 (0)	18 (0)	264 (1)	340 (13)	60 (1)
S. aureus	159 (1)	3,794 (2)	9,624 (48)	1,193 (45)	1,042 (20)
β-haemolytic *Streptococcus* spp. group A	63 (0)	70 (0)	469 (2)	86 (3)	383 (7)
β-haemolytic *Streptococcus* spp. group B	142 (1)	7,868 (4)	542 (3)	28 (1)	2,726 (51)
Other Gram-positives[4]	1,594 (11)	24,655 (12)	1,771 (9)	139 (5)	446 (8)

[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., Pantoea spp., Salmonella spp., Hafnia spp., Escherichia spp. (non-coli), Cronobacter spp.*

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

[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. dysgalactiae n.n.g., S. dysgalactiae subsp. equisimilis, β-haemolytic Streptococcus spp. group C, S. anginosus, β-haemolytic Streptococcus spp. group G, S. pneumoniae, S. mitis/S. oralis, C. perfringens.*

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 2021

	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	6	68	4	74	3	76	3	79
Antibiotic								
amoxicillin/ampicillin	31	34	-	-	17	20	-	-
co-amoxiclav - non-uuti	24	26	27 ↑	17	4	5	-	-
piperacillin-tazobactam	-	-	-	-	-	-	1	3
cefuroxime	4	7	5	11	1	1	-	-
cefotaxime/ceftriaxone - nonmen	2	3	3	3	0	0	-	-
ceftazidime	2	2	4	3	0	0	1	1
meropenem - nonmen	-	-	-	-	-	-	0	0
imipenem	-	-	-	-	-	-	0	5
ciprofloxacin	5	9	1 ↓	10 ↓	4	10	0	9
gentamicin	3	4	0	1	3	5	-	-
tobramycin	3	4	1	2	2	3	1	1
fosfomycin[1]	1	2	-	-	-	-	-	-
trimethoprim	18	20	8	15 ↓	26	31	-	-
co-trimoxazole	16	18	5	6 ↓	20	23	-	-
nitrofurantoin	0	2	-	-	-	-	-	-
Multidrug resistance								
HRMO[2]	3	4	3	4	1	3	0	1
multidrug resistance[3] - non-uuti	1	3	0	2	0	1	-	-

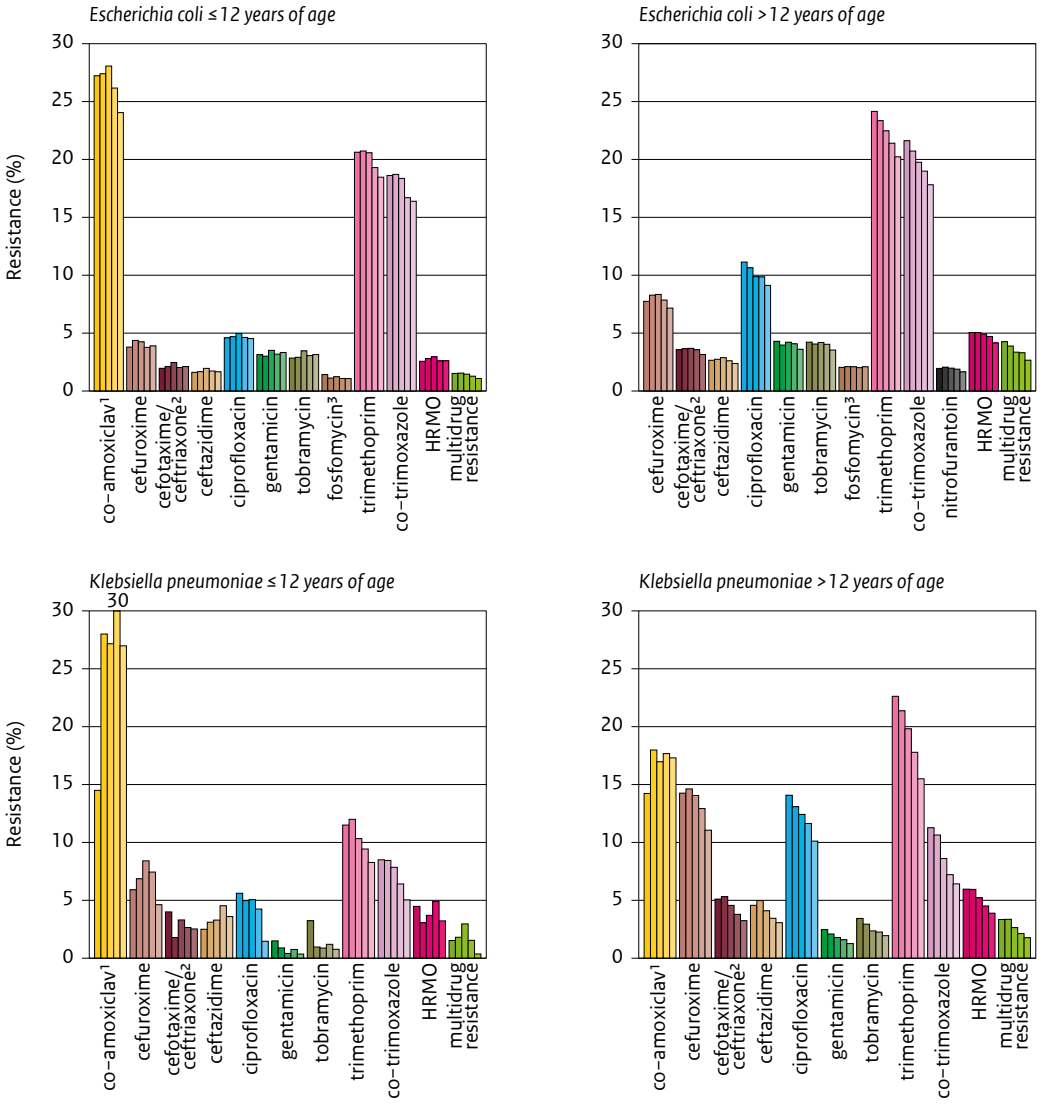
10 ↑	Significant and clinically relevant increasing trend since 2017.
10 ↓	Significant and clinically relevant decreasing trend since 2017.
10*	Trend not calculated because data from the years before 2021 did not meet the criteria for trend analysis.
10	No significant and clinically relevant time trend.

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

- = Resistance not calculated.
non-uuti = according to breakpoint for indications other than uncomplicated urinary tract infection.
nonmen = according to breakpoint for indications other than meningitis.

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.*
2 *Highly resistant microorganism (HRMO). For a definition of HRMO per species see section 4.1.1.*
3 *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.*

Figure 4.2.1 Trends in antibiotic resistance (from left to right 2017 to 2021) 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.

HRMO = highly resistant microorganism. For a definition of HRMO per species see section 4.1.1.

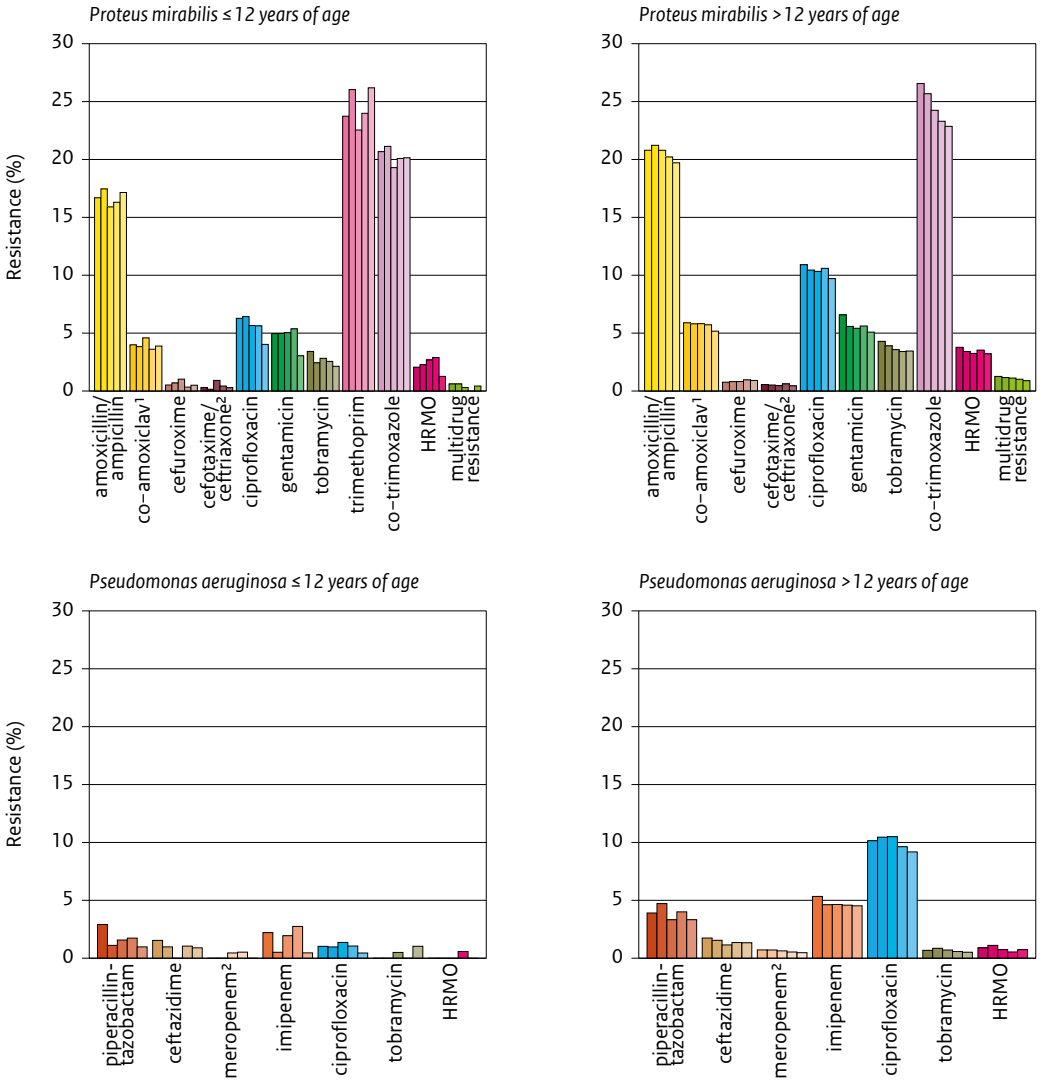
Multidrug resistance is defined as resistance to all following oral agents: co-amoxiclav (according to breakpoint for indications other than uncomplicated urinary tract infection), ciprofloxacin, and co-trimoxazole.

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 2017 to 2021) 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.

HRMO = highly resistant microorganism. For a definition of HRMO per species see section 4.1.1.

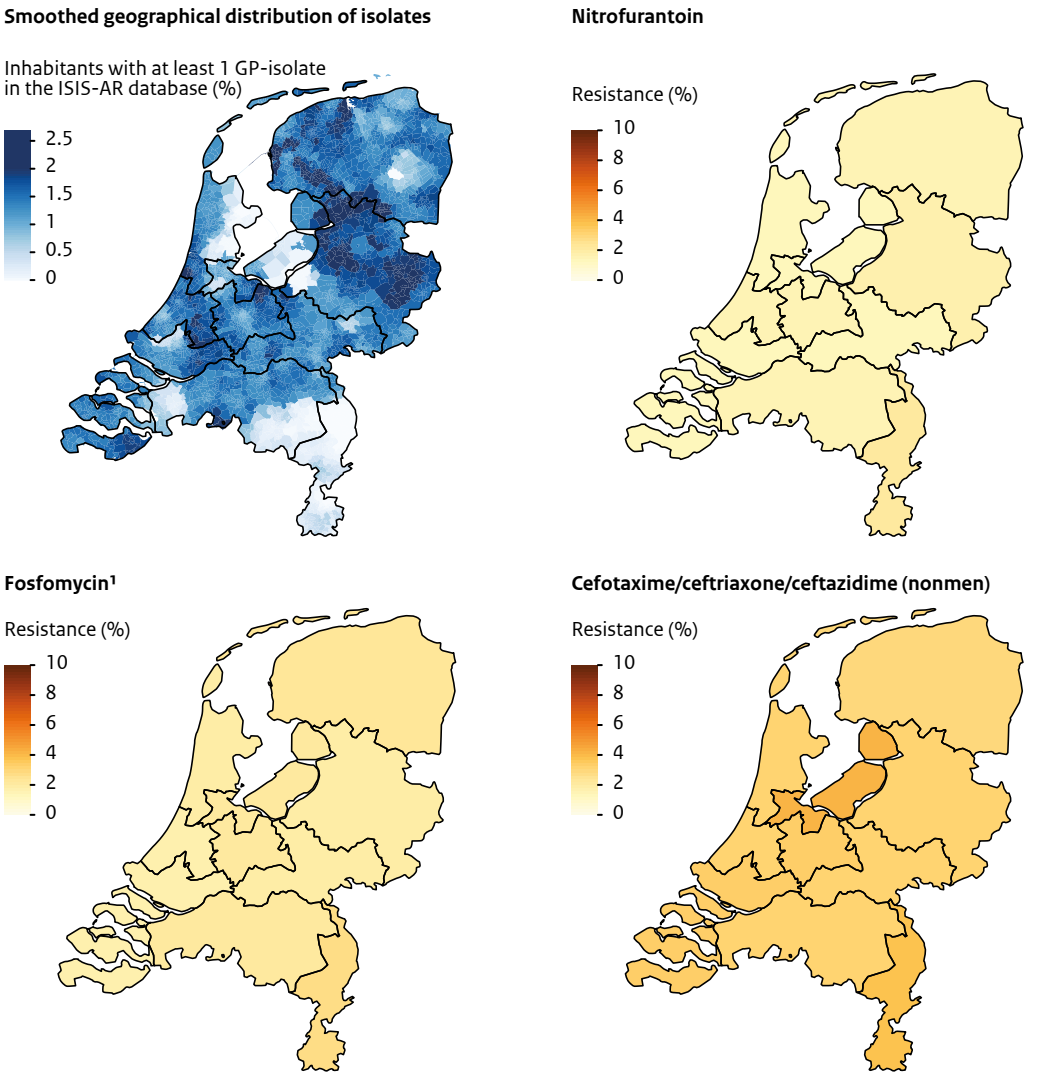
Multidrug resistance is defined as resistance to all following oral agents: co-amoxiclav (according to breakpoint for indications other than uncomplicated urinary tract infection), ciprofloxacin, and co-trimoxazole.

[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 2021



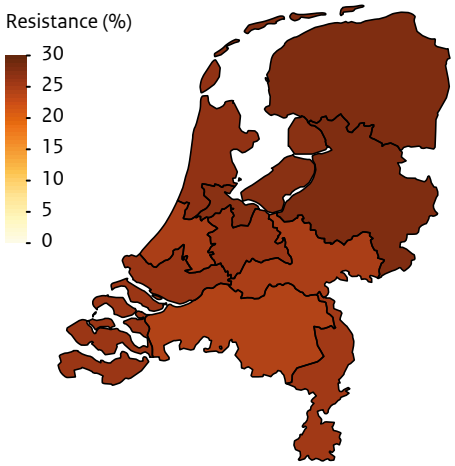
nonmen = according to breakpoint for indications other than meningitis.

Note: No statistically significant and clinically 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).

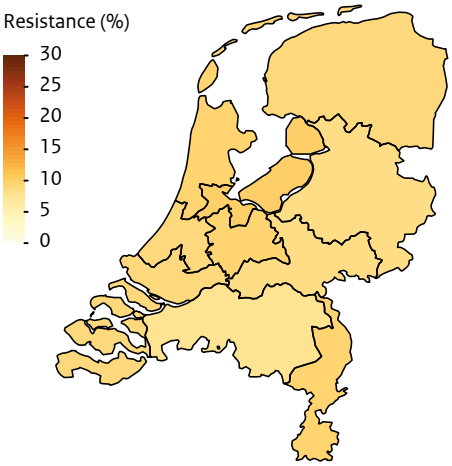
[1] *Resistance percentage calculated using an mic cut-off of 16 mg/L and a diameter cut-off of 24 mm. For more details see section 4.1.1.*

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 2021

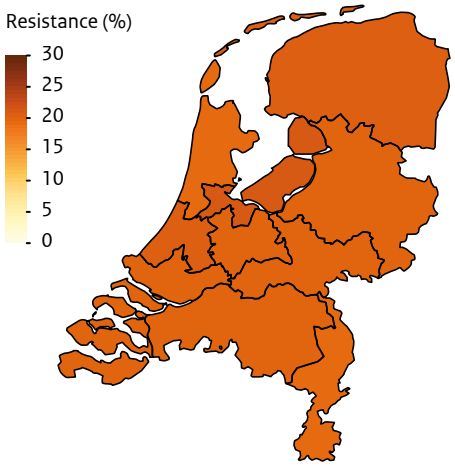
Co-amoxiclav (non-uuti)



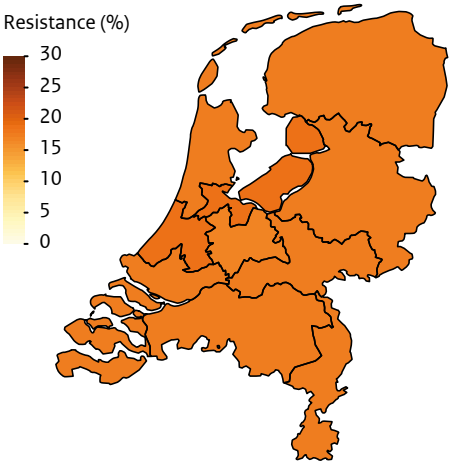
Ciprofloxacin



Trimethoprim



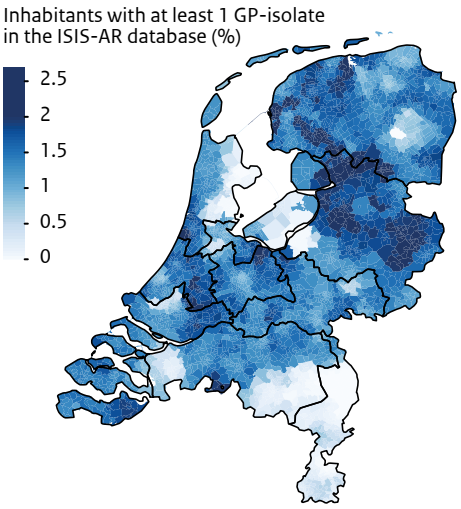
Co-trimoxazole



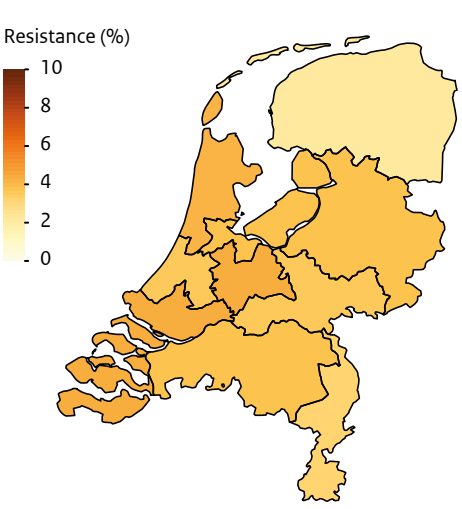
non-uuti = according to breakpoint for indications other than uncomplicated urinary tract infection.
Note: No statistically significant and clinically relevant differences of regional resistance levels were found for the selected antibiotics in comparison to all regions combined (for details see section 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 2021

Smoothed geographical distribution of isolates

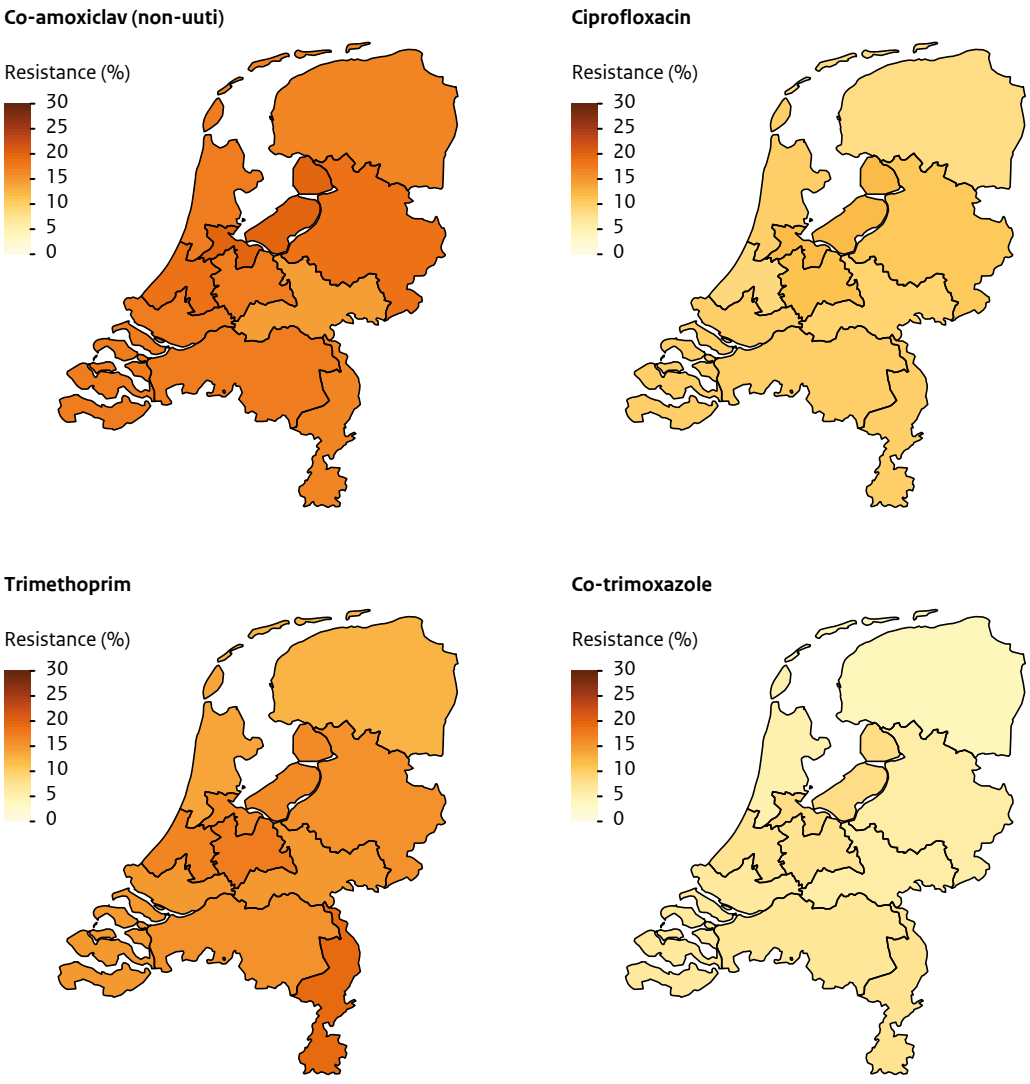


Cefotaxime/ceftriaxone/ceftazidime (nonmen)



nonmen = according to breakpoint for indications other than meningitis.
Note: No statistically significant and clinically 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 2021



non-uuti=according to breakpoint for indications other than uncomplicated urinary tract infection.
Note: No statistically significant and clinically 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 2021

	S. aureus
Antibiotic	
flucloxacillin[1]	3
ciprofloxacin[2]	3
erythromycin	14
clindamycin including inducible resistance[3]	12
doxycycline/tetracycline	4
fusidic acid	17
co-trimoxazole	2

10 ↑	Significant and clinically relevant increasing trend since 2017.
10 ↓	Significant and clinically relevant decreasing trend since 2017.
10*	Trend not calculated because data from the years before 2021 did not meet the criteria for trend analysis.
10	No significant and clinically relevant time trend.

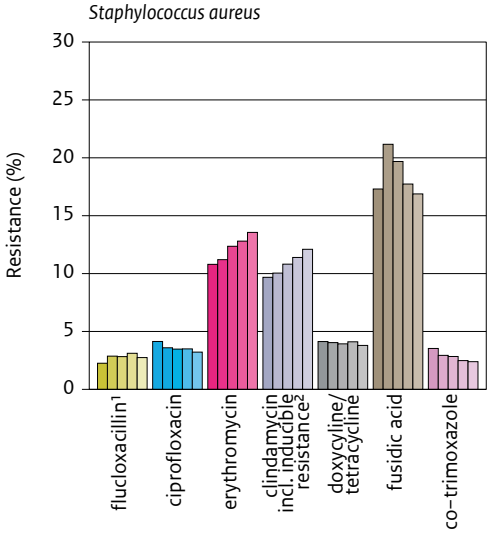
(For the criteria for trend analysis and the definition of a clinically relevant trend see section 4.1.1).

[1] *Resistance to flucloxacillin was estimated based on laboratory S/R interpretation for cefoxitin, or, if no cefoxitin test was available, for oxacillin/flucloxacillin (see section 4.1.1 for more detailed information).*

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

[3] *To estimate clindamycin resistance including inducible resistance, the laboratory S/I/R interpretation was used (see section 4.1.1 for more detailed information).*

Figure 4.2.4 Trends in antibiotic resistance (from left to right 2017 to 2021) among diagnostic wound or pus isolates of *S. aureus* from selected general practitioners' patients in ISIS-AR



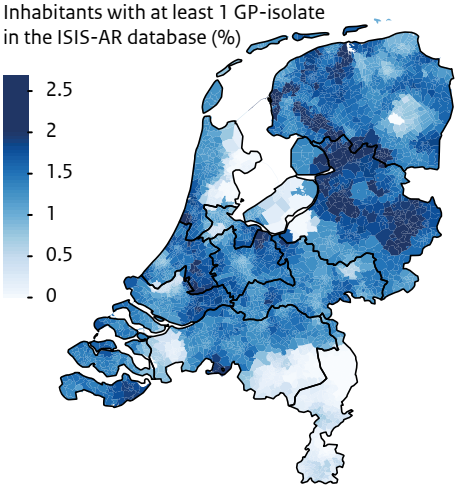
Only pathogen-agent combinations are shown for which the resistance levels were higher than 0.5% for at least one year and lower than 30% for at least three years.

[1] *Resistance to flucloxacillin was estimated based on laboratory S/R interpretation for cefoxitin, or, if no cefoxitin test was available, for oxacillin/flucloxacillin (see section 4.1.1 for more detailed information).*

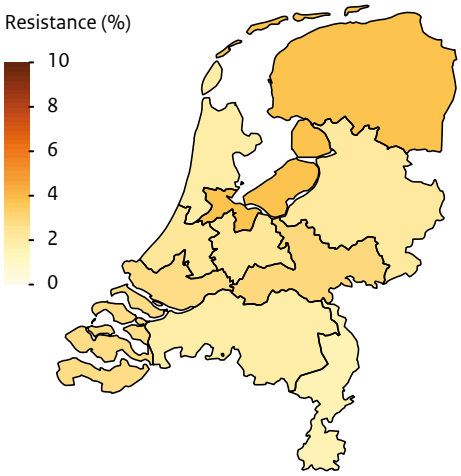
[2] *To estimate clindamycin resistance including inducible resistance, the laboratory S/I/R interpretation was used (see section 4.1.1 for more detailed information).*

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 flucloxacillin by regional cooperative network, ISIS-AR 2021

Smoothed geographical distribution of isolates



flucloxacillin[1]

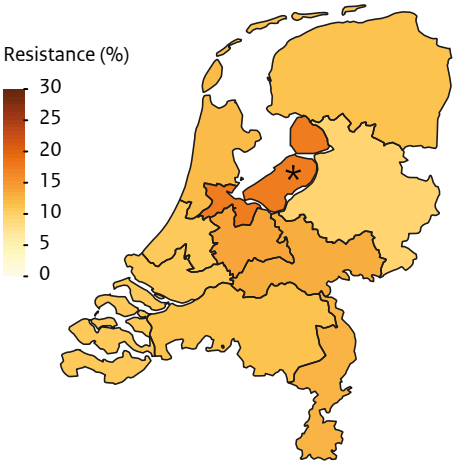


Note: No statistically significant and clinically relevant differences of regional resistance levels were found for the selected antibiotics in comparison to all regions combined (for details see section 4.1.1).

[1] *Resistance to flucloxacillin was estimated based on laboratory S/R interpretation for cefoxitin, or, if no cefoxitin test was available, for oxacillin/flucloxacillin (see section 4.1.1 for more detailed information)*

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 2021

Clindamycin incl. inducible resistance[1]



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

[1] *To estimate clindamycin resistance including inducible resistance, the laboratory S/R interpretation was used (see section 4.1.1 for more detailed information)*

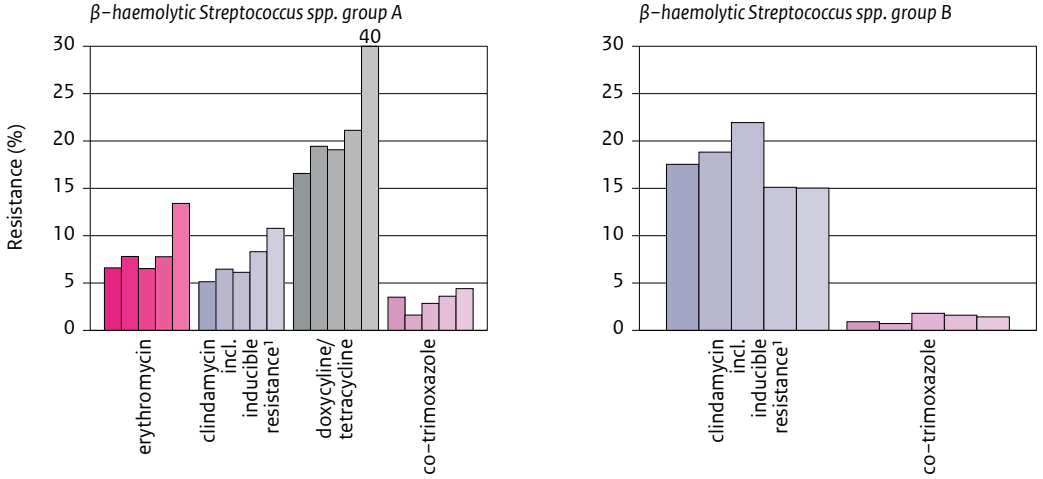
Table 4.2.4 Resistance levels (%) among diagnostic wound/pus, respiratory or genital isolates of β-haemolytic *Streptococcus* spp. group A and diagnostic urine or genital isolates of β-haemolytic *Streptococcus* spp. group B from selected general practitioners' patients, ISIS-AR 2021

	β-haemolytic *Streptococcus* spp. group A	β-haemolytic *Streptococcus* spp. group B
Antibiotic		
erythromycin	13 ↑	18*
clindamycin including inducible resistance[1]	11 ↑	15
doxycycline/tetracycline	40 ↑	75
co-trimoxazole	4	1

10 ↑	Significant and clinically relevant increasing trend since 2017.
10 ↓	Significant and clinically relevant decreasing trend since 2017.
10*	Trend not calculated because data from the years before 2021 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] *To estimate clindamycin resistance including inducible resistance, the laboratory S/I/R interpretation was used (see section 4.1.1 for more detailed information).*

Figure 4.2.6 Trends in antibiotic resistance (from left to right 2017 to 2021) 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.

[1] *To estimate clindamycin resistance including inducible resistance, the laboratory S/I/R interpretation was used (see section 4.1.1 for more detailed information).*

Key results

- The coverage of isolates from GP patients in the regional cooperative networks 'Noord-Holland West', 'Noord-Holland Oost/Flevoland', 'Noord-Brabant', and 'Limburgs infectiepreventie en antibioticaresistentie netwerk (LINK)' was low compared to other regional networks and regional resistance levels may be influenced by suboptimal representativeness.

Enterobacterales

- Resistance levels in selected GP patients aged >12 years were generally higher than in patients aged ≤12 years, except for **co-amoxiclav** (27% in patients ≤12 years old and 17% in patients >12 years old) in *K. pneumoniae*.
- For all *Enterobacterales*, resistance levels ≤10% were observed for **cefuroxime** (≤7%, except for *K. pneumoniae* in patients aged >12 years, 11%), **cefotaxime/ceftriaxone** (≤3%) **ceftazidime** (≤4%), **ciprofloxacin** (≤10%), **gentamicin** (≤5%), and **tobramycin** (≤4%). Additionally, resistance levels ≤10% were observed for **fosfomycin** (≤2%) and **nitrofurantoin** (≤2%) in *E. coli*; for **trimethoprim** (patients aged ≤ 12 years only, 8%) and **co-trimoxazole** (≤6%) in *K. pneumoniae*; and for **co-amoxiclav** (≤5%) in *P. mirabilis*.
- Resistance levels ≥20% were observed for **co-amoxiclav** (≥24%) in *E. coli* and *K. pneumoniae* (patients aged ≤12 years only); for **amoxicillin/ampicillin** (≥20%,) in *E. coli* and *P. mirabilis* (patients aged >12 years only) and **trimethoprim** (≥20%) in *E. coli* (patients aged >12 years only) and *P. mirabilis*; and for **co-trimoxazole** (≥20%) in *P. mirabilis*.
- There was a statistically significant and clinically relevant increase in resistance to **co-amoxiclav** (from 15% in 2017 to 27% in 2021) in *K. pneumoniae* in patients aged ≤12 years, especially in the first two years (from 15% in 2017 to 28% in 2018). Additionally, in *K. pneumoniae*, there was a statistically significant and clinically relevant decrease in resistance to **ciprofloxacin** (from 6% in 2017 to 1% in 2021 for patients aged ≤12 years and from 14% to 10% for patients aged >12 years), **trimethoprim** (from 23% to 15% for patients aged >12 years) and **co-trimoxazole** (from 11% to 6% for patients aged >12 years).
- The percentage of **HRMO** and **multidrug resistance** was ≤4% in all *Enterobacterales*.
- For *E. coli* and *K. pneumoniae*, no statistically significant and clinically relevant differences of regional resistance levels were found for the selected antibiotics in comparison to all regions combined.

***P. aeruginosa* (urine samples)**

- Resistance levels ≤10% were observed for **each of the selected agents** in both age groups (≤9%).
- For **none of the selected agents** a statistically significant and clinically relevant trend was observed.
- The percentage of **HRMO** was ≤1%.

***S. aureus* (wound or pus samples)**

- Resistance levels ≤10% were observed for **flucloxacillin** (3%), **ciprofloxacin** (3%), **doxycycline/ tetracycline** (4%), and **co-trimoxazole** (2%).
- For **none of the selected agents** a statistically significant and clinically relevant trend was observed.
- For *S. aureus*, a statistically significant and clinically relevant higher resistance percentage was found for **clindamycin incl. inducible resistance** in the regional cooperative network 'Noord-Holland Oost/Flevoland' (18% in the region versus 12% in all regions combined).

β-haemolytic *Streptococcus* spp. group A (wound/pus, respiratory, or genital samples) and group B (urine or genital samples)

- For both β-haemolytic *Streptococcus* spp. group A and group B, a resistance level of 10% or lower was observed for **co-trimoxazole** (≤4%).
- For both β-haemolytic *Streptococcus* spp. group A and group B, a resistance level of 20% or higher was observed for **doxycycline/tetracycline** (≥40%).
- There was a statistically significant and clinically relevant increase in resistance to **erythromycin** (from 7% in 2017 to 13% in 2021), **clindamycin including inducible resistance** (from 5% in 2017 to 11% in 2021), and **doxycycline/tetracycline** (from 17% in 2017 to 40% in 2021) in β-haemolytic *Streptococcus* spp. group A.

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 2021 is presented in table 4.3.1.1. The resistance levels for a selection of pathogens isolated from these patients in 2021 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 2021

	Lower respiratory tract	Urine	Wound or pus
Pathogen	**N (%)**	**N (%)**	**N (%)**
E. coli	363 (5)	18,645 (40)	1,988 (6)
K. pneumoniae	177 (2)	4,088 (9)	474 (2)
P. mirabilis	98 (1)	2,240 (5)	1,118 (4)
Other *Enterobacterales*[1]	716 (9)	6,709 (14)	3,552 (11)
P. aeruginosa	1,479 (18)	1,747 (4)	3,070 (10)
Other non-fermenters[2]	932 (12)	787 (2)	868 (3)
Other Gram-negatives[3]	2,006 (25)	23 (0)	773 (2)
S. aureus	1,569 (20)	1,703 (4)	12,927 (41)
Other Gram-positives[4]	681 (8)	10,399 (22)	6,622 (21)

[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., Salmonella spp., Escherichia spp. (non-coli), Cronobacter spp., Yersinia spp.*

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

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

[4] *In order of frequency: Enterococcus spp., ß-haemolytic Streptococcus spp. group B, S. anginosus, ß-haemolytic Streptococcus spp. group G, S. pneumoniae, ß-haemolytic Streptococcus spp. group C, ß-haemolytic Streptococcus spp. group A, S. mitis/S. oralis, S. dysgalactiae n.n.g., S. dysgalactiae subsp. equisimilis, 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 2021

	E. coli	*K. pneumoniae*	*P. mirabilis*	*P. aeruginosa*
Antibiotic				
amoxicillin/ampicillin	41	-	23	-
co-amoxiclav - non-uuti	31	19	7	-
piperacillin-tazobactam	4	14	0	5
cefuroxime	11	14	1	-
cefotaxime/ceftriaxone - nonmen	5	7 ↓	1	-
ceftazidime	4	6	0	3
meropenem/imipenem - nonmen	0	0	-	-
meropenem - nonmen	-	-	0	2
imipenem	-	-	-	5
ciprofloxacin	15	12	12	14
gentamicin	5	3	7	-
tobramycin	5	4	5	3
fosfomycin[1]	3	-	-	-
trimethoprim	25	19 ↓	31	-
co-trimoxazole	23	11 ↓	24	-
nitrofurantoin	2	-	-	-
Empiric therapy combinations				
gentamicin + co-amoxiclav - non-uuti	4	2	2	-
gentamicin + cefuroxime	2	2	0	-
gentamicin + cefotaxime/ceftriaxone - nonmen	1	2	0	-
ciprofloxacin + co-amoxiclav - non-uuti	9	5	2	-
ciprofloxacin + cefuroxime	5	8	1	-
ciprofloxacin + cefotaxime/ceftriaxone - nonmen	4	4	0	-
Multidrug resistance				
HRMO[2]	7	8 ↓	4	2
multidrug resistance[3]- non-uuti	5	3 ↓	1	-

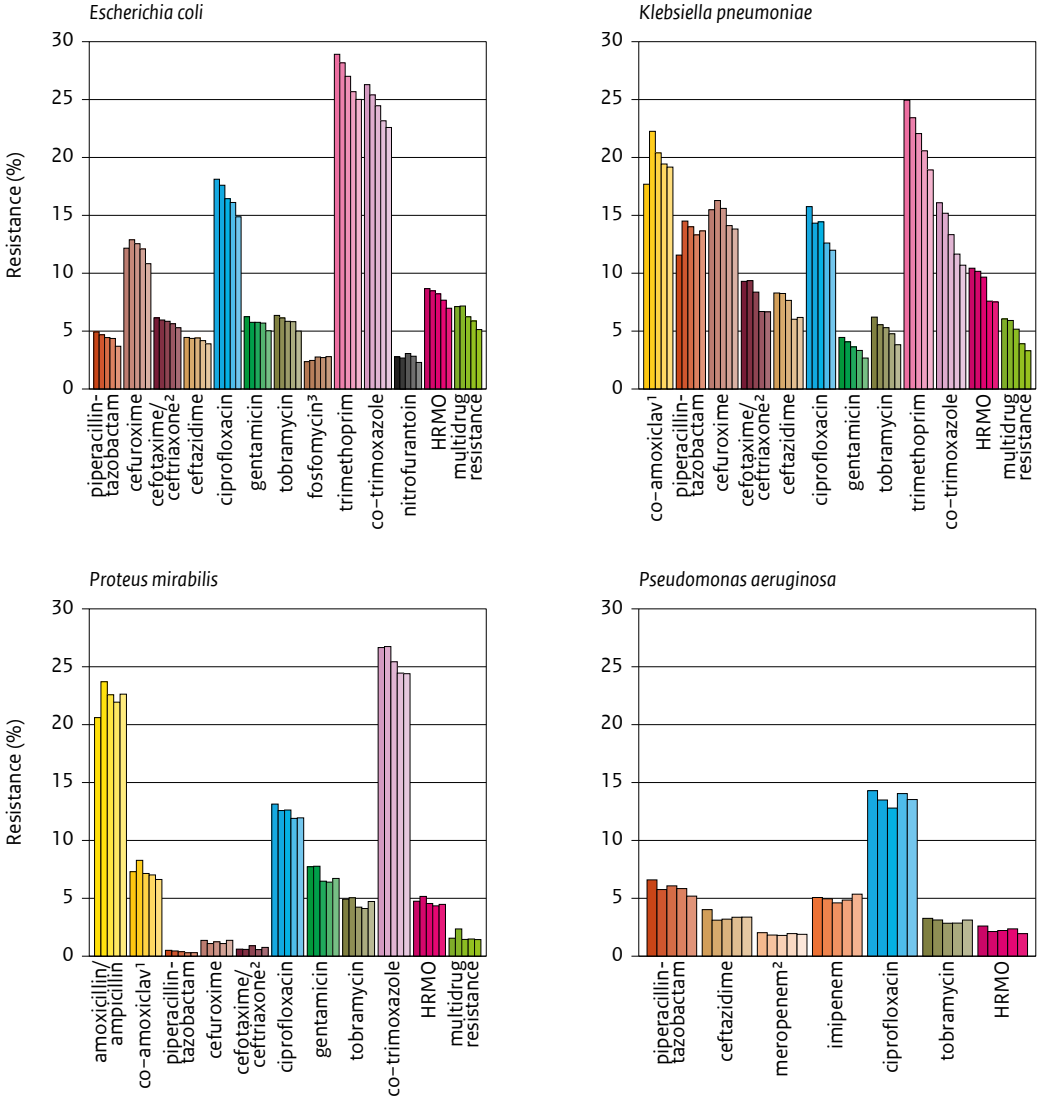
10 ↑	Significant and clinically relevant increasing trend since 2017.
10 ↓	Significant and clinically relevant decreasing trend since 2017.
10*	Trend not calculated because data from the years before 2021 did not meet the criteria for trend analysis.
10	No significant and clinically relevant time trend.

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

- = Resistance not calculated.
non-uuti = according to breakpoint for indications other than uncomplicated urinary tract infection.
nonmen = according to breakpoint for indications other than meningitis.

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.*
2 *Highly resistant microorganism (HRMO). For a definition of HRMO per species see section 4.1.1.*
3 *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.*

Figure 4.3.1.1 Trends in antibiotic resistance (from left to right 2017 to 2021) 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.

HRMO = highly resistant microorganism. For a definition of HRMO per species see section 4.1.1.

Multidrug resistance is 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.

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.*

Table 4.3.1.3 Resistance levels (%) among diagnostic isolates of *S. aureus* from patients attending outpatient departments, ISIS-AR 2021

	S. aureus
Antibiotic	
flucloxacillin[1]	2
ciprofloxacin[2]	4
gentamicin	1
erythromycin	16
clindamycin including inducible resistance[3]	14
doxycycline/tetracycline	4
fusidic acid	8
linezolid	0
co-trimoxazole	2
rifampicin	0

10 ↑	Significant and clinically relevant increasing trend since 2017.
10 ↓	Significant and clinically relevant decreasing trend since 2017.
10*	Trend not calculated because data from the years before 2021 did not meet the criteria for trend analysis.
10	No significant and clinically relevant time trend.

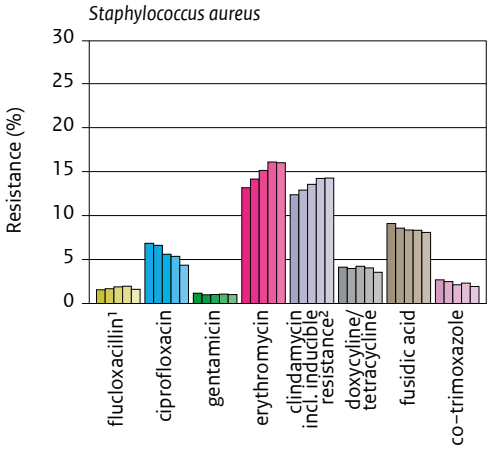
(For the criteria for trend analysis and the definition of a clinically relevant trend see section 4.1.1).

[1] *Resistance to flucloxacillin was estimated based on laboratory S/R interpretation for cefoxitin, or, if no cefoxitin test was available, for oxacillin/flucloxacillin (see section 4.1.1 for more detailed information).*

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

[3] *To estimate clindamycin resistance including inducible resistance, the laboratory S/I/R interpretation was used (see section 4.1.1 for more detailed information).*

Figure 4.3.1.2. Trends in antibiotic resistance (from left to right 2017 to 2021) 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.

[1] *Resistance to flucloxacillin was estimated based on laboratory S/R interpretation for cefoxitin, or, if no cefoxitin test was available, for oxacillin/flucloxacillin (see section 4.1.1 for more detailed information).*

[2] *To estimate clindamycin resistance including inducible resistance, the laboratory S/I/R interpretation was used (see section 4.1.1 for more detailed information).*

Key results

Enterobacterales

- For all *Enterobacterales*, resistance levels ≤10% were observed for **cefotaxime/ceftriaxone** (≤7%), **ceftazidime** (≤6%), **gentamicin** (≤7%), and **tobramycin** (≤5%). Resistance levels ≤10% were also observed for **meropenem/imipenem** (0%) in *E. coli* and *K. pneumoniae*; **piperacillin-tazobactam** (≤4%) in *E. coli* and *P. mirabilis*; **fosfomycin** (3%) and **nitrofurantoin** (2%) in *E. coli*; and **co-amoxiclav** (7%), **cefuroxime** (1%), and **meropenem/imipenem** (0%) in *P. mirabilis*.
- Resistance levels ≥20% were observed for **amoxicillin/ampicillin** (≥23%), **trimethoprim** (≥25%) and **co-trimoxazole** (≥23%) in *E. coli* and *P. mirabilis*; and for **co-amoxiclav** in *E. coli* (31%).
- A statistically significant and clinically relevant decrease in resistance was observed in *K. pneumoniae* for **cefotaxime/ceftriaxone** (according to the breakpoint for indications other than meningitis, from 9% to 7%), **trimethoprim** (from 25% to 19%), and **co-trimoxazole** (from 16% to 11%).
- For all *Enterobacterales* resistance levels ≤10% were observed for **each of the selected empiric therapy combinations**.
- For all *Enterobacterales*, the percentage **HRMO** was ≤8%, and the percentage of **multidrug resistance** was ≤5%. Furthermore, there was a statistically significant and clinically relevant decrease in resistance found in **HRMO** (from 10% in 2017 to 8% in 2021), and **multidrug resistance** (from 6% to 3%) in *K. pneumoniae*.

P. aeruginosa

- Resistance levels ≤10% were observed for **each of the selected agents** (≤5%), except for **ciprofloxacin** (14%).
- For **none of the selected agents** a statistically significant and clinically relevant trend was observed.
- The percentage of **HRMO** was 2%.

S. aureus

- Resistance levels ≤10% were observed for **each of the selected agents** (≤8%), except for **erythromycin** (16%) and **clindamycin including inducible resistance** (14%).
- For **none of the selected agents** a statistically significant and clinically relevant trend was observed.

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 2021 is presented in table 4.3.2.1. The resistance levels for a selection of pathogens isolated from these patients in 2021 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* and coagulase-negative *Staphylococcus* spp.), 4.3.2.5 (β-haemolytic *Streptococcus* spp. groups A, B, C, 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* and coagulase-negative *Staphylococcus* spp.), 4.3.2.4 (β-haemolytic *Streptococcus* spp. groups A, B, C, G, *S. anginosus*, and *S. mitis/S. oralis*), 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 2021

Pathogen	Blood or cerebrospinal fluid N (%)	Lower respiratory tract N (%)	Urine N (%)	Wound or pus N (%)
E. coli	4,182 (19)	735 (7)	19,312 (41)	3,466 (12)
K. pneumoniae	812 (4)	435 (4)	3,914 (8)	755 (3)
P. mirabilis	272 (1)	148 (1)	2,798 (6)	745 (3)
E. cloacae complex	345 (2)	366 (4)	1,247 (3)	1,227 (4)
Other *Enterobacterales*[1]	1,087 (5)	1,206 (12)	4,982 (11)	2,763 (9)
P. aeruginosa	451 (2)	1,335 (14)	2,429 (5)	1,643 (6)
Acinetobacter spp.	155 (1)	95 (1)	308 (1)	270 (1)
Other non-fermenters[2]	114 (1)	967 (10)	230 (0)	368 (1)
B. fragilis complex	309 (1)	0 (0)	12 (0)	624 (2)
Other Gram-negatives[3]	152 (1)	2,084 (21)	2 (0)	226 (1)
E. faecalis	673 (3)	21 (0)	5,033 (11)	1,731 (6)
E. faecium	439 (2)	19 (0)	1,667 (4)	1,252 (4)
S. aureus	2,149 (10)	1,602 (16)	1,477 (3)	7,390 (25)
CNS	8,645 (39)	12 (0)	468 (1)	3,618 (12)
β-haemolytic *Streptococcus* spp. group A	92 (0)	8 (0)	32 (0)	249 (1)
β-haemolytic *Streptococcus* spp. group B	324 (1)	74 (1)	1,227 (3)	662 (2)
β-haemolytic *Streptococcus* spp. group C	84 (0)	13 (0)	38 (0)	234 (1)
β-haemolytic *Streptococcus* spp. group G	163 (1)	17 (0)	70 (0)	311 (1)
S. anginosus	184 (1)	4 (0)	84 (0)	812 (3)
S. mitis/S. oralis	260 (1)	7 (0)	37 (0)	188 (1)
C. perfringens	70 (0)	0 (0)	0 (0)	126 (0)
Other Gram-positives[4]	1,197 (5)	729 (7)	1,878 (4)	949 (3)

CNS = Coagulase-negative Staphylococcus spp., including S. epidermidis.

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

[2] *In order of frequency: S. maltophilia, M. catarrhalis, 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: S. dysgalactiae n.n.g., S. dysgalactiae subsp. equisimilis, S. pneumoniae, A. urinae, Enterococcus spp. (non-faecalis, non-faecium), Staphylococcus spp. (non-aureus, non-CNS), 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 2021

	E. coli	*K. pneumoniae*	*P. mirabilis*	*E. cloacae* complex	*P. aeruginosa*	*Acinetobacter* spp.
Antibiotic						
amoxicillin/ampicillin	40	-	23	-	-	-
co-amoxiclav - non-uuti	31	21	6	-	-	-
piperacillin-tazobactam	4	16	0	-	6	-
cefuroxime	12	13	1	-	-	-
cefotaxime/ceftriaxone - nonmen	6	8	1	-	-	-
ceftazidime	4	8	0	-	4	-
meropenem/imipenem - nonmen	0	0	-	0	-	1 ↓
meropenem - nonmen	-	-	0	-	2	-
imipenem	-	-	-	-	5	-
ciprofloxacin	12	10	11	4	10	4
gentamicin	5	4	6	3	-	3
tobramycin	5	4 ↓	4	3	1	3
fosfomycin[1]	2	-	-	-	-	-
trimethoprim	23	15 ↓	31	7	-	-
co-trimoxazole	20	9 ↓	25	6	-	3
nitrofurantoin	1	-	-	-	-	-
Empiric therapy combinations						
gentamicin + co-amoxiclav -non-uuti	3	3	2	-	-	-
gentamicin + cefuroxime	2	3	0	-	-	-
gentamicin + cefotaxime/ceftriaxone - nonmen	1	3	0	-	-	-
tobramycin + ceftazidime	-	-	-	-	1	-
tobramycin + ciprofloxacin	-	-	-	-	1	-
ciprofloxacin + co-amoxiclav -non-uuti	7	6	2	-	-	-
ciprofloxacin + cefuroxime	5	7	0	-	-	-
ciprofloxacin + cefotaxime/ceftriaxone - nonmen	4	5	0	-	-	-
Multidrug resistance						
HRMO[2]	7	9	4	2	2	2
multidrug resistance[3] - non-uuti	4	4	1	-	-	-

10 ↑	Significant and clinically relevant increasing trend since 2017.
10 ↓	Significant and clinically relevant decreasing trend since 2017.
10*	Trend not calculated because data from the years before 2021 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.

non-uuti = according to breakpoint for indications other than uncomplicated urinary tract infection.

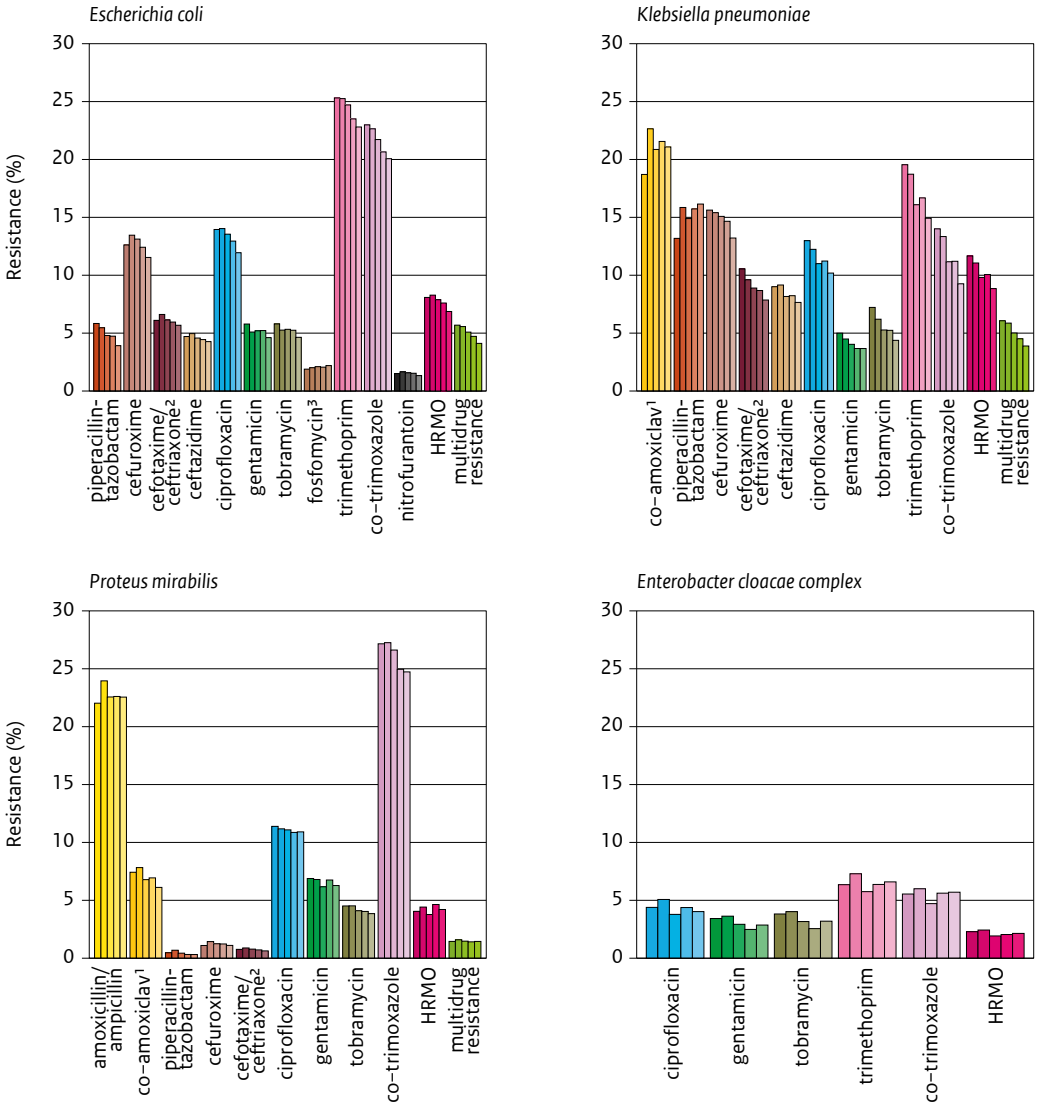
nonmen = according to breakpoint for indications other than meningitis.

[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.*

[2] *Highly resistant microorganism (HRMO). For a definition of HRMO per species see section 4.1.1.*

[3] *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.*

Figure 4.3.2.1 Trends in antibiotic resistance (from left to right 2017 to 2021) 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.

HRMO = highly resistant microorganism. For a definition of HRMO per species see section 4.1.1.

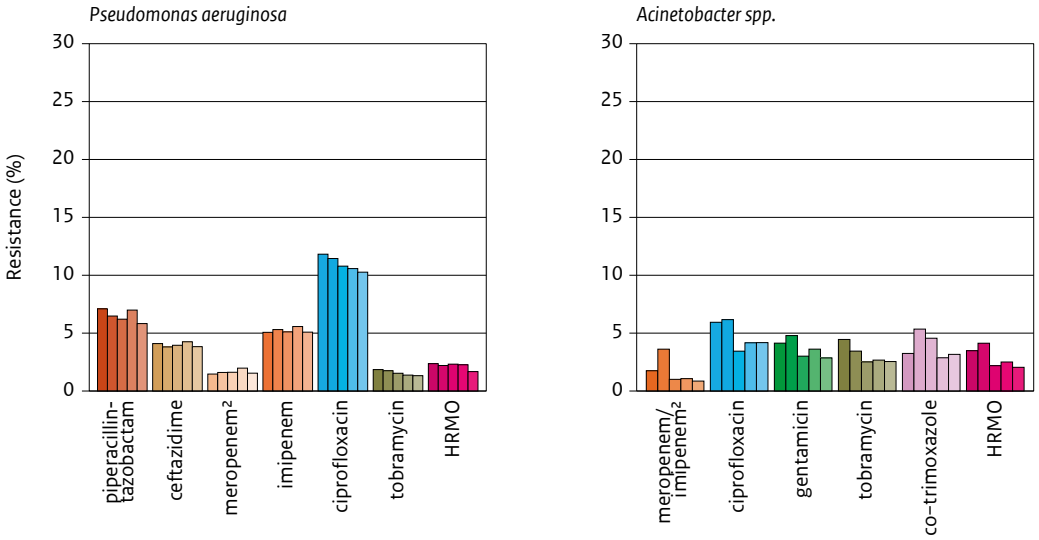
Multidrug resistance is defined as resistance to all following oral agents: co-amoxiclav (according to breakpoint for indications other than uncomplicated urinary tract infection), ciprofloxacin, and co-trimoxazole.

[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 4.3.2.1 (continued) Trends in antibiotic resistance (from left to right 2017 to 2021) 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.
HRMO = highly resistant microorganism. For a definition of HRMO per species see section 4.1.1.
Multidrug resistance is 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.
[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.*

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 2021

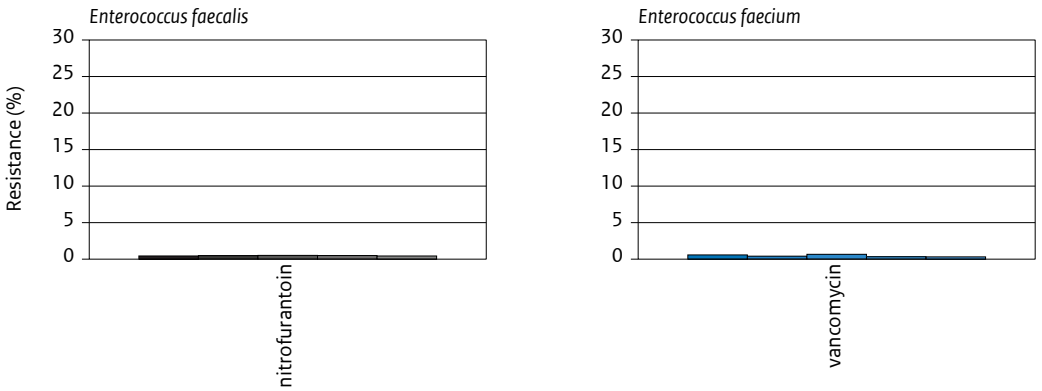
	E. faecalis	*E. faecium*
Antibiotic		
amoxicillin/ampicillin	-	87
vancomycin	0	0
linezolid	-	0
nitrofurantoin	0	-

10 ↑	Significant and clinically relevant increasing trend since 2017.
10 ↓	Significant and clinically relevant decreasing trend since 2017.
10*	Trend not calculated because data from the years before 2021 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 2017 to 2021) 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.

Table 4.3.2.4 Resistance levels (%) among diagnostic isolates of *S. aureus* and coagulase-negative *Staphylococcus* spp. from patients admitted to inpatient departments (excl. intensive care units), ISIS-AR 2021

	S. aureus	CNS
Antibiotic		
flucloxacillin[1]	2	40
ciprofloxacin[2]	5	27
gentamicin	1	25
erythromycin	15	41
clindamycin including inducible resistance[3]	13	29
doxycycline/tetracycline	3	15
fusidic acid	6	41
linezolid	0	0
co-trimoxazole	2	15
rifampicin	0	6 ↑

10 ↑	Significant and clinically relevant increasing trend since 2017.
10 ↓	Significant and clinically relevant decreasing trend since 2017.
10*	Trend not calculated because data from the years before 2021 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).

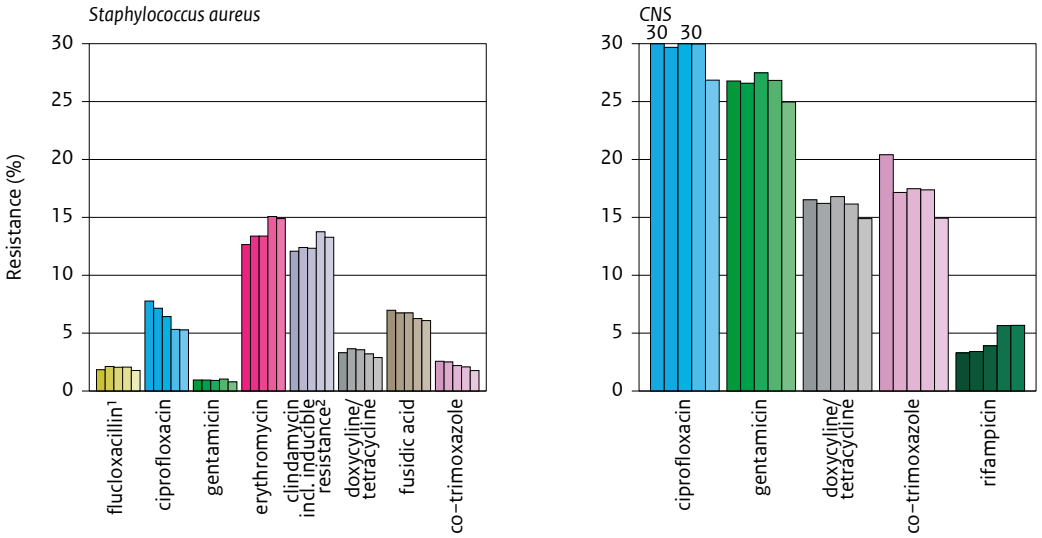
CNS = Coagulase-negative Staphylococcus spp., including S. epidermidis.

1 *Resistance to flucloxacillin was estimated based on laboratory S/R interpretation for cefoxitin, or, if no cefoxitin test was available, for oxacillin/flucloxacillin. Due to breakpoint changes in 2017, no test for trend could be conducted for CNS (see section 4.1.1 for more detailed information).*

2 *Resistance to ciprofloxacin is intended to be a class indicator for resistance to fluoroquinolones.*

3 *To estimate clindamycin resistance including inducible resistance, the laboratory S/I/R interpretation was used (see section 4.1.1 for more detailed information).*

Figure 4.3.2.3 Trends in antibiotic resistance (from left to right 2017 to 2021) among diagnostic isolates of *S. aureus* and coagulase-negative *Staphylococcus* spp. from patients admitted to inpatient departments (excl. intensive care units) in ISIS-AR



CNS=Coagulase-negative Staphylococcus spp., including S. epidermidis.

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.

[1] *Resistance to flucloxacillin was estimated based on laboratory S/R interpretation for cefoxitin, or, if no cefoxitin test was available, for oxacillin/flucloxacillin (see section 4.1.1 for more detailed information).*

[2] *To estimate clindamycin resistance including inducible resistance, the laboratory S/I/R interpretation was used (see section 4.1.1 for more detailed information).*

Table 4.3.2.5 Resistance levels (%) among diagnostic isolates of β-haemolytic *Streptococcus* spp. groups A,B,C,G, *S. anginosus*, and *S. mitis/S. oralis* from patients admitted to inpatient departments (excl. intensive care units), ISIS-AR 2021

	β-haemolytic *Streptococcus* spp. group A	β-haemolytic *Streptococcus* spp. group B	β-haemolytic *Streptococcus* spp. group C	β-haemolytic *Streptococcus* spp. group G	*S. anginosus*	*S. mitis/ S. oralis*
Antibiotic						
(benzyl)penicillin	-	-	-	-	0	5
(benzyl)penicillin (I)[1]	-	-	-	-	0	8
amoxicillin/ampicillin	-	-	-	-	-	8*
erythromycin	11 ↑	20*	5	18 ↑	-	-
clindamycin including inducible resistance[2]	9 ↑	17 ↓	8 ↑	15 ↑	7	7
doxycycline/tetracycline	31*	75*	-	33*	-	-
co-trimoxazole	3*	1	-	0	-	-

10 ↑	Significant and clinically relevant increasing trend since 2017.
10 ↓	Significant and clinically relevant decreasing trend since 2017.
10*	Trend not calculated because data from the years before 2021 did not meet the criteria for trend analysis.
10	No significant and clinically relevant time trend.

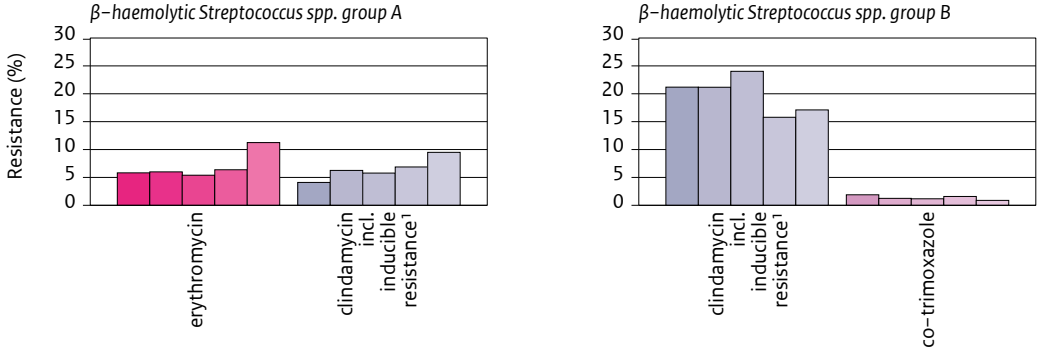
(For the criteria for trend analysis and the definition of a clinically relevant trend see section 4.1.1).

- = Resistance not calculated.

[1] *I is defined as susceptible, increased exposure, according to EUCAST definitions (https://www.eucast.org/newsiandr).*

[2] *To estimate clindamycin resistance including inducible resistance, the laboratory S/I/R interpretation was used (see section 4.1.1 for more detailed information).*

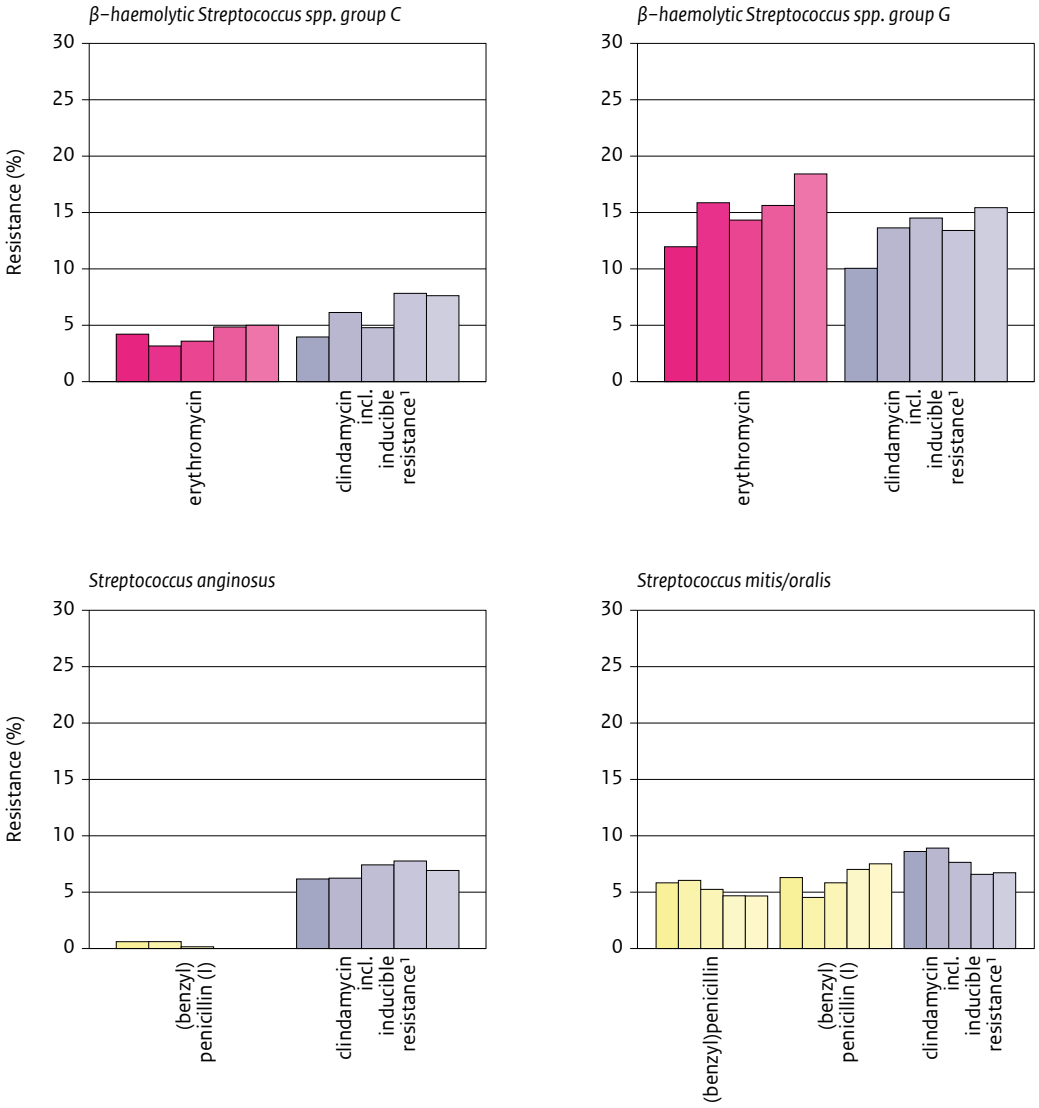
Figure 4.3.2.4 Trends in antibiotic resistance (from left to right 2017 to 2021) among diagnostic isolates of β-haemolytic *Streptococcus* spp. groups A,B,C,G, *S. anginosus*, and *S. mitis/S. oralis* from patients admitted to inpatient departments (excl. intensive care units) in ISIS-AR



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.

[1] *To estimate clindamycin resistance including inducible resistance, the laboratory S/I/R interpretation was used (see section 4.1.1 for more detailed information).*

Figure 4.3.2.4 (continued) Trends in antibiotic resistance (from left to right 2017 to 2021) among diagnostic isolates of β-haemolytic *Streptococcus* spp. groups A,B,C,G, *S. anginosus*, and *S. mitis/S. oralis* 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.

[1] *To estimate clindamycin resistance including inducible resistance, the laboratory S/I/R interpretation was used (see section 4.1.1 for more detailed information).*

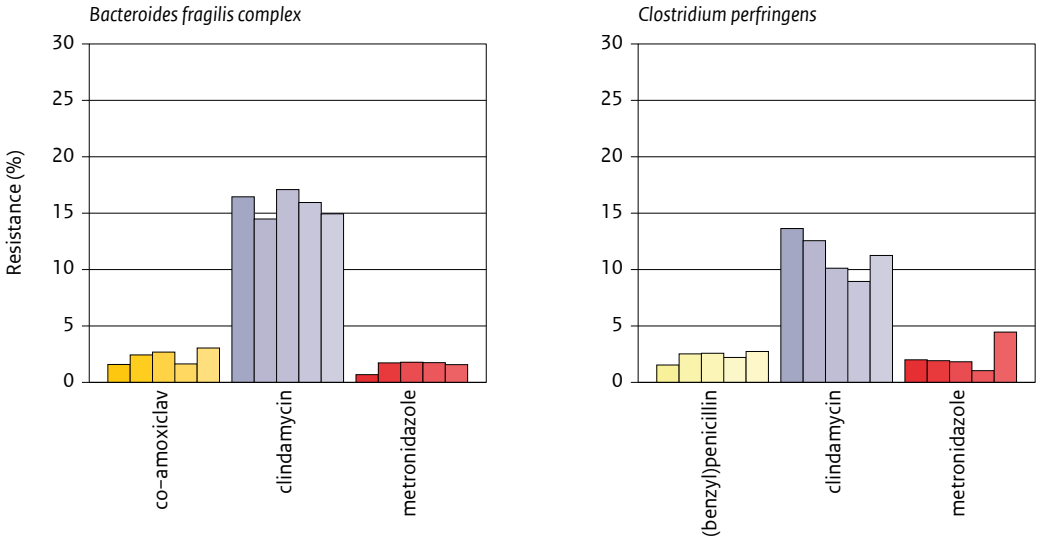
Table 4.3.2.6 Resistance levels (%) among diagnostic isolates of *B. fragilis* complex and *C. perfringens* from patients admitted to inpatient departments (excl. intensive care units), ISIS-AR 2021

	B. fragilis complex	*C. perfringens*
Antibiotic		
(benzyl)penicillin	-	3
co-amoxiclav	3	0
clindamycin	15	11
metronidazole	2	4

10 ↑	Significant and clinically relevant increasing trend since 2017.
10 ↓	Significant and clinically relevant decreasing trend since 2017.
10*	Trend not calculated because data from the years before 2021 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 2017 to 2021) 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.

Key results

Enterobacterales

- In all *Enterobacterales*, resistance was ≤10% for **cefotaxime/ceftriaxone** (≤8%), **ceftazidime** (≤8%), **gentamicin** (≤6%), and **tobramycin** (≤5%). Resistance was also ≤10% for **meropenem/imipenem** in *E. coli*, *K. pneumoniae* and *E. cloacae* complex (0%), **piperacillin-tazobactam** in *E. coli* and *P. mirabilis* (≤4%); for **ciprofloxacin** (≤10%) and **co-trimoxazole** (≤9%) in *K. pneumoniae* and *E. cloacae* complex; for **fosfomycin** (1%) and **nitrofurantoin** (1%) in *E. coli*; for **co-amoxiclav** (6%), **cefuroxime** (1%), and **meropenem** (0%) in *P. mirabilis*; and for **trimethoprim** in *E. cloacae* complex (7%).
- Resistance was ≥20% for **amoxicillin/ampicillin** (≥23%), **trimethoprim** (≥23%), and **co-trimoxazole** (≥20%) in *E. coli* and *P. mirabilis*; and for **co-amoxiclav** in *E. coli* and *K. pneumoniae* (≥21%).
- A statistically significant and clinically relevant decrease in resistance was observed for **tobramycin** (from 7% in 2017 to 4% in 2021), **trimethoprim** (from 20% to 15%) and **co-trimoxazole** (from 14% to 9%) in *K. pneumoniae*.
- Resistance was ≤7% for **empiric therapy combinations** in all *Enterobacterales*.
- The percentage **HRMO** and **multidrug resistance** was ≤9% in all *Enterobacterales*.

P. aeruginosa

- Resistance was ≤10% for **each of the selected agents**.
- For **none of the selected agents** a statistically significant and clinically relevant trend was observed.
- Resistance was 1% for **empiric therapy combinations**.
- The percentage **HRMO** was 2%.

***Acinetobacter* spp.**

- Resistance was ≤10% for **each of the selected agents** (≤4%).
- A statistically significant and clinically relevant decrease in resistance was observed for **meropenem/imipenem** according to the breakpoint for indications other than meningitis (from 2% in 2017 to 1% in 2021).
- The percentage **HRMO** was 2%.

E. faecalis* and *E. faecium

- Resistance was ≤10% for **vancomycin** (0% in both pathogens); for **nitrofurantoin** in *E. faecalis* (1%); and for **linezolid** in *E. faecium* (0%).
- Resistance was ≥20% for **amoxicillin/ampicillin** in *E. faecium* (87%).
- For **none of the selected agents** a statistically significant and clinically relevant trend was observed.

S. aureus

- Resistance was ≤10% for **each of the selected agents** (≤6%), except for **erythromycin** (15%) and **clindamycin including inducible resistance** (13%).
- For **none of the selected agents** a statistically significant and clinically relevant trend was observed.

Coagulase-negative *Staphylococcus* spp.

- Resistance was ≥20% for **each of the selected agents** (≥25%), except for **doxycycline/tetracycline** (15%), **linezolid** (0%), **co-trimoxazole** (15%) and **rifampicin** (5%).
- A statistically significant and clinically relevant increase in resistance was observed for **rifampicin** (from 3% in 2017 to 6% in 2021).

β-haemolytic *Streptococcus* spp. groups A, B, C, G

- Resistance levels ≤10% were observed for **co-trimoxazole** (≤3%) in β-haemolytic *Streptococcus* spp. groups A. B and G. In addition, resistance was ≤10% for **clindamycin including inducible resistance** in β-haemolytic *Streptococcus* spp. groups A and C (≤9%); and for **erythromycin** in β-haemolytic *Streptococcus* spp. Group C (5%).
- Resistance levels ≥20% were observed for **doxycycline/tetracycline** in β-haemolytic *Streptococcus* spp. groups A, B and G (≥31%) and for **erythromycin** in ß-haemolytic *Streptococcus* spp. Group B (20%).
- A statistically significant and clinically relevant increase in resistance was observed for **clindamycin including inducible resistance** in β-haemolytic *Streptococcus* spp. group A (from 4% in 2017 to 9% in 2021), group C (from 4% to 8%) and group G (from 10% to 15%); and for **erythromycin** in β-haemolytic *Streptococcus* spp. group A (from 6% to 11%) and group G (from 12% to 18%). A statistically significant and clinically relevant decrease was found for **clindamycin including inducible resistance** in β-haemolytic *Streptococcus* spp. group B (from 21% to 17%).

S. anginosus* and *S. mitis/S. oralis

- Resistance levels ≤10% were observed for **each of the selected agents** (≤8%). The percentage I for (benzyl)penicillin was 0% in *S. anginosus* and 8% in *S. mitis/S. oralis*.
- For **none of the selected agents** a statistically significant and clinically relevant trend was observed.

B. fragilis* complex and *C. perfringens

- Resistance was ≤10% for **each of the selected agents** (≤4%), except for clindamycin in both *B. fragilis complex* (15%) and *C. perfringens* (11%).
- For **none of the selected agents** a statistically significant and clinically relevant trend was observed.

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 2021 is presented in table 4.3.3.1. The resistance levels for a selection of pathogens isolated from these patients in 2021 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*), and 4.3.3.4 (*S. aureus* and coagulase-negative *Staphylococcus* spp.). 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.) and 4.3.3.2 (*S. aureus* and coagulase-negative *Staphylococcus* spp.). For *E. faecium* and *E. faecalis* trends, and for β-haemolytic *Streptococcus* spp. groups A, B, C, G, *S. anginosus*, *S. mitis/S. oralis*, *B. fragilis* complex, and *C. perfringens*, resistance levels and trends were not calculated because in 2021 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 2021

Pathogen	Blood or cerebrospinal fluid N (%)	Lower respiratory tract N (%)	Urine N (%)	Wound or pus N (%)
E. coli	169 (3)	397 (8)	473 (35)	300 (14)
K. pneumoniae	45 (1)	248 (5)	80 (6)	72 (3)
P. mirabilis	14 (0)	74 (1)	55 (4)	29 (1)
E. cloacae complex	40 (1)	247 (5)	27 (2)	87 (4)
Other *Enterobacterales*[1]	87 (2)	891 (18)	112 (8)	186 (9)
P. aeruginosa	63 (1)	363 (7)	84 (6)	115 (5)
Acinetobacter spp.	17 (0)	125 (2)	2 (0)	14 (1)
Other non-fermenters[2]	7 (0)	464 (9)	2 (0)	29 (1)
Other Gram-negatives[3]	35 (1)	339 (7)	1 (0)	58 (3)
E. faecalis	320 (6)	63 (1)	214 (16)	222 (10)
E. faecium	588 (11)	76 (1)	211 (16)	321 (15)
S. aureus	219 (4)	1,439 (28)	28 (2)	237 (11)
CNS	3,402 (66)	44 (1)	17 (1)	291 (13)
Other Gram-positives[4]	135 (3)	308 (6)	54 (4)	222 (10)

CNS = Coagulase-negative Staphylococcus spp., including S. epidermidis.

[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), Providencia spp., Pantoea spp., Yersinia spp., Salmonella spp., Escherichia spp. (non-coli), Cronobacter spp.*

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

[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, ß-haemolytic Streptococcus spp. group A, ß-haemolytic Streptococcus spp. group G, S. mitis/S. oralis, S. dysgalactiae n.n.g., S. anginosus, ß-haemolytic Streptococcus spp. group C, S. dysgalactiae subsp. equisimilis, Enterococcus spp. (non-faecalis, non-faecium), A. urinae, Staphylococcus spp. (non-aureus, non-CNS), 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 2021

	E. coli	*K. pneumoniae*	*P. mirabilis*	*E. cloacae* complex	*P. aeruginosa*	*Acinetobacter* spp.
Antibiotic						
amoxicillin/ampicillin	43	-	28	-	-	-
co-amoxiclav - non-uuti	34	25	9	-	-	-
piperacillin-tazobactam	6	17	1	-	13	-
cefuroxime	18	20	1	-	-	-
cefotaxime/ceftriaxone - nonmen	11	16	1	-	-	-
ceftazidime	8	15	1	-	8	-
meropenem/imipenem - nonmen	0	1	-	1	-	7
meropenem - nonmen	-	-	0	-	2	-
imipenem	-	-	-	-	7	-
ciprofloxacin	14	11	15	6	11	11
gentamicin	6	8	6	12	-	9
tobramycin	7	9	6	11	4	6
co-trimoxazole	21	13	28	9	-	12
Empiric therapy combinations						
gentamicin + co-amoxiclav - non-uuti	5	7	3	-	-	-
gentamicin + cefuroxime	4	8	1	-	-	-
gentamicin + cefotaxime/ceftriaxone - nonmen	3	8	1	-	-	-
tobramycin + ceftazidime	-	-	-	-	1	-
tobramycin + ciprofloxacin	-	-	-	-	3	-
ciprofloxacin + co-amoxiclav - non-uuti	9	7	4 ↑	-	-	-
ciprofloxacin + cefuroxime	8	9	1	-	-	-
ciprofloxacin + cefotaxime/ceftriaxone - nonmen	6	8	1	-	-	-
Multidrug resistance						
HRMO[1]	11	17	3	5	4	8
multidrug resistance[2] - non-uuti	6	6	3	-	-	-

10 ↑	Significant and clinically relevant increasing trend since 2017.
10 ↓	Significant and clinically relevant decreasing trend since 2017.
10*	Trend not calculated because data from the years before 2021 did not meet the criteria for trend analysis.
10	No significant and clinically relevant time trend.

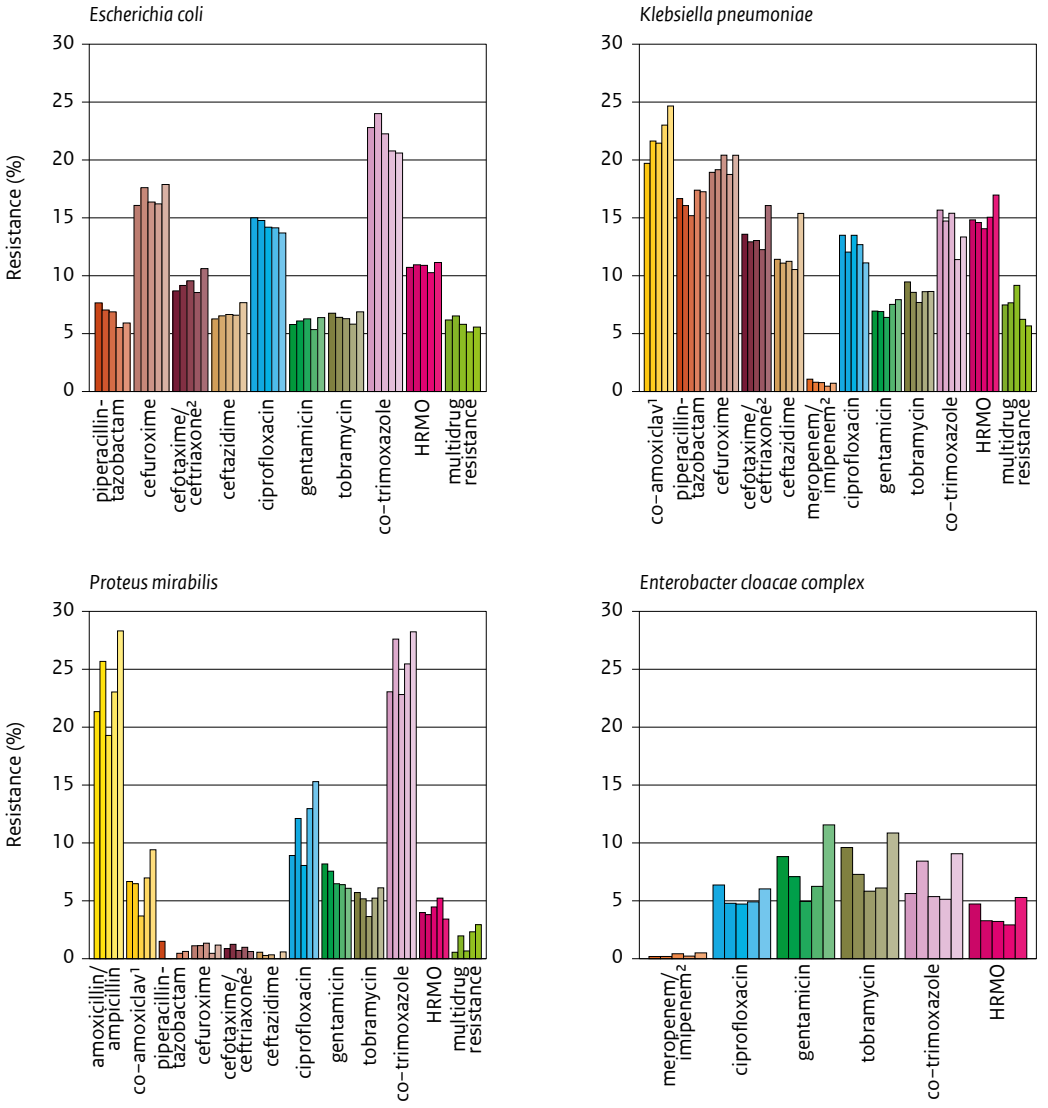
(For the criteria for trend analysis and the definition of a clinically relevant trend see section 4.1.1).

- = Resistance not calculated.
non-uuti = according to breakpoint for indications other than uncomplicated urinary tract infection.
nonmen = according to breakpoint for indications other than meningitis.

[1] *Highly resistant microorganism (HRMO). For a definition of HRMO per species see section 4.1.1.*

[2] *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.*

Figure 4.3.3.1 Trends in antibiotic resistance (from left to right 2017 to 2021) 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.

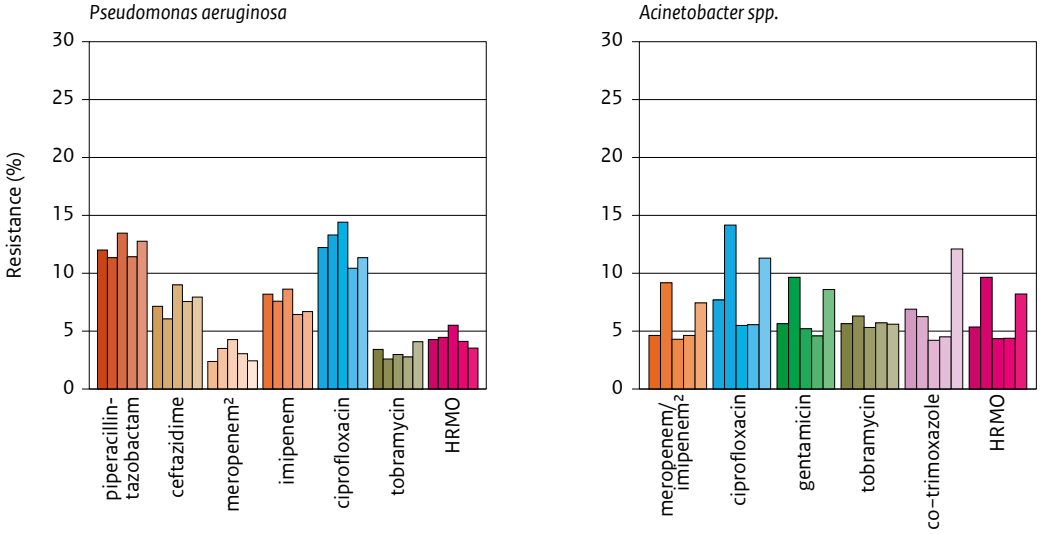
HRMO = highly resistant microorganism. For a definition of HRMO per species see section 4.1.1.

Multidrug resistance is 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.

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

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

Figure 4.3.3.1 (continued) Trends in antibiotic resistance (from left to right 2017 to 2021) 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.
HRMO = highly resistant microorganism. For a definition of HRMO per species see section 4.1.1.
Multidrug resistance is 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.
[1] *According to breakpoint for indications other than uncomplicated urinary tract infection*
[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 2021

	E. faecalis	*E. faecium*
Antibiotic		
amoxicillin/ampicillin	-	92
vancomycin	0	0
linezolid	-	0

10 ↑	Significant and clinically relevant increasing trend since 2017.
10 ↓	Significant and clinically relevant decreasing trend since 2017.
10*	Trend not calculated because data from the years before 2021 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* and coagulase-negative *Staphylococcus* spp. from patients admitted to intensive care units, ISIS-AR 2021

	S. aureus	CNS
Antibiotic		
flucloxacillin[1]	3	80 ↑
ciprofloxacin[2]	3 ↓	73 ↑
gentamicin	1 ↑	64 ↑
erythromycin	15 ↑	73 ↑
clindamycin including inducible resistance[3]	14	65 ↑
doxycycline/tetracycline	5	29 ↑
fusidic acid	4	44
linezolid	0	0
co-trimoxazole	2	28
rifampicin	0	21 ↑

10 ↑	Significant and clinically relevant increasing trend since 2017.
10 ↓	Significant and clinically relevant decreasing trend since 2017.
10*	Trend not calculated because data from the years before 2021 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).

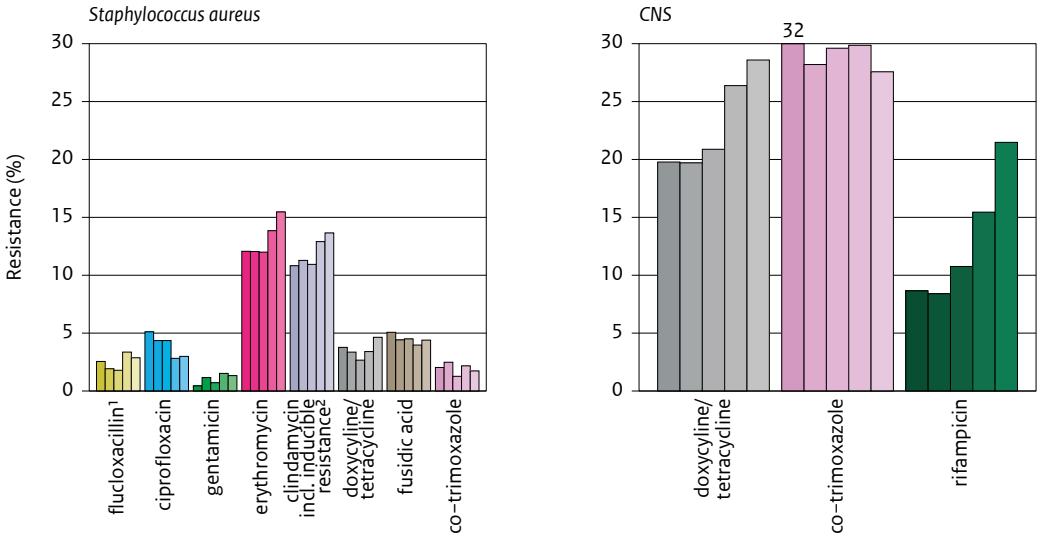
CNS = Coagulase-negative Staphylococcus spp., including S. epidermidis.

1 *Resistance to flucloxacillin was estimated based on laboratory S/R interpretation for cefoxitin, or, if no cefoxitin test was available, for oxacillin/flucloxacillin. Due to breakpoint changes in 2017, no test for trend could be conducted for CNS (see section 4.1.1 for more detailed information).*

2 *Resistance to ciprofloxacin is intended to be a class indicator for resistance to fluoroquinolones.*

3 *To estimate clindamycin resistance including inducible resistance, the laboratory S/I/R interpretation was used (see section 4.1.1 for more detailed information).*

Figure 4.3.3.2 Trends in antibiotic resistance (from left to right 2017 to 2021) among diagnostic isolates of *S. aureus* and coagulase-negative *Staphylococcus* spp. from patients admitted to intensive care units in ISIS-AR



CNS=Coagulase-negative Staphylococcus spp., including S. epidermidis.

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.

[1] *Resistance to flucloxacillin was estimated based on laboratory S/R interpretation for cefoxitin, or, if no cefoxitin test was available, for oxacillin/flucloxacillin (see section 4.1.1 for more detailed information).*

[2] *To estimate clindamycin resistance including inducible resistance, the laboratory S/I/R interpretation was used (see section 4.1.1 for more detailed information).*

Key results

Enterobacterales

- Resistance was ≤10% for **gentamicin** (≤8%) and **tobramycin** (≤9%) in *E. coli*, *K. pneumoniae* and *P. mirabilis*; and **meropenem/imipenem** (≤1%) in *E. coli*, *K. pneumoniae* and *E. cloacae* complex. Additionally, resistance levels ≤10% were observed for **piperacillin-tazobactam** (≤6%) and **ceftazidime** (≤8%) in *E. coli* and *P. mirabilis*; **co-amoxiclav** (9%), **cefuroxime** (1%), **cefotaxime/ ceftriaxone** (1%), and **meropenem** (0%) in *P. mirabilis*; and **ciprofloxacin** (6%) and **co-trimoxazole** (9%) in *E. cloacae* complex.
- Resistance levels ≥20% were observed for **co-amoxiclav** (≥25%) in *E. coli* and *K. pneumoniae*; **amoxicillin/ampicillin** (≥28%) and **co-trimoxazole** (≥21%) in *E. coli* and *P. mirabilis*; and **cefuroxime** (20%) in *K. pneumoniae*.
- For **none of the selected agents** a statistically significant and clinically relevant trend was observed.
- Resistance was ≤9% for **each of the selected empiric therapy combinations** in all *Enterobacterales*. Furthermore, there was a statistically significant and clinically relevant increase in resistance to **ciprofloxacin + co-amoxiclav** (from 1% in 2017 to 4% in 2021) in *P. mirabilis*.

- The percentage **HRMO** and **multidrug resistance** was ≤10% in Proteus and Enterobacter, and >10% in *E. coli* (11%) and *K. pneumoniae* (17%).

P. aeruginosa
- Resistance was ≤10% for **each of the selected agents** (≤8%), except for **piperacillin-tazobactam** (13%) and **ciprofloxacin** (11%).
- For **none of the selected agents** a statistically significant and clinically relevant trend was observed.
- Resistance was ≤3% for **empiric therapy combinations**.
- The percentage **HRMO** was 4%.

***Acinetobacter* spp.**
- Resistance was ≤10% for **each of the selected agents** (≤9%), except for **ciprofloxacin** (11%) and **co-trimoxazole** (12%).
- For **none of the selected agents** a statistically significant and clinically relevant trend was observed.
- The percentage **HRMO** was 8%.

E. faecalis* and *E. faecium
- Resistance was ≤10% for **vancomycin** (0% in both pathogens) and for **linezolid** in *E. faecium* (0%).
- Resistance was ≥20% for **amoxicillin/ampicillin** in *E. faecium* (92%).
- For **none of the selected agents** a statistically significant and clinically relevant trend was observed.

S. aureus
- Resistance was ≤10% for **each of the selected agents** (≤5%), except for **erythromycin** (15%) and **clindamycin including inducible resistance** (14%).
- A statistically significant and clinically relevant increase in resistance was observed for **erythromycin** (from 12% in 2017 to 15% in 2021) and **gentamicin** (from 0% to 1%). Furthermore, there was a statistically significant and clinically relevant decrease in resistance to **ciprofloxacin** (from 5% to 3%).

Coagulase-negative *Staphylococcus* spp.
- Resistance was ≥20% for **each of the selected agents** (≥21%), except for **linezolid** (0%).
- A statistically significant and clinically relevant increase in resistance was found for **flucloxacillin** (from 68% in 2017 to 80% in 2021), **ciprofloxacin** (from 55% to 73%), **gentamicin** (from 50% to 64%), **erythromycin** (from 62% to 73%), **clindamycin including inducible resistance** (from 53% to 65%), **doxycycline/tetracycline** (from 20% to 29%), and **rifampicin** (from 9% to 21%).

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 2021 is presented in table 4.3.4.1. Resistance levels for a selection of pathogens isolated from these patients in 2021 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* and coagulase-negative *Staphylococcus* spp.), 4.3.4.5 (β-haemolytic *Streptococcus* spp. groups A, B, C, 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, and *P. aeruginosa*), 4.3.4.2 (*E. faecium*), 4.3.4.3 (*S. aureus* and coagulase-negative *Staphylococcus* spp.), 4.3.4.4 (β-haemolytic *Streptococcus* spp. groups A, B, C, G, *S. anginosus*, and *S. mitis/S. oralis*), and 4.3.4.5 (*B. fragilis* complex). For *Acinetobacter* spp. trends, and for *C. perfringens* both resistance levels and trends were not calculated because in 2021 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, particularly for coagulase-negative *Staphylococcus* spp., 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 2021

Pathogen	Non-ICU N (%)	ICU N (%)
E. coli	5,866 (22)	164 (3)
K. pneumoniae	1,108 (4)	49 (1)
P. mirabilis	440 (2)	15 (0)
E. cloacae complex	446 (2)	54 (1)
Other *Enterobacterales*[1]	1,401 (5)	109 (2)
P. aeruginosa	587 (2)	82 (2)
Acinetobacter spp.	167 (1)	18 (0)
Other non-fermenters[2]	122 (0)	12 (0)
B. fragilis complex	341 (1)	25 (0)
Other Gram-negatives[3]	164 (1)	9 (0)
E. faecalis	870 (3)	336 (6)
E. faecium	537 (2)	629 (12)
S. aureus	2,671 (10)	238 (5)
CNS	9,431 (35)	3,333 (64)
ß-haemolytic *Streptococcus* spp. group A	106 (0)	1 (0)
ß-haemolytic *Streptococcus* spp. group B	374 (1)	13 (0)
ß-haemolytic *Streptococcus* spp. group C	101 (0)	1 (0)
ß-haemolytic *Streptococcus* spp. group G	189 (1)	2 (0)
S. anginosus	213 (1)	24 (0)
S. mitis/S. oralis	282 (1)	16 (0)
Other Gram-positives[4]	1,460 (5)	66 (1)

CNS = Coagulase-negative Staphylococcus spp., including S. epidermidis.

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

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

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

4 *In order of frequency: S. dysgalactiae subsp. equisimilis, S. dysgalactiae n.n.g., S. pneumoniae, Enterococcus spp. (non-faecalis, non-faecium), Staphylococcus spp. (non-aureus, non-CNS), A. urinae, C. perfringens, 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 2021

	E. coli	*K. pneumoniae*	*P. mirabilis*	*E. cloacae* complex	*P. aeruginosa*	*Acinetobacter* spp.
Antibiotic						
amoxicillin/ampicillin	41	-	22	-	-	-
co-amoxiclav - non-uuti	31	18	6	-	-	-
piperacillin-tazobactam	4	12	0	-	6	-
cefuroxime	11	12	0	-	-	-
cefotaxime/ceftriaxone - nonmen	6	9	0	-	-	-
ceftazidime	5	9	0	-	3	-
meropenem/imipenem - nonmen	0	0	-	0	-	0
meropenem - nonmen	-	-	0	-	1	-
imipenem	-	-	-	-	4	-
ciprofloxacin	13	10	13	5	6	2
gentamicin	5	4	5	5	-	6
tobramycin	5	6	3	4	0 ↓	4
co-trimoxazole	22	13	25	8	-	4
Empiric therapy combinations						
gentamicin + co-amoxiclav - non-uuti	4	4	1	-	-	-
gentamicin + cefuroxime	2	4	0	-	-	-
gentamicin + cefotaxime/ceftriaxone - nonmen	2	4	0	-	-	-
tobramycin + ceftazidime	-	-	-	-	0	-
tobramycin + ciprofloxacin	-	-	-	-	0 ↓	-
ciprofloxacin + co-amoxiclav - non-uuti	8	6	2	-	-	-
ciprofloxacin + cefuroxime	6	7	0	-	-	-
ciprofloxacin + cefotaxime/ceftriaxone - nonmen	4	6	0	-	-	-
Multidrug resistance						
HRMO[1]	8	10	3	3	1 ↓	2
multidrug resistance[2] - non-uuti	5	5	1	-	-	-

10 ↑	Significant and clinically relevant increasing trend since 2017.
10 ↓	Significant and clinically relevant decreasing trend since 2017.
10*	Trend not calculated because data from the years before 2021 did not meet the criteria for trend analysis.
10	No significant and clinically relevant time trend.

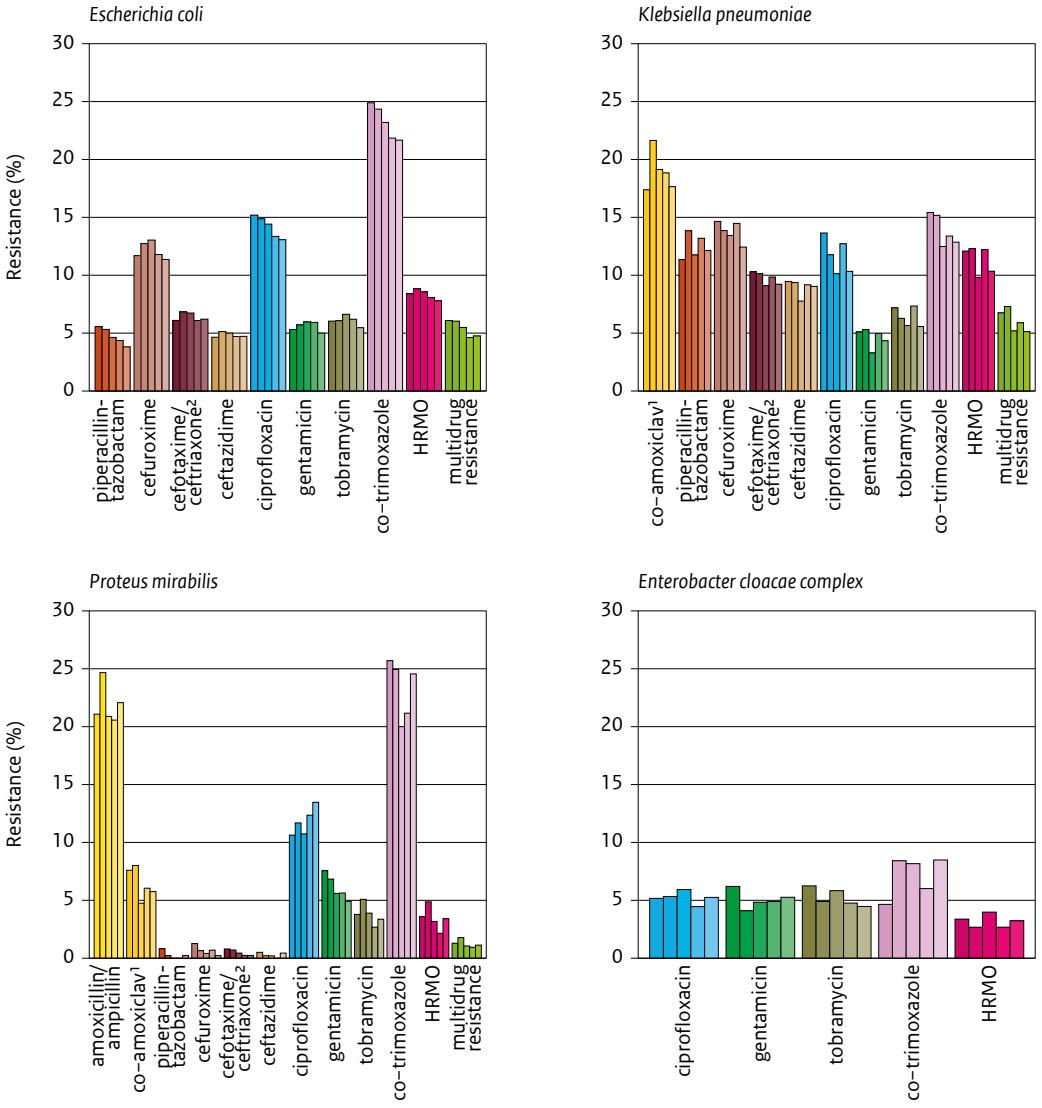
(For the criteria for trend analysis and the definition of a clinically relevant trend see section 4.1.1).

- = Resistance not calculated.
non-uuti = according to breakpoint for indications other than uncomplicated urinary tract infection.
nonmen = according to breakpoint for indications other than meningitis.

[1] *Highly resistant microorganism (HRMO). For a definition of HRMO per species see section 4.1.1.*

[2] *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.*

Figure 4.3.4.1 Trends in antibiotic resistance (from left to right 2017 to 2021) among diagnostic blood isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, and *P. aeruginosa* 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.

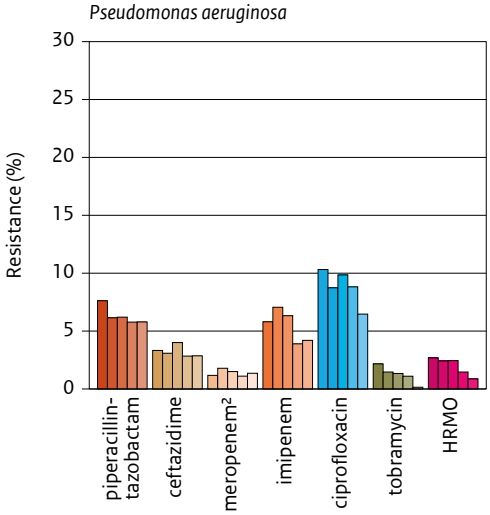
HRMO = highly resistant microorganism. For a definition of HRMO per species see section 4.1.1.

Multidrug resistance is 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.

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

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

Figure 4.3.4.1 (continued) Trends in antibiotic resistance (from left to right 2017 to 2021) among diagnostic blood isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, and *P. aeruginosa* 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.
HRMO = highly resistant microorganism. For a definition of HRMO per species see section 4.1.1.
Multidrug resistance is 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.
[1] *According to breakpoint for indications other than uncomplicated urinary tract infection*
[2] *According to breakpoint for indications other than meningitis.*

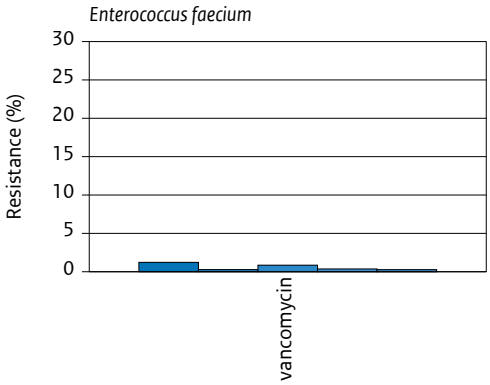
Table 4.3.4.3 Resistance levels (%) among diagnostic blood isolates of *E. faecalis* and *E. faecium* from patients admitted to inpatient departments (incl. intensive care units), ISIS-AR 2021

	E. faecalis	*E. faecium*
Antibiotic		
amoxicillin/ampicillin	-	91
vancomycin	0	0

10 ↑	Significant and clinically relevant increasing trend since 2017.
10 ↓	Significant and clinically relevant decreasing trend since 2017.
10*	Trend not calculated because data from the years before 2021 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 2017 to 2021) 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.

Table 4.3.4.4 Resistance levels (%) among diagnostic blood isolates of *S. aureus* and coagulase-negative *Staphylococcus* spp. from patients admitted to inpatient departments (incl. intensive care units), ISIS-AR 2021

	S. aureus	CNS
Antibiotic		
flucloxacillin[1]	2	50
ciprofloxacin[2]	5	36 ↑
gentamicin	0	33 ↑
erythromycin	13	50
clindamycin including inducible resistance[3]	12	36
doxycycline/tetracycline	3	20
linezolid	0	0
co-trimoxazole	2	16
rifampicin	0	9 ↑

10 ↑	Significant and clinically relevant increasing trend since 2017.
10 ↓	Significant and clinically relevant decreasing trend since 2017.
10*	Trend not calculated because data from the years before 2021 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).

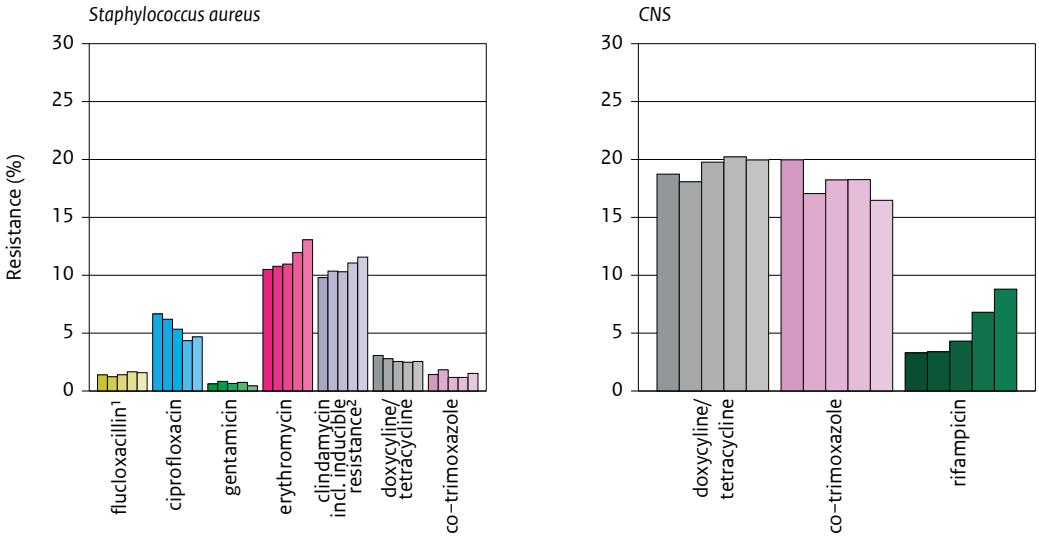
CNS = Coagulase-negative Staphylococcus spp., including S. epidermidis.

[1] *Resistance to flucloxacillin was estimated based on laboratory S/R interpretation for cefoxitin, or, if no cefoxitin test was available, for oxacillin/flucloxacillin. Due to breakpoint changes in 2017, no test for trend could be conducted for CNS (see section 4.1.1 for more detailed information).*

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

[3] *To estimate clindamycin resistance including inducible resistance, the laboratory S/I/R interpretation was used (see section 4.1.1 for more detailed information).*

Figure 4.3.4.3 Trends in antibiotic resistance (from left to right 2017 to 2021) among diagnostic blood isolates of *S. aureus* and coagulase-negative *Staphylococcus* spp. from patients admitted to inpatient departments (incl. intensive care units) in ISIS-AR



CNS=Coagulase-negative Staphylococcus spp., including S. epidermidis.

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.

[1] *Resistance to flucloxacillin was estimated based on laboratory S/R interpretation for cefoxitin, or, if no cefoxitin test was available, for oxacillin/flucloxacillin (see section 4.1.1 for more detailed information).*

[2] *To estimate clindamycin resistance including inducible resistance, the laboratory S/I/R interpretation was used (see section 4.1.1 for more detailed information).*

Table 4.3.4.5 Resistance levels (%) among diagnostic blood isolates of β-haemolytic *Streptococcus* spp. groups A,B,C,G, *S. anginosus*, and *S. mitis/S. oralis* from patients admitted to inpatient departments (incl. intensive care units), ISIS-AR 2021

	β-haemolytic *Streptococcus* spp. group A	β-haemolytic *Streptococcus* spp. group B	β-haemolytic *Streptococcus* spp. group C	β-haemolytic *Streptococcus* spp. group G	*S. anginosus*	*S. mitis/ S. oralis*
Antibiotic						
(benzyl)penicillin	-	-	-	-	0	4
(benzyl)penicillin (I)[1]	-	-	-	-	0	8
amoxicillin/ampicillin	-	-	-	-	0*	8*
erythromycin	8	22	-	18	-	-
clindamycin including inducible resistance[2]	8	20	9	13	8	5
doxycycline/tetracycline	30 ↑	77	-	32*	-	-
co-trimoxazole	2*	0	-	0*	-	-

10 ↑	Significant and clinically relevant increasing trend since 2017.
10 ↓	Significant and clinically relevant decreasing trend since 2017.
10*	Trend not calculated because data from the years before 2021 did not meet the criteria for trend analysis.
10	No significant and clinically relevant time trend.

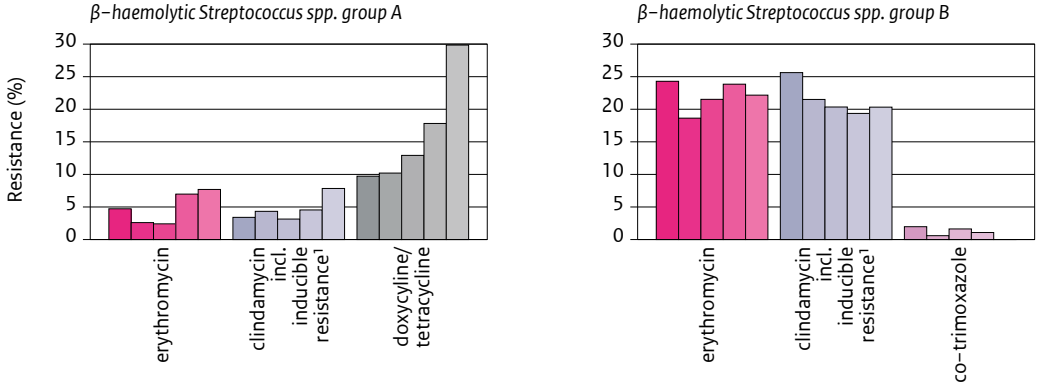
(For the criteria for trend analysis and the definition of a clinically relevant trend see section 4.1.1).

- = *Resistance not calculated.*

[1] *I is defined as susceptible, increased exposure, according to EUCAST definitions (https://www.eucast.org/newsiandr).*

[2] *To estimate clindamycin resistance including inducible resistance, the laboratory S/I/R interpretation was used (see section 4.1.1 for more detailed information).*

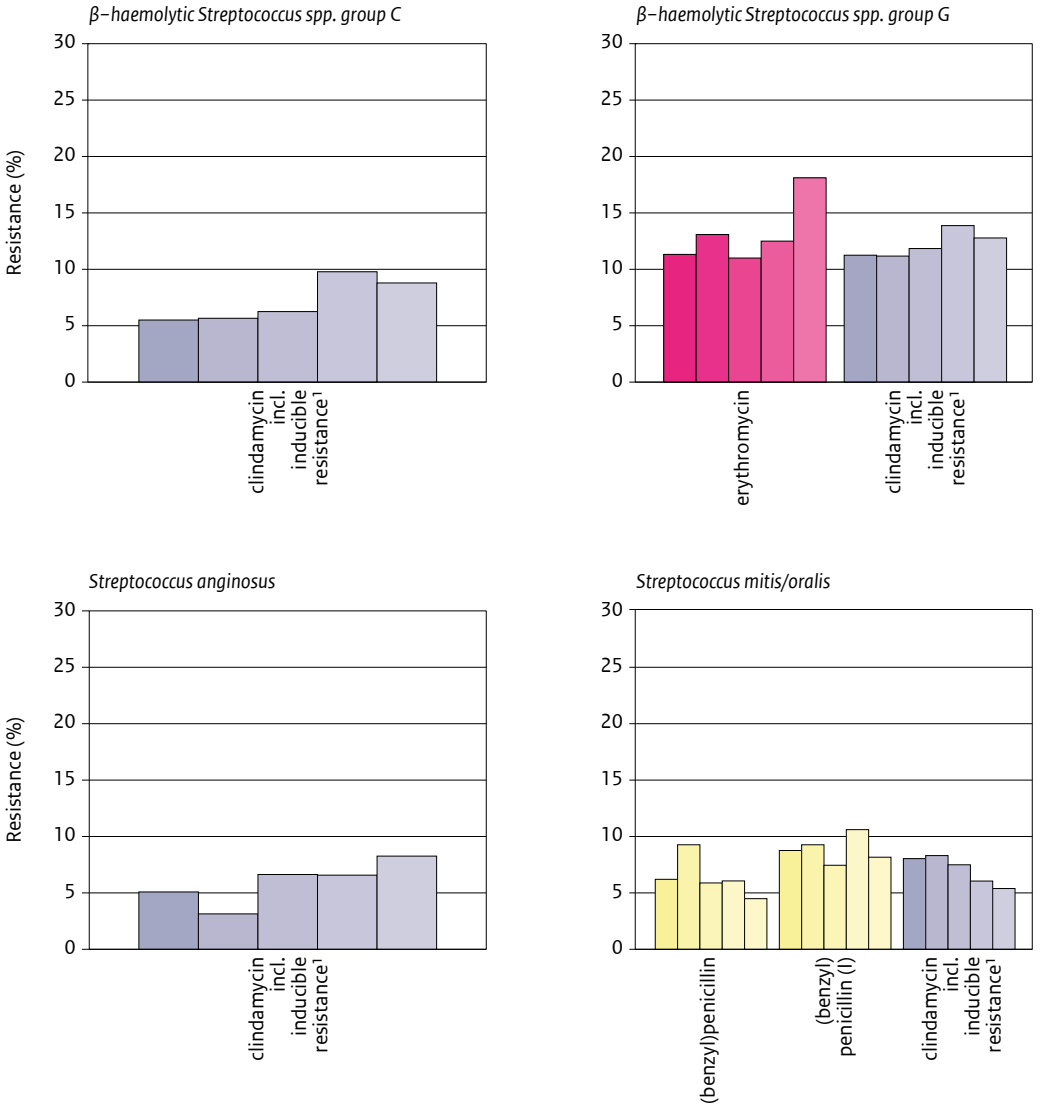
Figure 4.3.4.4 Trends in antibiotic resistance (from left to right 2017 to 2021) among diagnostic blood isolates of β-haemolytic *Streptococcus* spp. groups A,B,C,G, *S. anginosus*, and *S. mitis/S. oralis* from patients admitted to inpatient departments (incl. intensive care units) in ISIS-AR



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.

[1] *To estimate clindamycin resistance including inducible resistance, the laboratory S/I/R interpretation was used (see section 4.1.1 for more detailed information).*

Figure 4.3.4.4 (continued) Trends in antibiotic resistance (from left to right 2017 to 2021) among diagnostic blood isolates of β-haemolytic *Streptococcus* spp. groups A,B,C,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.

[1] *To estimate clindamycin resistance including inducible resistance, the laboratory S/I/R interpretation was used (see section 4.1.1 for more detailed information).*

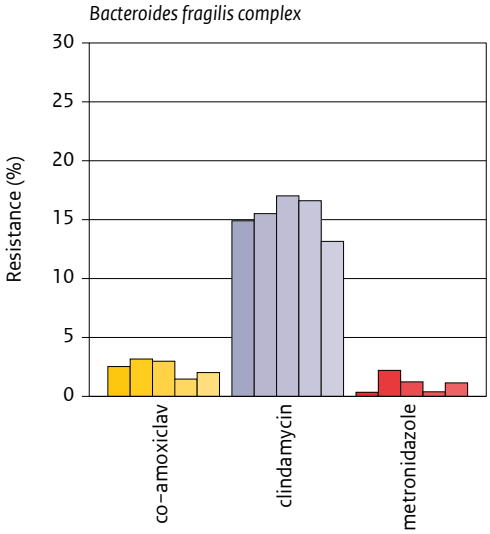
Table 4.3.4.6 Resistance levels (%) among diagnostic blood isolates of *B. fragilis* complex from patients admitted to inpatient departments (incl. intensive care units), ISIS-AR 2021

	***B. fragilis* complex**
Antibiotic	
co-amoxiclav	2
clindamycin	13
metronidazole	1

10 ↑	Significant and clinically relevant increasing trend since 2017.
10 ↓	Significant and clinically relevant decreasing trend since 2017.
10*	Trend not calculated because data from the years before 2021 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 2017 to 2021) 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.

Key results

- The majority (84%) of inpatient blood isolates (non-ICU and ICU departments combined) originated from non-ICU departments.

Enterobacterales

- In all *Enterobacterales*, resistance was ≤10% for **gentamicin** (≤5%), and **tobramycin** (≤6%). Additionally, resistance levels ≤10% were observed for **cefotaxime/ceftriaxone** (≤10%), and **ceftazidime** (≤9%) in *E. coli*, *K. pneumoniae* and *P. mirabilis*; **meropenem/imipenem** (0%) in *E. coli*, *K. pneumoniae* and *E. cloacae* complex; **piperacillin-tazobactam** in *E. coli* and *P. mirabilis* (≤4%); **ciprofloxacin** in *K. pneumoniae* and *E. cloacae* complex (≤10%); **co-amoxiclav** (6%), **cefuroxime** (0%), and **meropenem** (0%) in *P. mirabilis*; and for **co-trimoxazole** in *E. cloacae* complex (8%).
- Resistance was ≥20% for **amoxicillin/ampicillin** (≥22%) and **co-trimoxazole** (≥22%) in *E. coli* and *P. mirabilis*; and for **co-amoxiclav** in *E. coli* (31%).
- For **none of the selected agents** a statistically significant and clinically relevant trend was observed.
- Resistance was ≤8% for **each of the selected empiric therapy combinations** in all *Enterobacterales*.
- The percentage **HRMO** and **multidrug resistance** was ≤10% in all *Enterobacterales*.

P. aeruginosa

- Resistance levels ≤10% were observed for **each of the selected agents** (≤6%).
- A statistically significant and clinically relevant decrease in resistance was observed for **tobramycin** (from 2% in 2017 to 0% in 2021).
- Resistance was 0% for **each of the selected empiric therapy combinations**. A statistically significant and clinically relevant decrease in resistance was observed for **tobramycin + ciprofloxacin** (from 2% in 2017 to 0% in 2021).
- The percentage **HRMO** was 1%. A statistically significant and clinically relevant decrease in resistance was observed for **HRMO** (from 3% in 2017 to 1% in 2021).

***Acinetobacter* spp.**

- Resistance levels ≤10% were observed for **each of the selected agents** (≤6%).
- For **none of the selected agents** a statistically significant and clinically relevant trend was observed.
- The percentage **HRMO** was 2%.

E. faecalis* and *E. faecium

- Resistance was ≤10% for **vancomycin** (0% in both pathogens).
- Resistance was ≥20% for **amoxicillin/ampicillin** in *E. faecium* (91%).
- For **none of the selected agents** a statistically significant and clinically relevant trend was observed.

S. aureus

- Resistance levels ≤10% were observed for **each of the selected agents** (≤5%), except for **erythromycin** (13%) and **clindamycin including inducible resistance** (12%).
- For **none of the selected agents** a statistically significant and clinically relevant trend was observed.

Coagulase-negative *Staphylococcus* spp.

- Resistance levels ≥20% were observed for **each of the selected agents**, except for **linezolid** (0%), **co-trimoxazole** (16%), and **rifampicin** (9%).
- A statistically significant and clinically relevant increase in resistance was observed for **ciprofloxacin** (from 30% in 2017 to 36% in 2021), **gentamicin** (from 28% to 33%) and **rifampicin** (from 3% to 9%).

β-haemolytic *Streptococcus* spp. groups A, B, C, G

- Resistance was ≤10% for **co-trimoxazole** in β-haemolytic *Streptococcus* spp. group A, B and G (≤2%). Additionally, resistance was ≤10% for **clindamycin including inducible resistance** in β-haemolytic *Streptococcus* spp. groups A and C (≤9%); and for **erythromycin** in β-haemolytic *Streptococcus* spp. group A (8%).
- Resistance levels ≥20% were observed for **doxycycline/tetracycline** in β-haemolytic *Streptococcus* spp. group A, B and G (≥30%); for **erythromycin** (22%) and **clindamycin including inducible resistance** (20%) in β-haemolytic *Streptococcus* spp. group B.
- A statistically significant and clinically relevant increase in resistance was observed for **doxycycline/tetracycline** in ß-haemolytic *Streptococcus* spp. group A (from 10% in 2017 to 30% in 2021).

S. anginosus* and *S. mitis/S. oralis

- Resistance levels ≤10% were observed for **each of the selected agents** (≤8%). The percentage I for **(benzyl)penicillin** was 0% in *S. anginosus* and 8% in *S. mitis/S. oralis*.
- For **none of the selected agents** a statistically significant and clinically relevant trend was observed.

***B. fragilis* complex**

- Resistance was ≤10% for **co-amoxiclav** (2%) and **metronidazole** (1%).
- For **none of the selected agents** a statistically significant and clinically relevant trend was observed.

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 2021 is presented in table 4.3.5.1. Resistance levels for a selection of pathogens isolated from these patients in 2021 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 2021

	OPD	IPD
Pathogen	N (%)	N (%)
E. coli	9,858 (36)	1,717 (31)
K. pneumoniae	2,434 (9)	458 (8)
P. mirabilis	1,298 (5)	288 (5)
Other *Enterobacterales*[1]	4,480 (16)	1,002 (18)
P. aeruginosa	1,058 (4)	335 (6)
Other non-fermenters[2]	556 (2)	149 (3)
Other Gram-negatives[3]	10 (0)	4 (0)
E. faecalis	3,145 (12)	745 (13)
E. faecium	219 (1)	166 (3)
Other Gram-positives[4]	4,224 (15)	719 (13)

[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., Salmonella spp., Cronobacter spp., Escherichia spp. (non-coli).*

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

[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. group B, S. anginosus, ß-haemolytic Streptococcus spp. group G, S. pneumoniae, ß-haemolytic Streptococcus spp. group A, ß-haemolytic Streptococcus spp. group C, S. dysgalactiae n.n.g., S. mitis/S. oralis, S. dysgalactiae subsp. equisimilis, Enterococcus spp. (non-faecalis, non-faecium), C. perfringens, L. monocytogenes.*

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 2021

	E. coli		*K. pneumoniae*		*P. mirabilis*		*P. aeruginosa*	
	OPD	IPD	OPD	IPD	OPD	IPD	OPD	IPD
Antibiotic								
amoxicillin/ampicillin	42	46	-	-	22	22	-	-
co-amoxiclav - non-uuti	33	36	18	19	6	5	-	-
piperacillin-tazobactam	4	5	12	13	0	0	4	9
cefuroxime	11	18	14	10 ↓	1	2	-	-
cefotaxime/ceftriaxone - nonmen	6	9	6 ↓	8 ↓	1	1	-	-
ceftazidime	4	7	6 ↓	8 ↓	0	0	2	2
meropenem/imipenem - nonmen	0	0	0	0	-	-	-	-
meropenem - nonmen	-	-	-	-	0	0	1 ↑	2
imipenem	-	-	-	-	-	-	6	9
ciprofloxacin	19	23 ↓	13 ↓	13 ↓	14	18	15	18
gentamicin	5	7	2	5	7	7	-	-
tobramycin	5	7	3 ↓	5 ↓	5	3	2	1
fosfomycin[1]	3	3	-	-	-	-	-	-
trimethoprim	27	28	21 ↓	18 ↓	32	38	-	-
co-trimoxazole	25	25	11 ↓	16 ↓	25	30	-	-
nitrofurantoin	3	2	-	-	-	-	-	-
Empiric therapy combinations								
gentamicin + co-amoxiclav - non-uuti	4	6	2	4	2	3	-	-
gentamicin + cefuroxime	2	3	2	4	0	0	-	-
gentamicin + cefotaxime/ceftriaxone - nonmen	1	3	1	4	0	0	-	-
tobramycin + ceftazidime	-	-	-	-	-	-	0	1
tobramycin + ciprofloxacin	-	-	-	-	-	-	1	1
ciprofloxacin + co-amoxiclav - non-uuti	11	14	5	7 ↓	2	3	-	-
ciprofloxacin + cefuroxime	6	11	8	8	1	1	-	-
ciprofloxacin + cefotaxime/ceftriaxone - nonmen	4	7	4	7	1	1	-	-
Multidrug resistance								
HRMO[2]	8	11 ↓	7 ↓	9 ↓	5	6	1	2
multidrug resistance[3] - non-uuti	6	9	3 ↓	7	2	3	-	-

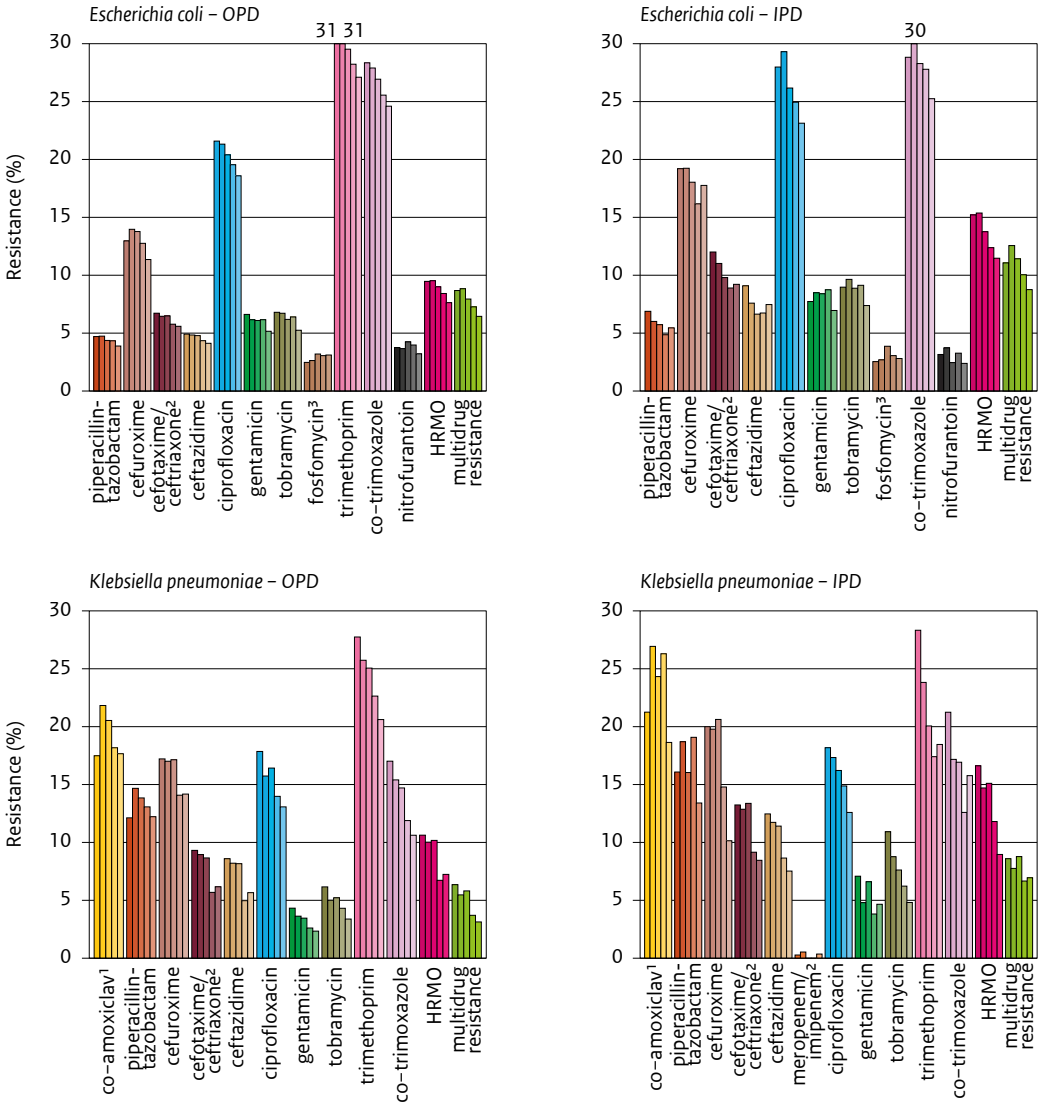
10 ↑	Significant and clinically relevant increasing trend since 2017.
10 ↓	Significant and clinically relevant decreasing trend since 2017.
10*	Trend not calculated because data from the years before 2021 did not meet the criteria for trend analysis.
10	No significant and clinically relevant time trend.

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

- = Resistance not calculated.
non-uuti = according to breakpoint for indications other than uncomplicated urinary tract infection.
nonmen = according to breakpoint for indications other than meningitis.

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.*
2 *Highly resistant microorganism (HRMO). For a definition of HRMO per species see section 4.1.1.*
3 *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.*

Figure 4.3.5.1 Trends in antibiotic resistance (from left to right 2017 to 2021) 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.

HRMO = highly resistant microorganism. For a definition of HRMO per species see section 4.1.1.

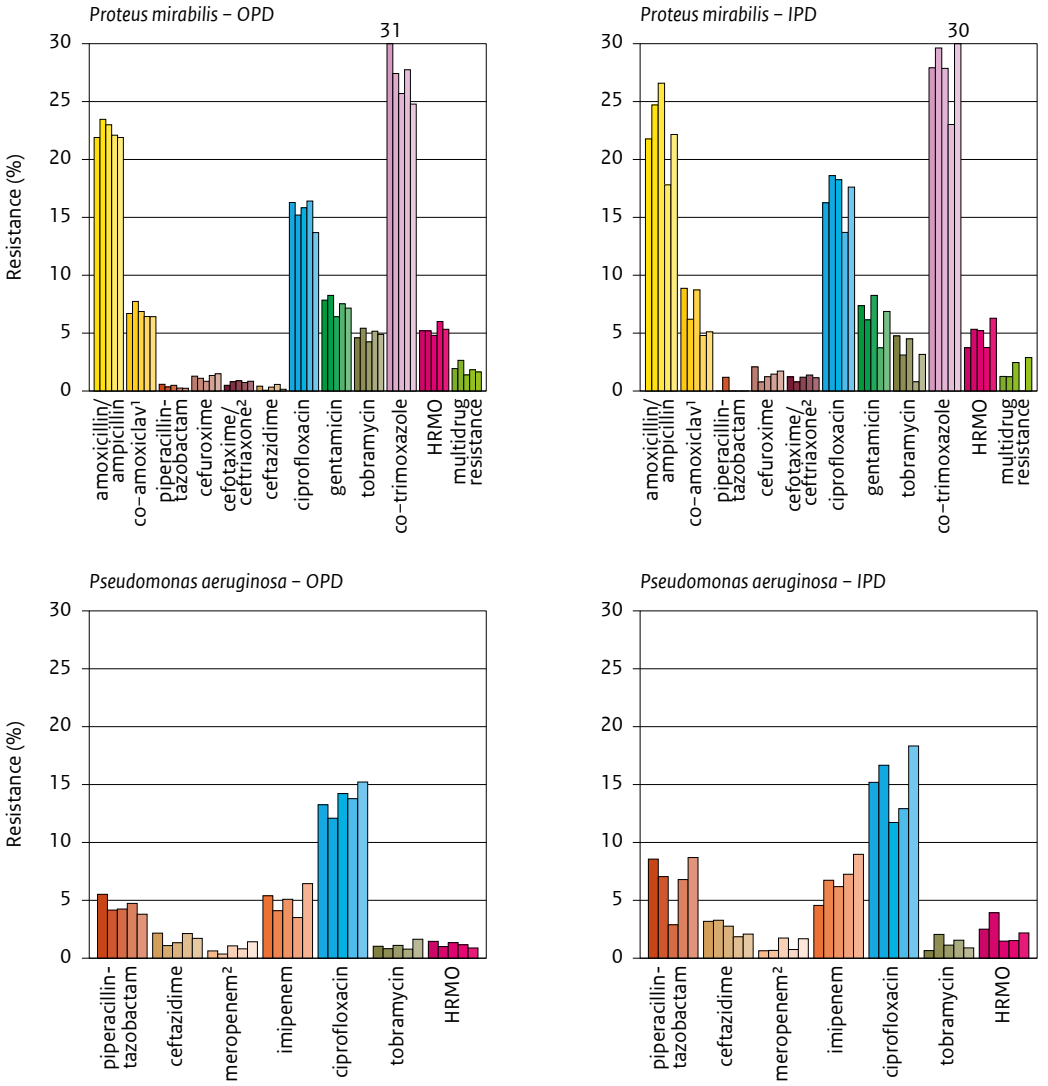
Multidrug resistance is defined as resistance to all following oral agents: co-amoxiclav (according to breakpoint for indications other than uncomplicated urinary tract infection), ciprofloxacin, and co-trimoxazole.

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 4.3.5.1 (continued) Trends in antibiotic resistance (from left to right 2017 to 2021) 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.

HRMO = highly resistant microorganism. For a definition of HRMO per species see section 4.1.1.

Multidrug resistance is 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.

[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.*

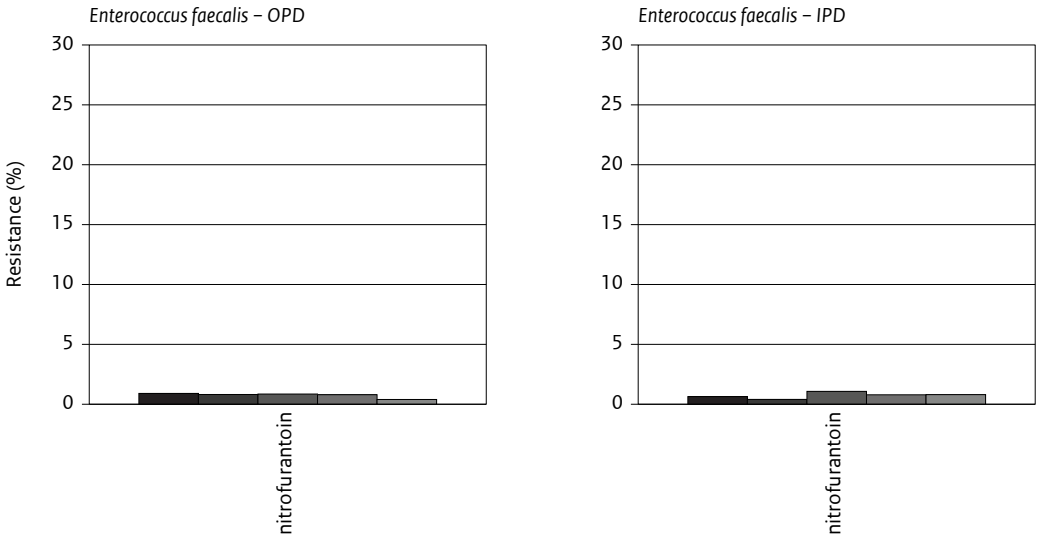
Table 4.3.5.3 Resistance levels (%) among diagnostic urine isolates of *E. faecalis* and *E. faecium* from patients attending urology outpatient departments (OPD) and patients admitted to urology inpatient departments (IPD), ISIS-AR 2021

	E. faecalis		*E. faecium*	
	OPD	IPD	OPD	IPD
Antibiotic				
amoxicillin/ampicillin	-	-	82	94
vancomycin	0	0	0	1
nitrofurantoin	0	1	-	-

10 ↑	Significant and clinically relevant increasing trend since 2017.
10 ↓	Significant and clinically relevant decreasing trend since 2017.
10*	Trend not calculated because data from the years before 2021 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).

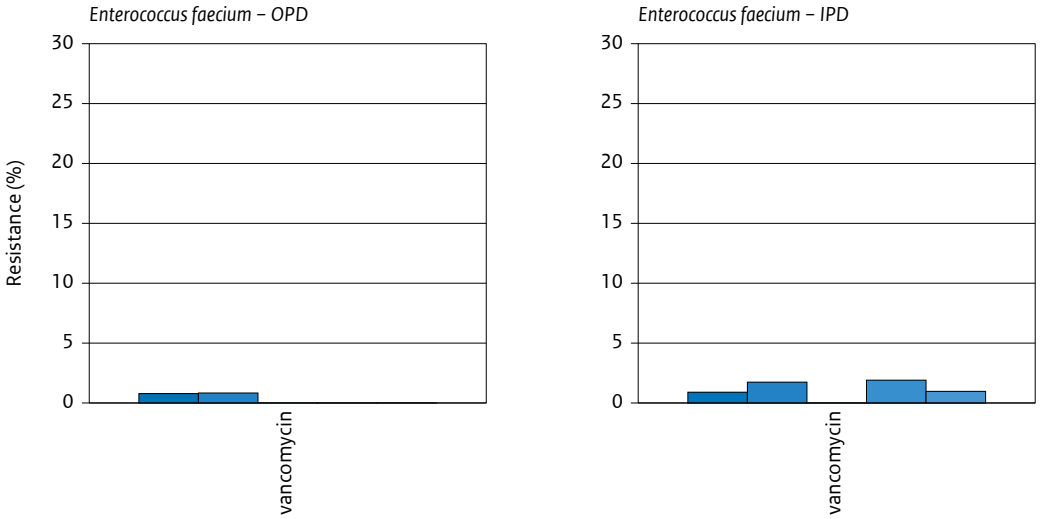
- = Resistance not calculated.

Figure 4.3.5.2 Trends in antibiotic resistance (from left to right 2017 to 2021) 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.

Figure 4.3.5.2 (continued) Trends in antibiotic resistance (from left to right 2017 to 2021) 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.

Key results

Enterobacterales

- In all *Enterobacterales*, resistance levels ≤10% were observed for **cefotaxime/ceftriaxone** (≤9%), **ceftazidime** (≤8%), **gentamicin** (≤7%), and **tobramycin** (≤7%). In addition, levels ≤10% were observed for **meropenem/imipenem** (0%) in *E. coli* and *K. pneumoniae*; for **piperacillin-tazobactam** in *E. coli* and *P. mirabilis* (≤5%); for **fosfomycin** (3%) and **nitrofurantoin** (≤3%) in *E. coli*; for **cefuroxime** in *K. pneumoniae* from IPD patients (10%); and for **co-amoxiclav** (≤6%), **cefuroxime** (≤2%), and **meropenem** (0%) in *P. mirabilis*.
- In all *Enterobacterales*, resistance levels ≥20% was observed for **trimethoprim** (≥21%, except in *K. pneumoniae* from IPD patients: 18%). Furthermore, resistance levels ≥20% were observed for **amoxicillin/ampicillin** (≥22%), and **co-trimoxazole** (≥25%) in *E. coli* and *P. mirabilis*; and for **co-amoxiclav** (≥33%) and **ciprofloxacin** in *E. coli* from IPD patients (23%).
- A statistically significant and clinically relevant decrease in resistance was observed for **ciprofloxacin** in *E. coli* from IPD patients (from 28% in 2017 to 23% in 2021). In addition, in *K. pneumoniae*, resistance decreased to a statistically significant and clinically relevant extent for **cefuroxime** in IPD (from 20% to 10%), **cefotaxime/ceftriaxone** (according to the breakpoint for indications other than meningitis, from 9% to 6% in OPD and from 13% to 8% in IPD), **ceftazidime** (from 9% to 6% in OPD and from 12% to 8% in IPD), **ciprofloxacin** (from 18% to 13% in both OPD and IPD), **tobramycin** (from 6% to 3% in OPD and from 11% to 5% in IPD), **trimethoprim** (from 28% to 21% in OPD and from 28% to 18% in IPD), and **co-trimoxazole** (from 17% to 11% in OPD and from 21% to 16% in IPD).

- For all *Enterobacterales*, resistance levels ≤10% were observed for **each of the selected empiric therapy combinations**, except for **ciprofloxacin + co-amoxiclav** (≥11%) in *E. coli*, and **ciprofloxacin + cefuroxime** (11%) in *E. coli* from IPD patients. In *K. pneumoniae*, a statistically significant and clinically relevant decrease in resistance was observed for **ciprofloxacin + co-amoxiclav** in IPD (from 11% in 2017 to 7% in 2021).
- The percentage of **HRMO** and **multidrug resistance** was ≤10% in all *Enterobacterales*, except for **HRMO** in *E. coli* (11%) in IPD patients. Furthermore, there was a statistically significant and clinically relevant decrease in resistance for **HRMO** in *E. coli* from IPD patients (from 15% in 2017 to 11% in 2021) and *K. pneumoniae* (from 11% to 7% in OPD and from 17% to 9% in IPD), and for **multidrug resistance** in *K. pneumoniae* from OPD patients (from 6% to 3%).

P. aeruginosa

- Resistance levels ≤10% were observed for **each of the selected agents** (≤9%), except for **ciprofloxacin** (≤18%).
- There was a statistically significant and clinically relevant increase in resistance to **meropenem** in OPD (according to the breakpoint for indications other than meningitis, from 0.6% in 2017 to 1.4% in 2021).
- Resistance to **empiric therapy combinations** was ≤1%.
- The percentage **HRMO** was ≤2%.

E. faecalis* and *E. faecium

- Resistance levels ≤10% were observed for **vancomycin** (≤1%) and **nitrofurantoin** (≤1%, presented for *E. faecalis* only).
- Resistance levels ≥20% was observed for **amoxicillin/ampicillin** in *E. faecium* (≥82%).
- For **none of the selected agents** a statistically significant and clinically relevant trend was observed.

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 2021 is presented in table 4.4.1. The resistance levels in 2021 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'.

Sampling policies in LTCFs 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 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 2021

	Urine	Wound or pus
Pathogen	**N (%)**	**N (%)**
E. coli	10,397 (40)	180 (7)
K. pneumoniae	2,682 (10)	55 (2)
P. mirabilis	2,880 (11)	188 (8)
Other *Enterobacterales*[1]	2,830 (11)	232 (10)
P. aeruginosa	1,333 (5)	264 (11)
Other non-fermenters[2]	241 (1)	42 (2)
Other Gram-negatives[3]	1 (0)	20 (1)
S. aureus	1,004 (4)	1,079 (45)
Other Gram-positives[4]	4,574 (18)	356 (15)

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

[4] *In order of frequency: Enterococcus spp., A. urinae, ß-haemolytic Streptococcus spp. group C, S. dysgalactiae n.n.g., S. dysgalactiae subsp. equisimilis, ß-haemolytic Streptococcus spp. group A, S. anginosus, ß-haemolytic Streptococcus spp. group B, ß-haemolytic Streptococcus spp. group G, 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 2021

	E. coli	*K. pneumoniae*	*P. mirabilis*	*P. aeruginosa*
Antibiotic				
amoxicillin/ampicillin	42	-	21	-
co-amoxiclav - non-uuti	34	26	6	-
piperacillin-tazobactam	5	21	0	5
cefuroxime	13	12	1	-
cefotaxime/ceftriaxone - nonmen	6	6	0	-
ceftazidime	5	6	0	3
meropenem/imipenem - nonmen	0	0	-	-
meropenem - nonmen	-	-	0	1
imipenem	-	-	-	5
ciprofloxacin	16	11	15	10
gentamicin	6	2	6	-
tobramycin	6	3	3	1
fosfomycin[1]	3	-	-	-
trimethoprim	21	16	33	-
co-trimoxazole	19	8	25	-
nitrofurantoin	3	-	-	-
Multidrug resistance				
HRMO[2]	9	7	4	1
multidrug resistance[3] - non-uuti	4	3	2	-

- = Resistance not calculated.
non-uuti = according to breakpoint for indications other than uncomplicated urinary tract infection.
nonmen = according to breakpoint for indications other than meningitis.

[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.*

[2] *Highly resistant microorganism (HRMO). For a definition of HRMO per species see section 4.1.1.*

[3] *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.*

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 2021

	S. aureus
Antibiotic	
flucloxacillin[1]	1
ciprofloxacin[2]	17
erythromycin	13
clindamycin including inducible resistance[3]	13
doxycycline/tetracycline	3
fusidic acid	9
co-trimoxazole	1

[1] *Resistance to flucloxacillin was estimated based on laboratory S/R interpretation for cefoxitin, or, if no cefoxitin test was available, for oxacillin/flucloxacillin (see section 4.1.1 for more detailed information).*

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

[3] *To estimate clindamycin resistance including inducible resistance, the laboratory S/I/R interpretation was used (see section 4.1.1 for more detailed information).*

Key results

***Enterobacterales* (urine samples)**

- For all *Enterobacterales*, resistance levels ≤10% were observed for **cefotaxime/ceftriaxone** (≤6%), **ceftazidime** (≤6%), **gentamicin** (≤6%), and **tobramycin** (≤6%). In addition, resistance levels ≤10% were also observed for **meropenem/imipenem** (0%) in *E. coli* and *K. pneumoniae*; for **piperacillin-tazobactam** in *E. coli* and *P. mirabilis* (≤5%); **fosfomycin** (3%), and **nitrofurantoin** (3%) in *E. coli*; for **co-trimoxazole** (8%) in *K. pneumoniae*; and for **co-amoxiclav** (6%), **cefuroxime** (1%), **meropenem** (0%) in *P. mirabilis*.
- Resistance levels ≥20% were observed for **co-amoxiclav** (≥26%) in *E. coli* and *K. pneumoniae*; and for **amoxicillin/ampicillin** (≥21%), and **trimethoprim** (≥21%) in *E. coli* and *P. mirabilis*. Additionally, resistance levels ≥20% were also observed for **piperacillin-tazobactam** (21%) in *K. pneumoniae*, and for **co-trimoxazole** in *P. mirabilis* (25%).
- The percentage of **HRMO** and **multidrug resistance** was ≤9% in all *Enterobacterales*.

***P. aeruginosa* (urine samples)**

- Resistance levels ≤10% were observed for **each of the selected agents**.
- The percentage of **HRMO** was 1%.

***S. aureus* (wound or pus samples)**

- Resistance levels ≤10% were observed for **flucloxacillin** (1%), **doxycycline/tetracycline** (3%), **fusidic acid** (9%), and **co-trimoxazole** (1%).

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 2021 is presented in table 4.5.1. Resistance levels for respiratory pathogens (*S. pneumoniae*, *H. influenzae*, and *M. catarrhalis*) in 2021 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, 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 2021

	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	81 (7)	1 (0)	783 (2)	1,442 (7)	79 (2)
Other Gram-positives[1]	198 (16)	1,166 (83)	20,188 (63)	4,951 (22)	3,082 (63)
H. influenzae	278 (22)	35 (3)	112 (0)	3,896 (18)	278 (6)
M. catarrhalis	103 (8)	20 (1)	19 (0)	1,051 (5)	96 (2)
Other non-fermenters[2]	267 (21)	19 (1)	942 (3)	4,325 (20)	373 (8)
Enterobacterales[3]	291 (23)	153 (11)	9,552 (30)	5,950 (27)	925 (19)
Other Gram-negatives[4]	24 (2)	3 (0)	434 (1)	429 (2)	62 (1)

[1] *In order of frequency: Staphylococcus spp., Enterococcus spp., ß-haemolytic Streptococcus spp. group C, S. anginosus, S. dysgalactiae n.n.g., ß-haemolytic Streptococcus spp. group A, ß-haemolytic Streptococcus spp. group B, S. mitis/S. oralis, ß-haemolytic Streptococcus spp. group G, S. dysgalactiae subsp. equisimilis, A. urinae, C. perfringens, 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., Pantoea spp., Hafnia spp., Salmonella spp., Providencia spp., Yersinia spp., Cronobacter spp., Shigella 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 *H. influenzae*, and *M. catarrhalis* from general practitioners' patients (GP), and diagnostic isolates of *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* from patients attending outpatient departments and patients admitted to inpatient departments (incl. intensive care units), ISIS-AR 2021

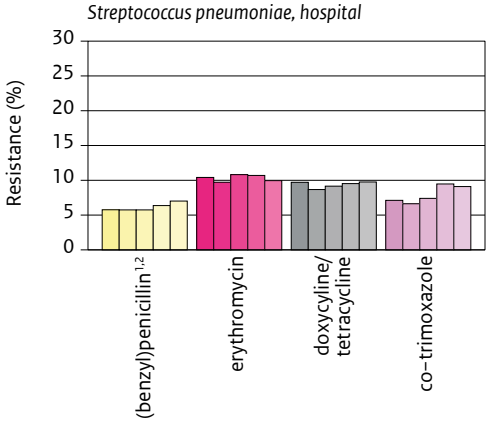
	S. pneumoniae	*H. influenzae*		*M. catarrhalis*	
	Hospital	GP	Hospital	GP	Hospital
Antibiotic					
(benzyl)penicillin[1] - nonmen	0	-	-	-	-
(benzyl)penicillin[1] - men	7	-	-	-	-
co-amoxiclav	-	18 ↑	15 ↑	2	3 ↑
erythromycin	10	-	-	1*	3
doxycycline/tetracycline	10	0	1	1	1
co-trimoxazole	9 ↑	24	26	2	5

10 ↑	Significant and clinically relevant increasing trend since 2017.
10 ↓	Significant and clinically relevant decreasing trend since 2017.
10*	Trend not calculated because data from the years before 2021 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).

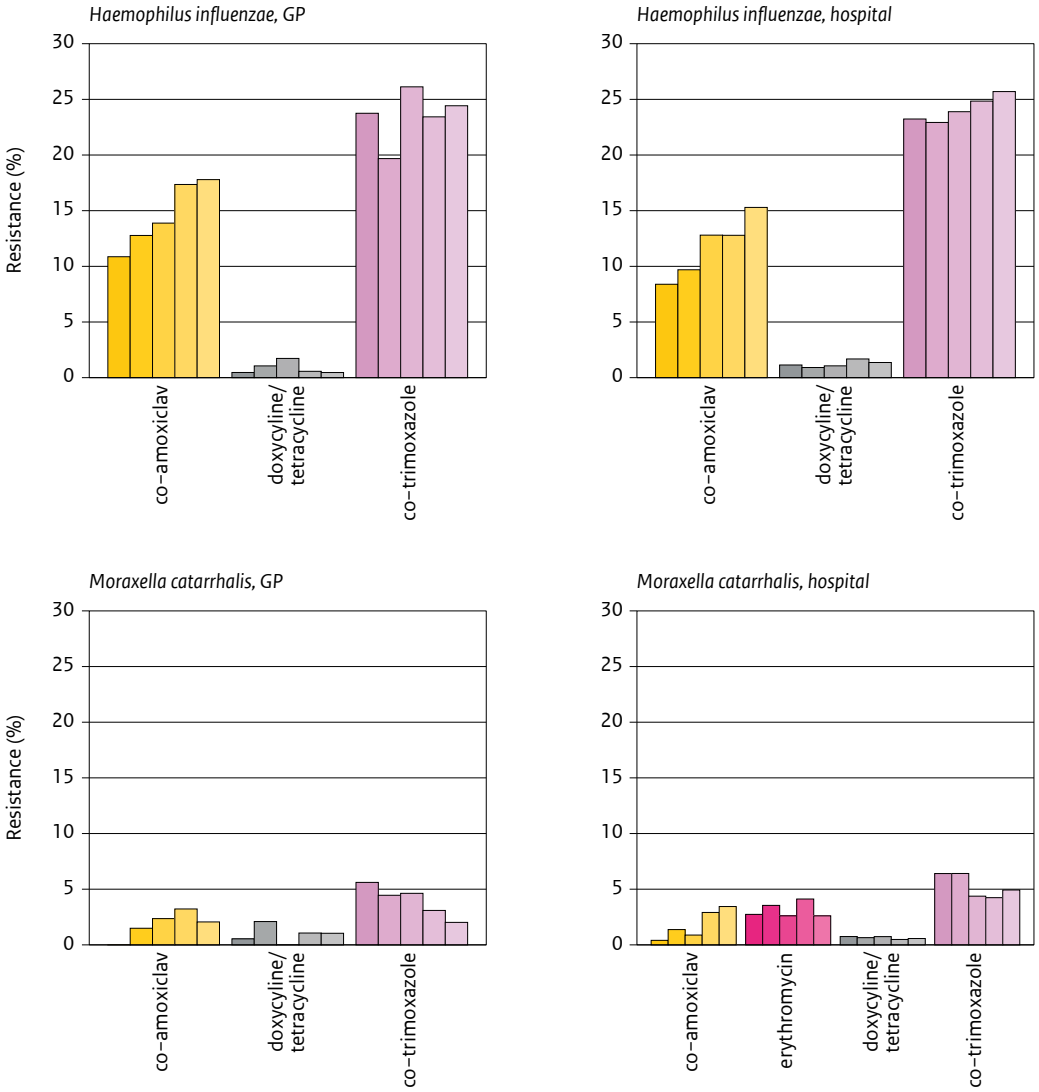
- = Resistance not calculated.
nonmen = according to breakpoint for indications other than meningitis.
men = according to breakpoint for meningitis.
[1] *Resistance to (benzyl)penicillin was estimated based on reinterpretation of oxacillin test values, or, if the result for oxacillin was I or R, on reinterpreatation 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.*

Figure 4.5.1 Trends in antibiotic resistance (from left to right 2017 to 2021) 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.
[1] *According to breakpoint for 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.*

Figure 4.5.1 (continued) Trends in antibiotic resistance (from left to right 2017 to 2021) 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.

[1] *According to breakpoint for 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.*

Key results

S. pneumoniae

- Resistance levels for GP patients could not be shown, because the number of isolates was too low.
- Resistance levels ≤10% were observed for **each of the selected agents** in hospital patients.
- A statistically significant and clinically relevant increase in resistance was observed for **co-trimoxazole** in hospital patients (from 7% in 2017 to 9% in 2021).

H. influenzae

- Resistance levels ≤10% was observed for **doxycycline/tetracycline** (≤1%).
- Resistance levels ≥20% were observed for **co-trimoxazole** (≥24%).
- A statistically significant and clinically relevant increase in resistance was observed for **co-amoxiclav** in both GP patients (from 11% in 2017 to 18% in 2021) and hospital patients (from 8% to 15%).

M. catarrhalis

- Resistance levels ≤10% was observed for **each of the selected agents** (≤5%).
- A statistically significant and clinically relevant increase in resistance was observed for **co-amoxiclav** in hospital patients (from 0% in 2017 to 3% in 2021).

4.6 Antimicrobial resistance in *Helicobacter pylori* infections

Introduction

H. pylori is a Gram-negative curved bacterium that resides only on the gastric epithelium. Primary colonization often occurs during childhood and can last a lifetime. The global prevalence of *H. pylori* carriage is estimated to range between 20%-30% in Northern and Central European countries, to over 70% in parts of Asia, Africa and Southern Europe.[1] *H. pylori* has been found an important factor in the etiology of a wide range of gastric disorders including peptic ulcer disease, chronic gastritis, Mucosa-Associated Lymphoid Tissue (MALT) lymphoma, and gastric cancer.[2] In the past decades, this highly prevalent infection has been treated with various antimicrobial regimens, and concerns about antimicrobial resistance in this pathogen are rising, also in the Netherlands.[3,4] In this section we describe (trends in) antimicrobial resistance to a selection of agents frequently used for treatment of *H. pylori* infection in the Netherlands during the period 2017-2021.

Methods

Data from 30 laboratories for which continuous data from 2017 to 2021 were available in the ISIS-AR database, were considered for analysis. We included isolates of *H. pylori* from all specimen types (as we could not distinguish gastric specimens specifically) and their antimicrobial susceptibility test (AST) data for amoxicillin/ampicillin, levofloxacin, clarithromycin, doxycycline/tetracycline, and metronidazole in the years 2017-2021. If multiple isolates per patient per year were available, we selected the first, to avoid repeated sampling causing bias in the results. To avoid bias due to selective testing of antibiotics, for each agent we included only data from laboratories that tested at least 50% of isolates for that specific agent in each year. To avoid bias due to differences in breakpoint guidelines and expert rules used in the participating laboratories, we reinterpreted MIC values according to EUCAST clinical breakpoints version 11.0, 2021. Laboratories for which less than 80% of MIC values could be reinterpreted in one or more years were excluded from analysis. Using logistic regression models on the resulting antimicrobial susceptibility categories (S/I/R), we calculated resistance ('R') percentages and linear time trends for the selected antibiotics, and for combined resistance to clarithromycin and metronidazole. Statistical significance and clinical relevance of trends were assessed using the criteria described in section 4.1.1.

Results

In total, 2 295 isolates from 30 laboratories were registered in the database for the selected time period. After the exclusion criteria were applied, the number of laboratories that could be included for the analysis ranged from 12 to 23 laboratories depending on the antimicrobial agent or combined resistance. Resistance to amoxicillin/ampicillin (7%) and doxycycline/tetracycline (1%) were lower than 10%, but resistance to levofloxacin (21%), clarithromycin (48%), and metronidazole (44%) were higher than 20% in 2021 (Table 4.6.1). Combined resistance to clarithromycin and metronidazole was 30%. Between 2017 and 2021, resistance increased to a statistically significant and clinically relevant extent for clarithromycin (from 42% to 48%), doxycycline/tetracycline (from 0% to 1%), metronidazole (from 40% to 44%), and clarithromycin + metronidazole (from 24% to 30%, Figure 4.6.1). However, it seems that the resistance for levofloxacin, clarithromycin, metronidazole and clarithromycin + metronidazole are stabilizing in the last three years. Although the trend for amoxicillin/ampicillin between 2017 and 2021 was not significant, between 2018 and 2021, resistance increased to a statistically significant and clinically relevant extent for amoxicillin/ampicillin (from 3% to 7%, Figure 4.6.1).

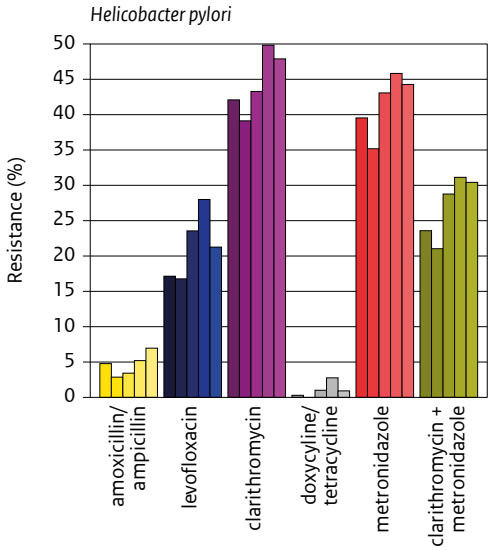
Table 4.6.1 Resistance levels (%) among isolates of *Helicobacter pylori*, ISIS-AR 2021

	Helicobacter pylori
Antibiotic	
amoxicillin/ampicillin	7
levofloxacin	21
clarithromycin	48 ↑
doxycycline/tetracycline	1 ↑
metronidazole	44 ↑
clarithromycin + metronidazole	30 ↑

10 ↑	Significant and clinically relevant increasing trend since 2017.
10 ↓	Significant and clinically relevant decreasing trend since 2017.
10*	Trend not calculated because data from the years before 2021 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 2017 to 2021) among isolates of *Helicobacter pylori* in ISIS-AR



Discussion

In *H. pylori*, substantial and increasing resistance levels were observed for clarithromycin, metronidazole, and for the combination of both agents. This finding is consistent with reports from other countries and challenges the international and national treatment guidelines.[4-7] However, the resistance percentages presented in this section should be interpreted with caution. For the culture of *H. pylori* and subsequent phenotypical antimicrobial susceptibly testing a biopsy from the gastric epithelium is required. This is not a standard procedure as *H. pylori* infection is primary 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. The resistance percentages presented in this section are therefore expected to be an overestimation of resistance in the general population. Nonetheless, the results are considered to provide a valid estimate of resistance in patients presenting with *H. pylori* infections in hospitals. In addition, the increasing time trend is expected to be valid for both populations and is alarming. Consequently, several initiatives are ongoing to get more insight in the clinical relevance of increased resistance for treatment of patients in primary healthcare and to consider alternative treatment options for multidrug resistant *H. pylori*.

References

1 Hooi JKY, Lai WY, Ng WK, Suen MMY, Underwood FE, Tanyingoh D, Malfertheiner P, Graham DY, Wong VWS, Wu JCY, Chan FKL, Sung JJY, Kaplan GG, Ng SC. Global Prevalence of *Helicobacter pylori* Infection: Systematic Review and Meta-Analysis. Gastroenterology. 2017 Aug;153(2):420-429. doi: 10.1053/j.gastro.2017.04.022. Epub 2017 Apr 27. PMID: 28456631.

2 Kusters JG, van Vliet AH, Kuipers EJ. Pathogenesis of *Helicobacter pylori* infection. Clin Microbiol Rev. 2006 Jul;19(3):449-90. doi: 10.1128/CMR.00054-05. PMID: 16847081; PMCID: PMC1539101.

3 Ruiter R, Wunderink HF, Veenendaal RA, Visser LG, de Boer MGJ. *Helicobacter pylori* resistance in the Netherlands: a growing problem? Neth J Med. 2017 Nov;75(9):394-398. PMID: 29219812.

4 Veenendaal RA, Woudt SHS, Schoffelen AF, de Boer MGJ, van den Brink G, Molendijk I, Kuijper EJ, namens de ISIS-AR-studiegroep. Verontrustende toename van antibioticaresistentie bij *Helicobacter pylori*. Ned Tijdschr Geneeskd. 2022 166:D6434.

5 Savoldi A, Carrara E, Graham DY, Conti M, Tacconelli E. Prevalence of Antibiotic Resistance in *Helicobacter pylori*: A Systematic Review and Meta-analysis in World Health Organization Regions. Gastroenterology. 2018 Nov;155(5):1372-1382.e17. doi: 10.1053/j.gastro.2018.07.007. Epub 2018 Jul 7. PMID: 29990487; PMCID: PMC6905086.

6 Megraud F, Bruyndonckx R, Coenen S, Wittkop L, Huang TD, Hoebeke M, Bénéjat L, Lehours P, Goossens H, Glupczynski Y; European *Helicobacter pylori* Antimicrobial Susceptibility Testing Working Group. *Helicobacter pylori* resistance to antibiotics in Europe in 2018 and its relationship to antibiotic consumption in the community. Gut. 2021 Apr 9:gutjnl-2021-324032. doi: 10.1136/gutjnl-2021-324032. Epub ahead of print. PMID: 33837118.

7 De Jongh E, De Wit NJ, Smeink P, van der Weele GM, Wesseler GH. NHG-standaard: Maagklachten (M36), Versie 5.0; maart 2021. (available from: richtlijnen.nhg.org/standaarden/maagklachten, accessed 21 April 2021).

4.7 Highly resistant microorganisms

4.7.1 Carbapenem-resistant and carbapenemase-producing *Enterobacterales*

Introduction

Carbapenem-resistant *Enterobacterales* (CRE) and carbapenemase-producing *Enterobacterales* (CPE), particularly *Klebsiella pneumoniae* and *Escherichia coli*, have been reported all over the world. Because carbapenems represent a drug of last resort for treatment of many enterobacterial infections, resistance poses a significant challenge to clinicians and negatively impacts patient care.[1] CRE were first described in Europe 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, and up to 2021 the Dutch national guideline suggested a gradient strip test as the first step in further investigation of isolates with automated elevated MIC.[5] This chapter describes the prevalence and confirmatory testing of CRE in the Netherlands, and molecular epidemiology of CPE. This information is 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).[6]

Prevalence and confirmatory testing of CRE in the Netherlands

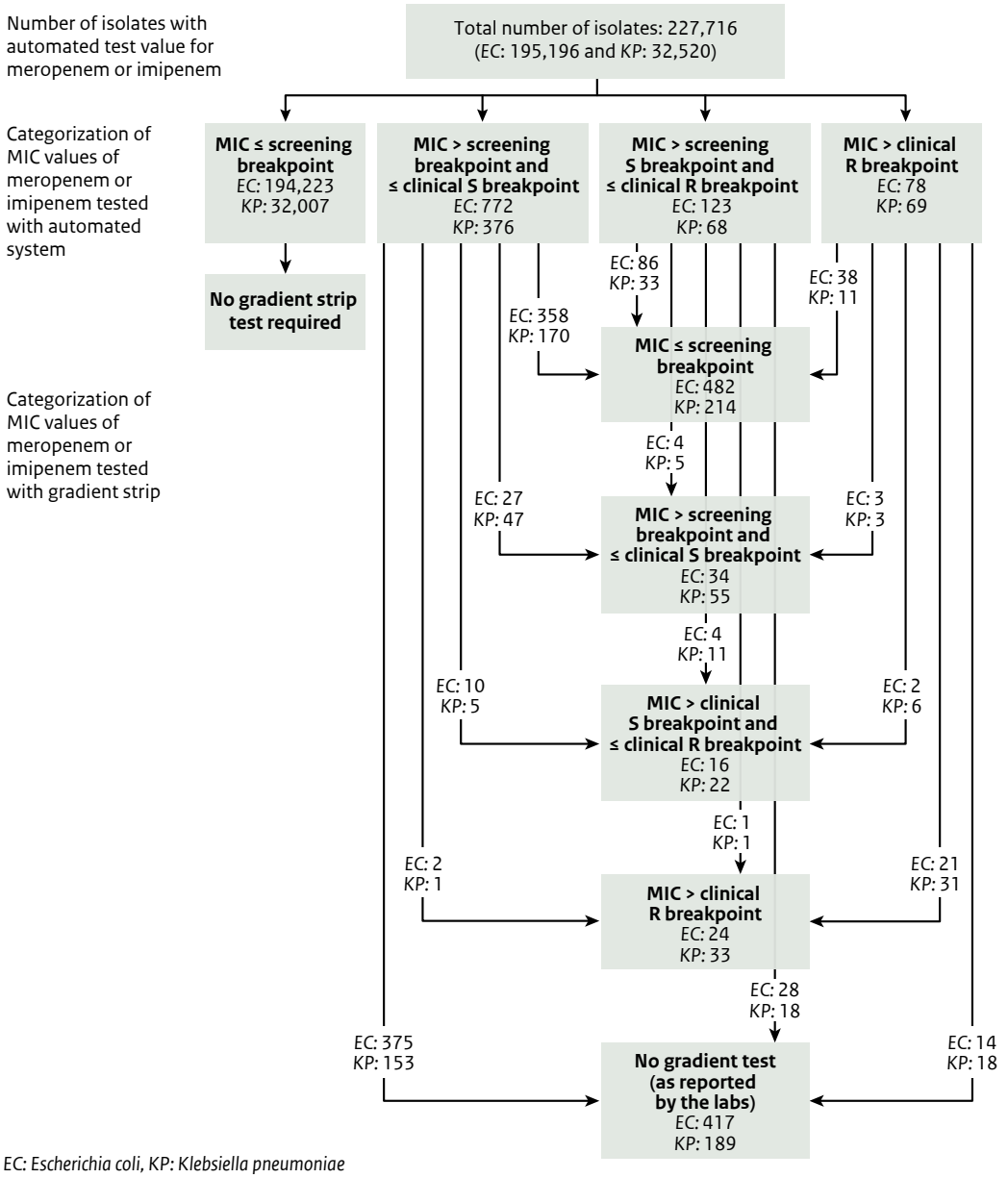
Methods

These analyses focus on *E. coli* and *K. pneumoniae* as the most prevalent *Enterobacterales* species. We searched the ISIS-AR database (years 2017-2021) for diagnostic and non-diagnostic isolates that were tested for meropenem and/or imipenem by an automated system. Several breakpoints are used in this chapter: i) the screening breakpoint as defined by the Dutch national guideline[5] (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 (referred to as elevated MIC)
iii) MIC > the clinical S breakpoint and < the clinical R breakpoint, or
iv) MIC > the clinical R breakpoint.

Subsequently, for isolates with elevated automated MIC (i.e., > the screening breakpoint), 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 species per year: an isolate with a gradient strip test was prioritized over an isolate with an automated test only. If, subsequently, multiple isolates were eligible for inclusion, we prioritized the most resistant isolate. Based on data of isolates from 37 laboratories, we calculated numbers of isolates with automated MIC in the respective categories in 2021. Subsequently, isolates with elevated automated MIC were categorized into the same categories as previously mentioned, according to gradient strip test results. Based on data from 33 laboratories that continuously submitted data to ISIS-AR from 2017 to 2021, we assessed the percentage of isolates with i) elevated MIC, in automated testing and gradient strip test confirmed separately, and ii) elevated automated MIC that underwent further testing, by year.

Figure 4.7.1.1 Results of automated and gradient strip testing of carbapenem susceptibility in *E. coli* and *K. pneumoniae* in 2021, according to NVMM guideline Laboratory detection of highly resistant microorganisms (version 2.0, 2012) in 37 laboratories participating in ISIS-AR



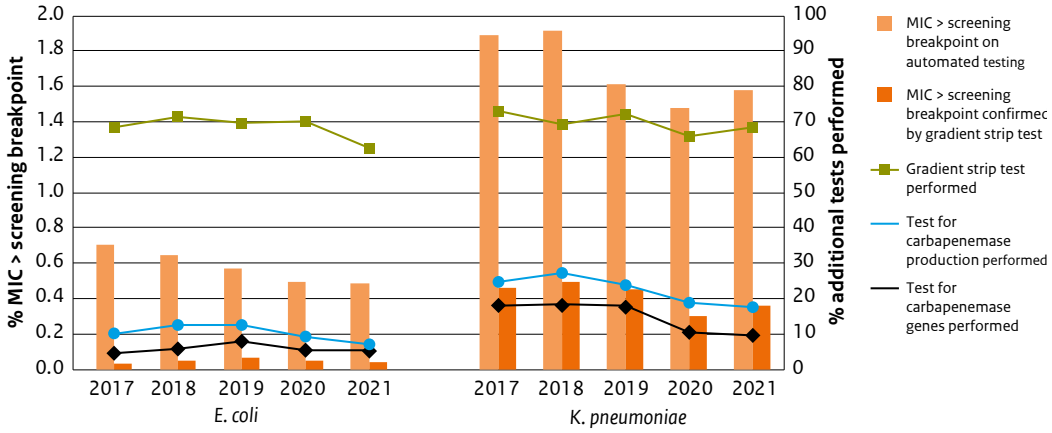
EC: Escherichia coli, KP: Klebsiella pneumoniae
Screening breakpoint: meropenem 0.25 mg/L, imipenem 1 mg/L
Clinical S breakpoint: meropenem 2 mg/L, imipenem 2 mg/L
Clinical R breakpoint: meropenem 8 mg/L, imipenem 4 mg/L
One isolate per patient per species was selected: the most completely tested and most resistant isolate (refer to Methods section)

Results

Absolute numbers of isolates and categorization according to automated and gradient strip test MICs in 2021 are presented in Figure 4.7.1.1. Of a total number of 227,716 isolates with an automated test value for meropenem or imipenem (195,196 *E. coli* and 32,520 *K. pneumoniae*), an elevated MIC on automated testing was found in 0.5% of isolates (1,486). The gradient strip method (performed in 59% of isolates with elevated MIC) confirmed elevated carbapenem MIC values in 21% (184/880) of tested isolates: 13% (74/556) of *E. coli* and 34% (110/324) of *K. pneumoniae*. Among the 973 *E. coli* isolates with an elevated MIC based on automated testing, 40 (4.1%) had an MIC above the clinical S breakpoint for the gradient strip test, of which 24 (2.5%) had an MIC above the clinical R breakpoint. Among the 513 *K. pneumoniae* isolates with an elevated MIC based on automated testing, these values were 55 (10.7%) and 33 (6.4%), respectively. Thus, gradient strip test-confirmed carbapenem resistance was calculated to be 0.01% in *E. coli* (24/195,196) and 0.10% (33/32,520) in *K. pneumoniae*.

The overall prevalence of *E. coli* strains with gradient strip test-confirmed MIC > the screening breakpoint has gradually increased between 2017 and 2019 in *E. coli* from 0.04% to 0.07% and decreased to 0.05% in 2020 and 0.04% in 2021 (Figure 4.7.1.2). For *K. pneumoniae* with gradient strip test-confirmed MIC > the screening breakpoint, the overall prevalence was stable from 2017 to 2019 at 0.50%, decreased to 0.30% in 2020 and increased again to 0.36% in 2021 (Figure 4.7.1.2). The use of gradient strip tests to confirm elevated automated carbapenem MIC values decreased from 68% in 2017 to 62% in 2021 in *E. coli* (statistically significant), and from 73% to 68% in *K. pneumoniae* (not statistically significant). In 2021, phenotypic tests for carbapenemase production were performed in 7% of *E. coli* isolates and in 18% of *K. pneumoniae* isolates with elevated MIC on automated testing, which is a decrease compared to 2017-2019 in both species. The percentage of tests for carbapenemase genes in 2021 was 5% in *E. coli* and 10% in *K. pneumoniae*, which is comparable to 2020 but lower than the percentage found in 2017-2019 (Figure 4.7.1.2).

Figure 4.7.1.2 (Additional testing of) elevated carbapenem MIC (%) in *E. coli* and *K. pneumoniae* by year, in 33 laboratories, ISIS-AR 2017-2021



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.
One isolate per patient per species was selected: the most completely tested and most resistant isolate (refer to Methods section).

Discussion

An elevated carbapenem MIC on automated testing was found in an overall 0.5% of *E. coli* and *K. pneumoniae* isolates in 2021. The actual percentage of gradient strip test-confirmed elevated MIC is much lower and is also influenced by the specificity of the automated systems and possibly by the sensitivity of the gradient strip tests. From 2021 on, the revised Dutch national guideline does not recommend the use of the gradient strip test anymore to confirm an elevated carbapenem MIC measured by an automated system.[7] The percentage of isolates with elevated automated MIC with a gradient strip test performed has decreased since 2017, especially in *E. coli*. Of note, the proportion of isolates with elevated automated MIC that underwent phenotypic or genotypic testing for carbapenemase production or genes, was lower in 2020 and 2021 compared to 2019. Potentially this relates to the COVID-19 pandemic, which caused patient populations to shift and possibly led to increased routine screening in ICU's.

Molecular epidemiology

Methods

For the enhanced surveillance of CPE by the RIVM, Dutch laboratories are requested to submit *Enterobacterales* isolates with an MIC for meropenem of >0.25 mg/L and/or an MIC for imipenem >1 mg/L and/or carbapenemase production and/or a detected carbapenemase encoding gene. For the surveillance the Type-Ned system is used, with the restriction that the laboratory can only send the first isolate from a person within a 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 third-generation 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 gene combination per person for the period 2017-2021 (based on sampling date and allele based on NGS). This included the first isolates belonging to known genetic clusters but excluded the additional isolates from the known genetic clusters. In contrast to the analyses from previous years, samples without a person ID (n=18) were not excluded but were included for further analysis if it was confirmed it represented a unique person, based on sex, age and postal code. Based on whole-genome multi-locus sequence typing (wgMLST), closely genetically related (20-25 allelic distance) *E. coli*, *K. pneumoniae*, *E. cloacae* complex and *C. freundii* complex 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 typically ≤20 alleles (25 for *E. coli*). Assigning clusters started in 2018, but includes all sequenced isolates available from the national surveillance since 2014. Except the first isolate, clusters of multiple isolates from the same patient, including over different years and/or submitted by different laboratories, were not counted.

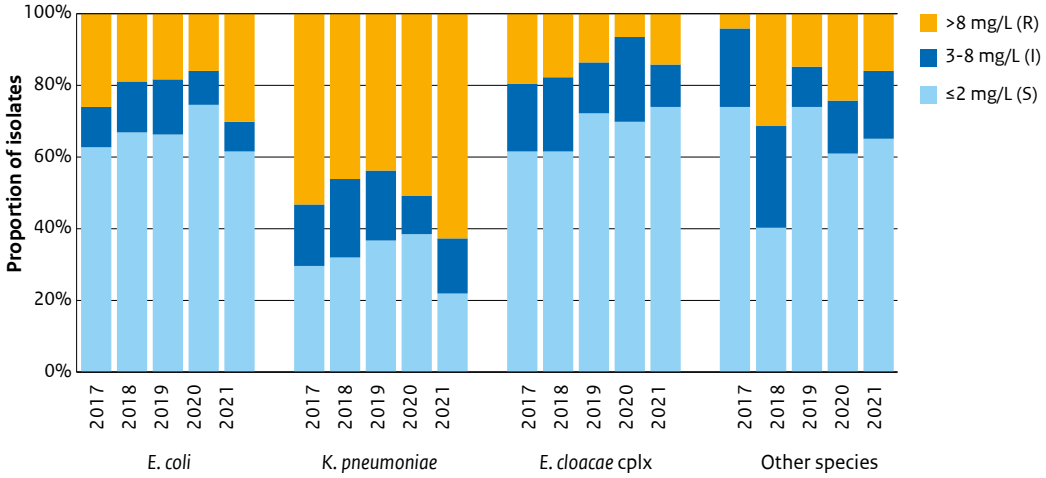
From 1 July 2019 onwards, CPE is mandatorily notifiable and since then epidemiological patient data are collected by Municipal Health Services (MHS) and entered into the national system for notifiable diseases (OSIRIS). Only notifications with a sampling date between 1 January and 31 December 2021 with status 'definite' are included in this chapter. Questionnaire data was analysed on person level and not on isolate level.

Finally, the SO-ZI/AMR database was interrogated for CPE outbreaks that were reported in 2021.

Results

Carbapenemase-production was confirmed in 242 *Enterobacterales* isolates (unique species/carbapenemase allele combinations per person) obtained in 2021 from 209 patients. The isolates were submitted to the RIVM by 40 of the 55 Dutch medical microbiology laboratories. The mean age of the patients was 60 years and 58% was male. Seventeen patients (8%) were diagnosed to have COVID-19. The number of unique carbapenemase-producing isolates submitted to the RIVM increased from 244 in 2017, to 397 in 2019 and then decreased to 225 and 242 in 2020 and 2021, respectively. This decrease can most likely be attributed to the COVID-19 pandemic and the altered patient population in hospitals at that time.
Of the 242 CPE isolates only the first genetic cluster isolate is included, and the additional 34 genetic cluster isolates are not further described. Of the remaining 208 isolates, 76 (37%) were *Klebsiella pneumoniae* complex, 70 (34%) *Escherichia coli*, and 30 (14%) *Enterobacter cloacae* complex and the remaining 32 (15%) isolates belonged to other species. When the EUCAST clinical breakpoints were applied, 76/208 (37%) had an MIC for meropenem above the cut-off of 8 mg/L. The fraction of meropenem-resistant isolates changed over this four-year time period (Figure 4.7.1.3).

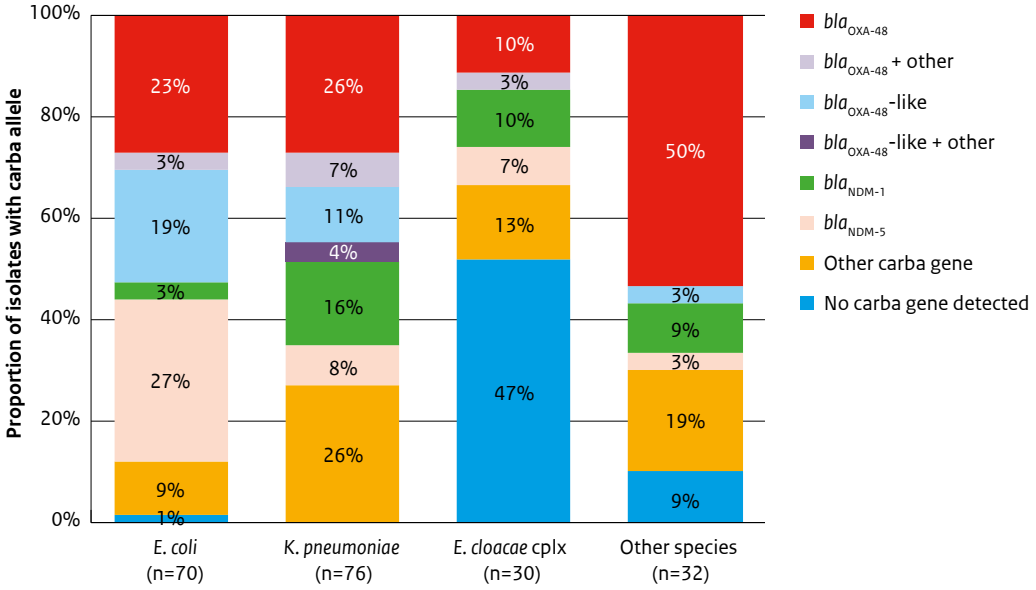
Figure 4.7.1.3 Distribution of meropenem susceptibility of CIM+ isolates of *Enterobacterales* isolates submitted with a sampling date in 2017-2021



As in previous years, the bla_{OXA-48} gene was the most frequently identified carbapenemase-encoding gene in CPE isolates cultured and submitted in 2021. The bla_{OXA-48} allele, either alone or in combination with another carbapenemase-encoding gene, was present in 26%, 34% and 13% of the *E. coli*, *K. pneumoniae* and *E. cloacae* complex, respectively (Figure 4.7.1.4). In *E. coli*, 27% of the isolates carried bla_{NDM-5} and the gene was found in 8% of the *K. pneumoniae* isolates. Conversely, bla_{NDM-1} was found predominantly in *K. pneumoniae* isolates (16%) and only in 3% of the *E. coli* isolates. The bla_{OXA-48}-like alleles ($bla_{OXA-181}$, $bla_{OXA-232}$, and $bla_{OXA-244}$) were found in 19% and 11% of the *E. coli* and *K. pneumoniae* isolates, respectively. Of the CPE analysed in 2021, 42% (88/208) carried a bla_{OXA-48} or bla_{OXA-48}-like gene. In 2021, there was a substantial decrease of submitted isolates carrying the $bla_{OXA-244}$ allele. This bla_{OXA-48}-like allele was found in *E. coli* only, comprising 7/70 (10%) of all carbapenemase-producing *E. coli* isolates submitted in 2021, compared to

33% (30/91) in 2020 and 7% (10/138) in 2019. All *bla*$_{OXA-244}$ *E. coli* isolates submitted in 2021 had MICs for meropenem ≤2 mg/L. Some of these isolates were genetically highly related, but there was no indication of transmission, as the isolates were spread over four different genetic clusters. Five percent (11/208) of the CPE carried two carbapenemase-encoding genes. In 18/208 (9%) no carbapenemase-encoding gene was detected. Of these isolates, 14 (78%) were *Enterobacter* spp. and 4 (22%) 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.

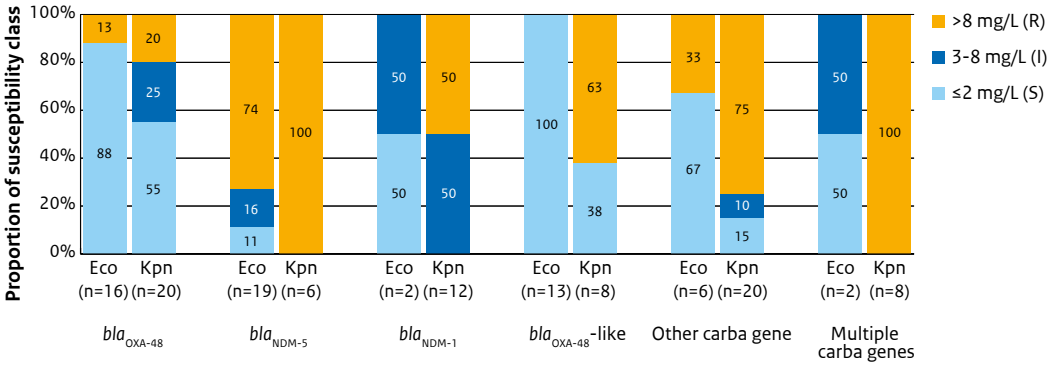
Figure 4.7.1.4 Distribution of carbapenemase-encoding genes in carbapenemase producing isolates submitted with a sampling date in 2021



bla$_{OXA-48}$*-like denotes the bla*$_{OXA-48}$ *gene variants bla*$_{OXA-181}$, *bla*$_{OXA-232}$, *bla*$_{OXA-244}$ *and bla*$_{OXA-245}$.
Carba gene is short for carbapenemase-encoding gene.

There was a strong correlation between the MIC for meropenem and the presence of particular species/carbapenemase-encoding allele combinations (Figure 4.7.1.5). In general, a larger proportion of the *K. pneumoniae* isolates (59%, 44/74) was meropenem resistant compared to the *E. coli* isolates (31%, 18/58), irrespective of the carbapenemase-encoding genes present. Only two *E. coli* isolates (2/16, 13%) carrying *bla*$_{OXA-48}$ had an MIC above the clinical breakpoint for meropenem resistance (MIC >8 mg/L). Twenty percent of the *K. pneumoniae* carrying *bla*$_{OXA-48}$ were meropenem resistant.

Figure 4.7.1.5 Relationship between the MIC for meropenem and the carbapenemase-coding genes in *E. coli* and *K. pneumoniae* isolates submitted with a sampling date in 2021



bla$_{OXA-48}$-like denotes the bla$_{OXA-48}$ gene variants bla$_{OXA-181}$, bla$_{OXA-232}$, bla$_{OXA-244}$ and bla$_{OXA-245}$.
Carba gene is short for carbapenemase-encoding gene.

Since the end of 2019, genetic cluster numbers for CPE are reported in Type-Ned. Cluster designation is available for *E. coli*, *K. pneumoniae* complex, *E. cloacae* complex, *C. freundii* complex and since 2021 for *Serratia marcescens*. Between 2017 and 2021, 536 of the 1348 (40%) of isolates of these 5 species-groups fell in one of 169 clusters. The largest clusters concerned *C. freundii* complex NDM-5 with 49 isolates, *E. cloacae* complex OXA-48 with 24 isolates, *E. coli* OXA-48 with 17 isolates and *E. coli* OXA-244 with 16 isolates. MMLs are notified by email that isolates they submitted within a period of one year are part of a genetic cluster. Of the five new clusters in 2021, two concerned multi-institutional genetic clusters, i.e., genetically highly similar isolates that were submitted by different MMLs.

Additional epidemiological questionnaire data was available in OSIRIS for 187 CPE positive persons with a sampling date in between 1 January 2021 and 31 December 2021 (Table 4.7.1.1). For 172 of the 187 definite notifications (92%) one or more isolates were identified in the Type-Ned database. For 38 persons in Type-Ned no corresponding notification could be identified in OSIRIS.
Screening was the reason for sampling in 73% of the persons in 2021, compared to 71% in 2020 and 69% in 2019. Hospitalization abroad for at least 24 hours within the previous two months was the most common reported risk factor for the presence of CPE (n=70, 37% of all notifications, 8% in persons with a diagnostic isolate, and 49% in persons with a screening isolate), with Turkey (n=24) and Morocco (n=9) leading the list of countries reported (Table 4.7.1.1. and Figure 4.7.1.6.). Most patients with a diagnostic isolate had no common risk factors identified (73%), while in persons screened 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 this was only 29%. Among persons with a diagnostic isolate, the most common reported infection was a urinary tract infection (44%, 21/48).

Table 4.7.1.1 Epidemiological data of notifications of persons carrying CPE (data from OSIRIS with sampling date 1 January-31 December 2021)[a]

Characteristic	Diagnostic and screening combined[b] n (%)	Diagnostic n (%)	Screening n (%)
Any questionnaire data available	187	48	136
Sample taking location			
At home or by a general practitioner	41 (22)	12 (25)	29 (21)
Emergency department	21 (11)	6 (13)	14 (10)
Outpatient department	25 (13)	8 (17)	17 (13)
Non-ICU	62 (33)	12 (25)	50 (37)
ICU	15 (8)	2 (4)	12 (9)
Hospital other/unknown department	19 (10)	8 (17)	10 (7)
Unknown	4 (2)	0 (0)	4 (3)
Residence			
Living independently	148 (79)	39 (81)	108 (79)
Rehabilitation centre	11 (6)	3 (6)	7 (5)
Nursing or elderly home	11 (6)	4 (8)	7 (5)
Asylum seekers centre	7 (4)	1 (2)	6 (4)
Facilities for small-scale housing for elderly	1 (1)	0 (0)	1 (1)
Other/unknown	9 (5)	1 (2)	7 (5)
Invasive medical procedure/diagnostics			
No	59 (32)	19 (40)	40 (29)
Surgery	45 (24)	13 (54)	32 (51)
Other (including invasive procedure like endoscopy, cystoscopy, urinary catheter, renal dialysis)	42 (23)	11 (23)	27 (20)
Unknown	41 (22)	5 (10)	33 (24)
Risk factors			
No risk factor known/unknown	78 (42)	35 (73)	40 (29)
Hospitalization abroad >24 hours during the previous two months	70 (37)	4 (8)	66 (49)
Hospitalized in a country in:			
West Asia (including Turkey)	28/70 (40)	2/4 (50)	26/66 (39)
North Africa	15/70 (21)	0 (0)	15/66 (23)
South Europe	12/70 (17)	1/4 (50)	11/66 (17)
South Asia	6/70 (9)	0 (0)	6/66 (9)
West Europe	3/70 (4)	0 (0)	3/66 (5)
Other region of the world/unknown	6/70 (9)	1/4 (50)	5/66 (8)

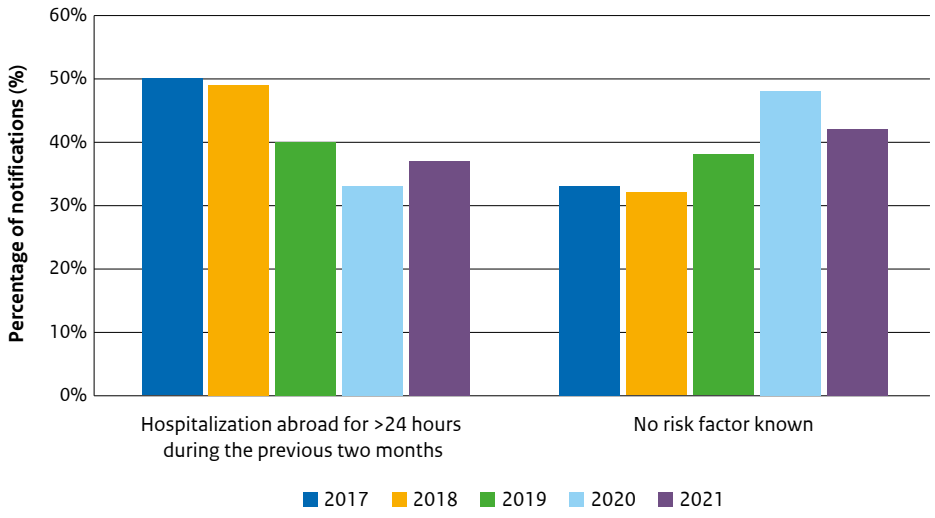
Table 4.7.1.1 (continued) Epidemiological data of notifications of persons carrying CPE (data from OSIRIS with sampling date 1 January-31 December 2021)[a]

Characteristic	Diagnostic and screening combined[b] n (%)	Diagnostic n (%)	Screening n (%)
Already known carrier of CPE	11 (6)	1 (2)	10 (7)
Received care in a department of a healthcare facility with an ongoing outbreak of CPE in the previous two months	5 (3)	1 (2)	4 (3)
Contact with a hospital abroad in the last year in a different way than >24 hours during the previous two months	9 (5)	3 (6)	6 (4)
Travelling abroad in the past twelve months without hospitalization or visiting a hospital	14 (7)	4 (8)	10 (7)

a Numbers and percentages are reported on person level with available questionnaire data for the particular characteristic unless otherwise indicated.

b Including persons for whom the reason for sampling was unknown.

Figure 4.7.1.6 Hospitalization abroad for at least 24 hours during the previous two months and no risk factor known among CPE positive persons, Type-Ned and OSIRIS, 2017-2021[a]



a Data with a sampling date from 1 January 2017-30 June 2019 from Type-Ned and from 1 July 2019-31 December 2021 from OSIRIS.

In 2021, one new outbreak with carbapenemase-producing *Enterobacterales* in a long-term care facility was reported to SO-ZI/AMR (Table 4.7.1.2). See chapter 4.7.6 for more details about SO-ZI/AMR.

Table 4.7.1.2 Outbreaks reported in 2021 to the Early warning and response meeting of Healthcare-associated Infections and Antimicrobial Resistance (SO-ZI/AMR)

Region	Main organism	Gene	No of patients
Noord-Holland West	*C. freundii*	NDM	2

Discussion

In 2020 and 2021, the number of carbapenemase-producing *Enterobacterales* isolates that was submitted to the RIVM was considerably lower than in previous years. This decrease most likely is the indirect result of the COVID-19 pandemic associated measures, such as travel restrictions and a reduction in regular healthcare. However, the fraction considered resistant for meropenem based on the EUCAST clinical breakpoints remained unchanged. No major shifts in the distribution of the composition carbapenemase-producing *Enterobacterales* were seen. The introduction of next-generation sequencing and third-generation sequencing on all carbapenemase-producing isolates now allows the identification of genetic clusters that may indicate transmission within and between healthcare centers.

Genetic clustering does not prove direct transmission or an outbreak. Also isolates that cluster together based on wgMLST may still be different in plasmid content and/or resistome. For some genetic clusters, sampling dates are several years apart. To identify direct transmission, additional patient information would be needed.

It is unknown whether or not all relevant CPE isolates are submitted to Type-Ned. The introduction of the mandatory notification of CPE led to more insight into the completeness of Type-Ned: 92% of the definite notifications have a corresponding isolate in Type-Ned. Remarkably, a substantial number of CPE isolates of positive persons are submitted to Type-Ned without a corresponding notification, which may be the result of several causes: the notification is not definite yet, the notification criteria are not exactly the same as the criteria to submit an isolate to Type-Ned, an MML did not notify the MHS or an MML did notify the MHS but the case was not reported to the RIVM for some reason.

Conclusions

- The overall percentage of *E. coli* and *K. pneumoniae* isolates with elevated carbapenem MIC values (i.e., > the screening breakpoint) on automated testing was 0.5% in 2021. Among isolates with an elevated MIC on automated testing, 6.4% had an MIC > the clinical S breakpoint and 3.8% had an MIC > the clinical R breakpoint on gradient strip testing.
- The percentage of *E. coli* and *K. pneumoniae* isolates with elevated carbapenem MIC values (i.e., > the screening breakpoint) on automated testing decreased between 2017 and 2020, with a slight increase again in 2021 in *K. pneumoniae*. However, the percentage of isolates with a gradient strip test-confirmed elevated MIC has increased between 2017 and 2019, but was lower in 2020 and 2021 compared to 2019.
- Confirmatory testing of elevated MIC values with a gradient strip method has decreased since 2017.
- The use of tests for carbapenemase production (phenotypic) or carbapenemase genes has increased between 2017 and 2018, but then decreased again to levels below those found in 2017.
- During the COVID-19 pandemic, the number of CPE submitted to the RIVM in 2021 has decreased with 39% compared to 2019, which is most likely the result of reduced travel and a reduction in regular healthcare in this period.

- The predominant carbapenemase-producing *Enterobacterales* species were *E. coli*, *K. pneumoniae* and species belonging to the *E. cloacae* complex.
- The most frequently identified carbapenemase encoding genes in *Enterobacterales* were bla_{OXA-48}, bla_{OXA-48}-like genes, bla_{NDM-1} and bla_{NDM-5}.
- The MIC for meropenem was generally higher for *K. pneumoniae* than for *E. coli* isolates harbouring $bla_{OXA-048}$ or bla_{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, *C. freundii* complex, and *Serratia marcescens* isolates found between 2017 and 2021, 40% could be categorized into one of 169 genetic clusters.
- Five new genetic clusters arose in 2021, two of which concerned multi-institutional genetic clusters. All new genetic clusters comprise two isolates only.
- Seventy-three percent of CPE cases were identified upon routine screening or targeted screening because of suspected CPE carriage.
- In 37% there is a relation with hospitalization abroad for more than 24 hours during the preceding two months, and it therefore is the main risk factor for CPE in the Netherlands. Turkey and Morocco are the countries that are most often reported.
- In 42% of the CPE positive persons no known risk factor was identified. In 51% of these cases, cultures were taken for screening purposes and 45% because of a diagnostic reason.

References

1 Tangden T, Giske CG. Global dissemination of extensively drug-resistant carbapenemase-producing *Enterobacteriaceae*: clinical perspectives on detection, treatment and infection control. J Intern Med. 2015 May;277(5):501-12.

2 Canton R, Akova M, Carmeli Y, Giske CG, Glupczynski Y, Gniadkowski M, Livermore DM, Miriagou V, Naas T, Rossolini GM, Samuelsen O, Seifert H, Woodford N, Nordmann P; European Network on Carbapenemases. Rapid evolution and spread of carbapenemases among *Enterobacteriaceae* in Europe. Clin Microbiol Infect. 2012 May;18(5):413-31.

3 Glasner C, Albiger B, Buist G, Tambić Andrasević A, Canton R, Carmeli Y, Friedrich AW, Giske CG, Glupczynski Y, Gniadkowski M, Livermore DM, Nordmann P, Poirel L, Rossolini GM, Seifert H, Vatopoulos A, Walsh T, Woodford N, Donker T, Monnet DL, Grundmann H; European Survey on Carbapenemase-Producing *Enterobacteriaceae* (EuSCAPE) Working Group. Carbapenemase producing *Enterobacteriaceae* in Europe: a survey among national experts from 39 countries, February 2013. Euro Surveill. 2013 Jul 11;18(28). pii: 20525. Erratum in: Euro Surveill. 2013;18. pii: 20575. Euro Surveill. 2014;19(47): pii=20972.

4 Nordmann P, Poirel L. The difficult-to-control spread of carbapenemase producers among *Enterobacteriaceae* worldwide. Clin Microbiol Infect. 2014 Sep;20(9):821-30.

5 NVMM Guideline Laboratory detection of highly resistant microorganisms, version 2.0, 2012.

6 Wielders CCH, Schouls LM, Woudt SHS, Notermans DW, Hendrickx APA, Bakker J, Kuijper EJ, Schoffelen AF, de Greeff SC; Infectious Diseases Surveillance Information System-Antimicrobial Resistance (ISIS-AR) Study Group; Dutch CPE Surveillance Study Group. Epidemiology of carbapenem-resistant and carbapenemase-producing Enterobacterales in the Netherlands 2017-2019. Antimicrob Resist Infect Control. 2022;11(1):57. doi: 10.1186/s13756-022-01097-9.

7 NVMM Guideline Laboratory detection of highly resistant microorganisms, 2021.

8 van der Zwaluw K, de Haan A, Pluister GN, Bootsma HJ, de Neeling AJ, Schouls LM. The carbapenem inactivation method (CIM), a simple and low-cost alternative for the Carba NP test to assess phenotypic carbapenemase activity in gram-negative rods. PloS One. 2015;10(3): e0123690. doi: 10.1371/journal.pone.0123690.

9 van der Zwaluw K, Witteveen S, Wielders L, van Santen M, Landman F, de Haan A, Schouls LM, Bosch T, Dutch CPE surveillance Study Group. Molecular characteristics of carbapenemase-producing Enterobacterales in the Netherlands; results of the 2014-2018 national laboratory surveillance. Clin Microbiol Infect. 2020. doi: 10.1016/j.cmi.2020.01.027.

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. Between 2012 and 2018, in-depth analysis of the evolutionary relatedness of *E. faecium* genotypes on a population level using multi locus sequence typing (MLST) was performed by UMC Utrecht.

Methods

VRE_{fm} outbreaks are reported through the Early warning and response meeting of Healthcare associated Infections and Antimicrobial Resistance (SO-ZI/AMR, see section 4.7.6). In 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, or, if these tests were lacking, on re-interpretation of test-values for amoxicillin/ampicillin and vancomycin according to EUCAST 2021, with VRE_{fm} being defined as resistant to amoxicillin/ampicillin and vancomycin. Both diagnostic isolates (i.e. infection-related and thus non-screening samples) and screening isolates (predominantly rectal swabs) were included. Numbers are based on data from 34 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 2021, 8 outbreaks with VRE_{fm} were reported in the Netherlands in SO-ZI/AMR (see section 4.7.6), all in hospitals, with a median reported number of 17 patients involved (range, 4 – 62 patients). This number is slightly higher than the 5 outbreaks reported in 2020, but much lower than the 19 outbreaks reported in 2019, and 10 to 15 outbreaks per year in the years before. In total, since the start of SO-ZI/AMR in April 2012, 119 outbreaks with VRE_{fm} have been reported in the Netherlands. The contribution of VRE_{fm} outbreaks 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 in general practitioner's office and (outpatient and inpatient) hospital departments in 2021 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.

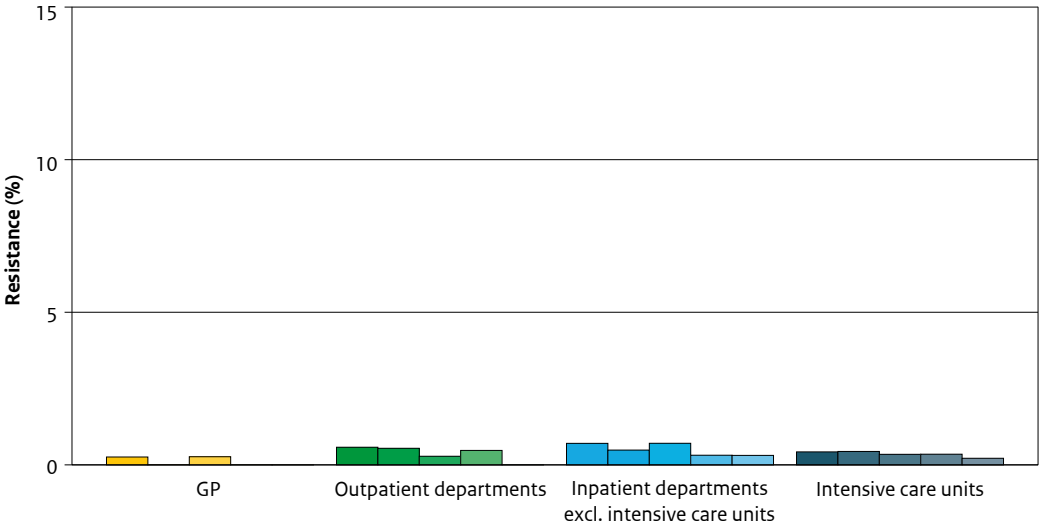
The absolute numbers of VRE_{fm} isolates from screening samples of inpatient hospital departments (including intensive care units), from 34 laboratories continuously reporting to ISIS-AR show a range of 87-130 positive isolates per year.

Table 4.7.2.1 Vancomycin-resistant *E. faecium* (VRE_{fm}) in diagnostic isolates in the Netherlands in 2021, based on ISIS-AR data

Type of setting	Tested isolates, N	VRE_{fm}, N (%)
General practitioner	482	0 (0)
Outpatient departments	430	0 (0)
Inpatient departments excl. intensive care units	2,574	8 (0.3)
Intensive care units	924	2 (0.2)
Total	**4,410**	**10 (0.2)**

Numbers are based on a selection of 34 laboratories.
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 2021, 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 2017 to 2021), based on ISIS-AR data



Numbers are based on a selection of 34 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 2021, with VRE_{fm} being defined as resistant to amoxicillin/ampicillin and vancomycin.

Table 4.7.2.2 Absolute numbers of positive vancomycin-resistant screening *E. faecium* (VRE_{fm}) isolates in the Netherlands, 2017-2021, based on ISIS-AR data

Year	Inpatient departments excluding intensive care units	Intensive care units	Total (Inpatient departments including intensive care units)
2017	131	23	154
2018	100	17	117
2019	109	13	122
2020	74	13	87
2021	101	29	130

Numbers are based on a selection of 34 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.
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 2021, with VRE_{fm} being defined as resistant to amoxicillin/ampicillin and vancomycin.

Discussion

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 since 2018. The number of reported VRE_{fm} outbreaks in 2021 was higher than the low number in 2020, but still lower compared to the previous years. This could be a result of the COVID-19 pandemic, which led to downscaling non-urgent healthcare in hospitals and a change in infection prevention measures. Although the number of screenings isolates that were obtained 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.[1,2] In 2016 in the EU/EEA (excluding the United Kingdom), the population-weighted mean percentage of invasive VRE_{fm} was 11.6% and increased significantly to 16.8% in 2020.[1] National percentages ranged from 0.0% to 56.6% and only 11 of the 29 EU/EEA countries reported percentages below 5%. Unlike resistance percentages for several other bacterial species-antimicrobial group combinations, there was no distinct geographical pattern for VRE among the included countries. Likewise, a recent retrospective observational study on vancomycin resistance in *E. faecium* and *E. faecalis* isolates from patients with bloodstream infections in the EU/EEA using data from the European Antimicrobial Resistance Surveillance Network (EARS-Net) database from 2012 to 2018, revealed that proportions of VRE_{fm} increased from 8.1% (95%CI 6.7–9.7%) in 2012 to 19.0% (95% CI 16.8–21.5%) in 2018.[3] Rising VRE_{fm} proportions were observed across all (Northern, Eastern, Southern and Western) European regions. Although it is known that enterococci are capable to develop resistance towards last resort antibiotics such as daptomycin, linezolid and/or tigecycline[4], a retrospective observational study on *E. faecium* also using data retrieved from the EARS-Net database, revealed that the population-weighted mean proportion of linezolid resistance in VRE_{fm} between 2014 and 2018 in the EU/EEA was 1.6% (95% CI 1.33-2.03%) and there was no temporal change.[5]
A national surveillance to monitor the emergence of VRE_{fm} and its resistance mechanisms, may be necessary.[4,6] In the coming year, the need for a national VRE_{fm} surveillance system will be evaluated and reconsidered.

Conclusions

- The number of reported hospital outbreaks with VRE_{fm} in 2021 was higher than in 2020, but still lower compared to 2019 and earlier, 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.
- The absolute number of positive screening VRE_{fm} isolates remains more or less stable over the years, with an exception of 2020, when the number of reported outbreaks was very low as well.
- There are no national data available on the molecular epidemiology of VRE_{fm} in Dutch hospitals.

References

1 https://www.ecdc.europa.eu/en/publications-data/antimicrobial-resistance-surveillance-europe-2022-2020-data

2 Markwart et al. The rise in vancomycin-resistant *Enterococcus faecium* in Germany: data from the German Antimicrobial Resistance Surveillance (ARS). Antimicrob Resist Infect Control. 2019 Aug 28;8:147.

3 Ayobami et al. The ongoing challenge of vancomycin-resistant *Enterococcus faecium* and *Enterococcus faecalis* in Europe: an epidemiological analysis of bloodstream infections. Emerging Microbes & Infections 2020, 9:1, 1180-1193.

4 Bender et al. Update on prevalence and mechanisms of resistance to linezolid, tigecycline and daptomycin in enterococci in Europe: Towards a common nomenclature. Drug Resist Updat. 2018 Sep;40:25-39.

5 Markwart et al. Low Proportion of Linezolid and Daptomycin Resistance Among Bloodborne Vancomycin-Resistant *Enterococcus faecium* and Methicillin-Resistant Staphylococcus aureus Infections in Europe. Front Microbiol. 2021 May 31;12:664199. doi: 10.3389/fmicb.2021.664199. eCollection 2021.

6 Babu Rajendran et al. Mandatory surveillance and outbreaks reporting of the WHO priority pathogens for research & discovery of new antibiotics in European countries. Clin Microbiol Infect. 2019 Dec 5. pii: S1198-743X(19)30620-2.

4.7.3 Methicillin-resistant *Staphylococcus aureus* (MRSA)

Introduction

The Netherlands is a country with still a low MRSA prevalence. This is most probably explained by the strict infection prevention rules ("search and destroy" MRSA policy) and the low use of antibiotics. The ISIS-AR database contains, among others, information regarding MRSA culture results from routine practices in medical microbiology laboratories. To monitor the occurrence of MRSA and the molecular characteristics of circulating MRSA types more in-depth, an enhanced MRSA surveillance at a national level was started in 1989 by the RIVM.

Methods

Prevalence

From the ISIS-AR database, *S. aureus* isolates, including MRSA, that were sampled during the five most recent years (2017 to 2021) were identified. Numbers are based on data from 34 laboratories that continuously reported complete data to the ISIS-AR database during the selected period. The first diagnostic *S. aureus* isolate per patient 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 analysis was conducted for *S. aureus* isolates from blood only.

Molecular results and epidemiology

For the enhanced MRSA surveillance, Dutch laboratories are requested to submit identified MRSA isolates using the Type-Ned system for molecular typing by multiple-locus variable number of tandem repeat analysis (MLVA). Since 2020, only 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 MRSA-screening patient materials, i.e. swabs from throat, nose, perineum and/or rectum). Livestock-associated MRSA (LA-MRSA) are defined as isolates from the MLVA-complex MC0398.
The data from the molecular surveillance were based on the first MRSA isolate per person sampled in the period 2017 to 2021, to investigate trends in molecular results, 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, *S. aureus* lacking a *mecA* or *mecC* gene, samples that could not be typed by MLVA, and isolates without a person ID were also excluded from analysis.
Since 2017, as part of the enhanced surveillance, an epidemiological questionnaire on patient characteristics is requested to be completed by the general practitioner, medical microbiologist, or infection control nurse, depending on the location of sampling. For the epidemiological analyses the same inclusion criteria were used as for the molecular analyses. Questionnaires related to isolates from employees in a healthcare facility that were screened as part of a local screening programme were excluded. Epidemiological data in this section are described for 2021 and compared with previous years, for all isolates combined and by reason for sampling.

Results

Prevalence

In ISIS-AR, the proportion of diagnostic isolates of *S. aureus* in 2021 that was identified as MRSA was 2% (n=551/29,592). The percentages were similar among the various types of departments, except for intensive care units in which the prevalence was 3% (Table 4.7.3.1). In blood isolates only, the prevalence of MRSA in 2021 was 2% (n=43/2,839). Figure 4.7.3.1 shows the trends in MRSA from 2017 to 2021 in all diagnostic isolates, which were quite stable, except in intensive care units in which the prevalence increased from ~2% in the first three years (n=34/1378 in 2017, 30/1,468 in 2018, and 20/1,312 in 2019) to ~3% in 2020 and 2021 (n=47/1,329 and 45/1,673, respectively).

Molecular results

The RIVM received 2,577 *S. aureus* isolates in 2021 that were *mecA* or *mecC* gene positive, from human origin and with a known personal identification number, submitted by 51 medical microbiology laboratories (MML). As only the first isolate per person for the period 2017-2021 was used, 2,311 isolates from 2,311 persons were included for further analyses. The absolute number of MRSA isolates included in the analysis in 2021 is comparable as in 2020 (2,379 isolates), but lower than in the years 2017-2019 (3,309, 3,152, and 3,300 isolates, respectively), which is most likely attributable to the COVID-19 pandemic.

In 2021, the persons from whom MRSA was cultured had a median age of 47 (0 - 99) years and 1,282 (55%) were male. The majority of the isolates were cultured from samples submitted to the MML from hospitals (n= 1,458; 63%), followed by GPs (n= 665; 29%) and nursing or elderly homes (n= 83; 4%). Based on culture methods and origin of the samples, 64% (n= 1,475) of the isolates were submitted as being screening samples representing swabs from nose, throat, perineum and rectum. A total of 821 isolates (36%) were categorised as diagnostic isolates, of which the majority were cultured from wound material or pus (n= 565; 69%), and 41 diagnostic isolates were cultured from blood (5%). The above-mentioned numbers are comparable to previous years.

For 2021, the MRSA population could be divided into 645 MLVA-types. The majority (590 MLVA-types, 2,215 isolates) could be grouped into 22 MLVA-complexes (MCs). For 54 MLVA-types (96 isolates) no MC could be assigned. The most frequently identified MC in 2021 was MC0398 (n= 537; 23%), also known as LA-MRSA, followed by MC0005 (n= 334; 14%) and MC0008 (n= 310; 13%). Notable, the proportion of MC0398 was higher in screening isolates (29%) than in diagnostic isolates (13%), like in previous years.

During the 2017-2021 surveillance period, there has been a trend of increasing proportion of screening isolates belonging to MC0008 and MC1933 and of diagnostic isolates belonging to MC0008 and MC0001 (Figure 4.7.3.2).

Of the 2,311 MRSA isolates submitted in 2021, 479 (21%) were Panton-Valentine Leukocidin (PVL) positive, which is lower than in the years 2017-2020 (respectively 22%, 25%, 25% and 28%). Of the 479 PVL positive isolates submitted in 2021, 230 (48%) were screening isolates and 245 (51%) were diagnostic isolates. The proportion of PVL positivity was higher in diagnostic isolates (245/821; 30%) than in screening isolates (230/1475; 16%). MC0008, MC0030 and MC0621 had the highest proportion of PVL positive isolates during the surveillance period 2017-2021 in, both, diagnostic and screening samples (Figure 4.7.3.3).

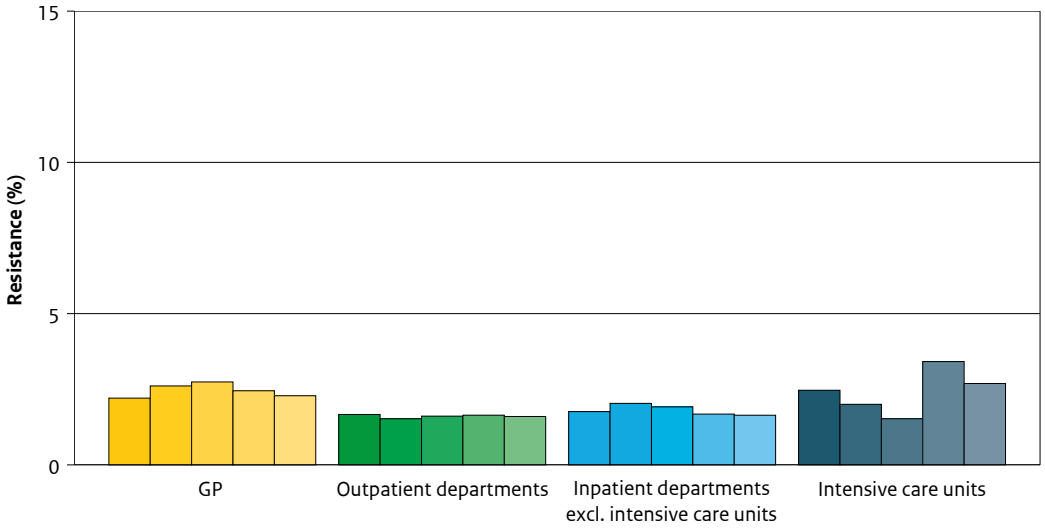
In 2021, 13 non-MLVA-typeable isolates, submitted as MRSA, were shown to be *Staphylococcus argenteus*. Of these, ten (77%) were categorised as screening isolates, one as diagnostic isolate (8%), and the remaining two could not be categorised into either screening or diagnostic.

Table 4.7.3.1 Methicillin-resistant *S. aureus* (MRSA) in the Netherlands in 2021, based on ISIS-AR data

Type of department	Tested isolates, N	MRSA, N(%)
GP	8,050	184 (2)
Outpatient departments	10,001	160 (2)
Inpatient departments excl. intensive care units	9,868	162 (2)
Intensive care units	1,673	45 (3)
Total	**29,592**	**551 (2)**

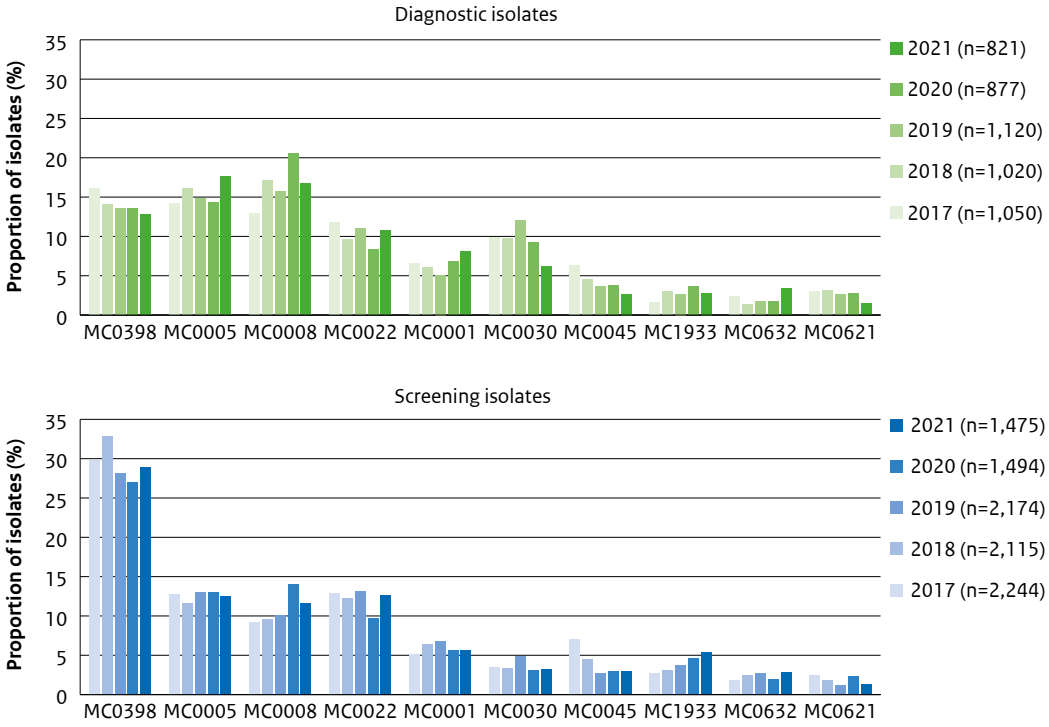
Numbers are based on a selection of 34 laboratories.
The first diagnostic S. aureus isolate per patient was selected.
The prevalence of MRSA isolates was based on positivity of confirmation tests (presence of mecA gene or pbp2), or, if these tests were lacking, on laboratory S/R interpretation for cefoxitin. If no data on a cefoxitin test was available, the prevalence was based on laboratory S/R interpretation of flucloxacillin/oxacillin.

Figure 4.7.3.1 Trends in Methicillin-resistant *S. aureus* (MRSA) in the Netherlands (from left to right 2017 to 2021), based on ISIS-AR data



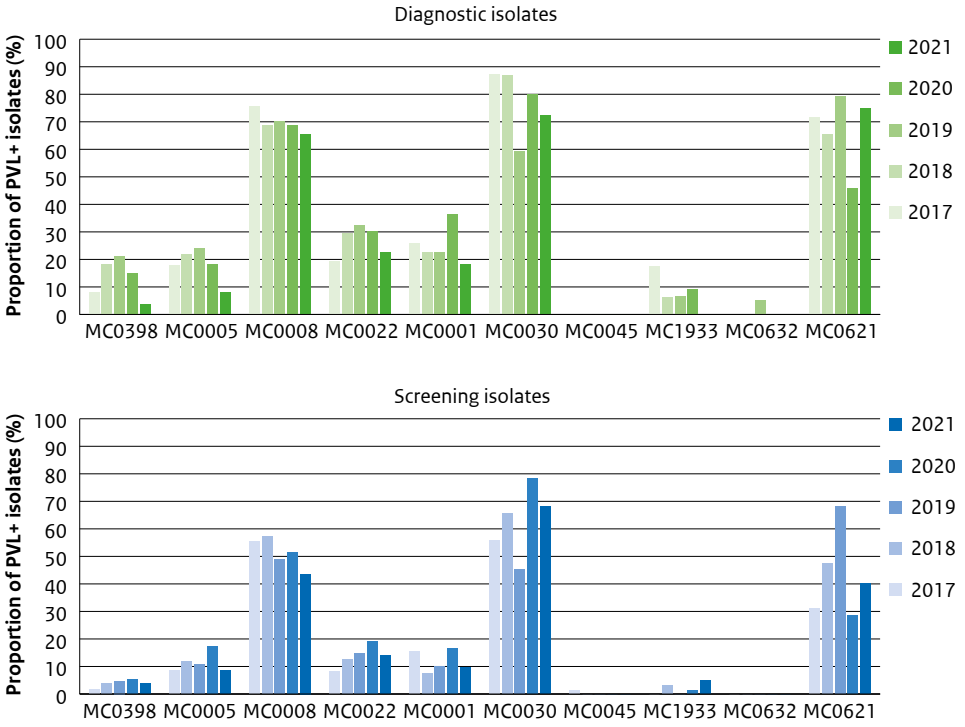
Numbers are based on a selection of 34 laboratories.
The first diagnostic S. aureus isolate per patient per year was selected.
The prevalence of MRSA isolates was based on positivity of confirmation tests (presence of mecA gene or pbp2), or, if these tests were lacking, on laboratory S/R interpretation for cefoxitin. If no data on a cefoxitin test was available, the prevalence was based on laboratory S/R interpretation of flucloxacillin/oxacillin.

Figure 4.7.3.2 Temporal trends of the ten most frequently identified MLVA complexes of MRSA in the Netherlands (2017 to 2021) among diagnostic and screening isolates, based on the 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 2017 to 2021 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.

Figure 4.7.3.3 Temporal changes of PVL positivity among the ten most frequently identified MLVA complexes of MRSA in the Netherlands (2017 to 2021), based on the 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 2017 to 2021 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.

Additional epidemiological questionnaire data for 2021, at least with regard to the reason for sampling, was available for 85% (n=1,967/2,311) of persons. For 69 (4%) 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 they were excluded from further analyses (n=1,898 remaining). Approximately two-third of patients (67%) were sampled in the hospital. Based on the information in the epidemiological questionnaire, in 43% (n=824/1,898) of patients a sample was taken for diagnostic reasons. For 50% (n=945) of patients the reason for sampling was screening/active surveillance, and for 7% (n=129) of patients the reason was unknown. For 350 patients no further information was available leaving 1,548 patients for the analysis of epidemiological characteristics.

In Table 4.7.3.2 a selection of the epidemiological data on included patients is summarized. For persons with a sample taken in the hospital, 40% were sampled in outpatient departments, 30% in inpatient departments and 8% was sampled during their stay in the Intensive Care Unit. In the group of patients that were sampled for screening/active surveillance, the large majority met the WIP risk category 1, 2, or 3[1] (87%), whereas in diagnostic isolates this proportion was much lower (40%), but increased from 14% in 2017 and 16% in 2018 to 26% in 2019 and 32% in 2020 and 2021. Work-related exposure to livestock animals was reported for 7% of patients with diagnostic samples and 27% of patients with samples that were taken for screening/active surveillance. The main group of livestock animals to which this group was exposed were pigs (78%), and from 96% of patients with a livestock related profession a LA-MRSA was sampled. Out of all patients with LA-MRSA, 37% (n=28/76) of patients with diagnostic samples, and 75% (n=149/200) of patients that were sampled for screening/active surveillance, were patients with work related exposure to livestock animals. The number of patients for whom hospitalisation abroad for at least 24 hours during the previous two months was recorded was much lower in 2020 and 2021 than in the years before (n=161/2,560 in 2017, 152/2,054 in 2018, 156/1,697 in 2019, 47/1,036 in 2020, and 55/950 in 2021), probably because of the travel restrictions during the COVID-19 pandemic. The main geographic regions of hospitalisation in 2021 were Western Asia (including Turkey) with 29% and Western Europe with 20%. Turkey was the country most frequently reported (n=13/55, 24%). In 345/942 (37%) patients an underlying illness was reported (underlying illness unknown in 606 patients), with diabetes being the most frequent illness in 123/345 (36%) patients.

Discussion

Within the ISIS-AR database all routine cultures from medical microbiological laboratories are collected. However, this can introduce bias due to selective sampling by general practitioners and to a lesser extent in hospital departments. Blood isolates are taken routinely in case of suspected bloodstream infection or meningitis, with case definitions based on uniform guidelines, and are, therefore, considered to be the least biased.

Within ISIS-AR an increase was found in the proportion MRSA in ICUs in 2020 and 2021, although the proportion in 2021 was closer to the results from 2017-2019. The explanation of this finding is currently unclear. Probably the changes in population characteristics of hospitalised patients during the COVID-19 pandemic play a role. Increased transmission is not a likely explanation since no large clusters were found based on the molecular data of the enhanced MRSA surveillance.

Within the enhanced MRSA surveillance, information on the reason for culturing is still missing for 15% to 21% of the isolates. Therefore, distinction between screening and diagnostic isolates within the analyses on molecular results, is solely based on the material and origin of the samples and misclassification of screening and diagnostic isolates might have occurred. MRSA screening isolates originate from specific PCRs or selective cultures for MRSA and cannot be used to calculate the percentage of MRSA among all *S. aureus*. The most common MLVA-complex found in the enhanced surveillance still is MC0398 (LA-MRSA). This is probably due to the national policy, where persons with exposure to livestock are actively screened for MRSA carriage. Finally, no correction for outbreaks could be made for the description of trends in the molecular epidemiology of MRSA (i.e., more than one isolate per outbreak could be included). Less than 1% of the isolates submitted as MRSA were S. argenteus. The ESCMID study group for Staphylococci and Staphylococcal diseases (ESGS) recommends this species, which is part of the *S. aureus* complex, to be handled according to the same protocols as MRSA.

Table 4.7.3.2. Epidemiological data of 1,548 MRSA positive persons (excluding employees of healthcare facilities) with a genotyped isolate in the enhanced MRSA surveillance system, with a sampling date in 2021

Characteristic	Diagnostic and screening combined[a]		Diagnostic		Screening/active surveillance	
	Data available (N)	n (%)	Data available (N)	n (%)	Data available (N)	n (%)
Proven COVID-19 infection	594	55 (9)	205	28 (14)	253	20 (8)
Sample taking location (hospital only)						
Outpatient departments	1,076	430 (40)	493	220 (45)	570	203 (36)
Inpatient departments (excluding Intensive Care Units)	1,076	328 (30)	493	159 (32)	570	168 (29)
Intensive Care Units	1,076	83 (8)	493	30 (6)	570	51 (9)
Other/unknown	1,076	235 (22)	493	84 (17)	570	148 (26)
Risk factors						
Meeting WIP[1] risk category 1,2 or 3[b,c]	1,304	871 (67)	556	221 (40)	721	629 (87)
Work-related exposure to livestock animals	1,038	185 (18)	458	31 (7)	558	152 (27)
Pigs	185	145 (78)	31	15 (48)	152	128 (84)
Cattle	185	38 (21)	31	15 (48)	152	23 (15)
Other/unknown	185	2 (1)	31	1 (3)	152	1 (1)
Hospitalization abroad >24 hours during the previous two months	950	55 (6)	433	6 (1)	504	47 (9)
Western Asia (including Turkey)	55	16 (29)	6	3 (50)	47	11 (23)
Western Europe	55	11 (20)	6	0 (0)	47	11 (23)
Southern Europe	55	3 (5)	6	0 (0)	47	3 (6)
Other/unknown country	55	25 (45)	6	3 (50)	47	22 (47)
Living in asylum centre	1,408	101 (7)	634	11 (2)	741	87 (12)

WIP: Working Party in Infection Control.

a Including persons for whom the reason for sampling was unknown.

b This question did not appear in all questionnaires and is therefore not completed for all MRSA positive persons.

c 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.

Conclusions

- Within ISIS-AR, the proportion of *S. aureus* that was MRSA positive was 2%.
- LA-MRSA MC0398 is still the predominant MRSA clade in the Dutch enhanced MRSA surveillance, constituting 29% of all screening isolates and 13% of all diagnostic isolates.
- In 2021, 30% of the diagnostic isolates carried the PVL-encoding genes, whereas 16% of the screening isolates were PVL positive. MC0008, MC0030 and MC0621 isolates had the highest proportion of PVL-positivity.
- The Dutch national MRSA surveillance has been expanded to include other methicillin-resistant species of the *S. aureus* complex, such as *S. argenteus*.
- In 43% of MRSA positive patients, the samples were taken for diagnostic reasons and this proportion is increasing over the years.
- The majority of patients with samples that were taken for screening/active surveillance, met WIP-category 1,2, or 3[1] (87%), with the main risk factor being work-related exposure to livestock animals (27%).

References

[1] Dutch Working Party on Infection Control (WIP) MRSA guidelines. 2012; available from: www.wip.nl.

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* (CRPA) as 'priority 1: critical'.[1]

Methods

Data on carbapenem-resistant and carbapenemase-producing *P. aeruginosa* (CPPA) were obtained from two different surveillance systems: ISIS-AR and the national surveillance on CPPA via Type-Ned CPE/CPPA, which started in 2020.

From the ISIS-AR database for each patient the first *P. aeruginosa* isolate per year was extracted. To avoid overestimation of the percentage CRPA 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. First, the number of phenotypical carbapenem-resistant isolates was determined (based on re-interpretation according to EUCAST 2021 using the breakpoint for imipenem of a minimal inhibitory concentration (MIC) >4 mg/L and/or the non-meningitis breakpoint for meropenem of an MIC >8 mg/L). Subsequently, for those isolates that were tested for either carbapenemase production (phenotypically) or for carbapenemase genes (genotypically) the percentage of carbapenemase-producing *P. aeruginosa* was estimated. In addition, the percentage *P. aeruginosa* that was MDR was calculated. Multidrug resistance was defined as resistance to ≥3 antimicrobial groups among fluoroquinolones, aminoglycosides, carbapenems, ceftazidime, and piperacillin-tazobactam. Only isolates which were tested for all five (groups of) antimicrobials were included in the latter analysis. The numbers were based on a selection of 34 laboratories (out of a total of 51 laboratories in the Netherlands) which provided complete data on the last five years (2017 to 2021).

In 2020 the national surveillance for CPPA was started, and medical microbiology laboratories (MMLs) could send *P. aeruginosa* isolates to the RIVM via Type-Ned CPE/CPPA for additional analyses if the isolates had an MIC for meropenem >2 mg/L or an MIC for imipenem >4 mg/L, or produced a carbapenemase or carried a gene encoding a carbapenemase. Submitted isolates were analyzed to confirm the species by MALDI-ToF. Carbapenem resistance was determined by assessing the MIC for meropenem by Etest. Carbapenemase production was evaluated by the carbapenemase inactivation method (CIM)[2] and the presence of carbapenemase-encoding genes (bla_{NDM}, bla_{KPC}, bla_{IMP}, bla_{VIM}, bla_{OXA}) by multiplex PCR. All carbapenemase-producing isolates were subjected to next-generation sequencing (NGS) and third generation sequencing (TGS). The NGS data was used to assess the genetic relationship between isolates and the presence of antibiotic resistance genes.

Results

A search in the 2021 ISIS-AR database revealed that 5% (757/15,011) of the diagnostic *P. aeruginosa* isolates were phenotypically resistant to carbapenems (Table 4.7.4.1). Approximately 3-4% of both the total number and of the carbapenem-resistant diagnostic *P. aeruginosa* isolates, were from diagnostic samples from ICUs. The observed proportion of resistance appears to be relatively stable over the 2017-2021 time period, except for a sharp decrease in the proportion of carbapenem-resistance in *P. aeruginosa* isolates from ICUs since 2020 (Figure 4.7.4.1). Of the total number of 757 carbapenem-resistant *P. aeruginosa* isolates, for 48 (6%) isolates obtained from 17 laboratories, data on tests for carbapenemase production were available, of which 8 (17%) showed a positive result.

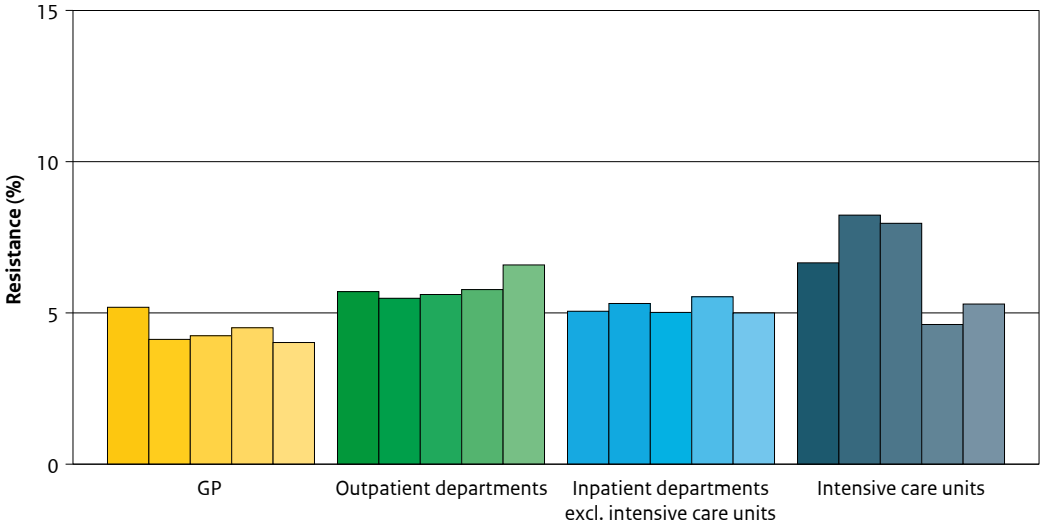
Additional analyses in the 2021 ISIS-AR database showed that 1% (n=197) of 14,266 diagnostic *P. aeruginosa* isolates tested for all five (groups of) antimicrobials included in the MDR definition, were MDR (Table 4.7.4.2) and 64% (126/197) of the MDR isolates were phenotypically resistant to carbapenems.

Table 4.7.4.1 Phenotypical carbapenem-resistant *P. aeruginosa* in the Netherlands in 2021, based on ISIS-AR data

Type of department	*P. aeruginosa*, N	Phenotypical carbapenem resistant *P. aeruginosa*, N(%)
GP	5,818	234 (4)
Outpatient departments	3,902	257 (7)
Inpatient departments excl. intensive care units	4,781	239 (5)
Intensive care units	510	27 (5)
Total	**15,011**	**757 (5)**

Numbers are based on a selection of 34 laboratories.
The first diagnostic P. aeruginosa isolate per patient was selected.
Phenotypical carbapenem resistance was defined as resistant to meropenem and/or imipenem, based on reinterpretation of test-values according to EUCAST 2021 using the non-meningitis clinical breakpoint.

Figure 4.7.4.1 Phenotypical carbapenem-resistant *P. aeruginosa* compared to the total number of *P. aeruginosa* isolates in the Netherlands (from left to right 2017 to 2021), based on ISIS-AR data



Numbers are based on a selection of 34 laboratories.
The first diagnostic P. aeruginosa isolate per patient per year was selected.
Phenotypical carbapenem resistance was defined as resistant to meropenem and/or imipenem, based on reinterpretation of test-values according to EUCAST 2021 using the non-meningitis clinical breakpoint.

Table 4.7.4.2 Multidrug resistant (MDR) *P. aeruginosa* in the Netherlands in 2021, and phenotypical carbapenem-resistant *P. aeruginosa* in relation to MDR *P. aeruginosa*, based on ISIS-AR data

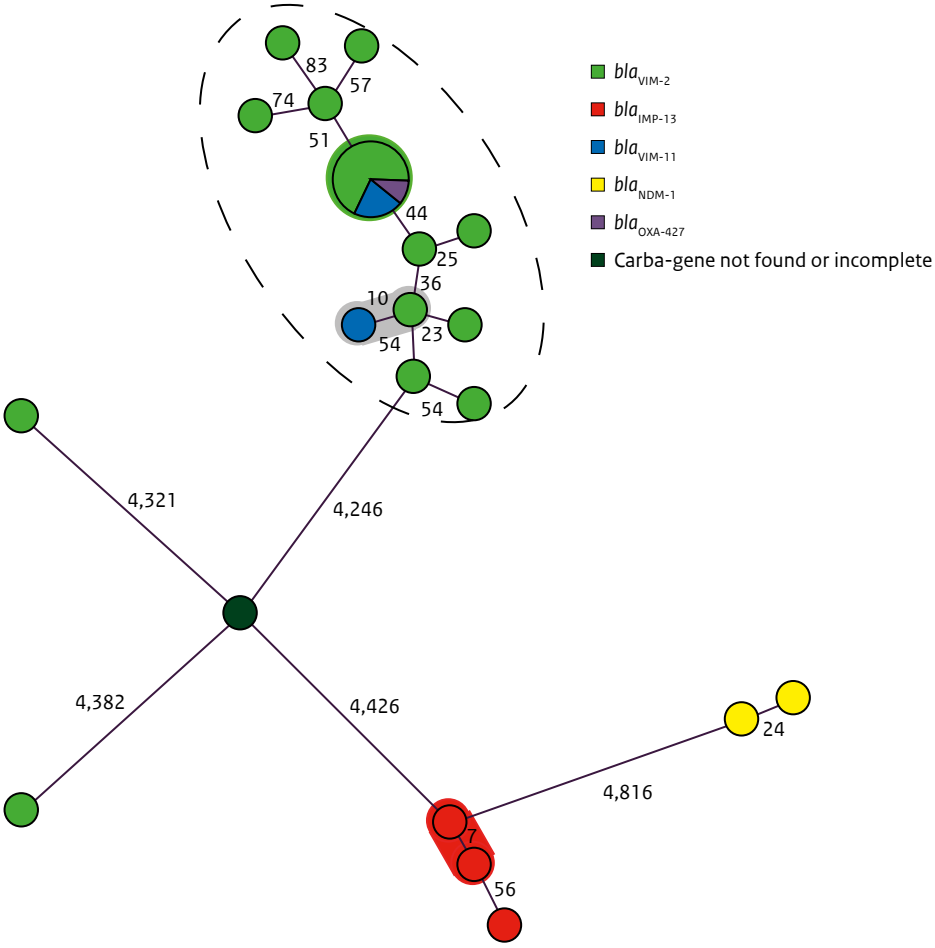
Type of department	*P. aeruginosa*, N	MDR *P. aeruginosa*, N(%)	Phenotypical carbapenem resistant *P. aeruginosa* in relation to MDR *P. aeruginosa*, N(%)
GP	5,574	23 (0)	14 (61)
Outpatient departments	3,704	97 (3)	61 (63)
Inpatient departments excl. intensive care units	4,510	58 (1)	39 (67)
Intensive care units	478	19 (4)	12 (63)
Total	**14,266**	**197 (1)**	**126 (64)**

Numbers are based on a selection of 34 laboratories.
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 2021 using the non-meningitis clinical breakpoint.
The proportion (%) of carbapenem resistance was compared to multidrug resistance.

The RIVM received 80 *P. aeruginosa* isolates via Type-Ned CPE/CPPA from 80 patients who were sampled in 2021; these isolates were submitted by 31 MMLs. Thirty of these isolates (37.5%, 30/80, one isolate per person), submitted by 12 MMLs, produced carbapenemase. None of the isolates that were negative for carbapenemase production, as determined by the CIM test, yielded a PCR product for bla_{NDM}, bla_{KPC}, bla_{IMP}, bla_{VIM}, or bla_{OXA}. Analysis of NGS data of all CPPA isolates revealed that 17 of the 27 positive isolates (63%) carried a bla_{VIM-2} allele. Of the remaining isolates, three carried bla_{VIM-11} (11%), three bla_{IMP-13} (11%), two bla_{NDM-1} (7%), a single isolate carried $bla_{OXA-427}$, and in two isolates (7%) no carbapenemase-encoding gene could be identified. The genetic relations were assessed by performing whole-genome multiple-locus sequence typing (wgMLST) (Figure 4.7.4.2). This revealed that most of the bla_{VIM-2} isolates resided in a group of genetically closely related bla_{VIM-2} carrying isolates, designated as Group 1.[3] There also were 3 genetic clusters (of ≥2 isolates differing ≤15 wgMLST alleles), representing possible transmission events among 8 institutions. Of the CPPA isolates 73% (22/30) had MICs for meropenem above the clinical (non-meningitis) breakpoint (Table 4.7.4.3), whereas 50% (25/50) of the *P. aeruginosa* not producing carbapenemase had MICs above the clinical breakpoint. The following sample materials were reported: eleven CPPA were from screening swabs, three isolates from blood, seven from sputum/bronchoalveolar lavage fluid and five from urine samples. The majority (27/30) of the CPPA were obtained from materials submitted by hospitals.
Additional epidemiological questionnaire data in Type-Ned CPE/CPPA were available for 17 of the 30 CPPA positive persons, which were all from hospital patients. In 12 (71%) of the complete questionnaires, screening was mentioned as the reason for taking the sample. In one of these 12 patients though, the sampling material from which the isolate was submitted to Type-Ned CPE/CPPA was blood, which makes screening as a sampling reason very unlikely. Three patients (18%) were admitted to the intensive care unit at the moment of sampling, 12 (71%) were admitted on a non-ICU hospital department, and two (12%) samples were taken at the outpatient department. Three persons (18%) had been admitted >24 hours in a hospital abroad in the previous two months (two in Greece and one in Turkey) and two (12%) had been

hospitalized in the previous two months in another healthcare institute with a known CPPA-outbreak. Twelve patients (71%) had (severe) co-morbidities, including four patients with malignancies, and two with bronchiectasis / cystic fibrosis. Seven patients (41%) were reported to have been using antibiotics in the two months before sampling date, of whom four had been using carbapenems and three vancomycin. More than half (9/17, 53%) of the patients had been hospitalized for more than 48 hours prior to the moment of sampling.

Figure 4.7.4.2 Minimum spanning tree of wgMLST analysis of 27 CPPA isolates from patients sampled in 2021



Each circle represents one or more (larger circle) CPPA isolates.
The lines connecting the circles denote the allelic distance.
The various carbapenemase encoding alleles are indicated in color.
The halos surrounding the circles denote genetic clusters (allelic distance ≤25).
The blaVIM-2 Group 1 isolates are indicated by the ellipse.

Table 4.7.4.3 Distribution of carbapenemase-encoding genes based on NGS in carbapenemase-producing *P. aeruginosa* isolates received via Type-Ned CPE/CPPA by the RIVM in 2021

MIC meropenem	Carbapenemase encoding alleles							Total
	bla_{VIM-2}	bla_{VIM-11}	$bla_{OXA-427}$	bla_{NDM-1}	bla_{IMP-13}	No NGS	No carba allele found	
≤ 2 mg/L (S)								0
3-8 mg/L (I)	4				1	2	1	8 (27%)
>8 mg/L (R)	13	3	1	2	2	1	1	22 (73%)
Total	**17**	**7**	**1**	**2**	**3**	**3**	**2**	**30**

NB: "No NGS" indicates that next-generation sequencing has not been performed.

Discussion

In 2021, in ISIS-AR, 5% of *P. aeruginosa* in diagnostic isolates were phenotypically resistant to carbapenems. For only 6% of these isolates, data on carbapenemase tests (phenotypically or genotypically) performed by the participating MMLs, were available in the ISIS-AR database. Of the 48 phenotypical carbapenem-resistant isolates with test results, 8 were positive for carbapenemase production. Because not all phenotypical carbapenem-resistant isolates are routinely tested on carbapenemase production or carbapenemase genes in the MMLs and such results are also not always routinely included in the data submitted to the surveillance system, the percentage of carbapenemase-producing *P. aeruginosa* will likely be underestimated. In addition, 1% of *P. aeruginosa* in diagnostic isolates were MDR, of which 64% were phenotypically resistant to carbapenems. The proportion of phenotypically carbapenem-resistant *P. aeruginosa* in ICUs was remarkably lower in 2020 and 2021 compared to the preceding three years, while these proportions in the other types of departments did not change. Due to the COVID-19 pandemic in 2020 and 2021, patient characteristics and infection prevention measures especially in ICUs were different from the years before, which might have resulted in, for example, lower transmission of and lower numbers of infections with carbapenem-resistant *P. aeruginosa*. Further research to explore these findings will be performed.

More than half of the CPPA submitted via Type-Ned CPE/CPPA carried the bla_{VIM-2} allele. Only 73% of the CPPA isolates had MICs for meropenem above the clinical (non-meningitis) breakpoint. The 2021 results were similar to those of 2020. Of the submitted isolates not producing carbapenemase, 50% had MICs for meropenem above the clinical (non-meningitis) breakpoint. It is likely this resistance is caused by other mechanisms than carbapenemase production, such as reduced cell membrane permeability, increased efflux pump activity, or AmpC activity.

For the majority of the CPPA positive persons in Type-Ned CPE/CPPA the available epidemiological data did not register a known risk factor for CPPA as described in the WIP guideline[4], i.e. previous (<2 months ago) hospitalization (>24 hrs) abroad or recent admission to a healthcare institution with a known CPPA-outbreak. Still, many of the patients had multiple co-morbidities, had been using antibiotics recently, and had been hospitalized for more than 48 hours prior to sampling, which might all be factors contributing to the risk of contracting CPPA colonization or infection.

Unfortunately, it is not yet possible to get a complete overview of carbapenem-resistant and carbapenemase-producing *P. aeruginosa* in the Netherlands, because laboratories not always routinely perform tests for carbapenemase production, and/or submitted only a selection of the relevant isolates and data to one or both of the surveillance systems ISIS-AR and Type-Ned CPE/CPPA in 2021. Therefore, the data as shown here are most likely an underestimation of the number present in the Netherlands.

Conclusions

- In 2021, 5% of the Dutch *P. aeruginosa* in diagnostic isolates were phenotypically resistant to carbapenems. Only for 6% of the phenotypically resistant *P. aeruginosa* isolates, information was reported on tests for carbapenemase production; of these 17% produced carbapenemase. In 2020 and 2021 the prevalence of carbapenem-resistant *P. aeruginosa* was lower than in previous years, and comparable to that in other departments, probably as an effect of the COVID-19 pandemic. One percent of the total number of *P. aeruginosa* isolates was MDR and 64% of these MDR isolates were carbapenem-resistant.
- The most predominant (63%) carbapenemase-encoding allele in carbapenemase-producing *P. aeruginosa* was bla_{VIM-2}.
- Only 73% of the carbapenemase-producing *P. aeruginosa* had MICs for meropenem above the EUCAST defined clinical (non-meningitis) breakpoints.
- For most of the CPPA patients no known risk factors for CPPA carriage were registered. The majority of patients had (severe) co-morbidities, and more than half of the patients had been hospitalized over 48 hours prior to sampling.
- Data from both ISIS-AR and Type-Ned CPE/CPPA could not give a complete overview of carbapenem-resistant and carbapenemase-producing *P. aeruginosa* in the Netherlands, because laboratories not always routinely perform tests for carbapenemase production, and/or submitted only a selection of the relevant isolates and data to one or both of the surveillance systems.

References

1 Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Evelina Tacconelli et al. Lancet Infect Dis 2018:18; 318–27 December 21, 2017 http://dx.doi.org/10.1016/S1473-3099(17)30753-3.

2 The Carbapenem Inactivation Method (CIM), a Simple and Low-Cost Alternative for the Carba NP Test to Assess Phenotypic Carbapenemase Activity in Gram-Negative Rods. Kim van der Zwaluw, Angela de Haan, Gerlinde N. Pluister, Hester J. Bootsma, Albert J. de Neeling, and Leo M. Schouls. PLoS One. 2015; 10(3): e0123690.

3 National surveillance pilot study unveils a multicenter, clonal outbreak of VIM-2-producing Pseudomonas aeruginosa ST111 in the Netherlands between 2015 and 2017. Janette Pirzadian et al. Sci Rep 2021;11(1):21015, doi: 10.1038/s41598-021-00205-w.

4 Dutch Working Party on Infection Control (WIP) HRMO guidelines. 2011; available from: https://www.rivm.nl/werkgroep-infectie-preventie-wip/wip-richtlijnen.

4.7.5 Extended spectrum beta-lactamases

Introduction

Extended spectrum beta-lactamase producing *Enterobacterales* (ESBL-E) have become a major concern worldwide. The prevalence of ESBL-E carriage has increased rapidly, even in countries known for prudent antibiotic use.[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 2021 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 site, i.e. general practice (GP), outpatient departments, inpatient departments and intensive care units (ICUs), in 2021. Trends in ESBL percentages (from left to right 2017 to 2021) among clinical isolates of *E. coli* and *K. pneumoniae* by site are shown in figure 4.7.5.1. The percentages of ESBL have slightly decreased for *E. coli* and *K. pneumoniae* from 2019 to 2021 for all sites, except for the ICUs. In contrast, there is a notably sharp increase in ESBL percentage from 2019 to 2021 for *K. pneumoniae* in ICUs from 12 to 15%.

Table 4.7.5.1 Extended spectrum beta-lactamase (ESBL) producing *E. coli* in the Netherlands in 2021, based on ISIS-AR data

Type of department	Tested isolates, N	ESBL
GP	113,787	3,254 (3)
Outpatient departments	16,865	746 (4)
Inpatient departments excl. intensive care units	24,794	1,263 (5)
Intensive care units	1,014	88 (9)
Total	**156,460**	**5,351 (3)**

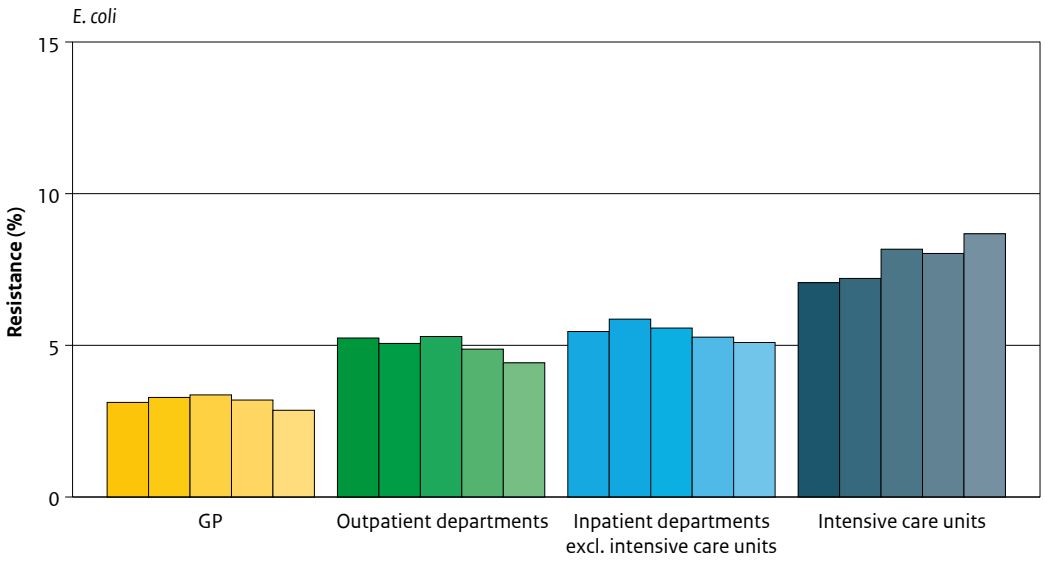
Numbers are based on a selection of 34 laboratories.
The first diagnostic E. coli isolate per patient was selected.
The percentage of ESBL producing E. coli was estimated based on positivity of confirmation tests, or, if data from these tests were lacking, resistance for third generation cephalosporins (cefotaxime/ceftriaxone/ceftazidime), based on re-interpretation of testvalues according to the meningitis breakpoint of EUCAST 2021.

Table 4.7.5.2 Extended spectrum beta-lactamase (ESBL) producing *K. pneumoniae* in the Netherlands in 2021, based on ISIS-AR data

Type of department	Tested isolates, N	ESBL
GP	15,385	496 (3)
Outpatient departments	3,865	237 (6)
Inpatient departments excl. intensive care units	5,304	414 (8)
Intensive care units	356	53 (15)
Total	**24,910**	**1,200 (5)**

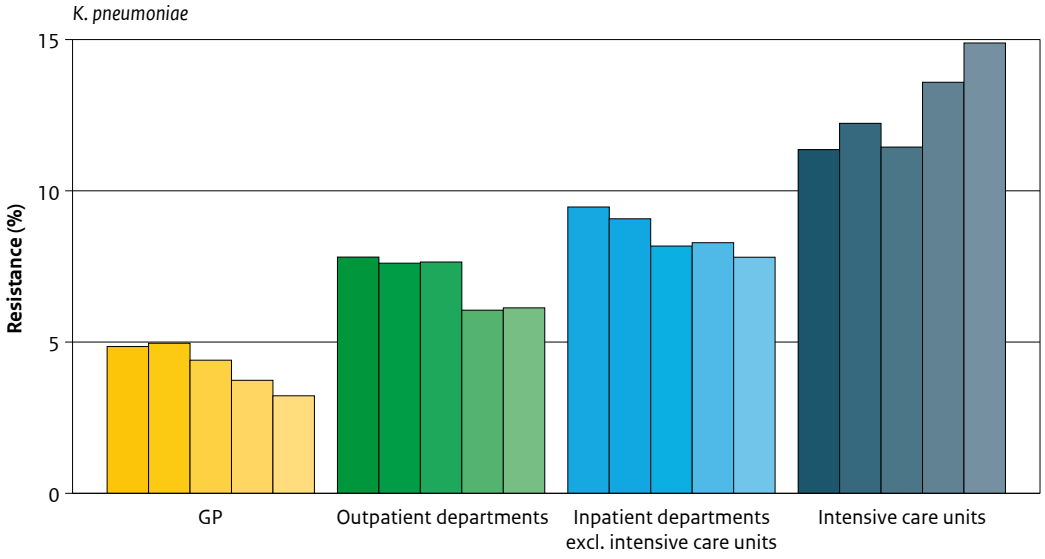
Numbers are based on a selection of 34 laboratories.
The first diagnostic K. pneumoniae isolate per patient was selected.
The percentage of ESBL producing K. pneumoniae was estimated based on positivity of confirmation tests, or, if data from these tests were lacking, resistance for third generation cephalosporins (cefotaxime/ceftriaxone/ceftazidime), based on re-interpretation of testvalues according to the meningitis breakpoint of EUCAST 2021.

Figure 4.7.5.1 Trends in extended spectrum beta-lactamase (ESBL) producing *E. coli* and *K. pneumoniae* in the Netherlands (from left to right 2017 to 2021), based on ISIS-AR data



Numbers are based on a selection of 34 laboratories.
The first diagnostic E. coli and K. pneumoniae isolate per patient per year was selected.
The percentage of ESBL producing E. coli and K. pneumoniae was estimated based on positivity of confirmation tests, or, if data from these tests were lacking, resistance for third generation cephalosporins (cefotaxime/ceftriaxone/ceftazidime), based on re-interpretation of testvalues according to the meningitis breakpoint of EUCAST 2021.

Figure 4.7.5.1 (continued) Trends in extended spectrum beta-lactamase (ESBL) producing *E. coli* and *K. pneumoniae* in the Netherlands (from left to right 2017 to 2021), based on ISIS-AR data



Numbers are based on a selection of 34 laboratories.
The first diagnostic E. coli and K. pneumoniae isolate per patient per year was selected.
The percentage of ESBL producing E. coli and K. pneumoniae was estimated based on positivity of confirmation tests, or, if data from these tests were lacking, resistance for third generation cephalosporins (cefotaxime/ceftriaxone/ceftazidime), based on re-interpretation of testvalues according to the meningitis breakpoint of EUCAST 2021.

Discussion

A slight decrease of the proportion of ESBL-producing *Enterobacterales* was found from 2019-2021 at all sites, except for ICUs. In France, a decrease in the proportion of ESBL in *E. coli* infections during the COVID-19 pandemic was found, which might be explained by factors such as reduced international travel due to COVID-19 pandemic restrictions, less antimicrobial usage by patients due to decreases of in-person visits by doctors and improved handhygiene.[2] Previous research has shown that international travel contributes substantially to the emergence of ESBL-producing *Enterobacterales* in the Dutch population.[3] The sharp increase of the proportion of ESBL-producing *K. pneumoniae* in ICUs in 2020 and 2021 compared to 2019 might also be related to the COVID-19 pandemic. The patient population in hospitals and especially in ICUs was different compared to previous years, with longer lengths-of-stay in ICUs potentially leading to an increase of resistant gram-negative bacteria through selection following antimicrobial treatment. Unfortunately, no additional epidemiological data on the patient or genotypical information of these isolates was available, and it is unknown if transmission of resistant strains between patients might have played a role.

Conclusions

- From 2019 to 2021, the percentages of ESBL for *E. coli* and *K. pneumoniae* slightly decreased in general practice (GP), outpatient departments and inpatient departments, while there was a sharp increase in the percentage of ESBL to 15% for *K. pneumoniae* in the intensive care units.

References

1 European Antimicrobial Resistance Surveillance Network (EARS-Net).

2 Olivier Lemenand O, Coeffic T, Thibaut S, Colomb Cotinat M, Caillon J and Birgand G. Decreasing proportion of extended-spectrum beta-lactamase among *E. coli* infections during the COVID-19 pandemic in France. Journal of Infection, 2021 Dec; 83: 664-670.

3 Arcilla MS, van Hattem JM, Haverkate MR, Bootsma MCJ, van Genderen PJJ, Goorhuis A, Grobusch MP, Lashof AMO, Molhoek N, Schultsz C, Stobberingh EE, Verbrugh HA, de Jong MD, Melles DC, Penders J. Import and spread of extended-spectrum β-lactamase-producing *Enterobacteriaceae* by international travellers (COMBAT study): a prospective, multicentre cohort study. Lancet Infect Dis. 2017 Jan;17(1):78-85.

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 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

In 2021, health care facilities sent outbreak notifications using a standardized webbased 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.

Results

Table 4.7.6.1 provides an overview of the 27 outbreaks reported in 2021. These were reported by 23 different healthcare institutions: 20 outbreaks in hospitals and 7 in LTCFs. Most outbreaks (n=22) ended in 2021 and 5 ended in 2022. As reported in the table, the most frequent reason for notification of an outbreak in a hospital was the imminent closure of wards (70%); a few were notified because transmission of outbreak strains was ongoing despite infection control measures. The median number of patients involved in outbreaks in hospitals (12) was higher as in the previous years (4 in 2020 and in 2019), and higher compared to LTCFs (4), and the maximum number of involved patients was almost four times as high in hospitals (62 vs 16).
VRE outbreaks were most often reported, all in a hospital setting. In contrast to earlier years, 5 methicillin-resistant *Staphylococcus aureus* (MRSA) outbreaks were reported in 2021. Still, in LTCFs MRSA was most often reported, although in 2021 the number was very low compared to previous years (11 in 2020, 17 in 2019). One outbreak of carbapenemase-producing *Enterobacterales* (various species) in a LTCF was reported, which was related to an earlier CPE outbreak in a nearby hospital. Five outbreaks of COVID-19 were reported to SO-ZI/AMR, of which 4 in hospitals.
Thirteen of the 27 reported outbreaks included more than 10 patients, which was almost half of the total number. Four outbreaks were classified as phase 2, meaning that the outbreaks lasted longer than expected. One of those was a long-lasting hospital outbreak with ESBL-positive and fluoroquinolone- and aminoglycoside-resistant (FAR) *K. pneumoniae* on a clinical haematology ward, which retrospectively had already started in October 2020. The outbreak was considered to be contained in March 2022, and had a duration of more than 500 days. The single-hospital outbreak strain (typed by whole generation

sequencing) was detected in 15 patients during the total period, of whom 3 patients had positive blood cultures with the outbreak strain. Two other outbreaks that were classified in phase 2 concerned VRE outbreaks in hospitals with 62 and 39 patients, respectively. The VRE outbreak with 62 colonized patients was spread over several clinical wards within the same hospital and had a total duration of 268 days. However, 6 different genetic strains, based on whole-genome sequencing, were detected, and thus different transmission chains were involved.
Finally, the fourth outbreak classified in phase 2 was an outbreak with carbapenemase-producing *Acinetobacter* spp. on an intensive care unit. Here extra advice by the SO-ZI/AMR consultation team was requested, since transmission was ongoing in spite of the infection prevention measures taken. Potential causes for ongoing transmission were discussed with the reporting medical microbiologist, and assistance by additional sequence typing of the involved strains was offered. These measures were considered to be helpful in managing the outbreak.
One outbreak in LTCF was reported which lasted 383 days. It concerned an outbreak of *Sarcoptes scabiei*, involving 16 patients and 15 healthcare workers. The outbreak was classified in phase 1 however, because of the high contamination rate and the long incubation period, it took a long time before this outbreak could be considered as contained.

Discussion

The total number of 27 outbreaks in 2021 was lower than the number in 2020 (34), and again remarkably lower than in 2017-2019, when 59 or 60 outbreaks were reported each year. Most likely, this is related to the COVID-19 pandemic and 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, although not very likely, it cannot be ruled out that in fact 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. The number of healthcare-associated outbreaks will be followed up in the coming years, and it remains to be seen if the number will increase again to the levels pre-COVID-19.
Although not being HRMO, 5 healthcare-associated outbreaks of COVID-19 were reported to the SO-ZI/AMR, 6 in hospitals and 1 in a long-term care facility. It is known (e.g. through the media) that much more healthcare-associated outbreaks of COVID-19 have taken place in 2021 and that the reported outbreaks to the SO-ZI/AMR are only a fraction of the true number of outbreaks.
In 2022, some changes in the SO-ZI/AMR will be implemented, concerning the criteria for reporting an outbreak and a renewed classification method, and a simplified web-based reporting system will be introduced.[2] The results of these changes will be evaluated in the coming years.

Table 4.7.6.1 Characteristics of outbreaks reported to the SO-ZI/AMR in 2021

	Hospitals n=20 n (%)	LTCFs n=7 n (%)	Total 2021 n=27 n (%)
Microorganism (resistance mechanism)[1]			
Enterococcus faecium (VRE)	8 (40)		8 (30)
Staphylococcus aureus (MRSA)	2 (10)	3 (43)	5 (19)
COVID-19	4 (20)	1 (14)	5 (19)
Sarcoptes scabiei	1 (5)	1 (14)	2 (7)
Enterobacterales (CPE) (various species)		1 (14)	1 (4)
Acinetobacter (CPA)	1 (5)		1 (4)
Enterobacter cloacae (ESBL)	1 (5)		1 (4)
Klebsiella pneumoniae (ESBL, FAR)	1 (5)		1 (4)
Escherichia coli (FAR)		1 (14)	1 (4)
Serratia marcescens	1 (5)		1 (4)
Norovirus	1 (5)		1 (4)
Reason of reporting			
threatening of ward closure	15 (75)	4 (57)	19 (70)
ongoing transmission	2 (10)		2 (7)
combination of both reasons above	1 (5)		1 (4)
HRMO outbreak (not in a hospital)		3 (43)	3 (11)
unknown	2 (10)		2 (7)
Highest level phase[2]			
phase 1	16 (80)	7 (100)	23 (85)
phase 2	4 (20)		4 (15)
Median number of patients (range)	12 (2-62)	4 (1-16)[3]	10 (1-62)[3]
Median duration outbreak in days from start or reporting date until end of the outbreak (range)	51 (4-523)	70 (11-383)	52 (4-523)
Request for help	1 (5)	0	1 (4)

n: number of outbreaks

1 *VRE=vancomycin-resistant Enterococcus faecium; MRSA=methicillin-resistant Staphylococcus aureus; CPE=carbepenemase-producing Enterobacterales; CPA=carbapenemase-producing Acinetobacter; ESBL=extended-spectrum β-lactamase producing; FAR=fluoroquinolone- and aminoglycoside-resistant*

2 *Based on this risk assessment (including updates after follow-up), outbreaks are categorized in one of six phases with 1 as lowest, 5 as highest risk. Once an outbreak is contained it is classified as phase 0. Phase 1: no further implications for (public) healthcare to be expected, the outbreak is expected to be contained soon. Phase 2: transmission of the outbreak strain continues despite appropriate infection control measures.*

3 *In one outbreak, one patient and one health care worker were involved.*

Conclusions

- On average two outbreaks a month were reported to the SO-ZI/AMR in 2021, which is lower than in 2020, and much lower as in the previous years, most likely due to the COVID-19 pandemic.
- Most outbreaks were classified as phase 1 and four hospital outbreaks as phase 2.
- The majority of the outbreaks were reported to SO-ZI/AMR within a month after detection.
- Most outbreaks were due to VRE (which were all reported by hospitals).
- The median number of patients involved in an outbreak was 10.
- Three outbreaks lasting longer than 180 days were reported.

References

1 Nederlandse Zorgautoriteit Beleidsregel BRMO-uitbraak - BR/REG-22116. Available from: https://puc.overheid.nl/nza/doc/PUC_641537_22/ [Accessed 26th April 2022]

2 https://www.rivm.nl/surveillance-van-infectieziekten/signalering-infectieziekten/signaleringsoverleg-zi-amr

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, Location AMC, Amsterdam. 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 2012-2021, a total of 340 strains from CSF (CSF and blood) and 722 strains from blood were included in the surveillance project of the NRLBM. The total number of isolates per year ranged between 186 (2018) to 25 (2021). Isolates from patients with a blood isolate and PCR-positive CSF sample are included in the CSF group. The MIC (minimal inhibitory concentration) 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 addition, the nucleotide sequence of *penA* coding for penicillin binding protein 2 (PBP2) was determined.[1,2] 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 2021, all 25 meningococcal isolates, isolated from CSF (n=7) and/or blood (n=18), were susceptible to penicillin, compared to 53 out of 54 (98%) isolates in 2020 (Table 4.8.1.1 and 4.8.1.2). None of the isolates was resistant to rifampicin. In accordance with phenotypic penicillin susceptibility, no alterations in the *penA* gene that are associated with decreased susceptibility to penicillin[2], were detected (Table 4.8.1.3).

Discussion

The breakpoint for phenotypic penicillin susceptibility was recently altered by EUCAST, whereby only 2 categories remain; isolates are either susceptible or resistant to penicillin. Based on these new criteria, it is clear that penicillin resistance in *N. meningitidis* isolates in the Netherlands is rare, with only 8 out of 1062 (0.8%) isolates displaying phenotypic penicillin resistance in the previous 10 years of which none in 2021. In addition, alterations in *penA* that are associated with resistance to penicillin were not detected in any of the isolates in 2021.

Table 4.8.1.1 Susceptibility of *N. meningitidis* isolated from CSF or CSF and blood to penicillin, 2012-2021

	Penicillin				
	MIC* ≤ 0.25		MIC* > 0.25		Total
	n	%	n	%	
2012	40	**97.5**	1	**2.4**	41
2013	41	**97.6**	1	**2.4**	42
2014	33	**99.9**	0	**0**	33
2015	32	**100.0**	0	**0**	32
2016	36	**100.0**	0	**0**	36
2017	46	**100.0**	0	**0**	46
2018	54	**98.2**	1	**1.8**	55
2019	33	**100.0**	0	**0**	33
2020	14	**93.4**	1	**6.6**	15
2021	7	**100**	0	**0**	7

* *MIC values in mg/L.*

Table 4.8.1.2 Susceptibility of *N. meningitidis* isolated from blood only to penicillin, 2012-2021

	Penicillin				
	MIC* ≤ 0.25		MIC* > 0.25		Total
	n	%	n	%	
2012	40	**100.0**	0	**0**	40
2013	69	**98.6**	1	**1.4**	70
2014	40	**100.0**	0	**0**	40
2015	52	**100.0**	0	**0**	52
2016	101	**100.0**	0	**0**	101
2017	128	**99.2**	1	**0.8**	129
2018	129	**98.5**	2	**1.5**	131
2019	102	**100.0**	0	**0**	102
2020	39	**100.0**	0	**0**	39
2021	18	**100.0**	0	**0**	18

* *MIC values in mg/L.*

Table 4.8.1.3 Alterations in the *penA* gene and penicillin susceptibility in *N. meningitidis*, 2021

	Number (%) of strains with penicillin MIC*	
Alterations *penA***	**MIC ≤ 0.25**	**MIC > 0.25**
Yes	0	0
No	25	0
Total	**25**	**0**

* *MIC values in mg/L.*

** *Resulting in five amino acids substitutions in PenA associated with non-susceptibility to penicillin.*[1]

Conclusions

- Number of invasive meningococcal disease cases decreased by 82% in 2021 compared to 2019 (pre-COVID-19).
- Penicillin resistance in *N. meningitidis* isolates is rare in the Netherlands.
- Resistance to rifampicin and ceftriaxone was not found in 2021.

References

1 Vázquez JA, Arreaza L, Block C, Ehrhard I, Gray SJ, Heuberger S, Hoffmann S,Kriz P, Nicolas P, Olcen P, Skoczynska A, Spanjaard L, Stefanelli P, Taha MK,Tzanakaki G. Interlaboratory comparison of agar dilution and Etest methods for determining the MICs of antibiotics used in management of *Neisseria meningitidis* infections. Antimicrob Agents Chemother. 2003;47:3430-4.

2 Taha MK, Vázquez JA, Hong E, Bennett DE, Bertrand S, Bukovski S, Cafferkey MT, Carion F, Christensen JJ, Diggle M, Edwards G, Enríquez R, Fazio C, Frosch M, Heuberger S, Hoffmann S, Jolley KA, Kadlubowski M, Kechrid A, Kesanopoulos K, Kriz P, Lambertsen L, Levenet I, Musilek M, Paragi M, Saguer A, Skoczynska A, Stefanelli P, Thulin S, Tzanakaki G, Unemo M, Vogel U, Zarantonelli ML. Target gene sequencing to characterize the penicillin G susceptibility of *Neisseria meningitidis*. Antimicrob Agents Chemother. 2007;51:2784-92. Epub 2007 May 21.

4.8.2 *Neisseria gonorrhoeae*

Introduction

Neisseria gonorrhoeae is a species of Gram-negative bacteria responsible for the sexually transmitted infection (STI) gonorrhoea. Gonorrhoea is the second most common bacterial 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. However, the susceptibility of gonococci to these cephalosporins has been decreasing. *Neisseria gonorrhoeae* has developed antimicrobial resistance to most drugs used for treatment in the past, including azithromycin, which is used as dual therapy in combination with ceftriaxone in some countries and can be used as an alternative treatment in patients allergic to ceftriaxone.

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 2021, 15 out of the 24 SHC participated in GRAS, which together accounted for 85% of SHC gonorrhoea diagnoses. Diagnosis of gonorrhoea is made by PCR on patients' materials. For GRAS, additional culture and susceptibility testing is performed using Etest. Isolates are tested for ciprofloxacin, cefotaxime, ceftriaxone, and azithromycin. Resistance levels are calculated using the EUCAST breakpoints for resistance.[1]

Results

The number of gonorrhoea diagnoses reported by SHC participating in GRAS has been between 5,000 and 6,000 since 2015, and increased to 6,784 diagnoses in 2021. The percentage of diagnoses including a susceptibility test has been stable around 39% since 2016 (39.3% in 2021, 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 52.9% in 2021. Resistance to cefotaxime has been slowly decreasing since 2012 and was 0.1% in 2021. For azithromycin, resistance steadily increased from 2.1% in 2012 to 10.8% in 2018, was stable around 10% in 2019 and 2020, but increased again to 18.0% in 2021. No resistance was reported to ceftriaxone (Figure 4.8.2.2).

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, 2012-2021

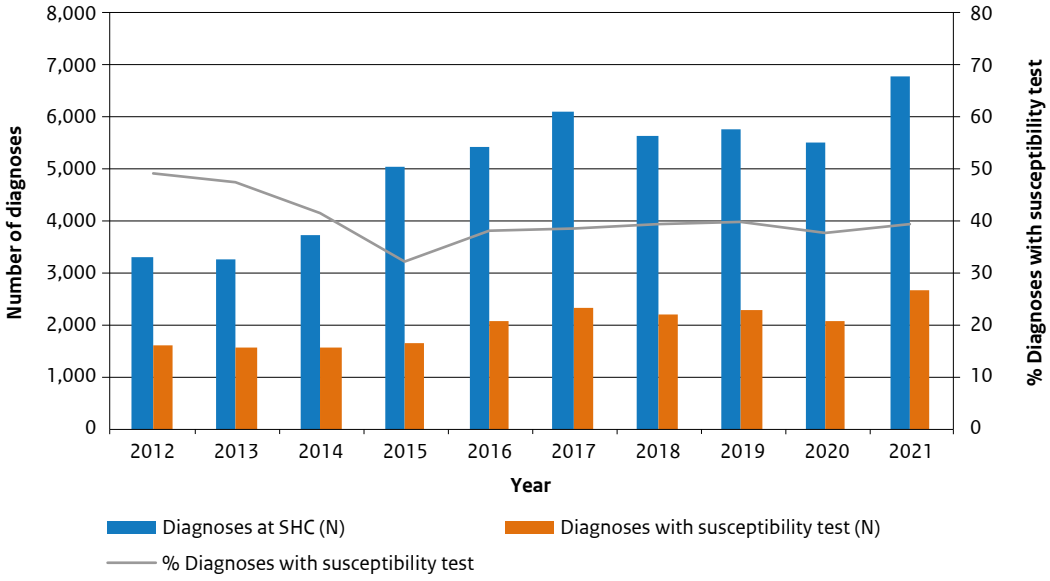
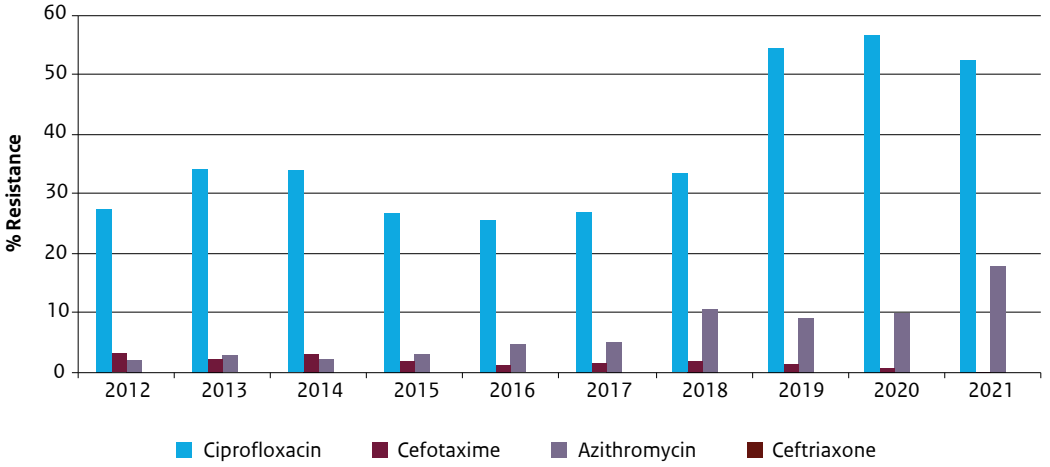


Figure 4.8.2.2 Trends in antimicrobial resistance among *Neisseria gonorrhoeae* (following EUCAST breakpoints) in the Netherlands, 2012-2021

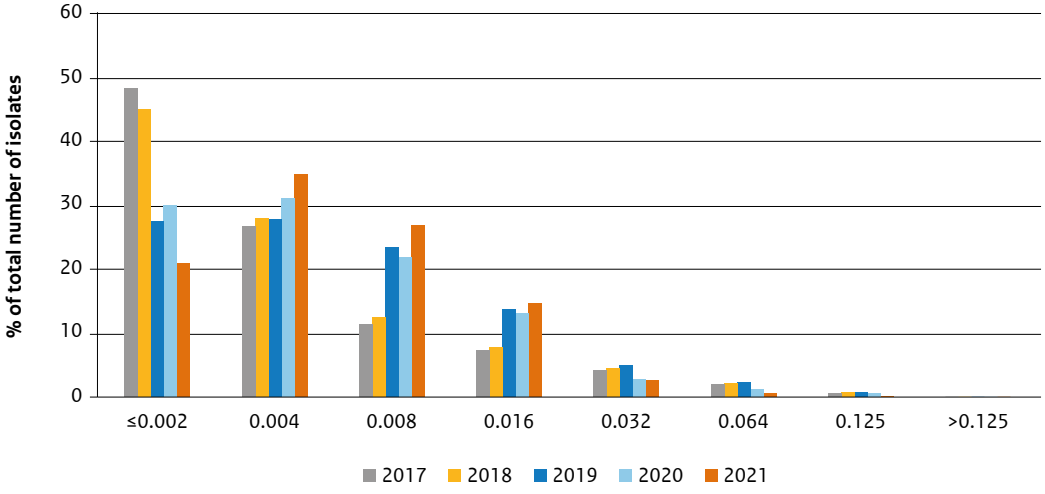


No resistance to ceftriaxone has been reported.

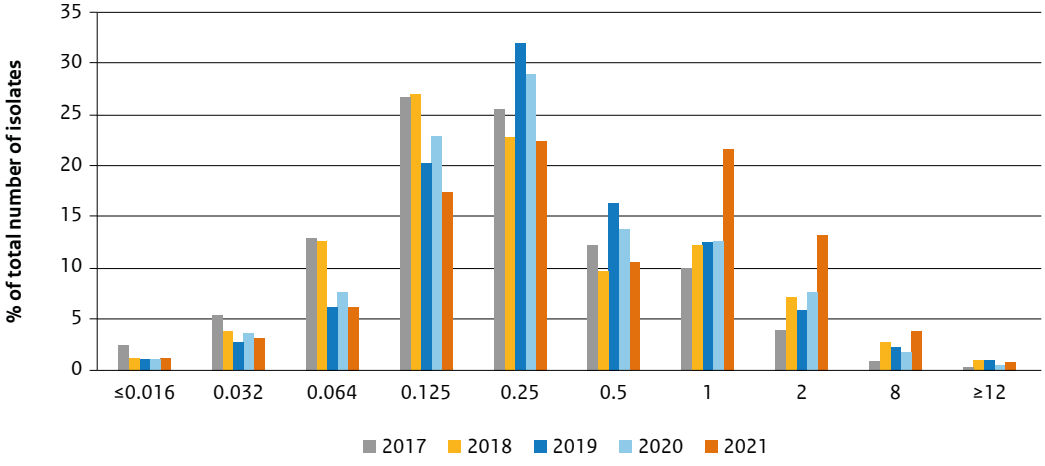
In the MIC distribution of ceftriaxone a shift was observed in 2019 where the proportion of isolates with an MIC <0.006 mg/L decreased and the proportion of isolates with slightly higher MIC values (MIC 0.006-0.016 mg/L) increased (Figure 4.8.2.3a). This continued in 2020 and 2021. For azithromycin a shift towards higher MICs is observed over time and in particular for 2021 (Figure 4.8.2.3b).

Figure 4.8.2.3 MIC distributions of ceftriaxone and azithromycin for *Neisseria gonorrhoeae*, 2017-2021

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 2021 in less than half (39.3%) of all gonorrhoea diagnoses at the SHC participating in GRAS resistance levels were measured by additional susceptibility testing. 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. Due to COVID-19, sexual health care at the SHC was downscaled in 2020 and, to a lesser extent, in 2021. The SHC performed stricter triaging and prioritized testing of persons at highest risk of STI and PrEP (Pre-Exposure Prophylaxis for HIV) care. The effect of this downscaling in testing had limited impact on the availability of susceptibility testing results in GRAS in 2020, and the number of gonorrhoea diagnoses included in GRAS increased again in 2021. Cultures were less often performed than before covid (68.1% of gonorrhoea patients in 2021 versus 77.5% in 2019), but the percentage of patients with reported susceptibility testing results was stable (39.3% in 2021 versus 39.8% in 2019). 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

- No resistance to ceftriaxone, the current first-line treatment for gonorrhoea, has been reported. However, the proportion of isolates with higher MIC values is increasing since 2019.
- Resistance to ciprofloxacin more than doubled since 2016, to 52.9% in 2021.
- MIC values for azithromycin continue to increase after a stable period between 2018-2020.

References

[1] The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 12.0, 2022. Available from http://www.eucast.org/clinical_breakpoints/.

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 natives; more than 75% of the TB cases is currently diagnosed in foreign-born persons. Compared to 2019, in 2020 the total number of reported TB cases declined with 17% to 622 cases and this may be related to the COVID-19 pandemic because of reduced immigration, less transmission and delayed diagnosis. In 2021 however, the total number of reported TB cases increased by 10% to 680 cases.

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 for therapy guidance of individual patients, investigations on 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 the investigations on TB transmission by Municipal Health Services. Before 2020, the resistance testing was based on the phenotypic approach using the Mycobacteria Growth Indicator Tubes (MGIT) and a few molecular tests. From 2020 on, all isolates are initially examined on the presence of resistance mutations in the nine major resistance genes in *M. tuberculosis*. In absence of such mutations, no further testing is performed. If resistance mutations are detected, phenotypic resistance testing is performed.[1,2,3] Because injectables are no longer part of the TB treatment regimen, we no longer determine the resistance against streptomycin. Since 2020 we also monitor the resistance to pyrazinamide, because the combination of the results of WGS and phenotypic testing yield more reliable results. Comparisons of molecular and phenotypic resistance testing have been described by Jajou *et al*[1] and Walker *et al*[2], this is the basis of the current algorithm of testing.

Results

In 2021, of the 680 notified cases, 472 (69%) represented bacteriologically confirmed cases, of which the isolates were received at the RIVM. Some form of resistance was detected in 10.3% (49/472) of the isolates tested. In total 9 multi drug resistant (MDR)-TB cases (defined as resistant to at least INH and rifampicin) and two mono rifampicin resistant (RR) cases, (defined as resistant to only rifampicin) were detected. These observations were initially done by detection of resistance mutations in WGS and confirmed by phenotypic resistance testing. Combined MDR and RR represented 2.3% of the cases in 2021, which was similar to the combined MDR and RR resistance in 2020 (1.7%).

Figure 4.8.3.1 Percentage combined antibiotic resistance for *M. tuberculosis* isolates 2006-2021

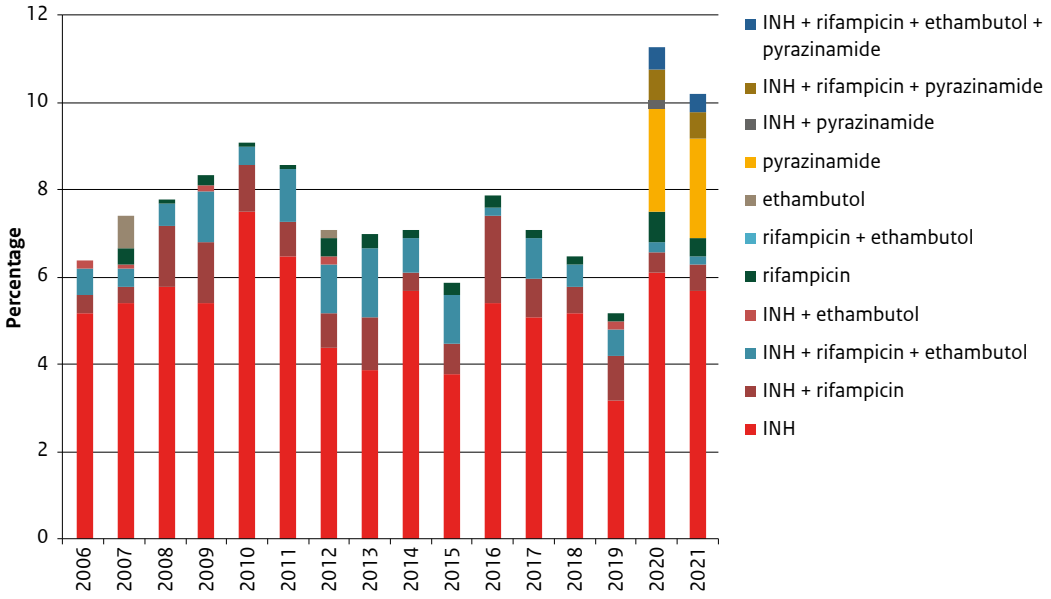
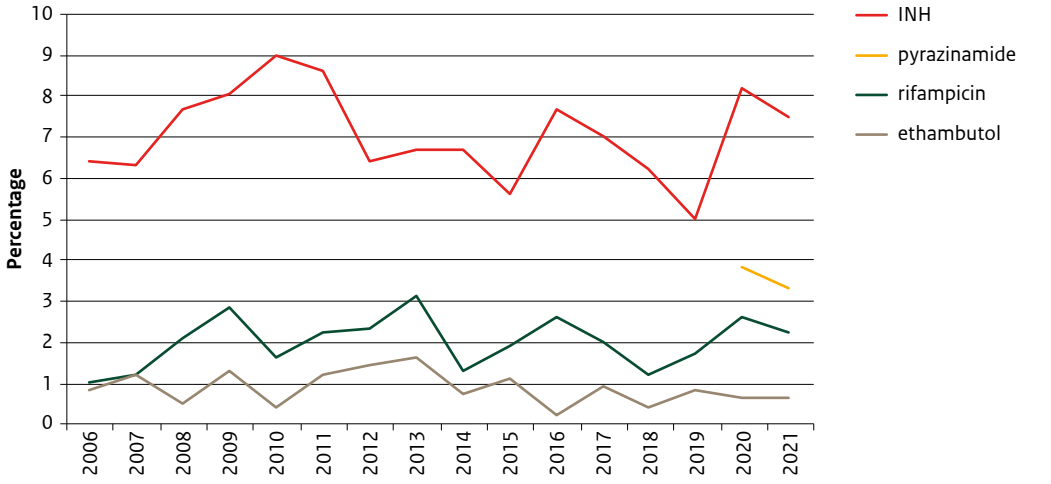


Figure 4.8.3.2 Percentage antibiotic resistance for *M. tuberculosis* isolates 2006-2021



Discussion

In 2021, 10.3% (49/472) of the isolates tested in the Netherlands revealed some form of resistance, compared to 11.3% (48/425) in 2020. This seems somewhat higher than the percentage observed in the years before. Although the number of multidrug resistant (including RR) isolates remained low in 2021 and amounted to 11 cases, due to the extended hospitalization of patients and the complicated treatment this problem continues to deserve special attention.

Worldwide, resistance is an important aspect in TB control. Because the vast majority of TB cases in The Netherlands is diagnosed in patients originating from high prevalence areas, it remains important to continue the structural surveillance on 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.
- MDR-TB remained stable in the recent years (average of 10 cases per year).
- There was a sharp decline in TB notification in 2020 (622 cases; 17% less), 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 was still influencing travelling and working.

References

1 Jajou R, van der Laan T, de Zwaan R, Kamst M, Mulder A, de Neeling A, Anthony R, van Soolingen D.J Antimicrob Chemother. 2019 Sep 1;74(9):2605-2616. doi: 10.1093/jac/dkz215.PMID: 31119271

2 Prediction of Susceptibility to First-Line Tuberculosis Drugs by DNA Sequencing
N Engl J Med. 2018 Oct 11;379(15):1403-1415. doi: 10.1056/NEJMoa1800474. Epub 2018 Sep 26.PMID: 30280646
Timothy M Walker et al.

3 WHO, *Catalogue of mutations in Mycobacterium tuberculosis complex and their association with drug resistance: supplementary document. supplementary document*. 2021, World Health Organization: Geneva.

4.8.4 Antiviral resistance

The plan of changing "antibacterial resistance (ABR)" surveillance programmes to "antimicrobial resistance (AMR)" will add to the existing influenza antiviral susceptibility surveillance. It will also enable surveillance of other viruses for reduced susceptibility to antivirals. Antiviral drugs are here defined as chemical compounds that interact with virus or host component essential for viral replication. Active and passive immunization using vaccines and antibodies are not subject of this chapter. Resistance to antivirals can be detected using phenotypic methods 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 agents 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.

For several viral infectious diseases enhanced surveillance programmes to monitor antiviral resistance may be conceivable and valuable.

Currently, the monitoring of **influenza** antiviral susceptibility is embedded in the surveillance of influenza by general practitioner (GP) sentinels, which is coordinated 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), and the surveillance of influenza viruses received from mainly hospital laboratories by the Erasmus Medical Centre or the RIVM locations of the NIC.[1] 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. High on the list of agents for treatment of COVID-19 is remdesivir, but susceptibility tests are performed on a very small scale. New treatments are currently being developed and preclinical laboratory tests are necessary to establish antiviral activity. In addition, preparedness for resistance development by the availability of appropriate tests is also required especially when antivirals with new mode of action become available.

RSV is a major cause of severe lower respiratory tract infections and hospitalization in infants under 1 year of age and its disease burden is similar to influenza in the frail elderly. Though the treatment of infections with RSV in infants and elderly is primarily supportive, new antiviral treatments are in development. Since the disease burden of RSV infection is high, surveillance and susceptibility tests for these new antiviral treatments aimed at monitoring the epidemiology and strain variation, are necessary.[2]

Herpes simplex virus (**HSV**) **infections** are associated with recurrent infections of the oral and genital regions, sometimes complicated with encephalitis, keratitis, and severe neonatal infections. Though some HSV infections cause sexually transmissible diseases, there is no mandatory reporting in the Netherlands and contact information is not advised since asymptomatic carriership is very common. Antiviral resistance has been described for acyclovir (and its orally bioavailable derivatives valacyclovir and famciclovir) and foscarnet. No information is available for cidofovir.

Infections with **cytomegalovirus (CMV)** mainly occur in immunocompromised patients and newborns. Both primary infections and reactivations occur, with systemic symptoms, pneumonia and hepatitis. Infections during pregnancy can lead to transfer to the unborn fetus (congenital CMV) or shortly after birth. The frequency of antiviral resistance to ganciclovir, foscarnet and cidofovir varies between 0% and 10% between different patient populations. Letermovir resistance has also been described. No consensus is available on when antiviral resistance to CMV should be suspected or tested.[3]

Liver infection with **hepatitis C Virus (HCV)** is mainly a blood transmissible disease, affecting specific subpopulations with a high, chronic burden of disease. It is a mandatory reporting disease (B2). The treatment options have been considerably expanded and include monitoring of antiviral resistance for genotypes in various disease stages.
Number of infections with **hepatitis B Virus (HBV)** decreased in the Netherlands due to the vaccination programme for specific subpopulations. There is a variety of HBV treatment guidelines, but not a specific guidance document in the Netherlands.[4] FDA approved antiviral agents for HBV include lamivudine, adefovir, entecavir, telbivudine, and tenofovir, known as nucleos(t)ide analogues, and interferon-α and (pegylated-) IFN-α therapy. Resistance markers have been reported for these antivirals, of which tenofovir and entecavir have the lowest risk of developing resistance.

Regarding **human immunodeficiency virus (HIV) infections**, there have been substantial changes and improvements in the use of antiretroviral drugs for its treatment and prevention, since the introduction of combination antiretroviral therapy (cART) in 1996. The current treatment guidelines recommend initiating cART as soon as possible for all people newly diagnosed with HIV, regardless of CD4 count. In the Netherlands, approximately 24,000 individuals are infected with HIV (see HIV monitoring). More than 85% receive antiviral treatment to improve the clinical outcome and prevent transmission of the disease. For HIV treatment, 20 agents belonging to 5 different antiviral drug classes are available and a combination of at least 3 different agents taken daily is advised, also to prevent resistance development. Therefore, monitoring of resistance development and its spread are import pillars of HIV treatment and prevention strategies, such as pre-exposure prophylaxis (PrEP).

Infections with **rubella**, **measles**, **mumps**, and **poliovirus/enterovirus** 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 (flaccid myelitis) similar to polio worldwide.[5] Currently, for these and other viruses, many new antivirals are being developed, are in clinical trials, or used off-market or experimentally.[6] It is important to assess both the clinical and public health impact of known and new antivirals with regards to antiviral resistance development for these and other viruses.

Conclusions

- In collaboration with national (NVMM and Nederlandse Werkgroep voor Klinische Virologie) and international stakeholders, a selection will be made for antiviral susceptibility surveillance programmes of specific viruses in 2023.
- Using the existing network of “Kiemsurveillance”, a start will be made with susceptibility testing of SARS-CoV-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
RSV	High	In development	No	No
Herpes simplex virus 1 and 2	High	Acyclovir and its derivates, foscarnet, cidofovir	No	No
Cytomegalovirus	High in immunocompromised patients, low in neonates	Acylcovir, ganciclovir, foscarnet, letermovir	No	No
Hepatitis B	Vaccination is recommended for specific populations	Various treatment guidelines with lamivudine, adefovir, entecavir, telbivudine, tenofovir, and (pegylated) interferon-α	No	No
Hepatitis C	High in specific subpopulations	See https://hcvrichtsnoer.nl/	No	Amsterdam UMC, Erasmus MC and UMCU
Mumps, measles, rubella and poliovirus/enterovirus	Low	In development	Part of vaccination programme monitoring	No
HIV	High	20 agents belonging to 5 different classes	Yes, by “HIV monitoring”	Yes

4.8.4.1 Influenza virus antiviral drug resistance

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 newly registered (2021) 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 and are therefore not summarized anymore in this update. Monitoring of NAI susceptibility of seasonal human influenza viruses is performed since the 2005/2006 winter season.[7] Monitoring of BXM susceptibility by monitoring amino acid substitutions in the polymerase acidic protein (PA) previously associated with reduced susceptibility against BXM has been added to the surveillance since the 2019/2020 season.

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 sources of viruses antiviral treatment of the patient, travel history and immune competence status in the 14 days preceding the time of specimen collection are asked to report. The footnotes to Table 4.8.4.2 list whether these characteristics for patients with antiviral reduced susceptible virus are known. Techniques currently used by the NIC in the Netherlands to monitor antiviral susceptibility include whole genome Nanopore sequencing and site-specific polymerase chain reaction (PCR) assays for known reduced inhibition markers for both NAIs and BXM. For a subset of influenza viruses, the susceptibility to NAIs is determined by the NIC using an enzyme inhibition assay, which generates a 50% inhibitory concentration of the drug (IC_{50}). The latter is done to confirm the impact of known markers for reduced antiviral susceptibility and to discover new markers. The use of antiviral drugs in the Netherlands 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 (91%) 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.[7,8] Table 4.8.4.2 displays an overview of the antiviral susceptibility of influenza viruses since the 2010/2011 influenza season. After the absence of influenza during the 2020/2021 season due to COVID-19 measures, influenza returned in the Netherlands in autumn 2021 and the seasonal epidemic started in week 9/2022 with A(H3N2) dominating up to week 12/2022. Each season, none or 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. Figure 4.8.4.1 shows the utilization of oseltamivir and zanamivir since 2010. Few, but increasing, number of oseltamivir prescriptions were observed during the 2021/2022 season so far, highly likely reflecting the very limited detection of influenza cases up to December 2021 due to COVID-19 control measures. SFK reported no prescriptions of zanamivir up to December 2021 during the 2021/2022 season. SFK reported no BXM prescriptions so far for the Netherlands since its EU authorization early 2021. Still, two A(H3N2) influenza viruses with PA-E23G marker for BXM mild reduced susceptibility were detected.

Discussion

In the Netherlands (Table 4.8.4.2), and globally, the proportion of NAI reduced susceptible influenza viruses remains very low.[9] 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 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. Few BXM reduced susceptible viruses were detected in the Netherlands, similar to the very low prevalence globally.[9]

Conclusions

- Over the last 12 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 or an amino acid substitution induced by virus isolation in cell culture.
- Prescriptions of oseltamivir remain low with sharp increases every influenza epidemic, except during the COVID-19 pandemic 2020/2021, similar to the 2013/2014 season.
- The Foundation for Pharmaceutical Statistics did not report prescriptions of zanamivir and baloxavir marboxil so far during the 2021/2022 season.

Table 4.8.4.2 (Highly) reduced inhibition of influenza viruses by NAIs and BXM in the Netherlands, 2010/2011 - 2021/2022[1]

	A(H3N2)		A(H1N1)pdm09		B	
Season	NAI	BXM	NAI	BXM	NAI	BXM
2010/2011	0/2	ND	0/58	ND	0/64	ND
2011/2012	0/257	ND	2/7 (29%)[2]	ND	0/10	ND
2012/2013	0/156	ND	3/125 (2.4%)[3]	ND	0/8	ND
2013/2014	2/220 (<1%)[4]	ND	1/150 (<1%)[5]	ND	0/4	ND
2014/2015	0/727	ND	1/130 (<1%)[6]	ND	0/42	ND
2015/2016	0/44	ND	1/1191(<1%)[7]	ND	1/69 (1%)[8]	ND
2016/2017	0/911	ND	2/11 (18%)[9]	ND	0/14	ND
2017/2018	0/355	ND	1/233(<1%)[10]	ND	0/156	ND
2018/2019	0/421	ND	3/331(<1%)[11]	ND	0/4	ND
2019/2020	0/242	0/114	0/151	0/39	0/16	0/1
2020/2021[12]	0/1	ND	ND	ND	ND	ND
2021/2022[13]	0/665	2/542[14]	0/95	0/84	0/4	0/3

[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, except for 2021/2022 for which the start was week 30/2021. 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] *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.*

[5] *One virus with highly reduced inhibition by oseltamivir due to the NA-H275Y amino acid substitution. 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.*

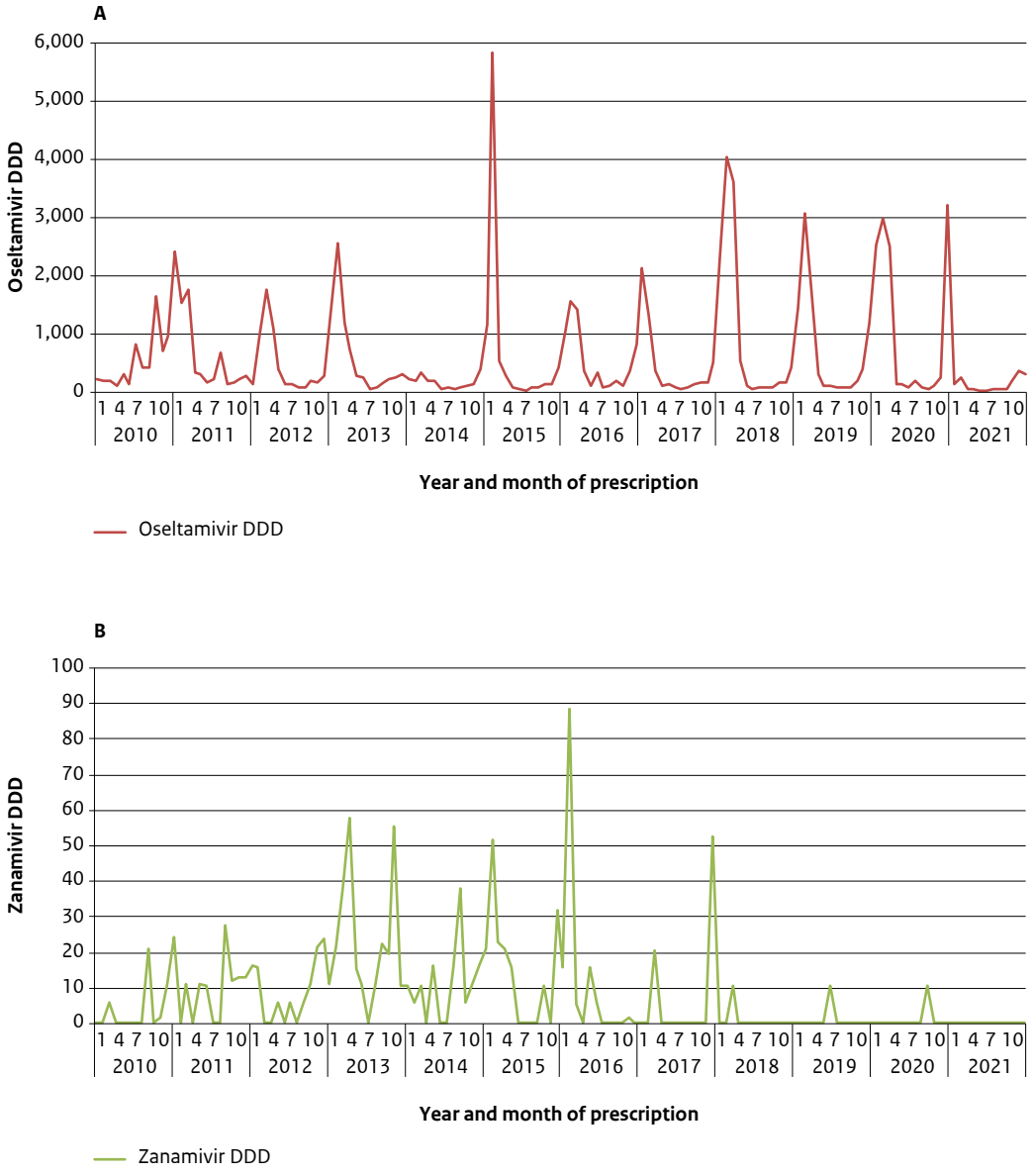
[11] *Three viruses with highly reduced inhibition by oseltamivir due to NA-H275Y (n=1) or mixture NA-275H/Y (n=2) amino acid substitution. Two patients were admitted to ICU of which one was treated with oseltamivir prior to specimen collection and the other had an unknown treatment status. One community patient had no prior treatment with oseltamivir.*

[12] *Early 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. There were no further influenza virus detections than the one A(H3N2) in the 2020/2021 season due to the COVID-19 pandemic and measures that also limited influenza virus circulation worldwide.*

[13] *Preliminary data up to week 12/2022; the season runs from week 30/2021 due to the very early start of circulation of influenza viruses in The Netherlands after lifting part of COVID-19 measures in summer 2021.*

[14] *Two viruses showed the amino acid substitution PA-E23G, previously associated with mild reduced susceptibility to baloxavir marboxil. One patient was hospitalized. The status of the other patient was unknown. For both patients no antiviral exposure data available.*

Figure 4.8.4.1 Prescriptions of oseltamivir (A) and zanamivir (B) in the Netherlands, 2010/2011 - 2021/2022. Shown are the Defined Daily Doses (ddd) cumulated by month. Data kindly provided by Foundation for Pharmaceutical Statistics (SFK), the Netherlands



References

1 NethMap 2021. Consumption of antimicrobial agents and antimicrobial resistance among medically important bacteria in the Netherlands / MARAN 2021. Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2020

2 Xing Y, Proesmans M. New therapies for acute RSV infections: where are we? Eur J Pediatr. 2019 Feb;178(2):131-138.

3 Ljungman P, de la Camara R, Robin C, Crocchiolo R, Einsele H, Hill JA, Hubacek P, Navarro D, Cordonnier C, Ward KN; 2017 European Conference on Infections in Leukaemia group. Guidelines for the management of cytomegalovirus infection in patients with haematological malignancies and after stem cell transplantation from the 2017 European Conference on Infections in Leukaemia (ECIL 7). Lancet Infect Dis. 2019 Aug;19(8):e260-e272.

4 Huang DQ, Lim SG. Hepatitis B: Who to treat? A critical review of international guidelines. Liver Int. 2020 Feb;40 Suppl 1:5-14.

5 Jelte Helfferich, Marjolein Knoester, Coretta C Van Leer-Buter, Rinze F Neuteboom, Linda C Meiners, Hubert G Niesters, Oebele F Brouwer. Acute flaccid myelitis and enterovirus D68: lessons from the past and present. Eur J Pediatr. 2019 Sep;178(9):1305-1315

6 Kimberley S M Benschop, Harrie G A M van der Avoort, Erwin Duizer, Marion P G Koopmans Antivirals against enteroviruses: a critical review from a public-health perspective. Antivir Ther 2015;20(2):121-30.

7 NethMap 2016. Consumption of antimicrobial agents and antimicrobial resistance among medically important bacteria in the Netherlands in 2015. National Institute for Public Health and the Environment, June 2016.

8 NethMap 2019. Consumption of antimicrobial agents and antimicrobial resistance among medically important bacteria in the Netherlands in 2018. National Institute for Public Health and the Environment, June 2019.

9 Govorkova EA, Takashita E, Daniels RS, Fujisaki S, Presser LD, Patel MC, Huang W, Lackenby A, Nguyen HT, Pereyaslov D, Rattigan A, Brown SK, Samaan M, Subbarao K, Wong S, Wang D, Webby RJ, Yen HL, Zhang W, Meijer A, Gubareva LV. Global update on the susceptibilities of human influenza viruses to neuraminidase inhibitors and the cap-dependent endonuclease inhibitor baloxavir, 2018-2020. Antiviral Res. 2022 Apr;200:105281. doi: 10.1016/j.antiviral.2022.105281. Epub 2022 Mar 12. PMID: 35292289.

4.8.5 Trends in antibiotic susceptibility profile of anaerobic bacteria isolated from human clinical specimens

Introduction

The antibiotic susceptibility profile of anaerobic bacteria differs between countries and regions. Due to the fastidious nature of anaerobic bacteria, the results of antibiotic susceptibility testing are often later available than that of aerobic bacteria. Therefore, antibiotic treatment of infections in which anaerobic bacteria are involved is often empiric, at least at the beginning. This makes it important to perform national surveillance studies, on a regular basis, to assess regional antibiotic susceptibility profile of anaerobes.
Here we report the antibiotic susceptibility profile of a variety of anaerobic bacteria isolated from clinical samples at the University Medical Center Groningen (UMCG) and the preliminary results of a national study focusing on *Bacteroides*, *Parabacteroides* and *Prevotella* clinical isolates, from 8 different centers within the Netherlands.

Methods

At the UMCG, anaerobic isolates from clinical infection-related samples, were identified using MALDI-TOF MS and MIC values were determined using Etest for amoxicillin (excluding *Bacteroides*, *Parabacteroides* and *Prevotella*), amoxicillin-clavulanic acid (only gram-negative anaerobes), clindamycin, meropenem (only *Bacteroides* and *Prevotella*) and metronidazole (excluding genera with intrinsic resistance). Only one isolate of each species per patient was included.
For the national study, 7 academic centers located throughout the Netherlands and one regional laboratory located in Noord-Brabant submitted each 20-25 *Bacteroides/Parabacteroides* and 10-15 *Prevotella* isolates from abscesses and normally sterile sites. Therefore, some isolates from the UMCG were included in both studies. MIC-values were determined using agar dilution and bacterial identity was confirmed using MALDI-TOF MS. Currently, MIC-values are available for amoxicillin, meropenem and metronidazole. For all data, the MIC_{50} and MIC_{90} were only determined when at least 10 isolates of a genus were available. Breakpoints according to EUCAST for anaerobic bacteria were updated in 2022 (v12.0), though for this analysis the breakpoints advised by EUCAST in 2021 (v11.0) were followed.

Results

Table 4.8.5.1 shows the MIC_{50}, MIC_{90} and the resistance percentage of different anaerobic genera isolated at the UMCG in 2021. In the last five years, an increase in amoxicillin resistance among peptostreptococci is observed, from 0% in 2017 to 7.7% in 2021, and for the first time amoxicillin resistance is reported among *Peptoniphilus* isolates. Furthermore, we noticed resistance to amoxicillin-clavulanic acid among *Prevotella* isolates for the first time, even though no meropenem resistance was observed. The 7 meropenem resistant *Bacteroides* isolates were all identified as *Bacteroides fragilis* and harbored the *cfiA* gene, as found with the MALDI-TOF MS subtyping module. Metronidazole resistance was observed among *Prevotella* (1.6%) and *Bacteroides* isolates (1.3%). These were two *Prevotella bivia* isolates and a *Bacteroides thetaiotaomicron* isolate.
The preliminary data from the national study (table 4.8.5.2) showed that resistance of *Prevotella* isolates to amoxicillin differs per hospital, ranging from 30.8% to 100%. The resistance rates for meropenem and metronidazole of *Prevotella* and *Bacteroides* isolates were mostly similar as the resistance rates reported for the UMCG isolates. However, we noticed that meropenem resistant *Bacteroides* isolates were only observed among isolates from the two university hospitals in Rotterdam and Leiden. Among the *Bacteroides* isolates from the UMCG included in the national study, no meropenem resistance was observed. However, among all *Bacteroides* isolates of 2021 we observed 4% resistance. None of the isolates from the UMCG or the national study showed resistance to both meropenem and metronidazole.

Discussion

In this study we not only report the antibiotic susceptibility profile of anaerobic isolates obtained from human clinical samples at the UMCG, but also from *Bacteroides* and *Prevotella* isolates obtained from 8 different centers in the Netherlands. Similar to previous years, the resistance rates differ per year, but no obvious increase or decrease in these rates is present.

The national study was initiated after several Dutch hospitals recovered a multi-drug resistant (MDR, resistant for three or more classes of antibiotics)[1] *B. fragilis* strain from clinical material which was resistant to several antibiotics, among which carbapenem and metronidazole[2]. Among the *Bacteroides* and *Prevotella* isolates from the national study and the UMCG that were collected in 2021, no MDR isolates were found. However, a metronidazole resistant *B. thetaiotaomicron* isolate from the national study was found with elevated MIC for meropenem of 4 mg/L.

In 2022, the EUCAST breakpoint for meropenem was lowered from 8 mg/L to 1 mg/L for *Bacteroides* and 0.25 mg/L for *Prevotella*. Due to this change, we may expect an increase in resistant isolates in the following years. Not only the breakpoints for meropenem were lowered, but also for other antibiotics, e.g. clindamycin. Therefore, we may expect not only an increase in the number resistant strains, but also an increase in strains which are determined to be MDR.

The finding of metronidazole resistant *Bacteroides* and *Prevotella* isolates or MDR strains is worrisome. We continue monitoring these trends and reveal the mechanisms behind it.

Conclusions

- Resistance rates of anaerobic bacteria differ per year.
- Meropenem resistance was observed in 4.9% of the *Bacteroides* isolates from the national surveillance and in 4.0% of all tested *Bacteroides* isolates in 2021 at the UMCG.
- Metronidazole resistance was observed among *Bacteroides* and *Prevotella* isolates, in the national and UMCG surveillance, varying from 0.6% to 2.1%.
- No differences in resistance rates for amoxicillin and metronidazole in *Bacteroides* isolates, between Dutch hospitals located in different regions.
- Resistance rates in *Prevotella* isolates, derived from different hospitals, for amoxicillin, differ between regions from 30.8% to 100%.

Table 4.8.5.1 The MIC_{50}, MIC_{90} and percentage resistance of different anaerobic genera, isolated at the UMCG from human clinical specimens in 2021, for different kind of antibiotics

	amoxicillin			amoxicillin/clavulanic acid			clindamycin			meropenem			metronidazole		
	MIC_{50}	MIC_{90}	%R	MIC_{50}	MIC_{90}	%R	MIC_{50}	MIC_{90}	%R	MIC_{50}	MIC_{90}	%R	MIC_{50}	MIC_{90}	%R
breakpoint	**> 2 mg/L**			**> 8 mg/L**			**> 4 mg/L**			**> 8 mg/L**			**> 4 mg/L**		
Gram-negative (n)															
Bacteroides spp. (174-175)[a]	n.d.[b]	n.d.	n.d.	0.25	2	2.3	3	>256	41.4	0.19	0.75	4	0.38	0.75	1.3
Bilophila spp. (11)	48	>256	100	0.38	3	0	0.5	24	18.2	n.d.	n.d.	n.d.	0.047	0.125	0
Dialister spp. (6)	n.d.	n.d.	33.3	n.d.	n.d.	0	n.d.	n.d.	0	n.d.	n.d.	n.d.	n.d.	n.d.	16.7
Fusobacterium spp. (40-41)[a]	0.023	0.094	2.4	0.5	4	2.5	0.047	0.5	2.4	n.d.	n.d.	n.d.	<0.016	0.064	0
Parabacteroides spp. (12)	n.d.	n.d.	n.d.	1	8	0	4	12	50	n.d.	n.d.	n.d.	0.38	1	0
Porphyromonas spp. (14 -15)[a]	0.023	0.38	0	0.25	4	0	0.016	>256	33.3	n.d.	n.d.	n.d.	0.047	0.125	0
Prevotella spp. (128)	n.d.	n.d.	n.d.	0.38	3	2.3	0.016	>256	23.4	0.032	0.094	0	0.19	0.75	1.6
Veillonella spp. (29)	0.38	1.5	3.4	0.38	2	0	0.125	0.38	0	n.d.	n.d.	n.d.	0.75	1.5	0
breakpoint	**> 8 mg/L**						**> 4 mg/L**						**> 4 mg/L**		
Gram-positive (n)															
Actinomyces spp. (148-149)[a]	0.125	0.5	0	n.d.	n.d.	n.d.	0.125	>256	11.4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Anaerococcus spp. (51)	0.032	0.125	0	n.d.	n.d.	n.d.	0.064	>256	17.6	n.d.	n.d.	n.d.	0.19	0.75	0
Atopobium spp. (9)	n.d.	n.d.	0	n.d.	n.d.	n.d.	n.d.	n.d.	11.1	n.d.	n.d.	n.d.	n.d.	n.d.	0
Bifidobacterium spp. (11-12)[a]	0.125	0.5	0	n.d.	n.d.	n.d.	0.094	1.5	9.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Clostridium spp. (44)	0.25	3	2.3	n.d.	n.d.	n.d.	2	8	29.5	n.d.	n.d.	n.d.	0.38	1	0
Eggerthella lenta (10)	1	1.5	0	n.d.	n.d.	n.d.	0.19	0.75	10	n.d.	n.d.	n.d.	0.125	0.25	0
Finegoldia magna (65)	0.19	0.38	0	n.d.	n.d.	n.d.	1	>256	18.5	n.d.	n.d.	n.d.	0.25	0.5	0
Parvimonas micra (39)	0.032	0.064	0	n.d.	n.d.	n.d.	0.38	6	12.8	n.d.	n.d.	n.d.	0.064	0.19	0
Peptoniphilus spp. (44)	0.047	0.19	2.3	n.d.	n.d.	n.d.	0.25	>256	13.6	n.d.	n.d.	n.d.	0.25	1	0
Peptostreptococcus spp. (13)	0.125	0.38	7.7	n.d.	n.d.	n.d.	0.047	0.38	0	n.d.	n.d.	n.d.	0.047	0.38	0
Cutibacterium spp. (197-198)[a]	0.094	0.25	0.5	n.d.	n.d.	n.d.	0.047	0.19	4.6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Slackia exigua (7)	n.d.	n.d.	0	n.d.	n.d.	n.d.	n.d.	n.d.	0	n.d.	n.d.	n.d.	n.d.	n.d.	0

a Not all isolates were tested for all antibiotics.
b Not determined.

Table 4.8.5.2 The MIC_{50}, MIC_{90} and percentage resistance of *Bacteroides* and *Prevotella* isolated from 8 different Dutch hospitals, for different kind of antibiotics

	MIC_{50} (mg/L)	MIC_{90} (mg/L)	% resistant
***Bacteroides* spp.**			
amoxicillin (n=162)	32	>256	98.1
metronidazole (n=161)	0.5	1	0.6
meropenem (n=143)	0.5	2	4.9
***Prevotella* spp.**			
amoxicillin (n=85)	8	128	55.3
metronidazole (n=96)	0.5	1	2.1
meropenem (n=75)	0.125	0.25	0

References

1 Magiokaros A-P, Srinivasan A, Cary RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JD, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL. Multidrug-resistant, extensively drug-resistant and pandrug- resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 2012;18:268-281

2 Boiten KE, Kuijper EJ, Smit LFE, Bode LGM, Schuele L, Schoffelen AF, Notermans DW, Woudt SHS, Winter HLJ, van Prehn J, Maat I, van Asten SAV, Wong MC, Rossen JWA, Veloo ACM. Genetic characterisation of multidrug-resistant *Bacteroides fragilis* clinical isolates in the Netherlands. Poster ECCMID 2021.

4.8.6 *Clostridioides difficile*

Introduction

Clostridioides difficile is an anaerobic, spore-forming bacterium which can colonise the intestine of humans and animals. Pathogenic *C. difficile* strains 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 programme started in May 2009 to monitor the incidence of *C. difficile* infections (CDI) in an endemic situation. Additionally, ad hoc typing service is offered for all microbiology laboratories in the Netherlands for typing of *C. difficile* isolates of patients with severe disease, or isolates from a suspected outbreak.

Methods

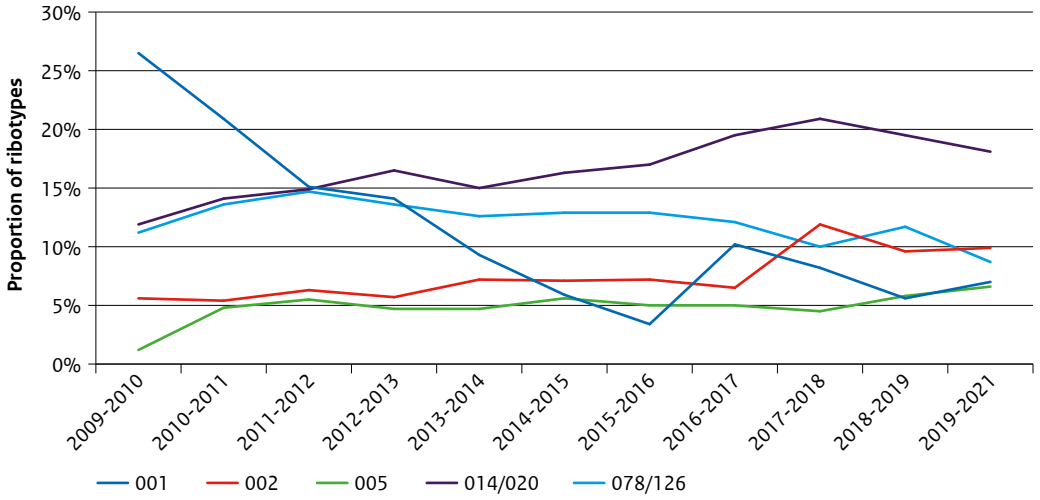
Hospitals participating in the sentinel surveillance are requested to include all hospitalised patients with clinical signs or symptoms of CDI in combination with a positive faeces test for *C. difficile* free toxins or a positive PCR for detection of toxigenic *C. difficile*. The assay or algorithm that is used to diagnose CDI, is chosen by the local laboratory. Laboratories that culture *C. difficile* (n=10) send strains to the laboratory of the LUMC. Other laboratories (n=9) send positive tested faecal samples. Some laboratories (n=3) send either faecal samples or strains.
All faecal samples are cultured and *C. difficile* isolates are characterised at the laboratory of the LUMC.
All *C. difficile* strains are further investigated by PCR-ribotyping. When an outbreak is suspected, strains are further investigated by MLVA or cgMLST.
Since the discovery of plasmid-mediated metronidazole resistance (pCD-METRO), we have implemented a PCR for pCD-METRO in our national sentinel surveillance programme. Isolates with a positive PCR result for pCD-METRO are tested for phenotypical metronidazole resistance using Etest.

Results

Twenty-two hospitals participated in the sentinel surveillance programme in the period May 2019 - January 2021. In the sentinel surveillance period May 2019 - January 2021, 1382 CDI patients were included and 1058 *C. difficile* isolates were ribotyped. The numbers of CDI per 10,000 patient-days per hospital are shown in Table 4.8.6.1 and are compared to the incidence rates of the preceding years.

Similarly as in the previous years, RT014/020 was the most frequently isolated ribotype, visualised in Figure 4.8.6.1. In the period May 2019 - January 2021, RT002 was the second most frequently isolated ribotype. Only two isolates were identified as the so-called “hypervirulent” PCR RT027 (0.2%; 95% CI 0.0-0.5). In the period between May 2019 - January 2021, no clusters of *C. difficile* in hospitals participating in the sentinel surveillance were reported.

Figure 4.8.6.1 Proportions of the 5 most common ribotypes in time in *C. difficile* sentinel surveillance samples



Since the implementation of sequence-based identification of metronidazole resistant strains of *C. difficile* at the LUMC laboratory in 2019, submitted strains are tested for the presence of pCD-METRO[1-3]. Since 2019, we have tested 3225 strains and identified 6 pCD-METRO positive isolates (0.18%), in line with previous results. Among these strains, we identified a PCR-positive strain from a symptomatic CDI patient belonging to the toxigenic RT005 that has not previously been reported to carry pCD-METRO. Using E-test, we confirmed that this strain was metronidazole non-susceptible (MIC = 3 mg/L). The patient had not responded to metronidazole treatment. A plasmid-negative RT005 strain isolated from the same patient at an earlier date was also identified but was phenotypically susceptible (MIC = 0.19 mg/L).
We received one *C. difficile* isolate (PCR RT151) from a patient with a severe CDI which was not detected by a commercially available PCR targeting toxin B gene (Cepheid). Whole genome sequence data confirmed the presence of mutations, most likely resulting to a primer mismatch. *C. difficile* RT151 is very rare and comprises less than 0.1% of all strains in our collection.

Discussion

The CDI incidence was 3.2 CDI cases per 10,000 patient-days, comparable to the incidence rates of previous years. Interestingly, there was an increased severity of CDI during the second COVID-19 wave in 2020. This may be caused by delayed diagnostics and decreased referral of patients.
Similar as the previous year, RT014/020 was the most frequently isolated ribotype. CDI-related mortality and the overall mortality in CDI patients were comparable to the previous year. The proportion of community-onset CDI cases was 37% (95% CI 33-41) at the start of the surveillance in 2009-2010, which was significantly lower than the proportion in 2019-2021, which was 45% (95% CI 43-48).
Of all tested strains, 0.18% contained pCD-METRO. For the first time, a metronidazole resistant toxigenic RT005 strain was found.
A very rare *C. difficile* RT151 was detected from a patient with severe CDI which was not detected by PCR due to a primer mismatch in toxin B gene.

Table 4.8.6.1 Data from the *C. difficile* sentinel surveillance for the period May 2019 - Jan 2021 compared to the data from preceding years

Surveillance period	2009-2010	2010-2011	2011-2012	2012-2013	2013-2014	2014-2015	2015-2016	2016-2017	2017-2018	2018-2019	2019-2021
Incidence											
Per 10,000 patient-days	2.7	2.8	2.9	2.9	2.9	3	3.1	3	2.9	3.1	3.2
Location of onset											
Within healthcare facility	63%	73%	69%	63%	64%	59%	58%	59%	55%	54%	55%
At home	37%	27%	31%	37%	36%	41%	42%	41%	45%	46%	45%
Course and outcome[1,2]											
Severe CDI[3]	28%	20%	27%	25%	21%	24%	21%	17%	20%	16%	21%
Uncomplicated course[4]	66%	86%	87%	88%	87%	86%	89%	87%	87%	90%	89%
Deaths contributable to CDI	4%	3%	4%	2%	3%	4%	2%	2%	3%	1%	2%
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%
N reported 027 outbreaks-sentinel surveillance	1	1	0	1	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

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 *Hospital R: outcome after 3 days instead of after 30 days.*

3 *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.*

4 *Uncomplicated course is defined as not admitted to the intensive care unit as a consequence of the Clostridioides difficile infection, no need for surgery as a consequence of the Clostridioides difficile infection and no death within 30 days after sample date.*

Conclusions

- CDI incidence is stable and outbreaks have not occurred in 2019-2021.
- From 1 January 2022 onwards, the National Reference Laboratory ended and some of the activities are continued in a new "Expertise Centre for CDI" located both in the LUMC and RIVM.

References

1 Smits WK, Lyras D, Lacy DB, Wilcox MH, Kuijper EJ. *Clostridium difficile* infection. Nature reviews Disease primers 2016; 2: 16020.

2 Boekhoud IM, Hornung BVH, Sevilla E, Harmanus C, Bos-Sanders I, Terveer EM, et al. Plasmid-mediated metronidazole resistance in *Clostridioides difficile*. Nat Commun. 2020 Jan 30;11(1):598.

3 Baktash A, Corver J, Harmanus C, Smits WK, Fawley W, Wilcox MH, Kumar N, Eyre DW, Indra A, Mellmann A, Kuijper EJ. Comparison of Whole-Genome Sequence-Based Methods and PCR Ribotyping for Subtyping of *Clostridioides difficile*.J Clin Microbiol. 2022 Feb 16;60(2):e0173721

4.8.7 *Aspergillus fumigatus*

Introduction

The saprobic mold *Aspergillus fumigatus* 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. Over the past decade invasive aspergillosis has been observed also in critically-ill patients, especially those with severe viral pneumonia caused by influenza virus and SARS-CoV-2. Triazoles 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 associated with environmental resistance selection through exposure to azole fungicides. TR-isolates are generally pan-azole resistant, with itraconazole showing no activity (MICs ≥16 mg/l) against isolates harboring TR_{34}/L98H and voriconazole showing no activity against TR_{46}/Y121F/T289A. 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 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 are sent to the reference laboratory for MIC-testing and sequence-analysis of the Cyp51A-gene. MIC testing is 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 2021 *A. fumigatus* isolates from 1,636 culture-positive patients were screened for triazole resistance, including 832 (range 103 to 209 per center) patients from UMCs and 804 (range 120 to 222 per center) patients from teaching hospitals. Overall 151 patients (9.2%) harbored a triazole-resistant isolate, with a resistance frequency of 11.7% (97 of 832 patients) in UMCs and 6.7% (54 of 804 patients) in teaching hospitals (Table 4.8.7.1). The resistance frequency in four UMCs was above 10%, which is the recommended threshold to consider changing empirical antifungal treatment regimen. The resistance frequency was lower in teaching hospitals with a range from 4.4% to 9%.

In total 196 azole-resistant isolates from 147 patients were analyzed for resistance mutations in the Cyp51A-gene. Overall in 28 isolates (14.3%) a wildtype Cyp51A-gene sequence was found, while in the remaining 168 isolates 23 different mutations were detected. The TR_{34}/L98H mutation was found in 96 of 196 (49%) isolates, while TR_{46}/Y121F/T289A was present in 50 (25.5%) isolates. Both TR_{34} and TR_{46} isolates were found to harbor additional short nucleotide polymorphisms (SNPs) or additional Cyp51A-gene mutations in 9 of 96 (9.4%) TR_{34} isolates, and 17 of 50 (34%) TR_{46} isolates. The implications of additional SNPs for the azole phenotype requires more study. However, two TR_{34} isolates contained an additional T289A mutation, a SNP commonly found in TR_{46} isolates. Unlike other TR_{34} isolates, which exhibit high resistance to itraconazole, these two isolates were susceptible to itraconazole and highly resistant to voriconazole.

Of the 151 patients with triazole-resistant *A. fumigatus*, 45 (29.8%) suffered from a structural lung disease (i.e. COPD or CF) and 34 (22.5%) had COVID-19 pneumonia as underlying condition.

Discussion

After a declining trend of triazole resistance frequency in 2019 and 2020, the year 2021 shows a similar resistance rate to 2019. The resistance frequency in teaching hospitals was 6.7% compared with 4.7% in 2020, while the frequency in the UMCs remained stable. Similar to previous years, resistance was dominated by TR-mediated resistance mutations, which accounted for 146 (74.5%) of detected resistance mechanisms. A concern is the number of variants that are being detected, in which the presence of additional SNPs or TR-variations may cause alteration of the azole phenotype. As molecular tests rely on the detection of core resistance mutations, such as TR_{34} or Y121F, the presence of these variations may be missed.[1] As a consequence the genotypic resistance test may not accurately predict the azole phenotype. Furthermore, the relevance of SNPs for the azole phenotype also needs to be demonstrated, as these may represent polymorphisms or otherwise be unrelated to azole resistance. In 2021, two isolates were detected that harbored a T289A mutation in the TR34 genetic background. The presence of this SNP appeared to be associated with a phenotype change from highly itraconazole-resistant to highly voriconazole-resistant. Although azole therapy is not recommended when azole-resistant infection is documented, in certain situations, such as CNS infection or long term ambulant (oral) therapy, azoles may remain the only option if tested susceptible. Looking back in the fungal culture collection of the mycology reference center, this variant was first detected in 2018 and has been repeatedly detected in the following years.

Table 4.8.7.1 Triazole resistance proportion in unselected clinical *A. fumigatus* isolates in 5 University Medical Centers and 5 teaching hospitals, 2018-2021

	2018		2019		2020		2021	
	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)
LUMC	120	25 (20.8)	90	14 (15.6)	83	8 (9.6)	103	7 (6.8)
Radboudumc	196	23 (11.7)	230	23 (10)	193	20 (10.4)	205	25 (12.2)
UMCG	238	34 (14.3)	230	27 (11.7)	181	31 (17.1)	209	28 (13.4)
AmsterdamUMC	81	13 (16)	51	6 (11.8)	172*	16 (9.3)	173	20 (11.6)
Total UMCs	**764**	**112 (14.7)**	**703**	**88 (12.5)**	**737**	**87 (11.8)**	**832**	**97 (11.7)**
Teaching hospitals								
Medisch Spectrum Twente	88	5 (5.7)	90	2 (2.2)	95	2 (2.1)	182	8 (4.4)
St Antonius Hospital	265	28 (10.6)	177	10 (5.7)	193	15 (7.8)	151	12 (7.9)
PAMM	81	4 (4.9)	147	8 (5.4)	150	3 (2)	129	6 (4.7)
CWZ	155	11 (7.1)	90	6 (6.7)	163	7 (4.3)	120	8 (6.7)
Isala	195	13 (6.7)	222	18 (8.1)	183	10 (5.5)	222	20 (9)
Total teaching hospitals	**784**	**50 (7.8)**	**726**	**42 (6.1)**	**784**	**37 (4.7)**	**804**	**54 (6.7)**

** Includes both VUmc and AMC, since 2020 AmsterdamUMC.*

Conclusions

- Triazole resistance frequency in 2021 was 11.7% in UMCs and 6.7% in teaching hospitals, which represents a resistance level similar to 2019.
- Overall the azole resistant *A. fumigatus* isolates harbored many different resistance variants most of which included TR-mediated mutations.
- Some variants, such as T289A mutation in a TR_{34} genetic background, cause a major azole resistance phenotype shift.

References

1 Scharmann U, Kirchhoff L, Hain A, Buer J, Koldehoff M, Steinmann J, Rath PM. Evaluation of three commercial PCR assays for the detection of azole-resistant *Aspergillus fumigatus* from respiratory samples of immunocompromised patients. J Fungi 2021;7:132.

5 Antimicrobial stewardship monitor in hospitals

5.1 Qualitative and organizational characteristics of Antimicrobial stewardship teams in the Netherlands

Methods and results

In 2021, a web-based survey was sent to all 71 acute care hospitals in the Netherlands to gain insight in the staffing and funding available for of the antimicrobial stewardship teams (A-teams). The results of the 60 responding hospitals (85%) 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 2017 and 2021

	2017	2018	2019	2020	2021
Survey response rate, N (%)*	64 (80%)	35 (45%)	39 (51%)	37 (51%)	60 (85%)
A-team characteristics					
Presence of an A-team in responding hospitals	94%	100%	97%	100%	100%
A-team consists of at least:					
≥1 clinical microbiologist	100%	100%	100%	97%	95%
≥1 hospital pharmacist	100%	100%	97%	100%	95%
≥1 infectious disease specialist	68%	86%	71%	76%	68%
≥1 nurse	10%	23%	21%	32%	18%
≥1 infection prevention specialist	14%	14%	16%	14%	15%
Time spent on stewardship per team, median [hours per week], (range)	12.0 (3-58)	34.0 (4-134)	21.0 (2-144)	not available	15.0 (0-98)
Budget provided by hospital board of directors	41%	79%	55%	54%	67%
Financial support, median [FTE], (range)	0.5 (0.05-1.5)	0.7 (0.1-3.1)	0.6 (0.05-3.30)	0.9 (0.1-2.6)	not available

* *Total number of hospitals in the Netherlands has changed. Total number of hospitals in 2017: 80, in 2018: 78, in 2019: 76, in 2020: 73, in 2021: 71.*

5.2 Quality of antimicrobial use

Methods

Participating hospitals

The antimicrobial stewardship monitor supports hospitals in obtaining information on the quality of antibiotic use. To this end, 20 Dutch acute care hospitals with interest to participate and sufficient data management capacity have been approached to participate in this specific data analysis.

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 medication prescriptions (both clinical and those started at discharge) for all patients admitted to the nursing wards with one or more antimicrobial prescriptions (ATC code starting with J01, J02, or J04). The 'basic set' further consisted of the date of admission, the date of discharge, the surgery date(s) (if applicable) and if possible the 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 in accordance with the local antibiotic guidelines. Here, data are shown from hospitals that provided complete data (i.e., a data set that contained antimicrobial prescriptions from all hospitalized patients) for 2020.

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 the 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, except when linked to a diagnosis; then the course(s) active on the day after admission was used as a proxy for empiric treatment. Intravenous to oral switch was defined as the start of a new oral antibiotic prescription between 24 hours before and 24 hours after discontinuation of intravenous empiric treatment. A course with aminoglycosides, piperacillin/tazobactam or meropenem that was started between 24 and 76 hours after initiation of an empiric cefuroxime/ceftriaxone course was considered escalation. Therapeutic drug monitoring (TDM) for antibiotic courses that were administered for >72 hours was considered performed if drug concentrations were measured at least once.

Results

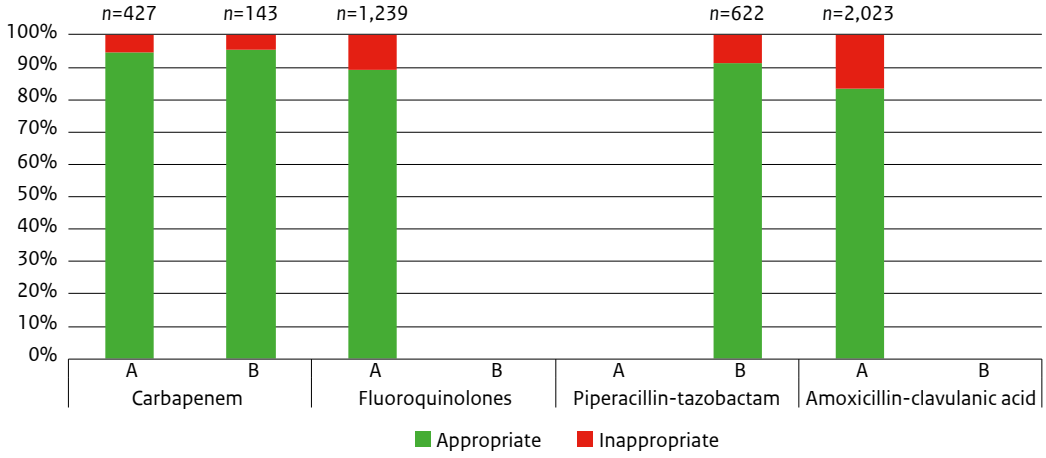
Participating hospitals

Seven hospitals participated and provided data for the basic set. For 2020, all hospitals provided data for the entire year, including two hospitals that provided the extensive data set.

Reserve antimicrobials

The percentage of carbapenem courses as part of total prescribed courses varied from 0.6% (143/25199) to 1.7% (427/24486), the percentage of quinolone courses from 2.7% (663/24695) to 7.0% (1074/15443) and glycopeptide courses from 0.4% (93/24695) to 2.6% (631/24486) between all seven hospitals. The percentage of amoxicillin/clavulanic acid courses as part of total prescribed courses varied from 6.7% (1030/15443) to 9.9% (2444/24695) and the percentage of piperacillin/tazobactam varied from 0 to 5.7% (1401/24486). Data on the appropriateness of the indication of reserve antibiotics to the local guideline was available for two hospitals and is shown in Figure 5.2.1.

Figure 5.2.1 Appropriateness of the indication of reserve antibiotic courses in two hospitals (A and B) in 2020

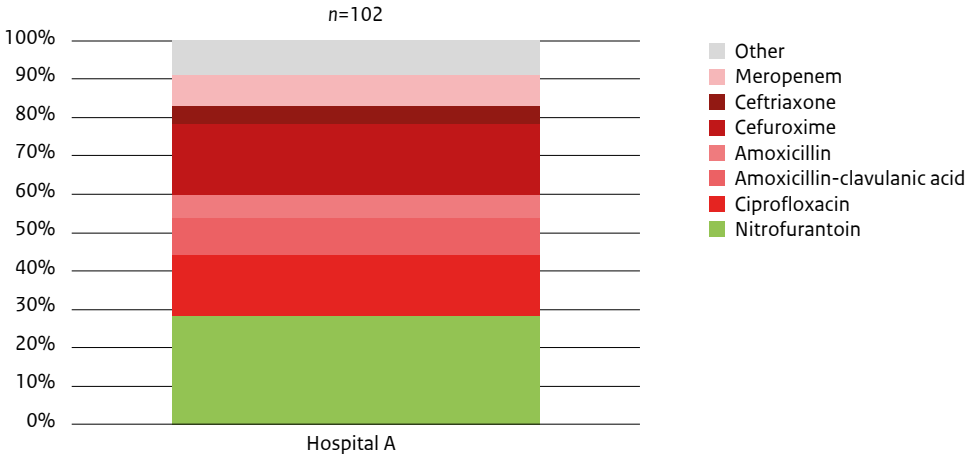


The percentage of antibiotic courses assessed is represented by the green (guideline adherent prescriptions) and red column (guideline inadherent prescriptions).
In hospital A, 13% of all carbapenem courses, 16% of all fluoroquinolone courses, and 14% of all amoxicillin-clavulanic acid courses were assessed.
In hospital B, 44% of all carbapenem courses, and 37% of all piperacillin-tazobactam courses were asssessed.
Total number of courses is displayed above the column.

Empiric treatment for urinary tract infections: an example from a mandatory indication-registration tool

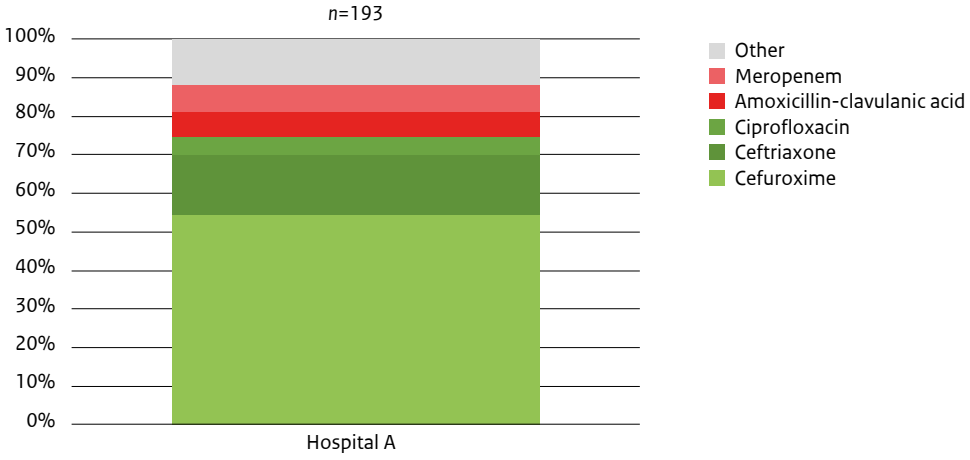
For one hospital, when prescribing an antimicrobial it was mandatory to choose the indication from a predefined list in the EMR. An example how to use these data for the assessment is shown in Figure 5.2.2. Of the 102 episodes of acute uncomplicated cystitis, 28% were empirically treated with a first choice agent recommended in the national SWAB guidelines (www.swabid.nl). Of the 193 episodes of complicated UTI, the guideline adherence rate was 75% and is shown in Figure 5.2.3.

Figure 5.2.2 Appropriateness of antibiotics for acute uncomplicated cystitis in one hospital (A) in 2020



Green colours: *in accordance with the guideline-recommended first choice agents*
Red colours: *discordant with the guideline*
Grey: antibiotics prescribed in less than 5% of cases
Total number of courses is displayed above the column.

Figure 5.2.3 Appropriateness of antibiotics for complicated UTI in one hospital (A) in 2020



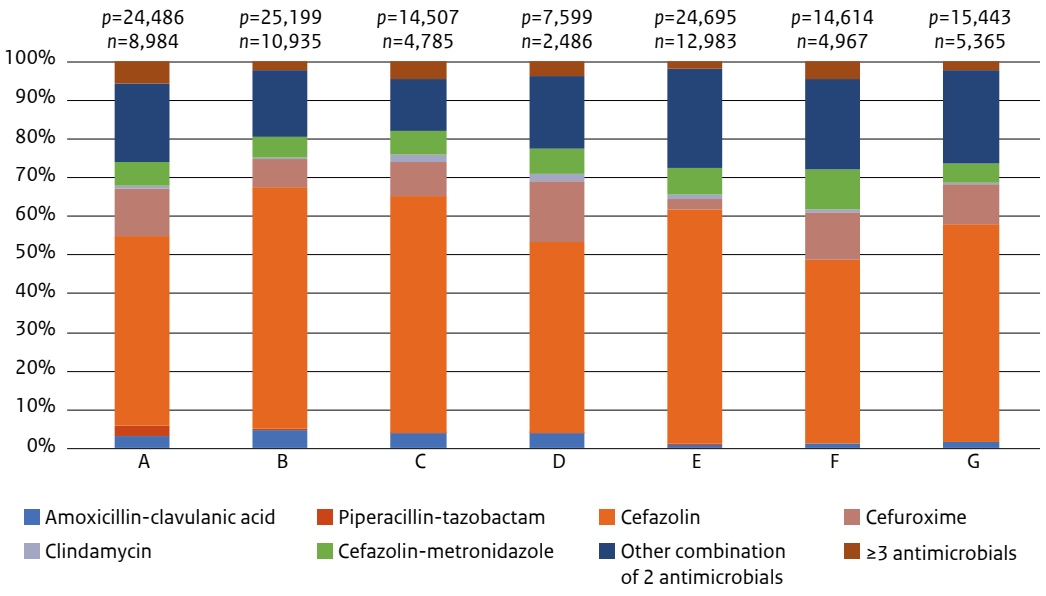
Green colours: *in accordance with the guideline-recommended first choice agents*
Red colours: *discordant with the guideline*
Grey: antibiotics prescribed in less than 5% of cases
Total number of courses is displayed above the column.

Surgical prophylaxis

The most commonly prescribed agents as preoperative prophylaxis, according to our proxy definition, in seven hospitals are summarized in Figure 5.2.4. Cefazolin was used as backbone of surgical antimicrobial prophylaxis in all seven hospitals.

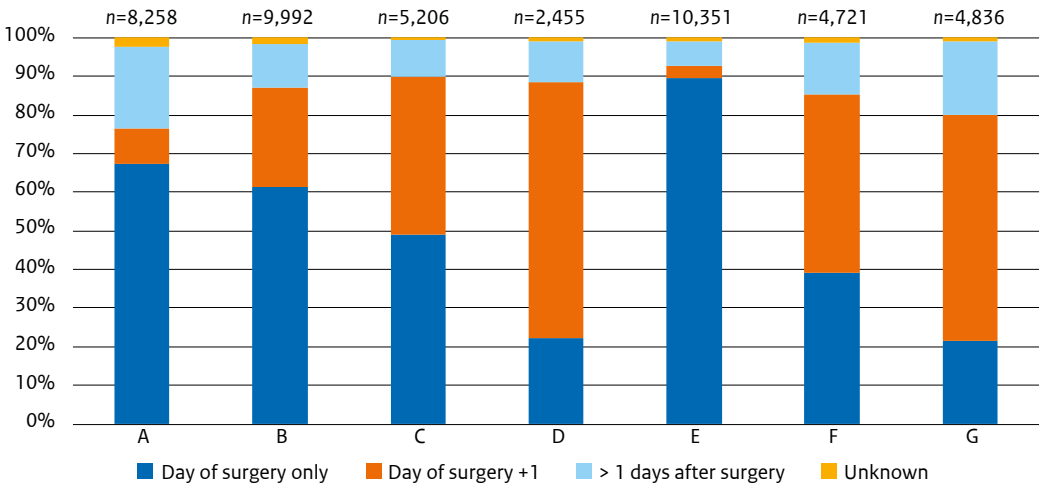
Figure 5.2.5 shows the duration of antimicrobial prophylaxis after surgery. Perioperative antimicrobial prophylaxis should generally be discontinued within 24 hours after surgery. On average, 85% (range 76-93%) of surgical antimicrobial prophylaxis courses were discontinued at the day of surgery or the day after.

Figure 5.2.4 Antibiotics used for surgical antimicrobial prophylaxis in seven hospitals (A-G) in 2020



Total number of courses used for surgical prophylaxis (n) and total number of prescribed antimicrobial courses (p) are displayed above the columns.

Figure 5.2.5 Distribution of the duration of surgical antimicrobial prophylaxis in seven hospitals (A-G) in 2020



Total number of courses is displayed above the columns.

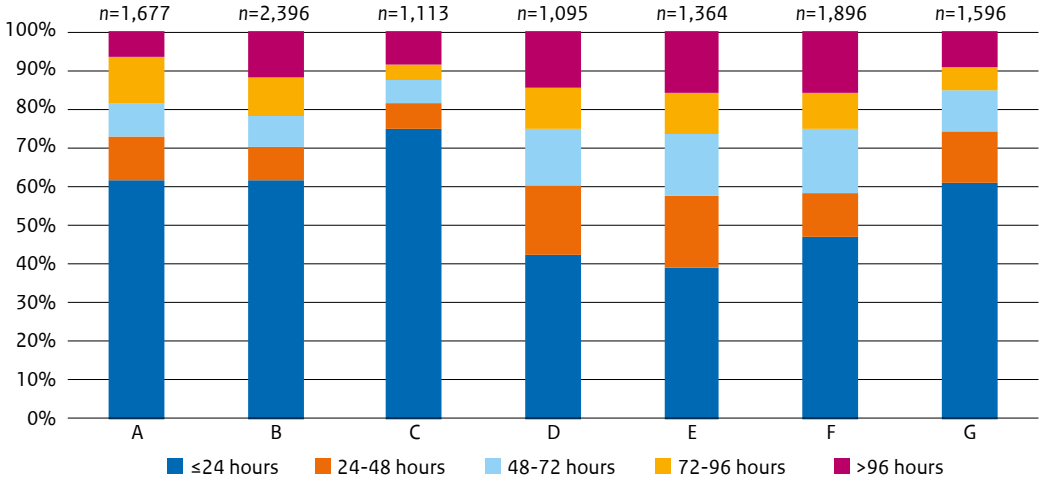
Intravenous to oral switch and escalation

Most hospitals in the Netherlands use either cefuroxime or ceftriaxone as empirical treatment of most infections, including sepsis of unknown origin. Figure 5.2.6 shows the duration of these antibiotic courses in the seven hospitals that provided these data. 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.

Sixty-nine percent (mean, range 62-76%) of these cefuroxime/ceftriaxone courses were discontinued without starting another course, while 17% (mean, range 11-24%) were switched to oral treatment and 14% (mean, range 10-27%) to other intravenous antibiotics between 24 hours before or after stop of cefuroxime/ceftriaxone (Figure 5.2.7). 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 (20% (mean, range 15-33%) versus 6% (mean, range 3-9%), data available for only 6 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 cefuroxime/ceftriaxone courses that had a duration of <24 hours (32% (mean, range 19-45%) versus 10% (mean, range 4-22%), data available for only 6 hospitals).

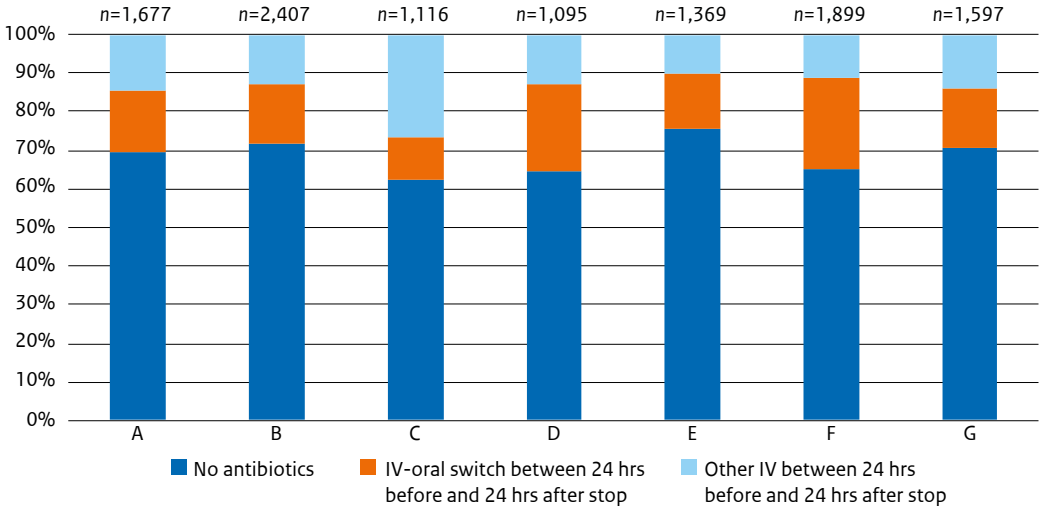
Empiric treatment of cefuroxime or ceftriaxone was only in a very small fraction of the patients escalated to an aminoglycoside-containing regimen, piperacillin/tazobactam or carbapenem, with little variation between the hospitals (Figure 5.2.8).

Figure 5.2.6 Duration of cefuroxime or ceftriaxone* courses started on the day of admission ('empiric treatment') in seven hospitals (A-G) in 2020



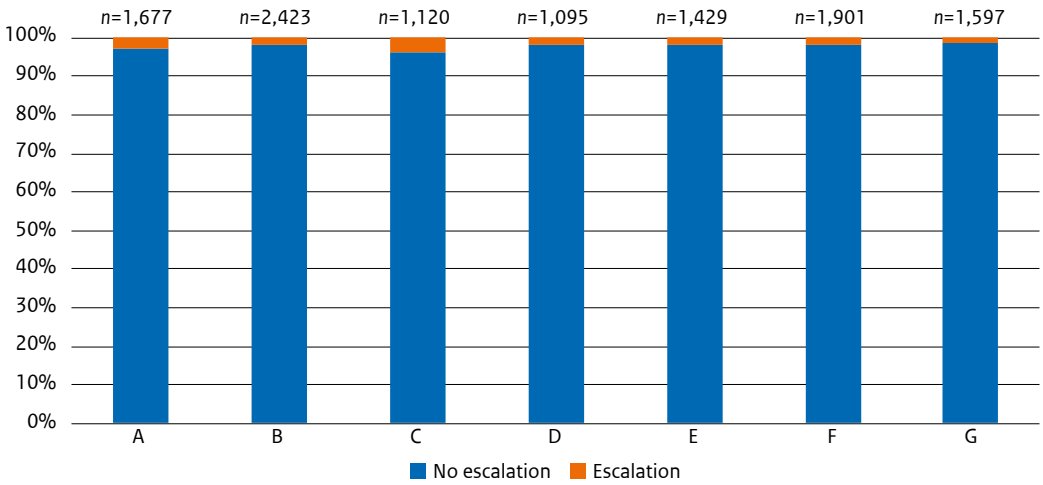
** Cefuroxime or ceftriaxone, depending on the preferred empiric treatment for sepsis of unknown origin.*
Total number of courses is displayed above the columns.

Figure 5.2.7 Discontinuation or change to oral or other intravenous antibiotic treatment of all cefuroxime or ceftriaxone courses started on the day of admission ('empiric treatment') in seven hospitals (A-G) in 2020



Total number of courses is displayed above the columns.

Figure 5.2.8 Percentage of empiric antibiotic cefuroxime 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 76 hours after initiation of cefuroxime/ceftriaxone in seven hospitals (A-G) in 2020

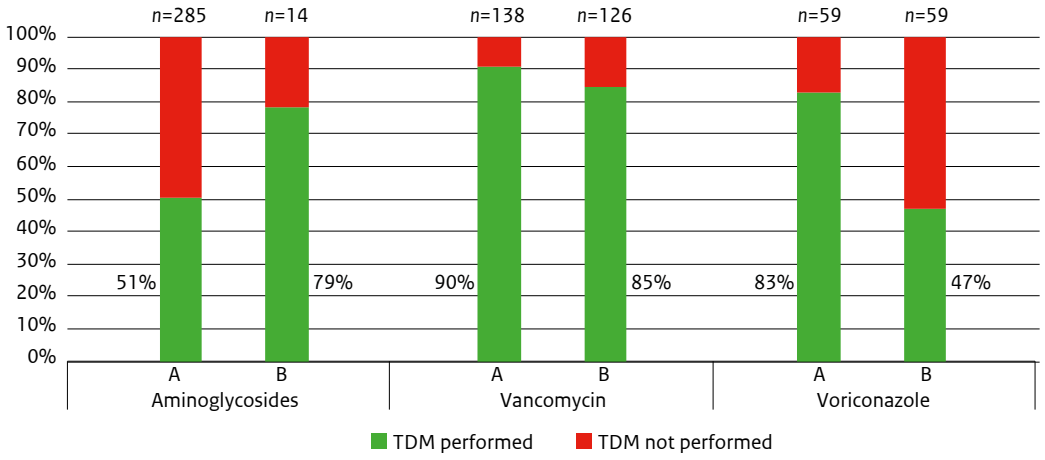


** Cefuroxime or ceftriaxone, depending on what the preferred empiric treatment for sepsis of unknown origin.*
Total number of courses is displayed above the columns.

Therapeutic drug monitoring

Two hospitals provided data on the performance of therapeutic drug monitoring (Figure 5.2.9). The number of antibiotic courses that were administered for >72 hours (total column) and where at least once drug concentrations were measured was consistently high for vancomycin: 90% and 85%. It varied, however, for aminoglycosides (51% and 79%) and for voriconazole (83% and 47%).

Figure 5.2.9 Number of antibiotic courses that were administered for >72 hours (total column) and where at least once drug concentrations were measured in two hospitals (A and B) in 2020



Total number of courses is displayed above the columns.

5.3 Discussion

For several years now, all hospitals have an A-team. The A-team's composition is more or less unchanged compared to previous years. In addition to a clinical microbiologist and a hospital pharmacist, approximately 70% of the A-teams have an infectious disease specialist and approximately 20% a nurse. There is no further increase in the number of A-teams with a nurse, although the much higher response rate this year makes comparison not straightforward. Equal to previous years, financial support remained on average less than the national staffing standard.

This is the first year 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. However, quality indicators are registered limitedly by A-teams in their EMR. Only for one hospital, data regarding appropriateness of empiric therapy for urinary tract infection was available. This is because compiling such figures requires prescriptions linked to an indication and a judgment on appropriateness by the A-team.

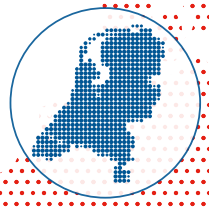
To overcome this limitation, we have also derived so-called 'proxy indicators' from the volume data. For certain metrics, it is immediately clear that they reflect the appropriateness of different aspects of antibiotic use. For example, we used an indicator that reflects the extent to which surgical prophylaxis was given after the operation, because postoperative continuation is never indicated. Still, it should be noted that the actual percentage of postoperative surgical prophylaxis is lower because we used a proxy indicator, which might include a number of therapeutic prescriptions. Similarly, the data on TDM are intuitive and actionable. On the other hand, the more descriptive data on the duration of IV courses and changes therein (e.g., start of another IV course, IV-oral switch, or escalation) are less easily directly linked to quality. However, the different outcomes show that there is practice variation and therefore potentially room for improvement. Future research should focus on how these data can be used for stewardship purposes.

Conclusions

- The composition of the A-team remained more or less the same: almost all consist of a clinical microbiologist, hospital pharmacist, two thirds of an infectious disease specialist and one fifth of a nurse.
- There has been no increase in time spent on antimicrobial stewardship in recent years and one-third of A-teams still received no funding from the hospital board.
- Seven (~10%) acute care hospitals extracted structured data from the electronic medical records and provided these to the interactive dashboard of the antimicrobial stewardship monitor.
- Based on prescriptions started on the day of surgery as a proxy for surgical prophylaxis, on average 85% (range 76-93%) of surgical antimicrobial prophylaxis courses were discontinued at the day of surgery or the day after.
- Seventeen percent (mean, range 11-24%) of the patients that received cefuroxime/ceftriaxone as empiric treatment upon admission were switched to oral treatment.

MARAN 2022

Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2021



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

SDa Autoriteit Diergeneesmiddelen

MARAN 2022

Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2021

June 2022

Contents

Colophon

This report is published under the acronym MARAN-2022 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) and the Netherlands Veterinary Medicines Institute (SDa). The information presented in MARAN-2022 is based on total sales data and animal specific usage of antimicrobial agents in animal husbandry and the occurrence of antimicrobial resistance and specific resistance genes in bacteria of animal origin and of relevance to public health.
MARAN-2022 is published in a combined back-to-back report with NETHMAP-2022. The combined report is available on the website of WBVR at www.wur.nl 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).

Editors

Dr. K.T. Veldman, Wageningen Bioveterinary Research (WBVR), Lelystad
Ing. B. Wit, Food and Consumer Product Safety Authority (NVWA), Utrecht
Dr. E. Franz, National Institute for Public Health and the Environment (RIVM), Bilthoven
Prof. Dr. D. Heederik, Netherlands Veterinary Medicines Institute (SDa), Utrecht

The following persons contributed to the writing of MARAN 2022

Part I Total sales of antibiotics and usage in livestock
Dr. I.M. van Geijlswijk, Prof. Dr. D. J.J. Heederik, P. Sanders Msc, SDa, Utrecht

Part II Resistance data
Dr. K.T. Veldman, Dr. M.S.M. Brouwer, WBVR, Lelystad
Ing. B. Wit, NVWA, Utrecht
Ir. B. A. Wullings, WFSR, Wageningen
Dr. E. Franz, Dr. R. Pijnacker, Dr. L. Mughini Gras, Dr. C. Dierikx, Dr. E. van Duijkeren, Dr. M. van der Beld
Dr. E. M. Broens, Prof. Dr. J. Wagenaar FD Utrecht

People involved in providing data for the surveillance of antimicrobial resistance

WBVR, Lelystad: Alieda van Essen-Zandbergen, Joop Testerink, Arie Kant, Yvon Geurts
WFSR, Wageningen: Michel Rapallini, Claire Schapendonk
RIVM, Bilthoven: Paul Hengeveld, Sjoerd Kuiling, Anjo Verbruggen, Kim van der Zwaluw
NVWA, Utrecht: Ben Wit, Petra Dop, Rianne Hagen-Lenselink

Acknowledgements

This study on antimicrobial resistance in food-animals was primarily financed by the Ministry of Agriculture, Nature and Food Quality through the project 'Antimicrobial Resistance Monitoring in Animals', grant number WOT-01-002-038.

The work of the Food and Consumer Product Safety Authority within the domain Microbiology is financed by the Ministry of Health, Welfare and Sport.
The work of the National Institute for Public Health and the Environment (RIVM) is financed by the Ministry of Health, Welfare and Sport.
The work of Wageningen Food Safety Research (WFSR) is financed by the Ministry of Health, Welfare and Sport.
The work of Netherlands Veterinary Medicines Institute is financed in equal amounts by public (Ministry of Agriculture, Nature and Food Quality) and private funds (animals production sectors for cattle, pigs, poultry and veal calves and the Dutch Royal Veterinary Association).
The authors thank Mr. Drs. J.F. Schutte, Drs. M. Poldermans and A de Goeij from FIDIN for providing detailed insight into the national sales data.

The authors thank Xerox/Osage for the layout.

1 Summary

Antibiotic Usage

In 2021 in total 145 tonnes of Antimicrobial Veterinary Medicinal Products (AVMPs) were sold, which is a decrease of 5.8% compared to 2020. A decrease in sales by 70.8% over the years 2009-2021 is attained (with 2009 considered a reference year by the Dutch Government). The decreased sales of AVMPs in the Netherlands in 2021 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 the unmonitored sectors. Use and sales of polymyxins decreased in 2021, overall decrease since 2011 is 77% in sales.

Antimicrobial resistance

In 2021, *Salmonella* Enteritidis (25%) followed by *S.* Typhimurium (19%) and monophasic *S.* Typhimurium (19%) were most frequently isolated from humans suffering from clinical salmonellosis. In pigs, the monophasic variant of *S.* Typhimurium (32%) and *S.* Derby (27%) dominated. In cattle, the most frequently identified serovars were *S.* Dublin (42%) and *S.* Typhimurium (27%). In broilers, *S.* Infantis (35%) and *S.* Paratyphi B var. Java (19%) dominated, while in layers *S.* Enteritidis (59%) and monophasic *S.* Typhimurium (15%) were the most common serovars. Over all serovars, the highest resistance proportions were observed for sulfamethoxazole (29.6%), tetracycline (26.6%) and ampicillin (24.5%), with approximately similar levels as in 2020. Serovars showing the highest levels of resistance were *S.* Infantis, *S.* Paratyphi B var. Java, monophasic *S.* Typhimurium variants, and *S.* Typhimurium, with resistance to ampicillin, tetracycline, sulfamethoxazole, trimethoprim, ciprofloxacin, and nalidixic acid reaching maximum levels of between 64% and 92%. Among *S.* Typhimurium, resistance to fluoroquinolones decreased considerably among human isolates, while it increased sharply among cattle isolates. Among *S.* Enteritidis, the fraction of resistance to ciprofloxacin and nalidixic acid among human isolates remained relatively stable but resistance to ampicillin and tetracycline decreased. In total, 10 (0.8%) ESBL-producing (human clinical) isolates were detected. In 2021, no carbapenemase-producing *Salmonella* were found. Due to a new legislation *Campylobacter jejuni* and *C. coli* isolates obtained from veal calves as well as *C. coli* from fattening pigs are included in the mandatory AMR monitoring program in livestock from 2021

onwards. In 2021, resistance proportions in *C. jejuni* isolates from caecal samples of broilers and meat thereof decreased but remained at a high level for quinolones and tetracycline. Resistance to macrolides was not detected in *C. jejuni* isolates from broilers and poultry meat, and was present at low levels in *C. coli* isolates from broilers and poultry meat. A notably higher level of macrolide resistance was observed in *C. coli* from veal calves. In human isolates, resistance proportions were higher in *C. coli* than in *C. jejuni*, but similar to 2020, these were overall lower in 2021 compared to previous years. This is most likely 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. Ciprofloxacin resistance in *Campylobacter* isolates from humans was again high in 2021, which is a concern for public health. It was, however, lower compared to 2017-2020. Resistance to erythromycin, first choice antibiotic in human medicine for campylobacteriosis, remained low.

In Shiga toxin-producing *E. coli* (STEC) O157, after a decrease in resistance for 2020, a tendency of increasing resistance towards the fluctuating levels of in 2018-2019 was observed. Resistance to the quinolones (ciprofloxacin and nalidixic acid) was very low in both (STEC) O157 and STEC/enteropathogenic *E. coli* (EPEC) non-O157 human isolates in 2021. Proportions of resistance were higher in human STEC/EPEC non-O157 *E. coli* than in STEC O157 for all antimicrobials, except gentamicin, tetracycline and sulfamethoxazole. No ESBL-producing isolates were detected in STEC O157, but a-typical enteropathogenic *E. coli* (aEPEC) O163 isolates from one case were confirmed as ESBL-producer carrying bla_{CMY-41}. Almost all STEC O146 isolates- associated with human infections linked to consumption of raw milk products from small ruminants - were pan-susceptible.

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, resistance in indicator *E. coli* from caecal samples further decreased to the lowest levels since 1998. In pigs and veal calves levels of resistance stabilised, whereas resistance in dairy cattle remained traditionally low. Resistance to third generation cephalosporins was very 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 with the low prevalence observed in pigs and veal calves. For almost all antibiotics tested, levels of resistance in *E. coli* from caecal samples of rosé veal calves were substantially lower than those from white veal calves. Resistance proportions in *E. coli* from pig and bovine meat were low compared to isolates from caeca. Low levels of resistance were observed in different types of retail meat as well as in imported meat. In vegetables, levels of resistance were very low for all antibiotic classes.

In 2021, only one confirmed ESBL-producing *E. coli* was detected through random isolation. Selective isolation of ESBL/pAmpC producing *E. coli* from broilers showed that after six years of reduction in prevalence, a plateau was reached. For the first time, whole-genome sequencing (WGS) was performed for all extended-spectrum cephalosporin resistant *E. coli* isolates from livestock and food products. WGS showed evidence of clonal transmission within livestock sectors and into the meat that these produce. The prevalence of ESBL-producing *Salmonella* isolated from human, livestock and food is considered low. In 2021, no carbapenemase-producing *Enterobacteriaceae* were detected in livestock and companion animals, but occasionally in imported food products. As in former years, the prevalence of *mcr* encoding *E. coli* was low in livestock and meat.

Within the study period, the MRSA prevalence varied substantially between the animal sectors: 89% on pig farms, 6% on dairy farms and no MRSA on broilers farms. On retail meat, the highest prevalence of MRSA was found on turkey meat, followed by lamb, chicken and veal.

It can be concluded that more than ten years of antibiotic reduction policies in the Netherlands has resulted in more than 70% reduction of sales of AVMPs for veterinary use. Antimicrobial resistance has decreased simultaneously in isolates from most livestock species. In spite of the AMU reduction a long-term increase of resistance is 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 2021

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 2021 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. 3.4% of the sold AVMPs (including all administration forms like tablets and injectables) is exclusively authorized for companion animals. AVMPs that are marketed in accordance with legal exemptions, such as products for minor species in small packages (article 3.7 Regeling diergeneesmiddelen) and those 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 85.6% of sales in 2021. This coverage fluctuates over the years, due to not yet monitored sectors (e.g. goats, 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 2021 in total 145 tonnes of AVMPs were sold, which is a decrease of 5.8% compared to 2020. A decrease in sales by 70.8% over the years 2009-2021 is attained (with 2009 considered a 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 2021. Antimicrobial use (in kg) in livestock sectors is presented as bars in which the use in different animal species can be distinguished. Liveweight of Dutch livestock was stable around 2500 ktonnes, which demonstrates that the trends in sales and use represent a true decrease of antibiotic use in animals since 2009. Veal calves (light blue) and pigs (green) used almost 80% of the total mass of all antibiotics sold 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 2021 between sales and usage in monitored sectors was 14% 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, tetracyclines and cephalosporins of 1st and 2nd generation).

Tetracyclines
Still the most sold when expressed in mass are the tetracyclines, decreasing with 3.4% in 2021 compared to 2020. The fraction of doxycycline (not specified in Figure 2) was in 2021 67.8% of the total sales of tetracyclines (63.2% in 2020, fluctuations between 31% and 69% in the years 2011-2019).

Penicillins
Second place in mass, sales of penicillins (including aminopenicillins) sharply decreased in 2021 compared to 2020, with 15.4%. The distribution of broad and narrow spectrum penicillins (in mass sold) is comparable to previous years with 71% aminopenicillins.

(Fluoro)quinolones

The sales of fluoroquinolones decreased again with 33kg (22%) in 2021. An overall reduction of 92.1% was realized since 2011. In 2021, 45% of the sales were applied in the monitored sectors. Extending monitoring to other animal species (as will be regulated with EU 2019/6) is warranted. The sales of quinolones (flumequine) decreased with 22.3% in 2021 when compared to 2020; these AVMPs are exclusively applied in food producing animals.

Cephalosporins

Sales of these AVMPs were relatively stable over the period 2015 to 2020, a relatively large increase in sales of 3rd and 4th generation cephalosporins was observed in 2019 followed by the lowest sales ever in 2020 (0.63kg). In 2021 a marked increase was observed in sales data, to 5.3kg.
Still a reduction of 99.4% of all cephalosporins sales has been achieved since 2011.

Polymyxins

Colistin sales decreased in 2021 with 16.9%, after some years of fluctuations in sales and use. The reduction since 2011 is 77%. 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. Main changes in AMU in the sectors (Figure 3) are seen in broilers and rabbits.

Figure 4 shows that in most sectors first choice antimicrobials (green and blue bars) are dominant. In all sectors, except for broilers and turkeys, this proportion of first choice AVMP's has attained a stable level, at 70-85%. Figure 4 also illustrates that use of fluoroquinolones (red bar) is the highest in turkeys, although a reduction of 78% has been observed since 2013. In veal calves, a large sector with the highest proportion of first choice AVMP's, a steady decrease in total use was observed until 2020, in 2021 the use remained the same.
In rabbits, the use of colistin was abandoned in 2020. Total AMU in this sector is still high but a reduction was attained in 2021, compared to the previous three years.

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 January 28th 2022. Sales data will have to be reported to EMA, as is already in place for most EU MS 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 2021 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 2021, overall decrease since 2011 is 77% in sales.

Table 1 Antimicrobial veterinary medicinal product sales from 1999-2021 in kg (thousands) (FIDIN, 2021)

year	'99	'00	'01	'02	'03	'04	'05	'06	'07	'08	'09	'10	'11	'12	'13	'14	'15	'16	'17	'18	'19	'20	'21
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
tetracyclines	162	194	200	214	216	256	292	301	321	257	251	217	157	102	80	69	82	62	68	65	51	49	47
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
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
(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
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
otherantibacterials	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
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

Figure 1 Mass balance of AVMPs sales data (black line, left y-axis) and use data (colored bars, left x-axis) (kg x 1000), combined with total live weight of the food animal population (dotted line, right y-axis, kg x 10^6) from 2009-2021

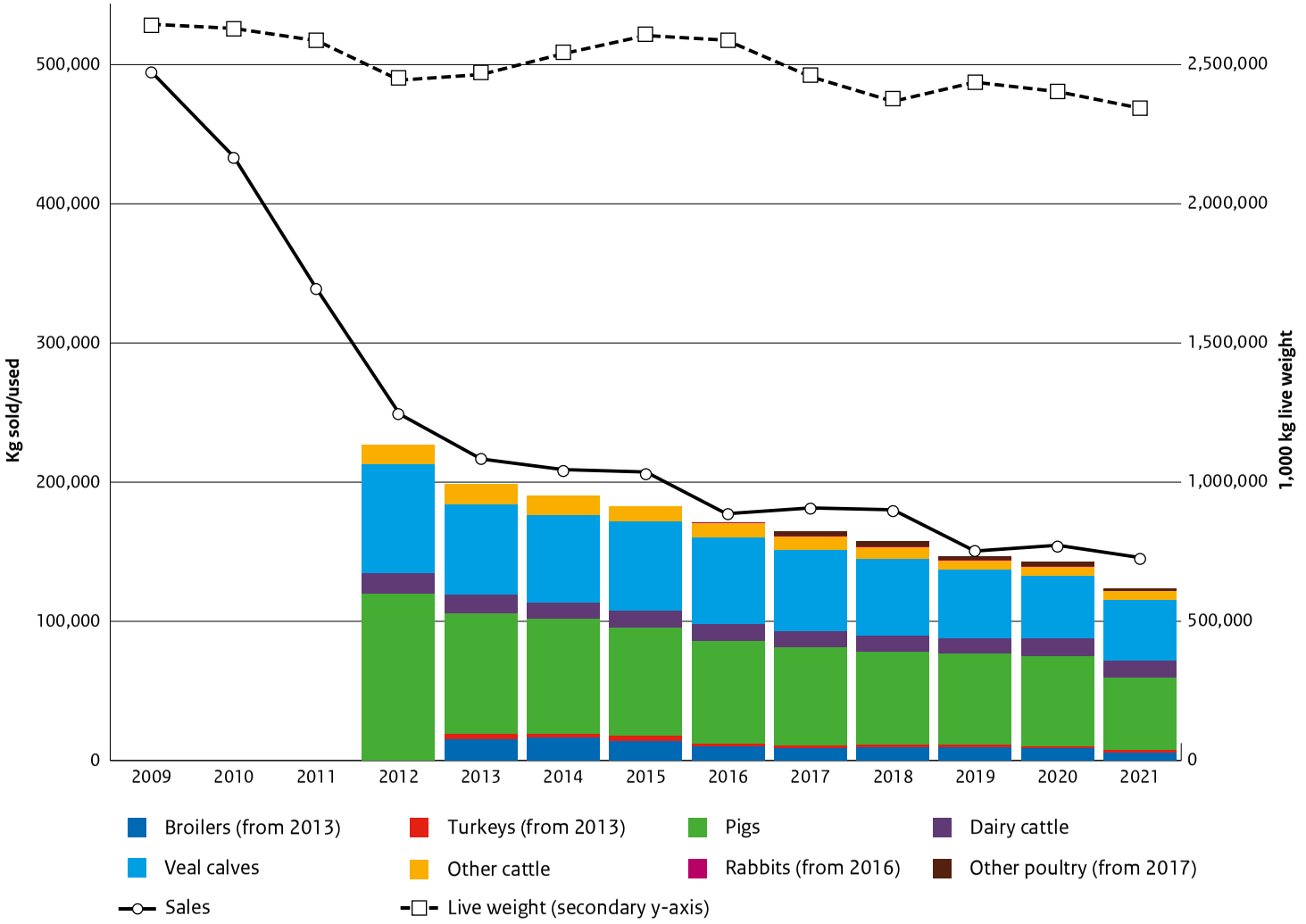


Figure 2 Antimicrobial Veterinary Medicinal Product sales by antibiotic class from 2011-2021 in kg (thousands)

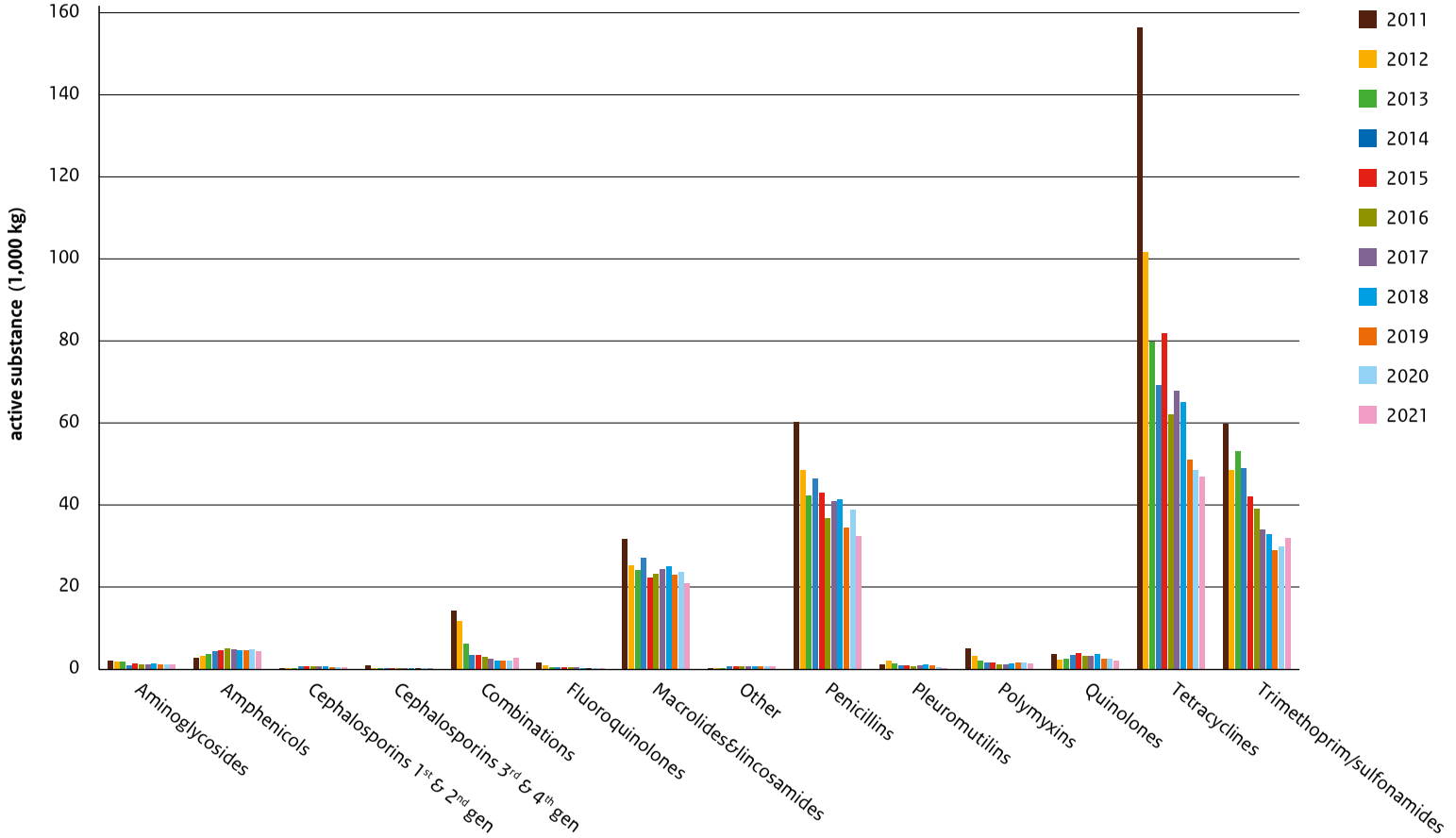


Figure 3 Number of animal-defined daily dosages per animal-year for rabbits (grey), turkeys (purple), veal calves (blue), broilers (orange), pigs (light green) and dairy cattle (dark green) farms as reported by LEI WUR-MARAN (years 2007-2010 as DD/AY) and by SDa (years 2011-2021 as $DDDA_{NAT}$) depicting point estimates (dots), 95% confidence limits (error bars), smoothed trend line (penalized spline) and 95% confidence limits for the spline (shaded area)

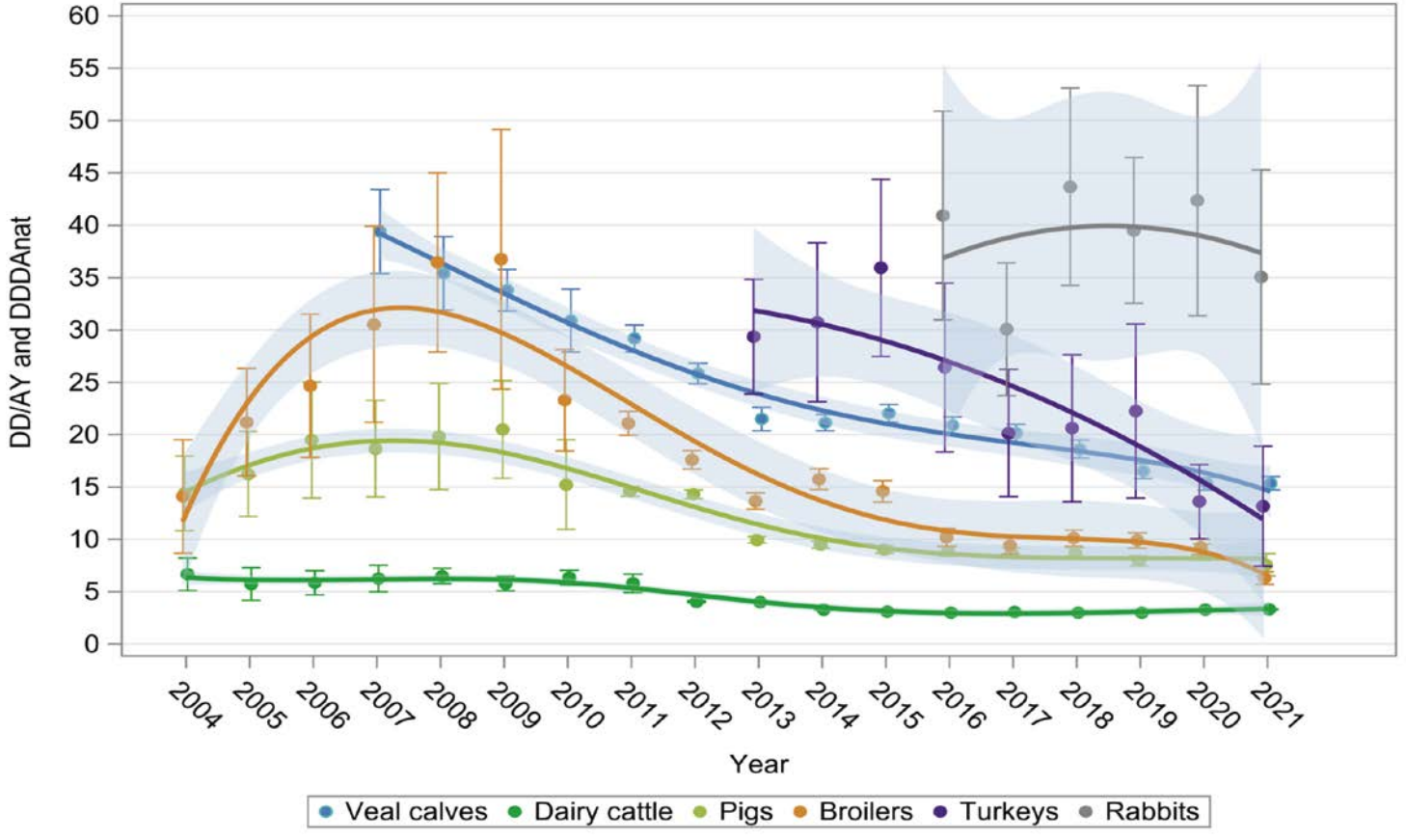
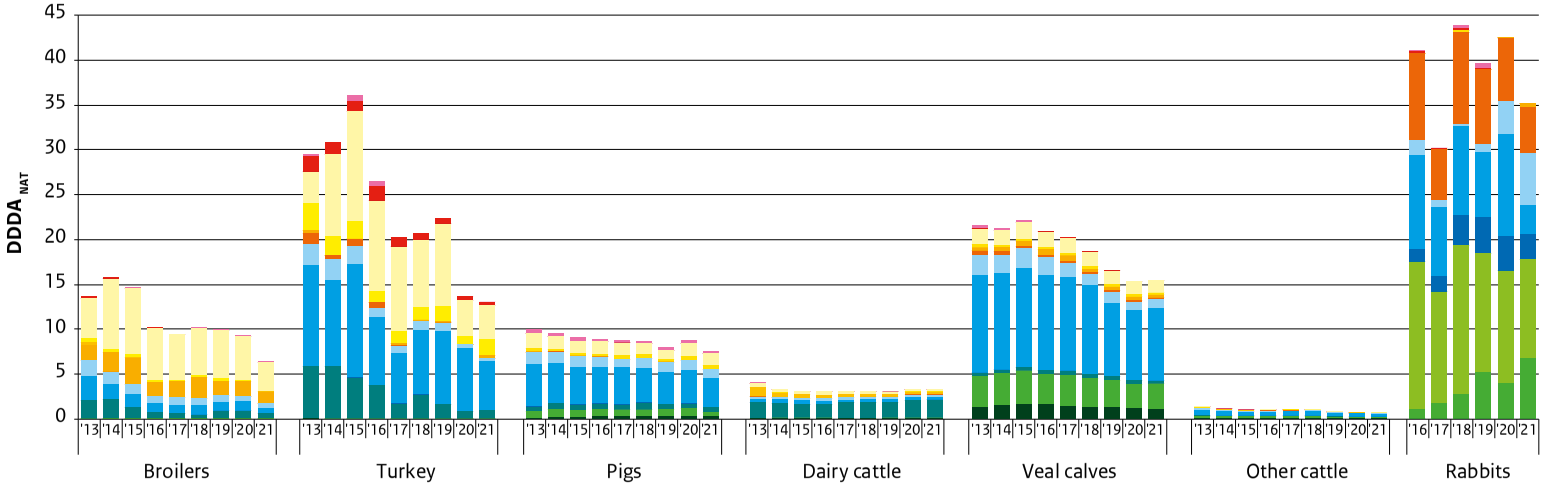


Figure 4 Number of $DDDA_{NAT}$ per animal-year of antimicrobial veterinary medicinal products specified by antibiotic class per animal sector over the years 2013-2021



3
Resistance data

This chapter describes susceptibility test results as determined in 2021 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. In 2021, S. Enteritidis (25%) followed by S. Typhimurium (19%) and monophasic S. Typhimurium (19%) were most frequently isolated from humans suffering from clinical salmonellosis.
2. In pigs, the monophasic variant of S. Typhimurium (32%) and S. Derby (27%) dominated. In cattle, the most frequently identified serovars were S. Dublin (42%) and S. Typhimurium (27%). In broilers, S. Infantis (35%) and S. Paratyphi B var. Java (19%) dominated, while in layers S. Enteritidis (59%) and monophasic S. Typhimurium (15%) were the most common serovars.
3. Over all serovars, the highest resistance proportions were observed for sulfamethoxazole (29.6%), tetracycline (26.6%) and ampicillin (24.5%), with approximately similar levels as in 2020.
4. Serovars showing the highest levels of resistance were S. Infantis, S. Paratyphi B var. Java, monophasic S. Typhimurium variants, and S. Typhimurium, with resistance to ampicillin, tetracycline, sulfamethoxazole, trimethoprim, ciprofloxacin, and nalidixic acid reaching maximum levels of between 64% and 92%.
5. Among S. Typhimurium, resistance to fluoroquinolones decreased considerably among human isolates, while it increased sharply among cattle isolates.
6. Among S. Enteritidis, the fraction of resistance to ciprofloxacin and nalidixic acid among human isolates remained relatively stable but resistance to ampicillin and tetracycline decreased.
7. In total, 10 (0.8%) ESBL-producing (human clinical) isolates were detected.
8. In 2021, no carbapenemase-producing *Salmonella* were found.

Salmonella prevalence

In the Netherlands, an extensive laboratory surveillance of human clinical *Salmonella* infections is carried out by the Dutch National Institute for Public Health and the Environment (RIVM). With this surveillance medical microbiological laboratories in the country are asked to send in isolates from human clinical cases to the RIVM where they are characterized and typed by whole-genome-sequencing (WGS). Table S01 shows a summary of the serotyping results of *Salmonella* isolated from humans and farm animals (pigs, cattle and poultry).

The most frequently isolated serovars from humans suffering from salmonellosis (N=679) in 2021 were the same as in previous years: *S.* Enteritidis (25%), followed by *S.* Typhimurium (19%) and monophasic *S.* Typhimurium (19%). The most frequent isolated serovars from pigs (N=129) were monophasic *S.* Typhimurium (32%), *S.* Derby (27%), and *S.* Typhimurium (12%). For cattle (N=55), these were *S.* Dublin (42%), and *S.* Typhimurium (27%). Isolates from broilers (N=263) were dominated by *S.* Infantis (35%), followed by *S.* Paratyphi B var. Java (19%) and *S.* Enteritidis (10%). Among laying hens (N=66), the most frequently isolated serovar were *S.* Enteritidis (59%) and monophasic *S.* Typhimurium (15%).

Table S01 Most prevalent *Salmonella* serotypes isolated in 2021 (in %) from humans, pigs (including pork), cattle (including beef), layers (including reproduction animals and eggs)

	Humans	Pigs	Cattle	Broiler	Layer
N Total obtained/received	**679**	**129**	**55**	**263**	**66**
N tested for AMR	**601**	**99**	**55**	**249**	**62**
Enteritidis	25	0	7	10	59
Typhimurium	19	12	27	4	2
Typhimurium monophasic	19	32	5	1	15
Braenderup	4	0	0	0	2
Infantis	3	2	0	35	5
Montevideo	3	0	0	1	0
Bovismorbificans	2	2	0	0	0
Newport	2	0	0	<1	0
Dublin	1	0	42	0	0
Virchow	1	0	2	3	0
Derby	1	27	0	0	3
Chester	1	0	0	0	0
Brandenburg	1	6	2	0	0
Typhi	1	0	0	0	0
Saintpaul	1	0	0	<1	0
Goldcoast	1	5	2	<1	0
Napoli	1	0	0	0	0
Goettingen	1	2	0	0	0
Coeln	1	0	0	0	0
Senftenberg	1	0	0	2	2
Kedougou	1	1	0	<1	0
Hadar	1	0	0	0	0
Give	1	0	0	0	0
Poona	<1	0	0	0	0
Paratyphi B. var. Java	<1	0	0	19	0
London	<1	2	0	0	0
Javiana	<1	0	0	0	0
Anatum	<1	0	0	0	0
OTHER	9	9	13	23	14

Resistance proportions overall

A selection of all human *Salmonella* isolates received by the RIVM from regional public health and other clinical laboratories (n = 601) was sent to WBVR for susceptibility testing. Moreover, 663 isolates from non-human sources were tested. These included isolates from broilers (n = 249), cattle (n = 55), pigs (n = 99), and layers (n = 62), as well as isolates from a diversity of other sources, including animal feed (n = 66), vegetables and other animals (e.g. sheep, goats, horses, rabbits, ducks, etc., n = 132). The non-human isolates included also 118 isolates from food products (e.g. meat, seafood, etc.) 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. Due to the implementation of the new European legislation on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria (2020/1729/EU) *Salmonella* isolates from caecal samples of pigs and veal calves collected at slaughter were included for the first time.

MIC-distributions and resistance percentages of 1264 *Salmonella* isolates from different sources tested for susceptibility in 2021 are presented in Table S02. Similarly to last years, the highest resistance proportions were again observed for (in decreasing order) sulfamethoxazole (30% in 2021, 26% in 2020), tetracycline (27% in 2021, 25% in 2020), ampicillin (25% in 2021, 22% in 2020), nalidixic acid (both 16% in 2021 and 2020), ciprofloxacin (16% in 2021, 16% in 2020), trimethoprim (12% in 2021, 12% in 2020) and chloramphenicol (7.9% in 2021, 6.7% in 2020). Similar to previous years, no resistance was detected to the carbapenem antibiotic meropenem. As in previous years, low proportions of resistance were found for tigecycline, azithromycin, cefotaxime, ceftazidime, and gentamicin.

Fluoroquinolone resistance

The class of fluoroquinolones is regarded as the treatment of choice for severe salmonellosis in 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, 16% of *Salmonella* isolates from 2021 demonstrated an acquired resistance phenotype for ciprofloxacin (Table S02), which is around the same as previous years. The highest levels of ciprofloxacin resistance among the most prevalent serovars was observed for *S.* Infantis, namely 64% in 2021 which seemed to have stabilized after a sharp increase from 45% in 2019 to 63% in 2020 (Table S03). The levels of resistance among *S.* Paratyphi B var. Java strongly (further) declined from 47% in 2019, 44% in 2020, to 30.8% in 2021. Ciprofloxacin resistance among *S.* Enteritidis remained stable (both 18% in 2021 and 2022). Resistance rates among *S.* Typhimurium declined substantially from 11% in 2020 to 4.7% in 2021, while among monophasic *S.* Typhimurium a slight increase was visible (15% in 2021, 12% in 2020).
Table S06 shows that the proportion of isolates resistant to ciprofloxacin in broiler meat, after a 1-year upsurge in 2020, continued to further decline (89% in 2017, 69% in 2018, 58% in 2019, 66% in 2020, 58% in 2021). These isolates (59% *S.* Infantis, 26% *S.* Paratyphi var. Java, 9% *S.* Havana) were obtained from broiler meat and broiler meat preparations from retail and meat industry. The high proportion of resistance to fluoroquinolones in poultry meat reflects the frequent usage of fluoroquinolones in the poultry production chain within EU.

Table So2 MIC distribution (in %) and resistance percentages (R%) for all *Salmonella* isolates (N=1,264) tested for antibiotic susceptibility during 2021

Salmonella N = 1,264	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							39.1	32.6	3.6	0.3			24.5						24.5	22.2 - 26.9
Cefotaxime					95.1	3.6	0.5			0.8									1.3	0.7 - 2.1
Ceftazidime					60.9	33.2	5.1	0.2	0.1	0.2	0.3								0.6	0.3 - 1.2
Gentamicin						90.4	5.4	0.6	0.2	0.1	0.2	3.2							3.6	2.7 - 4.7
Tetracycline								71.1	2.0	0.3	0.4	0.6	25.6						26.6	24.2 - 29.1
Sulfamethoxazole										36.2	19.2	9.4	4.7	0.9			29.6		29.6	27.6 - 32.2
Trimethoprim					67.7	17.7	2.1	0.2	0.2			12.1							12.3	10.6 - 14.3
Ciprofloxacin	28.9	52.5	3.1	2.1	8.0	4.1	0.9	0.2	0.1		0.2								15.5	13.6 - 17.6
Nalidixic acid									78.3	5.3	3.3	1.5	0.2	11.4					16.4	14.4 - 18.5
Chloramphenicol										85.9	6.2	0.7	0.2	7.0					7.9	6.5 - 9.5
Azithromycin*								2.0	29.6	60.6	6.3	0.8		0.7					1.5	0.9 - 2.3
Colistin**							76.9	12.8	6.6	3.7									-	-
Meropenem		71.8	28.2																0.0	0.0-0.3§
Tigecycline***					74.5	16.1	7.8	1.7	0.1										1.7	1.1 -2.6

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. Salmonella with elevated colistin MIC-values (colistin MIC > 2 mg/L for most Salmonella and MIC > 4 mg/L for Dublin and Enteritidis) were screened with PCR for the presence of mcr-genes (see section 4.3).*

*** *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.*

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 2021, the total number of genotypic confirmed ESBL *Salmonella* isolates was 10/1264 (0.8%) (compared to 6/1170 [0.5%] in 2020, and 24/1880 [1.3%] in 2019). These included, 1x human *S.* Infantis, 2x human *S.* Kentucky, 1x human *S.* Typhi, 3x human *S.* Typhimurium and 1x non-human (unknown source) *S.* Apeyeme and 2x non-human *S.* Minnesota.

Resistance proportions of the most prevalent serovars

Table S03 presents resistance percentages for the most prevalent serovars isolated in the Netherlands (all sources together) in 2021. There was considerable variation between the resistance profiles of the different serovars. For all antimicrobials tested, the most resistance serotypes (both with respect to absolute levels of resistance to individual antimicrobial and multi-drug resistance) were *S.* Infantis (very high levels of resistance to sulfamethoxazole, ciprofloxacin, nalidixic acid, tetracycline and high levels of resistance to trimethoprim), *S.* Paratyphi B var. Java (very high levels of resistance to trimethoprim and sulfamethoxazole; high levels of resistance to ciprofloxacin, nalidixic acid, and ampicillin), monophasic *S.* Typhimurium (very high levels of resistance to ampicillin, sulfamethoxazole, and tetracycline) and *S.* Typhimurium (high levels of resistance to ampicillin, tetracycline, sulfamethoxazole and chloramphenicol). *S.* Infantis and *S.* Paratyphi B var. Java are mainly poultry related while *S.* Typhimurium and its monophasic variant are mainly pig and cattle related.

Table S03 Resistance (%) of the most prevalent (>20 isolates) *Salmonella* serovars isolated in the Netherlands in 2021 (N tested)

	Enteritidis (227)	Typhimurium (191)	Typhimurium monophasic (177)	Infantis (120)	Paratyphi B vr. Java (52)	Dublin (34)	Derby (30)	Montevideo (27)	Braenderup (25)
Ampicillin	4.8	41.9	88.1	10.0	26.9	11.8	6.7	0.0	0.0
Cefotaxime	0.0	1.6	3.4	0.8	0.0	0.0	0.0	0.0	0.0
Ceftazidime	0.0	0.5	0.0	0.8	0.0	0.0	0.0	0.0	0.0
Gentamicin	0.4	10.5	6.2	0.8	0.0	0.0	0.0	0.0	4.0
Tetracycline	0.9	34.6	91.5	60.0	15.4	8.8	0.0	0.0	0.0
Sulfamethoxazole	4.4	36.6	86.4	63.3	55.8	11.8	3.3	0.0	0.0
Trimethoprim	0.0	9.4	15.3	28.3	88.5	8.8	3.3	0.0	0.0
Ciprofloxacin	17.6	4.7	15.3	64.2	30.8	2.9	0.0	0.0	4.0
Nalidixic acid	17.6	4.7	15.8	65.8	30.8	2.9	0.0	0.0	4.0
Chloramphenicol	0.4	17.8	18.6	5.8	0.0	11.8	0.0	0.0	0.0
Azithromycin	0.4	0.5	5.1	5.0	0.0	0.0	0.0	0.0	0.0
Meropenem	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Tigecycline	0.4	1.0	5.1	4.2	0.0	0.0	0.0	0.0	0.0

Resistance patterns of *S.* Typhimurium

The resistance patterns of *S.* Typhimurium are separately depicted in Table S04 and (Fig. S01). Resistance remained high for ampicillin, sulfamethoxazole, and tetracycline, especially among and in human and cattle isolates. Resistance to the clinically important drug cefotaxime was only detected among human isolates (2.4% in 2021 compared to 1.2% in 2020). The resistance percentage to fluoroquinolones decreased considerably among human isolates from 14.8% in 2020 to 5.6% in 2021. Also fractions of resistance against ampicillin and chloramphenicol decreased considerably. Increases in resistance proportions for *S.* Typhimurium were observed among isolates from cattle (Table S04 and Fig. S01), especially for ampicillin, ciprofloxacin, nalidixic acid and chloramphenicol. However, this might be related to the relatively small number of isolates per year and trends should be interpreted with care.
The monophasic variant of *S.* Typhimurium showed very high levels of resistance to ampicillin, tetracycline, and sulfamethoxazole among human and pig isolates, as well as isolates from other sources Table S04.

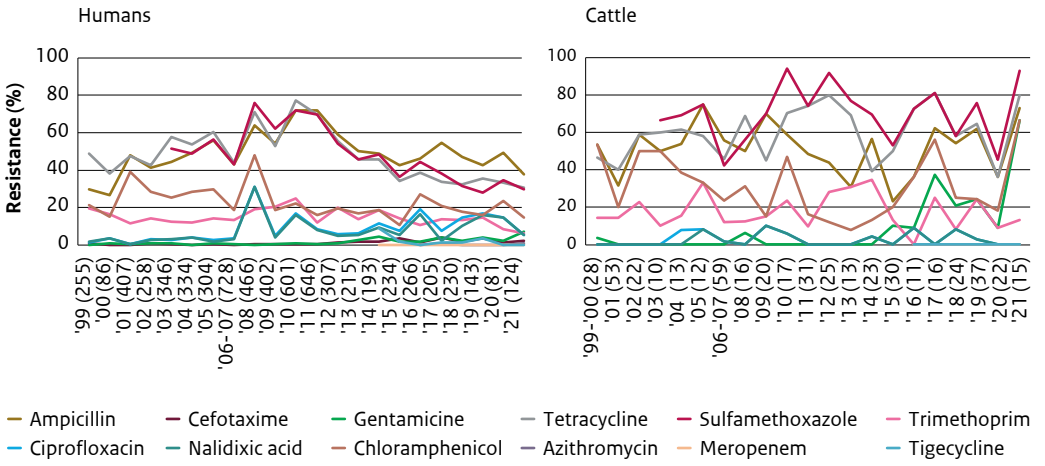
Table S04 Resistance percentages of *S.* Typhimurium (N tested) isolated from humans, cattle, pigs and other known sources in 2021

	S. Typhimurium (181)[a]			
	Humans (124)	Cattle (15)	Pig (16)	Other known sources (26)[b]
Ampicillin	37.9	73.3	68.8	26.9
Cefotaxime	2.4	0.0	0.0	0.0
Ceftazidime	0.8	0.0	0.0	0.0
Gentamicin	7.3	66.7	0.0	3.8
Tetracycline	30.6	80.0	37.5	19.2
Sulfamethoxazole	29.8	93.3	50.0	23.1
Trimethoprim	6.5	13.3	31.3	7.7
Ciprofloxacin	5.6	0.0	6.3	0.0
Nalidixic acid	5.6	0.0	6.3	0.0
Chloramphenicol	14.5	66.7	25.0	3.8
Azithromycin	0.8	0.0	0.0	0.0
Meropenem	0.0	0.0	0.0	0.0
Tigecycline	0.0	0.0	6.3	0.0

a Monophasic variant is exluded.

b Other known sources include broilers (11), layers (1), horses (5), grains/beans/seeds (2), goats (4), sheep (1) and feed (2).

Figure S01 Trends in resistance (%) of *S.* Typhimurium isolated from humans and food-animals in 1999-2021



Resistance proportions of *S.* Enteritidis

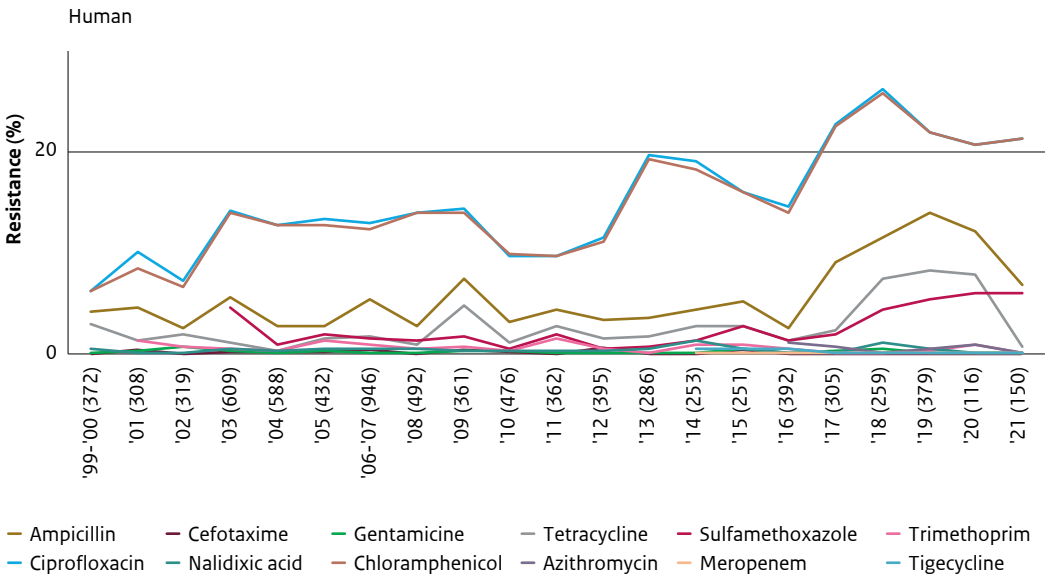
In the Netherlands, human infections caused by *S.* Enteritidis are mainly related to the consumption of contaminated eggs and, to a lesser extent, of poultry meat products or related to travel abroad.
Fractions of resistance in *S.* Enteritidis is relatively low compared to *S.* Typhimurium. Table S05 and Fig. S02 presents resistance proportions in *S.* Enteritidis isolates from human samples and other sources (including broilers, layers, cattle and very few food/feed isolates). Among human isolates, the resistance percentages for ciprofloxacin and nalidixic acid remained relatively stable (21% both in 2021 and 2020, 22% in 2019). Resistance to ampicillin (6.7% in 2021, 12% in 2020) and tetracycline (0.7% in 2021, 7.8% in 2020) decreased considerably. For all other antimicrobials, resistance proportions of human *S.* Enteritidis isolates were very low or not detected.
The most important resistance in the isolates from poultry were, alike the human isolates, against ciprofloxacin and nalidixic acid among layers (both 11% in 2021 compared to 13% in 2020) (Table S05).

Table S05 Resistance percentages of *S.* Enteritidis (N tested) isolated from humans and broilers in 2021

	S. Enteritidis (220)			
	Humans (150)	Layers (36)	Broilers (26)	Other known sources (8)[a]
Ampicillin	6.7	0.0	0.0	12.5
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	12.5
Tetracycline	0.7	0.0	0.0	12.5
Sulfamethoxazole	6.0	0.0	0.0	12.5
Trimethoprim	0.0	0.0	0.0	0.0
Ciprofloxacin	21.3	11.1	3.8	12.5
Nalidixic acid	21.3	11.1	3.8	12.5
Chloramphenicol	0.0	0.0	0.0	12.5
Azithromycin	0.0	0.0	0.0	12.5
Meropenem	0.0	0.0	0.0	0.0
Tigecycline	0.0	0.0	0.0	12.5

a Other sources include cattle (4), horses (1), rabbit (1), and feed (1).

Fig S02 Trends in resistance (%) of S. Enteritidis isolated from humans from 1999-2021



Salmonella from chicken meat, other meat sources and spices

Table S06 and Fig. S03 show resistance data of *Salmonella* isolates from broiler meat, other meat, and other products. In general, levels of resistance among isolates from broiler meat are similar to 2020, with high levels of resistance regarding ciprofloxacin (58.1%), nalidixic acid (59.5%), tetracycline (50%), sulfamethoxazole (60.8%), and trimethoprim (37.8%). Isolates from pork especially showed resistance to sulfamethoxazole (60.8%), ampicillin (52%), and tetracycline (48%).

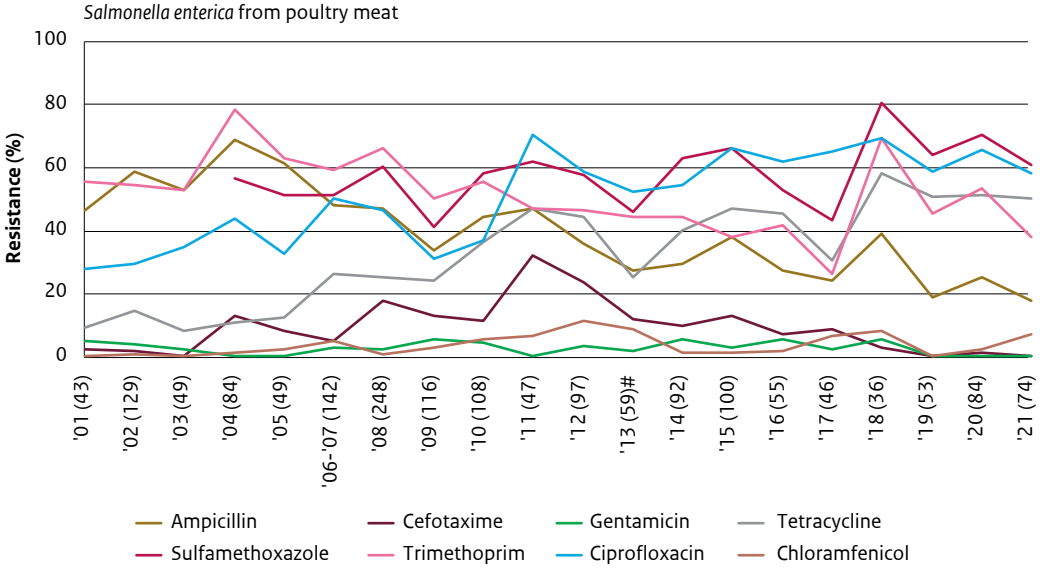
Table S06 Resistance (%) of *Salmonella enterica* isolated from different types of raw meat in the Netherlands in 2021

	Broiler meat[a]	Pork	Other meat[b]
	N = 73	N = 25	N =16
Ampicillin	17.6	52.0	12.5
Cefotaxime	0.0	0.0	0.0
Ceftazidime	0.0	0.0	0.0
Gentamicin	0.0	0.0	6.3
Tetracycline	50.0	48.0	12.5
Sulfamethoxazole	60.8	60.0	12.5
Trimethoprim	37.8	16.0	0.0
Ciprofloxacin	58.1	0.0	6.3
Nalidixic acid	59.5	4.0	6.3
Chloramphenicol	6.8	4.0	6.3
Azithromycin	8.1	0.0	6.3
Meropenem	0.0	0.0	0.0
Tigecycline	5.4	0.0	6.3

a Fresh chicken meat sampled at retail and chicken neck skin from verification projects.

b Other meat includes sheep/lamb (8), beef/calf (7), and rabbit (1).

Figure S03 Trends in resistance (%) of *Salmonella* enterica isolated from poultry meat in the Netherlands from 2001-2021



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 the new European legislation on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria (2020/1729/EU). It was decided to include isolates from both bovines and swine in addition to 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. Recently[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.

From 2019 onwards, data on human isolates were obtained from ISIS-AR (see chapter 4), 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.

Table C01 presents the MIC distributions and resistance percentages for all *Campylobacter jejuni* and *C. coli* strains isolated in 2021 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 the 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).

Table Co1 MIC distribution (in %) for *Campylobacter jejuni* (N = 354) and *C. coli* (N = 504) isolated from caecal samples of broilers in 2021

***C. jejuni*, broilers and veal calves**	**MIC (%) distribution mg/L**														**R%**	**95% CI**
(N = 354)	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**1024**		
Chloramphenicol					44.4	42.9	7.1	5.4	0.3	0.0					0.3	0.0 - 1.6
Ciprofloxacin	39.8	4.0	0.8	0.0	0.0	0.8	22.0	22.9	7.1	2.5					55.4	50.0 - 60.6
Ertapenem	74.3	18.9	4.2	1.1	0.3	1.1									2.5	1.2 - 4.8
Erythromycin				63.8	31.4	4.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0		0.0	0.0 - 1.0
Gentamicin		95.5	4.5	0.0											0.0	0.0 - 1.0
Tetracycline			22.9	2.3	0.3	0.0	1.7	4.2	3.7	16.7	48.3				74.9	70.0 - 79.3

***C. coli*, broilers, veal calves and pigs**	**MIC (%) distribution mg/L**														**R%**	**95% CI**
(N = 506)	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**1024**		
Chloramphenicol					7.3	43.1	37.0	12.5	0.2	0.0					0.2	0.0 - 1.1
Ciprofloxacin	43.5	10.5	2.0	0.0	0.0	4.0	13.8	15.2	10.5	0.6					44.1	39.7 - 48.5
Ertapenem	38.9	22.3	13.8	14.2	6.5	3.2	1.0								24.9	21.2 - 28.9
Erythromycin				36.6	30.2	18.0	3.8	0.2	0.2	0.0	0.0	0.6	1.0	9.5	11.5	8.8 - 14.6
Gentamicin		56.1	41.7	0.8					1.4						1.4	0.1 - 2.8
Tetracycline			18.0	2.8	1.0	0.0	0.8	1.4	1.8	10.9	63.4				78.3	74.4 - 81.8

Highlights

1. Due to a new legislation *C. jejuni* and *C. coli* isolates obtained from veal calves as well as *C. coli* from fattening pigs are included in the mandatory AMR monitoring program in livestock from 2021 onwards.
2. In 2021, resistance proportions in *C. jejuni* isolates from caecal samples of broilers and meat thereof decreased but remained at a high level for quinolones and tetracycline.
3. Resistance to macrolides was not detected in *C. jejuni* isolates from broilers and poultry meat, and was at low levels in *C. coli* isolates from broilers and poultry meat. A notably higher level of macrolide resistance was observed in *C. coli* from veal calves.
4. In human isolates, resistance proportions were higher in *C. coli* than in *C. jejuni*, but similar to 2020, these were overall lower in 2021 compared to previous years. This is most likely 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.
5. Ciprofloxacin resistance in *Campylobacter* isolates from humans was again high in 2021, which is a concern for public health. It was, however, lower compared to 2017-2020.
6. Resistance to erythromycin, first choice antibiotic in human medicine for campylobacteriosis, remained low.

Resistance proportions

As in former years, resistance proportions were high for ciprofloxacine and tetracycline in both *C. jejuni* and *C. coli* isolates (Table C01 and C02). In contrast, resistance against chloramphenicol, erythromycin, and gentamicin was rarely observed or completely absent in the different types of samples. Amongst *C. jejuni* isolates, resistance to erythromycin and gentamicin was not detected. For ertapenem, notable differences were observed in levels of resistance between *C. jejuni* and *C. coli* with higher resistance proportions in *C. coli* compared to *C. jejuni*. This issue is discussed in more detail later in this chapter.
Figure C01 presents the resistance levels of *C. jejuni* from poultry meat and broilers over the last 18 and 22 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 the resistance percentages for tetracycline over the years were approximately the same as ciprofloxacin resistance, with a similar trend. 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 which is most clearly observed in *C. coli* isolates from broilers.

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, but decreased from 68.9% in 2020 to 58.8% in 2021. Compared to 2020, the proportion of fluoroquinolone resistance in *C. jejuni* from poultry meat decreased to 63.3% which is similar to the resistance level in *C. jejuni* isolates from broilers in 2021 suggesting an overlap in the bacterial population examined from the different matrixes. In addition, similar resistance proportion are also observed in isolates from chicken neck skin samples (Table C02).

In 2021, the level of ciprofloxacin resistance in *C. coli* isolates from broilers was also high (78.6%), but showed a decreasing level compared to 2020 (91.7%). The proportion of resistance of *C. coli* isolates from poultry meat fluctuates more over time due to the low number of isolates found in the survey. In 2021 the observed proportion of ciprofloxacin resistance was high again with 88.6%.

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. Table C02 shows that no resistance was detected in *C. jejuni* from caecal samples of broilers, chicken and other poultry meat. In addition, no macrolide resistance was observed in *C. jejuni* from caecal samples of veal calves. Table C03 shows that between 1.9% and 2.5% (average: 2.1%) of human *C. jejuni* isolates were resistant for erythromycin in the period 2014-2021. It should be noted that for human isolates a lower breakpoint for resistance 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 (2.4%), pigs (3.5%) and broiler meat (8.3%) (table C02). Notably high levels of macrolide resistance were observed in *C. coli* isolates from caecal samples of veal calves (33.6%).

Carbapenems

Due to the new legislation, ertapenem was added to the mandatory antibiotic panel for testing *Campylobacter* as indicator for carbapenem resistance. Amongst *C. jejuni*, isolates exhibiting MICs above the tentative breakpoint (R: > 0.5 mg/L) were frequently detected resulting in resistance proportion varying from 0.5% in veal calves up to 12.3% in broilers (isolates from neck skin). Within *C. coli*, resistance proportions were higher compared to *C. jejuni* (except for pigs) varying between 2.8% in pigs and 76.2% in broilers. The biological meaning and the implications of these presumed high resistance proportion will be further assessed. This will include the evaluation of the tentative cut-off values for both *C. jejuni* and *C. coli* as well as molecular typing of the suspected ertapenem resistant isolates by Whole Genome Sequencing (WGS) to screen for potential resistance mechanisms involved.

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 chicken meat samples.
Figure C01 demonstrates a high similarity in resistance trends between *C. jejuni* obtained from caecal samples at slaughter and those obtained from retail meat. Resistance to tetracycline decreased, but remained high in 2021 in both broilers and poultry meat. Resistance percentages for ciprofloxacin have been high with fluctuation over the years. The resistance levels for erythromycin and gentamicin were very low to zero over the last 10 years. Moreover, resistance to erythromycin and gentamicin has not been detected in isolates from broilers, and poultry meat since 2020.
The resistance levels in *C. coli* isolates from broilers and poultry meat are presented in Figure C02. These levels show more fluctuation over years than levels of *C. jejuni*, which is most likely caused by the lower number of isolates in the survey. Resistance in *C. coli* from broilers and poultry meat could not be detected for gentamicin, which was also seen in the years before. Resistance levels for erythromycin in *C. coli* has been fluctuating substantially over the years. In 2021, resistance levels obtained in *C. coli* from broilers and broiler meat were similar to 2020 with 2.4% and 8.3%, respectively. Resistance percentages for

ciprofloxacin in broilers and poultry meat have been fluctuating at a high level since 2001, and were still high in 2021. 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 was detected in both *C. jejuni* and *C. coli* isolates from broilers and broiler meat.
Resistance to erythromycin was absent in *C. jejuni* isolates and rarely found in *C. coli* from broilers (2.4%), but more often in broiler meat (8.3%).
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. The resistance proportions of both *C. jejuni* and *C. coli* in broilers and poultry meat show similar trends, as can be seen in Figure C01 and Figure C02.

Figure C01 Trends in resistance (%) of *Campylobacter jejuni* isolated from broilers and chicken meat in the Netherlands

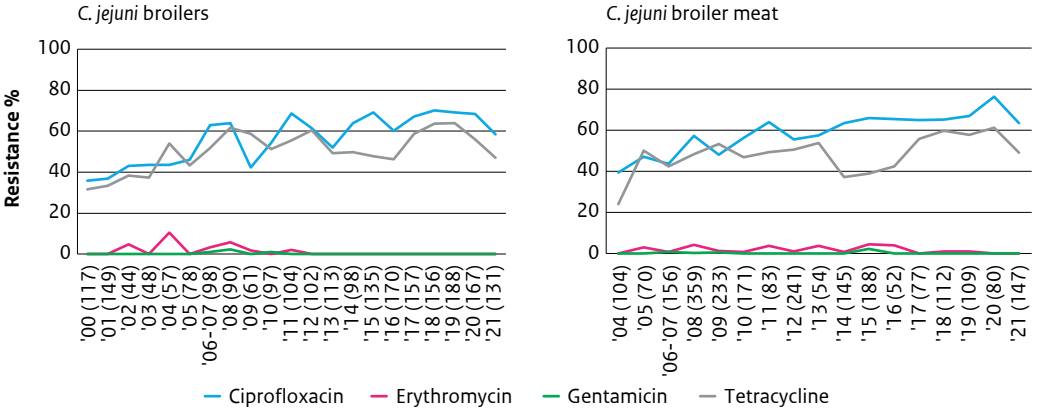


Figure Co2 Trends in resistance of *Campylobacter coli* isolated from broilers and chicken meat in the Netherlands

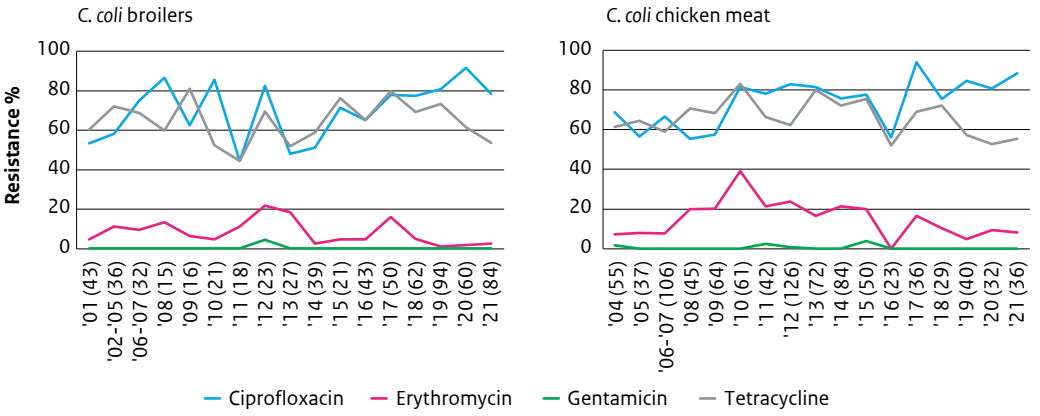


Table Co2 Resistance percentages of *C. jejuni* and *C. coli* isolated from faecal samples of livestock and meat in 2021

	C. jejuni (R%)					*C. coli* (R%)				
	Broilers	Chicken meat	Chicken nek skin	Poultry meat*	Veal calves	Broilers	Chicken meat	Chicken nek skin	Poultry meat*	Veal calves
	N = 131	N = 147	N = 65	N = 17	N = 222	N = 84	N = 36	N = 24	N = 137	N = 287
Chloramphenicol	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0
Ciprofloxacin	58.8	63.3	61.5	35.3	53.2	78.6	88.9	91.7	77.4	17.8
Ertapenem	6.1	6.8	12.3	5.9	0.5	76.2	25.0	54.2	39.4	2.8
Erythromycin	0.0	0.0	0.0	0.0	0.0	2.4	8.3	8.3	33.6	3.5
Gentamicin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.6	0.7
Tetracycline	47.3	49.0	56.9	23.5	91.0	53.6	55.6	54.2	95.6	77.4

* *Poultry meat: turkey, duck and guinea fowl*

Veal calves and fattening pigs

Due to the new legislation *Campylobacter* isolation of caecal samples of veal calves and fattening pigs was included in the AMR monitoring program in 2021. As a result, susceptibility testing was performed of 222 *C. jejuni* and 137 *C. coli* isolates collected from veal calves and 287 *C. coli* isolates from pigs. For tetracycline very high resistance levels were measured for *C. jejuni* (91.0%) and *C. coli* (95.6%) from veal calves as well as for *C. coli* from pigs (77.4%). Resistance levels were also high in veal calves for ciprofloxacin in both *C. jejuni* (53.2%) and *C. coli* (77.4%). Clearly lower levels of ciprofloxacin resistance were detected in *C. coli* from pigs (17.8%). In *C. jejuni* from veal calves resistance to ertapenem was rarely observed (0.5%) and undetected for chloramphenicol, erythromycin and gentamicin. In contrast, erythromycin resistance was frequently observed in *C. coli* from veal calves (33.6%), whereas resistance to chloramphenicol and gentamicin

was detected at low levels with 0.7% and 3.6%, respectively. As stated earlier, a notable high resistance proportion for ertapenem was observed in *C. coli* from veal calves (39.4%) which was substantially higher than in *C. coli* from pigs (2.8%). The implication of this outcome is not clear yet. Resistance to gentamicin and erythromycin was rare and chloramphenicol was not detected in *C. coli* from pigs.

Campylobacter in humans

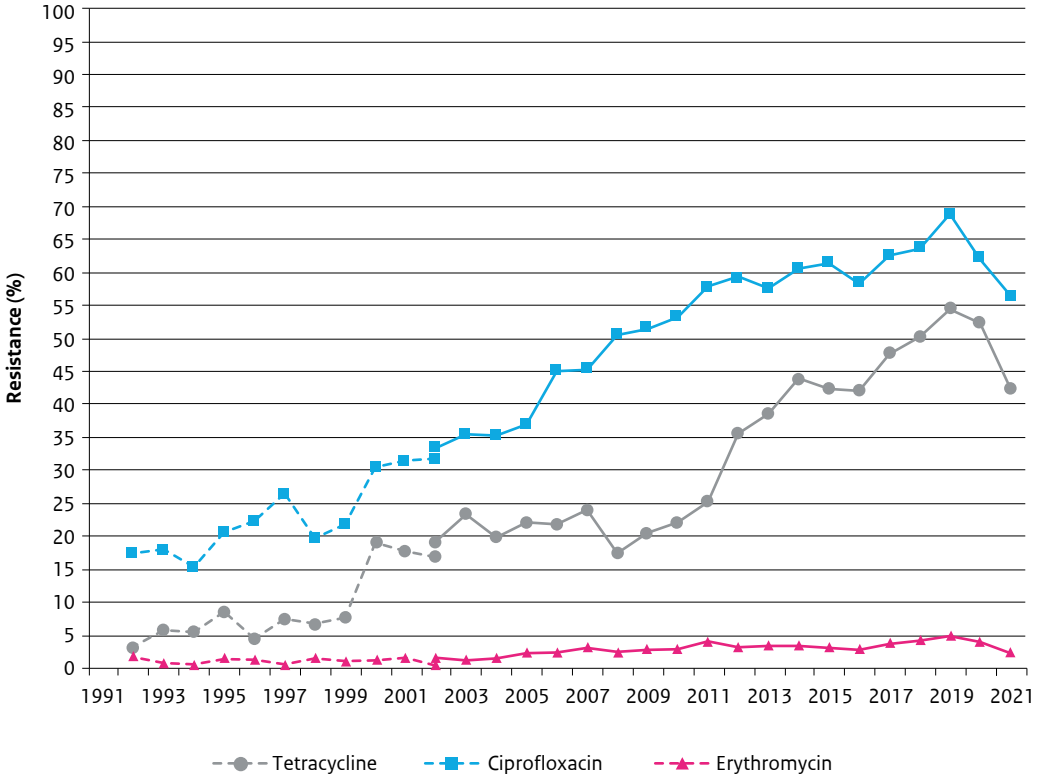
In 2021 an estimated 4219 campylobacteriosis cases occurred The Netherlands (based on 2692 reported cases in ISIS-AR with a national coverage of 64%). Similarly to 2020 this substantially lower compared to a median of 3780 reported cases between 2011-2019. Resistance levels in isolates from human patients 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, however, resistance levels for all measured antibiotics dropped, which dropped even further in 2021. This is most likely due to a substantial reduction in travel-related campylobacteriosis as a result of the COVID-19 lockdown (data on travel history not available), which is associated with higher resistance levels than domestically acquired campylobacteriosis. Because data on travel history it not available, this cannot be confirmed.

In 2021, the resistance levels for ciprofloxacin in human *Campylobacter* spp. isolates were still high with 56.5%, which is a public health concern. As shown in Figure C03, however, ciprofloxacin resistance in 2021 was substantially lower than before the COVID-19 pandemic, with resistance ranging between 62,6% and 68,9% in 2017-2019. Tetracycline resistance decreased to 42,4%, which is 10% lower than in 2020. Erythromycin resistance was 2.1% in 2021, which is lower than in the previous 15 years.
Table C03 shows the resistance levels for human *C. jejuni* and *C. coli* isolates since 2014. The resistance levels in human *Campylobacter* spp. isolates for all three antimicrobials show an increasing trend until 2019, and a reduction in resistance levels in 2020 and 2021. 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 2014 - 2021

	C. jejuni						*C. coli*					
	Fluoroquinolone		Tetracycline		Erythromycin		Fluoroquinolone		Tetracycline		Erythromycin	
	N	R%	N	R%	N	R%	N	R%	N	R%	N	R%
2014	2,084	63.2	1,919	44.9	2,068	2.1	143	72.7	131	56.5	141	22.0
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

Figure C03 Trends in resistance (%) of *Campylobacter* spp. isolated from humans between 1992 and 2021. The dashed line represents the sentinel surveillance between 1992 and 2002, the continuous line represents national surveillance data from 2002 onwards



3.1.3 Shiga-toxin producing *E. coli* (STEC) and atypical enteropathogenic *E. coli* (EPEC)

Highlights

1. In STEC O157, after a decrease in resistance for 2020, a tendency of increasing resistance towards the fluctuating levels of in 2018-2019 was observed.
2. Resistance to the quinolones (ciprofloxacin and nalidixic acid) was very low in both STEC O157 and STEC/EPEC non-O157 human isolates in 2021.
3. Proportions of resistance were higher in human STEC/EPEC non-O157 *E. coli* than in STEC O157 for all antimicrobials, except gentamicin, tetracycline and sulfamethoxazole.
4. No ESBL-producing isolates were detected in STEC O157, but O163 aEPEC isolates from one case were confirmed as ESBL-producer carrying bla_{CMY-41}.
5. Almost all STEC O146 isolates- associated with human infections linked to consumption of raw milk products from small ruminants - were pan-susceptible.

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 in assessing the risk of transmission of resistant bacteria, and resistance genes, from ruminants to humans.

In contrast to the years before 2020, in 2021 not only STEC O157 but a larger collection of isolates from human clinical cases (N = 306) consisting of multiple STEC/aEPEC/tEPEC[a] non-O157 serotypes were tested for susceptibility. The set consisted of 63 STEC O157 isolates, 243 STEC/EPEC non-O157 isolates: O146 (n=30), O26 (n=29), O63 (n=21), O145 (n=16), O103 (n=15), O91 (n=14), and other O-types (N=118). 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.

Compared to 2020, an increase in resistance proportions among STEC O157 similar to the fluctuating levels of 2018-2019 was observed for ampicillin, gentamicin, chloramphenicol and trimethoprim (Figure STEC01). Resistance proportions for tetracycline and sulfamethoxazole show a sharp increase in 2021 and reach the highest proportions since 1999. A slight increase for resistance to quinolones was observed for ciprofloxacin and nalidixic acid (both 3.2%) compared to the years 2018-2020. No ESBL-producing isolates were detected in 2021 among STEC O157.

a 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 1999-2021

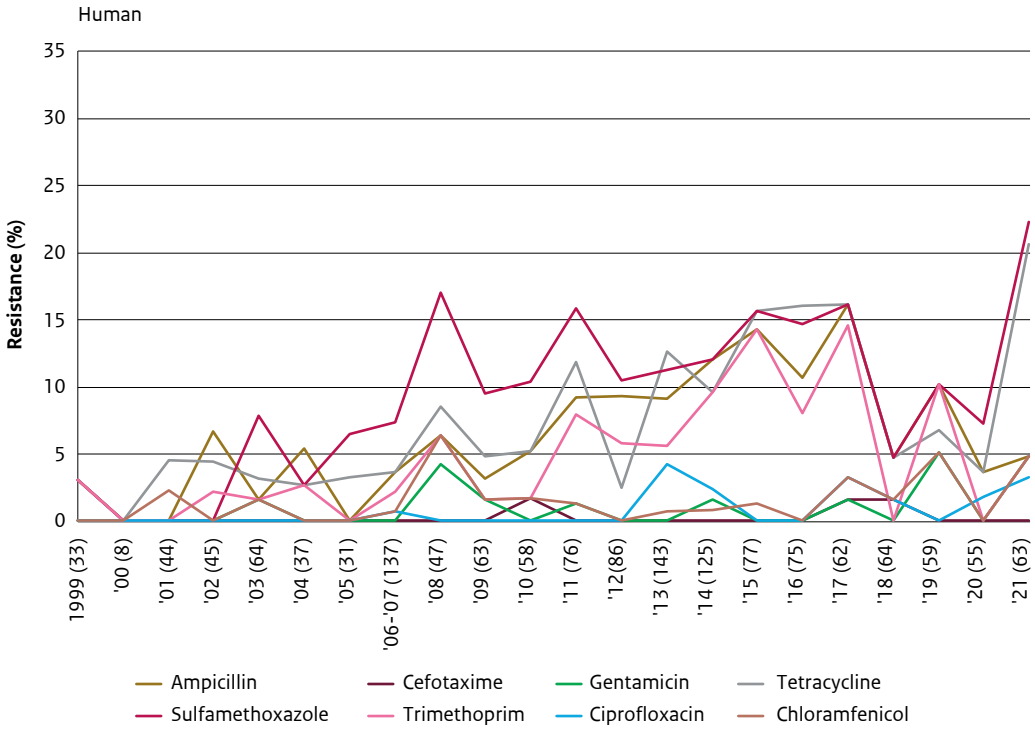


Table STEC01 MIC distribution (in %) and resistance percentages (R%) for *E. coli* STEC O157 (N=55) isolated from humans the Netherlands in 2020

E. coli N = 55	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							0.0	7.9	84.1	3.2	0.0	0.0	4.8						4.8	0.9-13.3
Cefotaxime					100.0	0.0	0.0	0.0	0.0										0.0	0.0-5.7
Ceftazidime					96.8	3.2	0.0	0.0	0.0	0.0									0.0	0.0-5.7
Gentamicin						84.1	9.5	1.6	0.0	1.6	1.6	1.6							4.8	0.9-13.3
Tetracycline								73.0	6.3	0.0	0.0	0.0	20.6						20.6	11.5-32.7
Sulfamethoxazole										77.8	0.0	0.0	0.0	0.0	0.0	0.0	22.2		22.2	12.7-34.5
Trimethoprim					92.1	3.2	0.0	0.0	0.0	0.0	0.0	4.8							4.8	0.9-13.3
Ciprofloxacin	92.1	4.8	0.0	0.0	1.6	1.6	0.0	0.0	0.0	0.0	0.0								3.2	0.4-11.0
Nalidixic acid									96.8	0.0		0.0	0.0	3.2					3.2	0.4-11.0
Chloramphenicol										92.1	3.2	0.0	0.0	4.8					4.8	0.9-13.3
Azithromycin								3.2	92.1	4.8	0.0	0.0	0.0						0.0	0.0-5.7
Colistine							98.4	1.6	0.0	0.0	0.0								0.0	0.0-5.7
Meropenem		98.4	1.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0								0.0	0.0-5.7
Tigecycline					100.0	0.0	0.0	0.0	0.0	0.0									0.0	0.0-5.7

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 with higher levels of resistance in O157 isolates for tetracycline and sulfamethoxazole and higher levels of resistance in non-O157 isolates for ampicillin, trimethoprim, ciprofloxacin and nalidixic acid, although none statistically different (p > 0.073). The STEC O157 isolates were slightly more often multidrug resistant[b] than non-O157 isolates, but not statistically significant (p=0.156).

Resistance to 3rd gen cephalosporins (cefotaxime and ceftazidime) and azithromycin were only detected in some non-O157 isolates tested with relatively low resistance proportions varying from 0.8 to 2.9%. Resistance against 3rd gen cephalosporins was detected in two O163 aEPEC isolates from the same patient at different sample dates. Resistance marker detection in the genome sequences confirmed the presence of bla_{CMY-41}. Most other types of resistance could not be clearly linked to specific serotypes, although multidrug resistance was more frequently observed amongst O26 isolates (p=0.024).

STEC O146 isolates are primarily associated with small ruminants as reservoir. In 2021, almost all STEC O146 isolates tested (n=30) were pan-susceptible with only one isolate exhibiting resistance against sulfamethoxazole and another multidrug resistant isolate exhibiting resistance against ampicillin, azithromycin, sulfamethoxazole and trimethoprim.
The detection of resistance against critically important antimicrobials within the non-O157 group indicates the additional value of monitoring resistance of a larger subset of pathogenic *E. coli.*

Table STEC02 Resistance percentages (R%) of pathogenic *E. coli* in the Netherlands in 2021

E. coli	O157 N = 63	Other serotypes N = 243
Ampicillin	4.8	6.6
Cefotaxime	0.0	0.8
Ceftazidime	0.0	0.8
Gentamicin	4.8	1.6
Tetracycline	20.6	12.8
Sulfamethoxazole	22.2	13.2
Trimethoprim	4.8	9.1
Ciprofloxacin	3.2	4.9
Nalidixic acid	3.2	4.9
Chloramphenicol	4.8	3.7
Azithromycin	0.0	2.9
Colistin	0.0	0.0
Meropenem	0.0	0.0
Tigecycline	0.0	0.0

b 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 have been 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 per cent 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 retail as well as imported frozen 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

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, resistance in indicator *E. coli* from caecal samples further decreased to the lowest levels since 1998. In pigs and veal calves levels of resistance stabilised, whereas resistance in dairy cattle remained traditionally low.
3. Resistance to third generation cephalosporins was very 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 with the low prevalence observed in pigs and veal calves.
5. For almost all 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. Resistance proportions in *E. coli* from pig and bovine meat were low compared to isolates from caeca.
7. Low levels of resistance were observed in different types of retail meat as well as in imported meat.
8. In vegetables, levels of resistance were very low for all antibiotic classes.

Resistance levels

Table Eco01 shows resistance levels, presented as MIC-distributions, of 1206 *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 2021. Table Eco02 presents resistance percentages per animal species. Trends in resistance levels from 1998 to 2021 are shown in Figure Eco01 and information on trends in multidrug resistance is shown in Figure Eco02.
Table Eco03 presents resistance percentages of 391 *E. coli* isolates collected from raw meat and vegetables at retail and import in the Netherlands in 2021. Figure Eco03 shows trends in resistance of *E. coli* in the Netherlands from 2002 to 2021 isolated from raw meat of bovine and pig.

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 was 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 fluoroquinolones in broilers and for chloramphenicol in white veal calves. Low resistance was noticed for amikacin, azithromycin and tigecycline. Resistance for colistin and meropenem was completely absent.

Table Eco01 MIC distribution (in %) and resistance percentages (R%) for all *E. coli* (N=1,206) isolated as indicator organism from intestines of food producing animals in the Netherlands in 2021

E. coli N = 1,206	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							4.1	24.0	44.9	6.1	0.0	0.2	20.6						20.9	18.6 - 23.3
Cefotaxime					99.8	0.0	0.0	0.1	0.0	0.1									0.2	0.0 - 0.6
Ceftazidime					93.6	6.3	0.0	0.1	0.0	0.0									0.1	0.0 - 0.5
Gentamicin						74.5	21.6	2.6	0.2	0.1	0.2	0.9							1.4	0.8 - 2.3
Tetracycline								67.0	7.2	0.4	0.5	0.8	24.0						25.4	22.9 - 27.9
Sulfamethoxazole										69.8	7.0	2.1	0.0	0.0	0.1	0.0	21.0		21.1	18.8 - 23.5
Trimethoprim					41.5	35.5	4.6	0.1	0.1	0.0	0.0	18.2							18.3	16.2 - 20.6
Ciprofloxacin	79.3	11.6	0.4	1.0	4.6	1.7	0.7	0.1	0.2	0.3	0.1								8.7	7.2 - 10.4
Nalidixic acid									90.5	2.2	0.4	0.1	1.2	5.6					7.3	5.9 - 8.9
Chloramphenicol										81.9	10.7	0.5	0.5	6.4					7.4	6.0 - 9.0
Azithromycin*								7.9	37.2	50.2	3.6	0.3	0.3	0.4					1.1	0.6 - 1.8
Colistin							98.9	1.1	0.0	0.0	0.0								0.0	0.0 - 0.3
Meropenem		99.8	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0								0.0	0.0 - 0.3
Tigecycline					96.8	3.1	0.1	0.0	0.0	0.0	0.0								0.1	0.0 - 0.5
Amikacine									96.3	3.3	0.4	0.0	0.0	0.0					0.4	0.1 - 1.0

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 (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.*

Fluoroquinolone resistance

Highest resistance levels for fluoroquinolones (FQ) were found in *E. coli* from broilers with 27.3% resistance to ciprofloxacin and 25.7% resistance to nalidixic acid (Table Eco02). Nevertheless, FQ resistance in broilers was lower than 30% for the first time since the beginning of the monitoring (Figure Eco01). In samples from other animal sectors FQ resistance was low or completely absent: 7.3% in white veal calves, 2.0% in pigs, 0.7% in dairy cattle, and undetected in isolates from rosé veal calves.

Resistance to fluoroquinolones in *E. coli* from meat was tested in 2021 for beef, pork, exotic meat and vegetable samples from retail in The Netherlands (Table Eco03). Due to the new legislation, imported meat has become a mandatory part of the sampling program from 2021 onwards. As a result, resistance levels of indicator *E. coli* collected from fresh retail meat as well as from imported frozen beef is reported separately in 2021 showing higher fluoroquinolone resistances in fresh retail beef compared to imported beef (Table Eco03). Figure Eco03 shows an increase of FQ resistance in bovine meat to 8.1% and, for the second year in a row, a complete absence of FQ resistance in pig meat.

Resistance against extended-spectrum cephalosporins (cefotaxime and ceftazidime)

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. In 2021 two randomly selected indicator *E. coli* isolates showed resistance to third generation cephalosporins (ESC-resistant *E. coli*). These isolates were both obtained in caecal samples of white veal calves resulting in 1% resistance to cefotaxime (Table Eco02). As the prevalence in selectively isolated ESC-R *E. coli* is highest in samples of white veal calves, the detection of this resistance in a low number of randomly isolated *E. coli* is not unsuspected. No ESC-resistant indicator *E. coli* were observed in randomly selected *E. coli* isolates from caecal samples of broilers, slaughter pigs, rosé veal calves and dairy cattle (Figure Eco01).

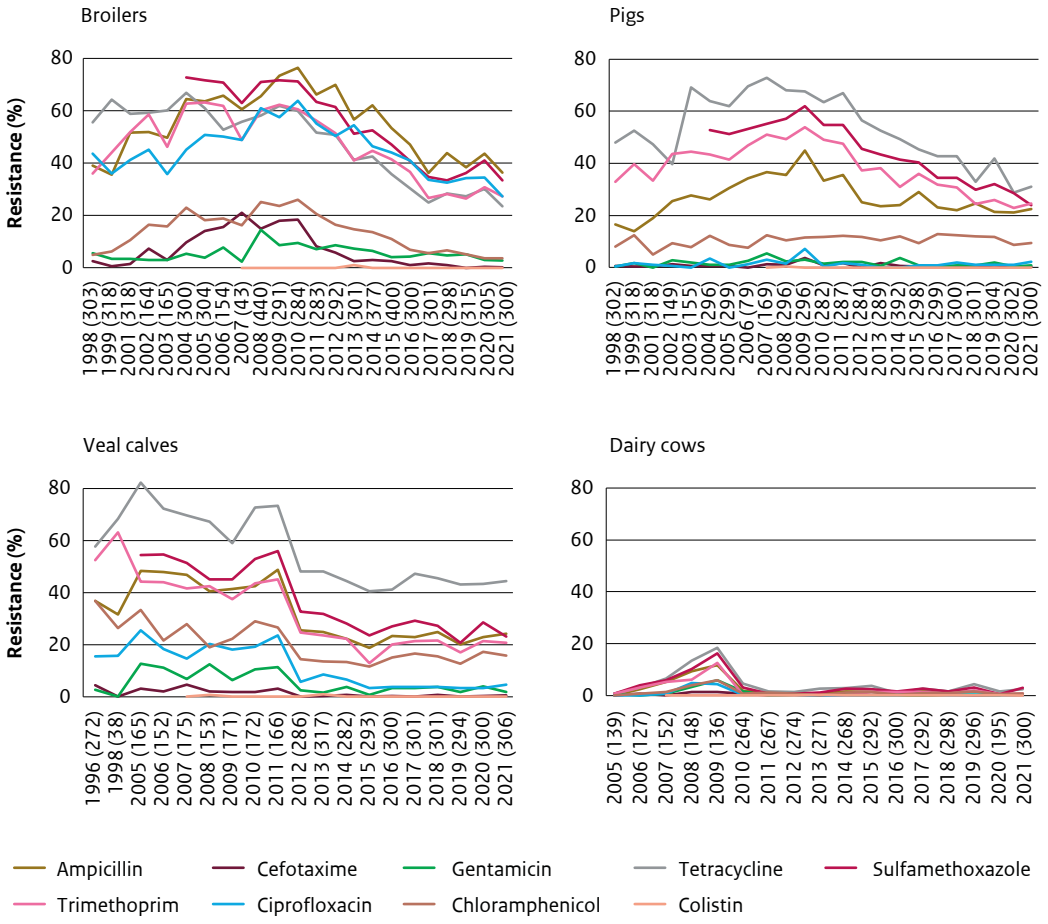
Active screening (by selective isolation)

In contrast to the low levels of 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 chicken

In 2021, the proportions of resistance decreased for all antimicrobial classes in commensal *E. coli* isolated from caecal samples of broiler chickens (Figure Eco01) with resistance levels below 40% for ampicillin (36.0%) and sulfamethoxazole (33.7%) and below 30% for tetracycline (23.3%), trimethoprim (27.0%) and ciprofloxacin (27.3%) resulting in the lowest resistance percentages measured since the beginning of the monitoring (Table Eco02 and Figure Eco01). Resistance to chloramphenicol and gentamicin was similarly low as in 2020. Resistance to azithromycin was detected in three isolates (1.0%). No resistance was observed for amikacin, cefotaxime, ceftazidime, colistin and meropenem.

Figure Eco01 Trends in proportion of resistance (%) of *E. coli* isolated from broilers, slaughter pigs, veal calves and dairy cattle in the Netherlands from 1996-2021



Slaughter pigs

The overall resistance proportion stabilised in slaughter pigs (Figure Eco01) with some fluctuation in resistance between the different antibiotic classes. For ampicillin, sulfamethoxazole, tetracycline and trimethoprim resistance levels stabilised between 20% and just above 30%. Chloramphenicol resistance remains stable, but for the second year below 10%. Continuous low levels of resistance are observed for ciprofloxacin and gentamicin. Resistance to cefotaxime, ceftazidime, meropenem, tigecycline and colistin was completely absent.

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, 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 2021, highest resistance levels in veal calves were observed for tetracycline (61.0% and 10.9% in white and rosé respectively), sulfamethoxazole (32.2% and 5.9%), trimethoprim (29.3% and 4.0%) and chloramphenicol (22.4% and 3.0%). In addition, low levels of resistance were observed for amikacin, azithromycin, cefotaxime and tigecycline in white veal calves, but not in rosé. (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.

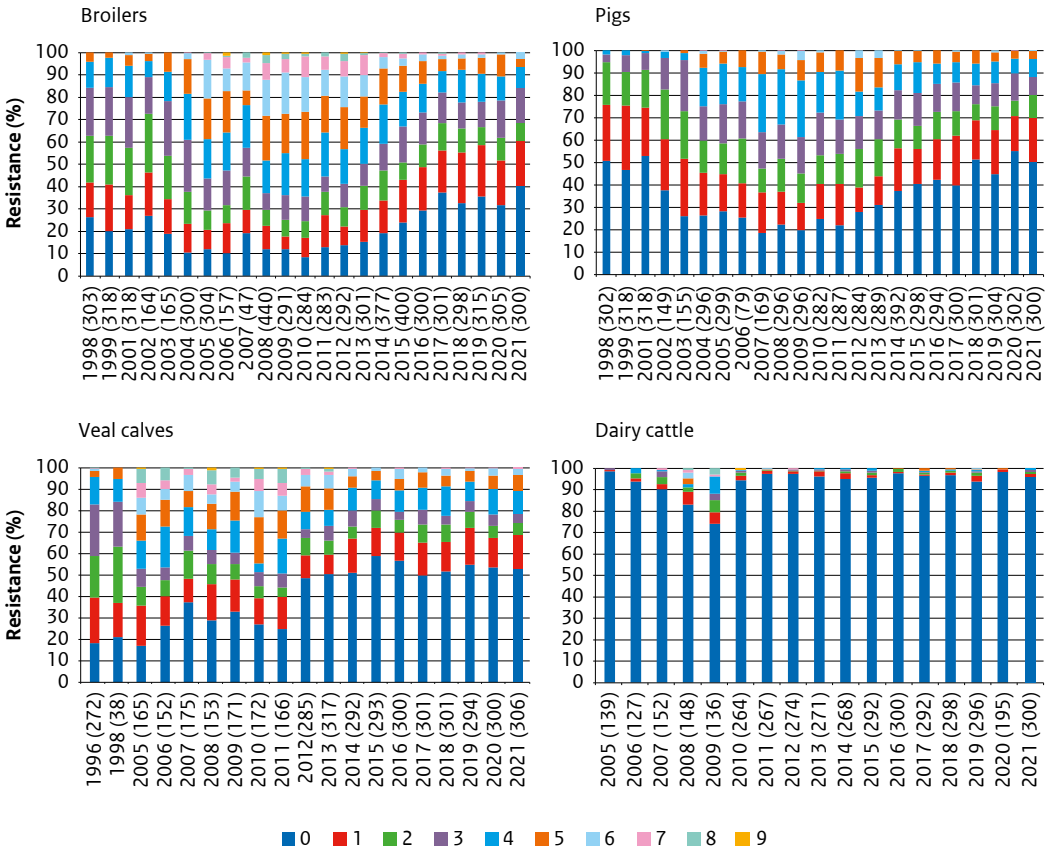
Table Eco02 Resistance percentages (%) of *E. coli* isolated from faecal samples of broilers, pigs, dairy cows, white veal calves and rosé veal calves in the Netherlands in 2021

Faecal samples	Broilers	Pigs	Dairy	Veal calves	
	N = 300	N = 300	N = 300	White, N = 205	Rosé, N = 101
Ampicillin	36.0	22.3	0.7	32.2	8.9
Cefotaxime	0.0	0.0	0.0	1.0	0.0
Ceftazidime	0.0	0.0	0.0	0.5	0.0
Gentamicin	2.7	0.7	0.0	3.4	0.0
Tetracycline	23.3	31.0	2.3	61.0	10.9
Sulfamethoxazole	33.7	24.0	3.0	32.2	5.9
Trimethoprim	27.0	24.3	1.0	29.3	4.0
Ciprofloxacin	27.3	2.0	0.7	7.3	0.0
Nalidixic acid	25.7	0.7	0.7	3.4	0.0
Chloramphenicol	3.3	9.3	0.7	22.4	3.0
Azithromycin	1.0	1.7	0.0	2.4	0.0
Colistin	0.0	0.0	0.0	0.0	0.0
Meropenem	0.0	0.0	0.0	0.0	0.0
Tigecycline	0.0	0.0	0.0	0.5	0.0
Amikacin	0.0	0.7	0.3	1.0	0.0

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), 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.

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 1998-2021



In general, the level of multidrug resistance (showing resistance to three or more classes of antimicrobials) stabilised in the last five years. In broilers, after a sudden peek in 2020 (38.0%), the proportion of multidrug resistance isolates declined to 31.3%, which is close to the levels measured between 2017 - 2019: 31.4% - 33.9%. The proportion of multidrug resistance in pigs further decreased to 20.0% being the lowest level measured since 2002. In veal calves the level of multidrug resistance was with 25.0% within range of the

levels measured between 2016 and 2020 (24.3% - 27.3%). As in former years, the proportion of multidrug resistant *E. coli* in dairy cattle was extremely low (1.3%) compared to the other animals species.
During the last decade, proportions of complete susceptibility have increased considerably in all animals species. Compared to 2020, the percentage of completely susceptible *E. coli* isolates sharply increased for broiler isolates to 40.0% being the highest measured since 1998. For pig and veal calf isolates the percentage of complete susceptibility decreased compared to 2020, but remained above 50% for both animals sectors (Figure Eco02).

E. coli in raw-meat and vegetables

Due to the new legislation (described above), meat products imported from outside the EU were included in the monitoring. Samples were collected at border control posts following the annual recommended frequency rates from 161 consignments of frozen beef imported from Africa, Australia, North-America and South-America as well as three consignments of imported pig meat from North-America. Due to the low number of pig samples collected from imported meat it was decided to merge these with samples of fresh retail meat. Table Eco03 presents resistance percentages of *E. coli* isolated from fresh bovine and pig meat sampled at retail, imported frozen bovine meat, exotic fresh meat (duck, quail, pheasant, dear, hare, horse and swine) as well as vegetables, sampled at retail by the Dutch Food and Consumer Product Safety Authority (NVWA). In 2021, isolates from poultry (broilers and turkeys) were not included. Meat from retail comprises meat produced in The Netherlands, but also in other EU countries. Vegetables originating from within and outside EU were sampled at retail. Overall, resistance levels were low in all types of retail meat examined. Notably, low levels of resistance was also observed in indicator *E. coli* obtained from imported bovine meat from outside the EU.

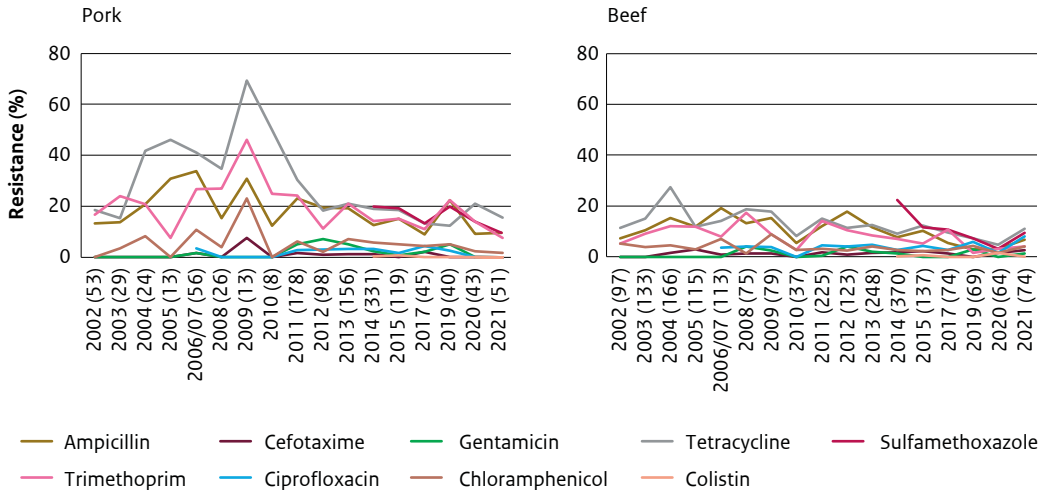
Table Eco03 Resistance percentages (R%) of *E. coli* isolated from raw meat and vegetables at retail in the Netherlands in 2021

Products	Bovine Fresh, retail N = 74	Bovine Imported N = 79	Pig N = 51	Exotic meat N = 74	Vegetables N = 113
Ampicillin	6,8	5,1	9,8	16,2	6,2
Cefotaxime	2,7	1,3	0,0	1,4	1,8
Ceftazidime	0,0	1,3	2,0	0,0	1,8
Gentamicin	1,4	1,3	0,0	2,7	0,0
Tetracycline	10,8	11,4	15,7	20,3	3,5
Sulfamethoxazole	9,5	7,6	9,8	8,1	2,7
Trimethoprim	4,1	3,8	7,8	8,1	0,9
Ciprofloxacin	8,1	1,3	0,0	6,8	2,7
Nalidixic acid	5,4	1,3	0,0	5,4	1,8
Chloramphenicol	4,1	3,8	2,0	4,1	0,0
Azithromycin	0,0	1,3	0,0	1,4	1,8
Colistin	0,0	0,0	0,0	0,0	0,0
Meropenem	0,0	0,0	0,0	0,0	0,0
Tigecycline	1,4	1,3	2,0	0,0	1,8
Amikacin	1,4	0,0	0,0	0,0	0,0

Fig Eco03 shows resistance rates were traditionally low in bovine meat with fluctuating resistance percentages below 5% for most antimicrobials tested. In pork, overall resistance rates were similarly low to bovine meat with complete absence of resistance to fluoroquinolones. In addition, in exotic meat higher levels of resistance were observed for ampicillin and tetracycline compared to bovine and pig meat. For most of the other antibiotic classes the resistance percentages in exotic meat were comparable to the other types of meat examined, table ECO03. A small number of indicator *E. coli* (n=2) from retail beef showed borderline resistance to cefotaxime, but not to ceftazidime. Only one ESC-R indicator *E. coli* was obtained from imported bovine meat. No cefotaxime resistance was detected in indicator *E. coli* isolates from pig meat. In addition, a small number of ESC-R *E. coli* was identified amongst indicator *E. coli* obtained from exotic (goose) meat (n=1) and vegetables (n=2).

In vegetables, resistance levels of *E. coli* isolates were very low, similar to former years. Percentages of resistance to ampicillin, chloramphenicol, 3rd generation cephalosporins, gentamicin, quinolones, tetracycline, trimethoprim and sulfamethoxazole were all below or equal to 5%.

Figure Eco03 Trends in resistance (%) of *E. coli* isolated from pork and beef in the Netherlands from 2002-2021



Reference

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.

2 Mughini-Gras L, van Pelt W, van der Voort M, Heck M, Friesema I, Franz E. 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). *Zoonoses Public Health.* Feb 2018;65(1):e8-e22.

3 European Food Safety A, European Centre for Disease P, Control. The European Union Summary Report on Antimicrobial Resistance in zoonotic and indicator bacteria from humans, animals and food in 2017/2018. *EFSA J.* Mar 2020;18(3):e06007.

4
Screening for ESBL, AmpC, carbapenemase-producing and colistin-resistant Enterobacteriaceae and MRSA in food-producing animals and meat in the Netherlands in 2021

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), for which resistance is highly prevalent in the Netherlands, or has been in the past, or for which prevalence is high or rising in countries abroad. Results include the non-selective and selective screening for ESBL/AmpC producing *E. coli* 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.

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 micro dilution. As WGS allows for an increased level of molecular analysis than was previously carried out, the method was adopted here for all selectively isolated ESBL and AmpC producing *E. coli*, colistin resistant *E. coli* and carbapenemase producing *Enterobacteriaceae*.

Highlights

- In 2021, only one confirmed ESBL-producing *E. coli* was detected through random isolation.
- Selective isolation of ESBL/pAmpC producing *E. coli* from broilers showed that after six years of reduction in prevalence, a plateau was reached.
- For the first time, whole-genome sequencing (WGS) was performed for all extended-spectrum cephalosporin resistant *E. coli* isolates from livestock and food products.
- WGS showed evidence of clonal transmission within livestock sectors and into the meat that these produce.
- The prevalence of ESBL-producing *Salmonella* isolated from human, livestock and food is considered low.
- In 2021, no carbapenemase-producing Enterobacteriaceae were detected in livestock and companion animals, but occasionally in imported food products.
- As in former years, the prevalence of *mcr* encoding *E. coli* was low in livestock and meat.
- Within the study period, the MRSA prevalence varied substantially between the animal sectors: 89% on pig farms, 6% on dairy farms and no MRSA on broilers farms.
- On retail meat, the highest prevalence of MRSA was found on turkey meat, followed by lamb, chicken and veal.

4.1 ESC-resistant Enterobacteriaceae

The extended spectrum cephalosporins (ESC) are classified as critically important for human medicine. The production of extended spectrum beta-lactamases (ESBLs) or AmpC beta-lactamases results in resistance to these antibiotics, with the distinction that hydrolytic activity of ESBLs is reduced by beta-lactamase inhibitors, while AmpC beta-lactamases are not. The initial focus on ESBLs for monitoring purposes is due to the genetic location of these genes, these are often encoded on plasmids which are accountable for their transmissibility amongst various bacterial species. AmpC genes can also be encoded on plasmids, referred to as plasmid-encoded AmpC for which the epidemiology is considered similar and these two groups of genes are further collectively referred to as ESBL/pAmpC. However, *E. coli* also encodes for a chromosomal AmpC gene which can result in ESC resistance due to a chromosomal mutation in the promotor of the gene. As this resistance is not transmissible, it is not included in the data presented for ESBL/pAmpC prevalence, although these are included when referring to ESBL-suspected or ESC-resistant *E. coli*.

Due to clinical importance for human medicine and the historic high prevalence, ESBL/pAmpC producing *E. coli* are monitored at two levels; in the non-selectively isolated *E. coli* that are also described in chapter 3, and using selectively isolated *E. coli*.

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 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 when a reduced susceptibility of the isolate is measured against the ESC cefotaxime and/or ceftazidime. After confirmation of the phenotype, whole genome sequencing (WGS) is performed using Illumina sequencing platforms. 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 (Bortolaia *et al.* 2020).

Figure ESBL01 shows the trends of randomly isolated ESC-resistant *E. coli* from 1998 until 2021. 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. In 2021, only two randomly selected ESC-resistant *E. coli* were isolated from veal calves. One isolate was shown to contain the $bla_{CTX\text{-}M\text{-}1}$ gene while the other displayed an elevated MIC for cefotaxime just above the breakpoint for which no resistance mechanism could be identified, see Table ESBL01.

Figure ESBL01 Trends in cefotaxime resistance (%) of *E. coli* randomly isolated from faeces of broilers, slaughter pigs, veal calves and dairy cows

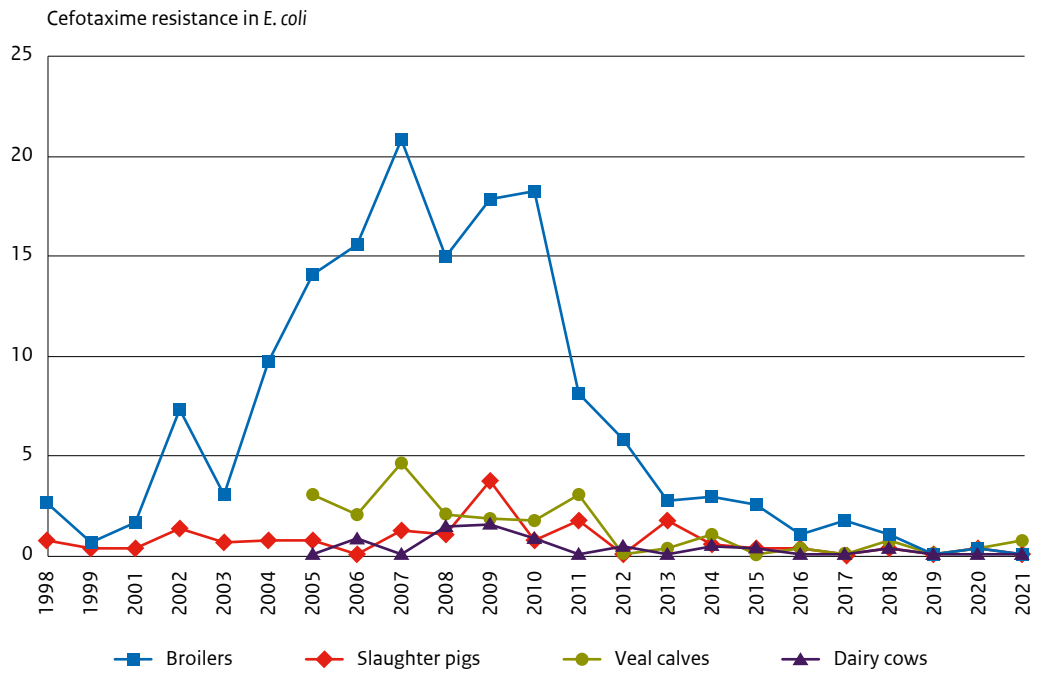


Table ESBL01 ESBL-genes found in *E. coli* isolates with reduced susceptibility to cefotaxime derived from broilers, veal calves, slaughter pigs, dairy cows and turkey (only 2011 and 2012) during 2007-2021

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	*n.t.*	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
Total	**275**	**32**	**32**	**8**	**6**	**350**	**139**	**19**	**3**	**39**	**2**	**45**	**12**	**44**	**22**	**29**		

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. 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).

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 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 beta-lactamase producing *Enterobacteriaceae*.

After confirmation of the phenotype, whole genome sequencing (WGS) is performed using Illumina sequencing platforms. 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 (Bortolaia *et al.* 2020).

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*. Table ESBL02 shows the number of ESC-resistant *E. coli* that were isolated in 2021 and the number of isolates that were confirmed as ESBL/pAmpC producing *E. coli* via WGS. The trends of confirmed ESBL/pAmpC producing *E. coli* over time 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 (Mughini-Gras *et al.* 2019).

In **broilers**, 11.3% of samples that were studied were confirmed to contain ESBL/pAmpC producing *E. coli.* This represents a minor increase compared to the prevalence measured in 2020 and represents the end of a period of seven years in which only reductions were seen. However, the increase is considered minor and is expected to be the start of a plateau in which minor fluctuations can be expected. As stated in previous years, the molecular typing of ESC-resistance in broilers shows fluctuations in the three most prevalent ESBL/pAmpC genes over time. While $bla_{CTX\text{-}M\text{-}1}$ was the most prevalent gene for several years, being 47.2% at a peak in 2015, in 2019 it represented only 25.9% of the ESC-resistant population, and in 2021 it was 35.3%. $bla_{CMY\text{-}2}$ also showed a relative reduction from 28.6% in 2014 to 6.5% in 2020, and 14.7% in 2021. Conversely, $bla_{SHV\text{-}12}$ represented only 9.9% of the ESC-resistant population in 2015 and has had a relative increase to 41.2% in 2021. The mechanisms behind these fluctuations are currently unknown.

Since 2014, the prevalence of ESBL/pAmpC in **slaughter pigs** has always been relatively low and quite stable compared to other livestock species, and was 9.3% in 2021. While each year several ESBL/pAmpC genes are detected, $bla_{CTX\text{-}M\text{-}1}$ is always the most prevalent gene. In slaughter pigs, the difference between ESBL-suspected (ESC-resistant) isolates is generally higher than for other livestock animals due to a higher prevalence of AmpC promotor mutations that cause ESC resistance. In 2021, 40.4% of ESC-resistant isolates was due to an AmpC promotor mutation.

The prevalence of ESBL/pAmpC producing *E. coli* in **dairy cows** has fluctuated little over time, and had a 10.3% prevalence in 2021. Rather similar to the situation in broilers, $bla_{CTX\text{-}M\text{-}1}$ was the most prevalent gene in the ESC-resistant population in dairy cows as well, with a peak of 41.3% in 2016 and dropping to 16.7% in 2021. Gradually, the gene $bla_{CTX\text{-}M\text{-}15}$ has taken over most of the ESC-resistant population and represents 47.6% in 2021, while the gene had not been detected in dairy cattle before 2015, when it was present at 9.1%.

Both in **rosé and white veal calves**, an increase in the prevalence of ESC-resistant *E. coli* was detected in 2016. For white veal calves, the prevalence in 2016 reached 33.9%, continuing to rise until 47.6% in 2016 and gradually declining since then, to 36.6% in 2021. In rosé veal calves, the prevalence appeared to drop from 28.7% in 2016 to 14.0% in 2019, but since then it increased again to 24.8% in 2021. Both in white and rosé veal calves, $bla_{CTX\text{-}M\text{-}1}$ was the most prevalent ESBL gene in the ESC-resistant *E. coli* population, but a somewhat similar pattern seems to occur as described for dairy cows. In white veal calves, both the population containing $bla_{CTX\text{-}M\text{-}1}$ and the population containing AmpC promotor mutations have decreased, to the advantage of $bla_{CTX\text{-}M\text{-}15}$ which increased from 14.3% in 2014 to 41.6% in 2021. In the ESC-resistant *E. coli* population in rosé veal calves, again the population containing $bla_{CTX\text{-}M\text{-}1}$ and the population containing AmpC promotor mutations have decreased, to the advantage of $bla_{CTX\text{-}M\text{-}15}$ which increased from 20.0% in 2014 to 50.0% in 2021, but here $bla_{CTX\text{-}M\text{-}14}$ also greatly fluctuates from 0% in 2014 to 33.3% in 2020 and 11.5% in 2021.

The differences over time for the various ESBL/pAmpC genes in each of the livestock species described above indicate that selective conditions probably change over time. These 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.

Table ESBL02 Prevalence of *E. coli* isolates showing reduced susceptibilty to cefotaxime derived from selective culturing of faecal samples from broilers, slaughter pigs, veal calves and dairy cows collected in 2021

	N samples	N suspected ESBL	% ESBL suspected	N confirmed ESBL	Prevalence (%) ESBL confirmed
Broilers	300	34	11.3	34	11.3
Pigs	300	47	15.7	28	9.3
Veal calves					
white	205	77	37.6	75	36.6
rosé	101	26	25.7	25	24.8
Dairy cows	302	42	13.9	31	10.3
Total	**1,208**	**226**	**17.7**	**193**	**16.0**

Figure ESBL02 Trends in prevalence of ESBL/AmpC-producing *E. coli* in faecal samples of broilers, pigs, white and rosé veal calves and dairy cows from 2014-2021 determined by using selective isolation

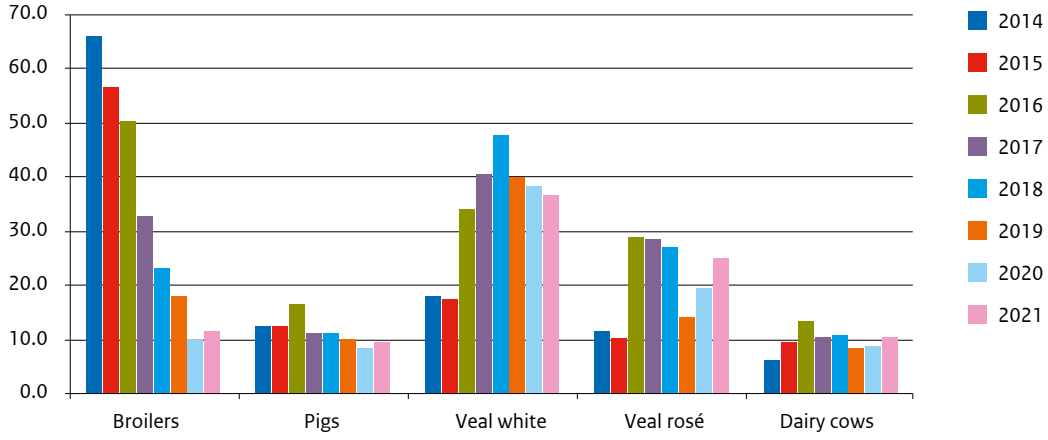


Table ESBL03 Beta-lactamases identified in *E. coli* derived from selective culturing of faecal samples of broilers, slaughter pigs, veal calves, and dairy cows in 2021

		Broilers	Slaughter pigs	Veal calves		Dairy cows	Total
				White	Rose		
CTX-M-1 group	CTX-M-1	12	17	23	6	7	65
	CTX-M-15	2	5	32	13	20	72
	CTX-M-32			7	1		8
	CTX-M-55	1	1	3			5
CTX-M-2 group	CTX-M-2						0
CTX-M-3 group	CTX-M-3			1			1
CTX-M-8/25 group	CTX-M-8			1			1
CTX-M-9 group	CTX-M-9		1				1
	CTX-M-14			2	3	1	6
	CTX-M-65				2	1	3
TEM	TEM-52c		2	6			8
SHV	SHV-12	14				2	16
CMY	CMY-2	5	2			2	9
Chromosomal *ampC*	*ampC*-type-3		19	2	1	9	31
Total		**34**	**47**	**77**	**26**	**42**	**226**

Results of selective isolation and molecular typing of ESC-resistant E. coli in raw meat and vegetables

Similar to samples from livestock, samples from food have an increased prevalence of ESC-resistant *E. coli* when selectively isolated, compared to isolates that were isolated randomly, as described in chapter 3. In table ESBL04, the number of ESC-resistant *E. coli* isolates are reported for the different food sources. Figure ESBL03 shows the trends over time of confirmed ESBL/pAmpC producing *E. coli* for those categories of meat for which monitoring of sufficient numbers of samples was done over time. As part of the updated EU legislation on monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria (2020/1729/EU), sampling of meat imported from outside the EU was included for the first time in 2021. In the different categories of meat, beef, lamb and pork have always had a low prevalence of ESC-resistant isolates since 2014. For **beef**, prevalence has fluctuated little between 0.4% and 2.3% and in 2021 this was 0.9%. Due to the low prevalence, the relative abundance of genes per year differs considerably but $bla_{CTX\text{-}M\text{-}1}$ was always the most prevalent gene at approximately 50%, until 2021 when for the first time $bla_{CTX\text{-}M\text{-}15}$ is slightly more abundant. In **lamb** meat the prevalence is mostly even lower and fluctuates between 0 and 5.1%, with 0.3% in 2021. This represents only a single *E. coli* isolate which contains the ESBL gene $bla_{CTX\text{-}M\text{-}55}$. Similarly in **pork** the prevalence has fluctuated between 0 and 2.7%, and was 1.2% in 2021. As for pigs, the AmpC chromosomal mutation is mostly present at similar abundance as $blaC_{TX\text{-}M\text{-}1}$, although in pork$bla_{CTX\text{-}M\text{-}55}$ is also detected most years.

Chicken meat had the highest prevalence of ESBL/pAmpC producing *E. coli* over the past years, similar as in broiler caecal samples. Here, the reduction in livestock has also lead to a reduction that is detected on the meat. In 2014, prevalence was 67.0% which was reduced to 9.0% in 2020. In 2021, the prevalence has increased again to 19.3%. Similar to the broiler caecal samples, the genes $bla_{CTX\text{-}M\text{-}1}$ and $bla_{CMY\text{-}2}$ were most abundant in 2014-2017 but their relative abundance went down in favor of *bla*SHV-12. In contrast to the broiler caecal samples, in chicken meat $bla_{CTX\text{-}M\text{-}55}$ has risen sharply in 2020 and 2021 and is now the second most abundant ESBL gene found in broiler meat while the third most abundant gene is $bla_{SHV\text{-}12}$.

Turkey is produced and consumed relatively little in the Netherlands, which is reflected in the lower number of samples and isolates collected from turkey meat. In 2021, only a single isolate was detected which contained $bla_{CTX\text{-}M\text{-}1}$.

In 2020, the screening of food was extended with **mushrooms** and **vegetables**. Both years, samples from mushrooms were found negative for ESC-resistant *E. coli*. Vegetables include salads and vegetables for raw consumption and chopped vegetables for cooking. Both in 2020 and 2021, prevalence in vegetables was very low, respectively 0.2 and 0.3%. In 2021, all three *E. coli* contained the gene $bla_{CTX\text{-}M\text{-}15}$.

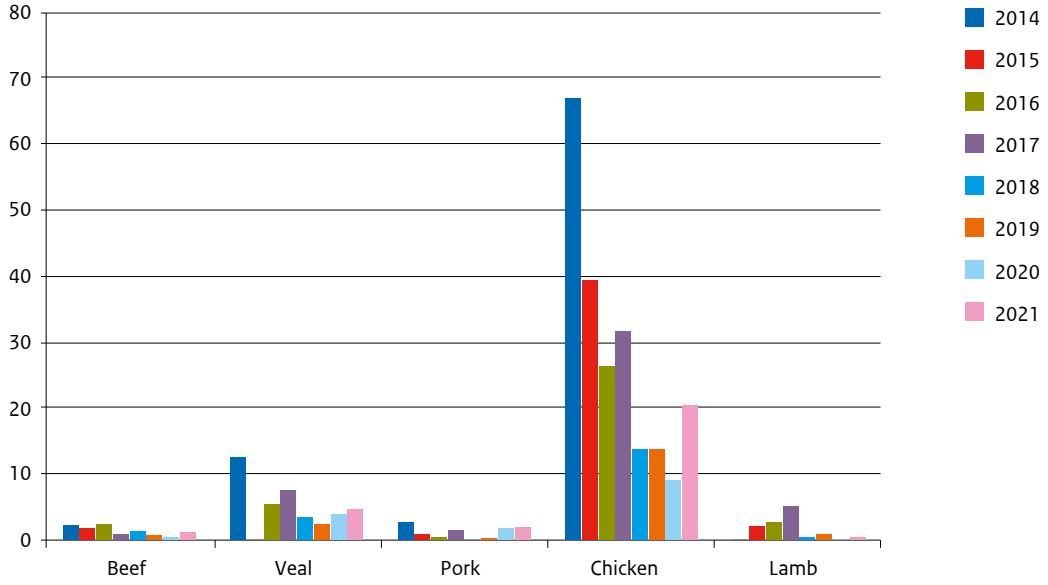
Since 2020, several sources of less common meat have been collectively reported as **exotic meat**. In 2021, this category included boar, deer, hare, quail, duck and fowl. The prevalence in this category is also considered low, 2.1% in 2021. The two *E. coli* isolates that were detected contained $bla_{CTX\text{-}M\text{-}1}$ and $bla_{CTX\text{-}M\text{-}15}$.

Imported **shrimp and fish** (tilapia and pangasius) have been monitored intermittently over the years. The prevalence of ESC-resistant *E. coli* is generally low and was 0.7% in 2021. The two isolates that were detected both contained the ESBL gene $bla_{CTX\text{-}M\text{-}55}$.

Table ESBL04 Prevalence of ESBL/AmpC-positive *E. coli* isolates from raw meat, vegetables and mushrooms in the Netherlands in 2021

Animal source	N screened	N ESBL/AmpC suspected	N ESBL/AmpC confirmed	% ESBL/AmpC positive
Beef	808	9	7	0.9%
Veal	305	14	11	3.6%
Pork	323	6	4	1.2%
Chicken	398	81	77	19.3%
Turkey	15	1	1	6.7%
Lamb	305	1	1	0.3%
Exotic meat	121	2	2	1.7%
Vegetables	1,593	7	5	0.3%
Mushrooms	104	0	0	0.0%
Imported aquaculture	306	2	2	0.7%
Total	**3,972**	**123**	**110**	**2.8%**

Figure ESBL03 Trends in prevalence of ESBL/AmpC-producing *E. coli* in fresh meat of broilers, pigs, veal calves, dairy cows and lambs from 2014-2021 determined by using selective isolation



Results of genomic comparisons of ESC-resistant E. coli from food and livestock

A comparison was performed of the complete DNA sequences of all selectively isolated ESC-resistant *E. coli* from livestock and food products. A total of 16 clusters were identified in which two or more isolates were considered clonal due to their high similarity, as previously described (Roer *et al.* 2019).

Three different clones were detected in pig and pork isolates, all encoding the chromosomal mutation in the AmpC promotor region that causes ESC resistance. One clone was detected in four pig isolates, one clone in two pig isolates and the last clone was detected in one pig and one pork isolate.

Nine different clones were detected between dairy cows, veal calves or veal meat. Five of these clones contain the ESBL gene $bla_{CTX\text{-}M\text{-}15}$, two of which were detected in a dairy cow and a veal calf, the third clone detected in one dairy cow and 2 veal calves, the fourth in two dairy cows and three veal calves and the last in one dairy cow and one veal meat isolate. Two clones were detected that contain the $bla_{CTX\text{-}M\text{-}32}$ gene, one clone in three veal calves and the other clone in two veal calves. Finally, two clones were detected that contain the chromosomal AmpC promotor mutation, one clone in three caecal samples of veal calves, the other in samples from two white veal calves.

The last four clones were detected in caecal samples from broilers and chicken meat. Two clones encode the $bla_{CTX\text{-}M\text{-}1}$ gene, one of which was detected in one broiler and three chicken meat samples, the other in two broiler samples. Two clones encode the $bla_{SHV\text{-}12}$ gene, one of which was detected in four broiler and one chicken meat sample, the other in two broiler samples.

Table ESBL05 Beta-lactamases identified in *E. coli* from raw meat products in the Netherlands in 2021

	ESBL gene	Chicken	Pork	Beef	Veal	Turkey	Lamb	Exotic meat	Vegetables	Imported aquaculture	Total
CTX-M-1 group	CTX-M-1	7	3	2	3	1		1			17
	CTX-M-15	1		4	4			1	3		13
	CTX-M-32	1			1						2
	CTX-M-55	18	1		1		1			2	23
CTX-M-2 group	CTX-M-2	5									5
CTX-M-8 group	CTX-M-8	7									7
	CTX-M-8; CTX-M-55	1									1
CTX-M-9 group	CTX-M-14			1	1						2
TEM	TEM-52	11									11
SHV	SHV-12	19			1						20
	SHV-12; TEM-52	1									1
CMY	CMY-2	6									6
ACT	ACT-7								1		1
DHA	DHA-1								1		1
Chromosomal *ampC*	*ampC*-type-3	1	2	2	3						8
Total		**78**	**6**	**9**	**14**	**1**	**1**	**2**	**5**	**2**	**118**

No sequencing studies are available in literature in which sequencing has been performed on such a large number of ESC resistant *E. coli* isolates from a monitoring program so it is difficult to put these results into context. While genomic links between isolates have previously been reported, generally these were performed in the context of cross-sectional studies. The fact that a numerous set of clones was detected within each livestock species and meat from that species indicates that transmission is likely to occur through the production chains. The future continuation of these sequencing efforts within the monitoring program will provide a more robust dataset for source attribution studies in the One Health context.

ESC-resistant *Salmonella*

In 2021, 1264 *Salmonella* isolates from humans and fresh meat produced in the EU were tested through selective isolation for resistance against ESC. A total of ten isolates were confirmed to produce ESBL/pAmpC, seven of which were from human isolates while three were non-human isolates of unknown origin. The prevalence of ESBL/pAmpC producing *Salmonella* is considered to be low. As expected, isolates have diverse backgrounds from different serovars, Table ESBL06. While the proportion of *S. enterica* serovar Typhimurium of 30% appears high, the low number of isolates that are detected prevent from drawing any firm conclusions. Molecular characterisation of the ESBL/pAmpC genes was performed by WGS at RIVM through a similar protocol as for ESBL/pAmpC producing *E. coli*, described above. The results show that genes from the CTX-M-9 groups were most prevalent, comparable to previous years, Table ESBL07.

Table ESBL06 Beta-lactamases identified in *Salmonella* in 2021 (7 human isolates and 3 non-human isolates of unknow origin)

Serovar	CTX-M-15	CTX-M-55	CTX-M-9	CTX-M-14[b]	SHV-12	CMY-2	Total
Apeyeme[a]		1					1
Infantis					1		1
Kentucky				2			2
Minnesota[a]						2	2
Typhi	1						1
Typhimurium			3				3
Total	1	1	3	2	1	2	10

a origin unknown (non-human)

Table ESBL07 Beta-lactamases identified in *Salmonella* isolates collected in 2007-2021

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
Total	**111**	**45**	**1**	**9**	**86**	**47**	**4**	**13**	**135**	**3**	**2**	**457**	**25,653**	**1.8**

a contains $bla_{CTX-M-1}$, $bla_{CTX-M-55}$, $bla_{CTX-M-15}$, $bla_{CTX-M-3}$ and a combination with bla_{CMY-2} (n=2, 2014, 2015).

b In 2008 one combination of $bla_{CTX-M-2}$ with bla_{TEM-52} was found in S. Paratyphi B var Java.

c contains $bla_{CTX-M-9}$, $bla_{CTX-M-14}$ and $bla_{CTX-M-65}$.

d In 2007 three S. Concord were found containing both bla_{SHV-12} and $bla_{CTX-M-15}$.

e In 2015 a combination of bla_{CMY-2} and bla_{TEM-52} was found in S. Oranienburg and a combination of bla_{CMY-2} with $bla_{CTX-M-1}$ 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.

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 ESBL/AmpC *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 2021.

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 2021, 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, centrifuged and DNA isolated from the pellet. A multiplex Real Time-PCR (In house) that can detect the most prevalent carbapenemase gene families (bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP} and bla_{OXA-48}) was used according to the manufacturer's instructions. If Real Time-PCR gave suspicious or positive results, a step-wise analysis was performed to confirm the results:

1. Five singleplex Real Time-PCR-tests were performed on purified DNA of the 5 individual samples of the pool;
2. If 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. On DNA from grown bacterial isolates PCR was done and genes were identified with Sanger sequencing.

Carbapenemase screening in 2021 (n=1218) resulted in ten bla_{OXA-48}-like positive faecal samples (0.8%) which is similar to 2020 with 0.5% bla_{OXA-48}-like positive samples. Positive samples were distributed over all animals species: veal calves (n=4), broilers (n=3), dairy cattle (n=2) and slaughter pigs (n=1). In five samples the presence of bla_{OXA-48}-carrying *Shewanella* was confirmed by bacterial culturing followed by PCR and sequencing: $bla_{OXA-48b}$ (n=3), $bla_{OXA-204}$ (n=1) and $bla_{OXA-252}$ (n=1). In the remaining five samples $bla_{OXA-4b8}$ (n=3), $bla_{OXA-181}$ (n=1) and $bla_{OXA-252}$ (n=1) were 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 seven years where bla_{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 (Zong 2012), these genes were considered of environmental origin and not a public health risk. Most importantly, no carbapenemase-producing *Enterobacteriaceae* were detected in faecal samples from livestock in the Netherlands in 2021. Screening for carbapenemase-producing isolates in faecal samples of food-producing animals will continue in 2022.

4.2.2 Monitoring in companion animals

Carbapenemase producing *Enterobacteriaceae* (CPE) in companion animals in Europe have been observed, but the prevalence is still relatively low. CPE have been found in pet dogs from Germany (Stolle *et al.* 2013) (Pulss *et al.* 2018), Spain (Gonzalez-Torralba *et al.* 2016), France (Melo *et al.* 2017), UK (Reynolds *et al.* 2019), Portugal (Brilhante *et al.* 2020) and Switzerland (Dazio *et al.* 2021). Monitoring to detect introduction of CPE in companion animals in the Netherlands was initiated in 2015. The screening for CPE comprised of an initial retrospective study and a prospective study. Until 2016, CPE had not been detected in the Netherlands (MARAN 2017). In 2017, the first case of a bla_{OXA-48} producing *E. coli*, isolated from a faecal dog sample, was reported (MARAN 2018). The faecal sample was submitted to the Veterinary Microbiological Diagnostic Center (VMDC) of Utrecht University for parasitology diagnostics. In 2018, two individual dog samples were found positive for *E. coli*, harboring bla_{OXA-48} and $bla_{OXA-181}$. Both samples originated from different parts of the Netherlands and were sent to the VMDC for parasitology diagnostics. In 2019 and 2020 the continued monitoring performed at the VMDC did not reveal CPE in samples of dogs and cats.

In 2021, 223 faecal samples of cats and dogs were examined. Samples were obtained through the 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.
In 2021, 103 faecal samples from dogs and 120 faecal samples from cats were screened. From each sample, 0.5 gram feces 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 bla_{NDM} (Manchanda *et al.* 2011), bla_{KPC} (Bradford *et al.* 2004), bla_{IMP} (Ellington *et al.* 2007), bla_{VIM} (Ellington *et al.* 2007), bla_{OXA}-group-23, -24, -51, -58(Voets *et al.* 2011) and bla_{OXA}-group-48(Poirel *et al.* 2004).

None of the faecal samples from cats and dogs showed growth on the selective plates (direct and after enrichment). This result indicates a low concentration of CPE present in the samples if any. PCR screening revealed two bla_{OXA}-suspected fragments in samples from dogs. Additional (selective) culturing of the samples was negative, so the resistance genes could not be linked to specific bacterial isolates. Sequencing of the PCR fragments revealed that one fragment was a bla_{OXA-10} (presumptive) from a *Pseudomonas* sp.; the other fragment turned out to be a bla_{OXA-58} from an *Acinetobacter* sp.. In conclusion: no carbapenemase-producing *Enterobacteriaceae* were detected in dogs and cats in 2021. Screening for carbapenemase-producing isolates in companion animals will continue in 2022.

4.2.3 Monitoring in imported seafood, seaweed and herbs

In 2021, 205 batches of frozen fish originating from fish farms in South-East Asia as well as 101 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, 283 batches of imported seaweed and 65 batches of herbs were screened for the presence of CPE. As a result, one carbapenemase-producing *Enterobacter asburiae* isolate carrying bla_{IMI-3} was detected in Coriander from Kenia. This is the second finding of CPE on imported vegetables indicating a wider spread of IMI-positive Enterobacter species in imported food products.

For the fifth year in a row, carbapenemase-producing *Enterobacteriaceae* were detected in batches of imported food. The monitoring of imported food will be continued in 2022 and extended to PCR screening in order to increase the sensitivity of the method.

4.3 Colistin resistance

In 2021, active screening for the presence of *mcr*-genes in caecal samples was continued using selective culturing and PCR. For this purpose, purified DNA of pooled BPW cultures (five samples per pool) from a total of 1212 faecal samples of Dutch livestock were tested with for the presence of *mcr*-1, *mcr*-2, *mcr*-3, *mcr*-4 and *mcr*-5 using an in house designed multiplex RT-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). 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 contrast to former years (2019 and 2020) when *mcr* was not found in broilers, most *mcr*-1 carrying *E. coli* were detected in broilers in 2021. As a result of the screening, *mcr*-1 positive *E. coli* were identified in eight samples (0.7%) consisting of caecal samples from six broilers (2.0%), one white veal calf (0.3%) and one slaughter pig (0.3%). The finding of a *Hafnia alvei* isolate carrying *mcr*-4.3 in a white veal calf (*mcr*-4.3 was also detected in veal calves in 2018 and 2019) indicates the continuing presence of this *mcr* variant in veal calves on a low level. Finally, no colistin resistant isolates were identified amongst the randomly selected indicator *E. coli* isolated from faecal samples of livestock and retail meat (Table Eco02 and Table Eco03).

4.4 MRSA surveillance in livestock and humans

Worldwide, 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 carriage of LA-MRSA is professional contact with livestock, especially pigs, poultry and veal calves (Graveland *et al.* 2011). Recently, however, the number of persons colonised or infected with LA-MRSA in The Netherlands who did not have direct contact with livestock, seemed to be increasing (Lekkerkerk *et al.* 2015). In 2018, a project on surveillance of MRSA in humans, livestock, and meat products was started. This project is a collaboration between NVWA, RIVM, WVBR 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 (MMLs) send MRSA isolates from carriers and from infected persons to the National Institute for Public Health and the Environment (RIVM). The objective of this project was 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 cows with mastitis
For the MRSA surveillance in livestock, each year one animal sector is monitored. For the year 2021 dairy cows and persons working and/or living on these farms were sampled.

Dairy farms
In 2021, a total of 181 dairy farms were investigated. Dust samples from the stables were taken as well as samples from the skin between the hindleg and the udder of the animals. In total 538 dust samples were taken, of which 18 (3.3%) were MRSA-positive. A total of 540 skin samples were investigated and 11 (2.0%) were MRSA-positive. MRSA was found in one or more dust samples on 10 farms (5.5%). On seven of these farms, both matrices (dust samples and skin samples) were MRSA positive. On one farm, all dust samples were MRSA-negative, but a skin sample of a cow was found MRSA positive. This resulted in an MRSA farm prevalence of 11/181 (6,1% (95%CI 3.4-10.6%)). This prevalence is comparable to that of a previous study at slaughterhouses performed in 2011/2012, in which it was shown that 16/411 dairy cows at the slaughterhouse were MRSA-positive, resulting in a prevalence of 3.9% (95% CI 2.0-5.8%) (van Duijkeren *et al.* 2014).

Persons working/living on dairy farms
Persons living and/or working on the dairy farms were asked to take a nasal swab on a voluntary basis. In total, 107 persons living and/or working on 60 of the 181 farms volunteered to send in a nasal swab. One person was MRSA-positive and this person worked on one of the MRSA-positive farms. The results of dairy farms are summarised in Table MRSA01 together with data from 2018/2019 (broiler farms) and from 2020 (pig farms).

Table MRSA01 Number of MRSA found on farms and in persons working/living on these farms from 2018-2021

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

NGS analyses MRSA from dairy farms

To date, 25 MRSA isolates from the dairy farms and the farmer were sequenced and most isolates were LA-MRSA ST398 (n=23). Two isolates had a new ST, which was closely related to ST398. All isolates carried the *mecA* gene. No MRSA carrying the *mecC* gene were identified. All isolates were negative for the Panton Valentine Leukocidin (PVL)-toxin gene, which encodes a cytotoxin and is associated with increased virulence.

NGS analyses MRSA from mastitis

Eighty-seven MRSA isolates (collected from 2010 to 2021) originating from cows with mastitis sent in by the Royal GD were sequenced and most isolates belonged to LA-MRSA ST398 (n=83). Other isolates belonged to ST1 (n=3) and another new *ST* (n=1). All isolates carried the *mecA* gene and all were negative for the PVL-toxin gene.

MRSA from caecal samples

In 2021, caecal samples from the national AMR surveillance were investigated. MRSA was found in 15/100 caecal samples from pigs, 31/70 caecal samples from white veal calves, 0/32 caecal samples from rosé veal calves, 1/102 caecal samples from broilers, and 1/102 caecal samples from dairy cattle (see Table MRSA02). Remarkably, MRSA was cultured from 44.3% of the white veal calves samples compared to 0% from the samples of rosé veal calves. The reason for this difference is unknown, but could be due to different management systems, including the higher antimicrobial consumption in white veal calves. Although MRSA was not found earlier on broiler farms (Table MRSA01), one caecal sample from broilers was MRSA positive, indicating that MRSA is present in Dutch broilers, but with a low prevalence (Table MRSA02).

Table MRSA02 Number of MRSA found in caecal samples collected in 2021

Animal	Positive samples (n)	Total (n)	Prevalence (%)	95%CI
Pigs	15	100	15.0	9-23
White veal calves	31	70	44.3	33-56
Rosé veal calves	0	32	0	0-11
Broilers	1	102	1.0	0-5
Dairy Cattle	1	102	1.0	0-5

MRSA on meat

MRSA was also found on meat. The prevalence on pork ranged between 5.9% in 2018 and 3.5% in 2020. For beef, the prevalence was 2.1% in 2018 and 3.8% in 2019. For veal the prevalence was 6.9%, for poultry meat 8.6% and for lamb 12.4% in 2021 (see table MRSA03). Generally, it is believed that contaminated meat is not an important transmission route for MRSA for the population at large. In some studies, food handling has been implicated as a transmission route (Ho, O'Donoghue, and Boost 2014) (Larsen *et al.* 2016).

Table MRSA03 Number of MRSA found on meat (products) from 2018-2021

	2018			2019			2020			2021		
	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	-
Beef	3	140	2.1	11	286	3.8	ns	ns	-	ns	ns	-
Veal	ns	ns	-	ns	ns	-	2	52	3.8	18	261	6.9
Poultry meat total	29	132	22	50	251	19.9	41	248	16.5	28	324	8.6
Chicken	26	129	20.2	41	237	17.3	36	234	15.4	25	310	8.1
Turkey	3	3	100	9	14	64.3	5	14	35.7	3	14	21.4
Lamb	ns	ns	-	ns	ns	-	ns	ns	-	37	299	12.4

ns: not sampled

Resistance levels of MRSA from livestock and meat

In 2021, susceptibility testing of MRSA was performed on a subset of isolates originating from caeca (veal calves and pigs), clinical masitis of cows, dairy farms, and meat (poultry, veal, small ruminants). The subset consisted of isolates from dust from dairy farms (n = 30), mastitis in dairy cows (n=93), pig caeca (n=15), poultry meat (n=32), sheep/goat meat (n=39), and veal calf caeca (n=22). MRSA isolates were tested for antimicrobial susceptibility with broth microdilution according to ISO standards using commercially available Sensititre plates (Thermofisher Scientific, panel EUST). The MIC-values were interpreted with ECOFFs as advised by EUCAST. Resistance percentages are depicted for each type of sample in table MRSA04.

As expected for MRSA, nearly all isolates tested resistant against (benzyl)penicillin and cefoxitin. High levels of resistance were also observed for tetracycline (up to 100% in chicken meat and in samples from veal calf caeca). This is in line with the known high levels (~100%) of tetracycline resistance in LA-MRSA. Also for trimethoprim, high levels of resistance were seen in isolates from mastitis, pig caeca, poultry meat, veal calf caeca, and veal calf meat (61.3% - 95.5%), while lower levels were observed in sheep/goat meat (13.5%). Remarkable was the high percentage of isolates resistant to fusidic acid (48.6%) in sheep/goat meat, as well as of those resistant to quinopristin/dalfopristin and tiamulin in poultry meat (both 65.6%). Finally, no resistance was detected in 2021 against the following antibiotics which are used to treat human infections: linezolid, mupirocin, rifampicin, or vancomycin.

Table MRSA04 Resistance percentages (R%) of MRSA isolated from dairy cattle (mastitis milk and dust), veal calves (caeca and meat), pigs (caeca), poultry (meat) and small ruminants (meat)

	Dairy cattle mastitis (N=93)	Dairy cattle dust (N=30)	Veal calves caeca (N=22)	Veal calves meat (N=18)	Pigs caeca (N=15)	Poultry meat (N=32)	Small ruminants meat (N=37)
Cefoxitin	98.9	100.0	100.0	100.0	100.0	96.9	100.0
Chloramphenicol	3.2	13.8	4.5	0.0	6.7	0.0	0.0
Ciprofloxacin	9.7	31.0	4.5	5.6	0.0	18.8	2.7
Clindamycin	19.4	34.5	72.7	27.8	53.3	78.1	8.1
Erythromycin	22.6	27.6	72.7	33.3	33.3	90.6	27.0
Fusidic acid	3.2	0.0	0.0	5.6	0.0	0.0	48.6
Gentamicin	45.2	37.9	81.8	50.0	33.3	3.1	45.9
Kanamycin	50.5	24.1	81.8	61.1	20.0	12.5	64.9
Linezolid	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mupirocin	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Penicillin	98.9	100.0	100.0	100.0	100.0	96.9	100.0
Quinupristin/dalfopristin	5.4	10.3	13.6	0.0	26.7	65.6	5.4
Rifampicin	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Streptomycine	29.0	13.8	4.5	5.6	6.7	31.3	21.6
Sulfamethoxazole	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Tetracycline	98.9	96.6	100.0	66.7	93.3	100.0	81.1
Tiamulin	4.3	6.9	4.5	11.1	33.3	65.6	5.4
Trimethoprim	61.3	55.2	95.5	72.2	80.0	62.5	13.5
Vancomycin	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Summary

In the study period 2018 – 2021, MRSA prevalence varied substantially between the sectors. A high MRSA prevalence was observed on pig farms (89.3%), whereas the MRSA prevalence was low on dairy farms (6.2%) and no MRSA were found on broilers farms. However, MRSA on broiler farms might have been underdetected by the inhibiting presence of coccidiostatica in the dust samples. On meat, the highest prevalence is found on turkey meat, followed by lamb, chicken and veal. Resistance against different classes of antibiotics often used in veterinary medicine was present in MRSA isolates from all sources. No resistance was found against antibiotics not authorized in food-producing animals (linezolid, vancomycin, mupirocin, rifampicin), while resistance to fusidic acid and chloramphenicol varied between the animal species.

References

Bortolaia, V., R. S. Kaas, E. Ruppe, M. C. Roberts, S. Schwarz, V. Cattoir, A. Philippon, R. L. Allesoe, A. R. Rebelo, A. F. Florensa, L. Fagelhauer, T. Chakraborty, B. Neumann, G. Werner, J. K. Bender, K. Stingl, M. Nguyen, J. Coppens, B. B. Xavier, S. Malhotra-Kumar, H. Westh, M. Pinholt, M. F. Anjum, N. A. Duggett, I. Kempf, S. Nykasenoja, S. Olkkola, K. Wieczorek, A. Amaro, L. Clemente, J. Mossong, S. Losch, C. Ragimbeau, O. Lund, and F. M. Aarestrup. 2020. 'ResFinder 4.0 for predictions of phenotypes from genotypes', *J Antimicrob Chemother*, 75: 3491-500.

Bradford, P. A., S. Bratu, C. Urban, M. Visalli, N. Mariano, D. Landman, J. J. Rahal, S. Brooks, S. Cebular, and J. Quale. 2004. 'Emergence of carbapenem-resistant Klebsiella species possessing the class A carbapenem-hydrolyzing KPC-2 and inhibitor-resistant TEM-30 beta-lactamases in New York City', *Clin Infect Dis*, 39: 55-60.

Brilhante, M., J. Menezes, A. Belas, C. Feudi, S. Schwarz, C. Pomba, and V. Perreten. 2020. 'OXA-181-Producing Extraintestinal Pathogenic Escherichia coli Sequence Type 410 Isolated from a Dog in Portugal', *Antimicrob Agents Chemother*, 64.

Dazio, V., A. Nigg, J. S. Schmidt, M. Brilhante, N. Mauri, S. P. Kuster, S. G. Brawand, G. Schupbach-Regula, B. Willi, A. Endimiani, V. Perreten, and S. Schuller. 2021. 'Acquisition and carriage of multidrug-resistant organisms in dogs and cats presented to small animal practices and clinics in Switzerland', *J Vet Intern Med*, 35: 970-79.

Ellington, M. J., J. Kistler, D. M. Livermore, and N. Woodford. 2007. 'Multiplex PCR for rapid detection of genes encoding acquired metallo-beta-lactamases', *J Antimicrob Chemother*, 59: 321-2.

Gonzalez-Torralba, A., J. Oteo, A. Asenjo, V. Bautista, E. Fuentes, and J. I. Alos. 2016. 'Survey of Carbapenemase-Producing *Enterobacteriaceae* in Companion Dogs in Madrid, Spain', *Antimicrob Agents Chemother*, 60: 2499-501.

Graveland, H., B. Duim, E. van Duijkeren, D. Heederik, and J. A. Wagenaar. 2011. 'Livestock-associated methicillin-resistant Staphylococcus aureus in animals and humans', *Int J Med Microbiol*, 301: 630-4.

Ho, J., M. M. O'Donoghue, and M. V. Boost. 2014. 'Occupational exposure to raw meat: a newly-recognized risk factor for Staphylococcus aureus nasal colonization amongst food handlers', *Int J Hyg Environ Health*, 217: 347-53.

Larsen, J., M. Stegger, P. S. Andersen, A. Petersen, A. R. Larsen, H. Westh, Y. Agerso, A. Fetsch, B. Kraushaar, A. Kasbohrer, A. T. Febetaler, S. Schwarz, C. Cuny, W. Witte, P. Butaye, O. Denis, M. Haenni, J. Y. Madec, E. Jouy, F. Laurent, A. Battisti, A. Franco, P. Alba, C. Mammina, A. Pantosti, M. Monaco, J. A. Wagenaar, E. de Boer, E. van Duijkeren, M. Heck, L. Dominguez, C. Torres, M. Zarazaga, L. B. Price, and R. L. Skov. 2016. 'Evidence for Human Adaptation and Foodborne Transmission of Livestock-Associated Methicillin-Resistant Staphylococcus aureus', *Clin Infect Dis*, 63: 1349-52.

Lekkerkerk, W. S., W. J. van Wamel, S. V. Snijders, R. J. Willems, E. van Duijkeren, E. M. Broens, J. A. Wagenaar, J. A. Lindsay, and M. C. Vos. 2015. 'What Is the Origin of Livestock-Associated Methicillin-Resistant Staphylococcus aureus Clonal Complex 398 Isolates from Humans without Livestock Contact? An Epidemiological and Genetic Analysis', *J Clin Microbiol*, 53: 1836-41.

Manchanda, V., S. Rai, S. Gupta, R. S. Rautela, R. Chopra, D. S. Rawat, N. Verma, N. P. Singh, I. R. Kaur, and P. Bhalla. 2011. 'Development of TaqMan real-time polymerase chain reaction for the detection of the newly emerging form of carbapenem resistance gene in clinical isolates of Escherichia coli, Klebsiella pneumoniae, and Acinetobacter baumannii', *Indian J Med Microbiol*, 29: 249-53.

Melo, L. C., M. N. Boisson, E. Saras, C. Medaille, H. J. Boulouis, J. Y. Madec, and M. Haenni. 2017. 'OXA-48-producing ST372 Escherichia coli in a French dog', *J Antimicrob Chemother*, 72: 1256-58.

Mughini-Gras, L., A. Dorado-Garcia, E. van Duijkeren, G. van den Bunt, C. M. Dierikx, M. J. M. Bonten, M. C. J. Bootsma, H. Schmitt, T. Hald, E. G. Evers, A. de Koeijer, W. van Pelt, E. Franz, D. J. Mevius, D. J. J. Heederik, and Esbl Attribution Consortium. 2019. 'Attributable sources of community-acquired carriage of Escherichia coli containing beta-lactam antibiotic resistance genes: a population-based modelling study', *Lancet Planet Health*, 3: e357-e69.

Poirel, L., C. Heritier, V. Tolun, and P. Nordmann. 2004. 'Emergence of oxacillinase-mediated resistance to imipenem in Klebsiella pneumoniae', *Antimicrob Agents Chemother*, 48: 15-22.

Pulss, S., I. Stolle, I. Stamm, U. Leidner, C. Heydel, T. Semmler, E. Prenger-Berninghoff, and C. Ewers. 2018. 'Multispecies and Clonal Dissemination of OXA-48 Carbapenemase in *Enterobacteriaceae* From Companion Animals in Germany, 2009-2016', *Front Microbiol*, 9: 1265.

Reynolds, M. E., H. T. T. Phan, S. George, A. T. M. Hubbard, N. Stoesser, I. E. Maciuca, D. W. Crook, and D. Timofte. 2019. 'Occurrence and characterization of Escherichia coli ST410 co-harbouring blaNDM-5, blaCMY-42 and blaTEM-190 in a dog from the UK', *J Antimicrob Chemother*, 74: 1207-11.

Roer, L., S. Overballe-Petersen, F. Hansen, T. B. Johannesen, M. Stegger, V. Bortolaia, P. Leekitcharoenphon, H. B. Korsgaard, A. M. Seyfarth, J. Mossong, P. Wattiau, C. Boland, D. S. Hansen, H. Hasman, A. M. Hammerum, and R. S. Hendriksen. 2019. 'ST131 fimH22 Escherichia coli isolate with a blaCMY-2/IncI1/ST12 plasmid obtained from a patient with bloodstream infection: highly similar to E. coli isolates of broiler origin', *J Antimicrob Chemother*, 74: 557-60.

Stolle, I., E. Prenger-Berninghoff, I. Stamm, S. Scheufen, E. Hassdenteufel, S. Guenther, A. Bethe, Y. Pfeifer, and C. Ewers. 2013. 'Emergence of OXA-48 carbapenemase-producing Escherichia coli and Klebsiella pneumoniae in dogs', *J Antimicrob Chemother*, 68: 2802-8.

van Duijkeren, E., P. D. Hengeveld, M. Albers, G. Pluister, P. Jacobs, L. Heres, and A. W. van de Giessen. 2014. 'Prevalence of methicillin-resistant Staphylococcus aureus carrying mecA or mecC in dairy cattle', *Vet Microbiol*, 171: 364-7.

Voets, G. M., A. C. Fluit, J. Scharringa, J. Cohen Stuart, and M. A. Leverstein-van Hall. 2011. 'A set of multiplex PCRs for genotypic detection of extended-spectrum beta-lactamases, carbapenemases, plasmid-mediated AmpC beta-lactamases and OXA beta-lactamases', *Int J Antimicrob Agents*, 37: 356-9.

Zong, Z. 2012. 'Discovery of bla(OXA-199), a chromosome-based bla(OXA-48)-like variant, in Shewanella xiamenensis', *PLoS One*, 7: e48280.