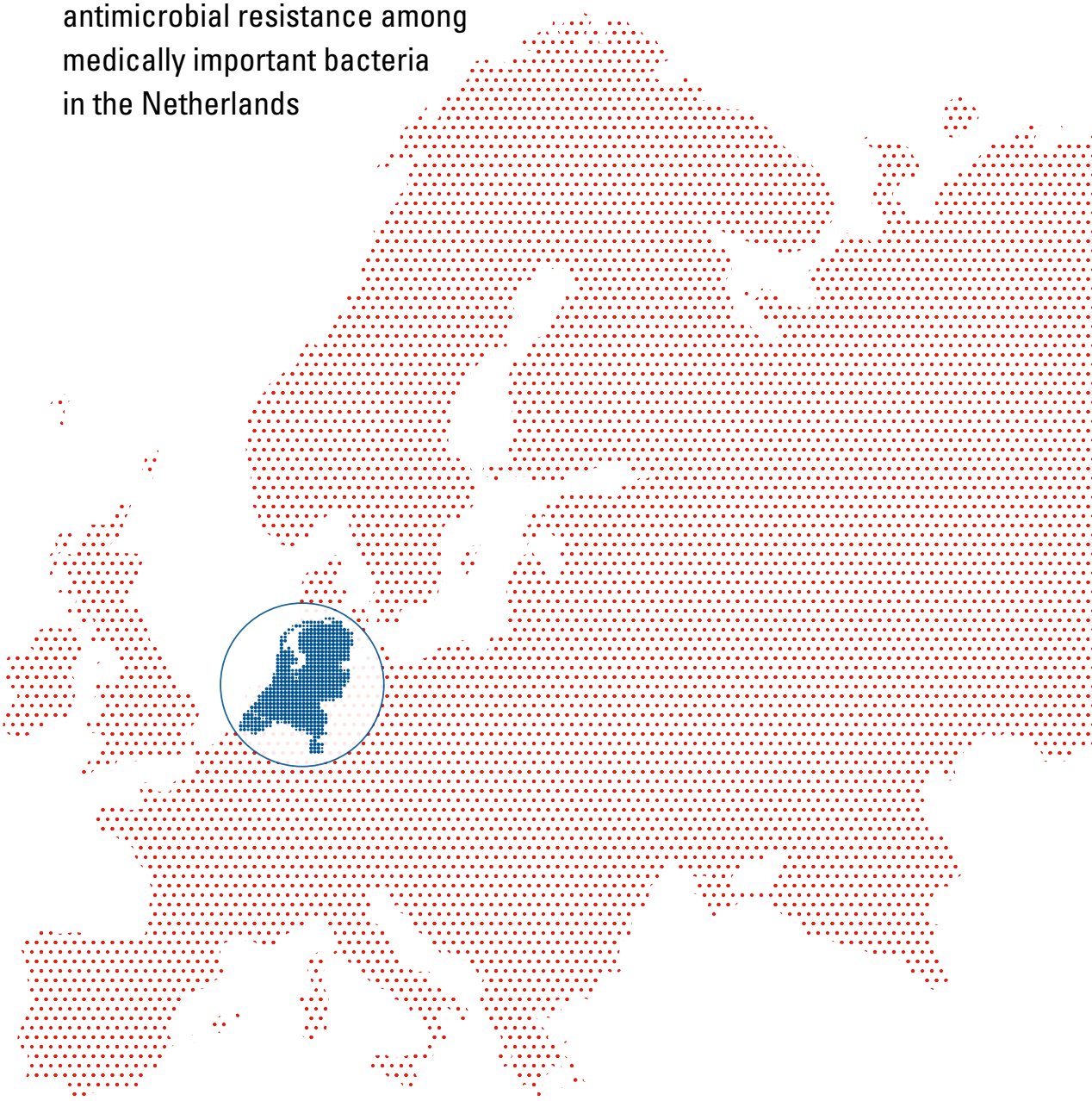
NethMap 2020

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



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

SWAB

NethMap 2020

Consumption of antimicrobial agents and
antimicrobial resistance
among medically important bacteria
in the Netherlands
in 2019

June 2020

In memoriam

Johan W. Mouton, b 03-11-1956 d 09-07-2019

On the 9th of July 2019, Johan W. Mouton, editor of NethMap since 2010, and a former president of SWAB, died from prostate cancer. He was only 62 years of age.

He was one of the driving forces in the surveillance of antimicrobial resistance and antibiotic use, not only in humans, but also in the veterinary sector and animal husbandry. Under his supervision, in 2012, the first combined publication of NethMap-MARAN (Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands) was published. He succeeded in presenting the vast amount of complex data in a clear and insightful way so that the report provided awareness and understanding of this important matter.

NethMap is considered one of the pillars of the successful Dutch strategy to control antimicrobial resistance. Knowing the facts about the epidemiology of antimicrobial resistance and antimicrobial use, was an important step forward. This to a large extent enabled optimization of antimicrobial use and the fight against antimicrobial resistance.

Above all, Johan was a great friend and we remember his enthusiasm and energy combined with his knowledge and passion for science. We miss him every day.

Synopsis

NethMap/MARAN-report

The number of bacteria that are resistant to antibiotics is increasing worldwide. In the Netherlands, that number is generally stable, and it is not at such a high level as in many other countries. There were hardly any increases in resistance found in 2019, and the resistance of some species of bacteria actually decreased in comparison to the previous years. The number of bacteria that are resistant to several different antibiotics and are therefore more difficult to treat is also not increasing. However, there is still reason to be vigilant in order to ensure that potential changes can be noticed in time.

To prevent resistance from developing, it is important to use antibiotics properly and only when necessary. General practitioners prescribed somewhat fewer courses of antibiotics in the past year compared to previous years. The overall use of antibiotics in hospitals increased somewhat.

Fewer antibiotics were prescribed for domestic farm animals in 2019 compared to 2018. In comparison to 2009, the reference year, the sale of antibiotics decreased by almost 70%. Almost no antibiotics that are important for treating infections in humans have been used for domestic farm animals in recent years. The level of antibiotic resistance in the various animal sectors remained the same or decreased somewhat in comparison to 2018. The percentage of ESBL-positive animals decreased further in all animal sectors. The biggest decrease in the percentage of ESBL-positive animals over the last 5 years was seen in broilers and on chicken meat. ESBLs are enzymes that can break down commonly used antibiotics such as penicillins.

In recent years, extra measures have been taken in the Netherlands to combat antibiotic resistance. These measures extend beyond the healthcare system because resistant bacteria also occur in animals, in foodstuffs and in the environment. That is why a 'One Health' approach is used in the Netherlands.
In the annual NethMap/MARAN 2020 report, various organisations collectively present their data on antibiotic use and resistance in the Netherlands, for humans as well as animals.

Keywords:
Antibiotic resistance, bacteria, antibiotic use, infection

Publiekssamenvatting

NethMap/MARAN-rapport

Wereldwijd neemt het aantal bacteriën die resistent zijn tegen antibiotica toe. In Nederland blijft dat aantal over het algemeen stabiel en is het minder hoog dan in veel andere landen. In 2019 zijn nauwelijks stijgingen in resistentie gevonden en bij sommige bacteriesoorten neemt de resistentie tegen bepaalde antibiotica zelfs iets af ten opzichte van de voorgaande jaren. Ook het aantal bacteriën dat resistent is tegen meerdere verschillende antibiotica tegelijkertijd, en daardoor moeilijker te behandelen, neemt niet toe. Er blijft altijd reden voor waakzaamheid, zodat veranderingen op tijd kunnen worden opgemerkt.

Om resistentie te voorkomen is het belangrijk om antibiotica op de juiste manier te gebruiken en alleen als het nodig is. Huisartsen schreven in het afgelopen jaar iets minder antibioticakuren voor dan de jaren daarvoor. In ziekenhuizen steeg het totale antibioticagebruik enigszins.

Voor landbouwhuisdieren is in 2019 minder antibiotica voorgeschreven dan in 2018. Ten opzichte van 2009, het referentiejaar, is de verkoop met bijna 70 procent verminderd. Voor landbouwhuisdieren zijn de afgelopen jaren bijna geen antibiotica gebruikt die belangrijk zijn om infecties bij de mens te behandelen. Ten opzichte van 2018 is de antibioticaresistentie in de verschillende diersectoren gelijk gebleven of licht afgenomen. Het percentage ESBL-positieve dieren is verder afgenomen in alle diersectoren. De grootste daling over de afgelopen 5 jaar van ESBL-producerende bacteriën wordt gezien bij vleeskuikens en op kippenvlees. ESBL zijn enzymen die veelgebruikte antibiotica kunnen afbreken, zoals penicillines.

In Nederland zijn de afgelopen jaren extra maatregelen genomen om antibioticaresistentie te bestrijden. Deze maatregelen reiken verder dan de gezondheidszorg omdat resistente bacteriën ook bij dieren, in voeding en in het milieu voorkomen. Daarom wordt in Nederland een ‘One Health’ aanpak gehanteerd. In de jaarlijkse rapportage NethMap/MARAN 2020 presenteren diverse organisaties gezamenlijk de gegevens over het antibioticagebruik en -resistentie in Nederland, zowel voor mensen als voor 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. The document was produced on behalf of the SWAB by the Studio of the RIVM.

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 2020 and earlier versions are also available from the website of the SWAB: www.swab.nl.

Members of CIb working on surveillance of antimicrobial resistance

Ing HJ Alblas
Dr Ir W Altorf-van der Kuil
Drs JM Aguilar Diaz
Dr Ir BHB van Benthem
Mrs L Blijboom
Drs I Bregman
Dr RD van Gaalen
Dr B de Gier
Dr Ir SC de Greeff
Mrs SR Groenendijk
Mrs K Halonen
Mrs J van Heereveld
Dr APA Hendrickx
Drs R Hertroys
Mrs TEM Hopmans
Mrs M Kamst-van Agterveld
Mrs TII van der Kooi
Dr MJ Korndewal
Prof Dr EJ Kuijper
Dr A Meijer
Drs JCM Monen
Dr DW Notermans
Drs WJ van den Reek
Dr AF Schoffelen
Dr LM Schouls
Prof Dr D van Soolingen
Drs M Visser
Dr CCH Wielders
Ir SHS Woudt

Members of SWAB's working group on surveillance of antimicrobial use

Dr S Natsch (chair)
Drs PDJ Bollen
Dr EMW van de Garde
Drs BJC Hendriks
Dr NGL Jager
Dr P de Klaver
Dr TBY Liem
Dr PD van der Linden
Drs M Lourens
S Mohsenian Naghani
Drs MMB Roukens
Dr AW van der Velden

Members of SWAB's working group on Antimicrobial Stewardship

Dr J ten Oever (chair)
S Balder
Dr ECH Boel
Prof Dr AW Friedrich
I Groothuis
Dr RM van Hest
Prof Dr MEJL Hulscher
Prof Dr BJ Kullberg
Dr PD van der Linden
Prof Dr JM Prins
Dr J Schouten
Dr KCE Sigaloff
Prof Dr B Sinha
Prof Dr A Verbon
Dr CM Verduin
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, Prof Dr MC Vos, 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 and Den Helder, NoordWest ziekenhuisgroep; Almelo and Hengelo, ziekenhuisgroep Twente; Almere, Flevoziekenhuis; Amersfoort, Meander MC; Amstelveen, ziekenhuis Amstelland; Amsterdam, AMC; Amsterdam, BovenIJ ziekenhuis; Amsterdam, OLVG Oost; Amsterdam, OLVG west; Amsterdam, VUMC; Amsterdam, Antoni van Leeuwenhoek ziekenhuis; Arnhem, Rijnstate; Assen, Wilhelmina ziekenhuis; Bergen op Zoom and Roosendaal, Bravis ziekenhuis; Beverwijk, Rode Kruis ziekenhuis; Boxmeer, Maasziekenhuis Pantein; Breda, Amphia ziekenhuis; Delft, Reinier de Graaf Groep; Den Bosch, Jeroen Bosch ziekenhuis; Den Haag, HAGA ziekenhuizen; Den Haag, HMC; Deventer, Deventer ziekenhuis; Dirksland, van Weel Bethesda ziekenhuis; Doetinchem, Slingerland ziekenhuis; Dordrecht, Albert Schweizer ziekenhuis; Ede, Gelderse vallei; Eindhoven, Maxima MC; Geldrop, St Anna ziekenhuis; Gorinchem, Rivas Zorggroep; Gouda, Groene hart ziekenhuis; Groningen, Martini ziekenhuis; Groningen, Ommelander ziekenhuis; Haarlem, Spaarne Gasthuis; Hardenberg, Ropcke Zweers; Harderwijk, St.Jansdal; Heerenveen, De Tjongerschans; Heerlen and Sittard, Zuyderland ziekenhuis; Hoogeveen and Emmen, Treant Zorggroep; Hoorn, Dijklander ziekenhuis; Leeuwarden, Medisch centrum Leeuwarden; Leiden, Alrijne ziekenhuis; Leiden, LUMC; Maastricht, MUMC; Meppel and Zwolle, Isala kliniek, 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; Sneek, Antonius ziekenhuis; Spijkenisse, Spijkenisse medisch centrum; Terneuzen, ZorgSaam; Tiel, Ziekenhuis Rivierenland; Tilburg, ETZ; Uden, Ziekenhuis Bernhoven; Utrecht, Diakonessenhuis; Utrecht, UMCU; Venlo, Vie Curi MC; Weert, St Jans Gasthuis; Zeeland, ADRZ; Zoetermeer, Lange Land ziekenhuis

Centres contributing to the surveillance of resistance to antimicrobial agents (ISIS-AR)

Alkmaar, Noordwest Ziekenhuisgroep; Amersfoort, Meander MC; Amsterdam, Atalmedial; Amsterdam, OLVG / Almere; Amsterdam, Streeklaboratorium Amsterdam – GGD; Amsterdam, VUmc; Apeldoorn, Gelre Ziekenhuizen; Bergen op Zoom, Bravis ziekenhuis, locatie Bergen op Zoom; 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; Heerlen, Zuyderland MC Heerlen; Hengelo, Laboratorium voor Medische Microbiologie Twente/Achterhoek; Hilversum, Centraal Bacteriologisch en Serologisch Laboratorium; Leeuwarden, Izore, Centrum Infectieziekten Friesland; Leiden, LUMC; Maastricht, Maastricht UMC+; Nieuwegein, St. Antonius Ziekenhuis; Nijmegen, Canisius Wilhelmina Ziekenhuis; Nijmegen, UMC St. Radboud; Roosendaal, Bravis ziekenhuis, locatie Roosendaal; Rotterdam, Erasmus MC; Rotterdam, Maasstad Ziekenhuis; 's-Gravenhage, Haaglanden Medisch Centrum / Alrijne Zorggroep; 's-Gravenhage, HagaZiekenhuis; 's-Gravenhage, MC Haaglanden Westeinde; 's-Hertogenbosch, Jeroen Bosch Ziekenhuis; Sittard-Geleen, Zuyderland MC; Terneuzen, Ziekenhuis ZorgSaam Zeeuws-Vlaanderen; 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 2020, the SWAB/RIVM report on the use of antibiotics, trends in antimicrobial resistance and antimicrobial stewardship programmes in the Netherlands in 2019 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), trends in antimicrobial resistance are monitored using routine antibiotic susceptibility testing data from microbiology laboratories in the Netherlands. Furthermore, the CIb subsidizes specific 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 2020 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 (HRMO's) receives attention in a separate chapter. 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 fifth consecutive year.

The 2020 pandemic of COVID-19 may have an impact on antimicrobial use and resistance; this warrants extra vigilance and analyses of data from the various AMR surveillance systems. We will report on this impact in the coming NethMap report 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 AF Schoffelen
Dr CM Verduin

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 2020) and the veterinary sector (MARAN 2020).

2.1 Most important trends in antimicrobial use

In outpatients

- In 2019 total systemic antibiotic use among outpatients has slightly decreased to 8.68 DDD/1,000 inhabitant days (DID).
- No major shifts in antibiotic use in outpatients have been observed except for beta-lactamase sensitive penicillins.
- The large increase of beta-lactamase sensitive penicillins, to pre-2018 levels, was probably driven by the resolved shortage in pheneticillin around April 2019.

In hospitals

- The inpatient use of antibiotics in 2018 slightly increased to 90.7 when expressed as DDD/100 patient-days and remained stable at 339.7 when expressed as DDD/100 admissions, probably indicating further intensification of the use of antibiotics in hospitals or a trend towards higher antibiotic dosing strategies in Dutch hospitals.
- The use of beta-lactamase resistant penicillins increased most and reached a level of 10.8 DDD/100 patient-days.
- The use of penicillins with extended spectrum has increased from 10.2 DDD/100 patient-days in 2017 to 11.1 DDD/100 patient-days in 2018.
- The use of fluoroquinolones decreased with 0.2 to 8.5 DDD/100 patient-days, mainly driven by reduction in use of ciprofloxacin.
- The use of first-, second- and third-generation cephalosporins has increased with 1.1, 2.1 and 0.5 DDD/100 patient-days, respectively.
- There are large differences in total antibiotic drug use between Dutch hospitals (range 54-144 DDD/100

patient-days). General hospitals used the least antibiotics (median 85.3 DDD/100 patient-days), where university hospitals reported the highest overall antibiotics use (91.5 DDD/100 patient-days).
- The use of antimycotics for systemic use has decreased from 13.6 in 2017 to 13.3 DDD/100 patient days in 2018.
- The use of antimycobacterials increased with 0.9 DDD/100 patient-days in 2018 and has now reached a level of 5.2 DDD/100 patient-days.
- Antibiotic use expressed as days of therapy (DOT)/100 patient-days informs on patient level exposure to antibiotics. Total inpatient use of antibiotics increased from 61.9 to 64.1 DOT/100 patient days in 2018
- The DOT/DDD ratio was highest (>1.5) for penicillins with extended spectrum, beta-lactamase resistant penicillins, tetracyclines and aminoglycosides. This indicates that for these antibiotics, higher doses, that exceed the DDD, are administered to patients
- PREZIES data showed that as in 2018 for surgical prophylaxis, cefazolin was used in the majority of cases in 2019. Use for medical prophylaxis was more diverse.

In long-term care facilities
- The mean use of antibiotics in long-term care facilities varies from year to year. In 2018, the mean of total systemic antibiotic use increased by 8.5 to 61.4 DDD/1,000 residents/day (range 25.5-142.5 DDD/1,000 residents/day).

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 central susceptibility testing, confirmation of important resistance mechanisms and molecular typing. In table 2.2.1 an overview is provided of surveillance programs that are included in NethMap 2020.

Table 2.2.1 Overview of antimicrobial resistance surveillance programs included in NethMap 2020

Surveillance program	Origin of isolates	availability	Sources 2019	Central or decentral susceptibility testing	Method of susceptibility testing
Surveillance program aimed at resistance surveillance in a wide range of pathogens					
ISIS-AR[1]	GP, Hospital, Nursing homes	2008-	47 laboratories	Decentral testing	Various methods used in routine susceptibility testing
Surveillance programs aimed at resistance surveillance in specific pathogens					
Neisseria meningitidis	Hospital	1994-	Nationwide	Central testing	Gradient testing
Neisseria gonorrhoeae	SHC	2006-	17 out of 24 SHC	Decentral testing	Gradient testing
Mycobacterium tuberculosis	General population	1993-	Nationwide	Primarily central testing	Agar dilution and BACTEC-MGIT 960 (liquid breakpoint)
Influenza antiviral drugs	community, GP, nursing home, hospital	2005-	NIVEL GP sentinels, SNIV nursing home 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-	1 lab	Central testing	Gradient testing
Clostridium difficile	Hospital, nursing homes	2005-	24 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, nursing homes	2008-	Nationwide	Central testing	MLVA, NGS
CPE	GP, hospital, nursing homes	2011-	Nationwide	Central testing	Gradient testing, Carba-PCR, NGS
CPPA	GP, hospital, nursing homes	2016-	Nationwide	Central testing	Gradient testing, multiplex PCR

ISIS-AR: Infectious disease Surveillance Information System on Antibiotic Resistance; 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

[1] *The 2020 pandemic of SARS-coronavirus-2 had a negative impact on the number of laboratories that was able to contribute data on antimicrobial resistance for the current edition of NethMap. Nevertheless, coverage and representativeness were still sufficient, and it is not expected that this has influenced the reliability of the resistance data that is presented.*

In GPs

- For most antimicrobials, there are no statistically significant and clinically relevant shifts in resistance levels since 2015.
- For isolates from urine cultures a distinction was 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 rates in the older age group were slightly higher than in the younger age group, except resistance of *K. pneumoniae* for co-amoxiclav which was higher in the age group below 12 years.
- Compared to 2015, there was a significant and clinically relevant increase in resistance to co-amoxiclav in *E. coli* and *K. pneumoniae* in both age groups, mainly due to a change in susceptibility testing method for co-amoxiclav in 2016. Compared tot 2018, there was no further increase in resistance to co-amoxiclav.
- Compared to 2015, the increase in ceftazidime resistance was statistically significant and clinically relevant in *E. coli* for patients aged ≤12 years (from 1% in 2015 to 2% in 2019), and in *K. pneumoniae* from patients aged ≤12 years (from 2% to 4%).
- ESBL percentages are low: 3% for *E. coli* and 4% for *K. pneumoniae*.
- Ciprofloxacin resistance in *K. pneumoniae* for both age groups increased statistically significant and clinically relevant (for patients aged ≤12 from 2% in 2015 to 6% in 2019 and for patients aged >12 years from 10% to 13%). Compared to 2018 ciprofloxacin resistance in *K. pneumoniae* was stable
- Resistance to trimethoprim and co-trimoxazole tends to decrease in the last 5 years for *E. coli* and *P. mirabilis*. In 2019 it is below 20% in *E. coli*, below 25% in *P. mirabilis* and below 10% in *K. pneumoniae*.
- The percentage of HRMO and multidrug resistance was ≤5% in all *Enterobacterales*, with a significant and clinically relevant increasing trend in multidrug resistance for *K. pneumoniae* isolates from patients aged ≤12 years (from 1% in 2015 to 4% in 2019). This increase is most likely caused by the increase in resistance to co-amoxiclav.
- In patients above 12 years of age, resistance of *P. aeruginosa* to ciprofloxacin is stable and around 11%.
- For *E. coli*, *K. pneumoniae*, and *S. aureus* resistance percentages are shown per region, these indicate that there are only minor differences in susceptibility.
- Clindamycin (inducible) resistance and resistance to macrolides in *S. aureus* rises every year, and is now more than 10%.
- In *S. aureus* a significant and clinically relevant increase in resistance to fusidic acid was found (from 16% in 2015 to 20% in 2019).

In hospitals

- Compared to 2015, overall resistance rates for many antimicrobials in *Enterobacterales* were similar.
- In all hospital departments, compared to 2015 resistance to co-amoxiclav in *E. coli* and *K. pneumoniae* in both age groups increased significantly and clinically relevant, mainly due to a change in susceptibility testing method for co-amoxiclav in 2016. However, when compared tot 2018, there was no further increase in resistance to co-amoxiclav.
- The rise of resistance in *K. pneumoniae* appears to have stopped. Resistance to all antimicrobials decreased in 2019, with the exception of piperacillin-tazobactam, rising to 9% in hospital inpatient departments. HRMO is stable at 10% in inpatient departments.
- When compared to other hospital departments, *Enterobacterales* isolates of urology patients (inpatient and outpatient) have higher levels of ciprofloxacin resistance. In urology inpatients the %HRMO is higher when compared to urology outpatient, and hospital outpatient and inpatient departments. This difference is most pronounced in *E. coli*, where ciprofloxacin resistance levels are 20% (in urology outpatient), versus 16% (hospital outpatient), and 25% (in urology inpatient) versus 14% (hospital inpatient).

- In 2019, Extended spectrum beta-lactamase (ESBL) producing *E. coli* further increased in all patient categories. ESBL-production in *K. pneumoniae* stabilised in all patient categories in 2019. The highest percentages ESBL are found in isolates from patients on the intensive care unit, 9% in E.coli and 12% in *K. pneumoniae*. ESBL percentages are still low when compared to other countries in Europe.
- In *E. coli*, resistance to ceftazidime increased to a statistically significant and clinically relevant extent in the last five years (from 3% to 5%).
- HRMO and multidrug resistance in *Enterobacterales* has stabilised in 2019, the percentage HRMO was ≤10% and the percentage of multidrug resistance was ≤6%.
- Resistance to empiric therapy combinations was ≤5% for all *Enterobacterales*.
- In unselected hospital patient departments resistance levels ≥20% were found for amoxicillin/ampicillin, trimethoprim and co-trimoxazole in *E. coli* and *P. mirabilis*, for co-amoxiclav in *E. coli* and *K. pneumoniae*, and for fosfomycin in *K. pneumoniae* and E. cloacae complex.
- For the first year we added data from ISIS-AR for the anaerobic pathogens *B. fragilis* and *C. perfringens*. Antimicrobial resistance in both species is low, with the exception of clindamycin resistance in *B. fragilis* of 15%.
- In intensive care units, in *E. coli*, resistance to ceftazidime increased to a statistically significant and clinically relevant extent in the last five years (from 4% to 7%). Furthermore, in *K. pneumoniae*, a statistically significant and clinically relevant increase in resistance was observed for piperacillin-tazobactam (from 6% in 2015 to 8% in 2019); this increase was primarily observed between 2015 and 2017. In *P. mirabilis*, a significant and clinically relevant decrease in resistance between 2015 and 2019 was observed for co-amoxiclav (from 13% to 6%) and for ciprofloxacin (from 16% to 9%).
- In intensive care units, resistance was ≤6% for all empiric therapy combinations in all *Enterobacterales*. A statistically significant and clinically relevant increase in resistance was observed for gentamicin + co-amoxiclav in *E. coli* (from 3% in 2015 to 5% in 2019), most likely caused by the high resistance rates to co-amoxiclav in this species.
- The percentage HRMO in intensive care units was 12% for *E. coli* and 14% for *K. pneumoniae*.
- In intensive care units, in *P. aeruginosa* resistance to ciprofloxacin (16%) and piperacillin-tazobactam (14%) is high and increasing.
- In blood isolates resistance was similar to resistance of isolates from all specimens combined in non-ICU departments and ICU-departments.

Specific pathogens and situations

- Vancomycin resistance (VRE) in infection related isolates of *E. faecium* remains low, and is below 1%. The number of outbreaks with VRE reported to SO-ZI/AMR was 19 in 2019. Constant evaluation of infection control measures to contain outbreaks is needed.
- In *S. pneumoniae,* the percentages of R and I+R results for (benzyl)penicillin, were ≤10% in GP patients and hospital patients: when taking intermediate resistant strains into account, then 6% of isolates in both patient groups have a reduced susceptibility.
- MRSA prevalence in diagnostic samples is 2% and remained stable over the past 5 years. The MRSA prevalence in blood culture isolates remained low, 1%.
- PVL positivity in MRSA increased over the years and is now 24%. In diagnostic isolates it is 40%, in screening isolates it is 17%.
- Remarkably: in 2019, PVL-genes were present in 8% of LA-MRSA isolates.
- Macrolide and clindamycin resistance in group B beta-haemolytic streptococci (GBS) from neonates with invasive infection is high. In 2019 erythromycin resistance was 22% and clindamycin resistance was 24%. Comparable resistance percentages were found in genital isolates of women aged 18-45.

- In 2019, the resistance levels for fluoroquinolone in human *Campylobacter* isolates were high again, and again increased compared with the year before (from 63.6% in 2018 to 68.9% in 2019)
- In human STEC O157 isolates proportions of resistance were higher than in 2018. Resistance to the quinolones (ciprofloxacin and nalidixic acid) and 3rd generation cephalosporins was not detected in human STEC O157 isolates in 2019.
- In gonococci, no resistance to ceftriaxone, the current first-line treatment was found. However, MIC values of ceftriaxone are increasing, and this trend is worrying and needs close surveillance. Resistance to azithromycin decreased in 2019 and is now below 10%, compared to 11% in 2018. Ciprofloxacin resistance showed a large increase and reached 55% in 2019.
- Data on antimicrobial susceptibility of anaerobic bacteria is limited. To gain more insight in resistance in anaerobic bacteria a more extensive surveillance program is needed.
- Resistance in *C. difficile* is rare, but 2 cases (0.2%) of plasmid-mediated resistance to metronidazole were found in the Netherlands. The emergence of metronidazole resistance and its clinical relevance is subject of study.
- In 2019, for the first time, azole resistance in *Aspergillus fumigatus* decreased to 12.5% in university hospitals and 6.1 % in teaching hospitals compared to the previous years.
- The proportion of *E. coli* and *K. pneumoniae* isolates with elevated carbapenem MIC values (i.e. > the screening breakpoint) on automated testing was 0.7% in 2019, and has remained stable over the past five years. The overall percentage of confirmed non-susceptible *E. coli* and *K. pneumoniae* was increased over the years but is still very low (In *E. coli* from 0.03% in 2015 to 0.08% in 2019 in *E. coli*, and in *K. pneumoniae* from 0.35% to 0.50%).
- In 2019, 363 unique carbapenemase-producing *Enterobacterales* isolates were obtained from 316 persons (mean age 62 years and 53% male) In 2018, this number was 306 unique carbapenemase-producing *Enterobacterales* isolates from 266 persons. Only 27% of those isolates had an MIC (for meropenem) above the clinical breakpoint.
- Targeted screening is the reason for sampling in 69% of CPE-positive persons. Hospitalization abroad for at least 24 hours during the previous two months was the most common risk factor for the presence of CPE.
- In 2019, 5% of *P. aeruginosa* in diagnostic isolates were phenotypically resistant to carbapenems. 2% of the *P. aeruginosa* isolates was MDR and 57% of these MDR isolates were carbapenem-resistant. The prevalence of carbapenem resistant *P. aeruginosa* is relatively highest in the ICU department. In a minority of phenotypically carbapenem resistant strains (8%), data on carbapenemase production was available, of which only 6% was positive.
- The predominant carbapenemase-encoding gene found in *P. aeruginosa* was *bla*VIM, found in 78% of strains submitted via Type-Ned CPE.
- In 2019, 59 outbreaks were reported to the Early warning and response meeting of Healthcare associated Infections and AntiMicrobial Resistance (SO-ZI/AMR). Most outbreaks were caused by MRSA and VRE. There were 2 outbreaks with carbapenemase-producing *Enterobacterales* (both in *K. pneumoniae*) and one outbreak with a carbapenemase-producing *P. aeruginosa* reported to SO-ZI/AMR. The risk of an outbreak for public health was estimated as low for all outbreaks in 2019 (57 outbreaks were phase 1, 2 outbreaks phase 2).

2.3 Antibiotic use and resistance in animals

Antimicrobial use

- Sales of antimicrobial veterinary medicinal products in 2019 (150 tonnes) decreased by 16.1 % compared to 2018 (179 tonnes). This means that the total reduction compared to the index year 2009 was almost 70%, which is the result of combined efforts of the authorities, the livestock sectors and the veterinarians.
- Antibiotic usage in veal calves and pigs decreased compared to 2018, while antibiotic use in dairy cattle and broilers was relatively stable at a low level over the last four years.
- In accordance with the recent WHO- classification of polymyxins as *Highest Priority Critically Important Antibiotic*, the Netherlands Veterinary Medicines Institute considers polymyxins as third choice drugs, and this antibiotic class is reported as such. The consequence is that similar as for fluoroquinolones and 3rd/4th generation cephalosporins, the target for its use from 2021 onwards will be no usage.

Antimicrobial resistance

- Overall, the highest resistance proportions in *Salmonella* were observed for tetracycline, sulfamethoxazole, ampicillin, and to a lesser extent for ciprofloxacin, nalidixic acid, and trimethoprim. These resistance patterns were most frequently found in the monophasic *S. Typhimurium*, *S. Infantis*, *S. Paratyphi B var. Java* from broilers, *S. Kentucky* (travel related), *S. Chester*, and to a lesser extent in *S. Typhimurium*.
- Among *Salmonella* isolates, only 19 isolates (1.0%) were confirmed ESBL-producers mainly from humans. No carbapenemase producing *Salmonella* were found in 2019.
- Proportions of resistance in *C. jejuni* isolates from caecal samples of broilers and meat thereof were traditionally high for quinolones and tetracycline and did not substantially change in 2019, compared to 2018. Resistance to macrolides was rarely detected in *C. jejuni* isolates from broilers and poultry meat, and was at low levels in *C. coli* isolates from broilers and poultry meat.
- Indicator *E. coli* isolated from randomly collected caecal samples of food animals at slaughter and meat thereof are most suited to study the effects of any interventions on antibiotic use.
- Among these indicator *E. coli* from animals and meat, resistance levels to ampicillin, tetracycline, sulfamethoxazole and trimethoprim were still relatively high in broilers, pigs, (white) veal calves and chicken and turkey meat.
- Resistance in indicator *E. coli* from caecal samples showed a tendency to stabilise in broilers, pigs and showed a slight decrease in veal calves. This is mostly in agreement with the use data reported.
- For the first time in twenty years no indicator *E. coli* isolates resistant to extended spectrum cephalosporins were detected in faecal samples from broilers, pigs, dairy cattle and veal calves.
- Resistance to fluoroquinolones was at the same level as in 2018, and was still commonly present in indicator *E. coli* from caecal samples of broilers and meat thereof.
- In 2019, a reduction in proportion of animals (prevalence determined with selective method) positive for ESBL/AmpC producing *E. coli* was observed in all livestock species compared to 2018. After a period of increasing trends of ESBL-carriers in veal calves, 2019 revealed a reduction in both rosé and in white veal calves. The largest reduction in the prevalence of ESBL/AmpC-producing *E. coli* has been achieved in broilers decreasing from 66.0% in 2014 to 17.9% in 2019, which can be considered a great success of the measures on reducing antimicrobial use initiated since 2011.
- In 2019, the *mcr-1* gene, encoding for colistin resistance, was identified at very low level (< 1%) in caecal samples from slaughter pigs and white veal calves. For the second year in row *mcr-4* was detected in white veal calves at low level (2%). No *mcr* genes were identified in *E. coli* isolated from broilers and in

chicken meat indicative for a further reduction of *mcr-1* in the broiler sector, although the use of colistin in broilers did increase again in 2019. This is important given the high priority of colistin for human medicine.

- The first results of a comparative study suggest an overall low genetic relatedness between LA-MRSA isolates from livestock (pigs and poultry) and humans. Moreover, the emergence of a more virulent (PVL-positive) LA-MRSA subclade is probably transmitted independent of livestock exposure.

2.4 Implications for therapy

Overall, resistance rates in the Netherlands are stable. The resistance rates in 2019 did not increase for most antibiotics and for many antibiotics there has even been a decrease. For now, the data on resistance look encouraging.
As already known for the last years, resistance to co-amoxiclav limits its usefulness in empiric therapy. There are significant differences in susceptibility by patient category. In particular for patients on the ICU, resistance levels are generally higher and routine culturing with antibiograms remains mandatory to tailor therapy to the individual patient. If broad spectrum therapy is initially chosen, antibiograms should be used to narrow down antimicrobial therapy to prevent even further emergence of resistance and cultures have to be repeated when indicated. Of note, EUCAST susceptibility breakpoints are based on the use of certain dosing regimens (to be found at www.eucast.org). The use of alternative (lower) dosing regimens should be used with care.
Of importance, resistance rates reported in NethMap are for one isolate per patient, and only the first one. Resistance of bacteria in the individual patient, especially those that stay longer in the hospital, is often significantly higher than reported here. On the other hand, resistance may be overestimated in GP and nursing home patients, since cultures are usually only performed after failure of initial therapy.
In the summary below, some of 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 UTI. Non-susceptible rates can be higher than resistance rates in some cases.

In GPs

- Resistance to nitrofurantoin and fosfomycin are still below 2% in *E. coli* indicating that use is suitable for uncomplicated urinary tract infections. High resistance rates and intrinsic resistance make fosfomycin unsuitable for *Klebsiella* therapy. Co-amoxiclav resistance in *E. coli* and *K. pneumoniae* are high, and its usefulness in the treatment of urinary tract infection in some patient categories is becoming more and more limited.
- Ciprofloxacin resistance in patients ≥12 years of age is stable in *Enterobacterales* and is around 10% for *E. coli* and *P. mirabilis*, and between 10-15% for *Klebsiella pneumoniae*. This should be taken into account when empiric ciprofloxacin therapy is considered for the treatment of complicated urinary tract infections.
- Clindamycin (inducible) resistance and resistance to macrolides in *S. aureus* rises every year, and is now more than 10%, which limits its usefulness in empiric therapy.
- Resistance percentages are available per region, these indicate that there are only minor differences in susceptibility between regions for some microorganisms and for some antibiotics and no regional adaptations in treatment guidelines are necessary.

In hospitals

Outpatient departments

- The levels of resistance in *Enterobacterales* preclude empirical treatment with oral agents for complicated UTI; culture, antibiograms and tailored therapy are necessary.
- Resistance levels are stable in all species, the rise in resistance of *K. pneumoniae* to many antimicrobial agents seen in the previous years appears to have stopped in 2019.
- HRMO and multidrug resistance in *Enterobacterales* has stabilised in 2019.

- 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.

Unselected hospital patient departments
- The rise of resistance in *K. pneumoniae* appears to have stopped. Nevertheless, patients suspected of infection with *K. pneumoniae* have a high risk of non-adequate treatment.
- For other *Enterobacterales*, it is encouraging to see that resistance to most antimicrobials is stable or even declining.
- Resistance to co-amoxiclav is high. The % resistance in 2019 for *E. coli* is 36% and in *K. pneumoniae* it is 21%. This renders the drug unsuitable for empiric therapy, unless it is combined with a second drug, for instance an aminoglycoside.
- For *P. aeruginosa* resistance is relatively low and stable for all antibiotics. If ciprofloxacin (resistance 12%) is considered as empiric therapy, combination with a second antipseudomonal agent should be considered.
- Combination therapy of a beta-lactam with an aminoglycoside is still the best suitable option for empirical treatment in serious infections with Gram-negative bacteria, unless a fluoroquinolone is specifically desired to cover specific pathogens.
- 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.
- Antimicrobial resistance in *B. fragilis* and *C. perfringens* is low, with the exception of clindamycin resistance in *B. fragilis* of 15%, limiting its use as part of empiric therapy in infections of the gastro-intestinal tract.

Intensive care units
- In *P. aeruginosa* the high and increasing resistance to ciprofloxacin and piperacillin-tazobactam indicates that culture and susceptibility testing are essential to guide therapy.
- Similar to patients on other wards, the level of resistance in *K. pneumoniae* is the main treatment challenge for patients on the intensive care. The %HRMO in *K. pneumoniae* was 14% in 2019 and there is a high risk of non-adequate empirical therapy.
- In *E. coli* resistance to cefotaxime/ceftriaxone (10%) and ceftazidime (7%) continues to rise over the years, most likely due to a rise in ESBL-production. Encouragingly, in *K. pneumoniae* this resistance decreased in 2019.
- Since species identification in Dutch laboratories is now usually very fast for positive cultures (within hours) due to the almost universal use of the MALDI-TOF and susceptibility still commonly requires overnight cultures, identification can have significant consequences for (empiric) therapy.
- Local resistance levels vary significantly between hospitals and even hospital wards, and also between moments in time. Tailored therapy and culture remain the mainstay of therapy.

Specific pathogens and situations
- In 2019, for the first time, azole resistance in *Aspergillus fumigatus* decreased to 12.5% in university hospitals and 6.1 % in teaching hospitals compared to the previous years. Susceptibility testing is required and azole monotherapy is not advised for empiric therapy.
- 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.
- Macrolide and clindamycin resistance in group B beta-haemolytic streptococci (GBS) from neonates with invasive infection is high. Intrapartum antibiotic prophylaxis with clindamycin should be reconsidered.

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, hospitals have established antimicrobial stewardship teams (A-teams) that are responsible for the implementation of an antimicrobial stewardship program. The antimicrobial stewardship monitor reports on 1) the stewardship activities employed by antimicrobial stewardship teams in hospitals and 2) the quality of antimicrobial use in hospitals.

The most important development concerning stewardship teams are:
- A-teams have become a universal part of the hospital
- Half of the A-teams have incorporated OPAT into their antimicrobial stewardship programs
- Barriers for the optimal functioning of A-teams is the lack of funding and formal IT-support

SWAB has continued the development and implementation of an antimicrobial stewardship monitor with the aim to provide benchmarked feedback reports. Three hospitals provided data by means of automatic data extraction from the electronic medical records (HIX; EPIC), which gave an impression on guideline adherence of antibiotics, the performance of iv-oral switch and therapeutic drug monitoring.

2.6 Implications for public health and health policy

Antibiotic resistance is a serious threat to public health in Europe, leading to increased healthcare costs, prolonged hospital stays, treatment failures and sometimes death.
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.Data from the European Antimicrobial Resistance Surveillance Network (EARS-Net) show that in Europe in 2018, carbapenem resistance in *E. coli* remained rare (<0.1%). However, several countries reported carbapenem resistance percentages above 10% for *K. pneumoniae*. Carbapenem resistance was also common in *Pseudomonas aeruginosa* and *Acinetobacter species*, and at higher percentages compared with *K. pneumoniae*. As a result, in these settings, only a limited number of therapeutic options are available such as colistin, often leading to more toxicity and side-effects. Furthermore, colistin resistance may develop in patients treated with this drug, which poses a substantial public health risk. Furthermore, for *K. pneumoniae*, 37.2% of the isolates reported to EARS-Net for 2018 were resistant to at least one of the surveyed antimicrobial groups (fluoroquinolones, aminoglycosides, third-generation cephalosporines, carbapenems), relevant significant increasing trends were noted for fluoroquinolones and carbapenems. In contrast, aminoglycoside resistance decreased significantly. Combined resistance to fluoroquinolones, third-generation cephalosporins and aminoglycosides is stable at 19.6%. In *E. coli*, an resistance to third-generation cephalosporins is high at 15.1% in 2018 and fluoroquinolones at 25.3%.

In the Netherlands, the prevalence of resistance of most pathogens is stable or even declining. Carbapenem resistance among *Enterobacterales* remained rare. The overall percentage of confirmed non-susceptible *E. coli* and *K. pneumoniae* was low (0.08% and 0.5%) and there was no significant increase in the last years.

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[1]. In December 2019 the program ended and a final report of the program was planned for the beginning of 2020 but has been postponed due to the covid-19 pandemic[2].
Several initiatives that started in the context of the program, will have effects on the implementation of the surveillance systems as presented in NethMap/MARAN. In 2019, the ten Regional Cooperative Networks concerning antimicrobial resistance became fully operative.

[1] https://www.rijksoverheid.nl/documenten/kamerstukken/2015/06/24/kamerbrief-over-aanpak-antibioticaresistentie
[2] https://www.rijksoverheid.nl/documenten/kamerstukken/2019/10/14/kamerbrief-over-aanpak-antibioticaresistentie

The target of these networks is to stimulate regional collaboration between all relevant stakeholders in healthcare settings, concerning the control of antibiotic resistance and HRMOs, infection prevention measures, antibiotic use, patient flows, and more. Various initiatives within the networks to reach these goals have been developed in the previous years, including the organization of a regional coordinating team and the start of regional stewardship programs.
Secondly, the project "Eenheid van Taal – Antimicrobial Resistance" aims to implement standardized communication of microbiological, clinical and epidemiological data between stakeholders. It kicked off successfully in 2017 and since April 2019, the first labs routinely submit their data on antimicrobial resistance testing to the national surveillance program (ISIS-AR) by using "Eenheid van Taal". If more laboratories will submit data according to this semantic standard with standardized data transfer, this will reduce errors in data handling and will enable more real-time surveillance on antimicrobial resistance in the Netherlands.

Conclusions

The data presented in NethMap/MARAN 2020 demonstrate that ongoing attention is needed to combat antibiotic resistance and optimize antimicrobial use in humans and animals. 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 further decreased and 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 close attention. In the Netherlands, outbreaks of drug-resistant micro-organisms are closely monitored and managed successfully. The procedures of SO-ZI/AMR with risk assessment, monitoring the course of the outbreak and (if asked for or essential) external expertise work very 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 need more attention. For instance, national surveillance of Enterococci is missing at the moment, and surveillance of resistance in anaerobic bacteria is still limited. The 2020 pandemic of SARS-coronavirus-2 may have an impact on antimicrobial resistance and antimicrobial use. This warrants extra vigilance and analyses of data from the various AMR surveillance systems in the coming period.

3 Use of antimicrobials

3.1 Outpatient antibiotic use

Methods

Data on outpatient antibiotic use in the Netherlands is annually 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] The data from 2019 is processed using these new DDDs. To enable comparison of the 2019 data with 2018, the data from 2018 are presented as they were in 2018, as well as using the 2019 DDDs.

Results

Total outpatient use of systemic antibiotics has slightly decreased from 8.90 DID in 2018 to 8.68 DID in 2019 (Table 3.1.1). Similar to previous years, the use of tetracyclines further decreased in 2019, to 1.83 DID. The use of beta-lactamase sensitive penicillins increased to pre-2018 levels, resulting in 0.16 DID. In 2019, the use of penicillins with extended spectrum decreased with 0.09 DID to 1.26 DID, mostly driven by a decreased amoxicillin use (Figure 3.1.1 and Figure 3.1.2A). Similar to previous years, the use of fluoroquinolones further decreased, to 0.67 DID (Figure 3.1.1 and Figure 3.1.2C).

Discussion

Total outpatient antibiotic use in the Netherlands slightly decreased in 2019. Tetracycline use declined further. The increase in the use of beta-lactamase sensitive penicillins was probably caused by the shortage of pheneticillin in 2018 which resolved around April 2019, resulting in a DID level in 2019 just below the levels in the years before 2018.

The decrease in fluoroquinolone use, especially ciprofloxacin, can be explained by the safety warning of the EMA for these drugs concerning side effects involving muscles, tendons or joints and the nervous system, which was published in March 2019[2].

Table 3.1.1 Ten years data on the use of antibiotics for systemic use (J01) in outpatients (DDD/1,000 inhabitant-days), 2010-2019 (source: SFK).

		DDD until 2018 (source: WHO)									DDD including changes as of 2019 (source: WHO)	
ATC Group*	Therapeutic group	2010	2011	2012	2013	2014	2015	2016	2017	2018	2018	2019
J01AA	Tetracyclines	2.67	2.60	2.49	2.33	2.23	2.25	2.10	1.98	1.94	1.94	1.83
J01CA	Penicillins with extended spectrum	1.81	1.91	1.94	1.99	1.94	2.13	2.08	1.94	2.02	1.35	1.26
J01CE	Beta-lactamase sensitive penicillins	0.37	0.35	0.33	0.31	0.30	0.23	0.24	0.22	0.07	0.07	0.16
J01CF	Beta-lactamase resistant penicillins	0.38	0.39	0.41	0.41	0.44	0.43	0.46	0.46	0.49	0.49	0.48
J01CR	Penicillins + beta-lactamase-inhibitors	1.80	1.82	1.82	1.67	1.55	1.56	1.52	1.42	1.42	0.95	0.93
J01D	Cephalosporins & carbapenems	0.04	0.04	0.04	0.04	0.04	0.04	0.03	0.03	0.03	0.03	0.03
J01EA	Trimethoprim and derivatives	0.20	0.20	0.19	0.17	0.16	0.14	0.14	0.13	0.13	0.13	0.12
J01EE	Sulphonamides + trimethoprim	0.35	0.34	0.33	0.29	0.28	0.28	0.28	0.29	0.30	0.30	0.33
J01FA	Macrolides	1.31	1.34	1.34	1.22	1.18	1.20	1.17	1.17	1.22	1.22	1.22
J01FF	Lincosamides	0.14	0.15	0.16	0.17	0.18	0.19	0.20	0.21	0.23	0.23	0.23
J01GB	Aminoglycosides	0.03	0.03	0.04	0.03	0.03	0.03	0.02	0.02	0.02	0.02	0.02
J01MA	Fluoroquinolones	0.85	0.82	0.80	0.76	0.79	0.77	0.75	0.73	0.73	0.73	0.67
J01XE	Nitrofuran derivatives	1.23	1.31	1.38	1.37	1.40	1.40	1.39	1.36	1.35	1.35	1.30
J01XX01	Fosfomycin	0.01	0.01	0.01	0.02	0.03	0.04	0.05	0.05	0.06	0.06	0.06
	others	0.05	0.05	0.05	0.04	0.04	0.04	0.02	0.05	0.04	0.04	0.03
J01	**Antibiotics for systemic use (total)**	**11.23**	**11.37**	**11.34**	**10.83**	**10.58**	**10.72**	**10.44**	**10.06**	**10.05**	**8.90**	**8.68**

* *From the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system*

Figure 3.1.1 Use of antibiotics for systemic use (J01) in outpatients at ATC-4 level, 2010-2019 (source: SFK).

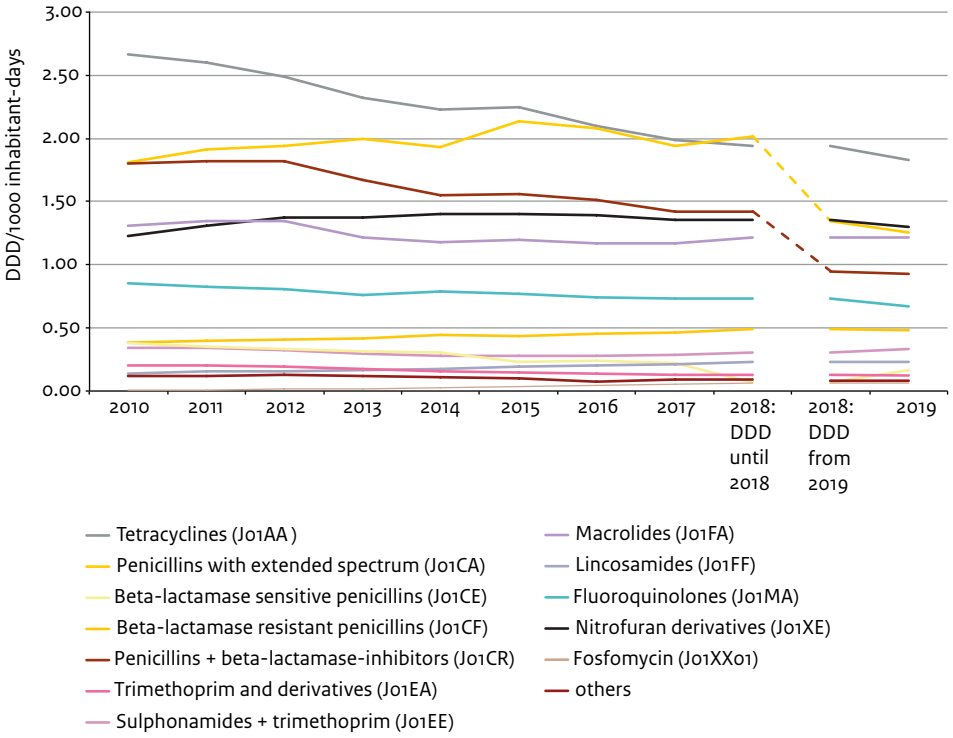
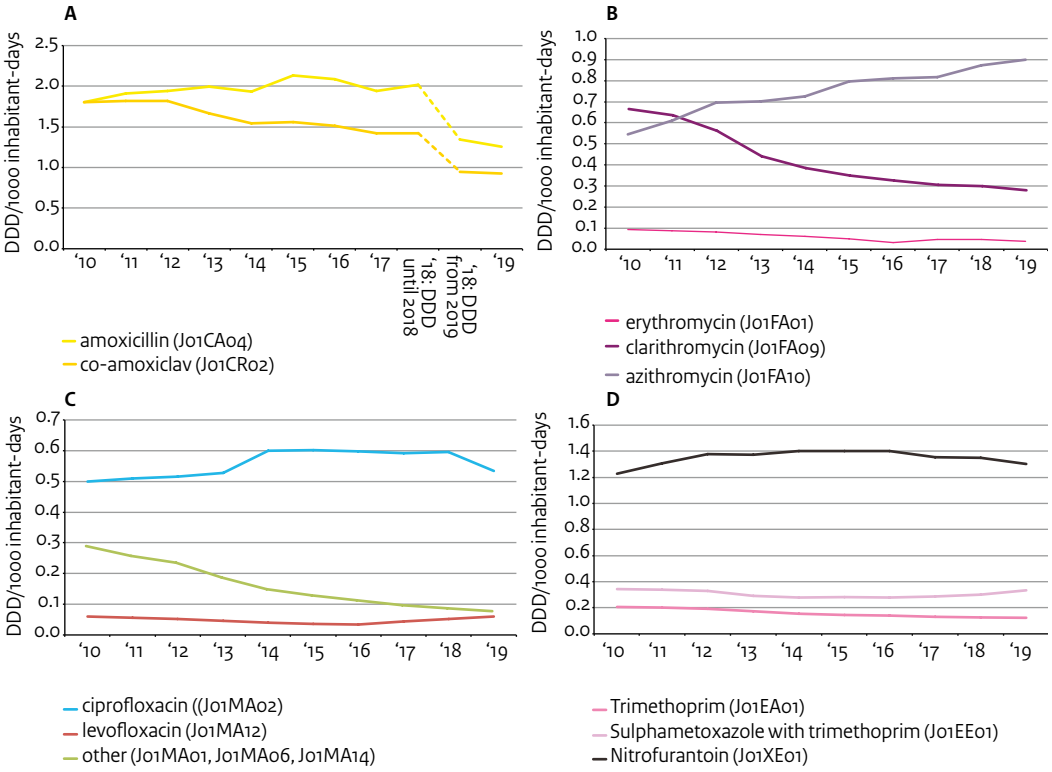


Figure 3.1.2 A-D Use of antibiotics for systemic use (J01) in outpatients at ATC-5 level, 2010-2019 (source: SFK).



3.2 Hospital care

3.2.1 Hospital antibiotic use in DDD

Methods

Data on the use of antibiotics in Dutch hospitals in 2018 were collected by means of a questionnaire distributed to all Dutch hospital pharmacies. DDD assigned per ATC-code and route of administration by the WHO in 2018[3] were extracted from the Dutch drug database (Z-index) on unit and product level, and used to calculate total antibiotic use as total amount of DDD per ATC-code. Use of antibiotics is expressed as DDD/100 patient-days and DDD/100 admissions. The number of patient-days is 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 ECDC. Data on the annual number of inhabitants in the Netherlands were obtained from Statistics Netherlands (CBS).

In addition, as in previous years, in 2019 Dutch hospitals collected detailed data on antibiotic usage (according to the methodology proposed by the ECDC), combined with the PREZIES point prevalence study on healthcare associated infections.[5] In the latter analysis, all patients admitted to the hospital on the day of survey were included, with the exception of patients on psychiatric wards and in the haemodialysis centre. Systemic antibacterials (ATC-code J01) and antimycotics (ATC-code J02) were included, with a maximum of three concomitant substances per patient.

Results

Data over 2018 were received from 61 hospitals (representing 54 different hospital organisations), together with the annual number of bed-days and admissions. The inpatient use of systemic antibiotics increased with 5.0 DDD/100 patient-days to 90.7 DDD/100 patient-days in 2018 (Table 3.2.1.1). Total inpatient use of systemic antibiotics remained the same compared to 2017; 340 DDD/100 admissions (Table 3.2.1.1). Total use of antibiotics for systemic use, calculated as DDD/1,000 inhabitant-days, also remained about the same; 0.94 in 2017 versus 0.93 in 2018 (Table 3.2.1.2).

The largest increases in antibiotic use were observed for penicillins with extended spectrum, beta-lactamase resistant penicillins and cephalosporins. Although in 2017 the use of penicillins with extended spectrum decreased, in 2018 its use increased to 11.1 DDD/100 patient-days (+8.4%). The use of beta-lactamase resistant penicillins increased to 10.8 DDD/100 patient-days (+12.3%) (Table 3.2.1.1). The increase in the use of penicillins in general is mainly driven by increase in use of amoxicillin (+0.9 DDD/100 patient-days) and flucloxacillin (+1.2 DDD/100 patient-days) (Figure 3.2.1.2). For all generations of cephalosporins the use increased in 2018; first-generation cephalosporins +21.5%, second-generation cephalosporins +36.1% and third-generation cephalosporins +7.6%.

Notable decreases were observed in the use of trimethoprim and derivatives, combinations of sulfonamides and trimethoprim and beta-lactamase sensitive penicillins, which decreased with 13.0%, 9.7% and 9.4%, respectively. Since 2016, a decrease in the use of fluoroquinolones was observed (Figure 3.2.1.1).

A large variation in systemic antibiotic drug use is seen between the different Dutch hospitals (Figure 3.2.1.3 and Figure 3.2.1.4). Considering site of care, in 2018, general hospitals had the lowest systemic antibiotic use (median 85.3 DDD/100 patient-days), whereas university hospitals reported the highest overall systemic antibiotic use (median 91.5 DDD/100 patient-days). The largest increase in use of systemic antibiotic was seen in university hospitals.
The use of second-generation cephalosporins is the highest in large teaching hospitals, compared to other types of hospitals. While the use of second-generation cephalosporins increased in large teaching hospitals and general hospitals, its use decreased in university hospitals in 2018. There was an increase in the use of first-generation cephalosporins in all types of hospitals (Figure 3.2.1.5), with 7.9%, 6.7% and 6.4% in large teaching, university and general hospitals respectively (Figure 3.2.1.6). Carbapenems, third generation cephalosporins, fluoroquinolones and glycopeptides are used to a larger extent in university hospitals, whereas most of the use of combinations of penicillins, penicillins with extended spectrum and lincosamides originates from general hospitals (Figure 3.2.1.6). There was a large decrease in the use of ciprofloxacin (-12.9%) in general hospitals (Figure 3.2.1.7).
The use of antimycotics further decreased in 2018, resulting in a use of 13.3 DDD/100 patient-days (-2.8%). The use of antimycobacterials increased over the past 10 years and reached 5.24 DDD/100 patient-days in 2018 (+21.5%). This included also rifampicin used for treatment of tuberculosis or as combination therapy for *S. aureus* infections. In particular the use of antivirals has increased, with 3.19 DDD/100 patient-days, and reached a level of 9.79 DDD/100 patient-days in 2018 (+48.3%). The use of neuraminidase inhibitors increased with 130% to 0.70 DDD/100-patient-days in 2018. Starting in 2010, the use of nucleosides (without reverse transcriptase inhibitors) is increasing every year, resulting in a level of 4.37 DDD/100 patient-days in 2018 (+46.2%) (Table 3.2.1.3).
Results of the point-prevalence study in 2019 (PREZIES data) were received from 21 hospitals, including 4828 patients of which 1685 received antibiotics, with a total of 2221 prescriptions. These numbers are similar to 2018. Antibiotic use divided by surgical versus medical prophylaxis and hospital versus community acquired infections is depicted in Figure 3.2.1.8. As in 2018, cefazolin was the most used antibiotic for surgical prophylaxis, which was used in 53% of cases in 2019. Use for medical prophylaxis was more diverse, with, similar to 2018, the highest use of trimethoprim/sulfamethoxazole. Antibiotic use for hospital and community acquired infections in 2019 is largely comparable to the distribution in 2018, although the use of ceftriaxone for community acquired infections increased from 8 to 13% in 2019.

Discussion

In 2018, antibiotic use in hospitals increased slightly when expressed as DDD/100 patient-days and remained stable when expressed as DDD/100 admissions. Shifts are observed from one subgroup of antibiotics to another, e.g. fluoroquinolones use has decreased, but the use of penicillins with extended spectrum, beta-lactamase resistant penicillins and cephalosporins continued to rise in 2018. There is a large variation in total antibiotic use between Dutch hospitals. Unfortunately, little is known about possible changes in hospital and patient characteristics which could influence the results in this surveillance.
The observed increase in use of first-generation of cephalosporins could be the result of higher doses of cefazolin for surgical prophylaxis.[6] Increase of second-generation cephalosporins might reflect a reaction to the adaption of the national treatment guideline for Community-Acquired Pneumonia in adults. Since 2017, a higher dose of cefuroxime is advised (1500 mg q8h instead of 750-1500 mg q8h)[7], where the DDDs of cefuroxime has not been altered by the WHO yet.

Table 3.2.1.1 Ten years use of antibiotics for systemic use (J01) in hospitals (DDD/100 patient-days), 2009-2018 (source: SWAB).

ATC group*	Therapeutic group	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
J01AA	Tetracyclines	1.64	1.67	1.84	1.74	1.75	1.90	1.89	1.96	1.97	2.05
J01CA	Penicillins with extended spectrum	7.57	7.25	7.31	7.62	7.95	8.42	9.24	10.88	10.22	11.08
J01CE	Beta-lactamase sensitive penicillins	1.60	1.54	1.52	1.74	1.86	2.40	2.39	2.55	2.50	2.26
J01CF	Beta-lactamase resistant penicillins	6.63	6.80	6.73	7.14	8.09	8.67	7.74	8.73	9.59	10.76
J01CR	Combinations of penicillins, incl. beta-lactamase-inhibitors	16.51	15.97	15.85	14.96	14.84	14.48	14.31	14.62	14.73	14.48
J01DB	First-generation cephalosporins	3.03	3.04	3.49	3.64	3.71	4.35	4.59	4.63	5.29	6.43
J01DC	Second-generation cephalosporins	3.60	3.42	3.68	4.09	4.68	4.98	5.33	5.75	5.87	7.99
J01DD	Third-generation cephalosporins	3.49	3.73	3.90	4.37	5.04	5.67	5.49	5.95	6.39	6.88
J01DH	Carbapenems	1.14	1.20	1.38	1.48	1.65	1.65	1.74	1.83	1.98	1.93
J01EA	Trimethoprim and derivatives	0.38	0.53	0.39	0.31	0.30	0.26	0.26	0.25	0.27	0.23
J01EE	Combinations of sulfonamides and trimethoprim, including derivatives	2.05	2.02	1.89	1.77	1.92	1.89	1.76	2.13	2.38	2.15
J01FA	Macrolides	2.63	2.66	2.86	2.81	2.64	2.88	2.74	2.97	2.82	2.66
J01FF	Lincosamides	2.38	2.34	2.29	2.21	2.30	2.30	2.35	2.45	2.43	2.54
J01GB	Aminoglycosides	4.18	4.06	3.95	3.26	3.55	3.57	3.66	3.70	3.62	3.76
J01MA	Fluoroquinolones	9.32	9.03	9.16	8.90	8.65	9.02	8.39	9.15	8.65	8.45
J01XA	Glycopeptides	1.26	1.25	1.28	1.36	1.49	1.59	1.60	1.62	1.72	1.73
J01XB	Polymyxins	0.22	0.39	0.22	0.16	0.23	0.19	0.23	0.23	0.24	0.14
J01XD	Imidazole derivatives	1.83	1.95	2.16	2.33	2.55	2.60	2.58	2.80	3.00	3.20
J01XE	Nitrofuran derivatives	1.11	1.19	1.24	1.22	1.30	1.55	1.42	1.67	1.73	1.63
J01XX	Other antibacterials	0.07	0.13	0.09	0.10	0.10	0.09	0.12	0.13	0.28	0.24
	Others**	0.27	0.12	0.07	0.10	0.08	0.07	0.07	0.07	0.08	0.10
J01	**Antibiotics for systemic use (total)**	**70.90**	**70.29**	**71.31**	**71.31**	**74.68**	**78.55**	**77.89**	**84.05**	**85.68**	**90.71**
	expressed in DDD/100 admissions:										
J01	**Antibiotics for systemic use (total)**	**321.3**	**315.9**	**306.4**	**295.7**	**307.8**	**326.0**	**330.1**	**326.1**	**340.2**	**339.7**

* *From the 2018 edition of the Anatomical Therapeutic Chemical (ATC) classification system*

** *J01DI, J01DF, J01EC and J01XC*

Table 3.2.1.2 Ten years data on the use of antibiotics for systemic use (J01) in hospital care (DDD/1,000 inhabitant-days), 2009-2018 (source: SWAB).

ATC Group*	Therapeutic group	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
J01AA	Tetracyclines	0.025	0.027	0.026	0.024	0.022	0.023	0.025	0.022	0.021	0.023
J01CA	Penicillins with extended spectrum	0.111	0.110	0.103	0.100	0.099	0.101	0.118	0.125	0.117	0.110
J01CE	Beta-lactamase sensitive penicillins	0.023	0.023	0.020	0.023	0.023	0.028	0.028	0.029	0.029	0.033
J01CF	Beta-lactamase resistant penicillins	0.093	0.097	0.089	0.093	0.100	0.105	0.097	0.102	0.103	0.105
J01CR	Penicillins + beta-lactamase-inhibitors	0.241	0.256	0.223	0.211	0.199	0.187	0.186	0.171	0.159	0.153
J01DB	First-generation cephalosporins	0.040	0.042	0.045	0.049	0.047	0.052	0.055	0.053	0.065	0.070
J01DC	Second-generation cephalosporins	0.051	0.055	0.050	0.052	0.055	0.058	0.065	0.066	0.067	0.070
J01DD	Third-generation cephalosporins	0.047	0.050	0.050	0.057	0.062	0.066	0.067	0.068	0.067	0.072
J01DH	Carbapenems	0.014	0.015	0.018	0.019	0.020	0.019	0.021	0.020	0.021	0.020
J01EA	Trimethoprim and derivatives	0.007	0.009	0.006	0.005	0.004	0.003	0.003	0.003	0.003	0.003
J01EE	Sulphonamides + trimethoprim	0.030	0.030	0.026	0.024	0.024	0.022	0.021	0.024	0.023	0.022
J01FA	Macrolides	0.039	0.041	0.037	0.038	0.034	0.034	0.034	0.034	0.030	0.030
J01FF	Lincosamides	0.033	0.035	0.032	0.031	0.032	0.028	0.030	0.028	0.027	0.026
J01GB	Aminoglycosides	0.055	0.058	0.054	0.044	0.045	0.044	0.046	0.043	0.037	0.037
J01MA	Fluoroquinolones	0.129	0.138	0.127	0.124	0.116	0.112	0.112	0.106	0.097	0.087
J01XA	Glycopeptide antibacterials	0.015	0.016	0.017	0.017	0.018	0.018	0.019	0.019	0.019	0.018
J01XB	Polymyxins	0.009	0.006	0.003	0.002	0.003	0.002	0.003	0.002	0.001	0.002
J01XD	Imidazole derivatives	0.026	0.030	0.027	0.029	0.030	0.030	0.032	0.032	0.034	0.033
J01XE	Nitrofuran derivatives	0.017	0.018	0.015	0.018	0.016	0.018	0.018	0.018	0.019	0.017
J01XX	Other antibiotics	0.002	0.002	0.001	0.002	0.002	0.001	0.002	0.002	0.003	0.003
	Others**	0.003	0.002	0.001	0.002	0.000	0.000	0.001	0.000	0.001	0.001
J01	**Antibiotics for systemic use (total)**	**1.008**	**1.061**	**0.971**	**0.963**	**0.950**	**0.953**	**0.982**	**0.968**	**0.942**	**0.934**

* *From the 2018 edition of the Anatomical Therapeutic Chemical (ATC) classification system*

** *J01DI, J01DF, J01EC and J01XC*

Figure 3.2.1.1 Use of antibiotics for systemic use (J01) in hospitals (DDD/100 patient-days) at ATC4 level, 2009-2018 (source: SWAB).

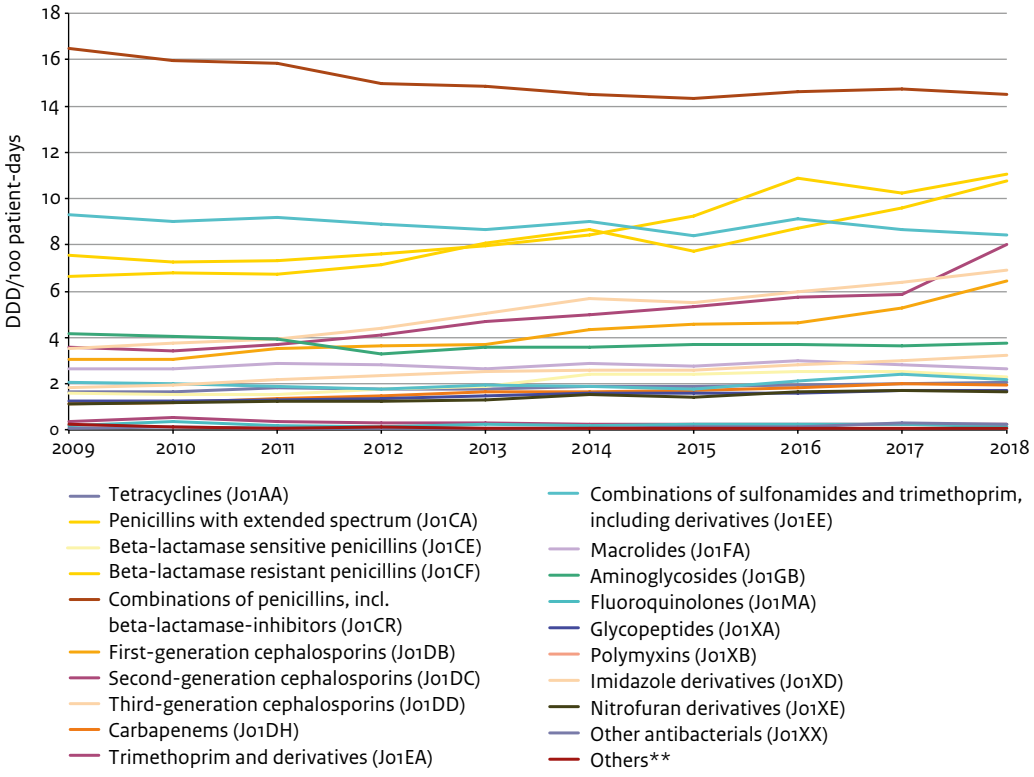


Figure 3.2.1.2 Use of beta-lactams, macrolides, aminoglycosides, fluoroquinolones and glycopeptides in hospitals expressed as DDD/100 patient-days (A) and DDD/100 admissions (B) at ATC-5 level, 2009-2018 (source: SWAB).

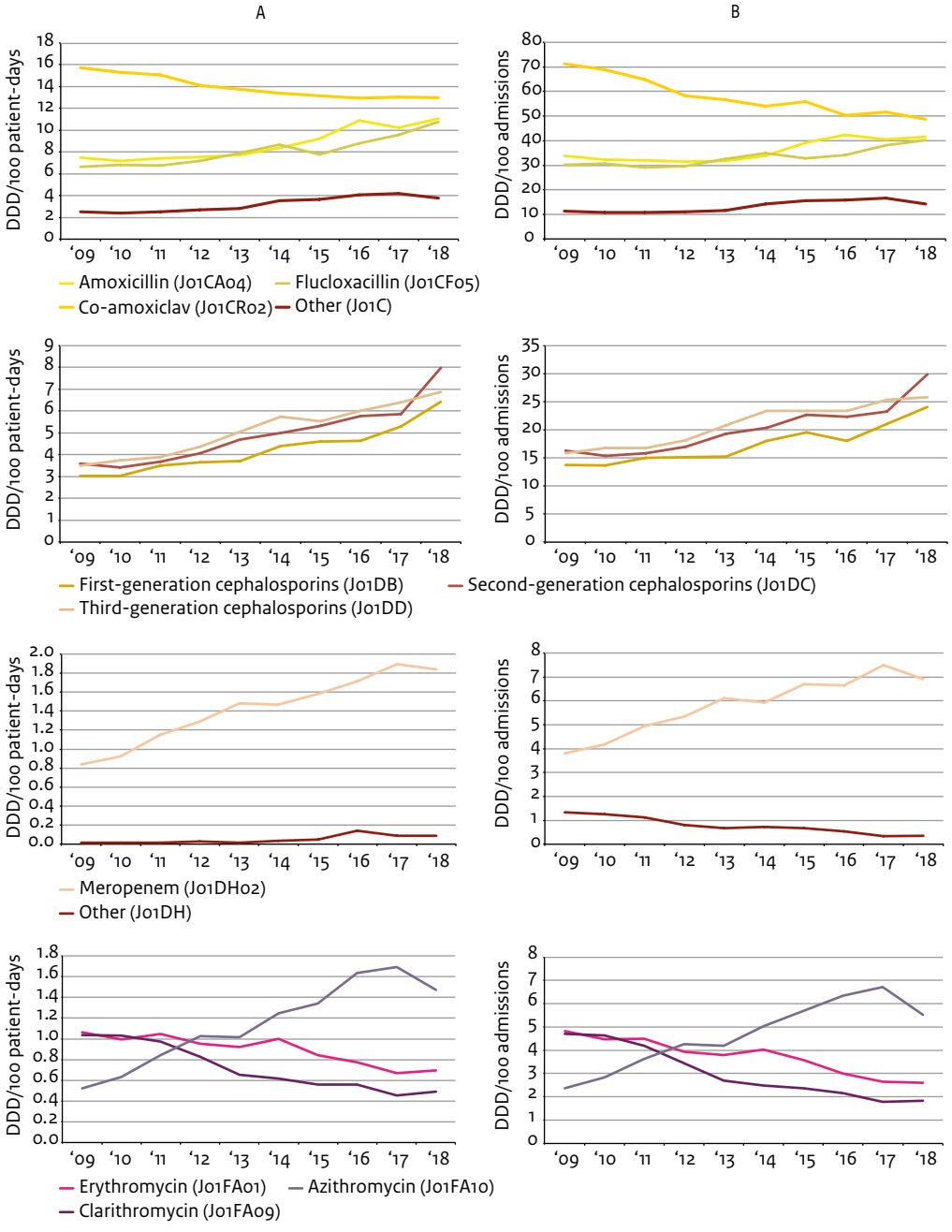


Figure 3.2.1.2 (continued) Use of beta-lactams, macrolides, aminoglycosides, fluoroquinolones and glycopeptides in hospitals expressed as DDD/100 patient-days (A) and DDD/100 admissions (B) at ATC-5 level, 2009-2018 (source: SWAB).

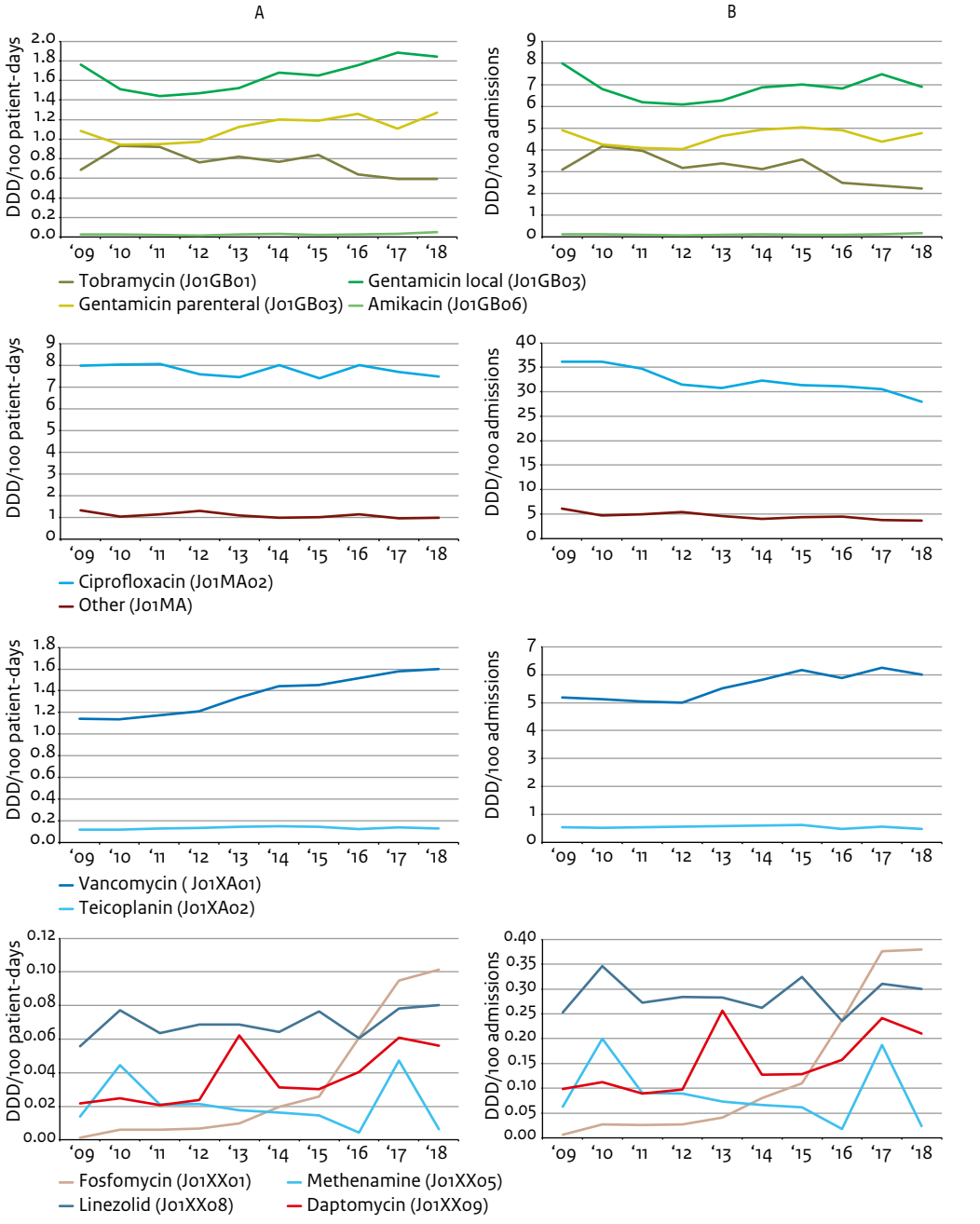


Figure 3.2.1.3 Total systemic antibiotic use (J01) and comparison across university, large teaching and general hospitals in 2018 (source: SWAB).

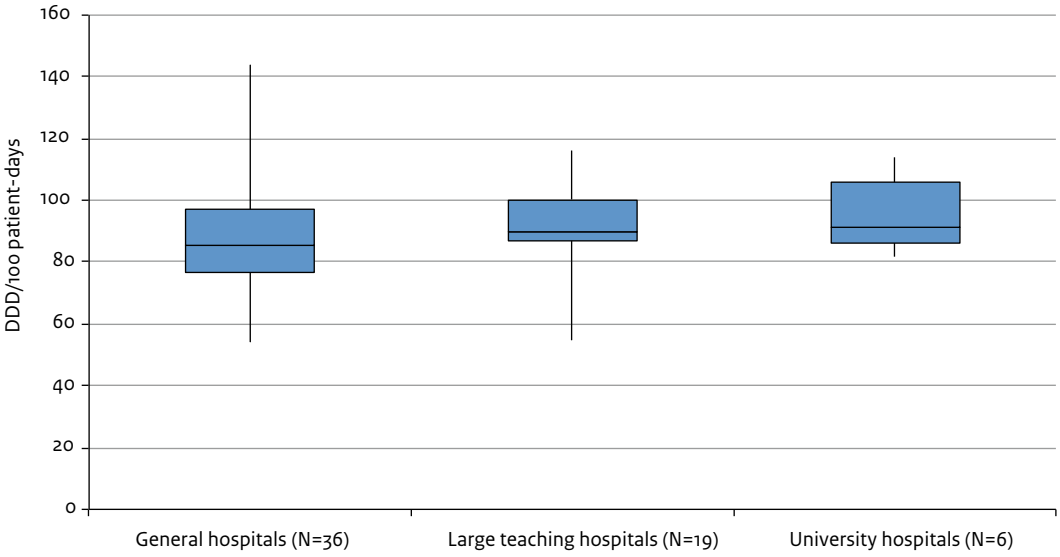


Figure 3.2.1.4 Comparison of the total systemic antibiotic drug use (J01) across Dutch hospitals in 2018 (source: SWAB).

Figure 3.2.1.5 Use of 1st, 2nd and 3th-generation cephalosporins in university, large teaching and general hospitals at ATC-5 level, 2010-2018 (source: SWAB).

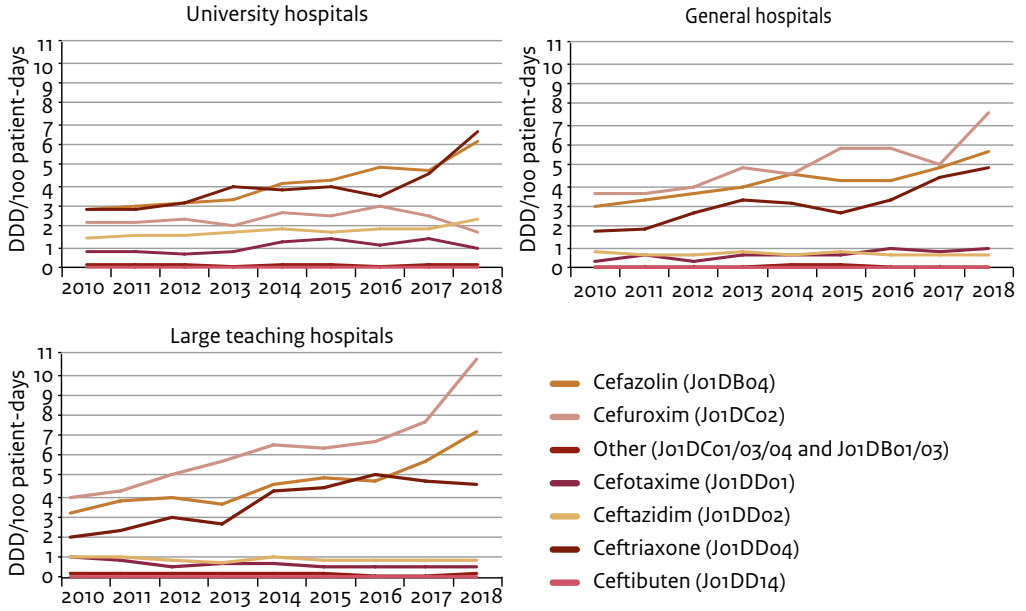


Figure 3.2.1.6 Distribution (%) of the use of antibiotics for systemic use (J01) in hospitals, 2018 (source: SWAB).

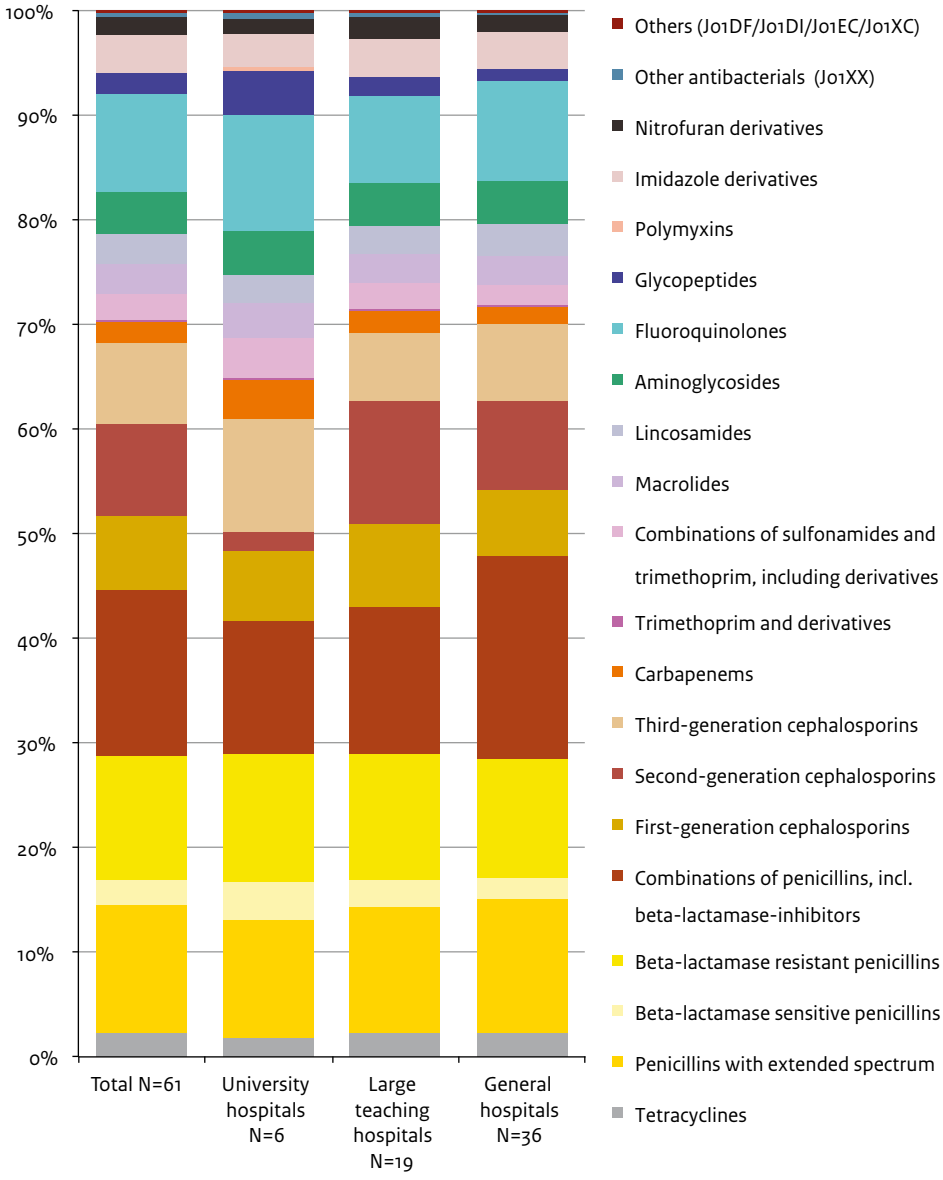


Figure 3.2.1.7 Use of cephalosporins (A), carbapenems (B), aminoglycosides (C), glycopeptides (D) and fluoroquinolones (E) in hospitals broken down by type of hospital, expressed as DDD/100 patient-days, 2009-2018 (source: SWAB).

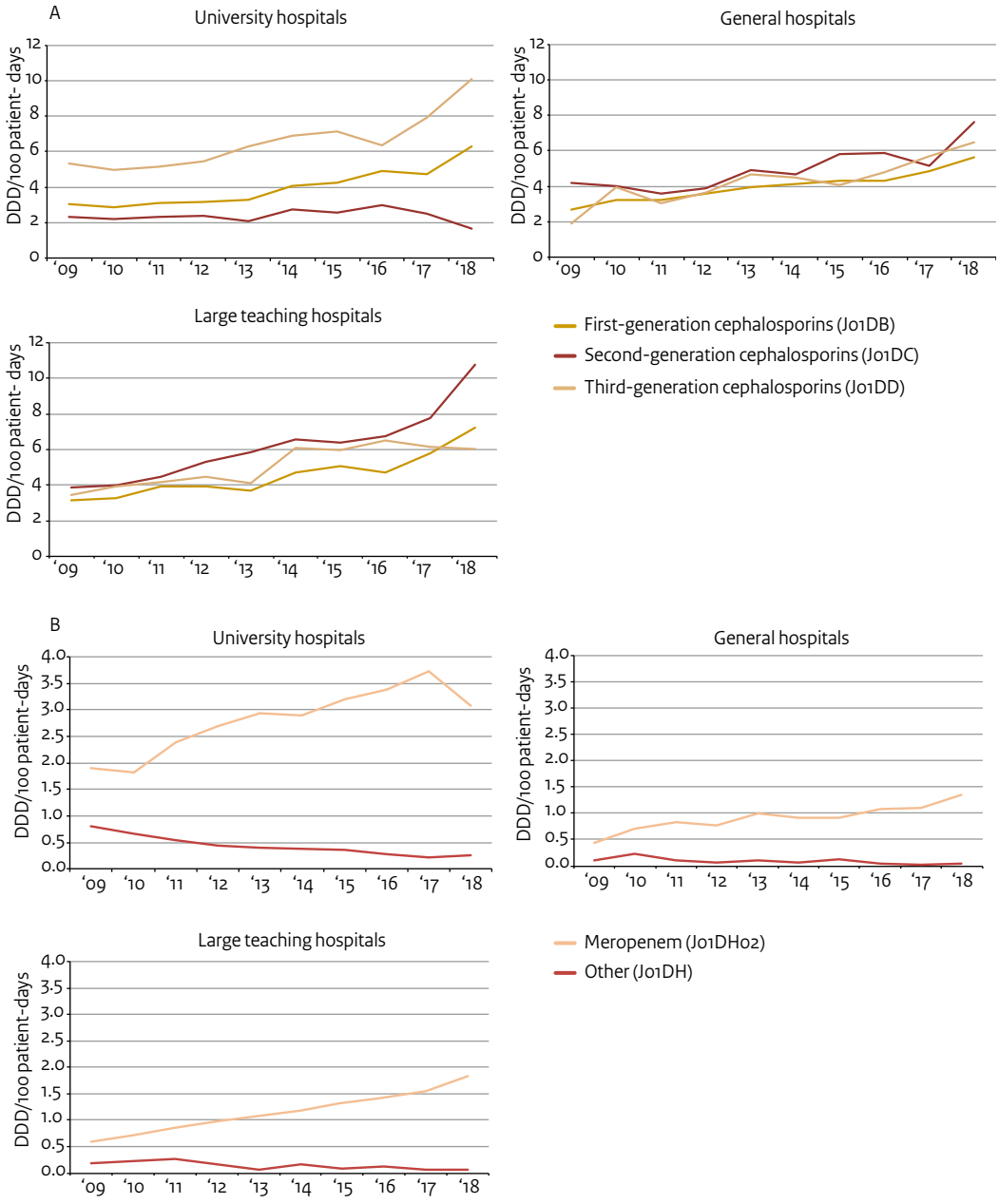


Figure 3.2.1.7 (continued) Use of cephalosporins (A), carbapenems (B), aminoglycosides (C), glycopeptides (D) and fluoroquinolones (E) in hospitals broken down by type of hospital, expressed as DDD/100 patient-days, 2009-2018 (source: SWAB).

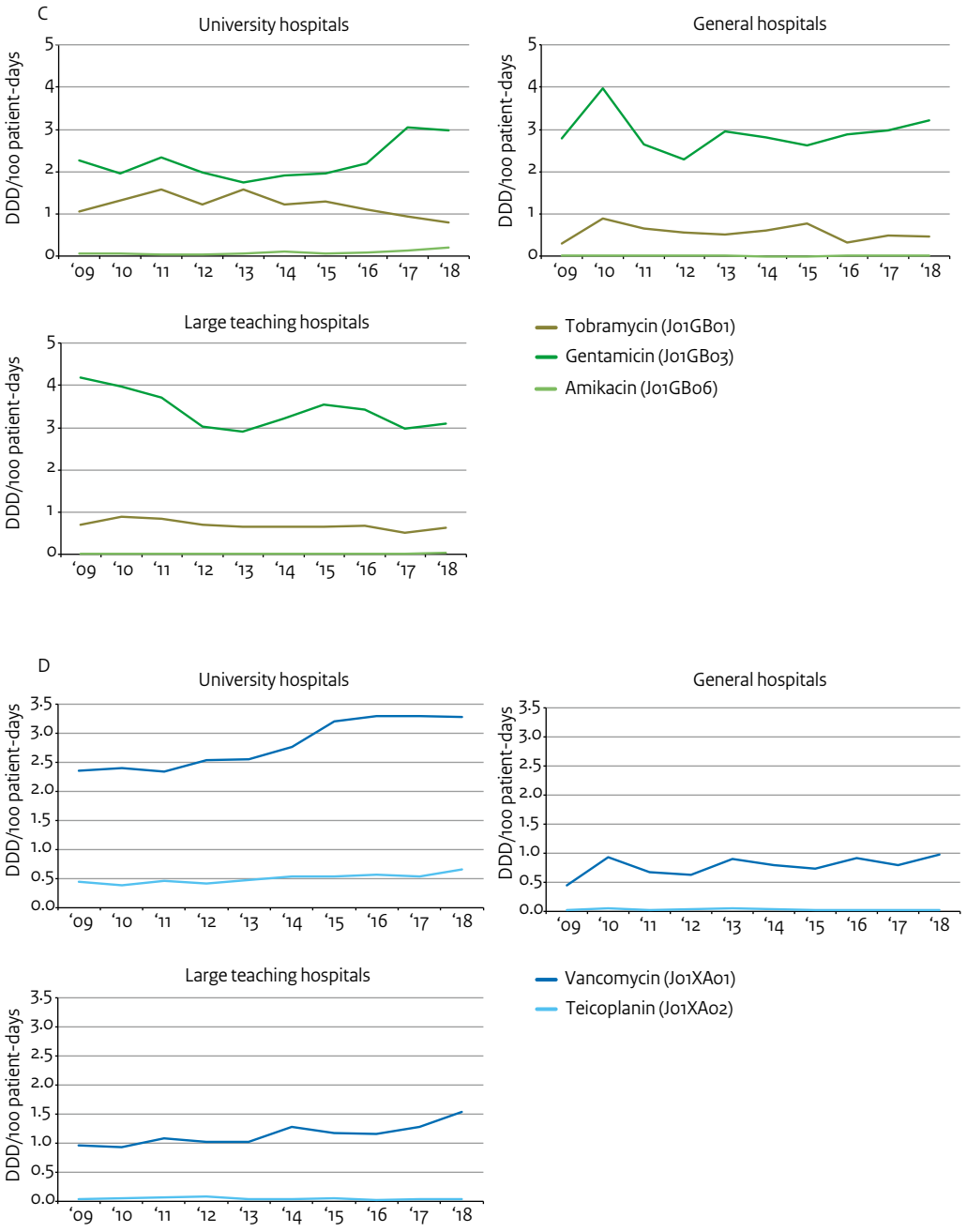


Figure 3.2.1.7 (continued) Use of cephalosporins (A), carbapenems (B), aminoglycosides (C), glycopeptides (D) and fluoroquinolones (E) in hospitals broken down by type of hospital, expressed as DDD/100 patient-days, 2009-2018 (source: SWAB).

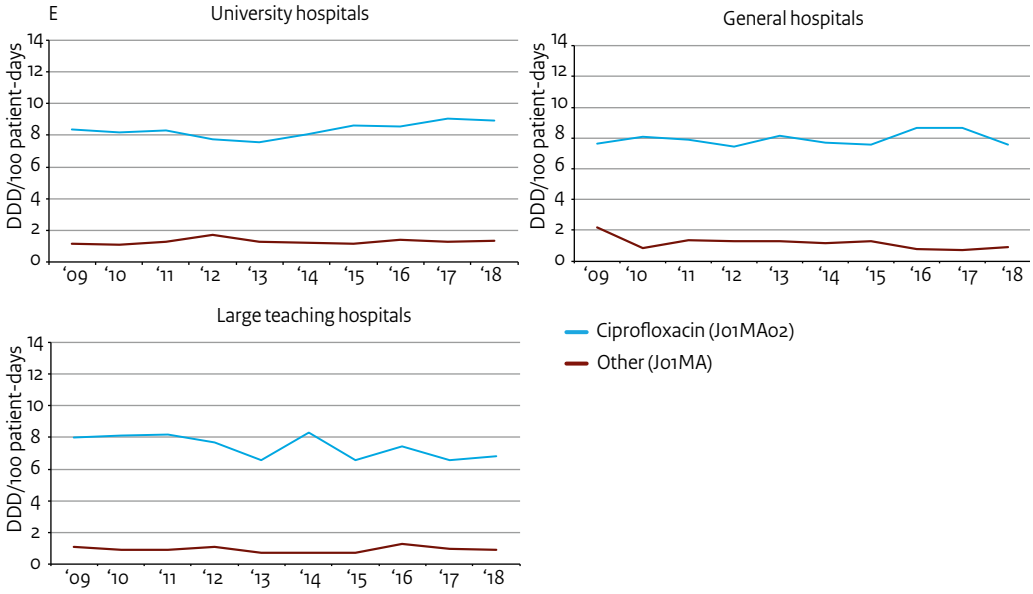


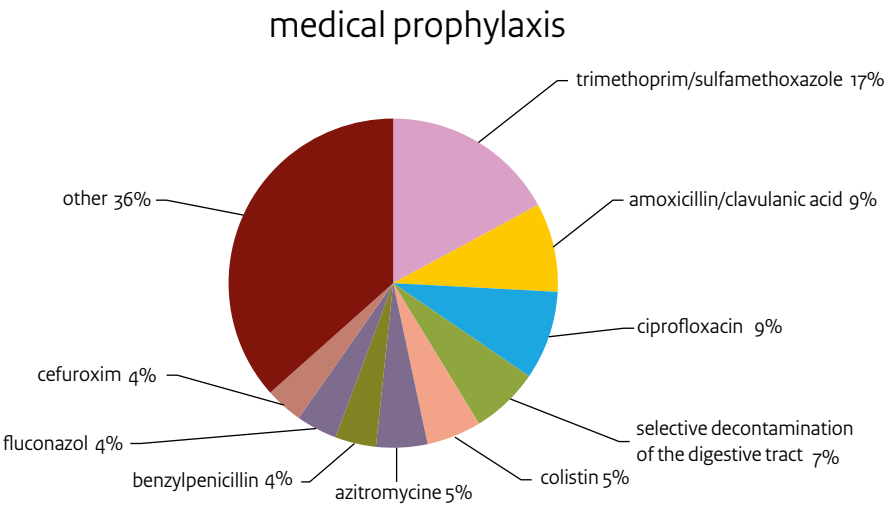
Table 3.2.1.3 Use of antimycotics, antimycobacterials and antivirals for systemic use (J02, J04, J05) in university hospitals (DDD/100 patient-days), 2009-2018 (source: SWAB).

ATC group *	Therapeutic group	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
J02AA01	Antibiotics (amphotericin B)	1.35	1.65	1.77	2.43	3.01	3.46	4.17	4.34	4.80	4.36
J02AB02	Imidazole derivatives (ketoconazole)	0.08	0.15	0.09	0.10	0.06	0.24	0.34	0.04	0.08	0.02
J02AC	Triazole derivatives	6.72	6.31	5.83	6.25	6.29	7.15	7.55	9.22	7.80	7.84
J02AX	Other antimycotics for systemic use (mainly echinocandines)	0.61	0.56	0.57	0.55	0.71	0.61	0.64	0.64	0.96	1.03
J02	**Antimycotics for systemic use (total)**	**8.77**	**8.66**	**8.26**	**9.33**	**10.06**	**11.47**	**12.70**	**14.23**	**13.63**	**13.25**
J04AA	Aminosalicylic acid and derivatives	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
J04AB	Antibiotics (mainly rifampicin)	1.27	1.41	1.56	1.24	1.43	1.39	1.33	1.13	1.69	1.89
J04AC	Hydrazides (mainly isoniazide)	0.40	0.34	0.30	0.40	0.57	0.56	0.35	0.30	0.67	0.98
J04AD	Thiocarbamide derivatives	0.00	0.00	0.01	0.00	0.00	0.00	0.12	0.14	0.01	0.02
J04AK	Other drugs for treatment of tuberculosis (pyrazinamide, ethambutol)	0.34	0.37	0.26	0.31	0.16	0.28	0.19	0.15	0.66	0.95
J04AM	Combinations of drugs for tuberculosis	0.00	0.00	0.00	0.01	0.02	0.04	0.07	0.11	0.15	0.22
J04BA	Drug for treatment of leprosy (dapson)	0.33	0.45	0.49	0.62	0.70	0.60	0.70	0.71	1.13	1.18
J04	**Antimycobacterials for systemic use (total)**	**2.35**	**2.58**	**2.62**	**2.57**	**2.88**	**2.87**	**2.76**	**2.55**	**4.31**	**5.24**
J05AB	Nucleosides excl. Reverse transcriptase inhibitors	2.22	2.02	2.18	2.24	2.33	2.71	2.76	2.97	2.99	4.37
J05AD	Phosphonic acid derivatives	0.13	0.10	0.10	0.15	0.12	0.16	0.14	0.20	0.20	0.31
J05AE	Protease inhibitors	0.75	0.78	0.55	0.81	0.63	0.40	0.33	0.30	0.31	0.25
J05AF	Nucleoside reverse transcriptase inhibitors	0.64	0.67	0.63	0.69	0.54	0.59	0.71	0.52	0.70	0.78
J05AG	Non-nucleoside reverse transcriptase inhibitors	0.23	0.22	0.14	0.18	0.16	0.18	0.23	0.22	0.26	0.23
J05AH	Neuraminidase inhibitors	n.a.#	0.21	0.42	0.19	0.49	0.16	0.30	0.43	0.31	0.70
J05AR	Antivirals for the treatment of HIV, combinations	0.55	0.76	0.69	0.91	0.89	0.94	0.95	0.99	1.12	1.96
J05AX	Other antivirals	0.06	0.15	0.17	0.24	0.29	0.22	0.33	0.46	0.72	1.18
J05	**Antivirals for systemic use (total)**	**4.59**	**4.91**	**4.89**	**5.41**	**5.47**	**5.37**	**5.75**	**6.09**	**6.60**	**9.79**

* *from the 2018 edition of the Anatomical Therapeutic Chemical (ATC) classification system*

\# *Total use not to be assessed because of alternative distribution during the pandemic*

Figure 3.2.1.8 Distribution of the use of antibiotics for systemic use (J01); results of the point-prevalence studies 2019 (source: PREZIES).



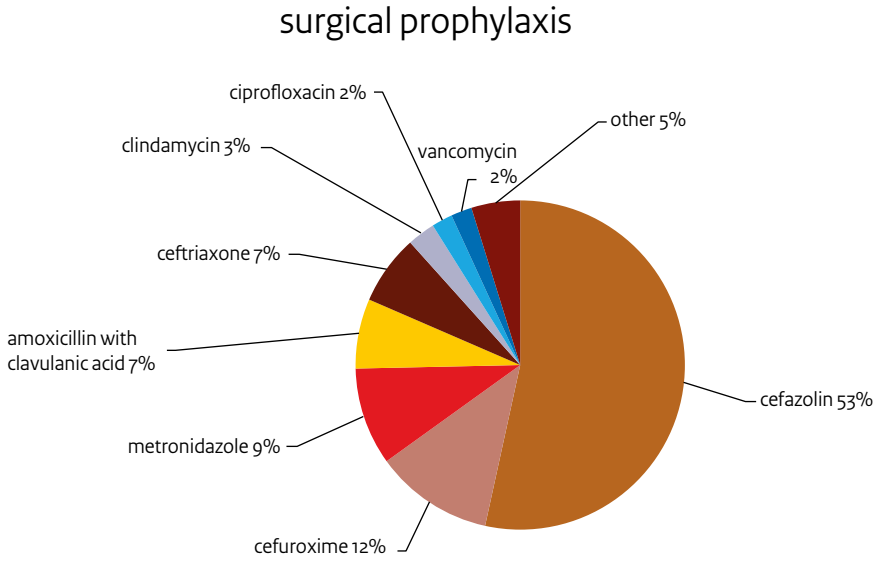
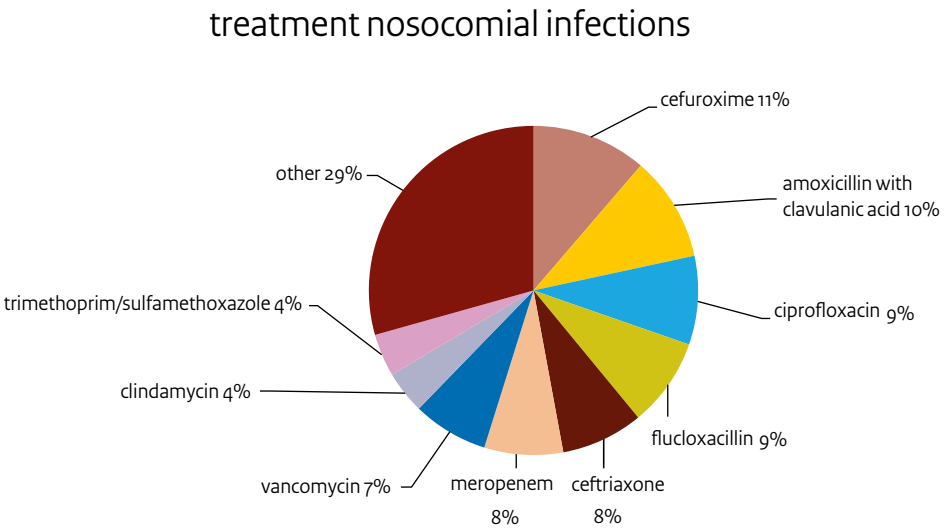
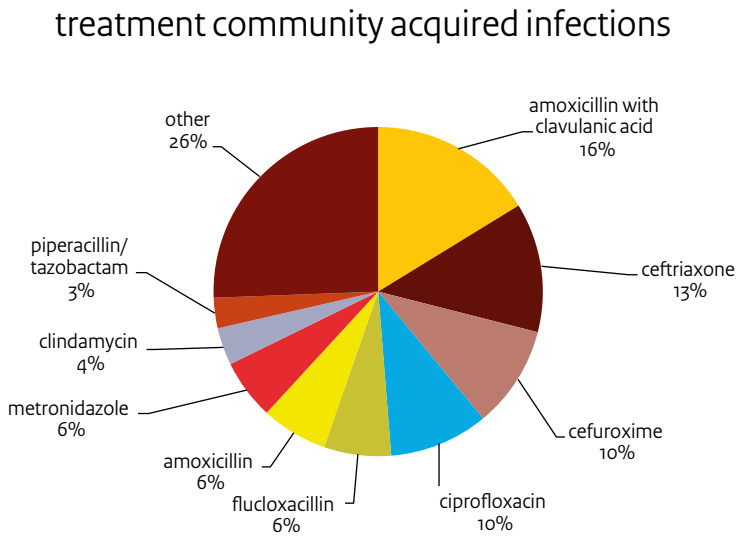


Figure 3.2.1.8 (continued) Distribution of the use of antibiotics for systemic use (J01); results of the point-prevalence studies 2019 (source: PREZIES).





3.2.2 Hospital antibiotic use in days of therapy (DOT)

Methods

Electronic prescriptions for antibiotics on patient level were extracted from Dutch hospital electronic prescribing systems over 2018. From these data the number of days of therapy (DOT) was calculated and expressed as DOT/100 patient-days, taking date of discharge into consideration. The method for calculation of the number of patient-days is described in Chapter 3.2.1. To compare these results to antibiotic use expressed in DDD a ratio dividing the number of DDD/100 patient-days by the numbers of DOT/100 patient-days per ATC4-code was calculated. A DDD/DOT-ratio <1 reflects the use of lower antibiotic dosages compared to the assigned DDD by the WHO, which could be due to prophylactic antibiotic use.

Results

Data over 2018 was evaluated for 31 hospitals (3 university hospitals, 11 large teaching hospitals and 17 general hospitals) compared to 11 hospitals in 2017. The number of DOT/100 patient-days for antibiotics restricted to in-hospital use is shown in Table 3.2.2.1. Total inpatient use of antibiotics, when calculated as DOT/100 patient-days, increased from 61.9 to 64.4 DOT/100 patient-days (+3.6%). The use of combinations of penicillins including beta-lactamase inhibitors, cephalosporins and fluoroquinolones, when calculated as DOT/100 patient-days was high compared to use of other systemic antibiotics. The lowest DOT/100 patient-days were seen in the use of polymyxins, trimethoprim and derivatives, tetracyclines, carbapenems and glycopeptides.

The DDD/DOT-ratio was highest (>1.5) for the use of penicillins with extended spectrum, beta-lactamase resistant penicillins, tetracyclines and aminoglycosides. The DDD/DOT-ratios for lincosamides, carbapenems and polymyxins were also above 1. The highest increases in DDD/DOT-ratios was seen for aminoglycosides (+1.47) and polymyxins (+0.89). In contrast, the DDD/DOT-ratio for imidazole derivates was the lowest (DDD/DOT-ratio 0.77).

Discussion

Antibiotic use expressed as DOT/100 patient-days informs on patient level exposure to antibiotics. Differences observed between antibiotic use expressed as DDD/100 patient-days and DOT/100 patient-days can be explained by differences between DDD and the actual prescribed daily antibiotic dose that is used in clinical practice. For penicillins, aminoglycosides and carbapenems higher doses, that exceed the DDD, are administered to individual patients. In the future, the course of the ratio between the DDD and DOT per 100 patient-days could provide more information on, for instance, potential dose inflation or extension of indications.

Table 3.2.2.1 Antibiotic use in hospitals expressed as days of therapy (DOT) /100 patient-days, DDD/100 patient-days and ratio DDD/DOT at ATC-4 level in 2018.

ATC Group*	Therapeutic group	DDD/100 patient-days	DOT/100 patient-days	Ratio DDD/DOT
J01AA	Tetracyclines	2.05	1.13	1.82
J01CA	Penicillins with extended spectrum	11.08	3.58	3.09
J01CE	Beta-lactamase sensitive penicillins	2.26	1.72	1.32
J01CF	Beta-lactamase resistant penicillins	10.76	3.16	3.40
J01CR	Combinations of penicillins, incl. beta-lactamase-inhibitors	14.48	10.97	1.32
J01DB	First-generation cephalosporins	6.43	7.53	0.85
J01DC	Second-generation cephalosporins	7.99	6.65	1.20
J01DD	Third-generation cephalosporins	6.88	5.78	1.19
J01DH	Carbapenems	1.93	1.32	1.46
J01EA	Trimethoprim and derivatives	0.23	0.28	0.84
J01EE	Combinations of sulfonamides and trimethoprim, including derivatives	2.15	2.53	0.85
J01FA	Macrolides	2.66	2.12	1.26
J01FF	Lincosamides	2.54	1.84	1.38
J01GB	Aminoglycosides	3.76	1.85	2.03
J01MA	Fluoroquinolones	8.45	6.58	1.28
J01XA	Glycopeptides	1.73	1.35	1.28
J01XB	Polymyxins	0.14	0.10	1.41
J01XD	Imidazole derivatives	3.20	4.17	0.77
J01XE	Nitrofuran derivatives	1.63	1.46	1.11
J01XX	Other antibacterials	0.24	0.24	1.00

* *From the 2018 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; the hospital pharmacies provided systemic antibiotic consumption data from long-term care facilities that their pharmacy is serving for 2018, collected over 365 days. The second source is the point prevalence study executed by the SNIV network of the RIVM in 2019, 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 is serving for 2018. 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 2019 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

Data obtained from hospital pharmacies serving LTCF: The antibiotic use of 12276 residents of long-term facilities was included in the data analysis for 2018.

The size of long-term facilities varied from 51-2000 residents per home, with a mean of 534 residents. Compared to 2017, the mean antibiotic use in long-term care facilities increased by 8.5 DDD/1,000 residents/day to 61.4 DDD/1,000 residents/day. The use varied highly between LTCF with a minimum of 25.5 and a maximum of 142.5 DDD/1,000 residents/day. Especially the use of tetracyclines, beta-lactamase resistant penicillins, combinations of penicillins (including beta-lactamase inhibitors) and fluoroquinolones increased (Table 3.3.1).

Figure 3.3.1 depicts antimicrobial medication used in the point prevalence study performed in 25 long-term care facilities of the SNIV network of RIVM in 2019. Of the 2530 residents that participated, 236 received antimicrobial medication, with a total of 260 prescriptions. Ketoconazole is the most frequently used antimicrobial drug; 35% of total prophylactic use and 33% of treatment use; followed by nitrofurantoin and amoxicillin-clavulanic acid. This is comparable to the results of the point prevalence study in 2018. The use of miconazole decreased in 2019, which was compensated by ketoconazole.

Discussion

Although the antibiotic use in long-term care facilities increased in 2018 when compared with 2017, it is within the range that was observed in the past years. The pattern of use is similar to 2017, with amoxicillin with clavulanic acid, fluoroquinolones and nitrofuran derivatives as the most widely used 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.

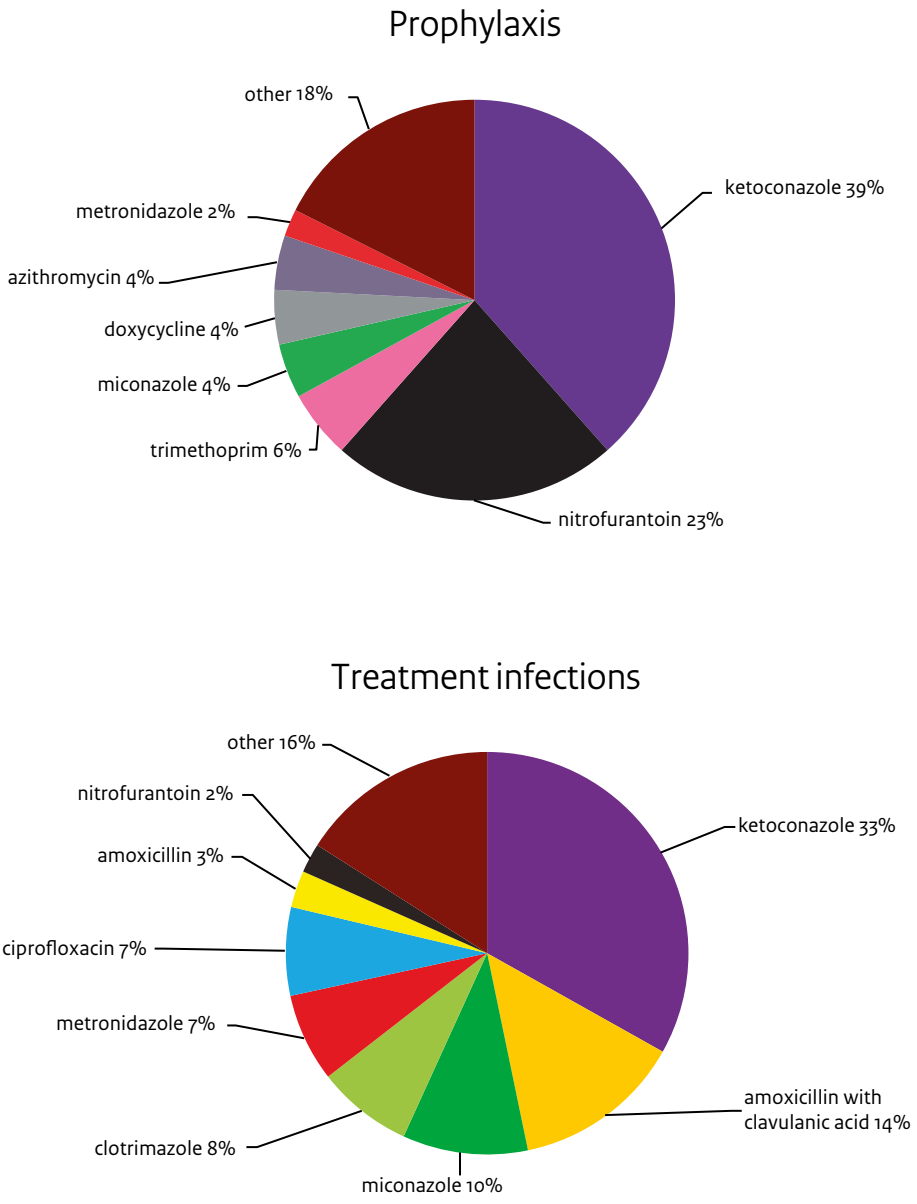
The point prevalence study also included topical antimycotics, therefore ketoconazole is reported to be high in Figure 3.3.1, contrary to the data of the SWAB surveillance. The increased use of ketoconazole in 2019 at the expense of miconazole might be explained by a difference in drug reimbursement and/or the increased knowledge on drug interactions with miconazole, compared with ketoconazole.

Table 3.3.1 Distribution of the use of antibiotics for systemic use (J01) in long-term care facilities, expressed as DDD/1,000 residents/day, 2011-2018 (source: SWAB).

ATC group*	Therapeutic group	2011	2012	2013	2014	2015	2016	2017	2018
J01AA	Tetracyclines	5.4	6.0	6.2	4.7	3.9	4.9	4.0	5.0
J01CA	Penicillins with extended spectrum	4.5	6.6	4.3	5.1	5.0	5.6	4.6	3.8
J01CE	Beta-lactamase sensitive penicillins	0.3	0.2	0.5	0.5	0.7	0.3	0.6	0.4
J01CF	Beta-lactamase resistant penicillins	2.5	3.7	1.7	1.4	2.3	1.8	2.2	3.3
J01CR	Combinations of penicillins, incl. beta-lactamase-inhibitors	18.8	18.8	19.5	16.3	17.9	16.1	15.5	18.0
J01DB	First-generation cephalosporins	0.0	0.0	0.0	0.1	0.1	0.0	0.2	0.1
J01DC	Second-generation cephalosporins	0.2	0.1	0.2	0.1	0.2	0.1	0.3	0.1
J01DD	Third-generation cephalosporins	0.5	1.0	0.6	0.6	0.8	0.4	0.5	0.4
J01DH	Carbapenems	0.1	0.0	0.0	0.0	0.1	0.0	0.1	0.1
J01EA	Trimethoprim and derivatives	2.2	2.3	2.4	1.9	1.4	1.6	1.6	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
J01FA	Macrolides	1.8	2.1	1.8	1.8	2.1	2.4	2.8	2.7
J01FF	Lincosamides	3.1	4.0	2.4	2.0	2.6	3.7	2.9	3.0
J01GB	Aminoglycosides	0.1	0.1	0.0	0.2	0.2	0.1	0.3	0.1
J01MA	Fluoroquinolones	10.3	10.7	8.3	8.4	8.9	8.2	6.9	8.7
J01XA	Glycopeptides	0.1	0.1	0.1	0.1	0.2	0.1	0.2	0.2
J01XB	Polymyxins	0.3	0.2	0.0	0.0	0.1	0.2	0.0	0.1
J01XD	Imidazole derivatives	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.0
J01XE	Nitrofuran derivatives	9.5	11.0	11.1	10.4	11.4	9.6	8.3	11.3
J01XX	other antibacterials	0.5	0.6	0.4	0.2	0.5	0.8	0.8	0.7
	others	0.4	0.1	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**

* *From the 2018 edition of the Anatomical Therapeutic Chemical (ATC) classification system*

Figure 3.3.1 Distribution of the use of antibiotics for systemic use (J01); results of the point-prevalence studies 2019 (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 16th March 2020]
2. European Medicines Agency. Disabling and potentially permanent side effects lead to suspension or restrictions of quinolone and fluoroquinolone antibiotics. Amsterdam, the Netherlands, 2019. Available from: https://www.ema.europa.eu/en/documents/referral/quinolone-fluoroquinolone-article-31-referral-disabling-potentially-permanent-side-effects-lead_en.pdf. [Accessed 16th March 2020]
3. WHO Collaborating Centre for Drug Statistics Methodology. ATC index with DDDs 2018. WHO Collaborating Centre; Oslo, Norway, 2017.
4. Kwint HM, Van der Linden PD, Roukens MMB et al. Intensification of antibiotic use within acute care hospitals in the Netherlands, J of antimicrob chemother 2012: 2283-2288.
5. The Dutch National Institute for Public Health and the Environment (RIVM). Over PREventie van ZIEkenhuisinfecties door Surveillance (PREZIES). Available from: https://www.rivm.nl/prezies/prevalentieonderzoek-ziekenhuizen/protocol-prevalentieonderzoek-ziekenhuizen. [Accessed 17th March 2020]
6. SWAB Stichting Werkgroep Antibioticabeleid. SWAB Richtlijn peri-operatieve profylaxe. Available from: https://swab.nl/swab/cms3.nsf/uploads/E21B508188787 4F4C1258442003B5315/$FILE/SWAB%20richtlijn%20perioperatieve%20profylaxe%20algemeen%20juli%202019%20def%20%2B%20specifieke%20adviezen.pdf. [Accessed 5th December 2019].
7. SWAB Stichting Werkgroep Antibioticabeleid. Management of Community-Acquired Pneumonia in Adults: 2016 Guideline Update From The Dutch Working Party on Antibiotic Policy (SWAB) and Dutch Association of Chest Physicians (NVALT). Available from: https://swab.nl/swab/cms3.nsf/uploads/6A6E127F9A2C1168C125816F004A013A/$FILE/CAP_SWAB_2017-DEF_R5.pdf. [Accessed 3rd December 2019].
8. The Dutch National Institute for Public Health and the Environment (RIVM). Protocol en dataspecificaties SNIV Prevalentieonderzoek Verpleeghuizen 2019. Available from: https://www.rivm.nl/sites/default/files/2018-11/Protocol%20en%20dataspecificaties%202018%20versie%201.0.pdf. [Accessed 17th March 2020].

4 ISIS-AR

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 Antibiotic 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 2019, 47 laboratories were connected to ISIS-AR, all performing antimicrobial susceptibility testing (AST) according to EUCAST guidelines. Out of these 47 laboratories, 30 provided complete data on the last five years (2015 to 2019). Four of these 30 laboratories exclusively served university hospitals; 24 laboratories served non-university hospitals, general practitioners, and long-term care facilities; and two laboratories exclusively served general practitioners and long-term care facilities. For the analyses in chapters 4.2 and 4.3 in which time trends were calculated, we selected only data from these 30 laboratories to avoid bias in time trends of resistance percentages due to incomplete data.
We calculated resistance percentages and linear time trends over the last five years (2015 to 2019) for selected clinically relevant pathogens in combination with their main antimicrobial treatment options. For some pathogens, resistance levels were only calculated for 2019 because criteria for calculation of time trends were not met (for details see paragraph on time trends below). For these calculations, we used data from 34 laboratories for which at least complete data on 2019 were available (five serving university hospitals, 26 serving non-university hospitals, general practitioners, and long-term care facilities; and three serving general practitioners and long-term care facilities only). For *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus / Staphylococcus argenteus* isolates from general practitioners' patients, we

conducted an extra analysis to calculate resistance to a selection of antibiotics in 2019 by regional cooperative network (for more information on regional cooperative networks see https://www.rivm.nl/antibioticaresistentie/nationale-aanpak-antibioticaresistentie/zorgnetwerken). For this analysis, we used data from a subset of 29 non-university laboratories for which at least complete data on 2019 were available.

Selection of isolates

We calculated resistance levels and, if applicable, time trends by site, i.e. general practices (patients aged ≤12 years and >12 years, separately), outpatient departments, inpatient departments (excl. intensive care units), intensive care units, urology departments (inpatient and outpatient, separately), and long-term care facilities. For a selection of antibiotics, we calculated resistance in isolates from general practitioners' patients by regional cooperative network. For general practices (chapter 4.2) and long-term care facilities (chapter 4.4), we selected urine isolates for analysis of resistance in *Enterobacterales* and *P. aeruginosa*, and wound or pus isolates for analysis of resistance in *Staphylococcus aureus / Staphylococcus argenteus*. For outpatient departments (chapter 4.3.1), inpatient departments (excl. intensive care units, chapter 4.3.2), and intensive care units (chapter 4.3.3), we calculated resistance levels based on 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, chapter 4.3.4). For urology departments (chapter 4.3.5), we selected only urine isolates. Finally, in chapter 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 higher respiratory tract and the lower respiratory tract for the analysis on GP 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 a separate analysis, 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 chapters 4.2 through 4.4, *S. argenteus* comprised 0.0 to 0.1% of the isolates from this complex. *S. schweitzeri*, the third member of the *S. aureus* complex, was not found in the ISIS-AR database.
Furthermore, the category wound or pus isolates consists of isolates from deep and superficial wounds, skin (excluding perineal swabs), pus (including pus from abscesses), 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.
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 in 2019 at least 100 isolates were available for analysis. If data on 100 or more isolates was available in 2019, but not in the years before, resistance percentages were only calculated for 2019 and no trends are shown. Furthermore, to avoid bias due to selective testing of antibiotics, 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 each 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 breakpoint guidelines and expert rules used in the participating laboratories, we first reinterpreted all crude test

values according to EUCAST breakpoints version 9.0. Because species specific breakpoints were not available for *S. argenteus*, breakpoints of *S. aureus* were used for reinterpretation, although these breakpoints were not validated for *S. argenteus*.
In 2016, a new testpanel for Gram-negative bacteria was introduced for the VITEK2 automated system (Biomérieux), which is the automated system used by most laboratories. In this testpanel, resistance to co-amoxiclav is tested according to EUCAST guidelines, using a fixed concentration (2 mg/L) of clavulanic acid, irrespective of the concentration of amoxicillin. Before the introduction of the new panel, resistance was tested according to the guidelines from the Clinical and Laboratory Standards Institute (CLSI), using a fixed 2:1 ratio between amoxicillin and clavulanic acid. The use of a fixed clavulanic acid concentration results in higher MIC values for co-amoxiclav. Reinterpretation does not take into account differences in test methods that result in higher test values, which may result in higher resistance levels for co-amoxiclav in Gram negative bacteria from 2016 onward. The magnitude of this effect may vary, depending on the microorganism.
Furthermore, for co-amoxiclav, the 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 resistance breakpoint of >32 mg/L. Therefore, in chapters 4.2 through 4.4, we only present resistance to co-amoxiclav according to the breakpoint for non-uncomplicated urinary tract infections.
For most included pathogens (*Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterobacter cloacae* complex, *Pseudomonas aeruginosa*, *Acinetobacter* spp., *Enterococcus faecalis*, *Enterococcus faecium*, *Staphylococcus aureus*, coagulase-negative *Staphylococcus* spp. including *Staphylococcus epidermidis*, *Bacteroides fragilis* complex, and *Clostridium perfringens*), at least 80% of the reported test values in each year were reinterpretable according to EUCAST clinical breakpoints version 9.0. Where reinterpretation was not possible, this was due to missing crude data or test values that were not compatible with the EUCAST breakpoints. For *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis*, less than 80% of test values could be reinterpreted. Therefore, for these pathogens we calculated resistance percentages based on S/I/R interpretations as reported by laboratories.

Because data on inducible clindamycin resistance tests were often not available in ISIS-AR, we calculated resistance levels for clindamycin including inducible resistance 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/I/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 susceptibility based on laboratory interpretation of oxacillin screen tests, or, if the result for oxacillin was I or R, on laboratory S/I/R interpretation for (benzyl)penicillin. Since there was no information on the breakpoint that was used by the laboratories for individual isolates (meningitis or non-meningitis breakpoint), the reported percentage I+R should be interpreted as the percentage that is resistant in case of meningitis. For non-meningitis indications, the percentage I+R should be interpreted as the percentage non-wild type.
For some antibiotic agents presented in this report, comparable resistance mechanisms exist, namely benzylpenicillin/penicillin, amoxicillin/ampicillin, cefotaxime/ceftriaxone, meropenem/imipenem (except for *P. aeruginosa* because of different resistance mechanisms for meropenem and imipenem), and doxycy-

cline/tetracycline. 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 (gentamicin + amoxicillin/ampicillin, gentamicin + co-amoxiclav, gentamicin + cefuroxime, gentamicin + cefotaxime/ceftriaxone, gentamicin + ceftazidime, gentamicin + piperacillin-tazobactam, 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 microorganisms.
To calculate the percentage of highly resistant microorganisms (HRMO), we used the definitions of the Working Group on Infection Prevention (WIP, https://www.rivm.nl/wip-richtlijn-brmo-bijzonder-resistente-micro-organismen-zkh). We considered *E. coli*, *K. pneumoniae*, and *P. mirabilis* to be an HRMO if they were 1) extended-spectrum beta-lactamase (ESBL)-producing, estimated by ESBL confirmatory tests, or, if no data on confirmatory tests were available, by resistance to cefotaxime/ceftriaxone and/or ceftazidime, 2) resistant to both fluoroquinolones and aminoglycosides, or 3) carbapenemase producing (CPE), estimated by confirmatory tests of carbapenemase production (both phenotypical and molecular), or, if no data on confirmatory tests were available, by resistance to meropenem or imipenem. 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, carbapenems, 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 imipenem or meropenem, or 2) resistant to both fluoroquinolones and aminoglycosides.
In addition, for *Enterobacterales* isolates from general practices, outpatient departments, urology departments, and long-term care facilities, we calculated multidrug resistance, which we defined as resistance to the oral agents co-amoxiclav, ciprofloxacin, and co-trimoxazole combined.
We compared resistance levels in general practitioners' patients within the regional cooperative networks with the resistance percentage in all regions combined. We considered a difference with a two-sided p-value of <0.05 statistically significant. We considered a difference that was larger than the square root of the national resistance percentage as clinically relevant. In the 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 2019, we calculated for chapters 4.2 and 4.3 time trends over the last five years (2015 to 2019) using logistic regression models. 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 (for criteria, see 'Calculation of resistance levels'-section above). 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-antibiotic combinations because EUCAST breakpoints for these combinations were not changed between 2015 and 2019. 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.

We considered two-sided p-values <0.05 to be statistically significant. When the absolute difference in predicted resistance from the logistic regression model between 2015 and 2019 was larger than the square root of the predicted resistance in 2015, we considered the trend to be clinically relevant. Statistically significant increasing trends that are considered to be clinically relevant are indicated in red, whereas decreasing trends that meet the same criteria are indicated in green. In addition, the resistance levels from 2015 to 2019 were shown in bar charts for each pathogen-agent combination for which the resistance levels were above 0.5% for at least one year and under 30% for at least two years.

4.1.2 Description of the ISIS-AR data

In this subsection, a number of 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 chapters 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 chapter 4.2 through 4.5 (see chapter 4.1.1 for inclusion criteria). In table 4.1.2.1, descriptive 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 chapter 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

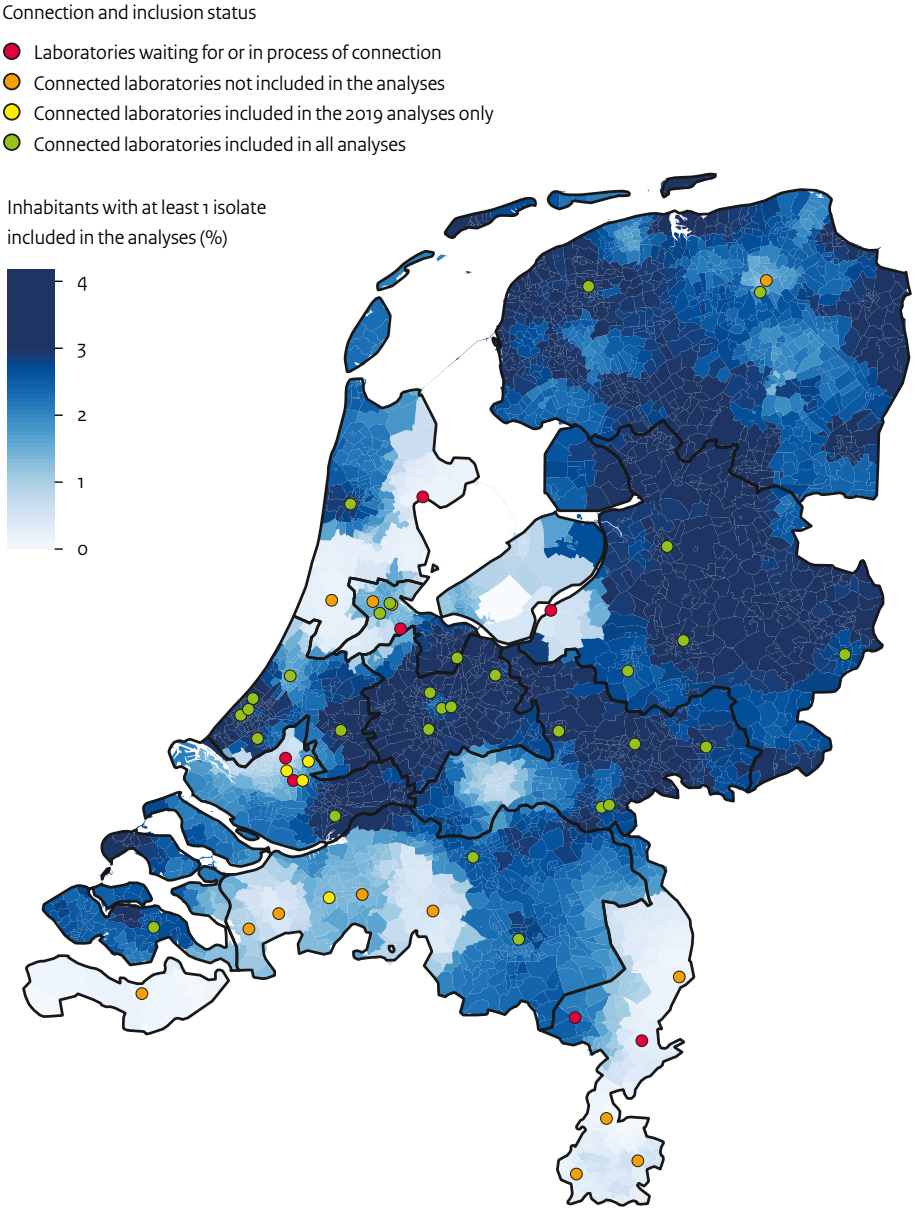


Table 4.1.2.1 Characteristics of 363,095 isolates included in the analyses in chapters 4.2 through 4.5, by pathogen

	E. coli	*K. pneumoniae*	*P. mirabilis*	*E. cloacae* complex	*P. aeruginosa*	*Acinetobacter* spp.	*E. faecalis*	*E. faecium*	*S. aureus*	CNS	*B. fragilis* complex	*C. perfringens*	*S. pneumoniae*	*H. influenzae*	*M. catarrhalis*
Total number of isolates	160,084	26,650	20,027	9,379	22,477	3,782	24,801	5,209	53,710	17,852	1,303	306	4,752	9,938	2,825
Sex of patient (%)															
Male	28	33	42	54	54	52	53	52	53	57	54	55	55	52	51
Female	72	67	58	46	46	48	47	48	47	43	46	45	45	48	49
Type of care (%)															
General practices	60	48	47	31	31	44	42	9	26	10	2	3	6	12	11
Outpatient departments	15	20	19	24	33	26	22	11	40	15	18	18	30	44	41
Inpatient departments (excl. Intensive Care Units)	20	25	23	37	29	24	30	61	29	60	72	71	56	39	41
Intensive Care Units	1	2	2	5	3	3	3	16	3	14	6	8	8	5	5
Long-term care facilities	4	5	9	3	4	2	4	3	2	1	1	1	1	1	2
Age category of patient in years (%)															
0-4	3	1	3	4	3	5	4	0	5	6	1	1	7	10	9
5-18	6	2	2	3	7	4	2	1	7	3	5	2	3	4	3
19-64	34	27	21	31	30	32	28	30	44	39	41	31	35	34	30
>65	57	70	74	63	61	60	66	69	44	52	53	67	55	51	59
Isolate source (%)															
Blood	3	4	2	4	2	3	3	12	5	45	25	25	28	1	1
Lower respiratory tract	1	4	2	10	17	9	0	1	9	0	0	0	56	80	85
Urine	89	84	81	53	40	59	83	51	13	17	2	1	1	0	0
Wound/Pus	4	6	13	29	38	25	12	33	59	31	70	69	10	11	8
Other	2	2	2	4	3	5	1	4	15	7	3	5	5	7	6
Type of hospital (hospital isolates only, %)															
General	39	35	39	30	31	25	33	23	32	28	34	37	37	33	31
Top clinical	48	49	48	48	47	52	53	54	50	45	46	50	50	50	56
University hospital	13	16	13	23	22	23	14	23	18	28	20	13	14	17	13

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

Key results

- 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)' (Figure 4.1.2.1).
- The distribution of included laboratories was reflected in the geographical distribution of isolates (Figure 4.1.2.1). The coverage was relatively high in the regions 'Noord Nederland', 'Euregio-Zwolle', 'Gelders Antibioticaresistentie & Infectiepreventie Netwerk' (GAIN)', 'Utrecht', 'Holland West', and 'Zuidwest NL'. In the other regions, the coverage was lower and less evenly distributed.
- *E. coli* (72%), *K. pneumoniae* (67%), and *P. mirabilis* (58%) were more often isolated from female patients than from male patients, likely because women are more prone to urinary tract infections. For the other pathogens, the percentage of male and female patients was similar.
- *E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, *P. aeruginosa*, *Acinetobacter* spp., *E. faecalis*, *S. aureus*, *H. influenzae*, and *M. catarrhalis* were most often isolated from patients receiving outpatient care (combined 54%-79%, depending on the pathogen), whereas a large part of *E. faecium* (77%), coagulase-negative *Staphylococcus* spp. (74%), *B. fragilis* complex (78%), *C. perfringens* (79%), and *S. pneumoniae* (64%) was isolated from inpatients.
- Most isolates originated from patients of 65 years and older (51-74%, depending on the pathogen). Only for *S. aureus* 44% of the isolates was from patients aged between 19 and 65 and 44% from those aged >65.
- *Enterobacterales*, *P. aeruginosa*, *Acinetobacter* spp., *E. faecalis*, and *E. faecium* were mainly isolated from urine (40-89%, depending on the pathogen), whereas *S. aureus*, *B. fragilis* complex, and *C. perfringens* were mainly isolated from wound or pus (59-70%, depending on the pathogen); coagulase-negative *Staphylococcus* spp. from blood (45%); and *H. influenzae*, *S. pneumoniae*, and *M. catarrhalis* from the lower respiratory tract (56-85%).
- Depending on the organism, 13 to 28% of the isolates originated from university hospital patients.

4.2 Primary care

The distribution of pathogens in diagnostic urine and wound or pus samples from general practitioners' (GP) patients is presented in table 4.2.1. The resistance levels in 2019 for *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* isolates from urine samples are presented in table 4.2.2 and for *S. aureus* isolates from wound or pus samples in table 4.2.3. 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. Five-year trends in resistance are shown in figure 4.2.1 (*E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa*) and figure 4.2.4 (*S. aureus*). 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 4.2.5 (*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* presenting at the GP. Therefore, 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 samples from selected general practitioners' patients, ISIS-AR 2019

Pathogen	Urine		Wound or pus
	Age≤12 N (%)	Age>12 N (%)	N (%)
E. coli	9,092 (72)	90,036 (55)	680 (3)
K. pneumoniae	232 (2)	12,867 (8)	208 (1)
P. mirabilis	621 (5)	8,796 (5)	488 (2)
Other *Enterobacterales*[1]	561 (4)	15,905 (10)	1,713 (9)
P. aeruginosa	211 (2)	3,949 (2)	2,991 (15)
Other non-fermenters[2]	134 (1)	2,446 (2)	627 (3)
Other Gram-negatives[3]	8 (0)	18 (0)	420 (2)
S. aureus	140 (1)	3,121 (2)	9,592 (48)
Other Gram-positives[4]	1,651 (13)	25,422 (16)	3,235 (16)

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

[2] *Acinetobacter spp., Pseudomonas spp. (non-aeruginosa), S. maltophilia, M. catarrhalis.*

[3] *H. parainfluenzae, H. influenzae, B. fragilis complex, H. pylori, N. meningitidis.*

[4] *Enterococ cus spp., S. pyogenes, S. anginosus, beta-haemolytic Streptococcus spp. gr G, S. oralis, S. agalactiae, S. pneumoniae, S. mitis, beta-haemolytic Streptococcus spp. gr C, S. dysgalactiae n.n.g., S. dysgalactiae subsp. equisimilis, Staphylococcus spp. (non-aureus complex), A. urinae, 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 2019

	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	67	5	74	3	76	4	79
Antibiotic								
amoxicillin/ampicillin	34	38	-	-	16	21	-	-
co-amoxiclav[1] - non-uuti	28	30	27	17	5	6	-	-
cefuroxime	4	8	8	14	1	1	-	-
cefotaxime/ceftriaxone	2	4	3	4	1	1	-	-
ceftazidime	2	3	4	4	0	0	0	1
ciprofloxacin	5	10	6	13	6	10	2	11
gentamicin	3	4	0	2	4	5	1	2
tobramycin	3	4	1	2	2	3	0	0
fosfomycin	1	1	8	28	7	16	-	-
trimethoprim	21	23	11	20	22	30	-	-
co-trimoxazole	18	20	9	9	19	24	-	-
nitrofurantoin	0	2	-	-	-	-	-	-
Multidrug resistance								
HRMO[2]	3	5	5	5	2	3	-	-
multidrug resistance[3] - non-uuti	1	3	4	3	0	1	-	-

10	Significant and clinically relevant increasing trend since 2015
10	Significant and clinically relevant decreasing trend since 2015
10	No significant and clinically relevant time trend

(For the definition of a clinically relevant trend see chapter 4.1.1)

- = Resistance not calculated.

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

[1] *During 2016, a new testpanel for Gram-negative bacteria, with co-amoxiclav concentrations being adapted to EUCAST testing guidelines, was introduced for the VITEK2 automated system. This results in higher MIC values for co-amoxiclav, which subsequently influence resistance from 2016 onward to higher levels than before (see chapter 4.1.1 for more detailed information).*

[2] *Highly resistant microorganism (HRMO), defined according to HRMO guideline of the WIP (https://www.rivm.nl/documenten/wip-richtlijn-brmo); for E. coli, K. pneumoniae, and P. mirabilis as one or more of the following: 1) extended-spectrum beta-lactamase (ESBL)-producing, estimated by ESBL confirmatory tests, or, if no data on confirmatory tests were available, by resistance to cefotaxime/ceftriaxone and/or ceftazidime, 2) resistant to both fluoroquinolones and aminoglycosides, or 3) carbapenemase producing (CPE), estimated by confirmatory tests of carbapenemase production (both phenotypical and molecular), or, if no data on confirmatory tests were available, by resistance to meropenem or imipenem.*

[3] *Defined as resistance to all of the following oral agents: co-amoxiclav, ciprofloxacin, and co-trimoxazole.*

Figure 4.2.1 Trends in antibiotic resistance (from left to right 2015 to 2019) 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

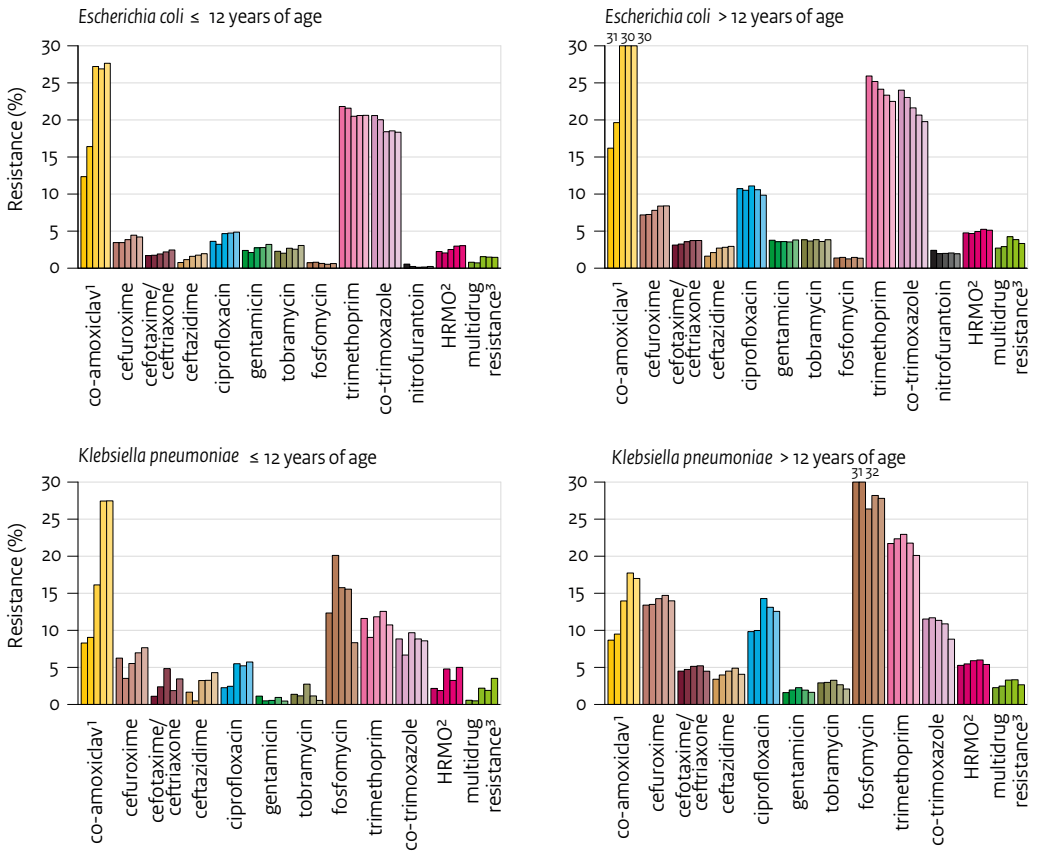
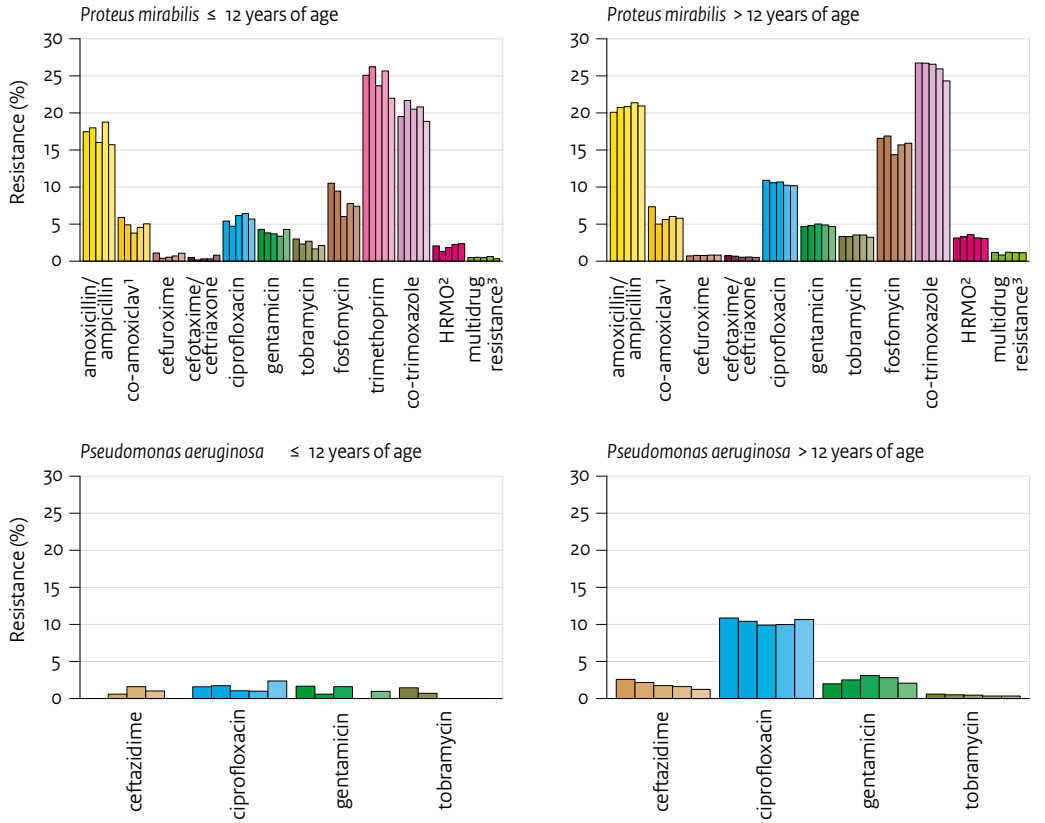


Figure 4.2.1 (continued) Trends in antibiotic resistance (from left to right 2015 to 2019) among diagnostic urine isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* from selected general practitioners' patients in ISIS-AR, by age category

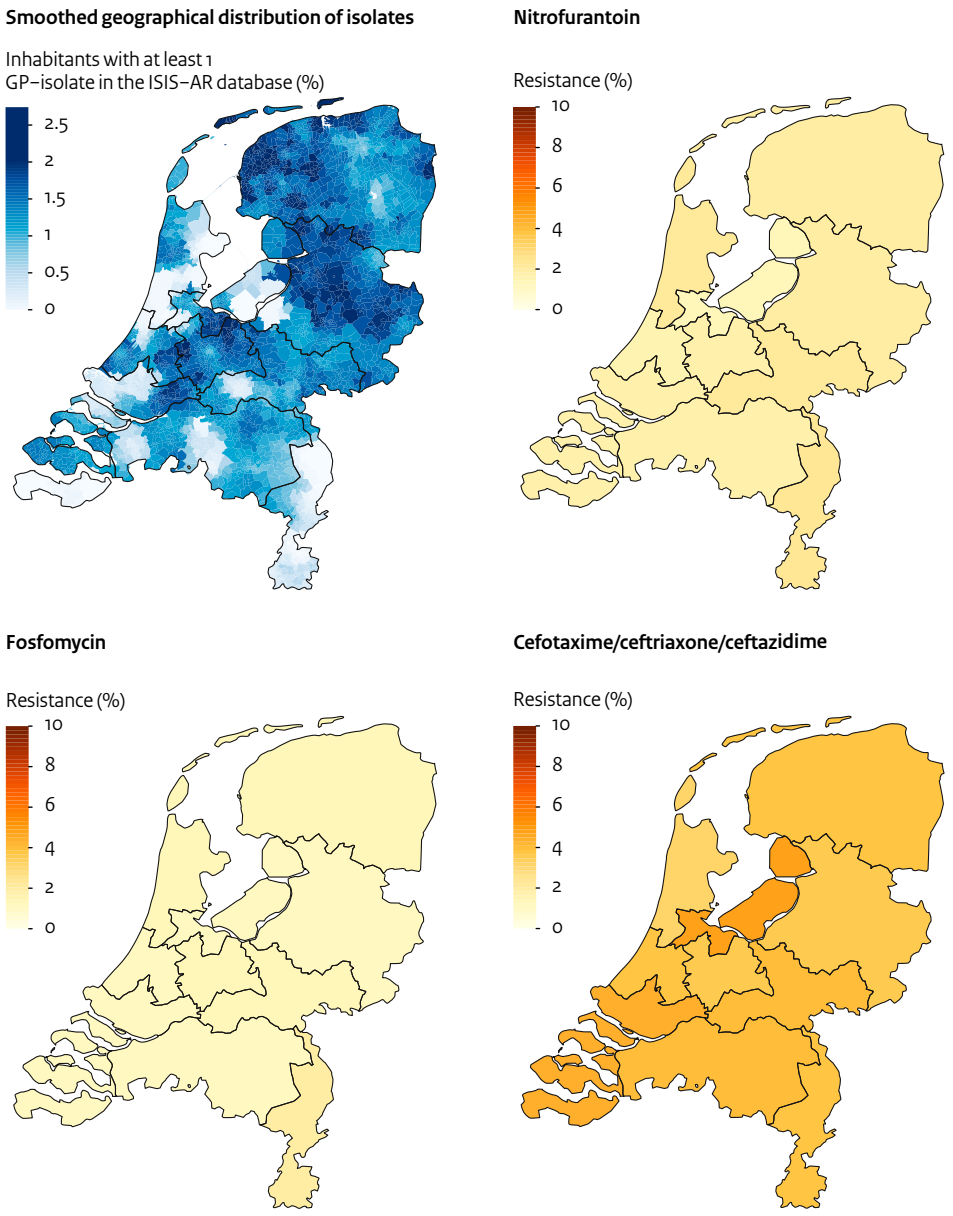


[1] *Resistance to co-amoxiclav was calculated according to the breakpoint for non-uncomplicated urinary tract infection. During 2016, a new testpanel for Gram-negative bacteria, with co-amoxiclav concentrations being adapted to EUCAST testing guidelines, was introduced for the VITEK2 automated system. This results in higher MIC values for co-amoxiclav, which subsequently influence resistance from 2016 onward to higher levels than before (see chapter 4.1.1 for more detailed information).*

[2] *Highly resistant microorganism (HRMO), defined according to HRMO guideline of the WIP (https://www.rivm.nl/documenten/wip-richtlijn-brmo); for E. coli, K. pneumoniae, and P. mirabilis as one or more of the following: 1) extended-spectrum beta-lactamase (ESBL)-producing, estimated by ESBL confirmatory tests, or, if no data on confirmatory tests were available, by resistance to cefotaxime/ceftriaxone and/or ceftazidime, 2) resistant to both fluoroquinolones and aminoglycosides, or 3) carbapenemase producing (CPE), estimated by confirmatory tests of carbapenemase production (both phenotypical and molecular), or, if no data on confirmatory tests were available, by resistance to meropenem or imipenem.*

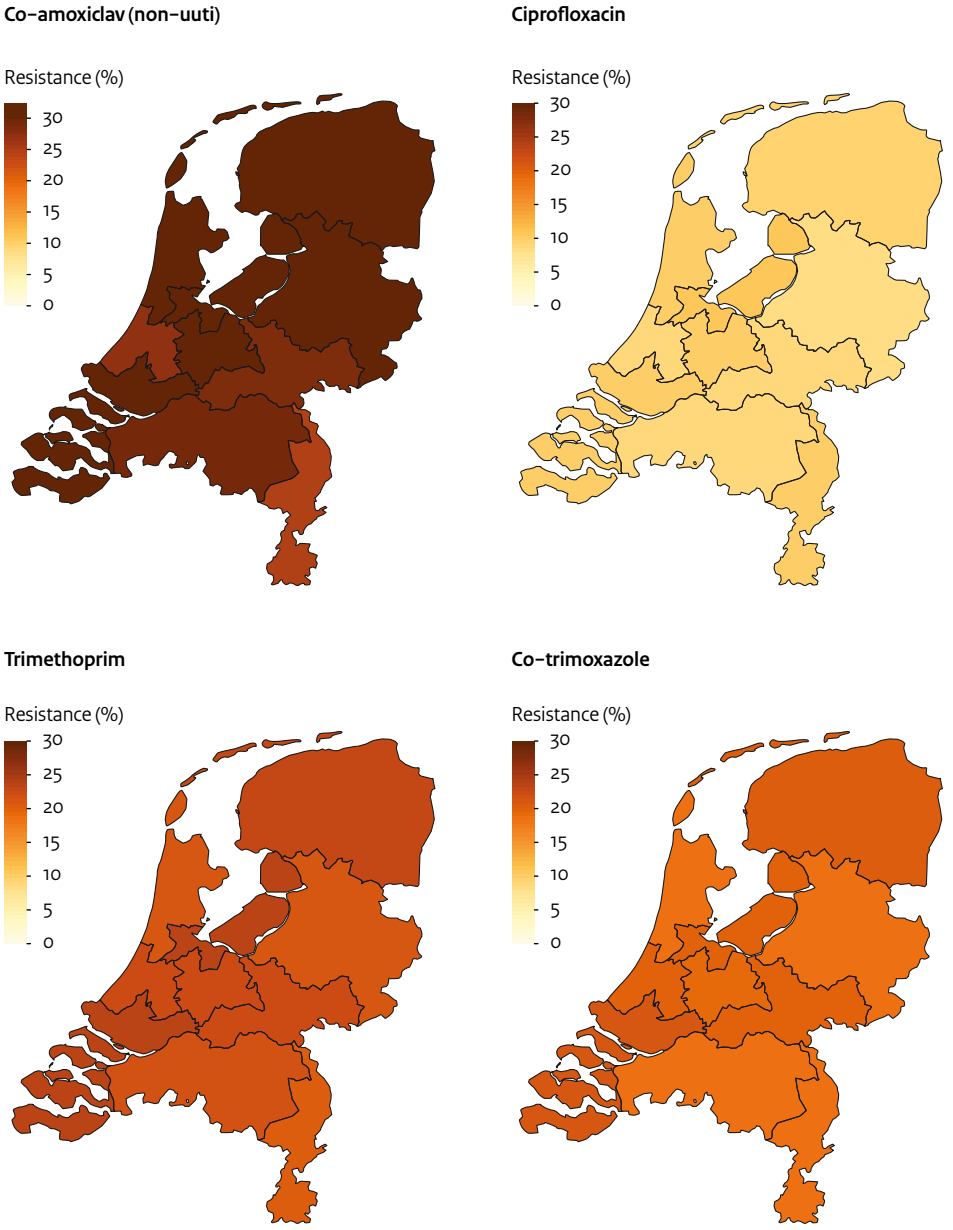
[3] *Defined as resistance to all of the following oral agents: co-amoxiclav (according to breakpoint for non-uncomplicated urinary tract infection), ciprofloxacin, and co-trimoxazole.*

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, and cefotaxime/ceftriaxone/ceftazidime by regional cooperative network, ISIS-AR 2019



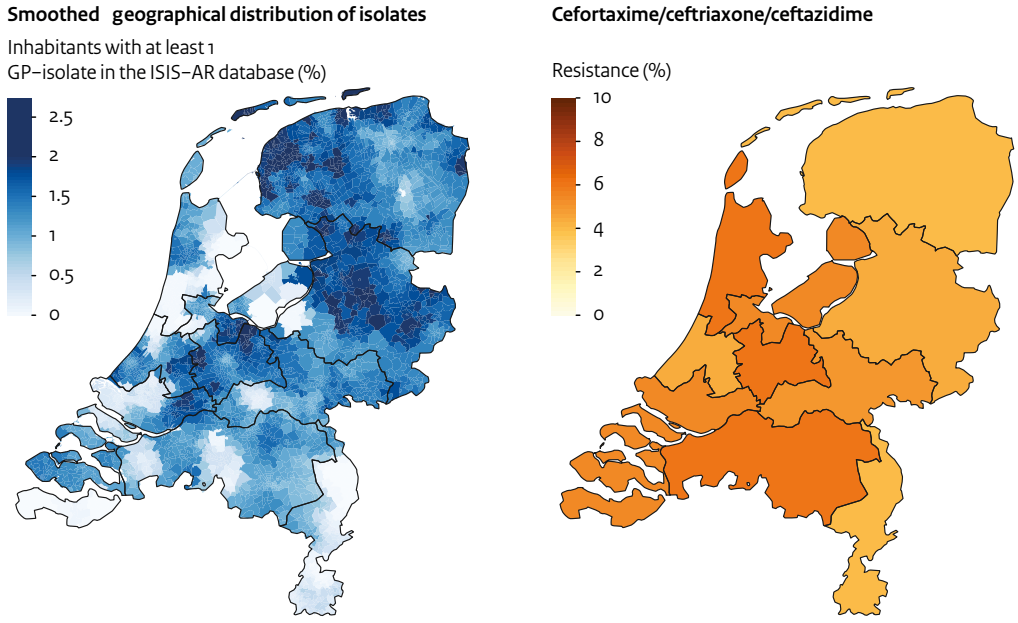
Note: No statistically significant and clinically relevant differences of regional resistance levels were found for the selected antibiotics in comparison to all regions combined (for details see chapter 4.1.1).

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 2019



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 chapter 4.1.1).
non-uuti=according to breakpoint for non-uncomplicated urinary tract infection.

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 2019



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 chapter 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, fosfomycin, trimethoprim, and co-trimoxazole from selected general practitioners' patients, by regional cooperative network, ISIS-AR 2019

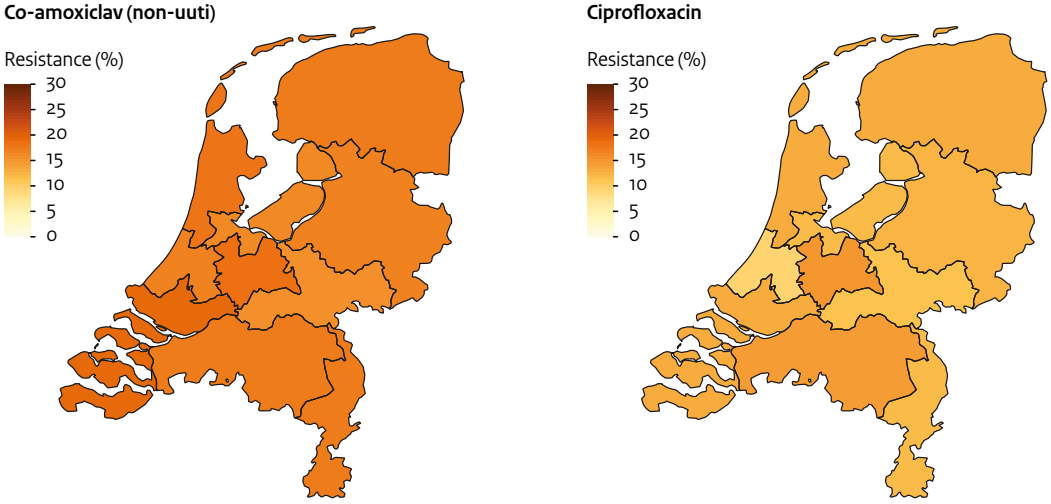
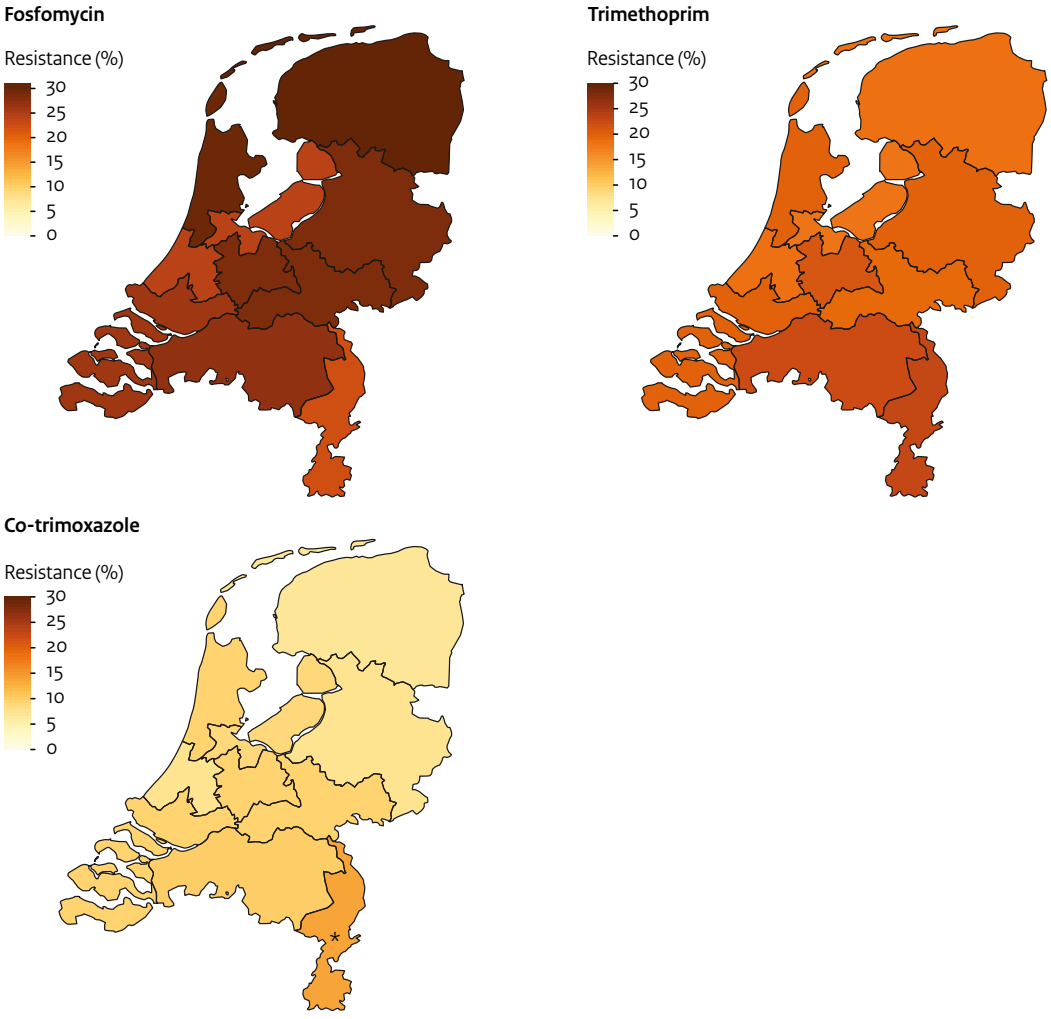


Figure 4.2.3b (continued) Resistance levels in diagnostic urinary *K. pneumoniae* isolates on a gradient scale between 0 and 30% for co-amoxiclav, ciprofloxacin, fosfomycin, trimethoprim, and co-trimoxazole from selected general practitioners' patients, by regional cooperative network, ISIS-AR 2019



* *Statistically significant and clinically relevant difference between resistance in the regional cooperative network and for all regions combined (for details see chapter 4.1.1). non-uuti=according to breakpoint for non-uncomplicated urinary tract infection.*

Table 4.2.3 Resistance levels (%) among diagnostic wound or pus isolates of *S. aureus* from selected general practitioners' patients, ISIS-AR 2019

	S. aureus
Antibiotic	
flucloxacillin[1]	3
ciprofloxacin[2]	3
erythromycin	12
clindamycin including inducible resistance[3]	11
doxycycline/tetracycline	4
fusidic acid	20
co-trimoxazole	3

10	Significant and clinically relevant increasing trend since 2015
10	Significant and clinically relevant decreasing trend since 2015
10	No significant and clinically relevant time trend

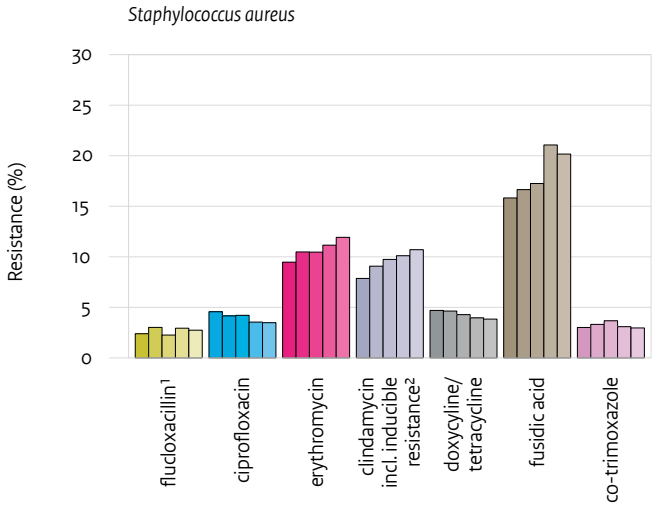
(For the definition of a clinically relevant trend see chapter 4.1.1)

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

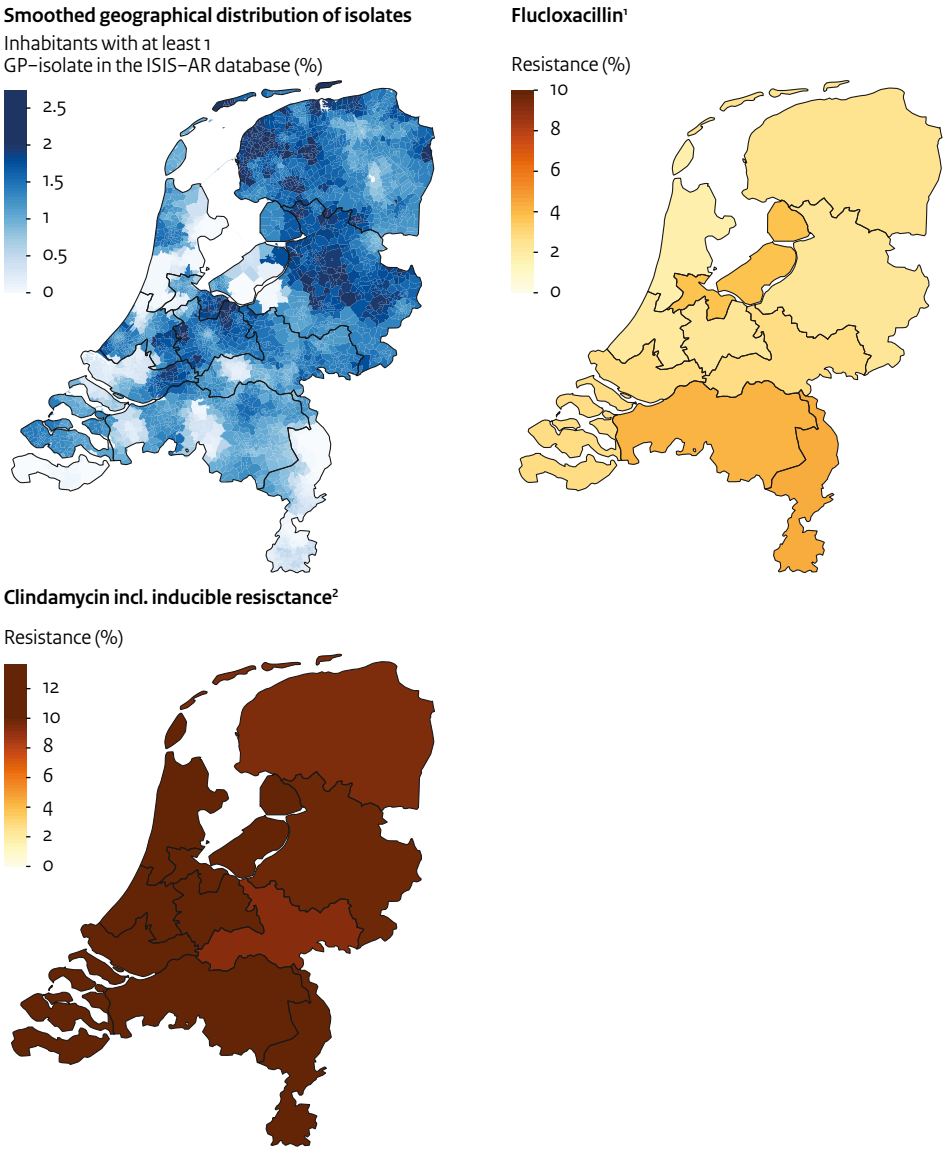
Figure 4.2.4 Trends in antibiotic resistance (from left to right 2015 to 2019) among diagnostic wound or pus isolates of *S. aureus* from selected general practitioners' patients in ISIS-AR.



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

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

Figure 4.2.5 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 and clindamycin including inducible resistance by regional cooperative network, ISIS-AR 2019



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 chapter 4.1.1).

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

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

Key results

The coverage of isolates from GP patients in the regional cooperative networks 'Noord-Holland West', 'Zuid-West NL', '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.
- For all *Enterobacterales*, resistance levels of 10% or lower were observed for cefotaxime/ceftriaxone (≤4%), ceftazidime (≤4%), gentamicin (≤5%), and tobramycin (≤4%). Resistance levels ≤10% were also found for ciprofloxacin (6%) and cefuroxime (8%) in *K. pneumoniae* in patients aged ≤12 years, and in *E. coli* (≤10%) and *P. mirabilis* (≤10%) in both age groups. Additionally, resistance levels ≤10% were found for fosfomycin (1%) and nitrofurantoin (≤2%) in *E. coli*, for co-trimoxazole (9%) and fosfomycin (patients aged ≤ 12 years only, 8%) in *K. pneumoniae*, and for co-amoxiclav (≤6%) and fosfomycin (patients aged ≤12 years only, 7%) in *P. mirabilis*.
- Resistance levels ≥20% were found for amoxicillin/ampicillin (≥34%), co-amoxiclav (≥28%), trimethoprim (≥21%), and co-trimoxazole (patients aged >12 years only, 20%) in *E. coli*; for co-amoxiclav (patients aged ≤12 years only, 27%), fosfomycin (patients aged>12 years only, 28%), and trimethoprim (patients aged>12 years only, 20%) in *K. pneumoniae*; and for amoxicillin/ampicillin (patients >12 years only, 21%),trimethoprim (≥22%), and co-trimoxazole (patients >12 years only, 24%) in *P. mirabilis*.
- There was a statistically significant and clinically relevant increase in resistance to co-amoxiclav in *E. coli* and *K. pneumoniae* in both age groups (In *E. coli* from 12% in 2015 to 28% in 2019 for patients aged ≤12 years and from 16% to 30% for patients aged >12 years; In *K. pneumoniae* from 8% to 27% and from 9% to 17% in the respective age groups), which may be partly due to the introduction of a new testpanel for the VITEK2 automated system in 2016 (for details see chapter 4.1.1). Statistically significant and clinically relevant increases in resistance were also found for ceftazidime in *E. coli* for patients aged ≤12 years (from 1% in 2015 to 2% in 2019), for ceftazidime in *K. pneumoniae* from patients aged ≤12 years (from 2% to 4%), and for ciprofloxacin in *K. pneumoniae* for both age groups (for patients aged ≤12 from 2% in 2015 to 6% in 2019 and for patients aged>12 years from 10% to 13%).
- The percentage of HRMO and multidrug resistance was ≤5% in all *Enterobacterales*, with a significant and clinically relevant increasing trend in multidrug resistance for *K. pneumoniae* isolates from patients aged ≤12 years (from 1% in 2015 to 4% in 2019).
- For *E. coli*, no statistically significant and clinically relevant differences of regional resistance levels were found for the selected antibiotics in comparison to all regions combined.
- For *K. pneumoniae*, a statistically significant and clinically relevant higher resistance percentage was found for co-trimoxazole in the regional cooperative network 'Limburgs infectiepreventie en antibioticaresistentie netwerk (LINK)' (13% in the region versus 9% in all regions combined).

P. aeruginosa

- Resistance levels ≤10% were found for each of the selected agents in both age groups, except for ciprofloxacin in patients aged >12 years (11%).

S. aureus

- Resistance levels of 10% or lower were observed for flucloxacillin (3%), ciprofloxacin (3%), doxycycline/tetracycline (4%), and co-trimoxazole (3%).
- A resistance level of 20% was found for fusidic acid.
- There was a significant and clinically relevant increase in resistance to fusidic acid (from 16% in 2015 to 20% in 2019).
- No statistically significant and clinically relevant differences of regional resistance levels were found for the selected antibiotics in comparison to all regions combined.

4.3 Hospital departments

In this section, resistance levels among isolates from patients in outpatient departments (chapter 4.3.1), inpatient departments (excluding intensive care units, chapter 4.3.2), and intensive care units (chapter 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 chapter 4.3.4 and for urine isolates from patients in urology departments (outpatient and inpatient departments) in chapter 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 is presented in table 4.3.1.1. The resistance levels for a selection of pathogens isolated from these patients in 2019 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*).

Among patients attending outpatient departments, the rate of sampling is higher than among GP patients. 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 2019

	Lower respiratory tract	Urine	Wound or pus
Pathogen	**N (%)**	**N (%)**	**N (%)**
E. coli	529 (5)	20,669 (43)	1,709 (5)
K. pneumoniae	249 (2)	4,289 (9)	394 (1)
P. mirabilis	141 (1)	2,421 (5)	1,032 (3)
Other *Enterobacterales*[1]	1,034 (9)	5,963 (12)	3,043 (10)
P. aeruginosa	1,403 (13)	1,811 (4)	3,382 (11)
Other non-fermenters[2]	1,505 (13)	744 (2)	879 (3)
Other Gram-negatives[3]	3,521 (31)	25 (0)	959 (3)
S. aureus	1,598 (14)	1,678 (3)	13,535 (42)
Other Gram-positives[4]	1,225 (11)	10,995 (23)	7,080 (22)

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

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

[3] *H. parainfluenzae, H. influenzae, B. fragilis complex, H. pylori, N. meningitidis.*

[4] *S. oralis, S. equi, S. agalactiae, S. pyogenes, S. dysgalactiae subsp. equisimilis, beta-haemolytic Streptococcus spp. gr C, S. mitis, S. pneumoniae, S. anginosus, S. dysgalactiae n.n.g., beta-haemolytic Streptococcus spp. gr G, Enterococcus spp., Staphylococcus spp. (non-aureus complex), 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 2019

	E. coli	*K. pneumoniae*	*P. mirabilis*	*P. aeruginosa*
Antibiotic				
amoxicillin/ampicillin	43	-	22	-
co-amoxiclav[1] - non-uuti	35	20	7	-
piperacillin-tazobactam	4	9	0	5
cefuroxime	13	16	1	-
cefotaxime/ceftriaxone	6	8	1	-
ceftazidime	4	7	0	2
meropenem/imipenem	0	0	-	-
meropenem	-	-	0	1
imipenem	-	-	-	4
ciprofloxacin	17	15	13	12
gentamicin	5	3	5	6
tobramycin	5	5	4	1
fosfomycin	2	25	15	-
trimethoprim	27	23	31	-
co-trimoxazole	24	13	26	-
nitrofurantoin	3	-	-	-
Empiric therapy combinations				
gentamicin + amoxicillin/ampicillin	5	-	4	-
gentamicin + co-amoxiclav - non-uuti	4	3	2	-
gentamicin + cefuroxime	2	3	0	-
gentamicin + cefotaxime/ceftriaxone	1	3	0	-
gentamicin + ceftazidime	1	2	0	1
Multidrug resistance				
HRMO[2]	8	10	4	3
multidrug resistance[3]- non-uuti	6	5	2	-

10	Significant and clinically relevant increasing trend since 2015
10	Significant and clinically relevant decreasing trend since 2015
10	No significant and clinically relevant time trend

(For the definition of a clinically relevant trend see chapter 4.1.1)

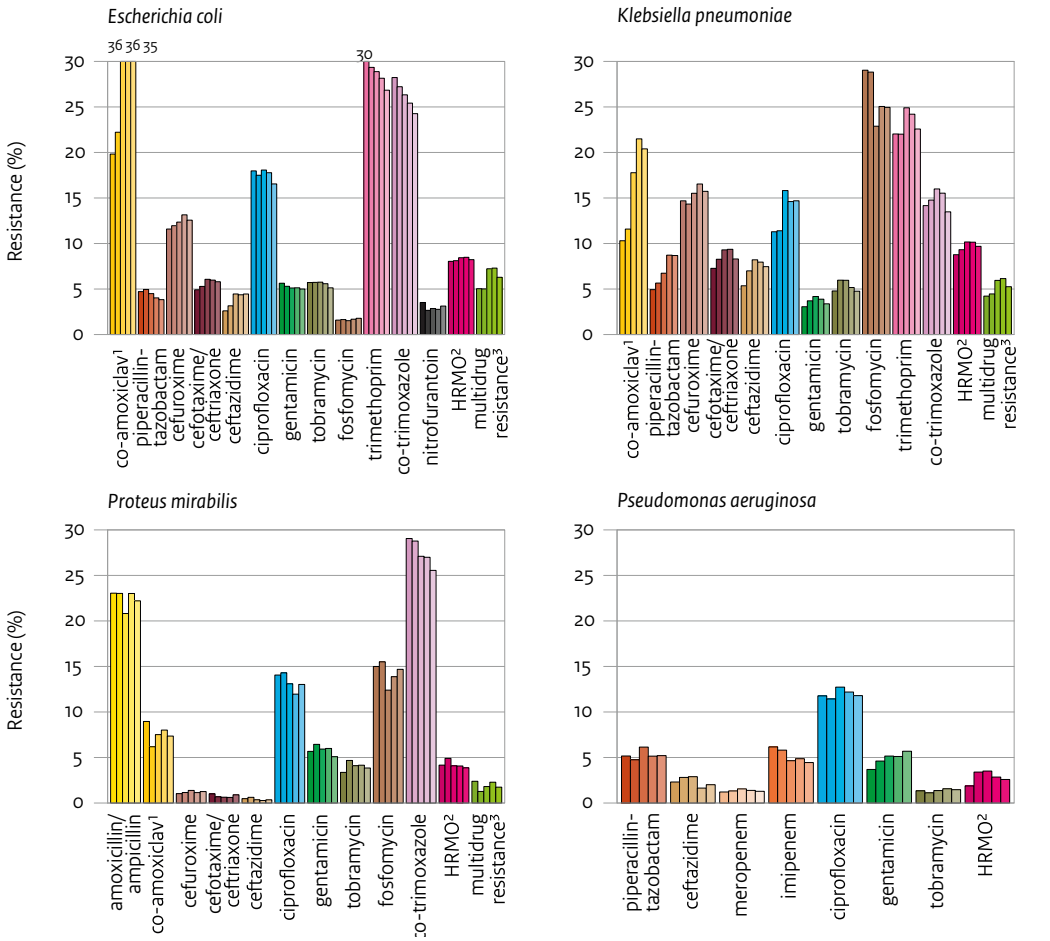
- = Resistance not calculated.
non-uuti = according to breakpoint for non-uncomplicated urinary tract infection.

1 *During 2016, a new testpanel for Gram-negative bacteria, with co-amoxiclav concentrations being adapted to EUCAST testing guidelines, was introduced for the VITEK2 automated system. This results in higher MIC values for co-amoxiclav, which subsequently influence resistance from 2016 onward to higher levels than before (see chapter 4.1.1 for more detailed information).*

2 *Highly resistant microorganism (HRMO), defined according to HRMO guideline of the WIP (https://www.rivm.nl/documenten/wip-richtlijn-brmo); for E. coli, K. pneumoniae, and P. mirabilis as one or more of the following: 1) extended-spectrum beta-lactamase (ESBL)-producing, estimated by ESBL confirmatory tests, or, if no data on confirmatory tests were available, by resistance to cefotaxime/ceftriaxone and/or ceftazidime, 2) resistant to both fluoroquinolones and aminoglycosides, or 3) carbapenemase producing (CPE), estimated by confirmatory tests of carbapenemase production (both phenotypical and molecular), or, if no data on confirmatory tests were available, by resistance to meropenem or imipenem; for P. aeruginosa as resistant to ≥3 antimicrobial groups among fluoroquinolones, aminoglycosides, carbapenems, ceftazidime, and piperacillin-tazobactam.*

3 *Defined as resistance to all of the following oral agents: co-amoxiclav, ciprofloxacin, and co-trimoxazole.*

Figure 4.3.1.1 Trends in antibiotic resistance (from left to right 2015 to 2019) among diagnostic isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* from patients attending outpatient departments in ISIS-AR



[1] *Resistance to co-amoxiclav was calculated according to the breakpoint for non-uncomplicated urinary tract infection. During 2016, a new testpanel for Gram-negative bacteria, with co-amoxiclav concentrations being adapted to EUCAST testing guidelines, was introduced for the VITEK2 automated system. This results in higher MIC values for co-amoxiclav, which subsequently influence resistance from 2016 onward to higher levels than before (see chapter 4.1.1 for more detailed information).*

[2] *Highly resistant microorganism (HRMO), defined according to HRMO guideline of the WIP (https://www.rivm.nl/documenten/wip-richtlijn-brmo); for E. coli, K. pneumoniae, and P. mirabilis as one or more of the following: 1) extended-spectrum beta-lactamase (ESBL)-producing, estimated by ESBL confirmatory tests, or, if no data on confirmatory tests were available, by resistance to cefotaxime/ceftriaxone and/or ceftazidime, 2) resistant to both fluoroquinolones and aminoglycosides, or 3) carbapenemase producing (CPE), estimated by confirmatory tests of carbapenemase production (both phenotypical and molecular), or, if no data on confirmatory tests were available, by resistance to meropenem or imipenem; for P. aeruginosa as resistant to ≥3 antimicrobial groups among fluoroquinolones, aminoglycosides, carbapenems, ceftazidime, and piperacillin-tazobactam.*

[3] *Defined as resistance to all of the following oral agents: co-amoxiclav (according to breakpoint for non-uncomplicated urinary tract infection), ciprofloxacin, and co-trimoxazole.*

Table 4.3.1.3 Resistance levels (%) among diagnostic isolates of *S. aureus* from patients attending outpatient departments, ISIS-AR 2019

	S. aureus
Antibiotic	
flucloxacillin[1]	2
ciprofloxacin[2]	6
gentamicin	1
erythromycin	15
clindamycin including inducible resistance[3]	14
doxycycline/tetracycline	4
fusidic acid	9
linezolid	0
co-trimoxazole	2
rifampicin	0

10	Significant and clinically relevant increasing trend since 2015
10	Significant and clinically relevant decreasing trend since 2015
10	No significant and clinically relevant time trend

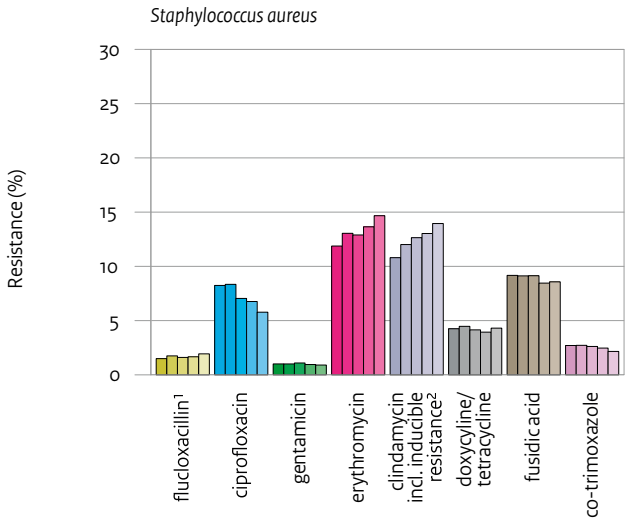
(For the definition of a clinically relevant trend see chapter 4.1.1)

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

Figure 4.3.1.2. Trends in antibiotic resistance (from left to right 2015 to 2019) among diagnostic isolates of *S. aureus* from patients attending outpatient departments in ISIS-AR



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

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

Key results

Enterobacterales

- For all *Enterobacterales*, resistance levels of 10% or lower were found for piperacillin-tazobactam (≤9%), cefotaxime/ceftriaxone (≤8%), ceftazidime (≤7%), gentamicin (≤5%), and tobramycin (≤5%). Resistance levels ≤10% were also found for fosfomycin (2%) and nitrofurantoin (3%) in *E. coli*; meropenem/imipenem in *E. coli* and *K. pneumoniae* (0%); and co-amoxiclav (7%), cefuroxime (1%), and meropenem (0%) in *P. mirabilis*.
- Resistance of 20% or higher was found for trimethoprim in all *Enterobacterales* (≥23%), for co-amoxiclav in *E. coli* and *K. pneumoniae* (≥20%), for amoxicillin/ampicillin (≥22%) and co-trimoxazole (≥24%) in *E. coli* and *P. mirabilis*, and for fosfomycin in *K. pneumoniae* (25%).
- A statistically significant and clinically relevant increase in resistance was observed for co-amoxiclav in *E. coli* (from 20% in 2015 to 35% in 2019) and in *K. pneumoniae* (from 10% to 20%), which may be partly due to the introduction of a new testpanel for the VITEK2 automated system in 2016 (for details see chapter 4.1.1). In *E. coli*, a statistically significant and clinically relevant increase in resistance was observed for ceftazidime in the last five years (from 3% in 2015 to 4% in 2019).

Furthermore, in *K. pneumoniae*, statistically significant and clinically relevant increasing trends were observed for piperacillin-tazobactam (from 5% in 2015 to 9% in 2019) and ciprofloxacin (from 11% to 15%).
- Resistance to empiric therapy combinations was ≤5% for all *Enterobacterales*.
- For all *Enterobacterales*, the percentage HRMO was ≤10% and the percentage of multidrug resistance was ≤6%.

P. aeruginosa
- Resistance levels of 10% or lower were observed for each of the selected agents (≤5%), except for ciprofloxacin (12%).

S. aureus
- Resistance levels of 10% or lower were observed for each of the selected agents (≤9%), except for erythromycin (15%) and clindamycin including inducible resistance (14%).

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) is presented in table 4.3.2.1. The resistance levels for a selection of pathogens isolated from these patients in 2019 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.), and 4.3.2.5 (*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.), and 4.3.2.4 (*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 2019

	Blood or cerebrospinal fluid	Lower respiratory tract	Urine	Wound or pus
Pathogen	**N (%)**	**N (%)**	**N (%)**	**N (%)**
E. coli	4,344 (22)	989 (7)	20,384 (43)	3,604 (12)
K. pneumoniae	839 (4)	531 (4)	3,954 (8)	750 (2)
P. mirabilis	297 (2)	207 (2)	3,047 (6)	827 (3)
E. cloacae complex	302 (2)	439 (3)	1,200 (3)	1,215 (4)
Other *Enterobacterales*[1]	1,006 (5)	1,426 (10)	4,582 (10)	2,554 (8)
P. aeruginosa	353 (2)	1,451 (11)	2,471 (5)	1,722 (6)
Acinetobacter spp.	75 (0)	100 (1)	301 (1)	279 (1)
Other non-fermenters[2]	68 (0)	1,546 (11)	196 (0)	333 (1)
B. fragilis complex	270 (1)	0 (0)	19 (0)	586 (2)
Other Gram-negatives[3]	223 (1)	3,708 (27)	11 (0)	279 (1)
E. faecalis	551 (3)	20 (0)	4,792 (10)	1,732 (6)
E. faecium	348 (2)	11 (0)	1,422 (3)	1,116 (4)
S. aureus	2,043 (11)	1,729 (13)	1,416 (3)	8,084 (27)
CNS	5,763 (30)	19 (0)	614 (1)	3,104 (10)
C. perfringens	70 (0)	0 (0)	2 (0)	133 (0)
Other Gram-positives[4]	2,902 (15)	1,409 (10)	3,220 (7)	3,968 (13)

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

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

[2] *M. catarrhalis, S. maltophilia, Pseudomonas spp. (non-aeruginosa), B. cepacia.*

[3] *H. parainfluenzae, H. influenzae, N. meningitidis, C. jejuni, H. pylori.*

[4] *S. pneumoniae, S. mitis, S. anginosus, beta-haemolytic Streptococcus spp. gr C, S. equi, S. agalactiae, S. oralis, S. pyogenes, S. dysgalactiae n.n.g., beta-haemolytic Streptococcus spp. gr G, S. dysgalactiae subsp. equisimilis, A. urinae, Enterococcus spp. (non-faecalis, non-faecium), Staphylococcus spp. (non-aureus complex, 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 2019

	E. coli	*K. pneumoniae*	*P. mirabilis*	*E. cloacae* complex	*P. aeruginosa*	*Acinetobacter* spp.
Antibiotic						
amoxicillin/ampicillin	44	-	23	-	-	-
co-amoxiclav[1] - non-uuti	36	21	7	-	-	-
piperacillin-tazobactam	4	9	0	-	6	-
cefuroxime	13	15	1	-	-	-
cefotaxime/ceftriaxone	6	9	1	-	-	-
ceftazidime	5	8	0	-	3	-
meropenem/imipenem	0	0	-	0	-	2
meropenem	-	-	0	-	1	-
imipenem	-	-	-	-	5	-
ciprofloxacin	14	11	12	4	10	5
gentamicin	5	4	5	2	3	3
tobramycin	5	5	4	3	1	2
fosfomycin	1	20	12	44*	-	-
trimethoprim	24	16	32	5	-	-
co-trimoxazole	22	12	26	6	-	4
nitrofurantoin	2	-	-	-	-	-
Empiric therapy combinations						
gentamicin + amoxicillin/ampicillin	4	-	4	-	-	-
gentamicin + co-amoxiclav - non-uuti	4	3	2	-	-	-
gentamicin + piperacillin-tazobactam	0	1	0	-	1	-
gentamicin + cefuroxime	2	3	0	-	-	-
gentamicin + cefotaxime/ceftriaxone	1	3	0	-	-	-
gentamicin + ceftazidime	1	3	0	-	0	-
tobramycin + ceftazidime	-	-	-	-	0	-
tobramycin + ciprofloxacin	-	-	-	-	1	-
Multidrug resistance						
HRMO[2]	8	10	3	2	2	3

10	Significant and clinically relevant increasing trend since 2015
10	Significant and clinically relevant decreasing trend since 2015
10	No significant and clinically relevant time trend

(For the definition of a clinically relevant trend see chapter 4.1.1)

- = Resistance not calculated.
non-uuti = according to breakpoint for non-uncomplicated urinary tract infection.
* *Trend not calculated because of a low number of tests in the years before 2019*
[1] *During 2016, a new testpanel for Gram-negative bacteria, with co-amoxiclav concentrations being adapted to EUCAST testing guidelines, was introduced for the VITEK2 automated system. This results in higher MIC values for co-amoxiclav, which subsequently influence resistance from 2016 onward to higher levels than before (see chapter 4.1.1 for more detailed information).*
[2] *Highly resistant microorganism (HRMO), defined according to HRMO guideline of the WIP (https://www.rivm.nl/documenten/wip-richtlijn-brmo); for E. coli, K. pneumoniae, and P. mirabilis as one or more of the following: 1) extended-spectrum beta-lactamase (ESBL)-producing, estimated by ESBL confirmatory tests, or, if no data on confirmatory tests were available, by resistance to cefotaxime/ceftriaxone and/or ceftazidime, 2) resistant to both fluoroquinolones and aminoglycosides, or 3) carbapenemase producing (CPE), estimated by confirmatory tests of carbapenemase production (both phenotypical and molecular), or, if no data on confirmatory tests were available, by resistance to meropenem or imipenem; for E. cloacae complex at least one or both of the situations 2 and 3 as described for the other Enterobacterales; for P. aeruginosa as resistant to ≥3 antimicrobial groups among fluoroquinolones, aminoglycosides, carbapenems, ceftazidime, and piperacillin-tazobactam; for Acinetobacter spp. as either one or both 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 imipenem or meropenem, or 2) resistant to both fluoroquinolones and aminoglycosides.*

Figure 4.3.2.1 Trends in antibiotic resistance (from left to right 2015 to 2019) 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

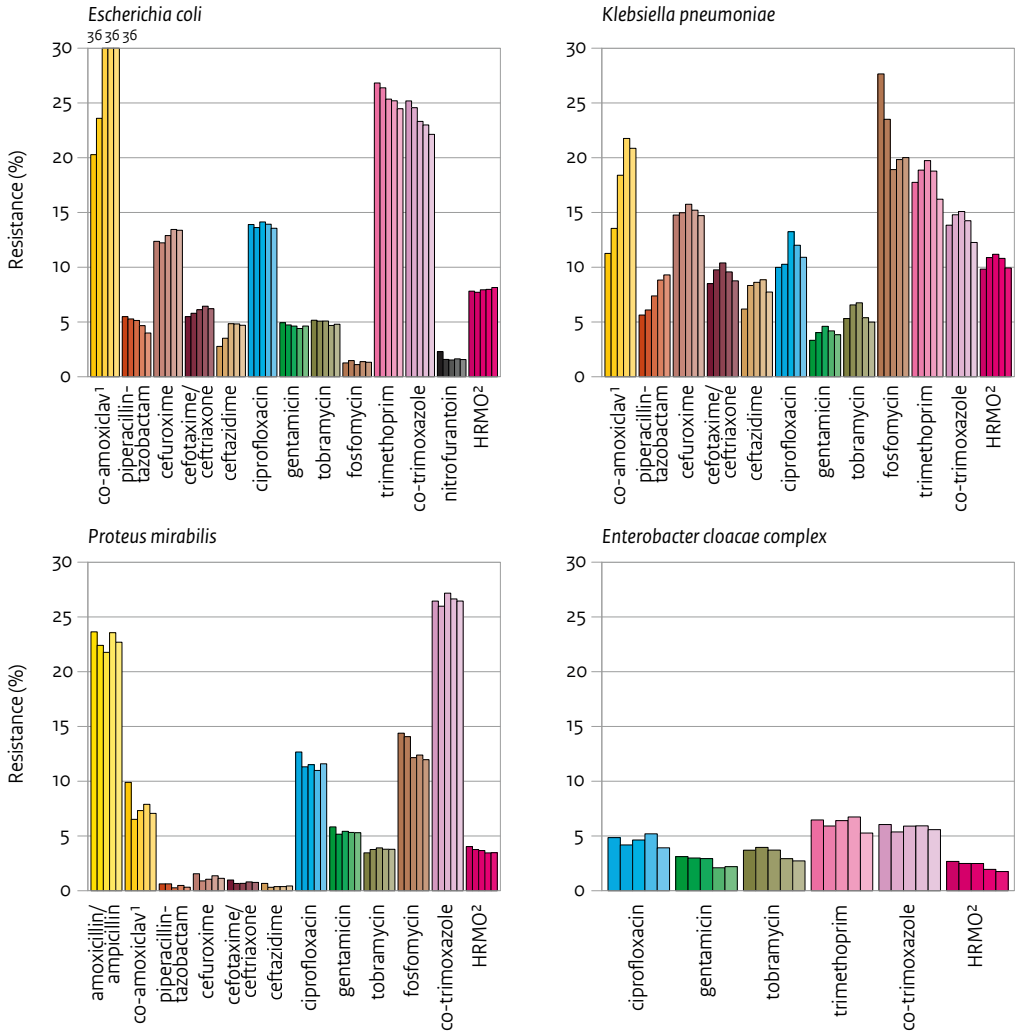
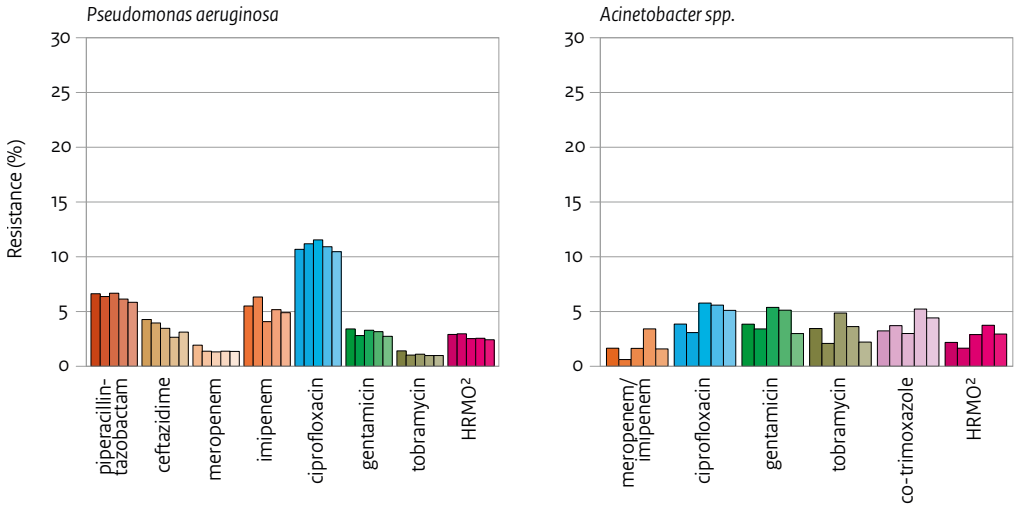


Figure 4.3.2.1 (continued) Trends in antibiotic resistance (from left to right 2015 to 2019) among diagnostic isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, *P. aeruginosa*, and *Acinetobacter* spp. from patients admitted to inpatient departments (excl. intensive care units) in ISIS-AR



[1] *Resistance to co-amoxiclav was calculated according to the breakpoint for non-uncomplicated urinary tract infection. During 2016, a new testpanel for Gram-negative bacteria, with co-amoxiclav concentrations being adapted to EUCAST testing guidelines, was introduced for the VITEK2 automated system. This results in higher MIC values for co-amoxiclav, which subsequently influence resistance from 2016 onward to higher levels than before (see chapter 4.1.1 for more detailed information).*

[2] *Highly resistant microorganism (HRMO), defined according to HRMO guideline of the WIP (https://www.rivm.nl/documenten/wip-richtlijn-brmo); for E. coli, K. pneumoniae, and P. mirabilis as one or more of the following: 1) extended-spectrum beta-lactamase (ESBL)-producing, estimated by ESBL confirmatory tests, or, if no data on confirmatory tests were available, by resistance to cefotaxime/ceftriaxone and/or ceftazidime, 2) resistant to both fluoroquinolones and aminoglycosides, or 3) carbapenemase producing (CPE), estimated by confirmatory tests of carbapenemase production (both phenotypical and molecular), or, if no data on confirmatory tests were available, by resistance to meropenem or imipenem; for E. cloacae complex at least one of the situations 2 and 3 as described for the other Enterobacterales; for P. aeruginosa as resistant to ≥3 antimicrobial groups among fluoroquinolones, aminoglycosides, carbapenems, ceftazidime, and piperacillin-tazobactam; for Acinetobacter spp. as either one or both 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 imipenem or meropenem, or 2) resistant to both fluoroquinolones and aminoglycosides.*

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 2019

	E. faecalis	*E. faecium*
Antibiotic		
amoxicillin/ampicillin	-	84
vancomycin	0	1
nitrofurantoin	0	-

10	Significant and clinically relevant increasing trend since 2015
10	Significant and clinically relevant decreasing trend since 2015
10	No significant and clinically relevant time trend

(For the definition of a clinically relevant trend see chapter 4.1.1)

- = *Resistance not calculated.*

Figure 4.3.2.2 Trends in antibiotic resistance (from left to right 2015 to 2019) among diagnostic isolates of *E. faecalis* and *E. faecium* from patients admitted to inpatient departments (excl. intensive care units) in ISIS-AR

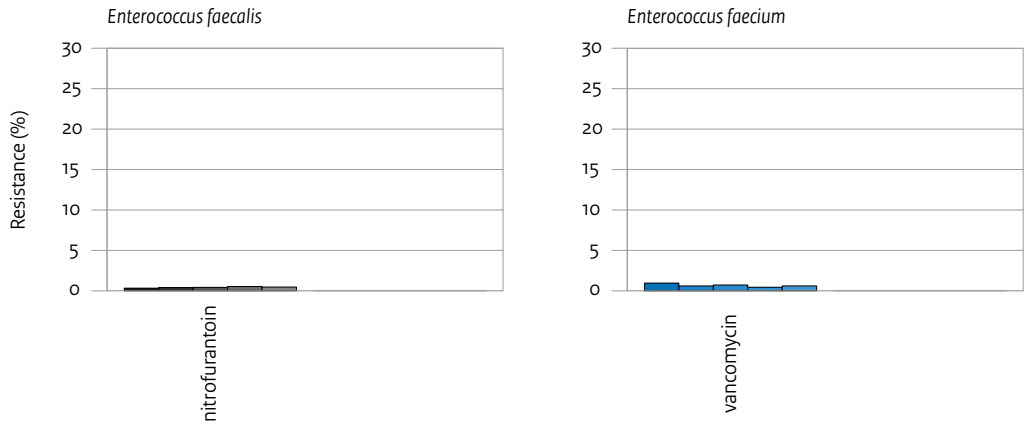


Table 4.3.2.4 Resistance levels (%) among diagnostic isolates of *S. aureus* and coagulase-negative *Staphylococcus* spp. from patients admitted to inpatient departments (excl. intensive care units), ISIS-AR 2019

	S. aureus	CNS
Antibiotic		
flucloxacillin[1]	2	41
ciprofloxacin[2]	7	31
gentamicin	1	26
erythromycin	13	43
clindamycin including inducible resistance[3]	13	31
doxycycline/tetracycline	3	17
fusidic acid	7	42
linezolid	0	0
co-trimoxazole	2	17
rifampicin	0	3

10	Significant and clinically relevant increasing trend since 2015
10	Significant and clinically relevant decreasing trend since 2015
10	No significant and clinically relevant time trend

(For the definition of a clinically relevant trend see chapter 4.1.1)

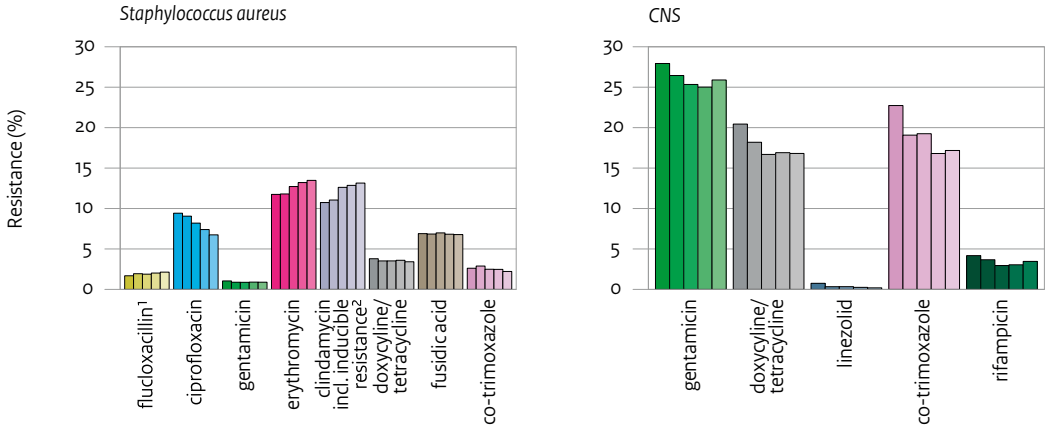
CNS=Coagulase-negative Staphylococcus spp., including S. epidermidis.

[1] *Resistance to flucloxacillin was estimated based on laboratory S/I/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 chapter 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 chapter 4.1.1 for more detailed information).*

Figure 4.3.2.3 Trends in antibiotic resistance (from left to right 2015 to 2019) 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.

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

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

Table 4.3.2.5 Resistance levels (%) among diagnostic isolates of *B. fragilis* complex and *C. perfringens* from patients admitted to inpatient departments (excl. intensive care units), ISIS-AR 2019

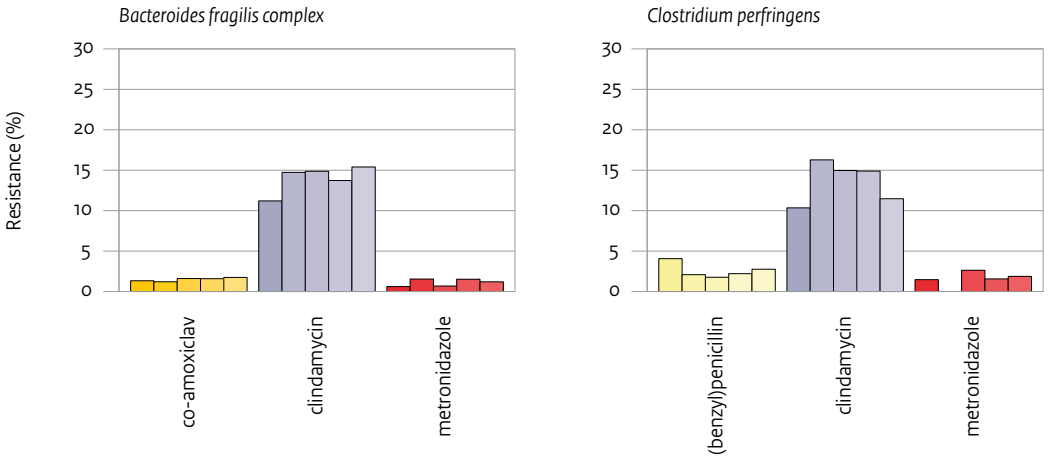
	B. fragilis complex	*C. perfringens*
Antibiotic		
(benzyl)penicillin	-	3
co-amoxiclav	2	0
clindamycin	15	11
metronidazol	1	2

10	Significant and clinically relevant increasing trend since 2015
10	Significant and clinically relevant decreasing trend since 2015
10	No significant and clinically relevant time trend

(For the definition of a clinically relevant trend see chapter 4.1.1)

- = Resistance not calculated.

Figure 4.3.2.4 Trends in antibiotic resistance (from left to right 2015 to 2019) among diagnostic isolates of *B. fragilis* complex and *C. perfringens* from patients admitted to inpatient departments (excl. intensive care units) in ISIS-AR



Key results

Enterobacterales

- In all *Enterobacterales*, resistance was ≤10% for piperacillin-tazobactam (≤9%), cefotaxime/ceftriaxone (≤9%), ceftazidime (≤8%), meropenem/imipenem (0%), gentamicin (≤5%), and tobramycin (≤5%). Resistance was ≤10% for fosfomycin (1%) and nitrofurantoin (2%) in *E. coli*; co-amoxiclav (7%), cefuroxime (1%), and meropenem (0%) in *P. mirabilis*; and ciprofloxacin (4%), trimethoprim (5%), and co-trimoxazole (6%) in *E. cloacae* complex.
- Resistance was ≥20% for amoxicillin/ampicillin (≥23%), trimethoprim (≥24%), and co-trimoxazole (≥22%) in *E. coli* and *P. mirabilis*; for co-amoxiclav in *E. coli* and *K. pneumoniae* (≥21%); and for fosfomycin in *K. pneumoniae* and *E. cloacae* complex (≥20%).
- A statistically significant and clinically relevant increase in resistance was observed for co-amoxiclav in *E. coli* (from 20% in 2015 to 36% in 2019) and *K. pneumoniae* (from 11% to 21%), which may be partly due to the introduction of a new testpanel for the VITEK2 automated system in 2016 (for details see chapter 4.1.1). In *E. coli*, resistance to ceftazidime also increased to a statistically significant and clinically relevant extent in the last five years (from 3% to 5%). Furthermore, in *K. pneumoniae*, a statistically significant and clinically relevant increase was observed for piperacillin-tazobactam (from 6% in 2015 to 9% in 2019). A statistically significant and clinically relevant decrease in resistance was observed for fosfomycin in *K. pneumoniae* (from 28% in 2015 to 20% in 2019).
- Resistance was ≤4% for empiric therapy combinations in all *Enterobacterales*.
- The percentage HRMO in all *Enterobacterales* was ≤10%.

P. aeruginosa

- Resistance was ≤10% for each of the selected agents.
- Resistance was ≤1% for empiric therapy combinations.

- The percentage HRMO was 2%.

Acinetobacter spp.
- Resistance was ≤10% for each of the selected agents.
- The percentage HRMO was 3%.
- A statistically significant and clinically relevant increase in resistance was observed for HRMO (from 2% in 2015 to 3% in 2019).

E. faecalis and E. faecium
- Resistance was ≤10% for vancomycin (≤1%) and nitrofurantoin (0%, calculated for *E. faecalis* only).
- Resistance was ≥20% for amoxicillin/ampicillin in *E. faecium* (84%).

S. aureus
- Resistance was ≤10% for each of the selected agents (≤7%), except for erythromycin and clindamycin including inducible resistance (both 13%).

Coagulase-negative Staphylococcus spp.
- Resistance was ≥20% for each of the selected agents, except for doxycycline/tetracycline (17%), linezolid (0%), co-trimoxazole (17%), and rifampicin (3%).
- A statistically significant and clinically relevant decrease in resistance was observed for co-trimoxazole (from 23% in 2015 to 17% in 2019).

B. fragilis complex and C. perfringens
- Resistance was ≤10% for each of the selected agents (≤3%), except for clindamycin (15% in *B. fragilis* complex, 11% in *C. perfringens*).

4.3.3 Intensive Care Units

The distribution of pathogens from diagnostic samples (blood or cerebrospinal fluid, lower respiratory tract, urine, and wound or pus) from patients admitted to intensive care units is presented in table 4.3.3.1. The resistance levels for a selection of pathogens isolated from these patients in 2019 are presented in tables 4.3.3.2 (*E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, and *P. aeruginosa*), 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, and *P. aeruginosa*), 4.3.3.2 (*E. faecium*), and 4.3.3.3 (*S. aureus* and coagulase-negative *Staphylococcus* spp.). For *Acinetobacter* spp., *B. fragilis* complex, and *C. perfringens* resistance levels and trends were not calculated because in 2019 less than 100 isolates were available for analysis.

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 2019

	Blood or cerebrospinal fluid	Lower respiratory tract	Urine	Wound or pus
Pathogen	**N (%)**	**N (%)**	**N (%)**	**N (%)**
E. coli	234 (9)	403 (10)	609 (38)	466 (16)
K. pneumoniae	68 (3)	217 (5)	134 (8)	89 (3)
P. mirabilis	13 (0)	82 (2)	106 (7)	79 (3)
E. cloacae complex	29 (1)	194 (5)	35 (2)	134 (5)
Other *Enterobacterales*[1]	96 (4)	659 (16)	159 (10)	247 (9)
P. aeruginosa	43 (2)	270 (7)	94 (6)	180 (6)
Acinetobacter spp.	12 (0)	47 (1)	4 (0)	18 (1)
Other non-fermenters[2]	9 (0)	327 (8)	3 (0)	54 (2)
B. fragilis complex	14 (1)	2 (0)	2 (0)	49 (2)
Other Gram-negatives[3]	20 (1)	507 (13)	0 (0)	12 (0)
E. faecalis	99 (4)	29 (1)	178 (11)	248 (9)
E. faecium	184 (7)	52 (1)	115 (7)	366 (13)
S. aureus	240 (9)	820 (20)	33 (2)	307 (11)
CNS	1,390 (53)	16 (0)	53 (3)	288 (10)
C. perfringens	1 (0)	0 (0)	0 (0)	21 (1)
Other Gram-positives[4]	174 (7)	394 (10)	78 (5)	282 (10)

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

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

[2] *S. maltophilia, M. catarrhalis, Pseudomonas spp. (non-aeruginosa), B. cepacia.*

[3] *H. influenzae, H. parainfluenzae, N. meningitidis, H. pylori.*

[4] *S. pneumoniae, S. mitis, S. anginosus, beta-haemolytic Streptococcus spp. gr C, S. equi, S. agalactiae, S. oralis, S. dysgalactiae n.n.g., S. pyogenes, beta-haemolytic Streptococcus spp. gr G, S. dysgalactiae subsp. equisimilis, Enterococcus spp. (non-faecalis, non-faecium), A. urinae, Staphylococcus spp. (non-aureus complex, non-CNS), L. monocytogenes.*

Table 4.3.3.2 Resistance levels (%) among diagnostic isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, *P. aeruginosa* from patients admitted to intensive care units, ISIS-AR 2019

	E. coli	*K. pneumoniae*	*P. mirabilis*	*E. cloacae* complex	*P. aeruginosa*
Antibiotic					
amoxicillin/ampicillin	46	-	21	-	-
co-amoxiclav[1] - non-uuti	39	21	6	-	-
piperacillin-tazobactam	6	8	0	-	14
cefuroxime	17	20	1	-	-
cefotaxime/ceftriaxone	10	13	1	-	-
ceftazidime	7	10	0	-	8
meropenem/imipenem	0	1	-	1	-
meropenem	-	-	0	-	5
imipenem	-	-	-	-	10
ciprofloxacin	15	12	9	5	16
gentamicin	6	7	8	5	6
tobramycin	6	8	4	5	4
co-trimoxazole	23	13	24	5	-
Empiric therapy combinations					
gentamicin + amoxicillin/ampicillin	5	-	5	-	-
gentamicin + co-amoxiclav - non-uuti	5	6	3	-	-
gentamicin + piperacillin-tazobactam	1	2	0	-	3
gentamicin + cefuroxime	3	6	0	-	-
gentamicin + cefotaxime/ceftriaxone	3	6	0	-	-
gentamicin + ceftazidime	2	5	0	-	1
tobramycin + ceftazidime	-	-	-	-	1
tobramycin + ciprofloxacin	-	-	-	-	3
Multidrug resistance					
HRMO[2]	12	14	4	3	5

10	Significant and clinically relevant increasing trend since 2015
10	Significant and clinically relevant decreasing trend since 2015
10	No significant and clinically relevant time trend

(For the definition of a clinically relevant trend see chapter 4.1.1)

- = Resistance not calculated.

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

[1] *During 2016, a new testpanel for Gram-negative bacteria, with co-amoxiclav concentrations being adapted to EUCAST testing guidelines, was introduced for the VITEK2 automated system. This results in higher MIC values for co-amoxiclav, which subsequently influence resistance from 2016 onward to higher levels than before (see chapter 4.1.1 for more detailed information).*

[2] *Highly resistant microorganism (HRMO), defined according to HRMO guideline of the WIP (https://www.rivm.nl/documenten/wip-richtlijn-brmo); for E. coli, K. pneumoniae, and P. mirabilis as one or more of the following: 1) extended-spectrum beta-lactamase (ESBL)-producing, estimated by ESBL confirmatory tests, or, if no data on confirmatory tests were available, by resistance to cefotaxime/ceftriaxone and/or ceftazidime, 2) resistant to both fluoroquinolones and aminoglycosides, or 3) carbapenemase producing (CPE), estimated by confirmatory tests of carbapenemase production (both phenotypical and molecular), or, if no data on confirmatory tests were available, by resistance to meropenem or imipenem; for E. cloacae complex at least one or both of the situations 2 and 3 as described for the other Enterobacterales; for P. aeruginosa as resistant to ≥3 antimicrobial groups among fluoroquinolones, aminoglycosides, carbapenems, ceftazidime, and piperacillin-tazobactam*

Figure 4.3.3.1 Trends in antibiotic resistance (from left to right 2015 to 2019) among diagnostic isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, and *P. aeruginosa* from patients admitted to intensive care units in ISIS-AR

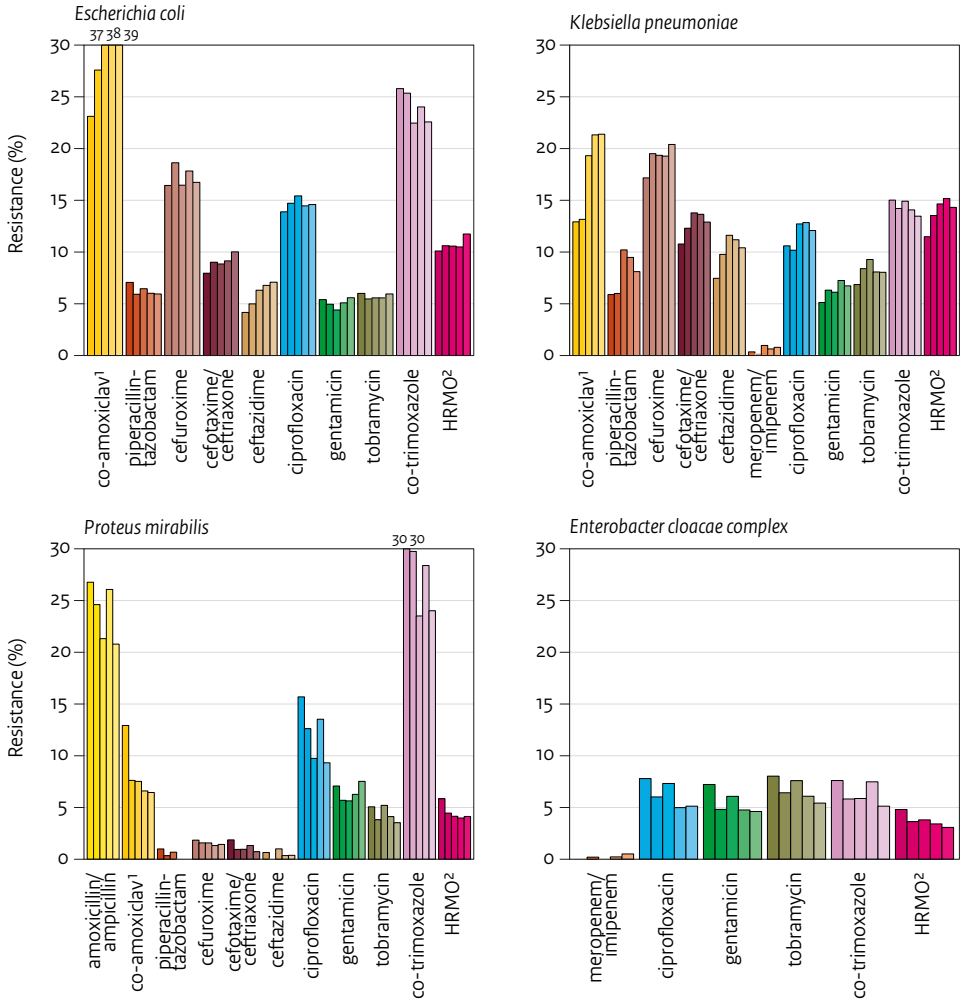
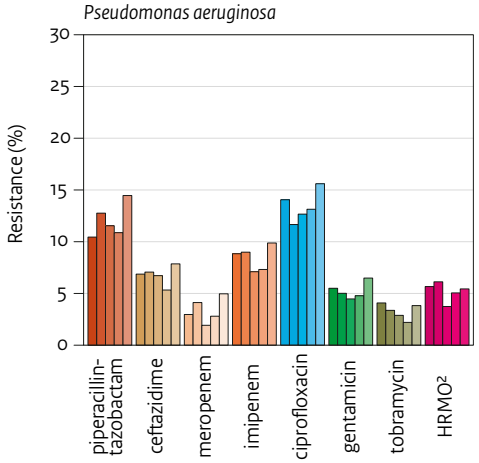


Figure 4.3.3.1 (continued) Trends in antibiotic resistance (from left to right 2015 to 2019) among diagnostic isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, and *P. aeruginosa* from patients admitted to intensive care units in ISIS-AR



[1] *Resistance to co-amoxiclav was calculated according to the breakpoint for non-uncomplicated urinary tract infection. During 2016, a new testpanel for Gram-negative bacteria, with co-amoxiclav concentrations being adapted to EUCAST testing guidelines, was introduced for the VITEK2 automated system. This results in higher MIC values for co-amoxiclav, which subsequently influence resistance from 2016 onward to higher levels than before (see chapter 4.1.1 for more detailed information).*

[2] *Highly resistant microorganism (HRMO), defined according to HRMO guideline of the WIP (https://www.rivm.nl/documenten/wip-richtlijn-brmo); for E. coli, K. pneumoniae, and P. mirabilis as one or more of the following: 1) extended-spectrum beta-lactamase (ESBL)-producing, estimated by ESBL confirmatory tests, or, if no data on confirmatory tests were available, by resistance to cefotaxime/ceftriaxone and/or ceftazidime, 2) resistant to both fluoroquinolones and aminoglycosides, or 3) carbapenemase producing (CPE), estimated by confirmatory tests of carbapenemase production (both phenotypical and molecular), or, if no data on confirmatory tests were available, by resistance to meropenem or imipenem; for E. cloacae complex at least one of the situations 2 and 3 as described for the other Enterobacterales; for P. aeruginosa as resistant to ≥3 antimicrobial groups among fluoroquinolones, aminoglycosides, carbapenems, ceftazidime, and piperacillin-tazobactam.*

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 2019

	E. faecalis	*E. faecium*
Antibiotic		
amoxicillin/ampicillin	-	87
vancomycin	0	1

10	Significant and clinically relevant increasing trend since 2015
10	Significant and clinically relevant decreasing trend since 2015
10	No significant and clinically relevant time trend
	(For the definition of a clinically relevant trend see chapter 4.1.1)

- = Resistance not calculated.

Figure 4.3.3.2 Trends in antibiotic resistance (from left to right 2015 to 2019) among diagnostic isolates of *E. faecium* from patients admitted to intensive care units in ISIS-AR

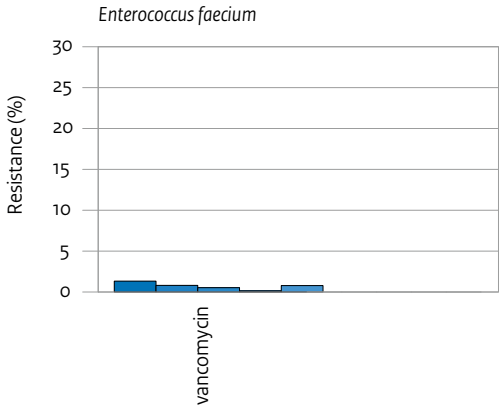


Table 4.3.3.4 Resistance levels (%) among diagnostic isolates of *S. aureus* and coagulase-negative *Staphylococcus* spp. from patients admitted to intensive care units, ISIS-AR 2019

	S. aureus	CNS
Antibiotic		
flucloxacillin[1]	2	74
ciprofloxacin[2]	5	60
gentamicin	1	55
erythromycin	12	65
clindamycin including inducible resistance[3]	12	57
doxycycline/tetracycline	3	22
linezolid	0	0
co-trimoxazole	1	30
rifampicin	0	9

10	Significant and clinically relevant increasing trend since 2015
10	Significant and clinically relevant decreasing trend since 2015
10	No significant and clinically relevant time trend

(For the definition of a clinically relevant trend see chapter 4.1.1)

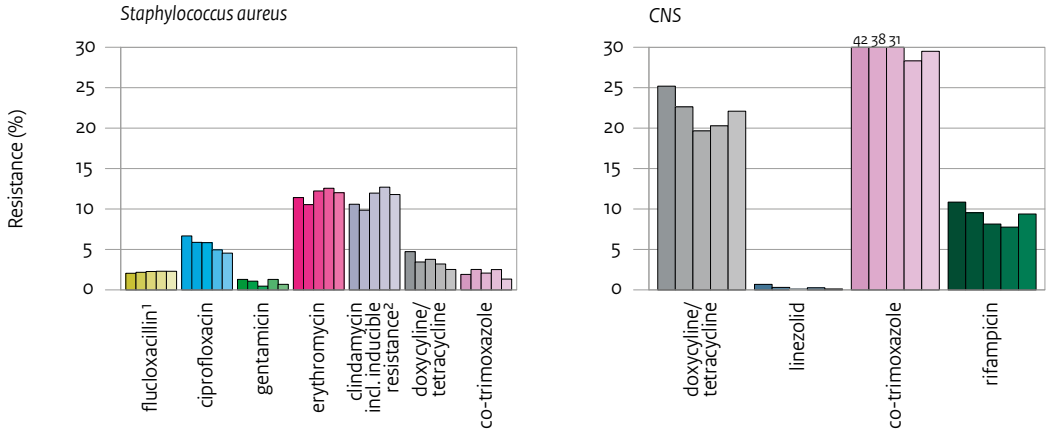
CNS=Coagulase-negative Staphylococcus spp., including S. epidermidis.

[1] *Resistance to flucloxacillin was estimated based on laboratory S/I/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 chapter 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 chapter 4.1.1 for more detailed information).*

Figure 4.3.3.3 Trends in antibiotic resistance (from left to right 2015 to 2019) 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.

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

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

Key results

Enterobacterales

- In all *Enterobacterales*, resistance was ≤10% for piperacillin-tazobactam (≤8%), ceftazidime (≤10%), meropenem/imipenem (≤1%), gentamicin (≤8%), and tobramycin (≤8%). Resistance was ≤10% for cefotaxime/ceftriaxone in *E. coli* and *P. mirabilis* (≤10%); co-amoxiclav (6%), cefuroxime (1%), and meropenem (0%) in *P. mirabilis*; ciprofloxacin in *P. mirabilis* and *E. cloacae* complex (≤9%); and co-trimoxazole in *E. cloacae* complex (5%).
- Resistance was ≥20% for co-amoxiclav (≥21%) in *E. coli* and *K. pneumoniae*, for cefuroxime (20%) in *K. pneumoniae*, and for amoxicillin/ampicillin (≥21%) and co-trimoxazole (≥23%) in *E. coli* and *P. mirabilis*.
- A statistically significant and clinically relevant increase in resistance was observed for co-amoxiclav in *E. coli* (from 23% in 2015 to 39% in 2019) and *K. pneumoniae* (from 13% to 21%), which may be partly due to the introduction of a new testpanel for the VITEK2 automated system in 2016 (for details see chapter 4.1.1). In *E. coli*, resistance to ceftazidime also increased to a statistically significant and clinically relevant extent in the last five years (from 4% to 7%). Furthermore, in *K. pneumoniae*, a statistically significant and clinically relevant increase in resistance was observed for piperacillin-tazobactam (from 6% in 2015 to 8% in 2019); this increase was primarily observed between 2015 and 2017. In *P. mirabilis*, a significant and clinically relevant decrease in resistance between 2015 and 2019 was observed for co-amoxiclav (from 13% to 6%) and for ciprofloxacin (from 16% to 9%).
- Resistance was ≤6% for empiric therapy combinations in all *Enterobacterales*. A statistically significant and clinically relevant increase in resistance was observed for gentamicin + co-amoxiclav in *E. coli*

(from 3% in 2015 to 5% in 2019).
- The percentage HRMO was ≤4%, except for *E. coli* (12%) and *K. pneumoniae* (14%).

P. aeruginosa
- Resistance was ≤10% for each of the selected agents, except for resistance to piperacillin-tazobactam (14%) and ciprofloxacin (16%).
- Resistance was ≤3% for empiric therapy combinations.
- The percentage HRMO was 5%.

E. faecalis and E. faecium
Resistance was ≤10% for vancomycin (≤1%).
Resistance was ≥20% for amoxicillin/ampicillin in *E. faecium* (87%).

S. aureus
- Resistance was ≤10% for each of the selected agents (≤5%), except for erythromycin and clindamycin including inducible resistance (both 12%).

Coagulase-negative Staphylococcus spp.
- Resistance was ≥20% for each of the selected agents (≥22%), except for linezolid (0%) and rifampicin (9%).
- A significant and clinically relevant decrease in resistance was found for co-trimoxazole (from 42% in 2015 to 30% in 2019).

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) is presented in table 4.3.4.1. Resistance levels for a selection of pathogens isolated from these patients in 2019 are presented in tables 4.3.4.2 (*E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, and *P. aeruginosa*), 4.3.4.3 (*E. faecalis* and *E. faecium*), 4.3.4.4 (*S. aureus* and coagulase-negative *Staphylococcus* spp.), and 4.3.4.5 (*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.), and 4.3.4.4 (*B. fragilis* complex). For *Acinetobacter* spp. and *C. perfringens* resistance levels and trends were not calculated because in 2019 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 2019

	Non-ICU	ICU
Pathogen	**N (%)**	**N (%)**
E. coli	5,543 (25)	260 (10)
K. pneumoniae	1,037 (5)	72 (3)
P. mirabilis	409 (2)	15 (1)
E. cloacae complex	365 (2)	34 (1)
Other *Enterobacterales*[1]	1,170 (5)	101 (4)
P. aeruginosa	461 (2)	51 (2)
Acinetobacter spp.	77 (0)	11 (0)
Other non-fermenters[2]	71 (0)	10 (0)
B. fragilis complex	280 (1)	15 (1)
Other Gram-negatives[3]	219 (1)	19 (1)
E. faecalis	673 (3)	100 (4)
E. faecium	408 (2)	220 (8)
S. aureus	2,404 (11)	204 (8)
CNS	5,695 (26)	1,353 (52)
C. perfringens	71 (0)	1 (0)
Other Gram-positives[4]	3,010 (14)	156 (6)

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

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

[2] *S. maltophilia, Pseudomonas spp. (non-aeruginosa), M. catarrhalis, B. cepacia.*

[3] *H. parainfluenzae, H. influenzae, N. meningitidis, C. jejuni.*

[4] *S. pneumoniae, S. mitis, S. anginosus, beta-haemolytic Streptococcus spp. gr C, S. equi, S. agalactiae, S. oralis, beta-haemolytic Streptococcus spp. gr G, S. pyogenes, S. dysgalactiae n.n.g., S. dysgalactiae subsp. equisimilis, Enterococcus spp. (non-faecalis, non-faecium), Staphylococcus spp. (non-aureus complex, non-CNS), A. urinae, L. monocytogenes.*

Table 4.3.4.2 Resistance levels (%) among diagnostic blood isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, and *P. aeruginosa* from patients admitted to inpatient departments (incl. intensive care units), ISIS-AR 2019

	E. coli	*K. pneumoniae*	*P. mirabilis*	*E. cloacae* complex	*P. aeruginosa*
Antibiotic					
amoxicillin/ampicillin	44	-	21	-	-
co-amoxiclav[1] - non-uuti	36	19	5	-	-
piperacillin-tazobactam	3	7	0	-	6
cefuroxime	13	13	0	-	-
cefotaxime/ceftriaxone	7	9	0	-	-
ceftazidime	5	7	0	-	4
meropenem/imipenem	0	0	-	0	-
meropenem	-	-	0	-	1
imipenem	-	-	-	-	5
ciprofloxacin	15	9	12	5	10
gentamicin	5	3	5	3	2
tobramycin	6	6	3	4	1
co-trimoxazole	23	12	22	6	-
Empiric therapy combinations					
gentamicin + amoxicillin/ampicillin	5	-	4	-	-
gentamicin + co-amoxiclav - non-uuti	5	3	1	-	-
gentamicin + piperacillin-tazobactam	1	1	0	-	1
gentamicin + cefuroxime	2	3	0	-	-
gentamicin + cefotaxime/ceftriaxone	2	3	0	-	-
gentamicin + ceftazidime	2	2	0	-	1
tobramycin + ceftazidime	-	-	-	-	0
tobramycin + ciprofloxacin	-	-	-	-	1
Multidrug resistance					
HRMO[2]	9	10	3	3	5

10	Significant and clinically relevant increasing trend since 2015
10	Significant and clinically relevant decreasing trend since 2015
10	No significant and clinically relevant time trend

(For the definition of a clinically relevant trend see chapter 4.1.1)

- = Resistance not calculated.

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

[1] *During 2016, a new testpanel for Gram-negative bacteria, with co-amoxiclav concentrations being adapted to EUCAST testing guidelines, was introduced for the VITEK2 automated system. This results in higher MIC values for co-amoxiclav, which subsequently influence resistance from 2016 onward to higher levels than before (see chapter 4.1.1 for more detailed information).*

[2] *Highly resistant microorganism (HRMO), defined according to HRMO guideline of the WIP (https://www.rivm.nl/documenten/wip-richtlijn-brmo); for E. coli, K. pneumoniae, and P. mirabilis as one or more of the following: 1) extended-spectrum beta-lactamase (ESBL)-producing, estimated by ESBL confirmatory tests, or, if no data on confirmatory tests were available, by resistance to cefotaxime/ceftriaxone and/or ceftazidime, 2) resistant to both fluoroquinolones and aminoglycosides, or 3) carbapenemase producing (CPE), estimated by confirmatory tests of carbapenemase production (both phenotypical and molecular), or, if no data on confirmatory tests were available, by resistance to meropenem or imipenem; for E. cloacae complex at least one or both of the situations 2 and 3 as described for the other Enterobacterales; for P. aeruginosa as resistant to ≥3 antimicrobial groups among fluoroquinolones, aminoglycosides, carbapenems, ceftazidime, and piperacillin-tazobactam.*

Figure 4.3.4.1 Trends in antibiotic resistance (from left to right 2015 to 2019) 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

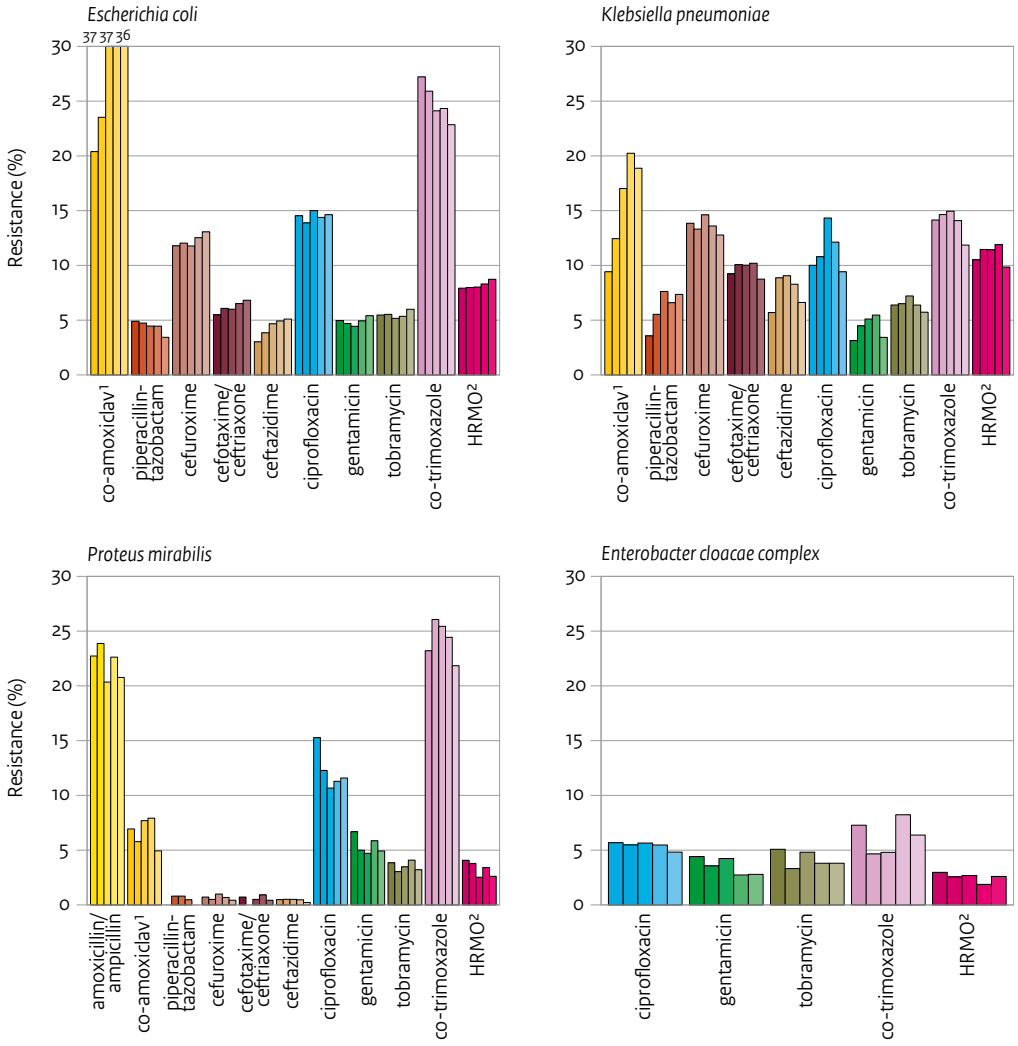
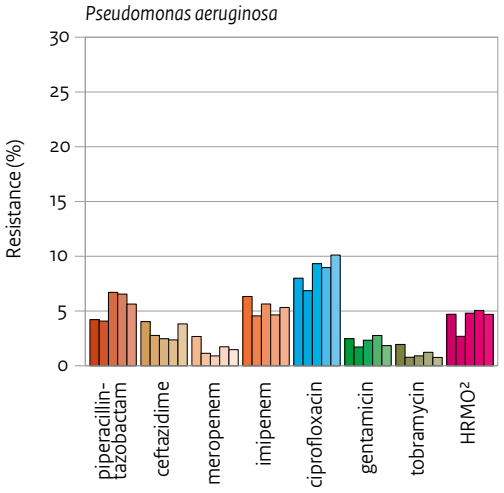


Figure 4.3.4.1 (continued) Trends in antibiotic resistance (from left to right 2015 to 2019) among diagnostic blood isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, and *P. aeruginosa* from patients admitted to inpatient departments (incl. intensive care units) in ISIS-AR



[1] *Resistance to co-amoxiclav was calculated according to the breakpoint for non-uncomplicated urinary tract infection. During 2016 a new testpanel for Gram-negative bacteria, with co-amoxiclav concentrations being adapted to EUCAST testing guidelines, was introduced for the VITEK2 automated system. This results in higher MIC values for co-amoxiclav, which subsequently influence resistance from 2016 onward to higher levels than before (see chapter 4.1.1 for more detailed information).*

[2] *Highly resistant microorganism (HRMO), defined according to HRMO guideline of the WIP (https://www.rivm.nl/documenten/wip-richtlijn-brmo); for E. coli, K. pneumoniae, and P. mirabilis as one or more of the following: 1) extended-spectrum beta-lactamase (ESBL)-producing, estimated by ESBL confirmatory tests, or, if no data on confirmatory tests were available, by resistance to cefotaxime/ceftriaxone and/or ceftazidime, 2) resistant to both fluoroquinolones and aminoglycosides, or 3) carbapenemase producing (CPE), estimated by confirmatory tests of carbapenemase production (both phenotypical and molecular), or, if no data on confirmatory tests were available, by resistance to meropenem or imipenem; for E. cloacae complex at least one of the situations 2 and 3 as described for the other Enterobacterales; for P. aeruginosa as resistant to ≥3 antimicrobial groups among fluoroquinolones, aminoglycosides, carbapenems, ceftazidime, and piperacillin-tazobactam.*

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 2019

	E. faecalis	*E. faecium*
Antibiotic		
amoxicillin/ampicillin	-	84
vancomycin	0	1

10	Significant and clinically relevant increasing trend since 2015
10	Significant and clinically relevant decreasing trend since 2015
10	No significant and clinically relevant time trend
	(For the definition of a clinically relevant trend see chapter 4.1.1)

- = Resistance not calculated.

Figure 4.3.4.2 Trends in antibiotic resistance (from left to right 2015 to 2019) among diagnostic blood isolates of *E. faecium* from patients admitted to inpatient departments (incl. intensive care units) in ISIS-AR

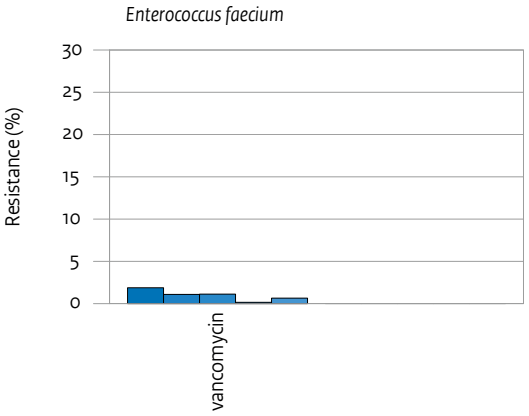


Table 4.3.4.4 Resistance levels (%) among diagnostic blood isolates of *S. aureus* and coagulase-negative *Staphylococcus* spp. from patients admitted to inpatient departments (incl. intensive care units), ISIS-AR 2019

	S. aureus	CNS
Antibiotic		
flucloxacillin[1]	1	47
ciprofloxacin[2]	6	33
gentamicin	1	29
erythromycin	11	47
clindamycin including inducible resistance[3]	11	34
doxycycline/tetracycline	3	20
linezolid	0	0
co-trimoxazole	1	18
rifampicin	0	3

10	Significant and clinically relevant increasing trend since 2015
10	Significant and clinically relevant decreasing trend since 2015
10	No significant and clinically relevant time trend

(For the definition of a clinically relevant trend see chapter 4.1.1)

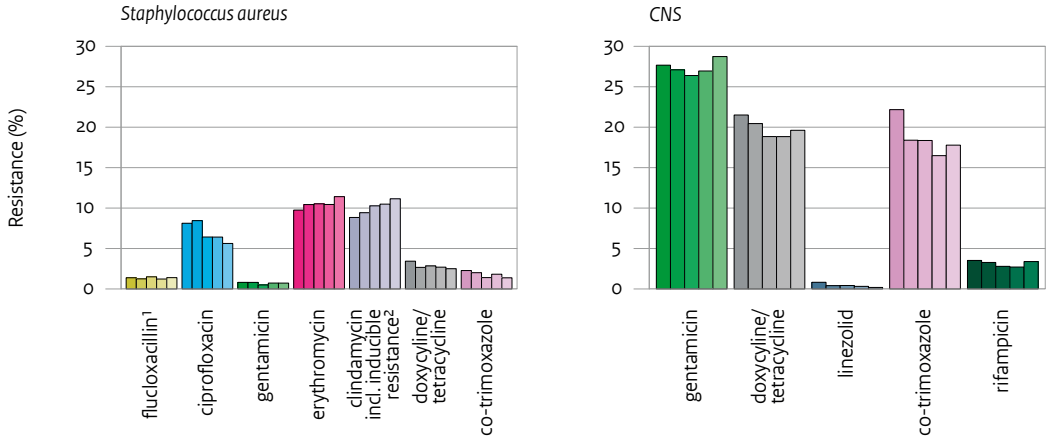
CNS=Coagulase-negative Staphylococcus spp., including S. epidermidis.

[1] *Resistance to flucloxacillin was estimated based on laboratory S/I/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 chapter 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 chapter 4.1.1 for more detailed information).*

Figure 4.3.4.3 Trends in antibiotic resistance (from left to right 2015 to 2019) 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.

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

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

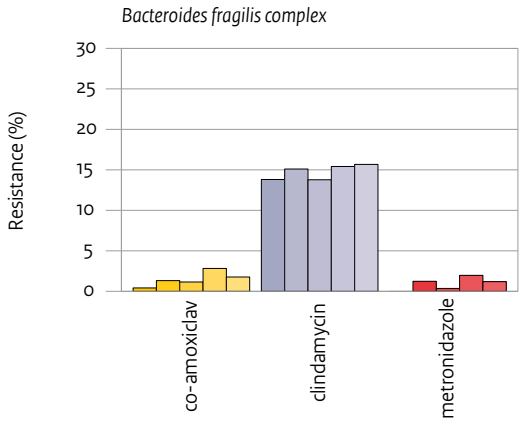
Table 4.3.4.5 Resistance levels (%) among diagnostic blood isolates of *B. fragilis* complex from patients admitted to inpatient departments (incl. intensive care units), ISIS-AR 2019

	***B. fragilis* complex**
Antibiotic	
co-amoxiclav	2
clindamycin	16
metronidazol	1

10	Significant and clinically relevant increasing trend since 2015
10	Significant and clinically relevant decreasing trend since 2015
10	No significant and clinically relevant time trend

(For the definition of a clinically relevant trend see chapter 4.1.1)

Figure 4.3.4.4 Trends in antibiotic resistance (from left to right 2015 to 2019) among diagnostic blood isolates of *B. fragilis* complex from patients admitted to inpatient departments (incl. intensive care units) in ISIS-AR



Key results

The majority (89%) of inpatient blood isolates (non-ICU and ICU departments combined) originated from non-ICU departments.

Enterobacterales

- In all *Enterobacterales*, resistance was ≤10% for piperacillin-tazobactam (≤7%), cefotaxime/ceftriaxone (≤9%), ceftazidime (≤7%), meropenem/imipenem (0%), gentamicin (≤5%), and tobramycin (≤6%). In addition, resistance was ≤10% for ciprofloxacin in *K. pneumoniae* and *E. cloacae* complex (≤9%); for co-amoxiclav (5%), cefuroxime (0%), and meropenem (0%) in *P. mirabilis*; and for co-trimoxazole (6%) in *E. cloacae* complex.
- Resistance was ≥20% for amoxicillin/ampicillin (≥21%) and co-trimoxazole (≥22%) in *E. coli* and *P. mirabilis*; and for co-amoxiclav in *E. coli* (36%).
- A statistically significant and clinically relevant increase in resistance was observed for co-amoxiclav in *E. coli* (from 20% in 2015 to 36% in 2019) and *K. pneumoniae* (from 9% to 19%), which may be partly due to the introduction of a new testpanel for the VITEK2 automated system in 2016 (for details see chapter 4.1.1). In *E. coli*, resistance to ceftazidime also increased to a statistically significant and clinically relevant extent in the last five years (from 3% to 5%). Furthermore, in *K. pneumoniae*, a statistically significant and clinically relevant increase was observed for piperacillin-tazobactam (from 4% in 2015 to 7% in 2019).
- Resistance was ≤5% for empiric therapy combinations in all *Enterobacterales*. In *E. coli*, resistance to gentamicin + co-amoxiclav increased to a statistically significant and clinically relevant extent (from 3% in 2015 to 5% in 2019).

- The percentage HRMO in all *Enterobacterales* was ≤10%.

P. aeruginosa
- Resistance levels ≤10% were observed for each of the selected agents.
- Resistance to empiric therapy combinations was ≤1%.
- The percentage HRMO was 5%.

E. faecalis and E. faecium
- Resistance levels ≤10% were found for vancomycin (≤1%).
- Resistance ≥20% was observed for amoxicillin/ampicillin in *E. faecium* (84%).
- A statistically significant and clinically relevant decreasing trend in resistance was observed for vancomycin in *E. faecium* (from 2% in 2015 to 1% in 2019).

S. aureus
- Resistance levels ≤10% were observed for each of the selected agents (≤6%), except for erythromycin and clindamycin including inducible resistance (both 11%).

Coagulase-negative Staphylococcus spp.
- Resistance levels ≥20% were observed for each of the selected agents, except for linezolid (0%), co-trimoxazole (18%), and rifampicin (3%).

B. fragilis complex
- Resistance levels ≤10% were observed for co-amoxiclav (2%) and metronidazol (1%).

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) is presented in table 4.3.5.1. Resistance levels for a selection of pathogens isolated from these patients in 2019 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*).

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 2019

	OPD	IPD
Pathogen	**N (%)**	**N (%)**
E. coli	11,551 (39)	2,096 (32)
K. pneumoniae	2,668 (9)	480 (7)
P. mirabilis	1,458 (5)	373 (6)
Other *Enterobacterales*[1]	4,104 (14)	1,077 (17)
P. aeruginosa	1,094 (4)	393 (6)
Other non-fermenters[2]	556 (2)	172 (3)
Other Gram-negatives[3]	10 (0)	14 (0)
E. faecalis	3,210 (11)	826 (13)
E. faecium	203 (1)	136 (2)
Other Gram-positives[4]	4,760 (16)	916 (14)

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

[2] *Acinetobacter spp., S. maltophilia, Pseudomonas spp. (non-aeruginosa).*

[3] *B. fragilis complex, H. parainfluenzae, H. influenzae.*

[4] *Staphylococcus spp., beta-haemolytic Streptococcus spp. gr C, S. dysgalactiae n.n.g., S. dysgalactiae subsp. equisimilis, S. pneumoniae, S. anginosus, S. pyogenes, S. oralis, beta-haemolytic Streptococcus spp. gr G, S. agalactiae, S. mitis, A. urinae, Enterococcus spp. (non-faecalis, non-faecium).*

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 2019

	E. coli		*K. pneumoniae*		*P. mirabilis*		*P. aeruginosa*	
	OPD	IPD	OPD	IPD	OPD	IPD	OPD	IPD
Antibiotic								
amoxicillin/ampicillin	45	49	-	-	23	27	-	-
co-amoxiclav[1] - non-uuti	36	40	21	22	7	8	-	-
piperacillin-tazobactam	4	5	9	9	0	0	4	4
cefuroxime	14	16	17	17	1	1	-	-
cefotaxime/ceftriaxone	6	9	9	11	1	0	-	-
ceftazidime	5	6	8	9	0	0	1	2
meropenem/imipenem	0	0	0	0	-	-	-	-
meropenem	-	-	-	-	0	0	1	2
imipenem	-	-	-	-	-	-	5*	4*
ciprofloxacin	20	25	17	14	16	18	14	10
gentamicin	5	7	3	5	6	7	2	2
tobramycin	6	8	5	6	4	4	1	0
fosfomycin	2	2	28	22	18	16	-	-
trimethoprim	29	30	25	20	33	38	-	-
co-trimoxazole	26	29	15	16	26	30	-	-
nitrofurantoin	4	3	-	-	-	-	-	-
Empiric therapy combinations								
gentamicin + amoxicillin/ampicillin	5	7	-	-	5	6	-	-
gentamicin + co-amoxiclav - non-uuti	5	6	3	4	2	2	-	-
gentamicin + piperacillin-tazobactam	-	1	-	1	-	0	0	0
gentamicin + cefuroxime	2	3	2	3	0	0	-	-
gentamicin + cefotaxime/ceftriaxone	1	3	2	3	0	0	-	-
gentamicin + ceftazidime	1	2	2	3	0	0	0	0
tobramycin + ceftazidime	-	-	-	-	-	-	0	0
tobramycin + ciprofloxacin	-	-	-	-	-	-	1	0
Multidrug resistance								
HRMO[2]	9	12	10	12	4	4	1	2
multidrug resistance[3] - non-uuti	8	11	6	7	2	3	-	-

10	Significant and clinically relevant increasing trend since 2015
10	Significant and clinically relevant decreasing trend since 2015
10	No significant and clinically relevant time trend

(For the definition of a clinically relevant trend see chapter 4.1.1)

- = Resistance not calculated.

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

* *Trend not calculated because of a low number of tests in the years before 2019.*

[1] *During 2016, a new testpanel for Gram-negative bacteria, with co-amoxiclav concentrations being adapted to EUCAST testing guidelines, was introduced for the VITEK2 automated system. This results in higher MIC values for co-amoxiclav, which subsequently influence resistance from 2016 onward to higher levels than before (see chapter 4.1.1 for more detailed information).*

[2] *Highly resistant microorganism (HRMO), defined according to HRMO guideline of the WIP (https://www.rivm.nl/documenten/wip-richtlijn-brmo); for E. coli, K. pneumoniae, and P. mirabilis as one or more of the following: 1) extended-spectrum beta-lactamase (ESBL)-producing, estimated by ESBL confirmatory tests, or, if no data on confirmatory tests were available, by resistance to cefotaxime/ceftriaxone and/or ceftazidime, 2) resistant to both fluoroquinolones and aminoglycosides, or 3) carbapenemase producing (CPE), estimated by confirmatory tests of carbapenemase production (both phenotypical and molecular), or, if no data on confirmatory tests were available, by resistance to meropenem or imipenem; for P. aeruginosa as resistant to ≥3 antimicrobial groups among fluoroquinolones, aminoglycosides, carbapenems, ceftazidime, and piperacillin-tazobactam.*

[3] *Defined as resistance to all of the following oral agents: co-amoxiclav, ciprofloxacin, and co-trimoxazole.*

Figure 4.3.5.1 Trends in antibiotic resistance (from left to right 2015 to 2019) among diagnostic urine isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* from patients attending urology outpatient departments and patients admitted to urology inpatient departments in ISIS-AR

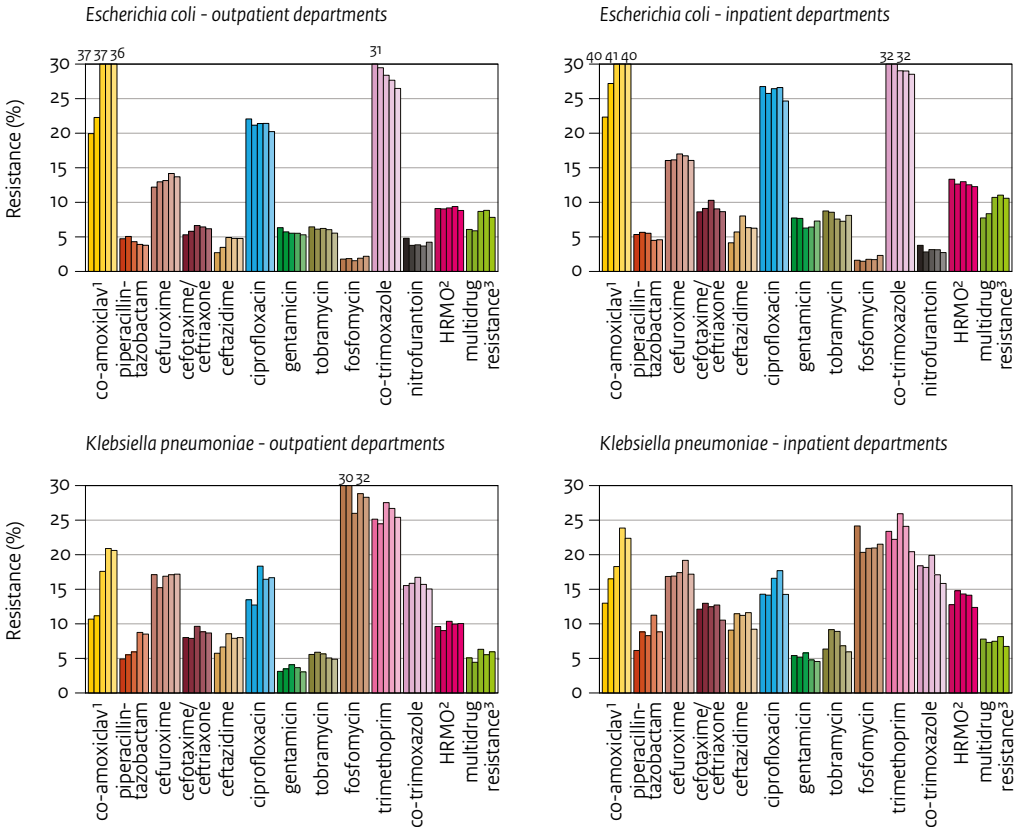
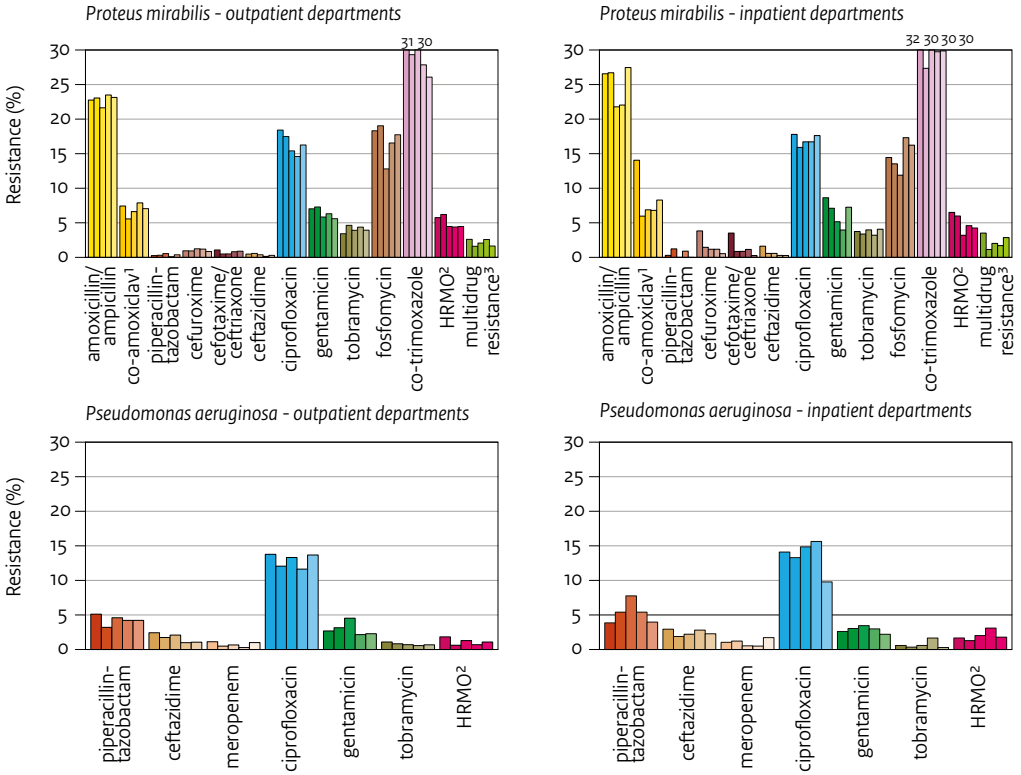


Figure 4.3.5.1 (continued) Trends in antibiotic resistance (from left to right 2015 to 2019) among diagnostic urine isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* from patients attending urology outpatient departments and patients admitted to urology inpatient departments in ISIS-AR



[1] *Resistance to co-amoxiclav was calculated according to the breakpoint for non-uncomplicated urinary tract infection. During 2016, a new testpanel for Gram-negative bacteria, with co-amoxiclav concentrations being adapted to EUCAST testing guidelines, was introduced for the VITEK2 automated system. This results in higher MIC values for co-amoxiclav, which subsequently influence resistance from 2016 onward to higher levels than before (see chapter 4.1.1 for more detailed information).*

[2] *Highly resistant microorganism (HRMO), defined according to HRMO guideline of the WIP (https://www.rivm.nl/documenten/wip-richtlijn-brmo); for E. coli, K. pneumoniae, and P. mirabilis as one or more of the following: 1) extended-spectrum beta-lactamase (ESBL)-producing, estimated by ESBL confirmatory tests, or, if no data on confirmatory tests were available, by resistance to cefotaxime/ceftriaxone and/or ceftazidime, 2) resistant to both fluoroquinolones and aminoglycosides, or 3) carbapenemase producing (CPE), estimated by confirmatory tests of carbapenemase production (both phenotypical and molecular), or, if no data on confirmatory tests were available, by resistance to meropenem or imipenem; for P. aeruginosa as resistant to ≥3 antimicrobial groups among fluoroquinolones, aminoglycosides, carbapenems, ceftazidime, and piperacillin-tazobactam.*

[3] *Defined as resistance to all of the following oral agents: co-amoxiclav (according to breakpoint for non-uncomplicated urinary tract infection), ciprofloxacin, and co-trimoxazole.*

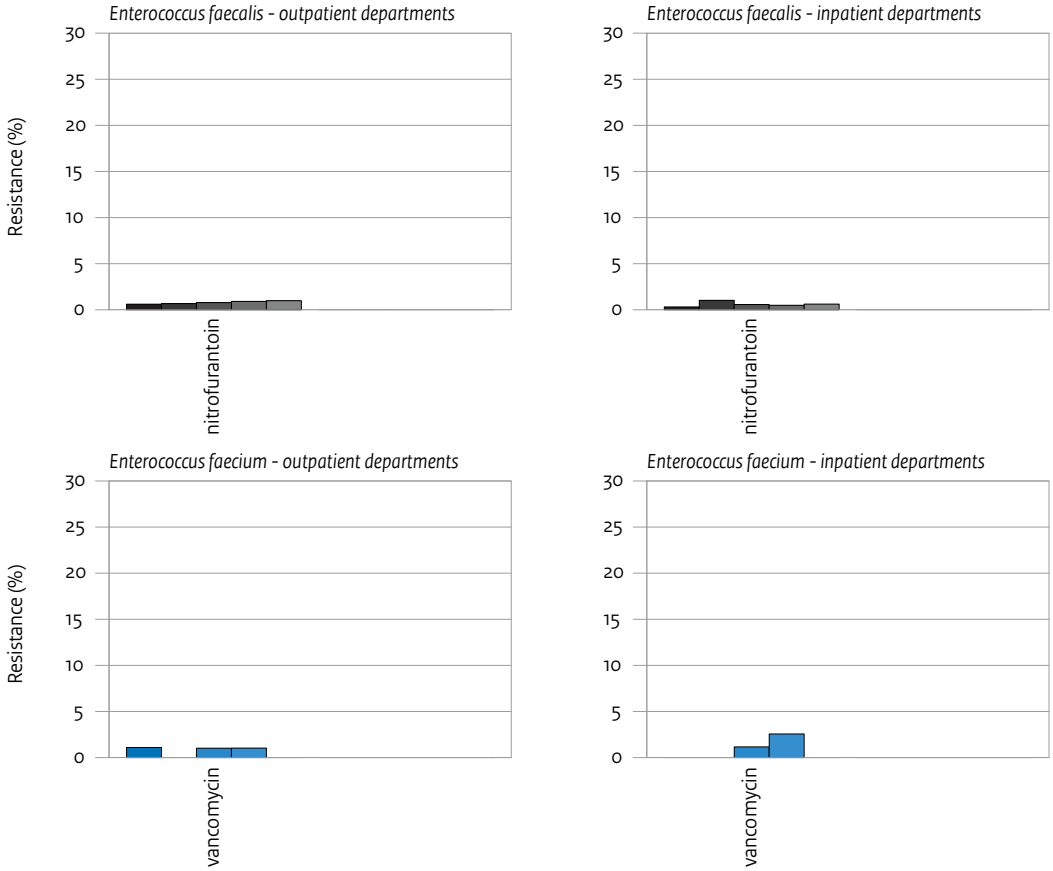
Table 4.3.5.3 Resistance levels (%) among diagnostic urine isolates of *E. faecalis* and *E. faecium* from patients attending urology outpatient departments (OPD) and patients admitted to urology inpatient departments (IPD), ISIS-AR 2019

	E. faecalis		*E. faecium*	
	OPD	IPD	OPD	IPD
Antibiotic				
amoxicillin/ampicillin	-	-	83	87
vancomycin	0	0	0	0
nitrofurantoin	1	1	-	-

10	Significant and clinically relevant increasing trend since 2015
10	Significant and clinically relevant decreasing trend since 2015
10	No significant and clinically relevant time trend

(For the definition of a clinically relevant trend see chapter 4.1.1)

Figure 4.3.5.2 Trends in antibiotic resistance (from left to right 2015 to 2019) among diagnostic urine isolates of *E. faecalis* and *E. faecium* from patients attending urology outpatient departments and patients admitted to urology inpatient departments in ISIS-AR



Key results

Enterobacterales

- In all *Enterobacterales*, resistance levels of 10% or lower were found for piperacillin-tazobactam (≤9%), cefotaxime/ceftriaxone (≤9%, except in *K. pneumoniae* from IPD patients: 11%), ceftazidime (≤9%), gentamicin (≤7%), and tobramycin (≤8%). In addition, levels of 10% or lower were found for meropenem/imipenem in *E. coli* and *K. pneumoniae* (0%); for fosfomycin (2%) and nitrofurantoin (≤4%) in *E. coli*; and for co-amoxiclav (≤8%), cefuroxime (1%), and meropenem (0%) in *P. mirabilis*.
- In all *Enterobacterales*, resistance of 20% or higher was observed for trimethoprim (≥20%). Furthermore, resistance of 20% or higher was found for co-amoxiclav in *E. coli* and *K. pneumoniae* (≥21%), for amoxicillin/ampicillin (≥23%) and co-trimoxazole (≥26%) in *E. coli* and *P. mirabilis*, for ciprofloxacin in *E. coli* (≥20%), and for fosfomycin in *K. pneumoniae* (≥22%).
- A statistically significant and clinically relevant increase in resistance was observed for co-amoxiclav in *E. coli* (from 20% in 2015 to 36% in 2019 in OPD, from 22% to 40% in IPD) and *K. pneumoniae* (from 11% to 21% in OPD, from 13% to 22% in IPD), which may be partly due to the introduction of a new testpanel for the VITEK2 automated system in 2016 (for details see chapter 4.1.1). In addition, in *E. coli*, a statistically significant and clinically relevant increasing trend was observed for ceftazidime in OPD (from 3% in 2015 to 5% in 2019). Furthermore, in *K. pneumoniae*, resistance increased to a statistically significant and clinically relevant extent for piperacillin-tazobactam in OPD (from 5% in 2015 to 9% in 2019), and ciprofloxacin in OPD (from 13% to 17%). In *P. mirabilis* from IPD patients, statistically significant and clinically relevant decreases in resistance were observed for co-amoxiclav (from 14% in 2015 to 8% in 2019), cefuroxime (from 4% to 1%), cefotaxime/ceftriaxone (from 4% to 0%), and ceftazidime (from 2% to 0%).
- Resistance to empiric therapy combinations was ≤7%. In *E. coli*, resistance to gentamicin + co-amoxiclav in IPD increased to a statistically significant and clinically relevant extent (from 4% to 6%). In *P. mirabilis* from IPD patients, significant and clinically relevant decreasing trends in resistance were observed for gentamicin + cefuroxime (from 2% to 0%) and gentamicin + cefotaxime/ceftriaxone (from 2% to 0%).
- The percentage of HRMO was ≤10% in all *Enterobacterales*, except in *E. coli* and *K. pneumoniae* from IPD patients (12%). The percentage of multidrug resistance was ≤8%, except in *E. coli* from IPD patients (11%). Multidrug resistance increased to a statistically significant and clinically relevant extent in *E. coli* (from 6% in 2015 to 8% in 2019 in OPD and from 8% to 11% in IPD).

P. aeruginosa

- Resistance levels of 10% or lower were found for each of the selected agents, except for ciprofloxacin in OPD (14%).
- Resistance to empiric therapy combinations and the percentage HRMO was ≤2%.

E. faecalis and E. faecium

- Resistance levels of 10% or lower were observed for vancomycin (0%) and nitrofurantoin (1%, presented for *E. faecalis* only).
- Resistance levels of 20% or higher were observed for amoxicillin/ampicillin in *E. faecium* (≥83%).
- In E. *faecium* from OPD patients, a statistically significant and clinically relevant increasing trend in resistance was observed for amoxicillin/ampicillin (from 65% in 2015 to 83% in 2019).

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) is presented in table 4.4.1. The resistance levels in 2019 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 2019

	Urine	Wound or pus
Pathogen	N (%)	N (%)
E. coli	6,474 (41)	128 (8)
K. pneumoniae	1,562 (10)	42 (3)
P. mirabilis	1,875 (12)	153 (9)
Other *Enterobacterales*[1]	1,504 (10)	116 (7)
P. aeruginosa	858 (5)	179 (11)
Other non-fermenters[2]	121 (1)	29 (2)
Other Gram-negatives[3]	0 (0)	19 (1)
S. aureus	572 (4)	704 (43)
Other Gram-positives[4]	2,646 (17)	252 (16)

[1] *Klebsiella spp. (non-pneumoniae), Citrobacter spp., Enterobacter spp., Morganella spp., Proteus spp. (non-mirabilis), Providencia spp., Serratia spp., Raoultella spp., Hafnia spp., Pantoea spp.*

[2] *Acinetobacter spp., Pseudomonas spp. (non-aeruginosa), S. maltophilia, M. catarrhalis.*

[3] *B. fragilis complex.*

[4] *Enterococcus spp., A. urinae, S. pyogenes, S. anginosus, beta-haemolytic Streptococcus spp. gr G, S. oralis, S. agalactiae, S. pneumoniae, beta-haemolytic Streptococcus spp. gr C, S. dysgalactiae n.n.g., S. mitis, S. dysgalactiae subsp. equisimilis, Staphylococcus spp. (non-aureus complex), 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 2019

	E. coli	*K. pneumoniae*	*P. mirabilis*	*P. aeruginosa*
Antibiotic				
amoxicillin/ampicillin	47	-	21	-
co-amoxiclav[1] - non-uuti	39	25	7	-
piperacillin-tazobactam	6	15	0	6
cefuroxime	15	15	1	-
cefotaxime/ceftriaxone	7	6	0	-
ceftazidime	5	5	0	3
meropenem/imipenem	0	0	-	-
meropenem	-	-	0	1
imipenem	-	-	-	4
ciprofloxacin	19	12	15	13
gentamicin	6	2	5	3
tobramycin	7	3	3	1
fosfomycin	2	27	17	-
trimethoprim	26	19	35	-
co-trimoxazole	23	10	26	-
nitrofurantoin	4	-	-	-
Multidrug resistance				
HRMO[2]	10	7	4	2
multidrug resistance[3] - non-uuti	6	3	1	-

- = Resistance not calculated.

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

[1] *During 2016, a new testpanel for Gram-negative bacteria, with co-amoxiclav concentrations being adapted to EUCAST testing guidelines, was introduced for the VITEK2 automated system. This results in higher MIC values for co-amoxiclav, which subsequently influence resistance from 2016 onward to higher levels than before (see chapter 4.1.1 for more detailed information).*

[2] *Highly resistant microorganism (HRMO), defined according to HRMO guideline of the WIP (https://www.rivm.nl/documenten/wip-richtlijn-brmo); for E. coli, K. pneumoniae, and P. mirabilis as one or more of the following: 1) extended-spectrum beta-lactamase (ESBL)-producing, estimated by ESBL confirmatory tests, or, if no data on confirmatory tests were available, by resistance to cefotaxime/ceftriaxone and/or ceftazidime, 2) resistant to both fluoroquinolones and aminoglycosides, or 3) carbapenemase producing (CPE), estimated by confirmatory tests of carbapenemase production (both phenotypical and molecular), or, if no data on confirmatory tests were available, by resistance to meropenem or imipenem; for P. aeruginosa as resistant to ≥3 antimicrobial groups among fluoroquinolones, aminoglycosides, carbapenems, ceftazidime, and piperacillin-tazobactam.*

[3] *Defined as resistance to all of the following oral agents: co-amoxiclav, 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 2019

	S. aureus
Antibiotic	
flucloxacillin[1]	2
ciprofloxacin[2]	19
erythromycin	12
clindamycin including inducible resistance[3]	11
doxycycline/tetracycline	3
fusidic acid	7
co-trimoxazole	3

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

Key results

Enterobacterales

- For all *Enterobacterales*, resistance levels of 10% or lower were found for cefotaxime/ceftriaxone (≤7%), ceftazidime (≤5%), gentamicin (≤6%), and tobramycin (≤7%). In addition, resistance levels of 10% or lower were also found for piperacillin-tazobactam (6%), meropenem/imipenem (0%), fosfomycin (2%), and nitrofurantoin (4%) in *E. coli*; for meropenem/imipenem (0%) and co-trimoxazole (10%) in *K. pneumoniae*; and for co-amoxiclav (7%), piperacillin-tazobactam (0%), cefuroxime (1%), and meropenem (0%) in *P. mirabilis*.
- In *E. coli* and *K. pneumoniae* resistance levels ≥20% were found for co-amoxiclav (≥25%). Additionally, resistance levels were ≥20% for amoxicillin/ampicillin (≥21%), trimethoprim (≥26%), and co-trimoxazole (≥23%) in *E. coli* and *P. mirabilis*; and for fosfomycin in *K. pneumoniae* (27%).
- In all *Enterobacterales*, the percentage of HRMO was ≤10% and the percentage of multidrug resistance was ≤6%.

P. aeruginosa

- Resistance levels for each of the selected agents were ≤6%, except for ciprofloxacin (13%).

S. aureus

- Resistance lower than 10% was found for flucloxacillin (2%), doxycycline/tetracycline (3%), fusidic acid (7%), and co-trimoxazole (3%).

4.5 Respiratory pathogens

In this section, the distribution of pathogens isolated from diagnostic lower and upper respiratory tract samples and resistance levels of respiratory pathogens (*S. pneumoniae*, *H. influenza*, and *M. catarrhalis*) are presented separately for general practitioners' (GP) patients and hospital patients (outpatients and inpatients, including intensive care patients). For GP patients, the pathogen distribution is presented in table 4.5.1 and resistance levels among diagnostic isolates are shown in table 4.5.2. Results for hospital patients are presented in tables 4.5.3 and 4.5.4, respectively.

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, a sample is taken for routine diagnostic purposes when a lower respiratory tract infection is suspected and therefore selective sampling bias is expected to be smaller compared with the GP setting. However, 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 former 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, ISIS-AR 2019

	Lower respiratory tract	Upper respiratory tract
Pathogen	**N (%)**	**N (%)**
S. pneumoniae	196 (7)	16 (1)
Other Gram-positives[1]	334 (13)	1,610 (81)
H. influenzae	810 (31)	68 (3)
M. catarrhalis	256 (10)	26 (1)
Other non-fermenters[2]	428 (16)	68 (3)
Enterobacterales[3]	542 (21)	178 (9)
Other Gram-negatives[4]	53 (2)	12 (1)

[1] *Staphylococcus spp., S. dysgalactiae n.n.g., beta-haemolytic Streptococcus spp. gr C, S. dysgalactiae subsp. equisimilis, S. pyogenes, S. anginosus, beta-haemolytic Streptococcus spp. gr G, S. agalactiae, S. mitis, Enterococcus spp., C. perfringens.*

[2] *Pseudomonas spp., S. maltophilia, Acinetobacter spp.*

[3] *Klebsiella spp., Escherichia spp., Serratia spp., Enterobacter spp., Citrobacter spp., Proteus spp., Raoultella spp., Pantoea spp., Hafnia spp., Morganella spp., Cronobacter spp., Providencia spp.*

[4] *H. parainfluenzae, N. meningitidis.*

Table 4.5.2 Resistance levels (%) among diagnostic isolates of *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* from general practitioners' patients, ISIS-AR 2019

	S. pneumoniae	*H. influenzae*	*M. catarrhalis*
Antibiotic			
(benzyl)penicillin (I+R)[1]	6	-	-
amoxicillin/ampicillin	-	24	-
co-amoxiclav	-	11	2
erythromycin	16	-	3
doxycycline/tetracycline	14	1	0
co-trimoxazole	13	23	5

- = Resistance not calculated.

[1] *Susceptibility to (benzyl)penicillin was estimated based on laboratory S/I/R interpretation for oxacillin, or, if the result for oxacillin was I or R, for (benzyl)penicillin (see chapter 4.1.1 for more detailed information). However, there was no information on the breakpoint (meningitis or non-meningitis breakpoint) that was used by the laboratories for individual isolates. The reported proportion I+R should be interpreted as the proportion that is resistant in case of meningitis. For non-meningitis indications, the percentage I+R should be interpreted as the percentage non-wild type.*

Table 4.5.3 Distribution of isolated pathogens in diagnostic blood or cerebrospinal fluid and respiratory samples from patients attending outpatient departments and patients admitted to inpatient departments (incl. intensive care units), ISIS-AR 2019

	Blood or cerebrospinal fluid	Lower respiratory tract	Upper respiratory tract
Pathogen	**N (%)**	**N (%)**	**N (%)**
S. pneumoniae	1,449 (5)	1,571 (8)	25 (1)
Other Gram-positives[1]	15,880 (54)	3,338 (17)	1,637 (56)
H. influenzae	164 (1)	4,162 (21)	97 (3)
M. catarrhalis	24 (0)	1,319 (7)	32 (1)
Other non-fermenters[2]	826 (3)	2,903 (15)	241 (8)
Enterobacterales[3]	10,793 (36)	5,837 (30)	851 (29)
Other Gram-negatives[4]	484 (2)	402 (2)	43 (1)

[1] *Staphylococcus spp., S. oralis, S. agalactiae, beta-haemolytic Streptococcus spp. gr C, S. equi, S. dysgalactiae subsp. equisimilis, S. mitis, S. pyogenes, S. anginosus, beta-haemolytic Streptococcus spp. gr G, S. dysgalactiae n.n.g., Enterococcus spp., C. perfringens, A. urinae, L. monocytogenes.*

[2] *Pseudomonas spp., S. maltophilia, Acinetobacter spp., B. cepacia.*

[3] *Escherichia spp., Klebsiella spp., Enterobacter spp., Serratia spp., Proteus spp., Citrobacter spp., Morganella spp., Raoultella spp., Salmonella spp., Hafnia spp., Pantoea spp., Providencia spp., Yersinia spp., Shigella spp.*

[4] *H. parainfluenzae, B. fragilis complex, N. meningitidis, C. jejuni.*

Table 4.5.4 Resistance levels (%) among 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 2019

	S. pneumoniae	*H. influenzae*	*M. catarrhalis*
Antibiotic			
(benzyl)penicillin (I+R)[1]	6	-	-
amoxicillin/ampicillin	-	26	-
co-amoxiclav	-	12	1
erythromycin	11	-	3
doxycycline/tetracycline	9	1	1
co-trimoxazole	9	23	3

- = Resistance not calculated.

[1] *Susceptibility to (benzyl)penicillin was estimated based on laboratory S/I/R interpretation for oxacillin, or, if the result for oxacillin was I or R, for (benzyl)penicillin (see chapter 4.1.1 for more detailed information). However, there was no information on the breakpoint (meningitis or non-meningitis breakpoint) that was used by the laboratories for individual isolates. The reported proportion I+R should be interpreted as the proportion that is resistant in case of meningitis. For non-meningitis indications, the percentage I+R should be interpreted as the percentage non-wild type.*

Key results

S. pneumoniae

- For (benzyl)penicillin, the percentage of isolates with laboratory interpretation I+R was ≤10% in both patient groups (6%). In hospital patients, resistance levels of 9% were found for both doxycycline/tetracycline and co-trimoxazole.

H. influenzae

- Resistance of 10% or lower was found for doxycycline/tetracycline in both patient groups (1%).
- Resistance levels of 20% or higher were found for amoxicillin/ampicillin (24% in GP patients and 26% in hospital patients) and for co-trimoxazole (23% in both patient groups).

M. catarrhalis

- Resistance to each of the selected agents was ≤5% in both patient groups.

4.6 Antibiotic resistance in invasive neonatal Group B *Streptococcus* infections

Introduction

In many countries, intrapartum antibiotics are administered to prevent neonatal Group B *Streptococcus* (GBS) infection, either based on risk factors or screening. In the Netherlands, intrapartum antibiotics are prescribed in an estimated 17% of all deliveries. Further, up to 7.4% of neonates in high-income countries receive empirical antibiotic treatment for neonatal sepsis.[1] Several reports have noted increasing antimicrobial resistance among microorganisms causing neonatal sepsis after intrapartum antibiotic treatment.[2-4] While resistance to benzylpenicillin, the first choice antibiotic for intrapartum antibiotic prophylaxis (IAP), is hardly ever observed in GBS, resistance to macrolides is increasing.[5] In addition, resistance of GBS to clindamycin, the prophylactic antibiotic of choice for women with penicillin allergy, is on the rise in several countries.[6, 7] In this section we describe trends in antibiotic resistance in invasive GBS infections in neonates in the Netherlands.

Methods

Data from 28 laboratories that provide diagnostics for hospitals and for which continuous data from 2015 to 2019 were available in the ISIS-AR database, were included in the analysis. We included isolates of beta-haemolytic *Streptococcus* spp. group B from blood or cerebrospinal fluid samples, and their antibiotic susceptibility test (AST) data for (benzyl)penicillin, vancomycin, erythromycin, and clindamycin (incl. inducible resistance), in the years 2015-2019. Subsequently, we selected isolates from patients aged 0-3 months old at the date of sampling and included only the first isolate per patient to avoid repeated sampling causing bias in the results. Using logistic regression models on the antimicrobial susceptibility categories (S/I/R) as reported by the laboratories, we calculated resistance ('R') percentages and linear time trends for the selected antibiotics. Statistical significance and clinical relevance of trends were assessed using the criteria described in chapter 4.1.1. To assess whether the resistance percentages found in invasive isolates from neonates were overestimated due to selection pressure asserted by antibiotic prophylaxis, we additionally assessed the resistance percentages of GBS in 2019 for clindamycin and erythromycin in isolates from genital samples from women aged 18-45 years old , for whom a culture was requested by a gynaecologist, obstetricist, or general practitioner. The majority of these women will not have received (intrapartum) antibiotics before the culture specimen was obtained.

Results

In total, 391 invasive isolates from neonates were included in the analysis. The majority (94%) of all first isolates were obtained from blood (Table 4.6.1). Resistance to (benzyl)penicillin and vancomycin were not observed in the selected time period (Figure 4.6.1). For erythromycin, there was an increase in resistance between 2015 and 2017 (from 16% to 30%), but a decrease to 22% in 2019. For clindamycin (including inducible resistance) a similar pattern was observed: resistance increased from 18% in 2015 to 32% in 2017, but decreased to 24% in 2019. In 4,037 genital isolates from women in 2019, resistance percentages were 20% for clindamycin and 21% for erythromycin (data not shown).

Discussion

In invasive GBS infections in neonatal patients, substantial resistance levels were observed for erythromycin and clindamycin (including inducible resistance). This finding is consistent with reports from other countries. For example, in the United Kingdom the observation of increasing clindamycin resistance in GBS isolates has resulted in a change in the intrapartum prophylactic antibiotic policy for women with penicillin allergy from clindamycin to vancomycin or cephalosporins.[8] However, the (trends in) resistance percentages presented in this section should be interpreted with caution, because of the relatively low number of isolates that was available for our analyses. Furthermore, GBS cultured from neonates with invasive disease could reflect a selection of strains with reduced susceptibility to the antibiotic used for prophylaxis. Such a selection bias would likely be limited, as the majority of neonatal invasive GBS disease patients are born without IAP having been administered.[9] Furthermore, in our analysis in genital isolates from women aged 18-45 years similar resistance percentages were found, suggesting that the resistance found in isolates causing invasive disease among neonates is not attributable to selection of resistant strains by prophylaxis administration. IAP programs to prevent neonatal GBS infection expose a large number of mothers and neonates to antibiotics, in order to prevent a serious but rare infection. Therefore, using the proper antibiotic to target GBS is of importance to maximize the effectiveness of this program.

References

1. Achten, N.B., et al. Sepsis calculator implementation reduces empiric antibiotics for suspected early-onset sepsis. Eur J Pediatr, 2018. 177(5): p. 741-746.
2. Bizzarro, M.J., et al. Changing patterns in neonatal Escherichia coli sepsis and ampicillin resistance in the era of intrapartum antibiotic prophylaxis. Pediatrics, 2008. 121(4): p. 689-96.
3. Ecker, K.L., et al. The impact of group B Streptococcus prophylaxis on early onset neonatal infections. J Neonatal Perinatal Med, 2013. 6(1): p. 37-44.
4. Bromiker, R., et al. Correlation of bacterial type and antibiotic sensitivity with maternal antibiotic exposure in early-onset neonatal sepsis. Neonatology, 2013. 103(1): p. 48-53.
5. Lopes, E., et al. Increasing macrolide resistance among Streptococcus agalactiae causing invasive disease in non-pregnant adults was driven by a single capsular-transformed lineage, Portugal, 2009 to 2015. Euro Surveill, 2018. 23(21).
6. Nanduri, S.A., et al. Epidemiology of Invasive Early-Onset and Late-Onset Group B Streptococcal Disease in the United States, 2006 to 2015: Multistate Laboratory and Population-Based Surveillance. JAMA Pediatr, 2019. 173(3): p. 224-233.
7. Shipitsyna, E., et al. Significant shifts in the distribution of vaccine capsular polysaccharide types and rates of antimicrobial resistance of perinatal group B streptococci within the last decade in St. Petersburg, Russia. Eur J Clin Microbiol Infect Dis, 2020.
8. Bevan, D., et al. Modelling the effect of the introduction of antenatal screening for group B Streptococcus (GBS) carriage in the UK. BMJ Open, 2019. 9(3): p. e024324.
9. Hahn, B., et al. Cost-effectiveness of maternal immunization against neonatal invasive Group B Streptococcus in the Netherlands. In preparation.

Table 4.6.1 Distribution of blood and cerebrospinal fluid isolates (N (%)) of beta-haemolytic *Streptococcus* spp. group B from neonatal patients by year, from 28 laboratories, ISIS-AR 2015-2019.

Specimen type	2015	2016	2017	2018	2019
Blood	84 (93)	65 (93)	65 (93)	77 (96)	76 (94)
Cerebrospinal fluid	6 (7)	5 (7)	5 (7)	3 (4)	5 (6)

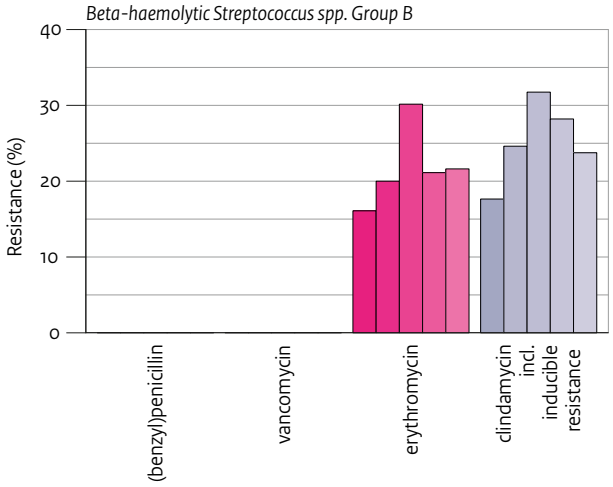
Table 4.6.2 Resistance levels (%) among blood and cerebrospinal fluid isolates of beta-haemolytic *Streptococcus* spp. group B from neonatal patients, ISIS-AR 2019.

	beta-haemolytic *Streptococcus* spp. group B
Antibiotic	
(benzyl)penicillin	0
vancomycin	0
erythromycin	22
clindamycin including inducible resistance	24

10	Significant and clinically relevant increasing trend since 2015
10	Significant and clinically relevant decreasing trend since 2015
10	No significant and clinically relevant time trend

(For the definition of a clinically relevant trend see chapter 4.1.1)

Figure 4.6.1 Trends in antibiotic resistance (from left to right 2015 to 2019) among blood and cerebrospinal fluid isolates of beta-haemolytic *Streptococcus* spp. group B from neonatal patients in ISIS-AR. Because of the relatively low number of isolates, (trends in) resistance percentages should be interpreted with caution.



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 significant challenges to clinicians and negatively impacts patient care.[1] CRE were first described in Europe in the early 2000's and their prevalence has increased since.[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 health care settings.[3] So far, CRE are mainly a problem in hospitals, 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 the Dutch national guideline suggests 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).

Prevalence and confirmatory testing of CRE in the Netherlands

Methods

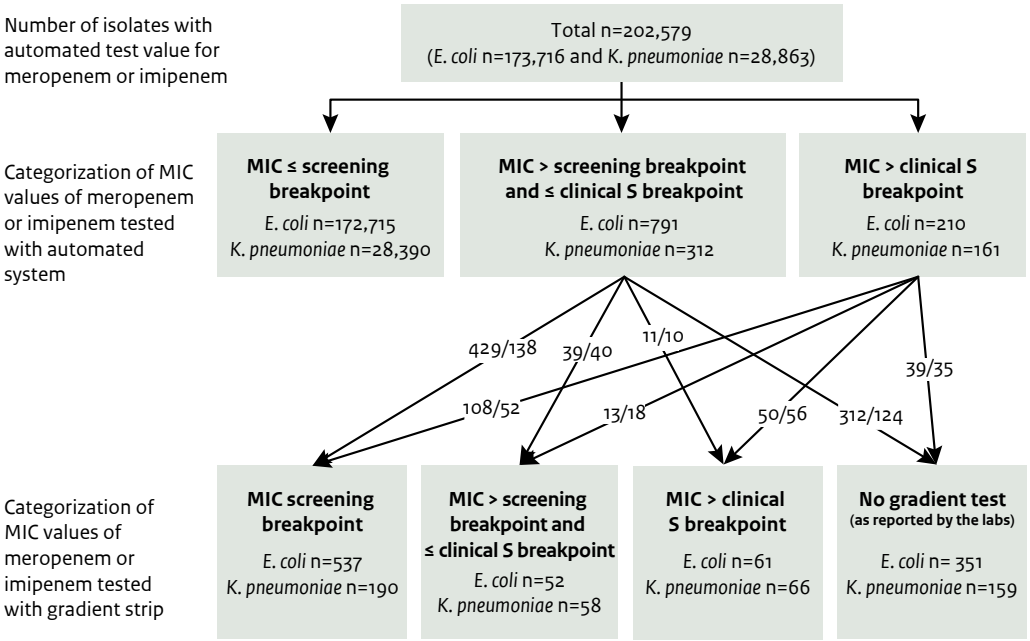
We searched the ISIS-AR database (years 2015-2019) for diagnostic and non-diagnostic *E. coli* and *K. pneumoniae* isolates, the two most prevalent *Enterobacterales* species, that were tested for meropenem and/or imipenem by automated system. The number of isolates of other *Enterobacterales* species were too small for a separate analysis and not included in this part of the chapter. Based on the crude automated test values, we categorized them as having either i) MIC ≤ the screening breakpoint as defined by the Dutch national guideline[1] (which is 0.25 mg/L for meropenem and 1 mg/L for imipenem), ii) MIC > the screening breakpoint and ≤ the EUCAST clinical S breakpoint (which is 2 mg/L for both imipenem and meropenem), or iii) MIC > the clinical S breakpoint. Subsequently, we searched the ISIS-AR and Type-Ned database for data on confirmatory tests (i.e. gradient strip tests and tests for carbapenemase production (phenotypic) or carbapenemase genes (genotypic)) for isolates with automated MIC > the screening breakpoint. We included only one isolate per patient per species: an isolate with a gradient strip test was prioritized over an isolate with an automated test only. Within those categories, we prioritized the most resistant isolate. Based on data of isolates from 38 laboratories, we calculated numbers of isolates with automated MIC in the respective categories in 2019. Subsequently, isolates with elevated automated MIC (i.e. > the screening breakpoint) were categorized by gradient strip test results. Based on data from 29 laboratories that continuously submitted data to ISIS-AR from 2015 to 2019, we assessed the percentage of isolates with i) elevated automated MIC that underwent further testing, and ii) gradient strip test confirmed elevated MIC, by year.

Results

Absolute numbers of isolates and categorization according to automated and gradient strip test MICs in 2019 are presented in Figure 4.7.1.1. Of a total number of 202,579 isolates with an automated test value for

meropenem or imipenem (173,716 *E. coli* and 28,863 *K. pneumoniae*), an elevated MIC on automated testing was found in 0.7% of isolates (1,474). Confirmatory testing using a gradient strip method (performed in 65.4% of isolates with elevated MIC) confirmed elevated carbapenem MIC values in 25% (237/964) of tested isolates (17% (113/650) of *E. coli* and 39% (124/314) of *K. pneumoniae*). Among 1,001 *E. coli* isolates with an elevated MIC on automated testing, 61 had an MIC > the clinical S breakpoint on gradient strip testing, versus 66 of 473 *K. pneumoniae* isolates.

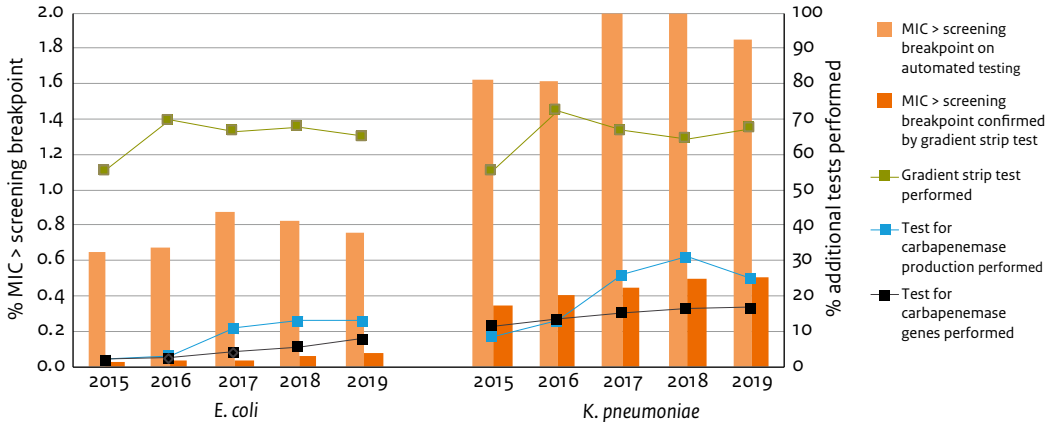
Figure 4.7.1.1 Results of automated and gradient strip testing of carbapenem susceptibility in *E. coli* and *K. pneumoniae* in 2019 , according to NVMM guideline Laboratory detection of highly resistant microorganisms (version 2.0, 2012) in 38 laboratories participating in ISIS-AR.



Screening breakpoint: meropenem 0.25 mg/L, imipenem 1 mg/L
Clinical S breakpoint: meropenem 2 mg/L, imipenem 2 mg/L

The overall prevalence of *E. coli* and *K. pneumoniae* strains with gradient strip test-confirmed MIC > the screening breakpoint has increased over the past five years (from 0.03% in 2015 to 0.08% in 2019 in *E. coli*, and from 0.35% to 0.50% in *K. pneumoniae*, Figure 4.7.1.2), which is worrying although it is still low. The use of gradient strip tests to confirm elevated automated carbapenem MIC values increased until 2016[6], but slightly decreased thereafter, to 65% in *E. coli* and 67% in *K. pneumoniae* in 2019. There was an increase in tests for carbapenemase production (from 2% in 2015 to 13% in 2019 in *E. coli* and from 9% to 25% in *K. pneumoniae*) and carbapenemase genes (from 2% to 8% in *E. coli* and from 12% to 17% in *K. pneumoniae*) in the past five years.

Figure 4.7.1.2 (Additional testing of) elevated carbapenem MIC (%) in *E. coli* and *K. pneumoniae* by year, in 29 laboratories, ISIS-AR 2015-2019.



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

Discussion

An elevated carbapenem MIC on automated testing was found in 0.7% of isolates in 2019. This is comparable with previous years. 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. The percentage of isolates with elevated automated MIC with a gradient strip test performed has slightly decreased since 2016. This is probably compensated by the observed increase in additional tests for carbapenemase production or carbapenemase genes in the past five years. This means that the vast majority of the suspected isolates is investigated further with one or more confirmatory tests, phenotypically and/or genotypically. It is important that confirmatory testing on both levels is performed, since phenotypic resistance does not always correlate with genotypic test results.

Molecular epidemiology

Methods

For the enhanced surveillance of CPE, Dutch laboratories are requested to submit isolates to the RIVM with an MIC for meropenem >0.25 mg/L and/or MIC for imipenem >1 mg/L and/or producing carbapenemase and/or a detected carbapenemase-coding 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 other *Enterobacterales* species/carbapenemase-encoding gene combinations. The RIVM confirms the species by MALDI-ToF, MIC for meropenem, carbapenemase production by carbapenemase inactivation method (CIM)[7], assesses the presence of carbapenemase-encoding genes by PCR (carba-PCR), and performs next-generation sequencing (NGS) for all isolates that are CIM positive.[8]
The data described in this chapter are based on the first unique CIM positive species/carbapenemase-encoding gene combination per person per year for the period 2017-2019 (based on sampling date and

allele based on NGS). This is different from the results published in NethMap in previous years, when species-gene combinations (gene based on carba-PCR) were used to identify unique combinations. Samples without a person ID were excluded from further analysis.
Up to 30 June 2019, epidemiological data on CPE isolates was collected using a questionnaire in Type-Ned. From 1 July 2019 onwards, CPE is mandatory notifiable[9] and since then the epidemiological data are collected by Municipal Health Services (MHS) and entered into the national system for notifiable diseases (OSIRIS). Only notifications with status 'definite' are included in this chapter ('authorised' (i.e. not complete/approved) notifications and notifications that do not meet the notification criteria are excluded). Questionnaire data was analyzed on person level and not on isolate level.
Finally, we summarise the CPE outbreaks that were reported to SO-ZI/AMR from 2017 to 2019.

Results

A total of 623 *Enterobacterales* isolates obtained in 2019, were submitted to the RIVM by 48 of the 55 Dutch medical microbiology laboratories. Among these were 363 unique carbapenemase-producing *Enterobacterales* isolates, obtained from 316 persons (mean age 62 years and 53% male). Of the 363 isolates 138 (38%) were *Escherichia coli*, 131 (36%) *Klebsiella pneumoniae*, 33 (9%) *Enterobacter cloacae* complex and the remaining 61 (17%) belonged to other species. When the EUCAST clinical breakpoints are applied, 97/363 (27%) have an MIC (for meropenem) above the cut-off of 8 mg/L. The number of unique isolates submitted to the RIVM increased from 234 in 2017, to 310 in 2018 and 363 in 2019. This amounts to a 17% increase of isolates submitted in 2019 compared to 2018 and 55% compared to 2017. However, neither the fraction carbapenemase-producing isolates nor the fraction of meropenem resistant isolates significantly changed over this three-year time period (Figure 4.7.1.3).

Figure 4.7.1.3 Carbapenemase production and meropenem sensitivity of *Enterobacterales* isolates submitted with a sampling date in 2017-2019. Panel A displays the carbapenemase production for the major species and panel B the distribution of meropenem resistant (CIM+) isolates.

A.

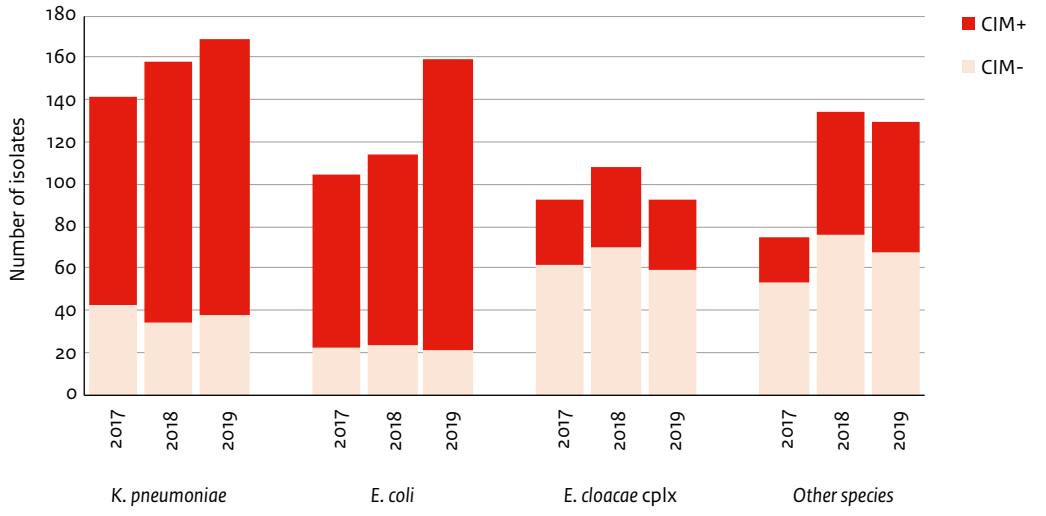
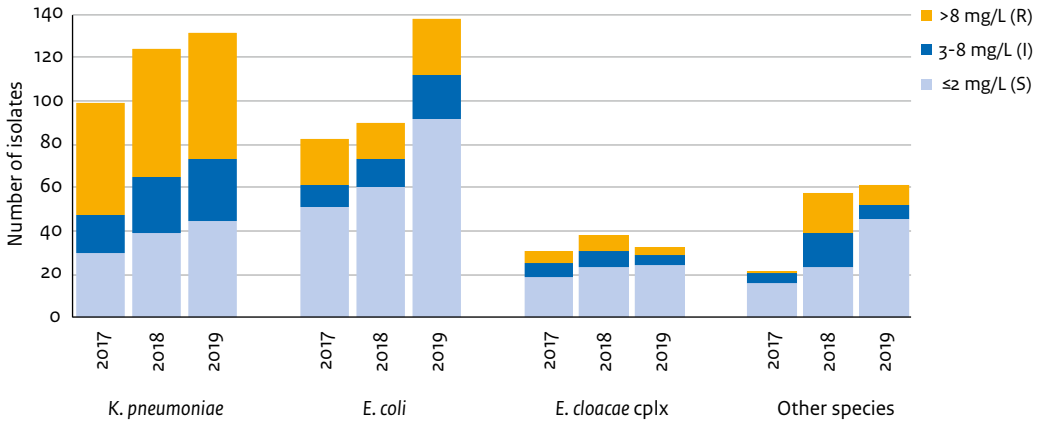


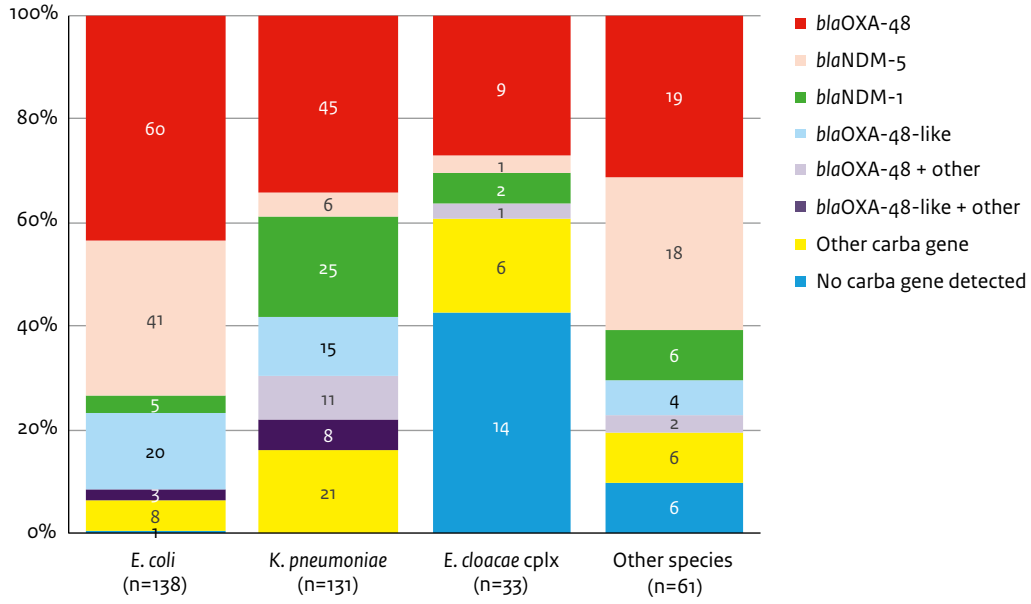
Figure 4.7.1.3 (continued) Carbapenemase production and meropenem sensitivity of *Enterobacterales* isolates submitted with a sampling date in 2017-2019. Panel A displays the carbapenemase production for the major species and panel B the distribution of meropenem resistant (CIM+) isolates.

B.



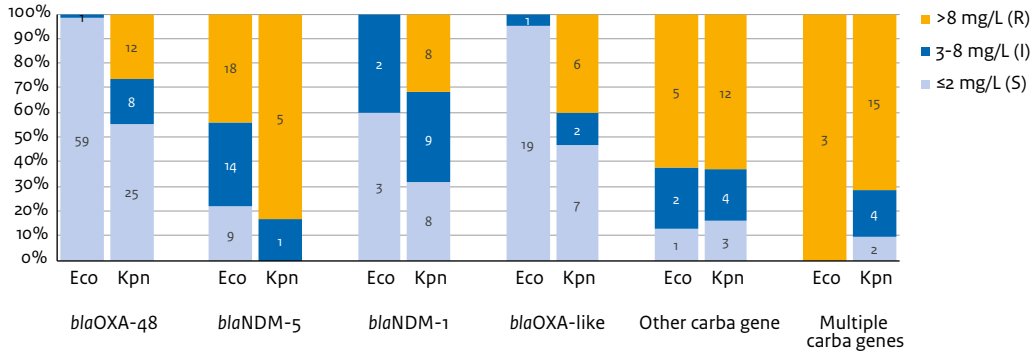
As in previous years, the *bla*OXA-48 gene was the most frequently identified carbapenemase-encoding gene in CPE isolates cultured and submitted in 2019. The *bla*OXA-48 allele, either alone or in combination with another carbapenemase-encoding gene, was present in 43%, 34% and 27% of the *E. coli*, *K. pneumoniae* and *E. cloacae* complex, respectively (Figure 4.7.1.4). In *E. coli*, 32% of the isolates carried *bla*NDM-5 and the gene was found in 13% of the *K. pneumoniae* isolates. Conversely, *bla*NDM-1 was found predominantly in *K. pneumoniae* isolates (24%) and only in 14% of the *E. coli* isolates. *bla*OXA-48-like alleles (*bla*OXA-181, *bla*OXA-232, *bla*OXA-244 and *bla*OXA-514) were found in 17% and 18% of *E. coli* and *K. pneumoniae*, respectively. In all, 54% (197/363) of all CPE analyzed in 2019 carried a *bla*OXA-48 or *bla*OXA-48-like gene. Seven percent (27/363) of the CPE carried two or more carbapenemase-encoding genes. The presence of multiple carbapenemase-encoding genes was most pronounced in *K. pneumoniae* (16%, 21/131) and in other species this occurred less frequent, e.g. only in 3/138 (2%) *E. coli* isolates. In 21 (10%) of the 363 CPE isolates cultured from patients in 2019 no carbapenemase-encoding gene was detected. Of these isolates 15 (71%) were *Enterobacter* spp. and 4 (19%) *Klebsiella aerogenes*, formerly classified as *Enterobacter aerogenes*. The nature of the apparent carbapenemase production in *Enterobacter* spp. is currently under investigation at the RIVM. There was a strong correlation between MIC for meropenem and the presence of particular species/carbapenemase-encoding gene combinations. None of the *E. coli* isolates carrying *bla*OXA-48 had MICs above the clinical breakpoint for meropenem resistance (MIC >8 mg/L; Figure 4.7.1.5). In contrast, 27% of the *K. pneumoniae* carrying *bla*OXA-48 were meropenem resistant. In general, a larger proportion of the *K. pneumoniae* isolates (44%, 58/131) were meropenem resistant compared to the *E. coli* isolates (19%, 26/138), irrespective of the carbapenemase-encoding genes present.

Figure 4.7.1.4 Distribution of carbapenemase-encoding genes in carbapenemase producing isolates submitted with a sampling date in 2019.



blaOXA-48-like denotes the blaOXA-48 gene variants blaOXA-181, blaOXA-232, blaOXA-244 and blaOXA-514
Other indicates one or more other carbapenemase-encoding genes

Figure 4.7.1.5 Relationship between MIC for meropenem and carbapenemase-coding genes in *E. coli* and *K. pneumoniae* isolates submitted with a sampling date in 2019.



Additional epidemiological questionnaire data was available in Type-Ned for 98/140 persons (70%) with a confirmed CPE isolate taken before 1 July 2019 (Table 4.7.1.1). Besides, one hundred and sixty-two CPE positive persons were reported in OSIRIS with a sampling date between 1 July (start of the mandatory notification) and 31 December 2019. For 154 of the 162 definite notifications (95%) one or more isolates were identified in the Type-Ned database, no isolate was found for eight notifications, and for 55 persons in Type-Ned no corresponding notification could be identified in OSIRIS.
Screening was the reason for taking the sample in 69% of the persons, which was 72% in 2017 and 2018. Hospitalization abroad for at least 24 hours within the previous two months was the most common risk factor for the presence of CPE (40%), with Turkey (n=20) and Morocco (n=14) leading the list of countries reported in both Type-Ned and OSIRIS. This was 50% in 2018 and 49% in 2017. No risk factor was identified in 38%, which was 34% in 2017 and 31% in 2018. When risk factors are assessed for patients with diagnostic isolates solely, hospitalization abroad for at least 24 hours within the previous two months was reported less often (21% in Type-Ned, 15% in OSIRIS, 17% overall) and the majority had no risk factor (66% in Type-Ned, 69% in OSIRIS, 68% overall). Among persons with a screening isolate, 51% overall (49% in Type-Ned and 52% in OSIRIS) had been hospitalized abroad for at least 24 hours during the previous two months and 25% (28% in Type-Ned and 24% in OSIRIS) had no risk factor.

In 2019, two new outbreaks with carbapenemase-producing *Enterobacterales* were reported to SO-ZI/AMR, see Table 4.7.1.2. In 2018 four outbreaks and in 2017 three new outbreaks were reported. Two outbreaks that started before 2019 ended in 2019: one outbreak caused by *Citrobacter freundii bla*NDM-5 in a hospital in Noord-Holland West (highest level phase 4) and one *K. pneumoniae* outbreak with *bla*NDM-1 and *bla*-OXA-232 in a hospital in Noord-Holland Oost / Flevoland (highest level phase 1). See chapter 4.7.6 for more details about SO-ZI/AMR.

Table 4.7.1.1 Epidemiological data of CPE positive persons with an isolate available in the enhanced surveillance system database Type-Ned (sampling date 1 January – 30 June 2019) and of notifications in OSIRIS (sampling date 1 July – 31 December 2019)[1].

Characteristic	Questionnaire isolate (Type-Ned)[1] 1 Jan – 30 Jun 2019 CPE positive persons n (%)[2]	Notification (OSIRIS)[1] 1 Jul – 31 Dec 2019 CPE positive persons n (%)[3]
Any questionnaire data available	98/140 (70)	162
Sample taking location		
Outpatient departments	45 (46)	NA
Inpatient departments (excluding Intensive Care Units)	22 (22)	NA
Intensive Care Units	8 (8)	NA
Other	23 (23)	NA
Reason for culturing		
Diagnostic	29 (30)	48 (30)
Screening	69 (70)	110 (68)
Other/unknown	NA	4 (2)
Colonisation with CPE or infection caused by CPE		
Colonisation	72 (73)	100 (62)[4]
Urinary tract infection	16 (16)	26 (16)[4]
Respiratory tract infection	5 (5)	3 (2)[4]
Sepsis/bacteraemia	0 (0)	4 (2)[4]
Other infection	5 (5)	10 (6)[4]
Unknown	0 (0)	24 (15)[4]
Residence		
Living independently	88 (90)	120 (74)
Nursing or elderly home	1 (1)	11 (7)
Facilities for small-scale housing for elderly	NA	6 (4)
Asylum seekers centre	4 (4)	4 (2)
Rehabilitation centre	1 (1)	4 (2)
Other/unknown	4 (4)	15 (9)
Underlying illness		
No underlying illness	51 (52)	NA
Malignancy/leukaemia or organ/bone marrow transplantation or immunosuppressive therapy (steroids/chemotherapy)	16 (16)	NA
Other	31 (32)	NA

Table 4.7.1.1 (continued) Epidemiological data of CPE positive persons with an isolate available in the enhanced surveillance system database Type-Ned (sampling date 1 January – 30 June 2019) and of notifications in OSIRIS (sampling date 1 July – 31 December 2019)[1].

Characteristic	Questionnaire isolate (Type-Ned)[1] 1 Jan – 30 Jun 2019 CPE positive persons n (%)[2]	Notification (OSIRIS)[1] 1 Jul – 31 Dec 2019 CPE positive persons n (%)[3]
Invasive medical procedure/diagnostics		
No	NA	81 (50)
Surgery	NA	37 (23)
Other (including invasive procedure like endoscopy, cystoscopy, urinary catheter, renal dialysis)	NA	30 (19)
Unknown	NA	14 (9)
Risk factors		
No risk factor known/unknown	38 (39)	62 (38)
Hospitalization abroad >24 hours during the previous two months	40 (41)	64 (40)
Hospitalized in a country in:		
North Africa	13/40 (33)	13/64 (20)
West Asia (including Turkey)	8/40 (20)	17/64 (27)
South Asia	7/40 (18)	7/64 (11)
South Europe	5/40 (13)	11/64 (17)
Other region of the world/unknown	7/40 (18)	16/64 (25)
Already known carrier of CPE	6 (6)	3 (2)
Received care in a department of another healthcare facility with an ongoing outbreak of CPE in the previous two months	1 (1)	3 (3)
Contact with a hospital abroad in the last year in a different way than >24 hours during the previous two months	7 (7)	20 (12)
Travelling abroad in the past six months (Type-Ned)/twelve months (OSIRIS) without hospitalization or visiting a hospital	4 (4)	11 (7)
Known CPE outbreak in own healthcare facility	1 (1)	7 (4)
Work-related exposure to livestock animals	1 (1)	NA

NA: not applicable

[1] *Data are presented separately for the two time periods because data collection of epidemiological data in Type-Ned was discontinued after 30 June 2019 due to the introduction of the mandatory notification for CPE on 1 July 2019.*

[2] *Numbers and percentages are reported on person level with available questionnaire data for the particular characteristic (n=98 as denominator) unless otherwise indicated.*

[3] *Numbers and percentages are reported on person level with available questionnaire data for the particular characteristic (n=162 as denominator) unless otherwise indicated.*

[4] *The total number is higher than 162 and the summed percentages higher than 100% because for some persons more than one answer was registered.*

Table 4.7.1.2 Outbreaks reported in 2017-2019 to the Early warning and response meeting of Hospital-acquired Infections and Antimicrobial Resistance (SO-ZI/AMR).

Year	Regional Care Networks for Antibiotic Resistance	Healthcare setting	Main species	Carbapenemase gene	Highest level phase*
2017	Euregio Zwolle	Elderly home	*E. coli*	*bla*VIM-1	1
	Noord-Brabant	Hospital	*K. pneumoniae*	*bla*KPC-3	1
	Zuidwest NL	Hospital	*E. cloacae*	*bla*OXA-48	2
2018	Noord-Holland West	Hospital	*C. freundii*	*bla*NDM-5	4
	Noord-Holland Oost / Flevoland	Hospital	*K. pneumoniae*	*bla*NDM-1 and *bla*OXA-232	1
	Noord-Brabant	Hospital	*K. pneumoniae*	*bla*OXA-48	1
	Zuidwest NL	Hospital	*K. pneumoniae*	*bla*NDM-5 and *bla*OXA-48	1
2019	Noord-Holland Oost / Flevoland	Hospital	*K. pneumoniae*	*bla*OXA-48	1
	Holland-West	Hospital	*K. pneumoniae*	*bla*OXA-48	1

* *The SO-ZI/AMR assesses the risk of the outbreak to public health, monitors the course of the outbreak and may advise a hospital to request external expertise. Based on this risk assessment (including updates based on 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. An outbreak (phase 1) that lasts more than 2 months is automatically categorized as phase 2. If a potential threat to the public health exists, the outbreak will be classified as phase 3; phase 4 and 5 describe potential management issues. See Chapter 4.7.6 for more details about SO-ZI/AMR.*

Discussion

In 2019, slightly more carbapenemase-producing *Enterobacterales* isolates were submitted to the RIVM than in 2017 and 2018. However, the fraction of isolates producing carbapenemase and 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 health care centers.

Since the end of 2019 cluster numbers are included in the Type-Ned database and in case multi-institutional clusters are detected, the respective MMLs are contacted by the RIVM to consent to share their name to the other MMLs involved in the cluster to enable collaboration in potential transmission control.

It is unknown if 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: 95% of the definite notifications have a corresponding isolate in Type-Ned. Remarkable is, however, that more CPE isolates of positive persons are submitted to Type-Ned without a corresponding notification, which may be the result of several causes: 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. It is not expected that these limitations have a major influence on trends of CPE in the Netherlands.

Finally, due to the differences in questions and answers between the Type-Ned and OSIRIS questionnaires it was decided to present data from both sources separately.

Conclusions

- The proportion of *E. coli* and *K. pneumoniae* isolates with elevated carbapenem MIC values (i.e. > the screening breakpoint) on automated testing has remained stable (around 0.7%) over the past five years.
- The overall percentage of *E. coli* and *K. pneumoniae* with an elevated MIC confirmed with a gradient strip test has increased over the past five years, but was still low overall (0.08% and 0.50% in 2019, respectively).
- Confirmatory testing of elevated MIC values with a gradient strip method has slightly decreased since 2016, but the use of tests for carbapenemase production (phenotypic) or carbapenemase genes has increased over the past five years.
- The number of CPE submitted to the RIVM in 2019 slightly increased compared to 2017 and 2018.
- 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 predominant carbapenemase-producing *Enterobacterales* species were *E. coli*, *K. pneumoniae* and species belonging to the *E. cloacae* complex.
- MIC for meropenem was generally higher for *K. pneumoniae* than for *E. coli* isolates harboring *bla*OXA-048 or *bla*OXA-48-like genes. Still, these isolates were more sensitive for meropenem than isolates carrying other carbapenemase-encoding genes.
- Targeted screening because of suspected CPE carriage is the reason for sampling in 69% of the CPE positive persons.
- In 40% there is a relation with hospitalization abroad for more than 24 hours during the last 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 38% of the CPE positive persons no known risk factor is present. Approximately 50% of these persons had cultures taken because of screening and 50% because of a diagnostic reason.

References

[1] Tängdén 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] Cantón R, Akóva M, Carmeli Y, Giske CG, Glupczynski Y, Gniadkowski M, Livermore DM, Miriagou V, Naas T, Rossolini GM, Samuelsen Ø, 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: EuroSurveill. 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] de Greeff SC, Mouton JW, NethMap 2018: Consumption of antimicrobial agents and antimicrobial resistance among medically important bacteria in the Netherlands / MARAN 2018: Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2017.

[7] 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.

[8] 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. Clini Microbiol Infect. 2020. doi: 10.1016/j.cmi.2020.01.027.

[9] Staatsblad van het Koninkrijk der Nederlanden. Besluit van 26 april 2019, houdende aanpassing van het Besluit publieke gezondheid vanwege een meldingsplicht voor Carbapenemaseproducerende Enterobacteriaceae. Staatsblad 2019, 184. 20 mei 2019. Available from: http://zoek.officielebekendmakingen.nl/stb-2019-184.pdf.

4.7.2 Vancomycin-resistant Enterococci

Introduction

In the last few years, a growing number of Dutch hospitals have been confronted with outbreaks of vancomycin-resistant *Enterococcus faecium* (VRE). From 2012 onwards, in-depth analysis of the evolutionary relatedness of *E. faecium* genotypes on a population level using Multi Locus Sequence Typing (MLST) was performed by the UMC Utrecht. Unfortunately, since 2018, centrally collected and aggregated national data on molecular typing of VRE are no longer available.

Methods

VRE outbreaks are reported through the Early warning and response meeting of Hospital-acquired 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 VRE in *E. faecium* isolates among patients in various healthcare settings in the Netherlands was determined. Only diagnostic isolates (i.e. infection-related and thus non-screening samples) from routine practice were included. Numbers are based on data from 30 laboratories in the Netherlands that continuously reported to the ISIS-AR database in the past five years. The first *E. faecium* isolate per patient was selected.

Results

In 2019, 19 outbreaks with VRE have been reported in the Netherlands in SO-ZI/AMR, all of them in hospitals, with a median reported number of 6 patients involved (range 2 – 37 patients). The annual number of outbreaks in the last few years fluctuates around 10-15 outbreaks per year. In total, since the start of SO-ZI/AMR in April 2012, 106 outbreaks with VRE have been reported in the Netherlands. The contribution of VRE outbreaks is substantial, with a proportion varying between 20 and 32% of all reported outbreaks in SO-ZI/AMR yearly.

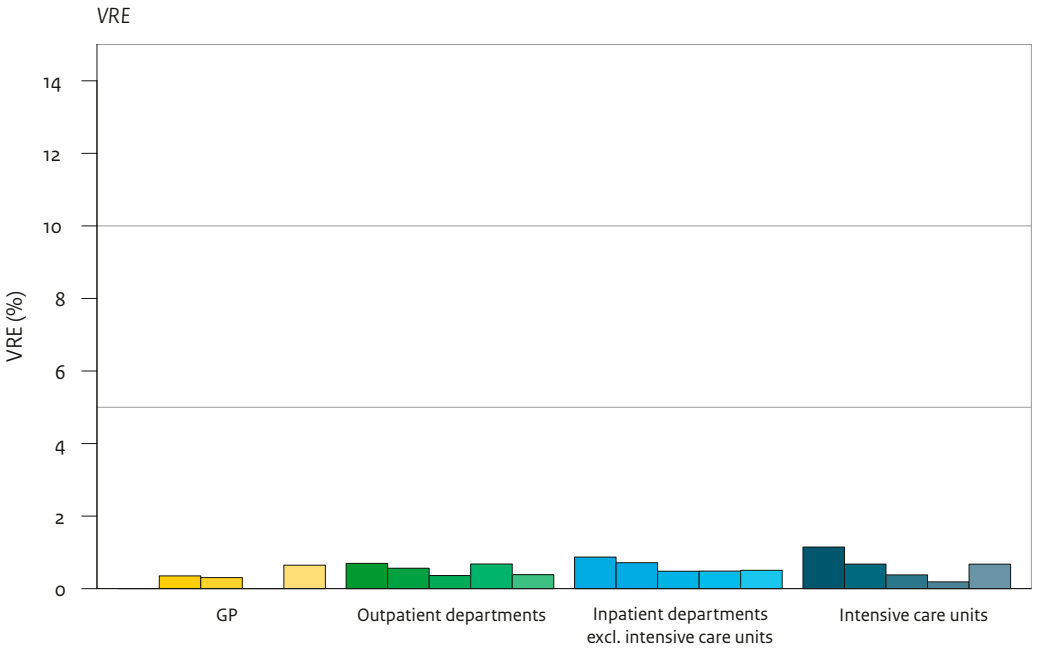
The percentage of VRE isolates in general practitioner patients and outpatient and inpatient hospital departments in 2019 in the Netherlands based on ISIS-AR is shown in table 4.7.2.1. Figure 4.7.2.1 shows the trends in vancomycin-resistance over the years. The number of diagnostic isolates with VRE was continuously low over the years.

Table 4.7.2.1 Vancomycin-resistant *E. faecium* (VRE) in the Netherlands in 2019 in diagnostic samples, based on ISIS-AR data.

Type of department	Tested isolates, N	VRE, N (%)
GP	309	2 (1)
Outpatient departments	259	1 (0)
Inpatient departments excluding intensive care units	1,777	9 (1)
Intensive care units	444	3 (1)
Total	**2,789**	**15 (1)**

Numbers are based on a selection of 30 laboratories.
The first diagnostic E. faecium isolate per patient was selected.
Based on re-interpretation according to EUCAST 2019.
The prevalence of VRE isolates was based on positivity of confirmation tests, or, if these tests were lacking, on laboratory S/I/R interpretation for amoxicillin/ampicillin and vancomycin, with VRE being defined as resistant to amoxicillin/ampicillin and vancomycin.

Figure 4.7.2.1 Trends in Vancomycin-resistant *E. faecium* (VRE) in the Netherlands (from left to right 2015 to 2019), based on ISIS-AR data.



Numbers are based on a selection of 30 laboratories.
The first diagnostic E. faecium isolate per patient per year was selected.
Based on re-interpretation according to EUCAST 2019.
The prevalence of VRE isolates was based on positivity of confirmation tests, or, if these tests were lacking, on laboratory S/I/R interpretation for amoxicillin/ampicillin and vancomycin, with VRE being defined as resistant to amoxicillin/ampicillin and vancomycin.

Discussion

Currently, there are no centrally collected data on molecular typing of VRE isolates or acquisition of novel resistance determinants by VRE in the Netherlands, even though the WHO marked VRE as a "high priority antibiotic resistant organism". Thus, there are no longer reliable data available on the molecular epidemiology of VRE in Dutch hospitals since 2018. The number of reported VRE outbreaks seems to be stable in the last few years, just as the low proportion of infection-related isolates with VRE in various healthcare settings. Notably, this is in contrast in the majority of European countries, where the number of invasive *E. faecium* isolates with resistance to vancomycin is considerably increasing in the past years.[1,2] In 2015 in the EU/EEA, the population-weighted mean percentage of invasive *E. faecium* with resistance to vancomycin was 10.5% and increased significantly to 17.3% in 2018.[1] The national percentages of invasive *E. faecium* isolates with resistance to vancomycin ranged from 0% in Iceland, Slovenia and Luxemburg to 59.1% in Cyprus. Twelve of the 30 reporting EU/EEA countries documented resistance percentages below 5%[1], including neighboring countries of the Netherlands such as Belgium, Luxemburg and France. The UK, Ireland, Denmark and Germany have higher (>12%) percentages of invasive VRE, and this percentage seems to increase in Germany and Denmark.[1,2,3] In addition, Enterococci have shown to be able to develop resistance towards last resort antibiotics such as daptomycin, linezolid and/or tigecycline.[4] Without a nation-wide surveillance to monitor the emergence of *E. faecium* with these resistance mechanisms, additional new resistances will be missed and may disseminate.[4,5]

Conclusions

- The contribution of hospital outbreaks with VRE is substantial and remains stable over the last few years.
- The proportion of VRE in infection-related isolates with *E. faecium* in various healthcare settings varies marginally below 1% and has not changed in the previous five years.
- There are no longer reliable data available on the molecular epidemiology of VRE in Dutch hospitals, which is a cause for great concern.

References

[1] https://www.ecdc.europa.eu/sites/default/files/documents/surveillance-antimicrobial-resistance-Europe-2018.pdf

[2] 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] WGS of 1058 *Enterococcus faecium* from Copenhagen, Denmark, reveals rapid clonal expansion of vancomycin-resistant clone ST80 combined with widespread dissemination of a *vanA*-containing plasmid and acquisition of a heterogeneous accessory genome. J Antimicrob Chemother. 2019 Jul 1;74(7):1776-1785

[4] 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] 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 still a country with a low MRSA prevalence. This is most probably explained by the strict "search and destroy" MRSA policy and the low use of antibiotics. The ISIS-AR database contains 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, at a national level enhanced MRSA surveillance was started in 1989 by the RIVM.

Methods

From the ISIS-AR database, *S. aureus* isolates, including MRSA, were identified for 2019. Numbers are based on data from 30 laboratories that continuously reported complete data to the ISIS-AR database during the five most recent years (2015 to 2019). Only the first *S. aureus* isolate per patient was selected.
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). Isolates in the database were categorized 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). Livestock-associated MRSA (LA-MRSA) is separately reported as MLVA-complex MC0398. From November 2016 on, next-generation sequencing (NGS) has been added to the enhanced MRSA surveillance for diagnostic isolates only.
The data from the molecular surveillance were based on the first MRSA isolate per person per year in the period 2008 to 2019 to investigate trends in molecular results, with the exception that the first diagnostic isolate is included when both a screening and a diagnostic sample are submitted from the same person in one year. Samples from non-human origin, *S. aureus* lacking a *mec* gene (*mecA* or *mecC*), samples that could not be typed by MLVA, and isolates without a person ID were also excluded from further analysis.
A questionnaire on patient characteristics is requested to be completed as part of the enhanced surveillance. Late November 2018, a new version of the epidemiological questionnaire was launched. Epidemiological data in this chapter are described for the period 2017 to 2019 and are analysed on person level per calendar year.

Results

Prevalence

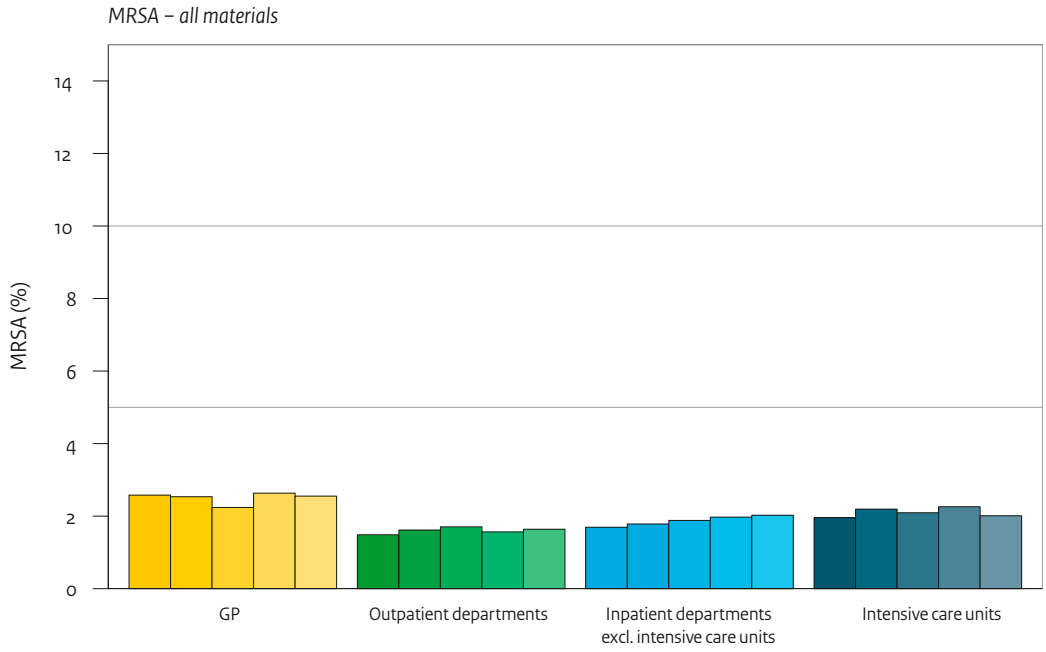
The proportion of *S. aureus* that is identified as MRSA amongst diagnostic isolates (including blood samples) based on ISIS-AR was 2% (621/30,661). The percentages were similar among the various types of departments (Table 4.7.3.1). Figure 4.7.3.1 shows the trends in MRSA from 2015 to 2019 in all diagnostic isolates, which seems to be quite stable. However, screening using selective culture media will strongly favor the isolation of MRSA over methicillin susceptible *S. aureus*. Therefore, the true MRSA prevalence in the population will be overestimated if based on all samples. In blood isolates, expected to be most unbiased, MRSA prevalence was 1.4% (35/2,586).

Table 4.7.3.1 Methicillin-resistant *S. aureus* (MRSA) in the Netherlands in 2019, based on ISIS-AR data.

Type of department	Tested isolates, N	MRSA, N(%)
GP	8,266	211 (3)
Outpatient departments	11,226	184 (2)
Inpatient departments excluding intensive care units	10,125	205 (2)
Intensive care units	1,044	21 (2)
Total	**30,661**	**621 (2)**

Numbers are based on a selection of 30 laboratories.
The first diagnostic S. aureus isolate per patient was selected.
Based on laboratory S/I/R interpretation.
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/I/R interpretation for cefoxitin. If no data on a cefoxitin test was available, the prevalence was based on laboratory S/I/R interpretation of flucloxacillin/oxacillin.

Figure 4.7.3.1 Trends in Methicillin-resistant *S. aureus* (MRSA) in the Netherlands (from left to right 2015 to 2019), based on ISIS-AR data.



Numbers are based on a selection of 30 laboratories.
The first diagnostic S. aureus isolate per patient per year was selected.
Based on laboratory S/I/R interpretation.
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/I/R interpretation for cefoxitin. If no data on a cefoxitin test was available, the prevalence was based on laboratory S/I/R interpretation of flucloxacillin/oxacillin.

Molecular results and epidemiology

A total of 3,789 genotyped isolates obtained in 2019 from 3,560 persons (mean age 44 years (standard deviation 25 years) and 1,822 (51.2%) male) submitted by 53 laboratories fulfilled the inclusion criteria (*S. aureus mecA* or *mecC* gene positive, from human origin with a known person ID). Thus, 3,560 isolates from single persons were used for further analysis.

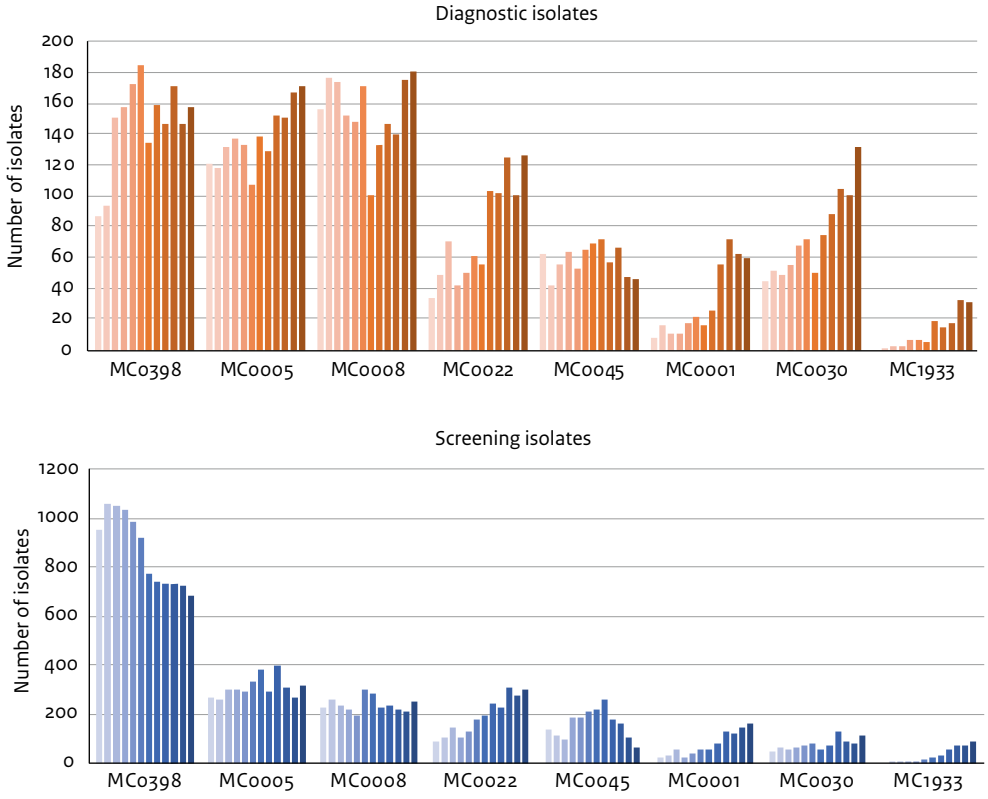
As in previous years, the majority of the 3,560 isolates were cultured from samples submitted to the MML from hospitals (n=2,101; 59%), followed by GPs (n=1,120; 31%) and nursing or elderly homes (n=203; 6%). Based on culture methods and origin of the samples, 68% (n=2,409) of the isolates were submitted as screening samples (mainly swabs of nose, throat and perineum) (Figure 4.7.3.2). A total of 1,142 samples (32%) were submitted as diagnostic sample with the majority being wound material or pus (844/1,142; 74%) and 38 blood samples (3%). For 9 samples (0.3%), the origin of the sample was unknown. All these proportions are similar to data from 2018.

For 2019, the MRSA population could be divided into 745 MLVA-types, which were grouped into 25 MLVA-complexes (MCs; 3,397 isolates). For 74 MLVA-types no MLVA-complex (163 isolates) could be assigned. The most frequently found MLVA-complex in 2019 was MC0398, also known as livestock-associated MRSA (LA-MRSA), which was detected in 846/3,560 (24%) of the isolates. Of all LA-MRSA isolates, 19% were diagnostic isolates (based on culture methods and origin of the samples), 81% were obtained from targeted screening, and for 0.4% it was unknown, comparable to previous years. The number of submitted LA-MRSA screening isolates has decreased over time from 949 isolates in 2008 to 686 in 2019. In contrast, the number diagnostic isolates increased from 87 in 2008 to 157 in 2019 (Figure 4.7.3.2).

In 2019 the proportion of isolates classified as diagnostic, was lowest for MC0398. However, these LA-MRSA isolates ranked third in absolute numbers of all diagnostic isolates among the Top8 MLVA-complexes in 2019. Conversely, the MC0030 complex had the highest proportion isolates classified as diagnostic (55%, 131/240), but ranked fourth in absolute numbers among the Top8 MLVA-complexes. During the 2008-2019 surveillance period, there has been a considerable increase in the prevalence of MC0022, MC0001 and MC0030 isolates, whereas the prevalence of MC0398 and MC0045 isolates has dropped. For MC0022 and MC0001 the increase in prevalence is seen both in screening and in diagnostic isolates. In contrast, the increase in MC0030 is predominantly in diagnostic isolates. The drop in MC0398 and MC0045 prevalence was predominantly in screening isolates and prevalence remained relatively stable in diagnostic isolates.

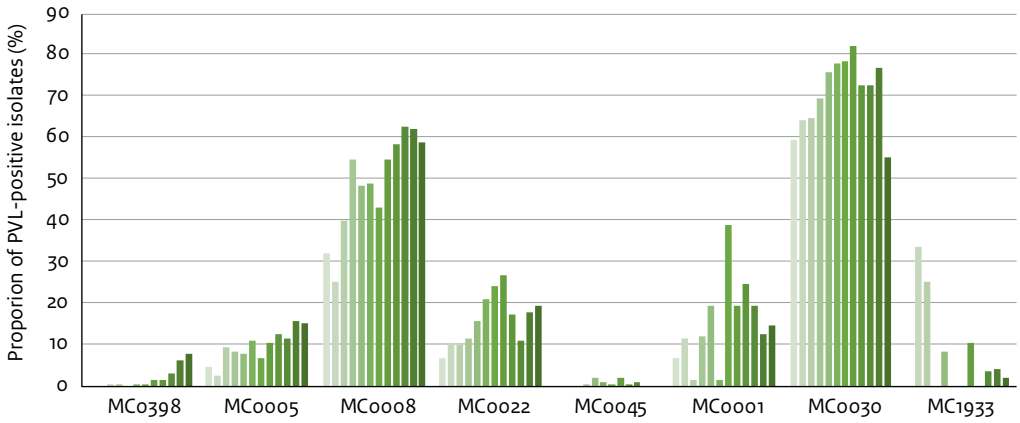
Panton-Valentine Leukocidin (PVL) positivity among all submitted MRSA isolates increased from 12% in 2008 to 18% in 2014 reaching 24% in 2019. In 2019, 40% (454/1,142) of the diagnostic isolates carried the PVL-encoding genes, whereas 17% (411/2,409) of the screening isolates were PVL positive. In 2019 MC0008 isolates had the highest proportion of PVL-positivity (59%, 254/430) (Figure 4.7.3.3). The most remarkable increase was in the MLVA-complex MC0398 (LA-MRSA), where PVL-positivity increased from 0% (no PVL-positive isolates) in 2008 to 8% (64/843) in 2019. Within MC0398, 81% (52/64) of the PVL-positive MC0398 isolates had MLVA-type MT0569.

Figure 4.7.3.2 Temporal trends of the eight most frequently identified MLVA complexes of MRSA in the Netherlands (2008 to 2019) among diagnostic and screening isolates, based on the enhanced MRSA surveillance data.



To better visualize the temporal changes, the Y-axes in the diagnostic and screening panels are different.
The first MRSA isolate per person per sampling year was selected.
The red bars represent the diagnostic isolates, the blue bars denote screening isolates.
Diagnostic indicates that the isolate was cultured from blood, cerebrospinal fluid, sputum, pus, urine or wound; screening isolates were cultured from swabs of nose, throat, perineum, rectum or insertion site.

Figure 4.7.3.3 Temporal changes of PVL-positivity among the eight most frequently identified MLVA complexes of MRSA in the Netherlands (2008 to 2019), 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 per year was selected.

Additional epidemiological questionnaire data for the period 2017 to 2019 was available for 8,453/10,334 (82%) persons. This was stable over the years: 2017: n=2,943/3,476 (85%), 2018: n=2,681/3,298 (81%), 2019: n=2,829/3,560 (79%). For 553 persons (7%) it was reported that they were an employee in a healthcare facility and for five persons it was unknown, so they were excluded from the data presented in Table 4.7.3.2. Targeted screening was the reason for taking the sample in 61% of the isolates, which decreased from 63% in 2017 to 61% in 2018 to 59% in 2019. As a consequence, the relative number of diagnostic isolates increased over the years. Hospitalization abroad for at least 24 hours during the previous two months was recorded for 449/7,846 persons (6%), which is similar for all three years. Turkey was most often mentioned as country of hospitalization (16% of all countries listed). Work-related exposure to livestock animals was reported for 12% of the persons, of which 95% had LA-MRSA which showed a slight decrease over time (98% in 2017, 95% in 2018 and 94% in 2019).
Of the patients with MRSA from diagnostic isolates, the large majority was previously not suspected for MRSA carriage. When risk factors are reviewed only for patients with diagnostic isolates, hospitalization abroad for at least 24 hours during the previous two months is reported only for 2% and the majority did not meet WIP risk category 1, 2 or 3[1] (73%), i.e. they were not suspected of MRSA carriage. In contrast, among screening isolates, 81% had been hospitalized abroad for at least 24 hours during the previous two months and 12% did not meet WIP risk category 1, 2 or 3[1].

Table 4.7.3.2 Epidemiological data of MRSA positive persons (excluding employees) with a genotyped isolate in the enhanced MRSA surveillance system with a sampling date from 2017 to 2019.

Characteristic	MRSA positive persons, n/N (%)
Questionnaire	
Any data available and no employee of healthcare facility	7,895/10,334 (76)
Sample taking location	
Outpatient departments	2,470/5,316 (46)
Inpatient departments (excluding Intensive Care Units)	1,754/5,316 (33)[a]
Intensive Care Units	189/5,316 (4)[a]
Other/unknown	903/5,316 (17)[a]
Reason for culturing	
Diagnostic	3,023/7,895 (38)
Screening	4,846/7,895 (61)
Unknown	26/7,895 (0)
Risk factors	
Work-related exposure to livestock animals	936/7,846 (12)
Pigs	644/936 (69)
Cattle	140/936 (15)
Hospitalization abroad >24 hours during the previous two months	449/7,846 (6)
Hospitalized in a country in:	
Western Asia (including Turkey)	87/449 (19)
Southern Europe	83/449 (18)
Western Europe	77/449 (17)
Asylum seeker living in asylum centre	390/7,846 (5)
Meeting WIP[1] risk category 1, 2 or 3[b]	4,251/6,858 (62)[c]

WIP: Working Party in Infection Control.

[a] *This question is only answered when the isolate is submitted by a hospital and is taken from a patient/client.*

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

[c] *This question did not appear in all questionnaires and is therefore not completed for all MRSA positive persons.*

Discussion

The distinction between screening and diagnostic isolates of the MRSA surveillance is solely based on the material and origin of the samples. Information on the reason for culturing is only available since the nationwide rollout of Type-Ned MRSA in November 2016 and for the period 2017 to 2019 still missing for 15% to 19% of the isolates. Therefore, some misclassification of screening and diagnostic isolates will 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*. In the ISIS-AR database, screening samples could potentially be misclassified as diagnostic samples, thereby falsely increasing the proportion of MRSA in diagnostic isolates.

The most common MLVA-complex found in the enhanced surveillance still is MC0398 (LA-MRSA). This is probably due to the search and destroy 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).

Conclusions

- The proportion of *S. aureus* that was MRSA positive in unbiased blood-culture isolates was 1.4%. The overall prevalence in diagnostic samples of other materials showed no increasing trend and remained around 2% (3% in general practices and 2% in outpatient departments, hospital departments, and Intensive Care Units).
- LA-MRSA is still the predominant MRSA clade in the Dutch enhanced MRSA surveillance. However, the absolute number of submitted LA-MRSA designated as screening isolates has decreased over time, while the number of diagnostic isolates increased. Still, compared to other MLVA complexes in 2019, the proportion of diagnostic isolates was lowest among MC0398.
- During the 2008-2019 surveillance interval there has been a considerable increase in the prevalence of MC0022, MC0001 and MC0030 isolates, whereas the prevalence of MC0398 and MC0045 isolates has dropped. This indicates that, although the genetic composition of the MRSA population is relatively stable, gradual shifts are occurring.
- PVL positivity among all submitted MRSA isolates increased from 12% in 2008 to 18% in 2014 reaching 24% in 2019. In 2019, 40% of the diagnostic isolates carried the PVL-encoding genes, whereas 17% of the screening isolates were PVL positive. MC0008 isolates had the highest proportion of PVL-positivity in 2019 (59%). In recent years the proportion of PVL-positive isolates found among LA-MRSA has been increasing, reaching 8% in 2019.
- Targeted screening because of suspected MRSA carriage is the reason for sampling in 61% of the MRSA positive persons, the remaining cultures are mainly taken because of a diagnostic reason.
- In 12% there is a relation with work-related exposure to livestock animals.
- A large proportion (38%) of the persons positive for MRSA does not seem to have a risk factor as defined in the WIP risk categories.

References

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

4.7.4 Carbapenemase-producing *Pseudomonas aeruginosa*

Introduction

Pseudomonas aeruginosa is one of the most common nosocomial pathogens that are intrinsically resistant to various antibiotics. The emergence of multidrug resistant (MDR) *P. aeruginosa* is a problem of global concern and in 2017, the World Health Organization classified carbapenem-resistant *P. aeruginosa* as 'priority 1: critical'[1].

Methods

For each patient the first *P. aeruginosa* isolate per year was extracted from the ISIS-AR database.
First, the number of phenotypical carbapenem resistant isolates was determined (based on re-interpretation according to EUCAST 2019). 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 multidrug resistant (MDR) was calculated. Multidrug resistance was defined as resistant 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. Numbers are based on a selection of 30 laboratories (out of a total of 55 laboratories in the Netherlands) which provided complete data on the last five years (2015 to 2019).
Although there is no national surveillance for carbapenemase-producing *P. aeruginosa* (CPPA), medical microbiology laboratories (MMLs) did send *P. aeruginosa* isolates to the RIVM via Type-Ned CPE, which is used for the national surveillance on carbapenemase-producing *Enterobacterales* (CPE), for additional analyses. As a courtesy submitted isolates were analyzed to confirm the species by MALDI-ToF.
Carbapenem resistance was determined by assessing minimal inhibitory concentrations (MIC) for meropenem by Etest. Carbapenemase production was evaluated by the carbapenemase inactivation method (CIM)[2] and the presence of carbapenemase-encoding genes by multiplex PCR. Since there is a need to assess the spread and resistance mechanisms of carbapenemase-producing *P. aeruginosa*, CPPA surveillance in the Netherlands will be started in 2020. This surveillance will include next-generation sequencing of CPPA and will provide a more structured data collection.

Results

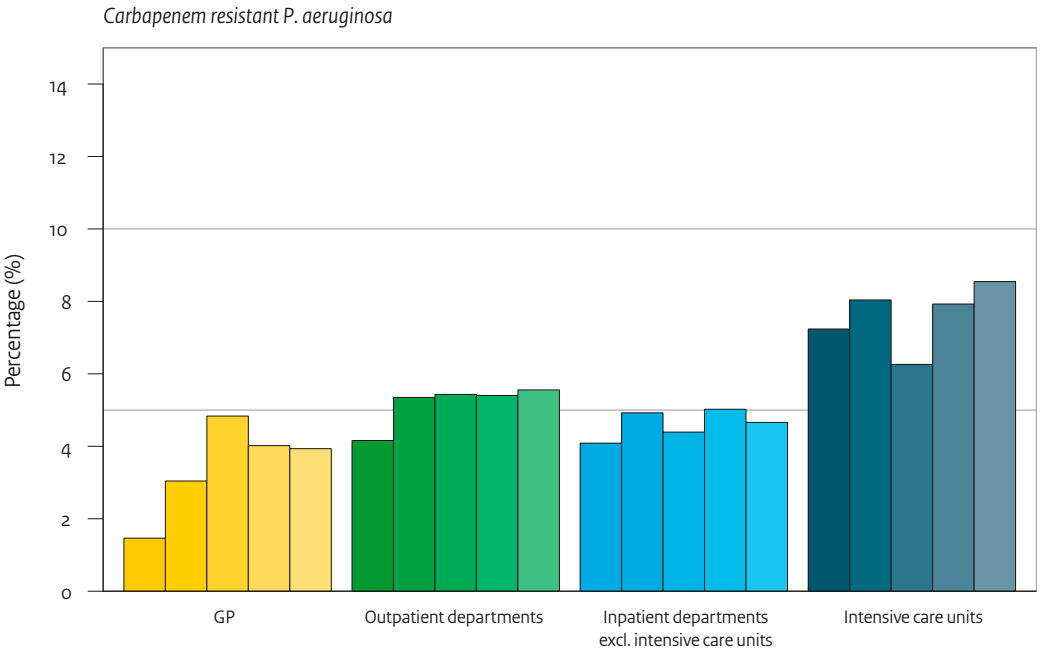
A search in the 2019 ISIS-AR database revealed that 5% (668/13,886) of the diagnostic (infection-related) *P. aeruginosa* isolates were phenotypically resistant to carbapenems (MIC >8 mg/L). This fraction was highest in isolates from ICUs (37/433; 9%) and lowest for isolates obtained from patients attending the general practitioner (180/4,575; 4%) (Table 4.7.4.1). The observed distribution appears to be relatively stable over the 2015-2019 time period (Figure 4.7.4.1). Of the total number of 668 carbapenem-resistant *P. aeruginosa* isolates, only 54 (8%) had available data on tests for carbapenemase production of which 3 (6%) showed a positive result.
Additional analyses in the 2019 ISIS-AR database showed that 2% (229/12,400) of the diagnostic (infection-related) *P. aeruginosa* isolates were MDR (Table 4.7.4.2). Approximately 57% (131/229) of the MDR isolates were phenotypically resistant to carbapenems (>8 mg/L).

Table 4.7.4.1 Phenotypical carbapenem-resistant *P. aeruginosa* in the Netherlands in 2019, based on ISIS-AR data.

Type of department	Tested isolates, N	Carbapenem-resistant *P. aeruginosa*, N(%)
GP	4,575	180 (4)
Outpatient departments	4,157	231 (6)
Inpatient departments excluding intensive care units	4,721	220 (5)
Intensive care units	433	37 (9)
Total	**13,886**	**668 (5)**

Numbers are based on a selection of 30 laboratories.
The first diagnostic P. aeruginosa isolate per patient was selected.
Based on re-interpretation according to EUCAST 2019.

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 2015 to 2019), based on ISIS-AR data.



Numbers are based on a selection of 30 laboratories.
The first diagnostic P. aeruginosa isolate per patient per year was selected.
Based on re-interpretation according to EUCAST 2019.

Table 4.7.4.2 Multidrug resistant MDR *P. aeruginosa* in the Netherlands in 2019, based on ISIS-AR data.

Type of department	Tested isolates, N	MDR *P. aeruginosa*, N(%)	Phenotypical carbapenem resistant MDR *P. aeruginosa*, N(%)
GP	4,268	36 (1)	14 (39)
Outpatient departments	3,725	95 (3)	60 (63)
Inpatient departments excluding intensive care units	4,049	80 (2)	44 (55)
Intensive care units	358	18 (5)	13 (72)
Total	**12,400**	**229 (2)**	**131 (57)**

Numbers are based on a selection of 30 laboratories.
The first diagnostic P. aeruginosa isolate per patient was selected.
Based on re-interpretation according to EUCAST 2019.
Multidrug resistance was defined as resistant to ≥3 antimicrobial groups among fluoroquinolones, aminoglycosides, carbapenems, ceftazidime, and piperacillin-tazobactam.
The proportion (%) of carbapenem resistance was compared to multidrug resistance.

The RIVM received 296 *P. aeruginosa* isolates via Type-Ned CPE from 286 patients sampled in 2019 submitted by 45 MMLs (Table 4.7.4.3). Of these isolates, 59 (21%, 59/286, one isolate per person) produced carbapenemase and were submitted by 26 MMLs. PCR revealed that the majority of the carbapenemase-producing isolates (46/59; 78%) carried a *bla*VIM gene. The remaining isolates carried *bla*IMP (14%), *bla*NDM (3%) and *bla*KPC (2%) and 2 isolates (3%) did not yield a PCR product. Isolates not producing carbapenemase as determined by the CIM test, did not yield a PCR product. Of the CPPA isolates 68% (40/59) had MICs for meropenem above the clinical breakpoint, whereas 38% (87/227) of the *P. aeruginosa* not producing carbapenemase had MICs above the breakpoint. For 22/59 (37%) of the patients tested positive for CPPA, completed questionnaires (originally designed for CPE) were available and this showed that 14/22 (64%) were clinical samples, three originated from ICU-patients.

Discussion

In 2019, 5% of *P. aeruginosa* in diagnostic isolates were phenotypically resistant to carbapenems. Of these isolates, for only 8%, data on carbapenemase tests (phenotypically or genotypically) were available in the ISIS-AR database. Of the 54 phenotypical carbapenem-resistant isolates with test results, 3 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 not always routinely included in the data submitted to the surveillance system, the percentage of carbapenemase producing *P. aeruginosa* may be biased. In addition, 2% of *P. aeruginosa* in diagnostic isolates were MDR, of which approximately 57% were phenotypically resistant to carbapenems.
The majority (78%, 46/59) of the CPPA submitted via Type-Ned CPE carried the *bla*VIM gene. Only 68% of the CPPA isolates had MICs for meropenem above the clinical breakpoint. The observed annual distribution was similar to that of the 2014-2018 period. Of the isolates not producing carbapenemase 38% were carbapenem-resistant. It is likely this is caused by other resistance mechanisms such as reduced cell wall permeability, increased efflux pump activity, AmpC activity etc.

Table 4.7.4.3 Distribution of carbapenemase-encoding genes based on PCR in carbapenemase-producing *P. aeruginosa* isolates received via Type-Ned CPE by the RIVM in 2019.

	Carbapenemase encoding gene					
MIC meropenem	**VIM**	**IMP**	**NDM**	**KPC**	**PCR-negative**	**Total (%)**
≤ 2 mg/L (S)	8				1	9 (15)
3-8 mg/L (I)	10					10 (17)
>8 mg/L (R)	28	8	2	1	1	40 (68)
Total	**46**	**8**	**2**	**1**	**2**	**59**

Conclusions

- In 2019, 5% of the Dutch *P. aeruginosa* in diagnostic isolates were phenotypically resistant to carbapenems. 2% of the *P. aeruginosa* isolates was MDR and 57% of these MDR isolates were carbapenem-resistant. The prevalence of carbapenem resistant *P. aeruginosa* is relatively highest in the ICU department.
- The most predominant (78%) carbapenemase-encoding gene in carbapenemase-producing *P. aeruginosa* was *bla*VIM.
- Only 68% of the carbapenemase-producing *P. aeruginosa* had MICs as measured by Etest interpreted as resistant according to the EUCAST clinical breakpoints.

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.

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 2019 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, in 2019. Trends in ESBL percentages (from left to right 2015 to 2019) among clinical isolates of *E. coli* and *K. pneumoniae* by site are shown in figure 4.7.5.1. The percentages of ESBL have increased for *E. coli* over the years with ESBL percentages between 3 and 9 % depending on type of department in 2019. For *K. pneumoniae* the percentages of ESBL increased between 2015-2018, with stabilization in 2019 with percentages between 4 and 12% depending on type of department. The data show an increase correlated with the complexity of care with highest ESBL percentages in the intensive care units. Despite the overall increase in ESBL-E prevalence in the Netherlands, percentages still remain low compared to many other countries in Europe.[1]

Table 4.7.5.1 Extended spectrum beta-lactamase (ESBL) producing *E. coli* in the Netherlands in 2019, based on ISIS-AR data.

Type of department	Tested isolates, N	ESBL
GP	92,287	3,092 (3)
Outpatient departments	17,390	949 (5)
Inpatient departments excluding intensive care units	24,102	1,417 (6)
Intensive care units	1,144	105 (9)
Total	**134,923**	**5,563 (4)**

Numbers are based on a selection of 30 laboratories.
The first diagnostic E. coli isolate per patient was selected.
Based on re-interpretation according to EUCAST 2019.
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).

Table 4.7.5.2 Extended spectrum beta-lactamase (ESBL) producing *K. pneumoniae* in the Netherlands in 2019, based on ISIS-AR data.

Type of department	Tested isolates, N	ESBL
GP	12,225	532 (4)
Outpatient departments	3,799	294 (8)
Inpatient departments excluding intensive care units	4,954	423 (9)
Intensive care units	351	41 (12)
Total	**21,329**	**1,290 (6)**

Numbers are based on a selection of 30 laboratories.
The first diagnostic K. pneumoniae isolate per microorganism per patient was selected.
Based on re-interpretation according to EUCAST 2019.
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).

Figure 4.7.5.1 Trends in extended spectrum beta-lactamase (ESBL) producing *E. coli* (a) and *K. pneumoniae* (b) in the Netherlands (from left to right 2015 to 2019), based on ISIS-AR data.

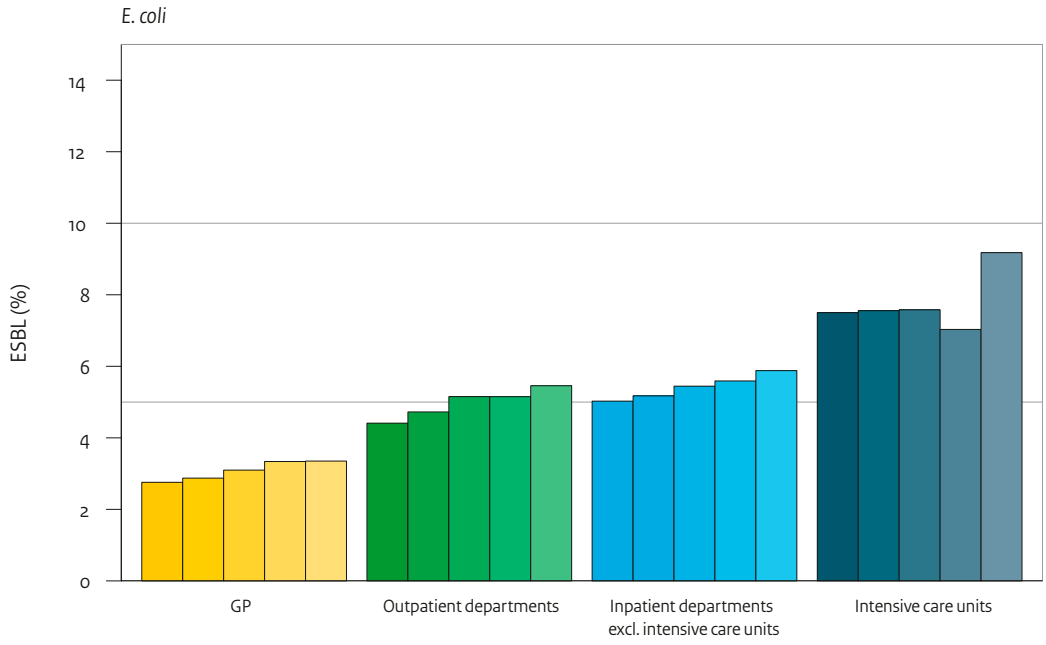
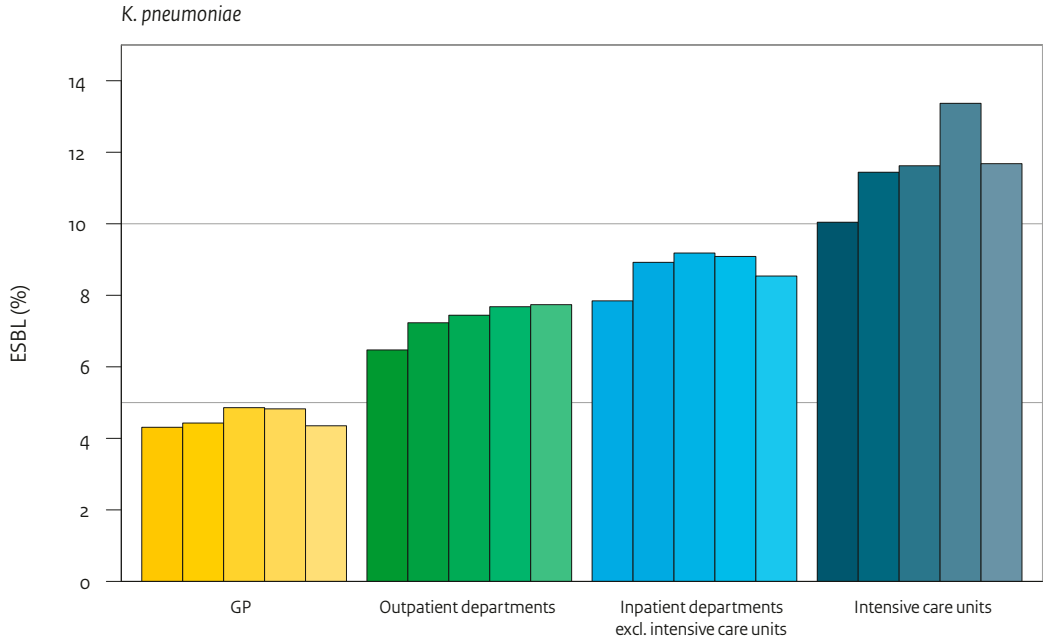


Figure 4.7.5.1 (continued) Trends in extended spectrum beta-lactamase (ESBL) producing *E. coli* (a) and *K. pneumoniae* (b) in the Netherlands (from left to right 2015 to 2019), based on ISIS-AR data.



Numbers are based on a selection of 30 laboratories.
The first diagnostic isolate per patient per year was selected.
Based on re-interpretation according to EUCAST 2019.
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).

Discussion

Extended-spectrum β-lactamases (ESBLs) are widespread in human and animal populations and in the environment. However, there seems to be no close link between ESBL genes and plasmid types of livestock (i.e. pigs, broilers and production animals (veal calves, dairy cattle, pigs, broilers and laying hens)) or their products and the general population.[2] Still, recent studies show substantial levels of ESBL/AMP-C carriage in the open horse population and in pets in the Netherlands.[3,4]
International travel remains a major risk factor for ESBL-E carriage in the Dutch population.[5] And human-to-human contact is shown to be the main driver for transmission of ESBL in the general population.[6]

Conclusions

- In 2019, the percentages of ESBL are between 3 and 9% for *E. coli* and between 4 and 12% for *K. pneumoniae*, with the highest percentages in the intensive care units.

References

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

[2] Dorado-García A, Smid JH, van Pelt W, et al. Molecular relatedness of ESBL/AmpC-producing Escherichia coli from humans, animals, food and the environment: a pooled analysis. J Antimicrob Chemother. 2018;73(2):339-347.

[3] Hordijk J, Farmakioti E, Smit LAM, Duim B, Graveland H, Theelen MJP, Wagenaar JA. Fecal Carriage of Extended-Spectrum-β-Lactamase/AmpC-Producing Escherichia coli in Horses. Appl Environ Microbiol. 2020 Apr 1;86(8).

[4] van den Bunt G, Fluit AC, Spaninks MP, Timmerman AJ, Geurts Y, Kant A, Scharringa J, Mevius D, Wagenaar JA, Bonten MJM, van Pelt W, Hordijk J. Faecal carriage, risk factors, acquisition and persistence of ESBL-producing Enterobacteriaceae in dogs and cats and co-carriage with humans belonging to the same household. J Antimicrob Chemother. 2020 Feb 1;75(2):342-350.

[5] Arcilla MS, Van Hattem JM, Bootsma MCJ, van Genderen PJJ, Goorhuis A, Grobusch MP, Klaassen CHW, Oude Lashof AM, Schultsz C, Stobberingh EE, de Jong MD, Penders J, Verbrugh HA, Melles DC. Prevalence and risk factors for carriage of ESBL-producing Enterobacteriaceae in a population of Dutch travellers: A cross-sectional study. Travel Med Infect Dis. 2020 Jan - Feb;33:101547.

[6] Mughini-Gras L, Dorado-García A, van Duijkeren E, et al. Attributable sources of community-acquired carriage of Escherichia coli containing beta-lactam antibiotic resistance genes: a population-based modelling study. The Lancet Planetary Health. 2019;3:357–369.

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 – in the acquisition of external expertise. 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. An outbreak (phase 1) that lasts more than 2 months is automatically categorized as phase 2. If a potential threat to the public health exists, the outbreak will be classified as phase 3; phase 4 and 5 describe potential management issues. An overview of active outbreaks is reported to professionals involved in infection prevention on a monthly basis.
Notifications are voluntary, but do not come without obligations. All hospitals have committed themselves to participate in SO-ZI/AMR. There is no official commitment from LTCFs yet to participate in SO-ZI/AMR. However, to benefit from a financial compensation rule introduced in 2017 to compensate for detection and control of all outbreaks in LTCF, outbreaks have to be reported to the SO-ZI/AMR.[1] In 2018 an external evaluation of the SO-ZI/AMR took place and based on the recommendations of this evaluation, the organization of the SO-ZI/AMR will be further optimized in the coming year.

Methods

Health care facilities send outbreak notifications using a standardized form to RIVM/NVMM (the Dutch Society of Medical Microbiology), where the information is copied into an MS Access database. Monthly updates are provided by institutions until the outbreak is considered ended.

Results

Table 4.7.6.1 provides an overview of the fifty-nine outbreaks reported in 2019. These were reported by 49 different healthcare institutions. These included 38 hospitals and 21 LTCFs. Most outbreaks (n=49) ended in 2019. As reported in the table, most frequent reasons for notification of an outbreak in a hospital was the imminent closure of wards (63%); 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 was only slightly higher compared to LTCFs, although the maximum number of involved patients was almost twice as high.

Methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) were most often reported, comparable to previous years. Four outbreaks with ESBL-producing *Enterobacterales* were reported, which was similar to previous years except for 2018 when the national Point Prevalence Survey in LTCF was conducted, aimed at detecting ESBL outbreaks in LTCFs.
Three outbreaks of carbapenemase-producing strains were reported (2 in hospitals, one in LTCF), compared to eight in 2018.

Table 4.7.6.1 Characteristics of outbreaks reported to the SO-ZI/AMR in 2019.

	Hospitals n=38 n (%)	LTCFs n=21 n (%)	Total 2019 n=59 n (%)
Microorganism (resistance mechanism)*			
Enterococcus faecium (VRE)	19 (50)	0	19 (32)
Staphylococcus aureus (MRSA)	9 (24)	17 (81)	26 (44)
Escherichia coli (ESBL)	0	1 (5)	1 (2)
Klebsiella pneumoniae (CP)	2 (5)	0	2 (3)
Klebsiella pneumoniae (ESBL)	2 (5)	0	2 (3)
Enterobacter cloacae (ESBL)	1 (3)	0	1 (2)
Pseudomonas aeruginosa (CP)	0	1 (5)	1 (2)
Norovirus	2 (5)	2 (10)	4 (7)
Other	3 (8)	0	3 (5)
Reason of reporting			
threatening of ward closure	24 (63)	4 (19)	28 (47)
ongoing transmission	2 (5)	0	2 (3)
combination of both	2 (5)	1 (5)	3 (5)
HRMO outbreak (not in a hospital)	0	14 (67)	14 (24)
unknown	10 (26)	2 (10)	12 (20)
Highest level phase			
phase 1	36 (95)	21 (100)	57 (97)
phase 2	2 (5)	0	2
phase 3	0	0	0
phase 4	0	0	0
phase 5	0	0	0
Median number of patients: (range)	4 (1-37)	3 (1-18)	3 (1-37)
Median duration outbreak in days from reporting date until end of the outbreak: (range)	59 (3-192)	53 (14-110)	56 (3-192)
Request for help	0	0	0

* *MRSA=methicillin-resistant Staphylococcus aureus; VRE=vancomycin-resistant Enterococcus faecium; ESBL=extended-spectrum beta-lactamase; CP=carbapenemase-producing*

Eleven outbreaks included more than 10 patients. The outbreaks classified as phase 2 comprised one ESBL *E. cloacae* outbreak and one VRE outbreak. Of the data available, the majority of the outbreaks appear to have been reported within a month after detection.
Three long-lasting outbreaks which had started in 2018, ended in 2019. Two of them were outbreaks with VRE both in a hospital, one with a duration of 579 days and 148 patients involved, and a second with a duration of 398 days and 180 patients involved. Both outbreaks had a maximum phase 3. The other outbreak comprised an NDM-producing strain of *C. freundii* in a hospital, with a duration of 572 days and involving 26 patients. Here the maximum phase was phase 4, but this was scaled down to phase 3 again after two months, and could be ended later in 2019 after careful infection control measures.

Discussion

In 2019, the number of outbreaks was similar as in previous years. The median number of patients involved in outbreaks in hospitals was lower than in previous years. For LTCFs, this number had not changed. Almost half of the outbreaks were MRSA outbreaks, of which two third was reported by an LTCF. The second most reported outbreaks were caused by VRE and were all reported by hospitals.

Conclusions

- On average five outbreaks a month were reported to the SO-ZI/AMR.
- Most outbreaks were classified as phase 1 and only two as phase 2.
- Three outbreaks by carbapenemase-producing strains were reported, two caused by *K. Pneumoniae* and one by *P. aeruginosa*.
- The majority of the outbreaks were reported to SO-ZI/AMR within a month after detection.
- Most outbreaks were due to MRSA (of which two third was reported by LTCFs) and VRE (all in hospitals).
- Most outbreaks were controlled within 2 months.
- The median number of patients involved in an outbreak was 3.

References

[1] Nederlandse Zorgautoriteit Beleidsregel BRMO-uitbraak - BR/REG-20117. Available from: https://puc.overheid.nl/nza/doc/PUC_278332_22/1/ [Accessed 20th May 2020]

4.8 Resistance in specific pathogens

4.8.1 *Neisseria meningitidis*

Introduction

Neisseria meningitidis isolates cultured from CSF and/or blood in microbiological laboratories in the Netherlands are submitted to the Netherlands Reference Laboratory for Bacterial Meningitis (NRLBM) at the Amsterdam UMC, Location AMC, Amsterdam. In *N. meningitidis*, the interpretation of the phenotypic susceptibility testing might not be fully reliable, because the susceptible/moderately susceptible break-point is exactly at the peak of the wild-type susceptibility distribution (0.06 mg/L). Since any MIC assay is not 100% reproducible, this likely results in a considerable number of minor and major interpretation errors. Therefore, the *penA* gene of all isolates was sequenced.

Methods

From 2010-2019, a total of 390 strains from cerebrospinal fluid (CSF) or CSF and blood and 817 strains from blood were included in the surveillance project of NRLBM. The MIC for penicillin was determined by Etest using MHF plates, incubation 18-24 h at 37°C under 5% Co_2. EUCAST criteria for resistance were applied (susceptible: MIC ≤0.06 mg/L; resistant: MIC >0.25 mg/L). In addition, the nucleotide sequence of *penA* coding for penicillin binding protein 2 was sequenced.[1,2] In case of moderate susceptibility or resistance to penicillin, susceptibility to ceftriaxone was also assessed by Etest using MHF plates, incubation 18-24 h at 37°C under 5% Co_2.

Results

In 2019 no isolates (n=136 tested) were resistant to penicillin, whereas 7.4% (2/27) of CSF (or CSF and blood) isolates and 10.1% (11/109) of the blood isolates were moderately susceptible to penicillin (MIC 0.06-0.25 mg/L). The proportion of isolates moderately susceptible to penicillin in 2019 was lower than in the previous years (table 4.8.1.1 and 4.8.1.2). The moderately susceptible isolates were not equally distributed among serogroups. Of those 13 moderately susceptible isolates from blood and/or CSF in 2019, five belonged to serogroup B (5/55; 9.1%), three to serogroup C (3/6; 50%), three to serogroup W (3/58; 5.2%) and two to serogroup Y (2/16; 12.5%). Resistance to ceftriaxone or rifampicin was not detected. Alterations in the *penA* gene, associated with non-susceptibility to penicillin[2], were detected in 8 (5.9%) of the 136 isolates. Of these isolates, one was phenotypically susceptible and 7 were moderately susceptible by Etest (table 4.8.1.3).
PenA genotyping yields more isolates (5.9%) resistant to penicillin as compared to phenotypic testing with Etest using EUCAST criteria (0%).

Discussion

Alterations in *penA* associated with resistance to penicillin are present in 6% of all isolates compared to 0% with Etest, showing a weak correlation between MIC to penicillin and alterations in *penA*. One or more of the following reasons may be involved: 1) other factors than *penA* alterations also confer non-susceptibility to penicillin; 2) a considerable number of minor interpretation errors occurs because the susceptible/moderately susceptible breakpoint lies at the peak of the wild-type susceptibility distribution; 3) this EUCAST breakpoint is too low and should be repositioned at 0.25 mg/L.

Conclusions

- Phenotypic penicillin resistance is sporadic (one strain in 2012, two strains in 2013, one strain in 2017, three in 2018 and none in 2019).
- In 2019 the proportion of moderately susceptible or resistant strains decreased compared to the previous year (from 25.4% (47/185) in 2018 to 9.6% (13/136) in 2019).
- Alterations in *penA* associated with non-susceptibility to penicillin are present in 6% of all isolates.
- Resistance to rifampicin and ceftriaxone was not found in 2019.

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 MK1, 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.

Table 4.8.1.1 Susceptibility of *N. meningitidis* isolated from CSF or CSF and blood to penicillin, 2010-2019.

	Penicillin*								
	MIC ≤ 0.064 sensitive		0.064 < MIC ≤ 0.25		0.25 < MIC ≤1.0		MIC >1.0		Total
	n	%	n	%	n	%	n	%	
2010	43	**81.1**	10	**18.9**	0	**0**	0	**0**	**53**
2011	29	**78.4**	8	**21.6**	0	**0**	0	**0**	**37**
2012	24	**58.5**	16	**39.0**	1	**2.4**	0	**0**	**41**
2013	35	**89.7**	3	**7.7**	1	**2.6**	0	**0**	**39**
2014	26	**83.9**	5	**16.1**	0	**0**	0	**0**	**31**
2015	31	**96.9**	1	**3.1**	0	**0**	0	**0**	**32**
2016	34	**89.5**	4	**10.5**	0	**0**	0	**0**	**38**
2017	37	**80.4**	9	**19.6**	0	**0**	0	**0**	**46**
2018	32	**69.6**	13	**28.3**	1	**2.2**	0	**0**	**46**
2019	25	**92.6**	2	**7.4**	0	**0**	0	**0**	**27**

* *MIC values in mg/L*

Table 4.8.1.2 Susceptibility of *N. meningitidis* isolated from blood only to penicillin, 2010-2019.

	Penicillin*								
	MIC ≤ 0.064 sensitive		0.064 < MIC ≤ 0.25		0.25< MIC ≤ 1.0		MIC >1.0		Total
	n	%	n	%	n	%	n	%	
2010	67	**84.8**	12	**15.2**	0	**0**	0	**0**	**79**
2011	34	**64.2**	19	**35.9**	0	**0**	0	**0**	**53**
2012	27	**67.5**	13	**32.5**	0	**0**	0	**0**	**40**
2013	53	**73.6**	18	**25.0**	1	**1.4**	0	**0**	**72**
2014	37	**88.1**	5	**11.9**	0	**0**	0	**0**	**42**
2015	46	**88.5**	6	**11.5**	0	**0**	0	**0**	**52**
2016	89	**87.3**	13	**12.7**	0	**0**	0	**0**	**102**
2017	104	**80.6**	24	**18.6**	1	**0.8**	0	**0**	**129**
2018	106	**76.3**	31	**22.3**	2	**1.4**	0	**0**	**139**
2019	98	**89.9**	11	**10.1**	0	**0**	0	**0**	**109**

* *MIC values in mg/L*

Table 4.8.1.3 Alterations in the *penA* gene and penicillin susceptibility in *N. meningitidis*, 2019.

	Number (%) of strains with penicillin MIC*:			
Alterations *penA* gene**	MIC ≤ 0.06 sensitive	0.064< MIC ≤ 0.25	0.25 < MIC ≤ 1.0	MIC >1.0
Yes	1	7	0	0
No	122	6	0	0
Total	**123**	**13**	**0**	**0**

* *MIC values in mg/L*

** *Resulting in five amino acids substitutions in PenA associated with non-susceptibility to penicillin1*

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 became the first-line therapy for gonorrhoea in 2003, and ceftriaxone in 2006. However, the susceptibility of gonococci to these cephalosporins has been decreasing and *Neisseria gonorrhoeae* has developed antimicrobial resistance to most drugs used for treatment, including azithromycin, which is used as an alternative treatment in patients allergic to ceftriaxone.

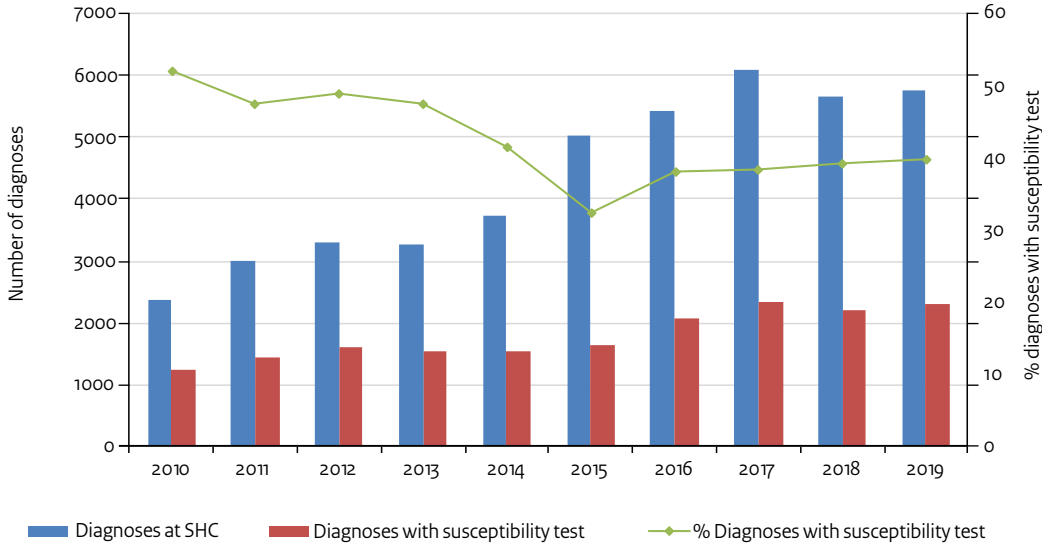
Methods

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. Seventeen out of 24 SHC participated in GRAS in 2019, which accounted for 84% 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. From 2006, isolates were tested for penicillin, tetracycline, ciprofloxacin and cefotaxime. In 2011, ceftriaxone, azithromycin and spectinomycin were added to the panel and testing for penicillin and tetracycline became optional. In 2014, testing for spectinomycin was also made optional. In 2015, penicillin and tetracycline were removed from the panel. Resistance levels are calculated using the EUCAST breakpoints for resistance[1]. In 2019, EUCAST altered the breakpoint for azithromycin resistance. The clinical breakpoint of MIC >0.5 mg/L was changed to an epidemiological cut-off value (ECOFF) of MIC >1.0 mg/L. Trends for azithromycin resistance have been altered retrospectively using the new ECOFF.

Results

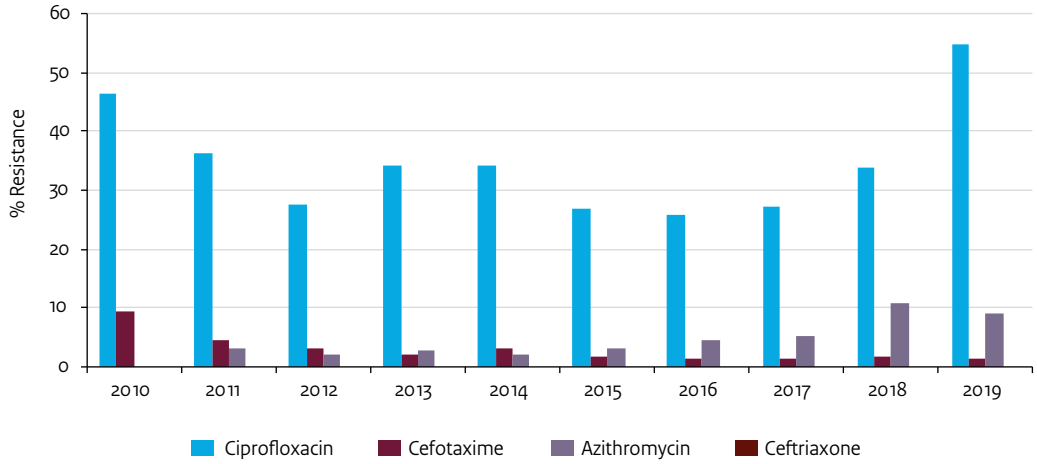
The number of gonorrhoea diagnoses at SHC participating in GRAS peaked in 2017 and is more or less stable after 2017 with 5648 diagnoses in 2018 and 5764 in 2019. The percentage of diagnoses including a susceptibility test has been stable around 39% for the past few years (39.8% in 2019) (Figure 4.8.2.1).

Figure 4.8.2.1 Number of gonorrhoea diagnoses and number and percentage of diagnoses including an antimicrobial susceptibility test at Sexual Health Centres participating in GRAS, 2010-2019.



Gonococcal resistance for ciprofloxacin decreased from 46.6% in 2010 to 25.8% in 2016, but increased again in the past few years. Especially in 2019 a large increase was seen in ciprofloxacin resistance levels, rising to 54.9%. Resistance levels for cefotaxime have been slowly decreasing since 2010, and have been stable around 1.5% since 2016. For azithromycin, resistance has steadily increased since 2012; from 2.1% to 10.8% in 2018. But in 2019 resistance to azithromycin slightly decreased to 9.3%. No resistance was reported for ceftriaxone (Figure 4.8.2.2).

Figure 4.8.2.2 Trends in antimicrobial resistance among *Neisseria gonorrhoeae* (following EUCAST breakpoints) in the Netherlands, 2010–2019.

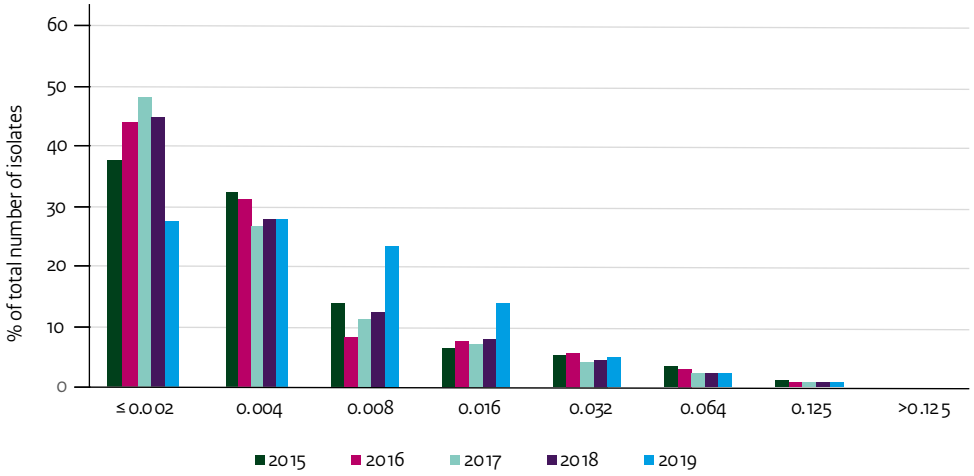


Ceftriaxone and azithromycin were added to the panel in 2011. No resistance to ceftriaxone has been reported.

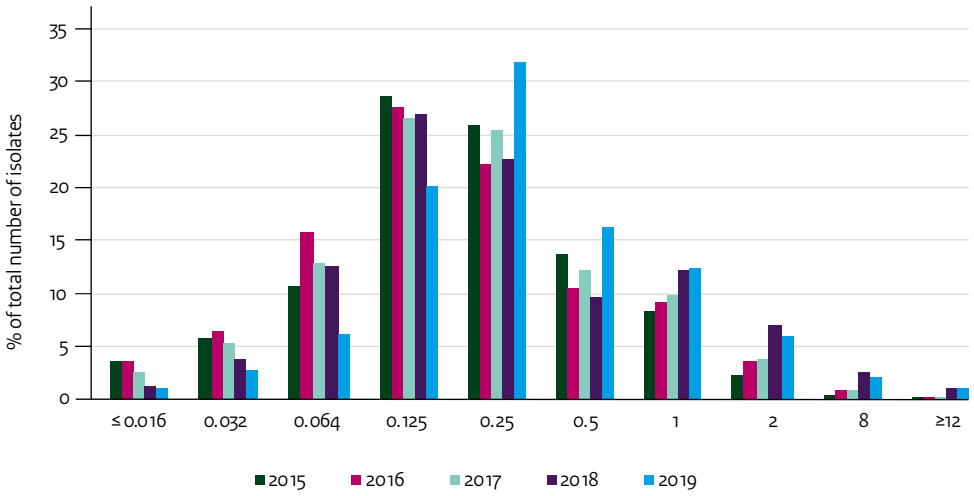
The MIC distribution of ceftriaxone is highly skewed to the right, and shows a unimodal shape. Until 2017, isolates seemed to become more susceptible for ceftriaxone. However, in 2018 and 2019 an increase can be seen in the proportion of isolates with reduced susceptibility (MIC 0.008 mg/L and 0.016 mg/L) (Figure 4.8.2.3a). Also for azithromycin the MIC distribution is shifting. In 2019 less isolates with high susceptibility were seen, and the proportions of isolates with reduced susceptibility increased (Figure 4.8.2.3b).

Figure 4.8.2.3 MIC distributions of ceftriaxone and azithromycin for *Neisseria gonorrhoeae*, 2015-2019.

a. MIC distribution for ceftriaxone



b. MIC distribution for azithromycin



Discussion

In 2019 in less than half (39.8%) 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.

In the Netherlands, the recommended treatment for gonorrhoea is a single injection with ceftriaxone (500 mg). Thus far, no ceftriaxone resistance had been reported. Yet, a few isolates have reached the borderline MIC value of 0.125 mg/L in the last years (2 cases in 2019). Clinical failure of gonorrhoea treatment has also not yet been reported in the Netherlands, but this is not structurally monitored.

Trends of decreasing susceptibility are observed for all antimicrobial agents monitored in GRAS. This calls for a continued effort to monitor trends and emergence of antimicrobial resistance in gonococci.

Conclusions

- The number of gonorrhoea diagnoses including susceptibility testing at the SHC remains relatively low (39.8%).
- Resistance for ciprofloxacin increased in the past few years to 54.9% in 2019.
- No resistance to ceftriaxone, the current first-line treatment, has been reported. However, higher proportions of isolates with (slightly) reduced susceptibility were seen in 2019 compared with previous years.
- All antimicrobials monitored in GRAS show trends of increasing MIC values.

References

[1] The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 10.0, 2020. Available from http://www.eucast.org/clinical_breakpoints/

4.8.3 *Mycobacterium tuberculosis*

Introduction

Of all infectious diseases, tuberculosis (TB) has the highest mortality worldwide. Although the incidence is slowly declining, it has been estimated that about one third of the global population is 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. Because of the increased influx of asylum seekers and immigrants, in 2016 there was an increase of about 3% in the notification of TB (886 cases). In 2018, the total number of TB cases declined to 797 cases and in 2019 to 759 cases.

Worldwide, there is a concern about the development of resistance, which hampers adequate treatment of tuberculosis. The majority of resistance testing of *M. tuberculosis* isolates in the Netherlands is performed at the RIVM and the results are used both for direct therapy guidance of individual patients and surveillance. The RIVM participates in the proficiency studies of the WHO for international WHO laboratories to monitor the quality of the resistance testing.

Around 30 laboratories in the Netherlands are involved in the diagnosis of TB and send all cultured *M. tuberculosis* isolates to the RIVM for epidemiological typing to support the investigations on TB transmission by Municipal Health Services. For a part of the strains also (sub) species identification and (molecular and/or) phenotypic resistance testing are performed.

Methods

The current drug susceptibility testing (DST) most often used is the WHO recommended mycobacteria growth indicator tube (MGIT) system. In this approach bacteria are incubated in the presence of critical concentrations of drugs and the MGIT incubator automatically monitors the (lack of) growth of the bacteria.

Since 2011, not all initial drug susceptibility testing for first line drugs is performed at the RIVM; a part (34%) of these tests is performed at regional or local microbiology laboratories. When resistance is observed however, this is reported to the national reference laboratory at the RIVM for verification and/or additional resistance testing.

Results

The presented data on 2019 is preliminary, as not all data is currently available. The *in vitro* generation time of *M. tuberculosis* is long and it therefore takes several weeks before cultures become positive, are sent to the RIVM, and the drug susceptibility testing has been finalized.

In the year 2019, 482 *M. tuberculosis* complex isolates were received at the RIVM for epidemiological typing, of which 319 (66%) were subjected to DST for first line drugs at the RIVM.

Figure 4.8.3.1 Trends in antibiotic resistance for *M. tuberculosis* 2004-2019.

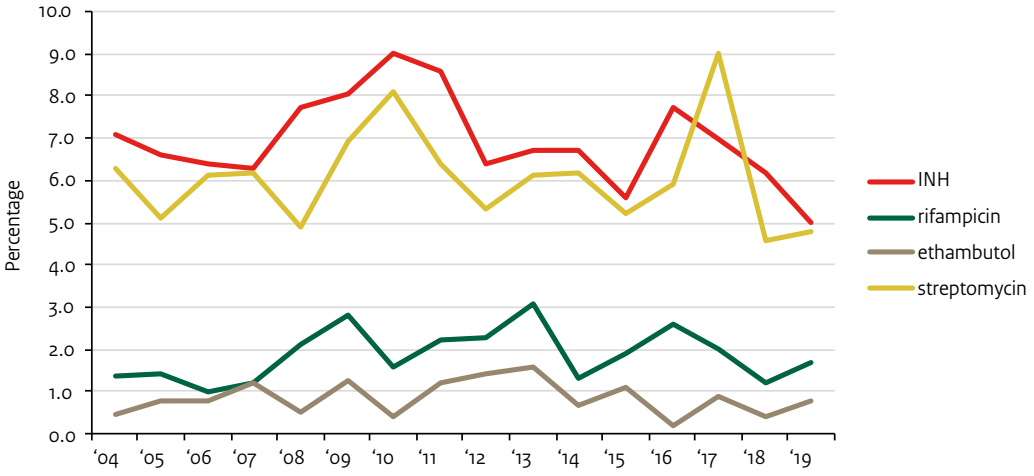
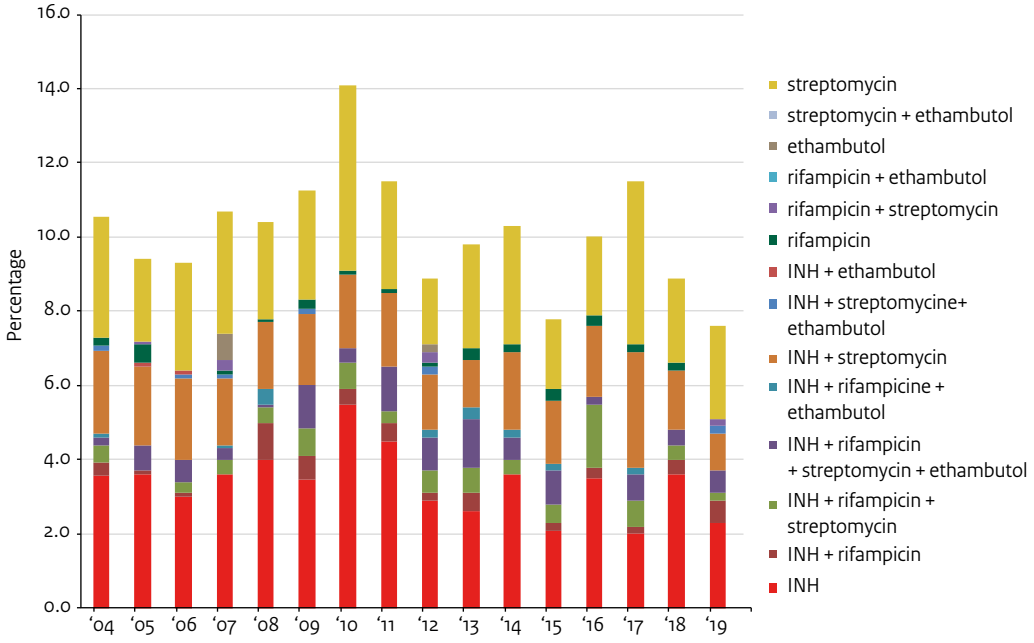


Figure 4.8.3.2 Trends in combined antibiotic resistance for *M. tuberculosis* 2004-2019.



In 2019, the number of TB notification cases was 759, of which 482 were confirmed *M. tuberculosis* complex cultures that were received at the RIVM for epidemiological typing and, in the majority of cases, resistance testing. It is expected there are still isolates of 2019 missing that will be received at the RIVM in the coming period.
In 2016 there was a clear increase in INH resistance to 7.7% (figure 4.8.3.1), but this decreased over the years to 5.0 % in 2019. In 2018, rifampicin resistance decreased to 1.1%, but increased in 2019 to 1.7% of the cases. In 2019, in 0.8% of the cases ethambutol resistance was detected, which is a slight increase in comparison to previous years.
In 2018, 6 MDR-TB cases, defined as resistance to at least INH and rifampicin, were diagnosed and one XDR-TB (in total 1.2%), defined as combined resistance to INH, rifampicin, an injectable antituberculosis drug, and a fluoroquinolone (figure 4.8.3.2). In 2019, 6 MDR-TB cases and one mono rifampicin resistant (RR), defined as resistance to only rifampicin, cases were detected. In addition, one XDR-TB case was diagnosed. Combined MDR, XDR and RR in 2019 in total was 1.6%.

Discussion

Worldwide, resistance is an important aspect of TB control. Because the vast majority of TB cases in the Netherlands are diagnosed in patients derived from high prevalence areas, it remains important to continue the surveillance on resistance. In 2017, the notification of TB declined with 11%, mainly due to a reduced number of newly arrived residents. In 2018, presumably due to variation in the composition of the group of asylum seekers there was a slight increase in the notification of TB. In the last year there was a minor decrease in the number of TB cases recorded.
In 2019, 7.6% (37/482) of the isolates tested in the Netherlands revealed some form of resistance. This seems a bit lower than the percentage observed in previous years. Although the number of multidrug resistant (including RR) isolates remained low and amounted to 8 cases, due to the extended hospitalization of patients and the cumbersome treatment this problem continues to deserve special attention.
In 2016, a new project was initiated at the RIVM on structural Whole Genome Sequencing (WGS) of *M. tuberculosis* isolates. The detection of mutations in the 9 major resistance genes appears a reliable predictor of resistance to first line drugs. From 2020 onwards WGS is performed to screen for resistance against first line drugs in *M. tuberculosis* isolates. In case no resistance mutations are observed, no additional phenotypic screening on resistance to first line drugs is performed.

Conclusions

- Resistance to the antibiotics to treat tuberculosis remained almost stable over the last 5 years, and showed a slight decrease in last years.
- MDR-TB remained stable in the recent years (average 8 each year).
- Occasionally, XDR-TB is diagnosed in the Netherlands. In 2019 there was one case of XDR-TB.
- Since 2018 around 800 cases of TB are notified each year. The total number in 2019 was 759.
- The notification and proportion of resistant cases is associated with the influx and composition of the group of asylum seekers and immigrants.

References

Evaluation of Mycobacteria Growth Indicator Tube (MGIT) for drug susceptibility testing for *Mycobacterium tuberculosis*. J.C. Palomino, H. Traore, K. Fissette, F. Portaels; Int J. Tuberc Lung Dis 1999 3(4) ;344-348.

Whole-genome sequencing for prediction of Mycobacterium tuberculosis drug susceptibility and resistance: a retrospective cohort study. Walker, Kohl, Omar, Hedge, Del Ojo Elias, Bradley, Iqbal, Feuerriegel, Niehaus, Wilson, Clifton, Kapatai, Ip, Bowden, Drobniewski, Allix-Béguec, Gaudin, Parkhill, Diel, Supply, Crook, Smith, Walker, Ismail, Niemann, Peto; Lancet Infect Dis. 2015 Oct;15(10):1193-202.

4.8.4 Influenza antiviral drugs

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 severe course of disease. In the Netherlands the M2 ion channel blockers (M2B) amantadine and rimantadine acting against type A viruses only, and the neuraminidase enzyme inhibitors (NAI) oseltamivir and zanamivir acting against both type A and B viruses, are approved. The M2B prevent uncoating of the virus in the cell and thereby virus replication whereas the NAI prevent release of progeny virus from the cell limiting 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.[1] Because EMA approval for Baloxavir marboxil (Xofluza®) (BXM), a cap-dependent acidic endonuclease inhibitor, is anticipated in 2020, monitoring of reduced susceptibility amino acid substitutions in the polymerase acidic protein (PA) has been added for 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). Viruses detected in hospital and peripheral laboratories are submitted to, and analysed at, the Erasmus Medical Centre location of the NIC. Techniques currently used in the Netherlands to monitor antiviral resistance include Sanger sequencing, whole genome Next Generation Sequencing or site-specific polymerase chain reaction (PCR) assays for known resistance markers for both NAIs and BXM. For a subset of influenza viruses, the susceptibility to NAIs is determined using an enzyme inhibition assay, which generates a 50% inhibitory concentration of the drug (IC_{50}).

Results

Findings for the influenza seasons 2005/2006 through 2009/2010 are presented in NethMap 2016 and for M2Bs up to 2018/2019 in NethMap 2019.[1,2] Table 4.8.4.1 displays an overview of the antiviral susceptibility of influenza viruses since the 2010/2011 influenza season. Figure 4.8.4.1 shows the prescriptions for oseltamivir and zanamivir since 2010. In the 2019/2020 season no viruses with evidence for NAI or BXM reduced inhibition were detected. Oseltamivir prescriptions increased sharply at the start of the 2019/2020 influenza epidemic similar to previous influenza epidemics. Zanamivir has not been prescribed up to January 2020 during the 2019/2020 season.

Discussion

In the Netherlands, and globally, the proportion of NAI reduced susceptible influenza viruses remains very low.[3] Except for the emergence and sustained worldwide circulation of oseltamivir reduced susceptible former seasonal A(H1N1) in 2007/2008 and some small clusters of oseltamivir reduced susceptible A(H1N1) pdm09 since 2009, most of the NAI reduced susceptible viruses 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. No markers for BXM reduced inhibition were detected, similar to the very low prevalence globally.[3]

Table 4.8.4.1 (Higly) reduced inhibition of influenza viruses by NAIs and BXM in the Netherlands, 2010/2011 - 2019/2020[1].

Season	A(H3N2)		A(H1N1)pdm09		B	
	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

[1] *Combined results obtained with phenotypic (virus isolates) and genotypic (clinical specimens) assays. Season defined as week 40 of the first year to week 39 of the following year. Abbreviations: NAI = neuraminidase inhibitor; BXM = baloxavir marboxil; ND = not done.*

[2] *Two viruses with highly reduced inhibition by oseltamivir due to the H25Y amino acid substitution, isolated from two epidemiological unlinked not treated patients returning from holiday at the Spanish coast.*

[3] *Three viruses with highly reduced inhibition by oseltamivir due to the H25Y 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 292R and 292K amino acid composition; R292K is associated with highly reduced inhibition for oseltamivir and zanamivir. No patient characteristics or viral exposure data available.*

[5] *One virus with highly reduced inhibition by oseltamivir due to the H275Y amino acid substitution. No patient characteristics or viral exposure data available.*

[6] *One virus with highly reduced inhibition by oseltamivir due to mixture 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 275H/Y amino acid substitution. No patient characteristics or viral exposure data available.*

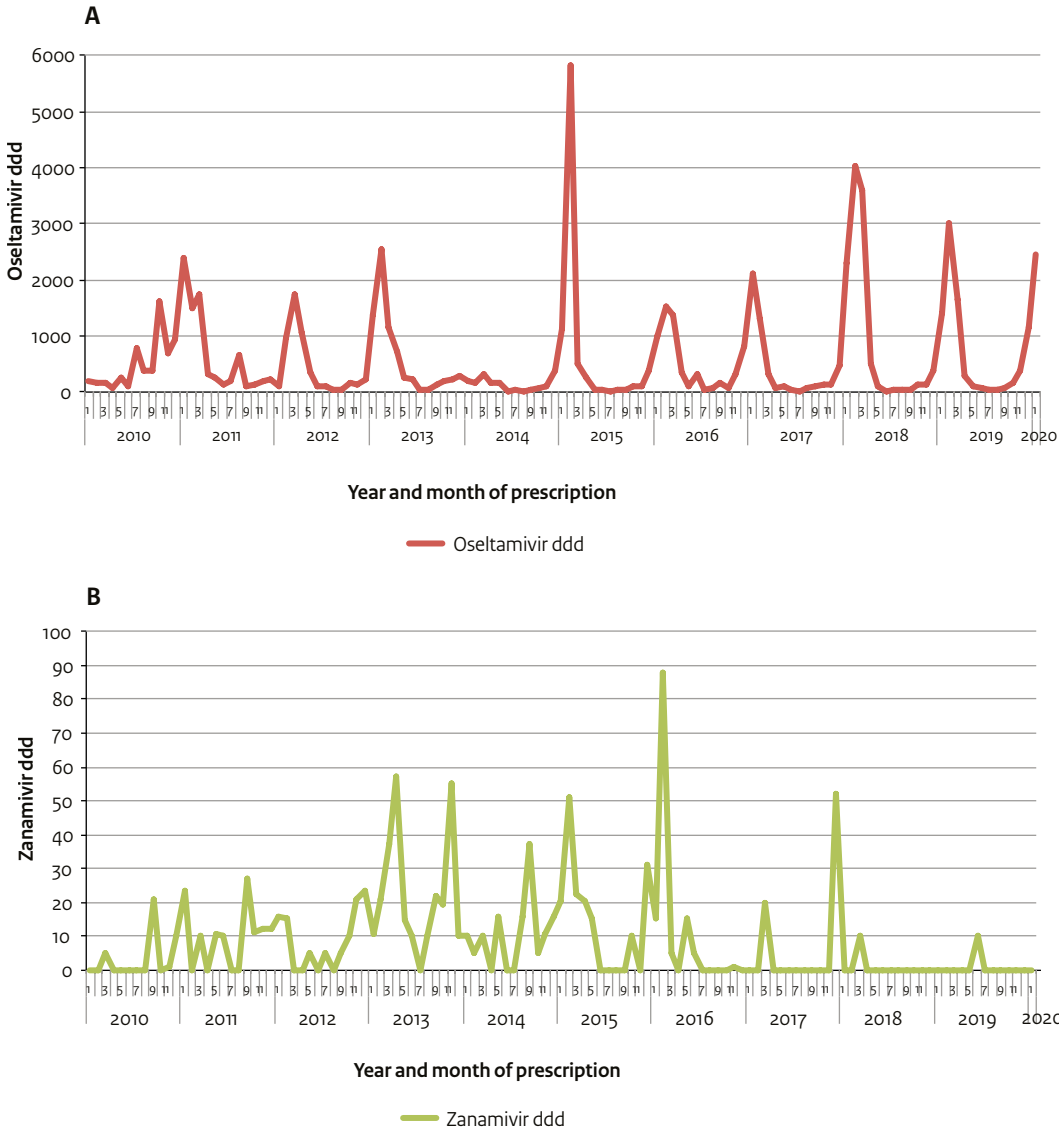
[8] *One virus with highly reduced inhibition by zanamivir and reduced inhibition by oseltamivir due to an E105K amino acid substitution. However, highly likely induced by virus isolation as in the clinical specimen this amino acid substitution was not detectable. The patient was not treated with antivirals prior to specimen collection.*

[9] *Two viruses from one patient taken 10 days apart with both highly reduced inhibition by oseltamivir due to a 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 275H/Y amino acid substitution. No patient characteristics or viral exposure data available.*

[11] *Three viruses with highly reduced inhibition by oseltamivir due to H275Y (n=1) or mixture 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.*

Figure 4.8.4.1 Prescriptions of oseltamivir (A) and zanamivir (B) in the Netherlands, 2010/2011 - 2019/2020. Shown are the Defined Daily Doses (ddd) cumulated by month. Data kindly provided by Foundation for Pharmaceutical Statistics (SFK), the Netherlands.



Conclusions

- Over the last 10 seasons type A and type B influenza viruses remained susceptible to the neuraminidase inhibitors oseltamivir and zanamivir.
- Sporadically, a neuraminidase inhibitor 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.
- Prescriptions of zanamivir remain very low.

References

[1] 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.

[2] 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.

[3] Takashita E, Daniels RS, Fujisaki S, Gregory V, Gubareva LV, Huang W, Hurt AC, Lackenby A, Nguyen HT, Pereyaslov D, Roe M, Samaan M, Subbarao K, Tse H, Wang D, Yen HL, Zhang W, Meijer A. Global update on the susceptibilities of human influenza viruses to neuraminidase inhibitors and the cap-dependent endonuclease inhibitor baloxavir, 2017-2018. Antiviral Res. 2020 Mar;175:104718.

4.8.5 The antibiotic susceptibility profile of anaerobic bacteria

Introduction

In recent years we noticed a change in the antibiotic susceptibility profile of certain anaerobic bacteria. Here we report the susceptibility profile of anaerobic bacteria isolated in 2019, at the University Medical Center Groningen (UMCG), from human clinical specimens. All isolates were considered to be clinically relevant and the antibiotic susceptibility profiles were compared with previous years.

Methods

All anaerobic isolates were identified using the Biotyper Matrix Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry [(MALDI-TOF MS), Bruker Daltonics, Bremen, Germany]. The MIC values for the tested antibiotics was obtained using Etest (bioMerieux, L'Etoile, France), after 48 hours of incubation at 37°C in an anaerobic atmosphere. Gram-negative anaerobic bacteria were tested for susceptibility against amoxicillin, amoxicillin-clavulanic acid, clindamycin, metronidazole and in case of *Bacteroides* or *Prevotella* also for meropenem. Gram-positive anaerobic bacteria were tested for resistance against amoxicillin, clindamycin and metronidazole. The latter antibiotic was not tested when the isolate was identified as *Actinomyces* or *Cutibacterium* (former *Propionibacterium*), both considered as intrinsically resistant to metronidazole. EUCAST breakpoints were used to assess whether an isolate was resistant or susceptible.

Results

The MIC_{50}, MIC_{90} and percentage resistance are shown in Table 4.8.5.1, in which they are presented as Gram-negative or Gram-positive anaerobic bacteria.

Gram-negative anaerobic bacteria

The two most commonly encountered genera were *Bacteroides* and *Prevotella*. Since >90% of the *Bacteroides* isolates are resistant for amoxicillin. The UMCG decided not to test for resistance against this antibiotic and isolates are assumed to be resistant. Among the *Prevotella* isolates 31.5% was resistant for amoxicillin and none were resistant for amoxicillin-clavulanic acid or meropenem. For *Bacteroides* 2.1% was resistant for amoxicillin-clavulanic acid, 1.6% for meropenem and 1% for metronidazole. Isolates resistant for the two latter antibiotics were identified as *Bacteroides fragilis*, but none of these isolates were resistant for both antibiotics. 1.6% of the *Prevotella* isolates, identified as *Prevotella nanceiensis* and *Prevotella timonensis*, were resistant for metronidazole. Among all other tested genera no metronidazole resistance was encountered. Amoxicillin resistance was highest among *Bilophila* spp. isolates, 85.7%, while clindamycin resistance was highest among *Parabacteroides* isolates, 72.7%. Among *Fusobacterium* isolates only resistance for clindamycin was observed and no resistance for the other tested antibiotics.
In Table 4.8.5.2 the percentage resistance per year is shown. This is the first year that we observed clindamycin resistance among *Fusobacterium* and *Bilophila* spp. The percentage resistance for the different antibiotics varied per year for all tested genera.

Gram-positive anaerobic bacteria

Resistance for amoxicillin was observed the genera *Parvimonas*, *Peptostreptococcus* and *Clostridium*, 2.3%, 2% and 2%, respectively. Clindamycin resistance was most prevalent among clostridia, *Peptoniphilus* and *Anaerococcus*. Two isolates (3.5%) of *Finegoldia magna*, one isolate of *Parvimonas micra* (2.3%) and one isolate of *Clostridium tertium* were resistant for metronidazole.

Compared to previous years (Table 4.8.5.3), this is the first year that we noticed amoxicillin resistance among *Parvimonas* and *Peptostreptococcus*. We also noticed an increase in clindamycin resistance among *Anaerococcus* and *Peptoniphilus* isolates.

Discussion

As in previous years we noticed that the resistance differs per year, which indicates that it is important to test the antibiotic susceptibility profile of anaerobic bacteria isolated from human clinical specimens. However, this year we encountered resistance for amoxicillin and metronidazole for the first time among genera of Gram-positive anaerobic cocci (GPAC). Penicillin resistance has been reported for GPAC isolates in other countries, as is metronidazole resistance among *Finegoldia* isolates.[1] As in 2018, one *Clostridium* isolate was resistant to metronidazole. The proportion of metronidazole resistance among *Bacteroides* and *Prevotella* isolates was similar as in previous years. The resistant isolates were identified as *Bacteroides fragilis*, *Prevotella timonensis* and *Prevotella nanceinsis*. Whole genome sequencing will be performed on metronidazole resistant isolates in order to assess whether *nim* genes are present and, if so, if they are located on a mobile genetic element. Recently a report signaled the presence of metronidazole and carbapenem resistant *Bacteroides fragilis* isolates in the Netherlands.[2] In 2019, none of such isolates were encountered at the UMCG.

In 2020, a few medical microbiological laboratories will collaborate in a project to set up an enhanced national surveillance system of antimicrobial resistance in anaerobic pathogens in the Netherlands.

Table 4.8.5.1 The MIC_{50}, MIC_{90} and percentage resistance of different anaerobic genera, isolated from human clinical specimens in 2019, for different kind of antibiotics.

	amoxicillin			amoxicillin- clavulanic acid			clindamycin			metronidazole			meropenem		
	MIC_{50}	MIC_{90}	%R[a]	MIC_{50}	MIC_{90}	%R	MIC_{50}	MIC_{90}	%R	MIC_{50}	MIC_{90}	%R	MIC_{50}	MIC_{90}	%R
Gram-negative															
Bacteroides spp. (190-193)[b]	n.d.[c]	n.d.	n.d.	0.5	3	2.1	2	>256	32.6	0.38	0.75	1	0.125	0.5	1.6
Bilophila spp. (13-14)	64	>256	85.7	1	4	7.1	0.5	1.5	7.7	0.047	0.125	0	n.d.	n.d.	n.d.
Fusobacterium spp. (24-25)	0.032	0.125	0	0.032	0.125	0	0.064	0.25	4	<0.016	0.032	0	n.d.	n.d.	n.d.
Parabacteroides spp. (8-11)	2	>256	37.5	2	8	7.3	6	48	72.7	0.38	1	0	n.d.	n.d.	n.d.
Porphyromonas spp. (18-19)	0.016	0.19	5.6	0.016	0.016	0	0.016	256	15.8	0.032	0.125	0	n.d.	n.d.	n.d.
Prevotella spp. (73-125)	0.064	>256	31.5	0.064	0.75	0	0.016	>256	16.1	0.125	1	1.6	0.032	0.094	0
Veillonella spp. (23-28)	0.38	1.5	4	0.5	1.5	0	0.125	0.38	0	0.75	2	0	n.d.	n.d.	n.d.
Gram-positive															
Actinomyces spp. (164)	0.125	0.38	0	n.d.	n.d.	n.d.	0.19	>256	15.2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Anaerococcus spp. (46)	0.023	0.094	0	n.d.	n.d.	n.d.	0.094	>256	26.1	0.125	0.5	0	n.d.	n.d.	n.d.
Atopobium spp. (8-9)	0.125	0.25	0	n.d.	n.d.	n.d.	0.38	2	0	0.19	0.38	0	n.d.	n.d.	n.d.
Clostridium spp. (50)	0.25	1	2	n.d.	n.d.	n.d.	3	>256	30	0.38	1	2	n.d.	n.d.	n.d.
Eggerthella spp. (15)	1	3	0	n.d.	n.d.	n.d.	0.25	0.5	0	0.125	0.5	0	n.d.	n.d.	n.d.
Finegoldia magna (57)	0.19	0.25	0	n.d.	n.d.	n.d.	0.75	32	17.5	0.19	0.38	3.5	n.d.	n.d.	n.d.
Parvimonas micra (44)	0.023	0.064	2.3	n.d.	n.d.	n.d.	0.125	6	11.4	0.064	0.25	2.3	n.d.	n.d.	n.d.
Peptoniphilus spp. (51)	0.016	0.064	0	n.d.	n.d.	n.d.	0.75	>256	29.4	0.25	0.75	0	n.d.	n.d.	n.d.
Peptostreptococcus spp. (19)	0.38	2	5.3	n.d.	n.d.	n.d.	0.19	0.5	5.3	0.125	0.25	0	n.d.	n.d.	n.d.
Cutibacterium spp. (220)	0.064	0.19	0	n.d.	n.d.	n.d.	0.047	0.125	2.3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

[a] *EUCAST breakpoints were applied to determine % resistance; amoxicillin MIC > 2 mg/L and MIC >8 mg/L for gram-negative and gram-positive anaerobic bacteria, respectively, clindamycin MIC >4 mg/L and metronidazole MIC >4 mg/L. For the gram-negative bacteria breakpoints of >8 mg/L was applied for amoxicillin-clavulanic acid and meropenem.*

[b] *Not all antibiotics are tested for each isolate.*

[c] *Not determined*

Table 4.8.5.2 An overview of the percentage resistance for different antibiotics within gram-negative anaerobic genera, per year.

		% resistance								
	Antibiotic	**2019**	**2018**	**2017**	**2016**	**2015**	**2014**	**2013**	**2012**	**2011**
Bacteroides spp.	amoxicillin	n.a.[a]	94	97	94	92	93	91	98	98
	amoxi-clav	2	1	1	0	1	2	0	0	1
	clindamycin	33	31	24	18	21	20	20	27	27
	metronidazole	1	0	1	1	0	2	0	0	0
	meropenem	2	3	0	1	n.a.	n.a.	n.a.	n.a.	n.a.
Parabacteroides spp.	amoxicillin	38	61	67	82	55	55	60	n.a.	n.a.
	amoxi-clav	7	22	0	6	17	9	0	n.a.	n.a.
	clindamycin	73	50	28	59	0	27	60	n.a.	n.a.
	metronidazole	0	0	0	0	0	0	0	n.a.	n.a.
Prevotella spp.	amoxicillin	32	49	41	52	41	51	60	33	42
	amoxi-clav	0	0	0	0	0	0	0	0	0
	clindamycin	16	6	9	13	17	11	4	10	8
	metronidazole	2	1	1	1	2	0	4	0	0
	meropenem	0	0	0	0	n.a.	n.a.	n.a.	n.a.	n.a.
Fusobacterium spp.	amoxicillin	0	16	24	3	6	0	16	9	22
	amoxi-clav	0	0	8	3	6	0	5	0	0
	clindamycin	4	0	0	0	0	0	0	0	0
	metronidazole	0	0	0	0	0	0	0	0	0
Porphyromonas spp.	amoxicillin	6	0	15	6	22	n.a.	n.a.	n.a.	n.a.
	amoxi-clav	0	0	0	0	0	n.a.	n.a.	n.a.	n.a.
	clindamycin	16	6	38	17	11	n.a.	n.a.	n.a.	n.a.
	metronidazole	0	0	0	0	0	n.a.	n.a.	n.a.	n.a.
Bilophila spp.	amoxicillin	86	100	86	100	78	n.a.	n.a.	n.a.	n.a.
	amoxi-clav	7	0	7	0	0	n.a.	n.a.	n.a.	n.a.
	clindamycin	8	0	0	0	0	n.a.	n.a.	n.a.	n.a.
	metronidazole	0	0	0	0	0	n.a.	n.a.	n.a.	n.a.
Veillonella spp.	amoxicillin	4	5	5	0	0	22	0	0	n.a.
	amoxi-clav	0	0	0	0	0	20	0	0	n.a.
	clindamycin	0	10	0	0	0	0	0	0	n.a.
	metronidazole	0	5	0	0	0	0	0	0	n.a.

[a] *Not available*

Table 4.8.5.3 An overview of the percentage resistance for different antibiotics within gram-positive anaerobic genera, per year.

		% resistance								
	Antibiotic	**2019**	**2018**	**2017**	**2016**	**2015**	**2014**	**2013**	**2012**	**2011**
Actinomyces spp.	amoxicillin	0	0	0	0	0	0	0	0	0
	clindamycin	15	12	5	7	7	11	0	0	8
Anaerococcus spp.	amoxicillin	0	0	0	n.a.a	n.a.	n.a.	n.a.	n.a.	n.a.
	clindamycin	26	11	13	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	metronidazole	0	0	0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Finegoldia magna	amoxicillin	0	0	0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	clindamycin	18	20	21	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	metronidazole	4	0	0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Parvimonas micra	amoxicillin	2	0	0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	clindamycin	11	8	4	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	metronidazole	2	0	0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Peptoniphilus spp.	amoxicillin	0	0	0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	clindamycin	29	16	17	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	metronidazole	0	0	0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Peptostreptococcus spp.	amoxicillin	5	0	0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	clindamycin	5	0	0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	metronidazole	0	0	0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Clostridium spp.	amoxicillin	2	0	3	14	7	14	0	10	0
	clindamycin	30	26	30	28	22	0	27	33	19
	metronidazole	2	2	0	0	0	0	0	0	0
Eggerthella lenta	amoxicillin	0	n.a.	0	0	0	n.a.	n.a.	n.a.	n.a.
	clindamycin	0	n.a.	0	0	0	n.a.	n.a.	n.a.	n.a.
	metronidazole	0	n.a.	0	0	0	n.a.	n.a.	n.a.	n.a.
Cutibacterium spp.	amoxicillin	0	0	0	0	0	0	0	0	0
	clindamycin	2	6	4	4	1	3	3	4	3

[a] *Not available*

Conclusions

- In contrast to previous years, amoxicillin resistance was not only encountered among gram-negative anaerobic bacteria, but also within certain genera of GPAC.
- Metronidazole resistance was observed in isolates belonging to the genera *Bacteroides*, *Prevotella*, *Parvimonas*, *Finegoldia* and *Clostridium*.
- The rate of resistance for metronidazole and meropenem among *Bacteroides* and *Prevotella* isolates remained similar when comparing it to previous years.
- None of the *Bacteroides* isolates was resistant to both metronidazole and meropenem.
- The rate of resistance for most tested antibiotics varies per year.

References

1 Veloo ACM, Bahar Tokman H, Jean-Pierre H, Dumont Y, Jeverica S, Lienhard R, Novak A, Rodloff A, Rotimi V, Wybo I, Nagy E. Antimicrobial susceptibility profiles of anaerobic bacteria, isolated from human clinical specimens, within different European and surrounding countries. A joint ESGAI study. Anaerobe 2020; 61:102111

2 Boiten KE, Kuijper EJ, Smit LFE, Bode LGM, Schule L, Schoffelen AF, Notermans D, Woudt SHS, Winter HLJ, van Prehn J, Veloo ACM. Pandrug resistant *Bacteroides fragilis* clinical isolates in the Netherlands; true or fiction. ESCCMID 2020, accepted as poster.

4.8.6 *Clostridioides difficile*

Introduction

The Centre for Infectious Disease Control (CIb) of the National Institute for Public Health and the Environment (RIVM) started a National Reference Laboratory for *Clostridioides (C.) difficile* at the Leiden University Medical Centre (LUMC) soon after recognition of fluoroquinolone resistant *C. difficile* PCR ribotype 027 outbreaks in 2005. Since then, this laboratory has offered ad hoc typing services for all microbiology laboratories in the Netherlands for typing of *C. difficile* isolates of patients with severe disease, or isolates from a suspected outbreak. Additionally, the Dutch sentinel *C. difficile* infections (CDI) surveillance programme has been initiated in 2009 in order to monitor CDI incidence rates and circulating ribotypes in an endemic situation. An annual report is published each year at the CIb website.[1] Antimicrobial susceptibility tests are regularly performed at the Reference laboratory and resistance to vancomycin, metronidazole and fidaxomicin was not detected until 2017. In December 2017, a clinical *C. difficile* isolate with PCR ribotype 020 was found (MIC=8 mg/L) in a patient who failed metronidazole treatment.[2] The stable metronidazole resistance correlated with the presence of a transferable plasmid which was not found in susceptible isolates.

Methods

In the period May 2018-May 2019, 24 acute care hospitals participated in the sentinel surveillance programme. In these hospitals, all hospitalized patients >2 years old with clinical signs and symptoms of CDI in combination with a positive test for *C. difficile* toxins or toxigenic *C. difficile* were included. Clinical data and outcomes after 30 days were registered. Isolates of all included CDI cases were sent to the LUMC for PCR ribotyping. Antibiotic resistance was determined by agar dilution for a selection of *C. difficile* sentinel surveillance isolates. Additionally, all submitted *C. difficile* isolates were subjected to a PCR assay to detect plasmid-associated metronidazole resistance.[2]

Results

From May 2018 to May 2019, a mean CDI incidence rate of 3.17 cases per 10,000 patient-days was found through sentinel surveillance. The most frequently encountered PCR ribotypes were 014/020 (20%) and 078/126 (12%). From May 2018 to May 2019, no outbreaks of *C. difficile* in hospitals participating in the sentinel surveillance were reported to the National Reference Laboratory.

Among samples submitted for ad hoc typing, PCR ribotype 014/020 was the predominant ribotype (15%), followed by PCR ribotype 002 (8%) and ribotype 015 (8%). No outbreaks were reported.

Antibiotic resistance was determined for 92 randomly selected *C. difficile* sentinel surveillance isolates from 24 different hospitals, collected between May 2018 and May 2019 (Table 4.8.6.1). Additional PCR ribotypes (n=56) included were PCR ribotypes 003, 005, 007, 010, 011, 012, 013, 015, 017, 021, 023, 026, 027, 029, 045, 050, 052, 057, 073, 081, 106, 114, 115, 116, 118, 122, 124, 150 and unknown ribotypes.

No resistance to vancomycin was detected using EUCAST ECOFF[3] cut-off levels of 2 mg/L, but there was resistance detected to metronidazole using EUCAST ECOFF cut-off levels of 2 mg/L in one non-toxigenic isolate with an MIC of 4, belonging to ribotype 010. Applying the PCR for plasmid-mediated metronidazole resistance, 2 of the 1002 tested strains were positive; except for the already mentioned non-toxigenic 010, another PCR ribotype 020 was found with an MIC of 6 mg/L.

Discussion

The epidemiology of CDI is comparable with previous years, except that *C. difficile* infections due to the hypervirulent PCR ribotype 027 are decreasing significantly compared to 2009-2014. Since 2015 a significant decrease has been observed. Resistance to antibiotics that are used for treatment of CDI is still very rare, though plasmid-mediated resistance to metronidazole (pCD-METRO) has been discovered in 2018. Using a newly developed PCR for detection of pCD-METRO, a large collection of human and animal strains were investigated. pCD-METRO was detected in toxigenic and non-toxigenic isolates from humans and animals in various countries. In 2018-2019, among clinical isolates sent to the Reference Laboratory only 2 were pCD-METRO positive. The presence of the plasmid always correlated with increased MIC levels to metronidazole. The clinical relevance of pCD-METRO is currently being studied.

Conclusions

- No resistance of *C. difficile* to vancomycin was found by agar dilution and only 1 isolate was found resistant to metronidazole with an MIC of 4 mg/L.
- Plasmid-mediated resistance to metronidazole (pCD-METRO) was found in 2 of 1002 tested clinical isolates.

References

1. Vendrik KEW, Baktash A., Crobach MJT, Harmanus C, Sanders IMJG, Berssenbrugge EKL, Kuijper EJ, Notermans DW, Greeff de SC, Albas J, Dissel v JT. 2019. Thirteenth Annual Report of the National Reference Laboratory for Clostridioides difficile and results of the sentinel surveillance. May 2018-May 2019.
2. Boekhoud IM, Hornung BVH, Sevilla E, Harmanus C, Bos-Sanders IMJG, Terveer EM, Bolea R, Corver J, Kuijper EJ, Smits WK. Plasmid-mediated metronidazole resistance in Clostridioides difficile. Nat Commun. 2020 Jan 30;11(1):598. doi: 10.1038/s41467-020-14382-1
3. EUCAST. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 10.0, 2020. Available at: http://www.eucast.org/clinical_breakpoints/.

Table 4.8.6.1 MIC_{50}, MIC_{90} and range (mg/L) of 92 *C. difficile* sentinel surveillance isolates.

	MIC_{50}	MIC_{90}	Range
Ribotype 001 (n = 3)			
Moxifloxacin	1	1	1 - 16
Metronidazole	0.25	0.25	0.25 - 0.5
Vancomycin	<0.06	<0.06	<0.06 - <0.06
Ribotype 002 (n = 6)			
Moxifloxacin	1	2	1 - 2
Metronidazole	0.25	0.25	0.125 - 0.25
Vancomycin	<0.06	0.125	<0.06 - 0.125
Ribotype 014 (n = 16)			
Moxifloxacin	1	2	1 - 32
Metronidazole	0.25	0.25	0.25 - 0.25
Vancomycin	<0.06	0.125	<0.06 - 0.25
Ribotype 078/126 (n = 11)			
Moxifloxacin	1	2	0.5 - 16
Metronidazole	0.25	0.25	<0.06 - 0.25
Vancomycin	0.125	0.125	<0.06 - 0.125
Other ribotypes (n = 56)			
Moxifloxacin	1	16	1 - 32
Metronidazole	0.25	0.25	0.06 - 4
Vancomycin	<0.06	0.125	<0.06 - 0.125

4.8.7 *Aspergillus fumigatus*

Introduction

Aspergillus fumigatus is a saprobic fungus that causes invasive and non-invasive diseases in humans depending on the immune status of the host. Acquired triazole resistance has emerged in *A. fumigatus*, due to mutations that most commonly affect the efficacy of all medical triazoles including itraconazole, voriconazole, isavuconazole and posaconazole. 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. Due to increasing azole resistance rates combination therapy is recommended 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 (VIPcheckTM, MediaProducts, Groningen, the Netherlands). Three agars contain medical triazoles, itraconazole, voriconazole and posaconazole, and one well acts as growth control. This method has been shown to be highly sensitive and specific to detect azole resistance.[1] Growth on the triazole containing well is highly indicative for resistance and these isolates, phenotypical resistant to one or more of the three triazoles, 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. 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 2019, *A. fumigatus* isolates from 1,429 culture-positive patients were screened for triazole resistance, including 703 (range 51 to 230 per center) patients from UMCs and 726 (range 90 to 222 per center) patients from teaching hospitals. Overall 130 patients (9.1%) harbored a triazole-resistant isolate, with a resistance proportion of 12.5% (88 of 703 patients) in UMCs and 6.1% (42 of 726 patients) in teaching hospitals (Table 4.8.7.1). In all UMCs the resistance proportion exceeded 10%, ranging from 10% in Radboudumc, Nijmegen to 17.8% in ErasmusMC, Rotterdam (Table 4.8.7.1). The resistance proportion was lower in teaching hospitals (range 2.2% to 8.1%), with all centers remaining below the 10% threshold. In total 167 isolates (of the 130 patients) showed phenotypical resistance to one or more of the three tested triazoles and were analyzed for resistance mutations in the *Cyp51A* gene. Environmental resistance mutations, i.e. TR_{34}/L98H and TR_{46}/Y121F/T289A, were most frequently present in isolates from all centers, accounting for 64% (107/167) and 12.6% (21/167) of the resistant isolates, respectively. Of isolates harboring a TR_{34}-mediated mutation, 31% showed a non-resistant phenotype for voriconazole, corresponding with a voriconazole MIC of ≤2 mg/L, while being phenotypically resistant to one or more of the other triazoles tested. Analysis of voriconazole resistant phenotype of *A. fumigatus* harboring TR_{34}/L98H between 2013 and 2018 showed a significant decrease of voriconazole resistance; the mean voriconazole MIC of TR_{34}/L98H isolates decreased from 8 mg/L in 2013 to 2 mg/L in 2018, and the voriconazole resistance frequency was 34% lower in 2018 compared with 2013 (P=0.0001; Figure 4.8.7.1)[2]. The range of voriconazole MICs in TR_{34}/L98H is very broad ranging between 0.5 mg/L and >16 mg/L.

In both UMCs and teaching hospitals the distribution of underlying diseases of patients with a triazole-resistant culture was similar with most patients suffering from chronic lung diseases (38%) and cystic fibrosis (20%).

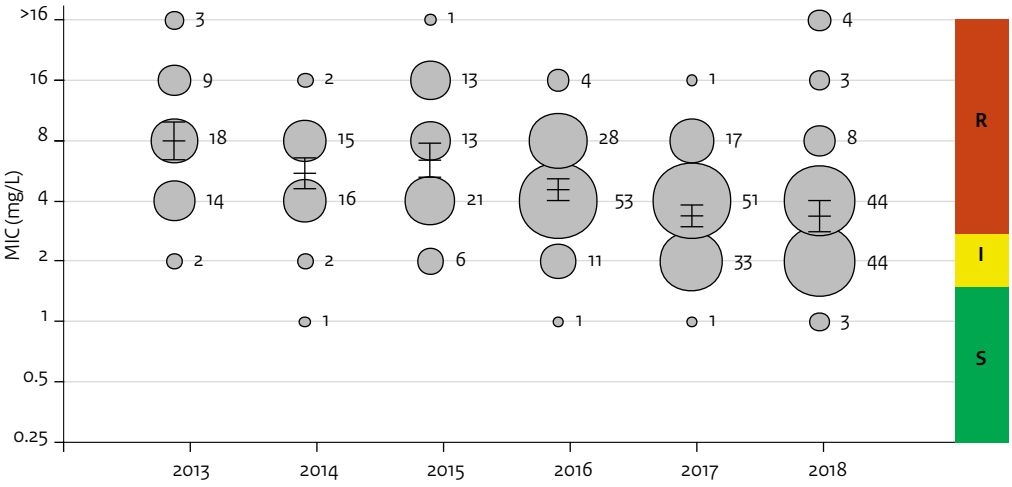
Table 4.8.7.1 Triazole resistance proportion in unselected clinical *A. fumigatus* isolates in 5 University Medical Centers, 2013 to 2019, and 5 teaching hospitals, 2018-2019.

	2013		2014		2015		2016		2017		2018		2019	
	Screened	AzoleR (%)	Screened	AzoleR (%)	Screened	AzoleR (%)	Screened	AzoleR (%)	Screened	AzoleR (%)	Screened	AzoleR (%)	Screened	AzoleR (%)
UMCs														
ErasmusMC	231	10 (4.3)	265	10 (3.8)	22	7 (31.8)*	186	24 (12.9)	147	19 (12.9)	129	17 (13.2)	102	18 (17.6)
LUMC	99	19 (19.2)	113	15 (13.3)	141	23 (16.3)	88	18 (20.5)	114	27 (23.7)	120	25 (20.8)	90	14 (15.6)
Radboudumc	123	6 (4.9)	143	7 (4.9)	145	12 (8.3)	210	20 (9.5)	198	21 (10.6)	196	23 (11.7)	230	23 (10)
UMCG	194	16 (8.2)	191	18 (9.4)	225	15 (6.7)	215	26 (12.1)	240	35 (14.6)	238	34 (14.3)	230	27 (11.7)
Vumc	113	8 (7.1)	104	9 (8.7)	89	14 (15.7)	85	13 (15.3)	75	12 (16)	81	13 (16)	51	6 (11.8)
Total UMCs	**760**	**58 (7.6)**	**814**	**59 (7.2)**	**600**	**64 (10.7)****	**784**	**101 (12.9)**	**774**	**114 (14.7)**	**764**	**112 (14.7)**	**703**	**88 (12.5)**
Teaching hospitals														
Medisch Spectrum Twente											88	5 (5.7)	90	2 (2.2)
St Antonius Hospital											265	28 (10.6)	177	10 (5.7)
PAMM											81	4 (4.9)	147	8 (5.4)
CWZ											155	11 (7.1)	90	6 (6.7)
Isala											195	13 (6.7)	222	18 (8.1)
Total teaching hospitals											**784**	**50 (7.8)**	**726**	**42 (6.1)**

* *Resistance was screened for in high risk patients only*

** *Resistance freequency was calculated based on the data of four centers*

Figure 4.8.7.1 Trends in voriconazole MIC distributions in *A. fumigatus* harboring TR_{34}/L98H.



Discussion

For the first time a decrease in azole resistance frequency was noted in clinical *A. fumigatus* isolates, although in all UMCs the resistance proportion exceeded the 10% threshold. It remains unclear why the frequency has decreased. Several studies have shown variation in resistance frequency over time in single centers.[3] As the total number of screened *A. fumigatus* isolates was lower compared with previous years, less favorable climatic factors might have played a role. Within the *A. fumigatus* isolates that harbor the TR_{34}/L98H mutation, the proportion of isolates that are resistant to voriconazole has decreased over time. The reason why more isolates have voriconazole MICs in the intermediate and susceptible range remains unknown. One explanation may be that the isolates acquire resistance mutations against other azole fungicides that effect the resistance to the medical azoles. According to the current guidelines, treatment with voriconazole monotherapy is not indicated if phenotypical resistance to one (or more) of the tested triazoles is found. Although lower voriconazole MICs may increase the role of voriconazole in treatment of *Aspergillus* diseases, it remains unclear if voriconazole-intermediate and voriconazole-susceptible TR_{34}/L98H isolates can be safely treated with voriconazole.

Conclusions

- Azole resistance frequency declined in comparison with previous years to 12.5% in UMCs and 6.1% in teaching hospitals.
- The azole resistance frequency remained ≥10% in the UMCs
- A decreasing trend in voriconazole MICs was observed in TR_{34}/L98H *A. fumigatus* isolates with 31% showing an intermediate or susceptible voriconazole MIC.

References

1 Arendrup MC, Verweij PE, Mouton JW, Lagrou K, Meletiadis J. Multi-center validation of four well azole agar plates as a screening method for detection of clinically relevant azole resistant *Aspergillus fumigatus*. J Antimicrob Chemother 2017;72:3325-33.

2 Lestrade PPA, Buil JB, van der Beek MT, Kuijper EJ, van Dijk K, Kampinga G, Rijnders BJA, Vonk A, de Greeff SC, Schoffelen AF, van Dissel J, Meis JF, Melchers WJG, Verweij PE. Paradoxal trends in azole-resistant *Aspergillus fumigatus* in a national multicenter surveillance program, 2013 – 2018. Emerg Infect Dis 2020;26:1447-55.

3 Buil JB, Snelders E, Bedin Denardi L, Melchers WJG, Verweij PE. Trends in azole resistance in *Aspergillus fumigatus*, the Netherlands, 1994–2016. Emerg Infect Dis 2019;25: 176-178.

5 Antimicrobial stewardship monitor in hospitals

Introduction

The antimicrobial stewardship monitor reports on 1) the stewardship activities employed by antimicrobial stewardship teams in hospitals and 2) the quality of antimicrobial use in hospitals.

5.1 Stewardship activities employed by antimicrobial stewardship teams in hospitals

Methods

In 2019, an electronic survey was sent to all 76 acute care hospitals in the Netherlands to assess stewardship activities employed by antimicrobial stewardship teams in hospitals. The survey was focused on the activities that A-teams undertake to measure and improve and was based on a systematic literature search including articles containing surveys on antimicrobial stewardship. It consisted of 39 questions categorized into four sections: 1) hospital characteristics; 2) organization of an antimicrobial stewardship program (ASP); 3) hospital resources for ASP; 4) stewardship activities. Results are presented as percentages of the responding hospitals. Trends were described comparing the data with the previous three years.

Results

Hospital characteristics, organization of and hospital resources for an antimicrobial stewardship program

Thirty-nine of 76 hospitals returned the survey, resulting in a response rate of 51%. The mean number of hospital beds was 552 (range 120-1080). Five (13%) of the hospitals were university hospitals, 24 (61%) were non-university teaching hospitals and 10 (26%) non-teaching hospitals. An A-team was present in all but one hospital. In that hospital preparations were being made to establish one. The 38 A-teams all included at least one medical microbiologist. Thirty-seven (97%) of the A-teams also included at least one hospital pharmacist and twenty-seven (71%) had at least one infectious disease specialist present in the

team. Eight (21%) of the A-teams also had a nurse employed, six (16%) an infection prevention specialist, and five (13%) a quality of care officer. Authorization by the hospital boards of directors had been granted to 90% of the A-teams. Six A-teams (16%) had antibiotic guardians (e.g. ambassadors) who propagate appropriate use of antimicrobials on all or nearly all wards and an additional nine (24%) had these on a limited number of wards. IT support was available for 33 (87%) of the A-teams, although IT formation had been officially allocated to only five (13%) of the A-teams and 11 (29%) of the A-teams explicitly indicated that they received only limited IT support. If available, IT support was mainly used for the following antimicrobial stewardship-related activities: selection of specified patients (82%), data reporting (70%), decision support (49%), and point prevalence survey (55%). Twenty-seven and 28 hospitals provided data on total time spent on stewardship-related activities and salary support, respectively. The time spent by the A-team was a mean of 31.2 and a median of 21.0 hours per week (range 2-144 hours). 55% of the hospital boards of directors provided a budget for the A-teams, with a mean financial support of 0.78 FTE and a median financial support of 0.55 FTE (range 0.05-3.30 FTE). Some organizational characteristics and resources are compared with 2016, 2017, and 2018 in Table 5.1.1.

Stewardship activities

Thirty-eight A-teams provided data on stewardship activities. Sixty-eight percent of the A-teams received at least annually reports on cumulative antimicrobial susceptibility provided by the medical microbiology laboratory. Eighty-two percent received reports on quantitative use of antimicrobials from the hospital pharmacy. Fifty-six percent of the A-teams performed a point prevalence survey to assess the appropriateness of antimicrobials use at least annually. Half of the A-teams had a structured outpatient parenteral antimicrobial therapy (OPAT) program and 18% had an allergy de-labeling service as part of their ASP. Twenty-five of the 38 A-teams (66%) used post-prescription review for several stewardship objectives, as summarized in Table 5.1.2 and compared to previous years in Table 5.1.1. All of these 25 A-teams provided individual recommendations on stewardship objectives. This was on average done by telephone by 93% (range per objective 80%-100%), face-to-face by 54% (range 24%-100%), and by computerized alerts or notes in the electronic medical chart by 62% (range 20%-100%) of the A-teams. Ninety-five percent of the A-teams had interventions in place to monitor and improve the use of restricted antimicrobials (Table 5.1.3). Table 5.1.4 summarizes the performance and monitoring of bedside consultation.

Table 5.1.1 Trends in A-team characteristics and monitoring between 2016 and 2019.

	2016	2017	2018	2019
Survey response rate, N (%)*	42 (48%)	64 (80%)	35 (45%)	39 (51%)
A-team characteristics				
Presence of an A-team	88%	94%	100%	97%
≥1 clinical microbiologist	100%	100%	100%	100%
≥1 hospital pharmacist	100%	100%	100%	97%
≥1 infectious disease specialist	70%	68%	86%	71%
≥1 nurse	5%	10%	23%	21%
≥1 infection prevention specialist	10%	14%	14%	16%
Time spent on stewardship per team, mean [hours per week], (range)	15.0 (1-47)	19.8 (3-58)	36.7 (4-134)	31.2 (2-144)

Table 5.1.1 (continued) Trends in A-team characteristics and monitoring between 2016 and 2019.

	2016	2017	2018	2019
Budget provided by hospital board of directors	39%	41%	79%	55%
Financial support, median [FTE], (range)	not available	0.5 (0.05-1.5)	0.7 (0.1 – 3.1)	0.6 (0.05-3.30)
*Occasional and continuous monitoring of***				
Restricted antimicrobials***	77%	91%	92%	95%
Guideline adherence empirical antimicrobial use	71%	28%****	51%	39%
IV-oral switch	76%	53%	80%	58%
De-escalation	71%	34%	40%	37%
Bedside consultation *S. aureus* bacteremia	53%	56%	72%	77%
Therapeutic drug monitoring	63%	65%	69%	44%
Correct diagnostics	58%	30%	34%	13%

* *total number of hospitals in the Netherlands has decreased. Total number of hospitals in 2016: 88, in 2017: 80, in 2018: 78, in 2019: 76*

** *meaning postprescription review for all objectives except bedside consultation and restricted antimicrobials*

*** *includes all types of interventions to improve the use of restricted antimicrobials*

**** *surveyed only for non-restricted antimicrobials in 2017*

Table 5.1.2 Number of hospitals that perform post-prescription review for stewardship activities (n=38).

	Total	Continuous (4-7 days a week)	Occasional (1-3 days a week)
No post prescription review, N (%)	13 (34)	n.a.	n.a.
Appropriateness of empirical antimicrobial use, N (%)	15 (39)	7 (18)	8 (21)
IV-oral switch, N (%)	22 (58)	16 (42)	6 (16)
De-escalation, N (%)	14 (37)	11 (29)	3 (8)
Discontinuation, N (%)	11 (29)	7 (18)	4 (11)
Therapeutic drug monitoring, N (%)	17 (44)	16 (42)	1 (3)
Correct diagnostics, N (%)	5 (13)	3 (8)	2 (5)
Surgical prophylaxis, N (%)	1 (3)	0 (0)	1 (3)

Table 5.1.3 Interventions in hospitals performed to monitor and improve the use of restricted antimicrobials (n=35).

Post-prescription review, N (%)	25 (66)
Education for residents, N (%)	17 (45)
Education for medical specialists, N (%)	11 (29)
Formulary restriction, N (%)	12 (32)
Computerized alert, N (%)	9 (24)
Check on diagnostics tests, N (%)	6 (16)
Post-authorization, N (%)	9 (24)
Pre-authorization, N (%)	8 (21)
Local opinion leaders, N (%)	2 (5)
Antibiotic checklist, N (%)	9 (24)
Antibiotic order forms, N (%)	2 (5)
Mandatory bedside consultation, N (%)	2 (5)
Stop orders, N (%)	1 (3)
No activities, N (%)	2 (5t)

Table 5.1.4 Patient categories for which the hospital agreed to perform a compulsory bedside consultation by an infectious disease specialist and for which A-teams monitor the performance.

	Compulsory bedside consultation, N (% of 38 hospitals)	Monitoring of performance of bedside consultation, N (% of hospitals with indication for consultation)
No recommended bedside consultation	7 (18)	Not applicable
Staphylococcus aureus bacteremia	30 (79)	23 (77)
Infective endocarditis	19 (50)	7 (37)
Prosthetic joint infection	8 (21)	2 (25)
Vascular prosthesis infection	8 (21)	1 (13)
Invasive fungal infection	15 (40)	4 (27)

5.2 Quality of antimicrobial use in hospitals

Methods

In 2019 ten hospitals with either HIX or EPIC and substantial datamanagement capacity available participated in this part of the antimicrobial stewardship monitor including the *S. aureus* bacteremia registry. Data acquisition was based on the extraction of data from the electronic medical records (EMR), although hospitals had the option of entering data manually in a web-based portal. The core of the data consisted of all the antimicrobial prescriptions at patient level. If recorded as structured data in the EMR, the judgment by the A-team on the appropriateness of the prescription or the possibility to switch from intravenous to oral administration were extracted as well. Additional data on the management of *S. aureus*

bacteremia could be provided. These included for example blood cultures, risk factors for complicated disease, non-microbiological diagnostics, non-antibiotic treatment, and final diagnosis. Data were used to calculate quality indicators (based on A-team's judgment) or to calculate metrics from the data on antimicrobial prescriptions that may potentially serve as proxy for the quality of antimicrobial use.

Results

Five hospitals provided data, three of which by means of automatic data extraction from the EMR, including one hospital that provided data on the management on *S. aureus* bacteremia (data not reported). The two other hospitals uploaded data in a web-based portal. This concerned data that was difficult to integrate into the automatically delivered data and is therefore not reported here.
The automatically extracted prescription data of the three hospitals concerned approximately the first six months of 2019, except for one hospital that provided data on 2018 and the first nine months of 2019. The number of unique patients varied from 4,531 to 5,439 per six months and the number of antimicrobial prescriptions from 8,811 to 14,447 per six months.

Restricted antimicrobials

Individual antimicrobial prescriptions were converted to uninterrupted courses ignoring dose and administration frequency. In a six months' time period, carbapenem courses varied from 66 to 399, amoxicillin-clavulanic acid courses from 874 to 1,402, chinolon courses from 655 to 1,401, and glycopeptide courses from 78 to 465 between the three hospitals. Data on adherence of antibiotic therapy to the local guideline was available for three hospitals. However, data from the one hospital that provided manually imputed data were limited and excluded from the analysis as shown in Figure 5.2.1.

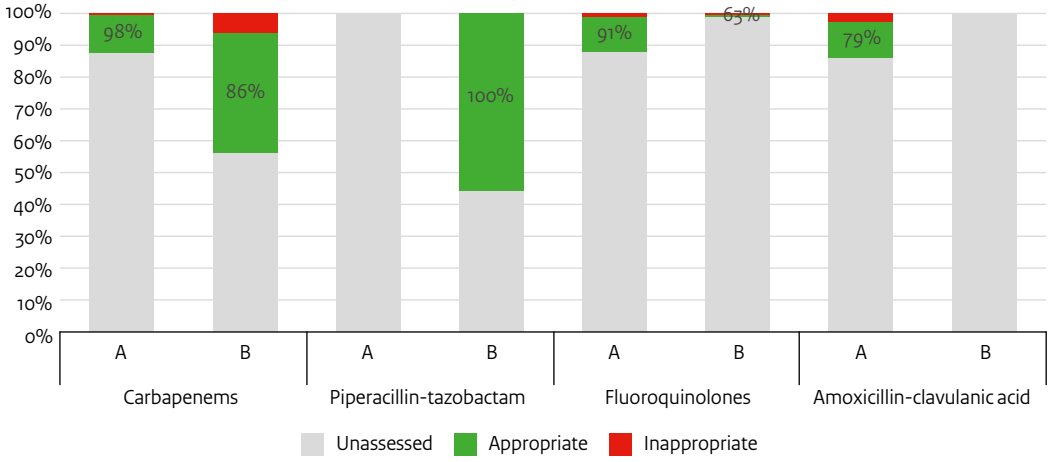
IV-oral switch

Figure 5.2.2. shows the proportion of antibiotic courses that were administered intravenously for ≤72 hours. For those courses it was calculated whether that course was followed by an oral antibiotic course within 24 hours after cessation (iv-oral switch). The remaining actions comprised discontinuation, escalation, or de-escalation of antibiotic treatment (not specified). The data on cephalosporins reflect the use of second generation cephalosporins (predominantly cefuroxim) in hospital B and C. In those hospitals cefuroxime is the backbone of empiric treatment of sepsis, severe pneumonia and gram-negative infections. Data on cephalosporins in hospital A relate to third generation cephaloporins. These consist of ceftriaxone (empiric treatment of sepsis, severe pneumonia and gram-negative infection), ceftazidime (febrile neutropenia), and cefotaxim (selective decontamination of the digestive tract).

Therapeutic drug monitoring (TDM)

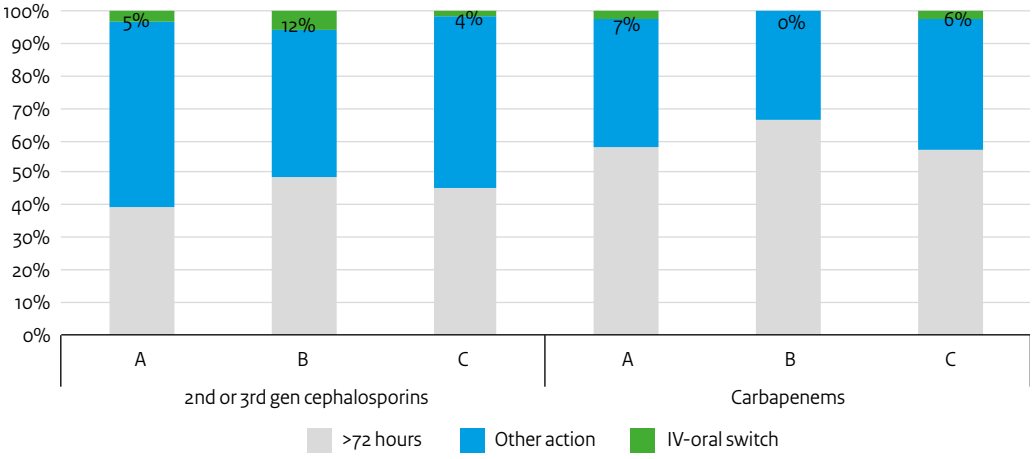
From one hospital the performance of drug concentration measurements were extracted from the EMR. In 25.2% of the aminoglycoside courses (n=1,032; 29.3% of all courses were given for more than 72 hours) drug concentrations were measured. This took place a median of two days (IQR 2-3) after the initiation of therapy. Vancomycin concentrations were measured during 60.5% of the courses (n=375) with a median of 2 days (IQR 1-2) after start. 49.4% of all glycopeptide courses (both vancomycin and teicoplanin) were given longer than 72 hours. Voriconazole concentrations were measured during 41.4% of courses (n=169) after a median of three days (IQR 2-4).

Figure 5.2.1 Adherence of antibiotic courses to the guidelines in two hospitals (A and B).



Grey column is percentage of antibiotic courses that was not assessed.
The percentage of antibiotic courses assessed is represented by the green (guideline adherent prescriptions) and red column (guideline inadherent prescriptions).
The percentages show the performance of the quality indicator "prescribe restricted antimicrobials according to the local guideline".

Figure 5.2.2 The proportion of antibiotic courses that were administered intravenously for >72 hours or were changed ≤72 hours in 3 hospitals (A, B and C).



The blue and the green column together correspond to the number of courses that were administered intravenously for ≤72 hours.
The green column and its percentage corresponds to the short intravenous courses that were switched to an oral antibiotic course.

Conclusions

- A-teams have become a universal part of the hospital
- Half of the A-teams have incorporated OPAT into their antimicrobial stewardship programs
- Barriers for the optimal functioning of A-teams is the lack of funding and formal IT-support

Discussion

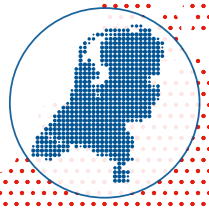
With the comment that there was a response rate of just over 50%, there has been a stabilization compared to last year in terms of the time spent by and focus on A-teams. Recent developments in antimicrobial stewardship have been picked up by some A-teams: 50% of the ASP harbor an OPAT program and 18% an allergy de-labeling service. The A-team's composition is also more or less unchanged. In addition to a clinical microbiologist and a hospital pharmacist, more than 70% of the A-teams have an infectious disease specialist and more than 20% a nurse. Financial support is insufficiently provided by the hospital boards of directors, just like formal IT-support.

Particularly in the light of the shortage of staff of the A-teams, it is essential to efficiently reuse data already documented in the EMR. The data presented are a follow-up on the pilot performed in 2017 that tested the feasibility to extract data from the EMR to assess the quality of antimicrobial use. The antimicrobial prescriptions are in particular the easiest to extract since all these are available as structured data in all hospitals. Traditionally, these are used to calculate quantity metrics. It is, however, difficult to identify improvement targets from these metrics. Adjustments of these quantity metrics might offer a solution and these adjusted quantity metrics can potentially serve a proxy for quality metrics, although this has yet to be shown. One example of the possibilities is shown for IV-oral switch. This process can be made clear by looking in a structured way at the route of administration of successive antibiotic courses (Figure 5.2.2.). Reuse of data also seems to have potential for determining the correct application of TDM. In the hospital that provided data on this stewardship objective, TDM seemed to be performed in almost all patients receiving prolonged courses of aminoglycosides and vancomycin. The other two hospitals gave priority to the extraction of antimicrobial prescription data, but this successful example and the availability of these data as structured data in the EMR are promising for wider application in the future.

A future challenge is to identify useful proxy indicators that are specific, e.g. for a certain infectious syndrome or type of indication (prophylaxis, empiric or directed treatment) and to incorporate specialism and reported indication in the proxy indicators. We are also working on the presence of a larger number of structured variables in the EMR so that we can also link A-team assessments to prescriptions. Finally, in the light of the shortage in IT support, we have made external IT support available to realize the intended increase in the number of participating hospitals.

MARAN 2020

Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2019



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 2020

Monitoring of Antimicrobial Resistance
and Antibiotic Usage in Animals in the Netherlands
in 2019

June 2020

Contents

Colophon

This report is published under the acronym MARAN-2020 by Wageningen Bioveterinary Research (WBVR) in collaboration with Wageningen Food Safety Research, the Food and Consumer Product Safety Authority (NVWA), the National Institute for Public Health and the Environment (RIVM), Utrecht University and the Netherlands Veterinary Medicines Institute (SDa). The information presented in MARAN-2020 is based on total sales data and animal specific usage of antimicrobial agents in animal husbandry and the occurrence of antimicrobial resistance in bacteria of animal origin and of relevance to public health.

MARAN-2020 is published in a combined back-to-back report with NETHMAP-2020. 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).

MARAN-2020 can be ordered from the secretariat of WBVR, p/a Houtribweg 39, 8221 RA Lelystad, The Netherlands.

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
Ir. B. Wullings, Wageningen Food Safety Research (WFSR), Wageningen
Prof. Dr. D. Heederik, Netherlands Veterinary Medicines Institute (SDa), Utrecht
Prof. Dr. D.J. Mevius, Faculty of Veterinary Medicine, Utrecht University

The following persons contributed to the writing of MARAN 2020

Part I Total sales of antibiotics and usage in livestock
Dr. I.M. van Geijlswijk, Prof. Dr. D. J.J. Heederik, Prof. Dr. D.J. Mevius, P. Sanders Msc, SDa, Utrecht
Part II Resistance data
Dr. M. Swanenburg, Dr. M.S.M. Brouwer, Dr. K.T. Veldman, WBVR, Lelystad
Ing. B. Wit, NVWA, Utrecht
Ir. B. Wullings, WFSR, Wageningen
Dr. E. Franz, Dr. R. Pijnacker, Dr. L. Mughini Gras, Dr. C. Dierikx, Dr. E. van Duijkeren, RIVM, Bilthoven
Dr. E. M. Broens, Prof. Dr. J. Wagenaar FD Utrecht

People involved in providing data for the surveillance of antimicrobial resistance

WBVR, Lelystad: Joop Testerink, Marga Japing, Arie Kant, Yvon Geurts, Alieda van Essen-Zandbergen,
WFSR, Wageningen: Michel Rapallini,
RIVM, Bilthoven: Thijs Bosch, Leo Schouls, Kim van der Zwaluw, Maaike van den Beld, Anjo Verbruggen,
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 Research in Animals', grant number WOT-01-002-03.02, project leader in 2019 Dr. K.T. Veldman.

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 and Drs. M. Poldermans from FIDIN for providing detailed insight into the national sales data.

1 Summary

Antibiotic Usage

Sales of antimicrobial veterinary medicinal products in 2019 (150 tonnes) decreased by 16.1 % compared to 2018 (179 tonnes). This means that the total reduction compared to the index year 2009 was almost 70%, which is the result of combined efforts of the authorities, the livestock sectors and the veterinarians. Antibiotic usage in veal calves and pigs decreased compared to 2018, while antibiotic use in dairy cattle and broilers was relatively stable at a low level over the last four years. Use in turkeys and rabbits shows substantial fluctuations and the goat sector is currently implementing a system for monitoring antibiotic use. The different livestock sectors each have a typical pattern in use of first, second and third choice antibiotics. In accordance with the recent WHO- classification of polymyxins as *Highest Priority Critically Important Antibiotic*, the Netherlands Veterinary Medicines Institute considers polymyxins as third choice drugs, and this antibiotic class is reported as such. The consequence would be that similar as for fluoroquinolones and 3rd/4th generation cephalosporins, the target for its use from 2021 onwards will be no usage.

Antimicrobial resistance

In 2019, S. Enteritidis (34%) followed by S. Typhimurium (12%) together with the monophasic variant of Typhimurium (S. enterica subspecies enterica 1,4,[5],12:i:-) (8%), were most frequently isolated from human clinical salmonellosis cases. In pigs, S. Typhimurium (36%) and the monophasic variant of S. Typhimurium (20%) dominated. In cattle, S. Typhimurium (35%) and S. Dublin (29%) were most commonly isolated. In poultry (including poultry products), the most frequently isolated serovars were S. Infantis (38%), S. Paratyphi B var. Java (S. Java, 11%) and S. Enteritidis (10%). Among laying hens, the most frequent isolated serotype was S. Enteritidis (31%), followed by S. Typhimurium (15%). This shows the complexity of the *Salmonella* epidemiology, with a variety of potential sources for human infection, including the Dutch food chain, but also travel and imported food products. Overall, the highest resistance proportions in *Salmonella* were observed for tetracycline, sulfamethoxazole, ampicillin, ciprofloxacin, nalidixic acid, and trimethoprim. Highest levels of resistance were found in the monophasic S. Typhimurium, S. Infantis, S. Paratyphi B var. Java from broilers, S. Kentucky (travel related), S. Chester, and to a lesser extent in S. Typhimurium. The highest levels of resistance among S. Enteritidis, the main serovar in human infections, were primarily those for fluoroquinolones (ciprofloxacin and nalidixic acid) in isolates from human and poultry sources. Only 24

(1,3%) ESBL suspected isolates were detected of which 19 isolates (1,0%) were confirmed ESBL-producers mainly from humans. No carbapenemase producing *Salmonella* were found in 2019.

Proportions of resistance in *C. jejuni* isolates from caecal samples of broilers and meat thereof were traditionally high for quinolones and tetracycline and did not substantially change in 2019, compared to 2018. Resistance to macrolides was rarely detected in *C. jejuni* isolates from broilers and poultry meat, and was at low levels in *C. coli* isolates from broilers and poultry meat. Overall, resistance proportions were higher in *C. coli* than in *C. jejuni* isolates. Ciprofloxacin resistance in *Campylobacter* isolates from human patients was again high in 2019 (with a substantial increase compared to 2018), which is a concern for public health. Resistance to erythromycin, first choice antibiotic in human medicine for campylobacteriosis, remained low.

The increasing tendency for resistance against ampicillin, sulfamethoxazole, tetracycline and trimethoprim in human STEC O157 isolates since 2009 did not continue in 2018 and 2019. Resistance to the quinolones (ciprofloxacin and nalidixic acid) and 3rd generation cephalosporins was not detected in human STEC O157 isolates in 2019.

Indicator *E. coli* isolated from randomly collected caecal samples of food animals at slaughter and meat thereof are most suited to study the effects of any interventions on antibiotic use. Among these indicator *E. coli* from animals and meat, resistance levels to ampicillin, tetracycline, sulfamethoxazole and trimethoprim were still relatively high in broilers, pigs, (white) veal calves and chicken and turkey meat. Resistance in indicator *E. coli* from caecal samples showed a tendency to stabilise in broilers, pigs and showed a slight decrease in veal calves. In dairy cattle resistance fluctuated at a low level. This is mostly in agreement with the use data reported. For the first time in twenty years no randomly selected indicator *E. coli* isolates resistant to extended spectrum cephalosporins were detected in faecal samples from broilers, pigs, dairy cattle and veal calves. Resistance to fluoroquinolones was at the same level as in 2018, and was still commonly present in indicator *E. coli* from caecal samples of broilers and meat thereof.

In 2019, a reduction in proportion of animals (prevalence determined with selective method) positive for ESBL/AmpC producing *E. coli* was observed in all livestock species compared to 2018. After a period of increasing prevalence of ESBL-carriers in veal calves, 2019 revealed a reduction in both rosé and white veal calves. The largest reduction in the prevalence of ESBL/AmpC-producing *E. coli* has been achieved in broilers decreasing from 66.0% in 2014 to 17.9% in 2019, which can be considered a great success of the measures on reducing antimicrobial use initiated since 2011.

The overall prevalence of ESBL/AmpC-producing *E. coli* stabilised at a low level (2.8%) in retail meat. After substantial reductions before 2018, the prevalence in broiler meat remained stable in 2019, with 13.7% of the meat being positive for ESBL/AmpC-producing *E. coli*. As in previous years, no carbapenemase-producing *Enterobacteriaceae* were detected in livestock and companion animals.

In 2019, the *mcr-1* gene, encoding for colistin resistance, was identified at very low level (< 1%) in caecal samples from slaughter pigs and white veal calves. For the second year in row *mcr-4* was detected in white veal calves at low level (2%). No *mcr* genes were identified in *E. coli* isolated from broilers and in chicken meat indicative for a further decrease of *mcr-1* in the broiler sector, although the use of colistin in broilers did increase again in 2019. This is important given the high priority of colistin for human medicine.

A comparative study using Whole Genome Sequencing of MRSA isolates revealed that most pig and poultry LA-MRSA isolates differed by more than 15 genes from human isolates from the national surveillance indicating an overall low genetic relatedness between isolates from livestock and humans. These first results suggest the emergence of a PVL-positive LA-MRSA subclade that is transmitted independent of livestock exposure. Further research into the genotypic and epidemiological characteristics of LA-MRSA isolates from livestock and humans is ongoing.

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 of this part of MARAN can be found in more detail in the annual reports of the SDa (https://www.autoriteitdiergeneesmiddelen.nl/en).

2.1 Total sales of veterinary antibiotics in the Netherlands 2019

Analysis of sales data

FIDIN, the federation of the Dutch veterinary pharmaceutical industry, provided sales data for all antimicrobial veterinary medicinal products on package level sold in 2019 in the Netherlands, as extracted from the Vetindex and supplemented with antimicrobial veterinary medicinal products (AVMPs) data of non-FIDIN members. These data are estimated to cover approximately 98% of all sales in the Netherlands. AVMPs that are marketed in accordance with the legal exemptions such as products for minor species in small packages (art 3.7 Regeling Diergeneesmiddelen) and those products that are imported form 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 use in the major livestock farming sectors (pigs, broilers, turkey, other poultry, veal calves, dairy- and other cattle, meat rabbits) covered 97.5% of sales in 2019.

Antimicrobial veterinary medicinal products are reported as active base substance mass (excluding mass of

salts and esters), including 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 on in a following paragraph.

Trends in total sales

Figure 1 and Table 1 show the trends in the total sales of antibiotics licenced for therapeutic use in animals in the Netherlands. Total sales decreased by 69.6 % over the years 2009-2019 implicating that the governmental 70% reduction goal has almost been attained.

Sales of antimicrobial veterinary medicinal products in 2019 (150 tonnes) showed a decrease of 16.1 % compared to 2018 (179 tonnes). The gap between sales data and usage in monitored sectors was 2.5% (Figure 2).

Figure 2 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) of the livestock sectors monitored from 2009 to 2019. Total 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. Figure 2 shows that in veal calves and pigs almost 80% of all antibiotic sold for therapy are used. The animals treated in these sectors are relatively large and therefore need more antibiotics per administration than small animals like broiler chickens. This demonstrates that sales data provide limited information about exposure of animals at risk. Use data as presented 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. However, expressing antibiotic use as Animal Defined-Daily Dosages (Figure 3), shows that the exposure of broilers is similar to that of pigs.

As demonstrated in Figure 3, antimicrobial sales by antibiotic groups show a fluctuating pattern over the years, with an overall decreasing tendency, and some variation from year to year (penicillins, tetracyclines and cephalosporins of 1st and 2nd generation).

Tetracyclines

The fraction of doxycycline (not specified in Figure 3) increased to the highest level ever with 68.6% of the total sales of tetracyclines (42% in 2018, fluctuations between 31% and 49% in the years 2011-2017).

Penicillins

Second place in mass, penicillin sales was stable in 2019 in comparison to 2018. The distribution of broad and narrow spectrum penicillins (in mass sold) has somewhat shifted to narrow spectrum, 70-30%.

(Fluoro)quinolones

The sales of fluoroquinolones decreased with 45kg (20%) in 2019. An overall reduction of 87.6% was realized in comparison with 2011. In 2019, 46% of the sales are applied in the monitored sectors. Extending the monitoring to other animal species (as will be regulated with EU 2019/6) is warranted. The sales of quinolones (flumequine) also decreased by 32% in 2019; these AVMPs are exclusively applied in food producing sectors.

Cephalosporins
Sales of these AVMPs were relatively stable over the period 2015 to 2018. A relatively large increase of sales of 3rd and 4th generation cephalosporins was observed in 2019 (the total mass sold is still less than 3kg). This increase is not associated with use in the monitored livestock sectors, implying use in companion animals, horses or unmonitored production sectors, such as goats.
A reduction of 99.7% of all cephalosporins sales has been achieved since 2011.

Polymyxins
Colistin sales increased again in 2019 with 13%; predominantly in weaned piglets (161 kg) and other poultry (parent animals) (59 kg).
Based on the recent classification of polymyxins as Highest Priority Critically Important Antimicrobials (CIAs) in the 6th revision of the WHO CIA list (2019), 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 for 2020 onwards will be 0 $DDDA_F$.
In the next chapter the use will be analyzed more in depth.

2.2 Usage in pigs, veal calves, cattle, broilers and turkeys in the Netherlands

Starting in 2004, AVMP consumption data derived from veterinarian's invoices were collected in the Netherlands for sentinel farms. These data were converted to the number of defined doses per animal year (DD/AY). The calculation method is similar to the method applied in human drug use. Applied antimicrobial veterinary medicinal products are converted to treated animal mass*days by national conversion factors (determined by the nationally authorized dosages and pharmacokinetics of the drug to compensate for duration of action) and related to animal mass present on a farm. Results are calculated for a period of a year and expressed as the number of days an average animal is treated in that year on that particular farm. The sentinel data (2004-2010) are weighted by farm related variables to obtain figures representative for the whole population of farms in a sector.

Since 2011, husbandry related antimicrobial consumption is monitored at all farms in the largest livestock sectors: pigs, veal calves, broilers, cattle (since 2012) and turkeys (since 2013). Since 2016 rabbits are also monitored but due to several continuance difficulties usage data are still not suitable for trend observations. Since 2017 also antimicrobial use in other poultry sectors than broilers and turkey ((grand)parents and layers) is made available to the SDa.

While the calculation method for treated body mass (numerator) is the same, totalized for all farms per sector, the denominator represents the whole sector, and this measure is referred to as Defined Daily Doses Animal ($DDDA_{NAT}$). Table 2 shows the biomass of the monitored animal sectors (pigs, veal calves, cattle, broilers, turkeys and rabbits), population data of broilers for 2018 have been corrected by Statistics Netherlands (CBS). In Table 3 the standardized animal weights are presented, as applied in the calculation of the denominator. In Table 4 the resulting DDDANAT are shown. In all sectors (dairy cattle, other cattle, veal valves, pigs, broilers and rabbits) but turkeys a reduction in consumption has been realized.

The trends in the number of defined daily dosages animal in veal calves, pigs, cattle, broilers and turkeys are depicted in Figure 4. Specification of applied antimicrobial groups in the different sectors including rabbits for 2015-2019 is presented in Figure 5. CBS data for number of animals are used in the calculations for broilers, turkeys, veal calves and rabbits, and EUROSTAT data for pigs and dairy cattle.

For benchmarking purposes, every farm in the Netherlands is periodically provided with the number of defined daily doses animal per year ($DDDA_F$) of the farm through internet portals of the sector's quality systems. Consumption is calculated with a detailed denominator, to facilitate benchmarking and avoid misclassification. Table 5 depicts the animal bodyweights applied in the calculation of the denominator of $DDDA_F$ by the SDa.

For more details in all animal sectors, annual reports of the SDa should be consulted (https://www.autoriteitdiergeneesmiddelen.nl/en).

Conclusion
Maximal transparency has been created since 2011 through monitoring antibiotics use by veterinarians and farmers. The decrease in sales of AVMPs in the Netherlands in 2019 is consistent with an overall decrease as observed in the use monitoring data. The calculation of consumption is based on national conversion factors (DDDAs) of authorized drugs.

The use of antibiotics of critical importance to human health care (especially cephalosporins of 3rd and 4th generation) is reduced to an absolute minimum, even in the unmonitored sectors. Use of polymyxins slightly increased in 2019.

Table 1 Antimicrobial veterinary medicinal product sales from 1999-2019 in kg (thousands) (FIDIN, 2019)

year	'99	'00	'01	'02	'03	'04	'05	'06	'07	'08	'09	'10	'11	'12	'13	'14	'15	'16	'17	'18	'19
betalactam antibiotics	35	36	38	38	36	43	51	57	61	70	73	71	66	54	45	48	45	39	42	43	36
tetracyclines	162	194	200	214	216	256	292	301	321	257	251	217	157	102	80	69	82	62	68	65	51
macrolides & lincosamides	10	15	17	19	17	23	28	42	55	52	46	39	34	26	25	28	23	23	25	25	23
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
(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
trimethoprim/ sulfonamides	72	80	92	92	88	91	91	93	99	100	92	78	58	48	53	49	42	39	34	33	29
other antibacterials	11	12	11	11	7	6	6	8	8	7	15	13	10	10	8.1	7.8	7.5	7.4	7.2	7.5	7.4
total sales	310	356	376	390	378	434	487	519	565	506	495	433	338	249	217	207	206	176	181	179	150

Figure 1 Antimicrobial Veterinary Medicinal Product (AVMP) sales 1999-2019 in kg (thousands)

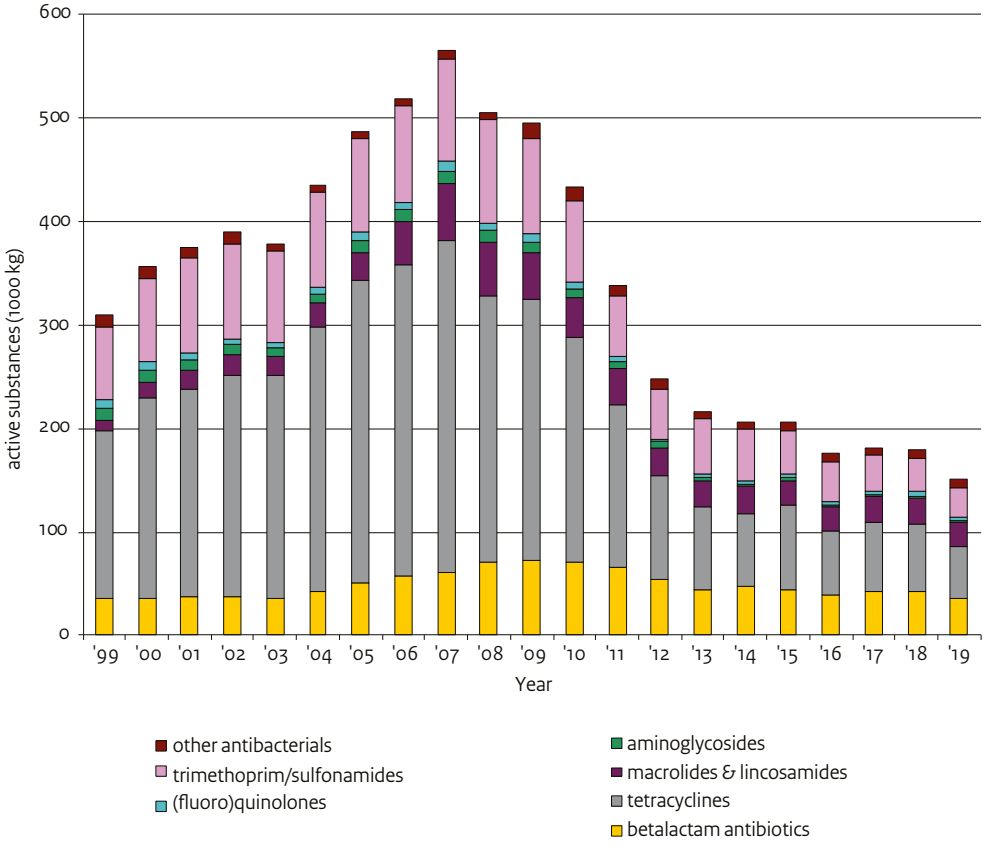


Table 2 Weight per sector in kg (thousands) for DDD_{NAT} calculation

Sector	2012	2018	2019
broilers	43.846	* 48,971	48.684
turkeys	4.961	3.338	3.190
pigs	710.688	662.266	692.233
diary cows	924.600	931.200	954.000
veal calves	156.602	174.934	183.266
other cattle	597.900	541.000	544.500
rabbits	872	866	922

* *corrected weight for broilers in 2018*

Table 3 Applied bodyweights for $DDDA_{NAT}$ calculation

species	category	Standard Weight (kg)
Veal Calves		172
Pigs	Piglets (< 20 kg)	10
	Sows	220
	Fattening pigs	70.2
	Other pigs	70
Broilers		1
Turkeys		6
Cattle	Dairy cows	600
	Other cows	500
Rabbits	Dow+kits	8.4
	Fattening rabbits	1.8
	Other rabbits	3.4

Figure 2 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 liveweight of the food animal population (dotted line, right y-axis, kg x 10^6) from 2009-2019.

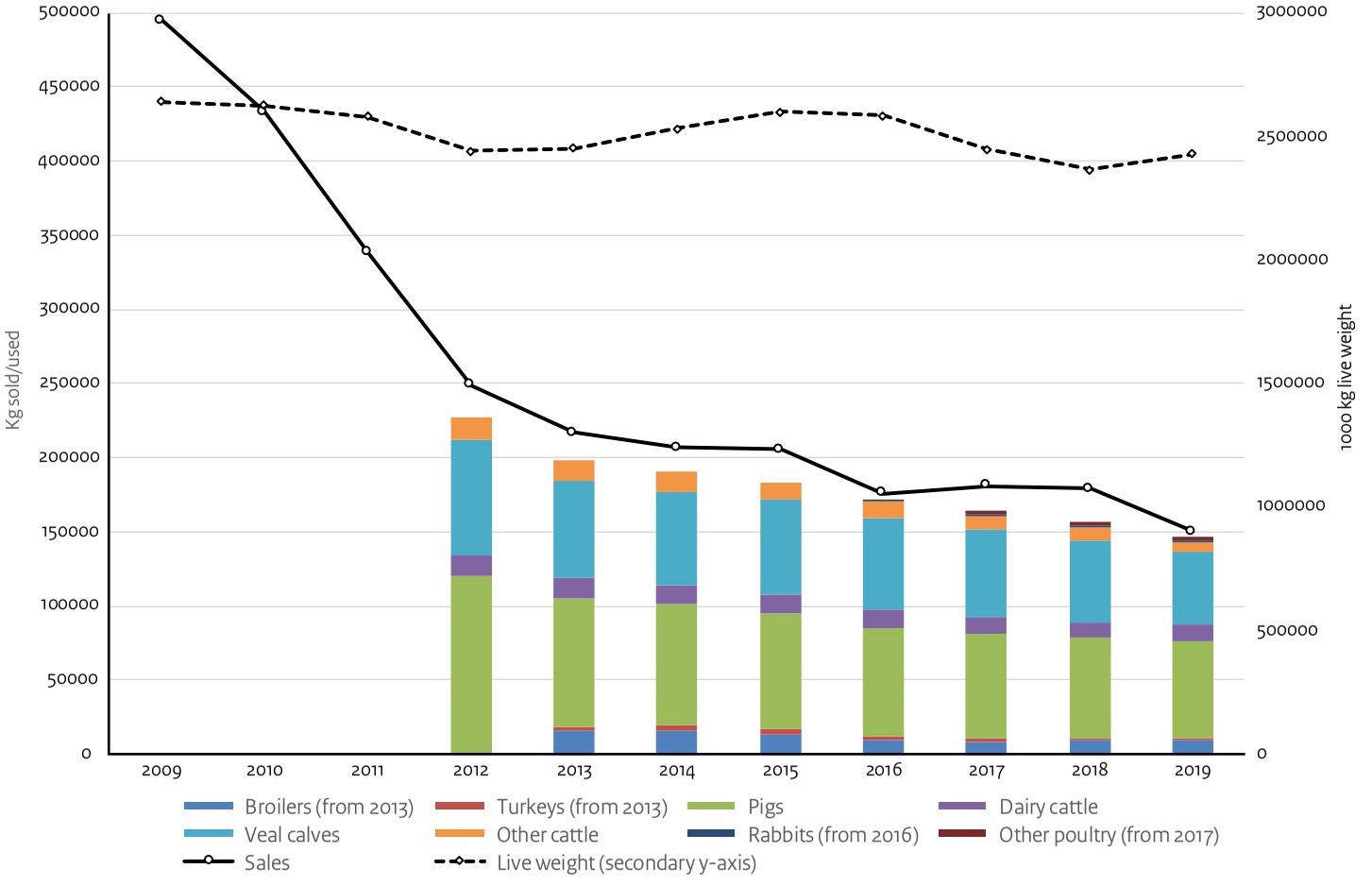


Figure 3 Antimicrobial Veterinary Medicinal Product sales by pharmaco-therapeutic class from 2011-2019 in kg (thousands)

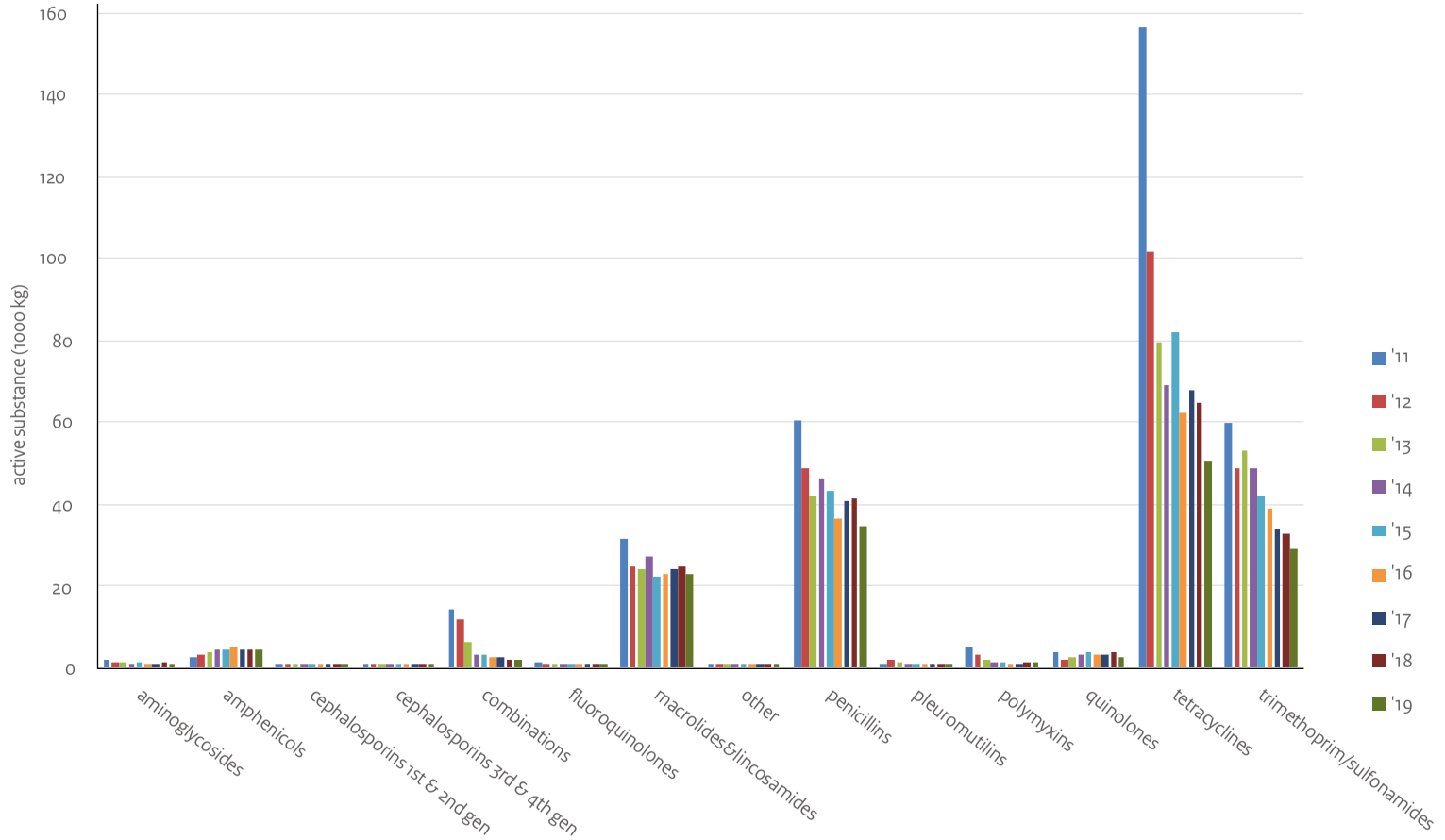


Table 4 Trends in $DDDA_{NAT}$ in the Netherlands in livestock 2015 - 2019

	Animalsector														
	Veal calves					Dairy cattle					Other cattle				
Year	2015	2016	2017	2018	2019	2015	2016	2017	2018	2019	2015	2016	2017	2018	2019
Number of farms with prescriptions	1978	1928	1868	1856	1841	17737	17529	17121	16499	15871	12971	12548	12790	12328	11614
Pharmacotherapeutic group															
First choice*	**18.99**	**17.94**	**17.30**	**16.09**	**14.15**	**2.27**	**2.23**	**2.35**	**2.40**	**2.39**	**0.86**	**0.91**	**0.92**	**0.94**	**0.71**
% 1st choice of total	86.1%	85.9%	85.9%	86.4%	85.6%	73.1%	74.0%	76.9%	79.0%	79.9%	86.0%	85.0%	84.2%	86.7%	85.5%
Amphenicols	1.63	1.59	1.44	1.33	1.28	0.06	0.06	0.05	0.05	0.05	0.10	0.11	0.11	0.10	0.08
Macrolides/lincosamides	3.70	3.35	3.43	3.21	3.05	0.09	0.06	0.05	0.05	0.06	0.15	0.15	0.16	0.14	0.11
Other	*	*	*	*	*	*	*	*	*	*	*	*	*	0.00	0.00
Penicillins	0.42	0.48	0.46	0.43	0.39	1.50	1.52	1.69	1.76	1.75	0.09	0.10	0.11	0.10	0.09
Pleuromutilins	*	*	*	*	*	*	*	*	*	*	*	*	*	0.00	0.00
Tetracyclines	11.01	10.47	10.35	9.86	8.23	0.37	0.35	0.32	0.32	0.30	0.42	0.44	0.45	0.53	0.38
Trimethoprim/sulfonamides	2.22	2.05	1.61	1.25	1.21	0.25	0.24	0.24	0.23	0.24	0.10	0.10	0.09	0.06	0.05
Second choice*	**2.86**	**2.85**	**2.78**	**2.50**	**2.35**	**0.83**	**0.77**	**0.70**	**0.63**	**0.59**	**0.13**	**0.16**	**0.17**	**0.14**	**0.12**
% 2nd choice of total	13.0%	13.7%	13.8%	13.4%	14.2%	26.6%	25.7%	22.8%	20.8%	19.9%	13.3%	14.6%	15.6%	12.9%	14.2%
Aminoglycosides	0.19	0.23	0.23	0.20	0.16	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00
Cefalosporins 1st & 2nd generation	*	*	*	*	*	0.02	0.03	0.03	0.03	0.03	0.00	0.00	0.00	0.00	0.00
Combinations	0.58	0.66	0.57	0.36	0.41	0.00	0.00	0.00	0.00	0.00	0.02	0.03	0.02	0.01	0.01
Macrolides/lincosamides	0.00	0.00	0.01	0.00	0.00	0.42	0.38	0.34	0.29	0.27	0.03	0.03	0.04	0.03	0.02
Penicillins	0.18	0.19	0.23	0.28	0.26	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.03	0.02
Quinolones	1.91	1.77	1.75	1.65	1.52	0.37	0.34	0.31	0.29	0.28	0.07	0.06	0.08	0.06	0.06
Third choice*	**0.21**	**0.09**	**0.06**	**0.04**	**0.02**	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**	**0.00**	**0.00**	**0.00**	**0.00**
% 3rd choice of total	0.9%	0.4%	0.3%	0.2%	0.1%	0.4%	0.3%	0.2%	0.2%	0.2%	0.7%	0.4%	0.2%	0.4%	0.3%
Cefalosporins 3rd & 4th generation	*	*	*	*	*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Fluoroquinolones	0.02	0.03	0.04	0.02	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Polymyxins	0.19	0.07	0.02	0.02	0.01	0.01	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
Total	**22.05**	**20.88**	**20.13**	**18.63**	**16.52**	**3.11**	**3.01**	**3.06**	**3.04**	**2.99**	**1.00**	**1.07**	**1.10**	**1.08**	**0.83**

**Categorization in first, second and third choice antimicrobials based on Dutch WVAB guideline 2018, with a modification for polymyxins.*

Table 4 (continued) Trends in $DDDA_{NAT}$ in the Netherlands in livestock 2015 - 2019

	Animalsector									
	Pigs					Broilers				
Year	2015	2016	2017	2018	2019	2015	2016	2017	2018	2019
Number of farms with prescriptions	5824	5462	5297	4975	4587	816	849	852	834	819
Pharmacotherapeutic group										
First choice*	**6.97**	**6.88**	**6.61**	**6.70**	**6.26**	**3.86**	**2.53**	**2.39**	**2.28**	**2.57**
% 1st choice of total	77.1%	77.5%	76.0%	77.2%	78.7%	26.5%	24.9%	25.4%	22.6%	26.0%
Amphenicols	0.18	0.24	0.25	0.25	0.26	*	*	*	*	*
Macrolides/lincosamides	0.78	0.82	0.76	0.77	0.84	0.10	0.04	0.04	0.03	0.02
Other	*	*	*	*	*	*	*	*	*	*
Penicillins	0.57	0.58	0.55	0.68	0.51	1.20	0.70	0.59	0.44	0.87
Pleuromutilins	0.08	0.07	0.09	0.12	0.09	*	*	*	*	*
Tetracyclines	4.14	4.07	4.05	3.86	3.54	1.49	1.01	0.95	1.04	0.90
Trimethoprim/sulfonamides	1.20	1.10	0.90	1.01	1.01	1.07	0.78	0.82	0.78	0.78
Second choice*	**1.69**	**1.71**	**1.83**	**1.67**	**1.36**	**10.60**	**7.55**	**6.96**	**7.74**	**7.24**
% 2nd choice of total	18.7%	19.3%	21.1%	19.3%	17.1%	72.7%	74.1%	73.7%	76.4%	73.1%
Aminoglycosides	0.01	0.00	0.01	0.03	0.03	0.02	0.01	0.03	0.02	0.01
Cefalosporins 1st & 2nd generation	*	*	*	*	*	*	*	*	*	*
Combinations	0.03	0.02	0.03	0.02	0.04	2.86	1.51	1.72	2.29	1.62
Macrolides/lincosamides	0.04	0.03	0.02	0.02	0.02	0.11	0.05	0.01	0.02	0.01
Penicillins	0.25	0.26	0.37	0.37	0.30	0.38	0.21	0.20	0.22	0.24
Quinolones	1.36	1.39	1.41	1.24	0.97	7.23	5.78	5.00	5.19	5.37
Third choice*	**0.38**	**0.28**	**0.26**	**0.31**	**0.34**	**0.13**	**0.11**	**0.08**	**0.10**	**0.09**
% 3rd choice of total	4.2%	3.2%	2.9%	3.6%	4.3%	0.9%	1.1%	0.9%	1.0%	0.9%
Cefalosporins 3rd & 4th generation	*	*	*	*	*	*	*	*	*	*
Fluoroquinolones	0.00	0.00	0.00	0.00	0.00	0.07	0.07	0.05	0.06	0.04
Polymyxins	0.38	0.28	0.26	0.31	0.34	0.06	0.04	0.03	0.04	0.05
Total	**9.03**	**8.87**	**8.70**	**8.68**	**7.96**	**14.59**	**10.19**	**9.44**	**10.13**	**9.90**

**Categorization in first, second and third choice antimicrobials based on Dutch WVAB guideline 2018, with a modification for polymyxins.*

Table 4 (continued) Trends in $DDDA_{NAT}$ in the Netherlands in livestock 2015 - 2019

Animalsector	Turkeys					Rabbits			
Year	**2015**	**2016**	**2017**	**2018**	**2019**	**2016**	**2017**	**2018**	**2019**
Number of farms with prescriptions	**40**	**47**	**45**	**39**	**43**	**41**	**49**	**40**	**36**
Pharmacotherapeutic group									
First choice*	**19.18**	**12.29**	**8.11**	**10.82**	**10.66**	**30.92**	**24.22**	**32.65**	**30.44**
% 1st choice of total	53.4%	46.5%	40.2%	52.5%	47.9%	**75.5%**	**80.6%**	**74.8%**	**77.1%**
Amphenicols	*	*	*	*	*	0.00	*	*	*
Macrolides/lincosamides	*	*	*	*	*	1.07	1.74	2.67	5.15
Other	*	*	*	*	*	16.37	12.36	16.55	13.25
Penicillins	4.49	3.70	1.64	2.62	1.61	*	*	0.00	*
Pleuromutilins	0.12	*	0.10	0.12	*	1.38	1.68	3.37	4.02
Tetracyclines	12.57	7.63	5.51	7.15	8.13	10.49	7.76	9.93	7.13
Trimethoprim/sulfonamides	2.01	0.95	0.86	0.93	0.93	1.62	0.69	0.13	0.89
Second choice*	**14.92**	**11.93**	**10.99**	**9.06**	**10.99**	**9.67**	**5.73**	**10.46**	**8.39**
% 2nd choice of total	41.5%	45.1%	54.5%	43.9%	49.4%	**23.6%**	**19.0%**	**24.0%**	**21.2%**
Aminoglycosides	0.71	0.69	0.05	0.00	*	9.66	5.73	10.22	8.33
Cefalosporins 1st & 2nd generation	*	*	*	*	*	*	*	*	*
Combinations	0.10	0.01	0.26	0.18	0.16	*	*	*	*
Macrolides/lincosamides	*	*	*	*	0.01	*	*	*	*
Penicillins	1.98	1.18	1.30	1.35	1.66	0.01	*	0.24	0.05
Quinolones	12.13	10.05	9.37	7.52	9.16	*	*	*	*
Third choice*	**1.84**	**2.21**	**1.06**	**0.75**	**0.61**	**0.34**	**0.12**	**0.57**	**0.68**
% 3rd choice of total	5.1%	8.4%	5.3%	3.6%	2.7%	**0.8%**	**0.4%**	**1.3%**	**1.7%**
Cefalosporins 3rd & 4th generation	*	*	*	*	*	*	*	*	*
Fluoroquinolones	1.20	1.60	1.06	0.75	0.59	0.25	0.12	0.29	0.11
Polymyxins	0.63	0.61	*	*	0.02	0.09	*	0.28	0.57
Total	**35.94**	**26.42**	**20.16**	**20.62**	**22.25**	**40.93**	**30.07**	**43.68**	**39.51**

**Categorization in first, second and third choice antimicrobials based on Dutch WVAB guideline 2018, with a modification for polymyxins.*

Figure 4 Animal-defined daily dosages for 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 2011-2019 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)

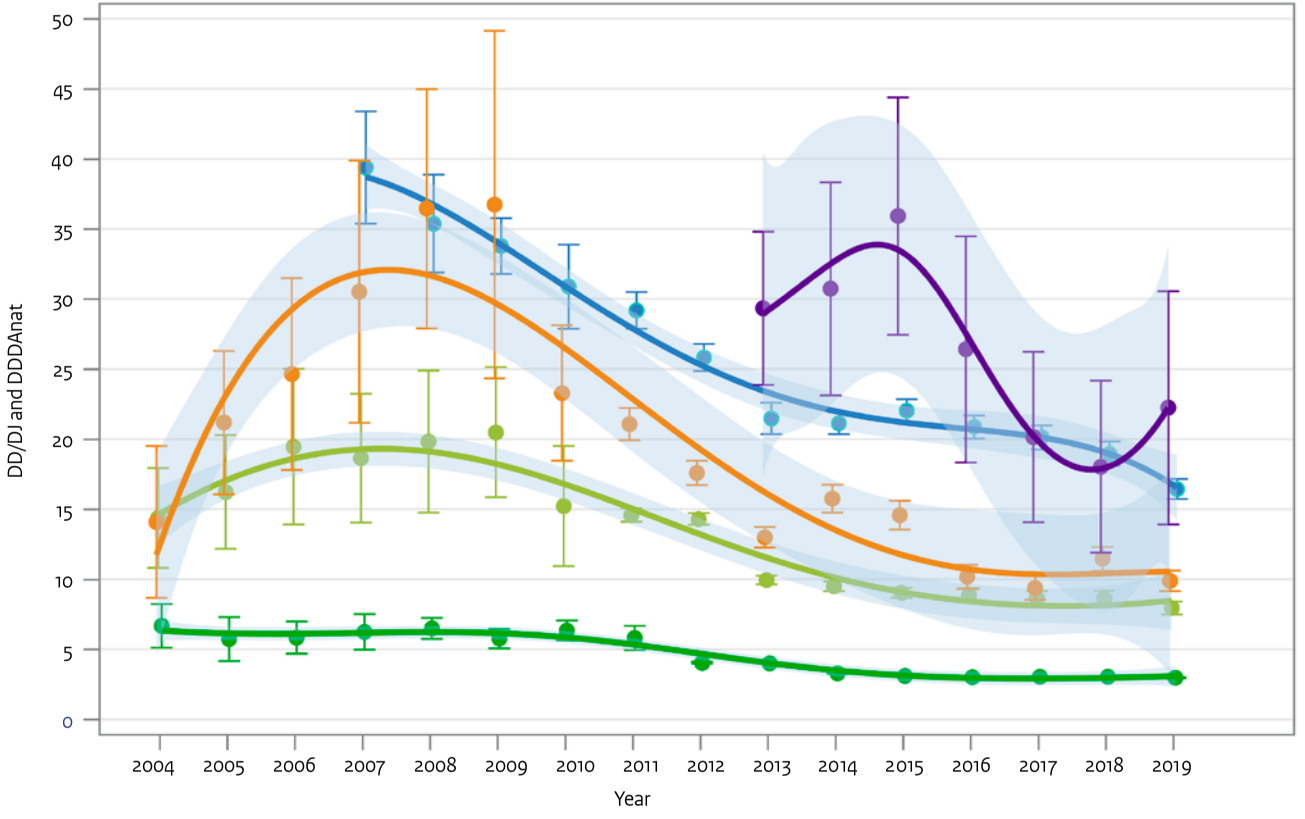
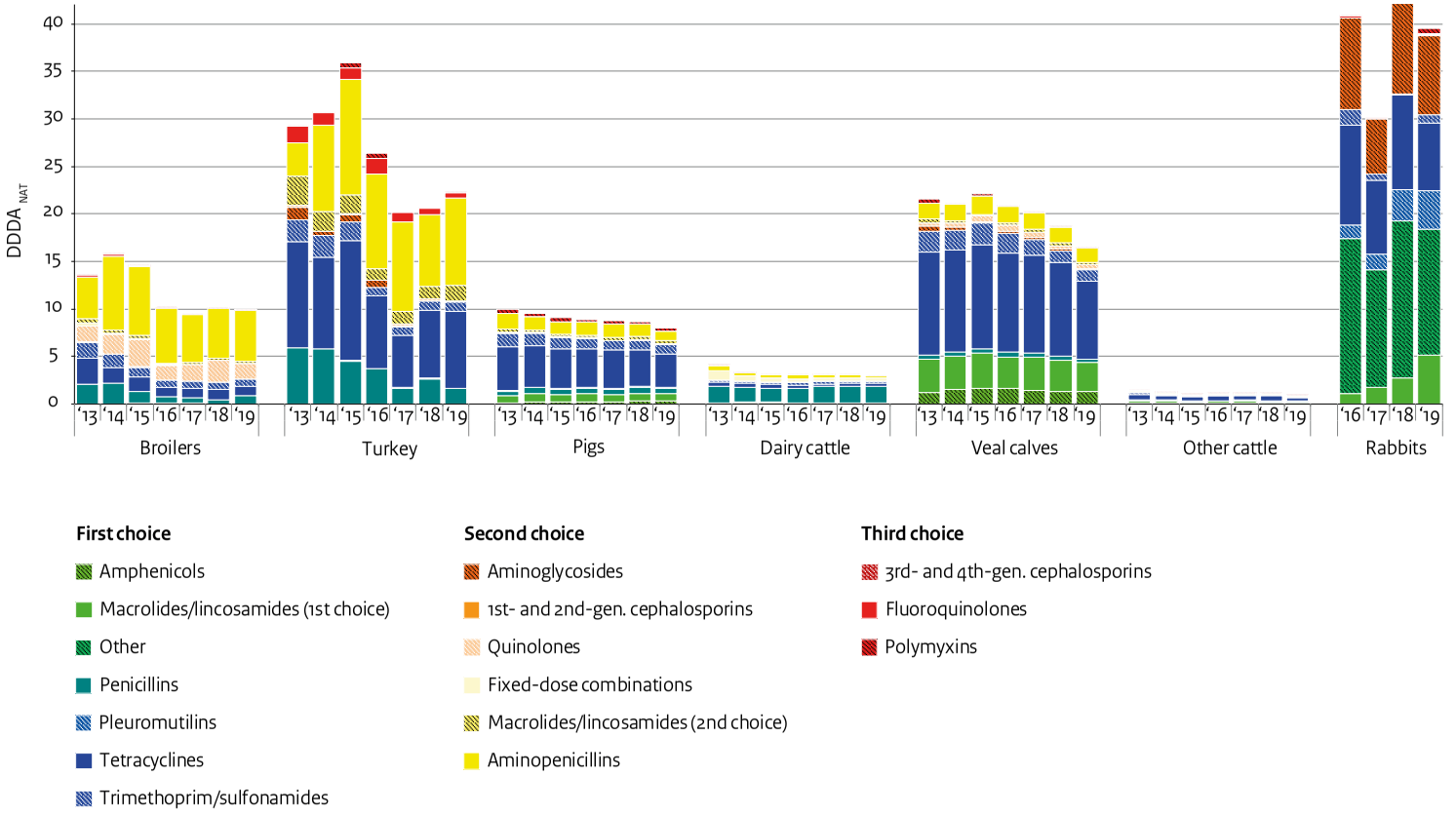


Table 5 Applied bodyweights for $DDDA_F$ calculation

species	category		specifications	age	Standard weight (kg)
Calves	White veal			0 - 222 days	160
	Red veal startup			0 - 98 days	77.5
	Red veal fattening			98 - 256 days	232.5
	Red veal combination			0 - 256 days	205
Pigs	Sows/piglets		Sows (all female animals after 1st insemination) and boars		220
			Suckling piglets	0 - 25 days	4.5
			Gilts	7 months - 1st insemination	135
	Weaned piglets			25 - 74 days	17.5
	Fattening pigs / gilts		Fattening pigs	74 days - 5 months	70
			gilts	74 days - 7 months	70
Broilers				0 - 42 days	1
Turkeys			male	0 - 20 weeks	10.5
			female	0 - 17 weeks	5.6
Cattle	Dairy cows		female	>2 years	600
	Suckler cows /		female	1-2 years	440
	Bulls for meat /		female	56 days - 1 year	235
	Rearing animals		female	<56 days	56.5
			male	>2 years	800
			male	1-2 years	628
			male	56 days - 1 year	283
			male	<56 days	79
Rabbits	Dow+kits		combined weight		8.4
			Dow	> 3-5 months	
			Kits	0 - 4.5 weeks	
	Fattening rabbits			4.5 - 13 weeks	1.8
	Other rabbits		female	11 weeks - 5 months	3.4

Figure 5 Number of $DDDA_{NAT}$ per animal-year of antimicrobial veterinary medicinal products specified by pharmaco-therapeutic groups per animal sector over the years 2013-2019



3 Resistance data

This chapter describes susceptibility test results as determined in 2019 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 patients suffering from clinically overt gastrointestinal infections, food-producing animals, food products of animal origin 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 2019, *S. Enteritidis* (34%) followed by *S. Typhimurium* (12%) together with the monophasic variant of Typhimurium: *S. enterica* subspecies enterica 1,4,[5],12:i:- (8%), were most frequently isolated from humans suffering from clinical salmonellosis.
2. In pigs, *S. Typhimurium* (36%) and the monophasic variant of *S. Typhimurium* (33%) dominated. In cattle, *S. Typhimurium* (35%) and *S. Dublin* (29%) were most commonly isolated. In poultry (including poultry products), the most frequently isolated serovars were *S. Infantis* (38%), *S. Paratyph*i B var. Java (*S. Java*, 11%) and *S. Enteritidis* (10%). Among laying hens, the most frequent isolated serotype was *S.*

Enteritidis (58%), followed by *S. Typhimurium* (9%).

3. Overall, the highest resistance proportions were observed for tetracycline, sulfamethoxazole, ampicillin, ciprofloxacin, nalidixic acid, and trimethoprim. The highest proportions of resistance were observed in the monophasic *S. Typhimurium*, *S. Infantis*, *S. Paratyph*i B var. Java from broilers, *S. Kentucky*, *S. Chester*, and to a lesser extent in *S. Typhimurium*.
4. The highest levels of resistance among *S. Enteritidis* were primarily those for fluoroquinolones (ciprofloxacin and nalidixic acid), while among *S. Typhimurium*, these were ampicillin, tetracycline, sulfamethoxazole, and trimethoprim.
5. In total 24 (1,3%) ESBL suspected isolates were detected mainly from humans of which 19 isolates (1,0%) were confirmed ESBL-producers.
6. In 2019 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). 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 in 2019 were the same as in previous years: *S. Enteritidis* (34%), followed by *S. Typhimurium* (12%) and its monophasic variant (*S.* 1,4,[5],12:i:-) (8%). *S. Typhimurium* and its monophasic variant were mainly isolated from pigs and cattle, but were also found in poultry. *S. Enteritidis* was mainly isolated from broilers, chicken meat and layers, and was not found in pigs in 2019 (Table S01).

The most frequent isolated serovar from pigs was *S. Typhimurium* (36%) and its monophasic variant (33%). For cattle, these were *S. Typhimurium* (35%) and *S. Dublin* (29%). Many different serovars were found in broilers (29% was listed in the "Other" group in Table S01) with the most isolated serovar being *S. Infantis* (38%), followed by *S. Paratyph*i B var. Java (11%) and *S. Enteritidis* (10%). Among laying hens, the most frequently isolated serovar was *S. Enteritidis* (58%), followed by *S. Typhimurium* (9%).

Table S01 Most prevalent *Salmonella* serotypes isolated in 2019 from humans, pigs (including pork), cattle (including beef), layers (including reproduction animals and eggs)

	Humans	Pigs	Cattle	Broiler	Layer
Total	**1256**	**133**	**105**	**166**	**45**
N tested	**1160**	**133**	**105**	**152**	**12**
Enteritidis	427		2	17	26
Typhimurium	153	48	37	6	4
1,4,5,12:i:-	102	44	13	1	
Infantis	31	6	2	63	3
Paratyphi B var. Java	24			18	2
Virchow	24				

Table S01 (continued) Most prevalent *Salmonella* serotypes isolated in 2019 from humans, pigs (including pork), cattle (including beef), layers (including reproduction animals and eggs)

	Humans	Pigs	Cattle	Broiler	Layer
Total	**1256**	**133**	**105**	**166**	**45**
N tested	**1160**	**133**	**105**	**152**	**12**
Chester	23				
Kentucky	20			2	1
Newport	20		4		
Stanley	20				
Muenchen	17				
Saintpaul	17		1		
Typhi	17				
Dublin	16	2	30		
Bovismorbificans	14				
Agona	12	1			2
Poona	11				
Goldcoast	10	3	1	3	
Braenderup	9			1	
Napoli	9				
Paratyphi B	9				
Brandenburg	8	3		2	
Coeln	8		1		
Derby	8	13		1	
London	8	4	2	1	
Ohio	8				
Corvallis	7			2	
Hadar	6				
Livingstone	6	2			
Montevideo	6				
Bredeney	5				
Panama	5	3			
Hvittingfoss	4				
Javiana	4				
Kottbus	4				
Paratyphi A	4				
Richmond	4				
Rissen	4	1		1	
Schwarzengrund	4		2		

Table S01 (continued) Most prevalent *Salmonella* serotypes isolated in 2019 from humans, pigs (including pork), cattle (including beef), layers (including reproduction animals and eggs)

	Humans	Pigs	Cattle	Broiler	Layer
Total	**1256**	**133**	**105**	**166**	**45**
N tested	**1160**	**133**	**105**	**152**	**12**
Weltevreden	4				
Bareilly	3				
Haifa	3				
Oranienburg	3				
Stanleyville	3				
Tennessee	3				
OTHER	149	3	10	48	7

Resistance proportions

A selection of all human *Salmonella* isolates received by the RIVM from regional public health and other clinical laboratories (N = 1160) was sent to WBVR for susceptibility testing. Moreover, 720 isolates from non-human sources were tested. These were mainly isolates from pigs (N = 133), cattle (N = 105), broilers (N = 152), and layers (N = 12), as well as isolates from a diversity of other sources, including animal feed (N = 185), food products (e.g. seafood, spices), and other animals (e.g. goats, horses). Non-human isolates were mainly sent to the RIVM by the Animal Health Service in Deventer from a diversity of surveillance programs and diagnostic activities for clinical infections in animals, or they were obtained from the NVWA (mainly non-clinical isolates) through its routine *Salmonella*-control activities on farms, slaughterhouses (e.g. EC/2073.2005 verification projects broiler neck skin) and at retail.

In November 2013, EU legislation on monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria (2013/652/EU) was implemented, including susceptibility testing of mandatory panels of antimicrobials. For the monitoring of *Salmonella* and *E. coli,* three antibiotic compounds (azithromycin, meropenem and tigecycline) used in human medicine, but not in veterinary practice, were added to the panel since the implementation of this legislation, and three antimicrobials of less importance for treatment of human infections (florfenicol, kanamycin and streptomycin) were removed from the panel (Table S02). Tigecycline is structurally related to tetracyclines, but has a broader spectrum of activity. Azithromycin is a potent macrolide and in human medicine often used instead of erythromycin for treatment of infections by Gram-positive bacteria, due to the effectiveness of a once-daily administration during a few days. Given its activity against Enterobacteriaceae and its favourable pharmacokinetics, it is also used for typhoidal *Salmonella* cases for which *in vivo* efficacy has been demonstrated. Meropenem belongs to the carbapenems, which are last resort antimicrobials that are used to treat infections with multi-drug resistant bacteria. In the past colistin has been used widespread in veterinary medicine for prevention and treatment of diarrhoeal diseases in livestock. In human medicine, colistin can be used for treatment of human infections with multidrug-resistant carbapenemase-producing bacteria. For this reason, the use of colistin in veterinary medicine has been reduced in Dutch livestock. Moreover, the finding of a plasmid-mediated colistin resistance gene (*mcr-family*) resulted in even more attention for this compound. Therefore, from 2020 onwards the SDa will consider and report it as third choice drug, comparable to fluoroquinolones and 3rd/4th generation cephalosporins (Chapter 2).

Table S02 MIC distribution (in %) and resistance percentages (R%) for all *Salmonella* isolates (N=1880) tested for antibiotic susceptibility during 2019.

Salmonella N = 1880	MIC (%) distribution mg/L																		R%	95% CI§
	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048		
Ampicillin							30.3	41.4	3.3	0.2	0.1			24.7					24.8	22.9 - 26.8
Cefotaxime					97.0	1.7	0.2	0.1		1.1									1.3	0.8 - 1.9
Ceftazidime						94.7	3.9	0.5	0.1	0.2	0.6								0.9	0.5 - 1.5
Gentamicin						83.7	12.4	0.6	0.2	0.3	0.4	0.5	1.9						3.3	2.6 - 4.3
Tetracycline								72.1	2.1	0.4	0.1	1.3	1.9	22.2					25.5	23.6 - 27.5
Sulfamethoxazole										31.3	35.4	6.4	2.2	0.3			0.2	24.3	24.4	22.5 - 26.4
Trimethoprim					77.0	11.7	0.5	0.1	0.1				10.6						10.7	9.4 - 12.2
Ciprofloxacin	30.2	50.7	2.0	1.2	7.4	6.1	1.3	0.1	0.1	0.3	0.6								17.0	15.4 - 18.9
Nalidixic acid									75.5	7.8	2.1	2.8	0.1	0.7	11.0				16.7	15.1 - 18.5
Chloramphenicol										87.5	5.5	0.4	0.3	0.6	5.9				7.1	5.9 - 8.3
Azithromycin*								0.6	53.3	42.0	3.7	0.2	0.2	0.1					0.4	0.2 - 0.8
Colistin**							7.0	70.0	15.0	8.0	0.1								-	-
Meropenem		75.3	24.6	0.1															0.0	0.0-0.2
Tigecycline***					54.9	37.4	5.6	1.7	0.3	0.2									2.1	1.6 - 2.9

The white areas indicate the dilution range tested for each antimicrobial agent. Values above this range indicate MIC values > the highest concentration in the range. Values at the lowest concentration tested indicate MIC-values ≤ the lowest concentration in the range. Vertical bars indicate the epidemiological cut-off values (ECOFF), used as breakpoints. If available, dashed bars indicate the clinical breakpoints. For ampicillin, ciprofloxacin and chloramphenicol the ECOFF and clinical breakpoints are identical.

* *tentative set ECOFF during the EURL AMR WP meeting on 25 April 2015 in Lyngby (DK).*

** *Because of differences in natural susceptibility for colistin between serovars there is no general Salmonella ECOFF available for colistin. For this reason the percentage of resistance is not depicted*

*** *Since 2019 the ECOFF is no longer available for Salmonella. The former defined ECOFF of EUCAST for tigecycline was used for monitoring purposes in 2018.*

§ One-sided, 97.5% confidence interval

Like in previous years, colistin resistance was not reported in *Salmonella* in 2019 (Table S02). That is because an epidemiological cut-off value that can be applied for all *Salmonella* serovars is lacking for colistin, which makes the results difficult to interpret. Using the former ECOFF of 2 mg/L (which is also the clinical breakpoint), resistance rates would have been highly influenced by differences in natural susceptibility (e.g. wild-type strains of *S. Enteritidis* and *S. Dublin* are less susceptible to colistin). As a result, colistin resistance would have been over-reported for *Salmonella*. Therefore, all *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).

MIC-distributions and resistance percentages of 1880 *Salmonella* isolates from different sources tested for susceptibility in 2019 are presented in Table S02. Overall, the resistance rates were approximately at the same level as the previous year. The highest resistance proportions were again observed for tetracycline, sulfamethoxazole, ampicillin, ciprofloxacin, nalidixic acid, trimethoprim and chloramphenicol. 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 (slightly increasing from 1% in 2018 to 2.1% in 2019), azithromycin (0.8% in 2018, 0.4% in 2019), cefotaxime, ceftazidime, and gentamicin.

Table S03 presents resistance percentages for the fourteen most prevalent serovars isolated in the Netherlands in 2019. There was considerable variation between the resistance profiles of the different serovars. Very high resistance proportions were observed for the monophasic variant of *S. Typhimurium* (>80% resistance to tetracycline, ampicillin and sulfamethoxazole) and *S. Paratyph*i B var. Java from broilers (100% resistance to trimethoprim, and high resistance levels for sulfamethoxazole, ciprofloxacin, nalidixic acid and ampicillin). High levels of resistance were observed for *S. Infantis* (tetracycline, sulfamethoxazole, ciprofloxacin and nalidixic acid), *S. Kentucky* (ampicillin, tetracycline, sulfamethoxazole, ciprofloxacin and nalidixic acid), *S. Chester* (tetracycline, sulfamethoxazole, trimethoprim, ciprofloxacin and nalidixic acid), and to a lesser extent for *S. Typhimurium* (ampicillin, tetracycline and sulfamethoxazole). All these serovars (except for *S. Kedougou* that was 100% susceptible to all antimicrobials) have acquired resistance against more than one antimicrobial. The serovars with the highest levels of multi-drug resistance in 2019 were *S. Infantis* (12/13), *S. Kentucky* (12/13), *S. Typhimurium* (11/13) and its monophasic variant (11/13). The most common pattern was resistance to ampicillin, sulfamethoxazole and tetracycline (ASuT).

Table S03 Resistance (%) of the fourteen most prevalent *Salmonella* serovars isolated in the Netherlands in 2019 (N tested).

	Enteritidis (418)	Typhimurium (261)	1,4,[5],12:i:- (148)	Infantis (111)	Dublin (52)	Paratyphi B var Java, human (24)	Paratyphi B var Java, broiler (15)	Livingstone (40)	Derby (32)	Newport (31)	Kentucky (30)	Kedougou (26)	Virchow (25)	Chester (24)	Montevideo (24)
Ampicillin	12.9	47.9	82.4	15.3	3.8	4.2	53.3	0.0	3.1	3.2	40.0	0.0	12.0	4.2	0.0
Cefotaxime	0.0	1.9	0.0	4.5	0.0	0.0	0.0	0.0	0.0	0.0	13.3	0.0	0.0	0.0	0.0
Ceftazidime	0.0	0.4	2.7	2.7	0.0	0.0	0.0	0.0	0.0	0.0	10.0	0.0	0.0	0.0	0.0
Gentamicin	0.0	7.3	9.5	2.7	0.0	4.2	6.7	0.0	0.0	0.0	30.0	0.0	12.0	0.0	0.0
Tetracycline	7.9	44.1	83.1	46.8	1.9	0.0	6.7	0.0	12.5	3.2	43.3	0.0	4.0	54.2	0.0
Sulfamethoxazole	4.8	39.1	85.8	48.6	3.8	4.2	60.0	0.0	12.5	0.0	40.0	0.0	4.0	58.3	4.2
Trimethoprim	0.2	22.2	16.2	29.7	1.9	4.2	100.0	0.0	12.5	0.0	10.0	0.0	4.0	54.2	4.2
Ciprofloxacin	21.5	11.9	13.5	45.0	1.9	4.2	46.7	2.5	0.0	9.7	53.3	0.0	16.0	62.5	4.2
Nalidixic acid	21.5	11.5	14.9	47.7	1.9	4.2	40.0	2.5	0.0	9.7	53.3	0.0	16.0	54.2	0.0
Chloramphenicol	0.5	17.6	20.3	9.9	3.8	0.0	0.0	0.0	0.0	0.0	10.0	0.0	0.0	12.5	0.0
Azithromycin	0.5	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.3	0.0	0.0	0.0	0.0
Meropenem	0.0	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Tigecycline	0.0	4.6	4.1	9.9	1.9	0.0	6.7	2.5	3.1	3.2	3.3	0.0	0.0	0.0	0.0

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, 17% of *Salmonella* isolates demonstrated an acquired resistance phenotype for ciprofloxacin (Table S02), which is around the same as in 2018 (17.7%). The highest levels of ciprofloxacin resistance among the most prevalent serovars were observed for *S. Chester* (63%), *S. Kentucky* (52%), *S. Paratyph*i B var. Java from broilers (47%), *S. Infantis* (45%), and *S. Enteritidis* (22%) (Table S03).

Table S06 shows that the proportion of isolates resistant to ciprofloxacin in chicken meat was still high in 2019 (58%) but showing a considerable decline over the last years (89% in 2017, 69% in 2018). These isolates 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 the EU.

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 2019, the total number of cefotaxime resistant (MIC > 0.5 mg/L) ESBL suspected *Salmonella* isolates was 24/1880 (1.3%), among nine different serovars, with 19 isolates from humans, two from feed samples, one from chicken, one from pigs, and one unknown. The main serovars were *S. Infantis* (N=5), *S. Typhimurium* (N=5), *S. Kentucky*, (N=4), and the monophasic variant of *S. Typhimurium* (N=3). In chicken meat samples, no ESBL-suspected isolate was found (Table S06).

S. Typhimurium

Table S01 shows that *S. Typhimurium* represented 12% (153/1256) of all human *Salmonella* isolates as characterized by the RIVM in 2019, which is considerably lower than previous years (2018: 19%, 2017:16%, 2016: 17%, 2015: 19%). *S. Typhimurium* is a common serovar in animals. If the monophasic Typhimurium variant is included, *S. Typhimurium* may be regarded as the most dominant serovar in humans and food-producing animals like pigs and cattle.

Table S04 shows that resistance in *S. Typhimurium* was very high for ampicillin, tetracycline, sulfamethoxazole in human, cattle and pig isolates. Resistance to chloramphenicol was especially high in cattle and to a lesser extent in pig and human isolates. Resistance to trimethoprim was especially high in pig and cattle isolates.

Table S04 Resistance percentages of *S. Typhimurium* (N tested) isolated from humans, cattle, pigs and other sources in 2019.

	S. Typhimurium **(261)**[a]			
	Humans (143)	**Cattle (37)**	**Pigs (48)**	**Other sources (33)**[b]
Ampicillin	42.7	62.2	56.3	42.4
Cefotaxime	3.5	0.0	0.0	0.0
Ceftazidime	0.7	0.0	0.0	0.0
Gentamicin	4.2	24.3	0.0	12.1
Tetracycline	35.7	64.9	52.1	45.5
Sulfamethoxazole	28.0	75.7	43.8	39.4
Trimethoprim	14.7	24.3	33.3	36.4
Ciprofloxacin	16.8	2.7	2.1	15.2
Nalidixic acid	16.1	2.7	4.2	12.1
Chloramphenicol	16.1	24.3	14.6	21.2
Azithromycin	0.0	0.0	0.0	0.0
Meropenem	0.0	0.0	0.0	0.0
Tigecycline	3.5	0.0	10.4	6.1

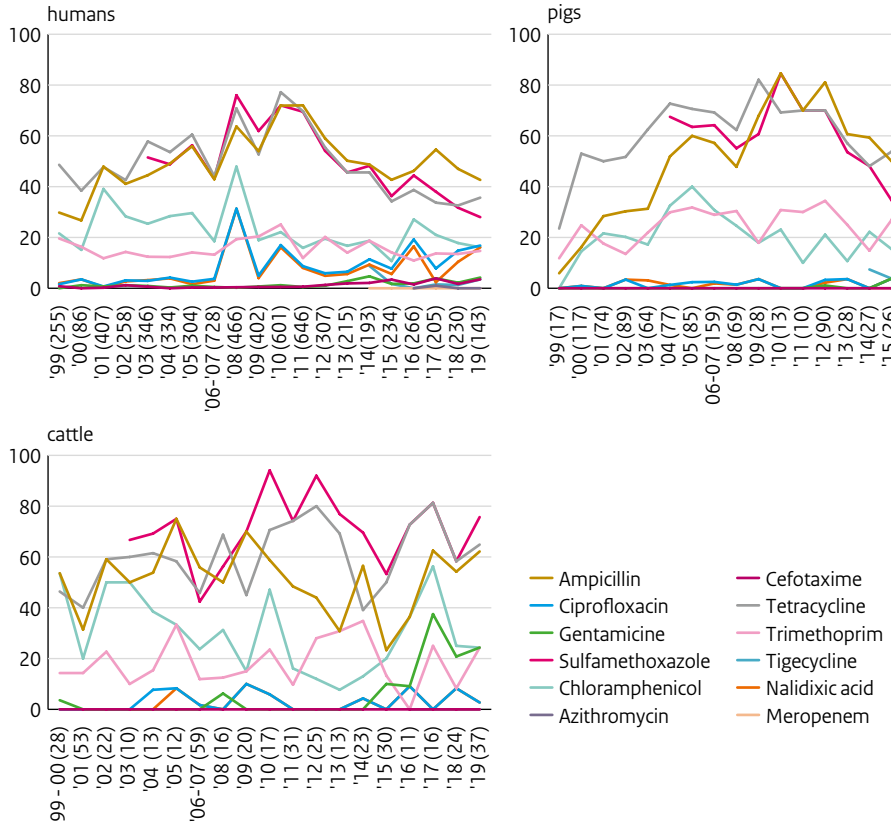
[a] *Monophasic variants (1,4,[5],12:i:-) are exluded.*

[b] *Other sources include broilers, layers, goats, horses, seafood and feed products.*

About 16% of the *S. Typhimurium* isolates exhibited the "quatro-resistance" profile Ampicillin-Chloramphenicol-Sulfamethoxazole-Tetracycline (ACSuT), which is the same as in 2018 but lower than in 2017 (20%) and 2016 (26%). Resistance to the clinically important drug cefotaxime was not detected in animal isolates and only at a low level in human isolates (0.7% compared to 1.7% in 2018). The resistance percentage to fluoroquinolones in human isolates was 16.8% in 2019, but varied in the last four years between 7.8% and 19.2%. In 2019, resistance to fluoroquinolones was found in one cattle and one pig isolate. In contrast to 2018, resistance to tigecycline in 2019 was not only observed in human isolates (N = 5), but also in pigs (N=5). These isolates tend to exhibit slightly elevated MIC-values caused by an unknown resistance mechanism (if any).

Resistance proportions in *S. Typhimurium* isolates from human samples showed an increasing tendency until 2010, after which they showed a tendency to decrease until 2013 (Figure S01). Since 2013, resistance proportions seem to fluctuate from year to year. In 2019, the resistance proportions for ampicillin, sulfamethoxazole, and chloramphenicol were lower than in 2018 and 2017. In contrast, resistance to ciprofloxacin increased over the last two years. Resistance proportions for cefotaxime and gentamicin, although being at low level, showed an increasing tendency as from 2011, and fluctuated since 2014 (Figure S01).

Figure S01 Trends in resistance (%) of *S. Typhimurium* isolated from humans and food-animals in 1999 - 2019.



Resistance proportions in *S. Typhimurium* isolates from pig and cattle samples (Figure S01) varied considerably over the years. These proportions seemed to decrease from 2013, but sharp increases were seen in 2016 and 2017 for the cattle as well as pig isolates. In 2018 and 2019, resistance for the major antimicrobials decreased among pig isolates, except for tetracycline. In contrast, among cattle isolates, most resistant proportions increased except for chloramphenicol. However, these figures should be interpreted with care, because of the relatively small number (horizontal axis in brackets) of isolates per year.

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 and travel abroad.

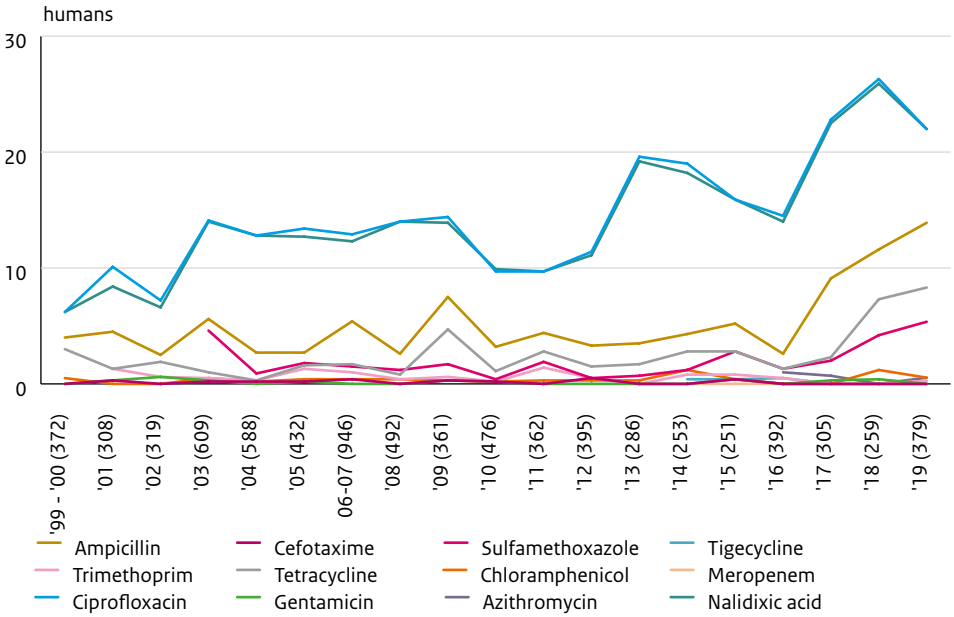
Table S03 shows that resistance in *S. Enteritidis* is relatively low, compared to many other public health relevant *Salmonella* serovars. Table S05 presents resistance proportions in *S. Enteritidis* isolates from human samples and other sources (including broilers, layers, goats, food and feed products). Among human isolates, the resistance percentage were relatively high for fluoroquinolones and nalidixic acid (both 22%) and to a lesser extent for ampicillin (13.9%), tetracycline (8.3%) and sulfamethoxazole (5.4%). For all other antimicrobials, resistance proportions of human *S. Enteritidis* isolates were very low or not detected. Resistance to fluoroquinolones decreased after two years of increase, while resistance to ampicillin, tetracycline and sulfamethoxazole showed an increasing trend since 2016 (Figure S02). The resistance percentages in the isolates of non-human sources were, alike the human isolates, relatively high for the fluoroquinolones and nalidixic acid (both 18%), which is very similar to the levels in 2018 (19%) Table S05. Lower resistance percentages were measured for ampicillin, tetracycline, and trimethoprim. Resistance to sulfamethoxazole was not observed among non-human isolates.

Table S05 Resistance percentages of *S. Enteritidis* (N tested) isolated from humans and broilers in 2019.

S. Enteritidis **(418)**		
	Humans (373)	**Other sources (45)[a]**
Ampicillin	13.9	4.4
Cefotaxime	0.0	0.0
Ceftazidime	0.0	0.0
Gentamicin	0.0	0.0
Tetracycline	8.3	4.4
Sulfamethoxazole	5.4	0.0
Trimethoprim	0.3	6.3
Ciprofloxacin	22.0	17.8
Nalidixic acid	22.0	17.8
Chloramphenicol	0.5	0.0
Azithromycin	0.5	0.0
Meropenem	0.0	0.0
Tigecycline	0.0	0.0

[a] *Other sources include broilers, layers, goats, duck, food and feed products.*

Figure S02 Trends in resistance (%) of *S. Enteritidis* isolated from humans from 1999 - 2019.



Salmonella from chicken meat, other meat sources and spices

Table S06 shows resistance data of *Salmonella* isolates from raw meat (chicken and other), herbs, spices and seafood. *S. Infantis* (57%) was the most prevalent serovar found in chicken meat in 2019 followed by *S. Paratyphi* B var. Java (19%). Isolates from other meat samples were resistant at lower levels than isolates from chicken meat (Table S06). Resistance proportions for the quinolones (ciprofloxacin and nalidixic acid) were high (both 58.5%) in isolates from chicken meat, but lower than in 2018 (both 69.4%). Borderline resistance to tigecycline was observed in 8 chicken meat isolates, 8 other meat isolates and one isolate from other products. Resistance to cephalosporins (cefotaxime or ceftazidime) was detected in one isolate from other meat, but absent among isolates from chicken meat and other products. Care should be taken with the interpretation of the resistance patterns of isolates obtained from other meat and other product samples because of the low numbers.

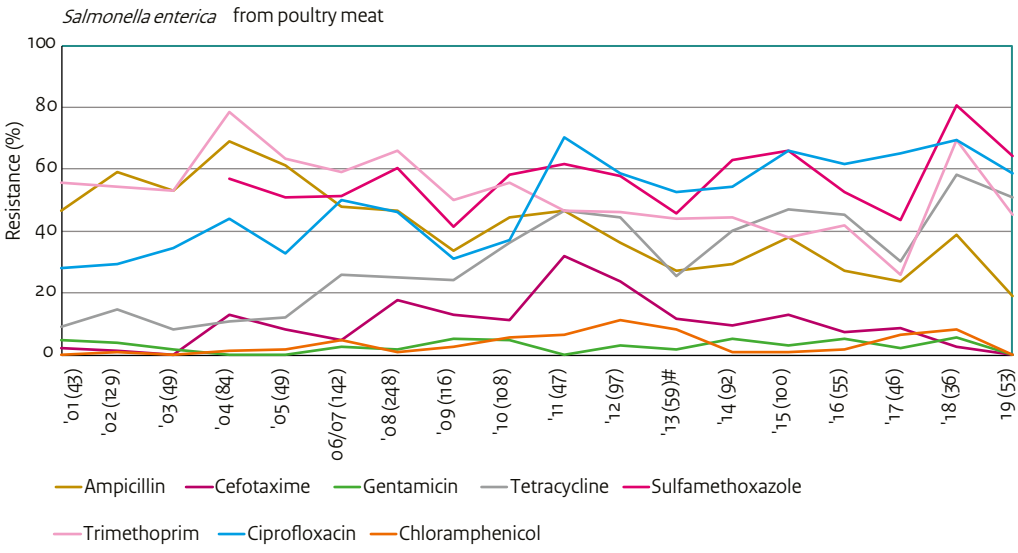
The overall resistance proportions of *Salmonella* isolates from poultry meat over the years fluctuate from year to year, with an overall increasing trend for ciprofloxacin, sulfamethoxazole, and tetracycline; and decreasing trends for trimethoprim, ampicillin, and cefotaxime (Figure S03). After an increase in resistance proportions in 2018 for ampicillin, tetracycline, trimethoprim, and sulfamethoxazole all these have decreased again in 2019. It should be noticed that the fluctuating resistance proportions during the years could be influenced by the varying proportions of retail broiler meat sampled per year originating from Dutch poultry farms and variation in proportion of serovars.

Table S06 Resistance (%) of Salmonella enterica isolated from different types of raw meat, herbs, spices and seafood in the Netherlands in 2019.

	Chicken meat[a]	Other meat[b]	Other products[c]
	N = 53	N = 37	N = 9
Ampicillin	18.9	27.0	11.1
Cefotaxime	0.0	2.7	0.0
Ceftazidime	0.0	2.7	0.0
Gentamicin	0.0	2.7	0.0
Tetracycline	50.9	32.4	11.1
Sulfamethoxazole	64.2	27.0	0.0
Trimethoprim	45.3	16.2	0.0
Ciprofloxacin	58.5	5.4	0.0
Nalidixic acid	58.5	5.4	0.0
Chloramphenicol	0.0	0.0	0.0
Azithromycin	0.0	0.0	0.0
Meropenem	0.0	0.0	0.0
Tigecycline	15.1	21.6	11.1

[a] *Fresh chicken meat sampled at retail and chiecken neck skin from verification projects*
[b] *Other meat includes pork (n = 27), beef (n = 3), veal calf (n = 6), and frog (n = 1)*
[c] *Other products includes seafood (n = 4) and spices (n = 5).*

Figure S03 Trends in resistance (%) of *Salmonella* enterica isolated from poultry meats in the Netherlands from 2001-2019.



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 from humans suffering from acute gastroenteritis. For 2019, 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 for the years 2014-2018. Differences were minor, with a yearly average difference between surveillance systems of 1.7% for ciprofloxacin, 3.8% for tetracycline, and 0.4% for erythromycin. As a result of prioritization and changes in legislation, from 2014 onwards the surveillance of antimicrobial resistance in *Campylobacter* focusses mainly on poultry (and poultry meat). No additional isolates from other animals species were collected in 2019.

Table Co1 presents the MIC distributions and resistance percentages for all *Campylobacter jejuni* and *C. coli* strains isolated in 2019 from caecal samples of broilers. Resistance percentages of *C. jejuni* and *C. coli* isolated from broilers and poultry meat are presented in Table Co2. Trends in resistance of *C. jejuni* and *C. coli* from broilers and poultry meat products over the last 16 to 19 years are presented in Figures Co1 and Co2.

Table C01 MIC distribution (in %) for Campylobacter jejuni (N = 188) and *C. coli* (N = 94) isolated from caecal samples of broilers in 2019.

C. jejuni, broilers	MIC (%) distribution mg/L												R%	95% CI
(N = 188)	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256		
Ciprofloxacin	28.2	2.1					18.1	41.5	10.1				69.7	62.3 - 76.2
Nalidixic acid				0.5	8.0	18.1	4.8	1.1		0.5	67.0		67.6	60.4 - 74.2
Erythromycin				64.9	34.6	0.5							0.0	0 - 1.9
Gentamicin	17.0	75.5	7.4										0.0	0 - 1.9
Streptomycin		0.5	22.3	61.2	8.5				7.4				7.4	4.1 - 12.2
Tetracycline			34.6	1.1	0.5			4.3	1.6	4.3	53.7		64.4	57.1 - 71.2

C. coli, broilers	MIC (%) distribution mg/L												R%	95% CI
(N = 94)	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256		
Ciprofloxacin	11.7	7.4				9.6	44.7	22.3	4.3				80.9	71.4 - 88.2
Nalidixic acid						8.5	8.5	2.1			80.9		80.9	71.4 - 88.2
Erythromycin				67.0	25.5	5.3	1.1					1.1	1.1	0 - 5.8
Gentamicin		59.6	39.4	1.1									0.0	0 - 3.9
Streptomycin				60.6	35.1				4.3				4.3	1.2 - 10.5
Tetracycline			23.4	2.1	1.1						73.4		73.4	63.3 - 82.0

National surveillance data for *Campylobacter* spp. isolated from humans are shown in Figure C03 (from 2002 onwards) and in Table C03 (from 2009 onwards).

Highlights

1. Resistance proportions in *C. jejuni* isolates from caecal samples of broilers and meat thereof were traditionally high for quinolones and tetracycline and did not substantially change in 2019, compared to 2018.
2. Resistance to macrolides was rarely detected in *C. jejuni* isolates from broilers and poultry meat, and was at low levels in *C. coli* isolates from broilers and poultry meat.
3. Overall, resistance proportions were higher in *C. coli* than in *C. jejuni* isolates.
4. Ciprofloxacin resistance in *Campylobacter* isolates from human patients was again high in 2019 (with a substantial increase compared to 2018), which is a concern for public health.
5. Resistance to erythromycin, first choice antibiotic in human medicine for campylobacteriosis, remained low.

Resistance proportions

EU legislation on monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria (2013/652/EU, implemented in November 2013) includes susceptibility testing of mandatory panels of antimicrobials. Since the start of the monitoring programme of *Campylobacter* spp., six out of twelve antimicrobials (ampicillin, chloramphenicol, clarithromycin, tulathromycin, sulfamethoxazole and neomycin) are no longer included. Most of the remaining antimicrobials in the panel (ciprofloxacin, gentamicin, erythromycin and tetracycline) represent antimicrobial classes, which are used in human medicine for treatment of campylobacteriosis.

In 2019, resistance proportions again were higher in *C. coli* than in *C. jejuni* isolates (Table C01 and C02), except for streptomycin. Resistance against gentamicin was not detected in any of the *C. jejuni* and *C. coli* isolates (Table C02).

As in previous years, the highest proportions of resistant *C. jejuni* and *C. coli* from broilers were found for tetracycline and the quinolones ciprofloxacin and nalidixic acid (Table C01). These high resistance proportions were found in isolates from both broilers and poultry meat, with the highest resistance proportions for the *C. coli* isolates (Table C02).

Figure C01 presents the resistance levels of *C. jejuni* from broilers and poultry meat over the last 17 to 20 years. The resistance levels for erythromycin, streptomycin and gentamicin were very low to zero over the last 10 years, but showed an increase in 2018 and 2019 for streptomycin (10.3% and 7.4% in broilers and 8.0% and 9.2% in poultry meat in 2018 and 2019, respectively). Resistance to erythromycin was not detected in isolates from broilers, and was 0.9% in isolates from poultry meat. Resistance to tetracycline showed an increasing trend in both broilers and poultry meat since 2014, and was in 2019 approximately at the same level as in 2018 (64.4% in broilers and 57.8% in poultry meat). Resistance percentages for ciprofloxacin had been high with some fluctuation over the years, and was again high in 2019 (69.7% in broilers, 67.0% in poultry meat).

The resistance levels in *C. coli* isolates from broilers and poultry meat are presented in Figure C02. These levels showed more fluctuation over years than levels of *C. jejuni*, which might be 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 and streptomycin in *C. coli* fluctuated quite a lot over the years. In 2019, the resistance levels for both erythromycin and streptomycin were lower than in 2018, with very low percentages in isolates from broilers (1.1% for erythromycin, 4.3% for streptomycin), and a bit higher percentages in isolates from poultry meat (16.7% for erythromycin, 11.1% for streptomycin). Resistance percentages for ciprofloxacin in broilers and poultry meat have been fluctuating at a high level since 2001, and were high again in 2019. Because of the relatively low number of *C. coli* isolates tested, these results might not be very representative. It can be seen in Figure C02 that the resistance percentages to tetracycline over the years seemed to follow the same trend at approximately the same percentages as ciprofloxacin resistance. However, since 2018, resistance levels for tetracycline showed some difference to the levels of ciprofloxacin. In poultry meat, the resistance level decreased from 72.4% in 2018 to 57.5% in 2019, whereas the resistance percentage in broilers increased from 69.4% in 2018 to 73.4% in 2019.

Fluoroquinolones

The high, yearly increasing, proportion of *Campylobacter* spp. isolates from animal origin resistant to the 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 quinolones remained at a continuously high level over the last 10 years, and was 69.7% in 2019. The proportion of fluoroquinolone resistance in *C. jejuni* from poultry meat was just as high (67.0% in 2019).
In 2019, again the *C. coli* isolates from broilers showed an increase of levels of ciprofloxacin resistance, with the level for the first time being over 80.0% (80.9%). The proportion of resistance of *C. coli* isolates from poultry meat fluctuates somewhat more over time due to the low number of isolates included in the survey. Resistance proportions in 2019 were almost 10% higher than in 2018 for both ciprofloxacin and nalidixic acid (both at 85.0%).

In 2019, the resistance levels for fluoroquinolone in human campylobacter isolates were high again, and were again increased compared with the year before (from 63.6% in 2018 to 68.9% in 2019). This continuously increasing trend of ciprofloxacin resistance in *Campylobacter* spp. isolated from human patients is shown in Figure C03.

Macrolides

Erythromycin, or other macrolides (clarithromycin), are the first-choice drugs for the treatment of campylobacteriosis in humans. In 2019, 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, and in only 0.9% of *C. jejuni* isolates from poultry meat. Table C03 shows that 2.2% of human *C. jejuni* isolates was resistant for erythromycin in the period 2014-2019. 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*).

In *C. coli* isolates from broilers and poultry meat, erythromycin resistance percentages were a bit higher than in *C. jejuni* isolates, but lower than in 2018. Resistance was detected in 1.1% of isolates from broilers and in 5.3% of isolates from poultry meat (table C02). 18.0% of human *C. coli* isolates was resistant for erythromycin in the period 2014-2019.

Figure Co1 Trends in resistance (%) of *Campylobacter jejuni* isolated from broilers and chicken meat in the Netherlands.

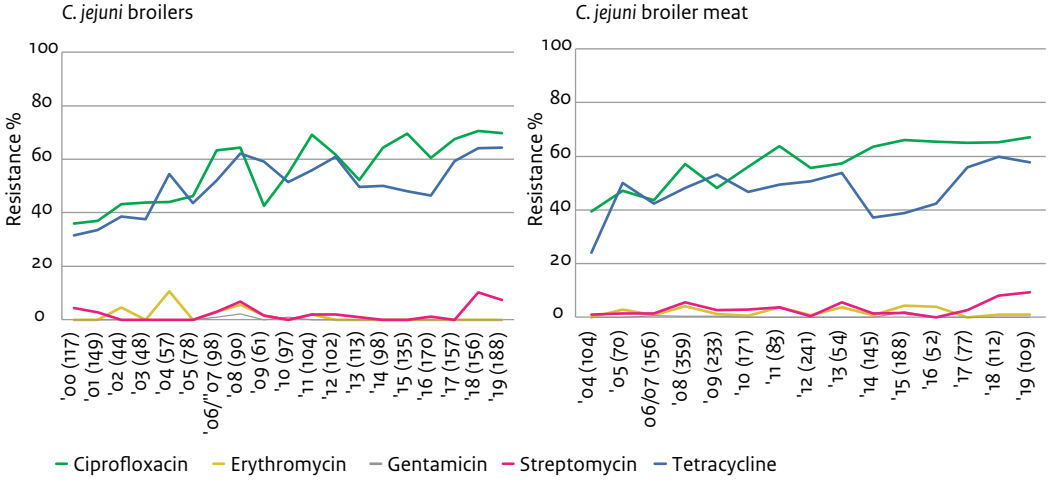
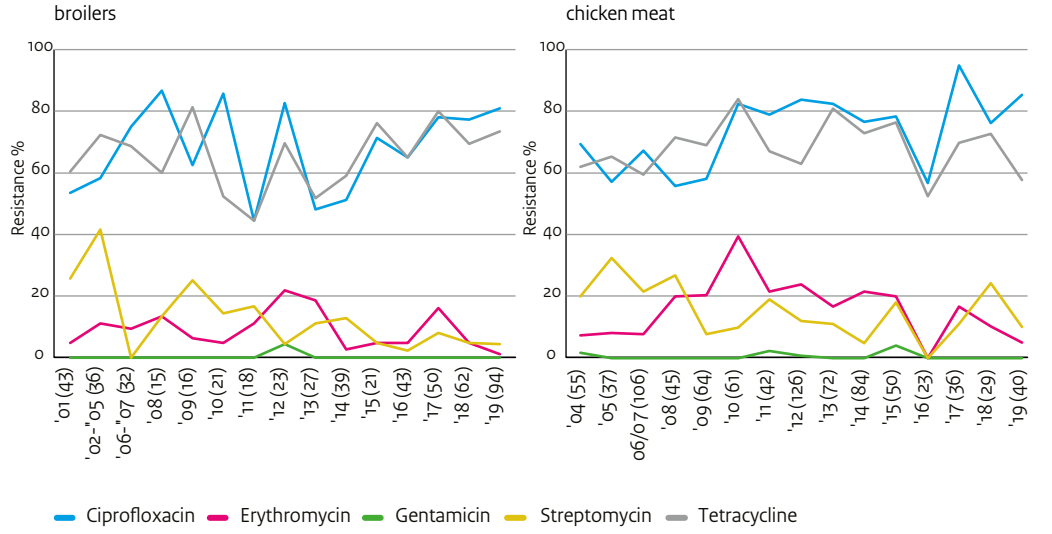


Figure Co2 Trends in resistance of *Campylobacter coli* isolated from broilers and chicken meat in the Netherlands.



Broiler chickens and poultry meat

In *Campylobacter* from poultry, resistance profiles were determined for isolates recovered from broilers as well as from chicken meat samples. No isolates were collected from laying hens, ducks and turkey meat, nor from other animal species.

Table Co2 shows that the proportions of resistance for tetracycline and the quinolones in *C. jejuni* isolates were at high levels for isolates from poultry meat, as well as for the isolates from caecal samples of broilers. The resistance levels for the *C. coli* isolates from broilers and poultry meat for tetracycline and quinolones were even higher. No resistance to gentamicin was detected in both *C. jejuni* and *C. coli* isolates. Resistance to erythromycin was also very low in *C. jejuni* isolates, but somewhat more frequently found in *C. coli*. Resistance to streptomycin was higher than to erythromycin in *C. jejuni* isolates from broilers and poultry meat, but still at low levels (7.4% and 9.2% respectively). In *C. coli* isolates from poultry meat, the proportion of isolates resistant to streptomycin was higher than in *C. jejuni* isolates (4.3% in broilers, 10.0% in poultry meat).

Table Co2 Resistance percentages of *C. jejuni* and *C. coli* isolated from faecal samples of broilers and from poultry meat in 2019.

	C. jejuni		*C. coli*	
	Broilers	Poultry meat	Broilers	Poultry meat
N =	188	109	94	40
Ciprofloxacin	69.7	67.0	80.9	85.0
Nalidixic acid	67.6	67.0	80.9	85.0
Erythromycin	0.0	0.9	1.1	5.0
Gentamicin	0.0	0.0	0.0	0.0
Streptomycin	7.4	9.2	4.3	10.0
Tetracycline	64.4	57.8	73.4	57.5

Higher resistance rates were observed for almost all antimicrobials in *C. coli* isolates from broilers and poultry 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 Co1 and Figure Co2.

Campylobacter in humans

Resistance levels in isolates from human patients were determined for ciprofloxacin, tetracycline and erythromycin, and are shown in Table Co3 and Figure Co3. Figure Co3 shows a continuously increasing trend of ciprofloxacin and tetracycline resistance, with an increase again in 2019 compared to 2018. Resistance to erythromycin seemed to stabilize around 3% in 2011-2015, but has since than increased to 4.7% in 2019.

Table Co3 shows the average resistance levels for human *Campylobacter* spp. isolates for the periods 2009-2013 and 2014-2019, and the resistance level per year since 2014. Because 2019 data were obtained from ISIS-AR, we could not stratify resistance proportions by travel history, as these data are not routinely collected within this surveillance system. The resistance levels in human *Campylobacter* spp. isolates for all

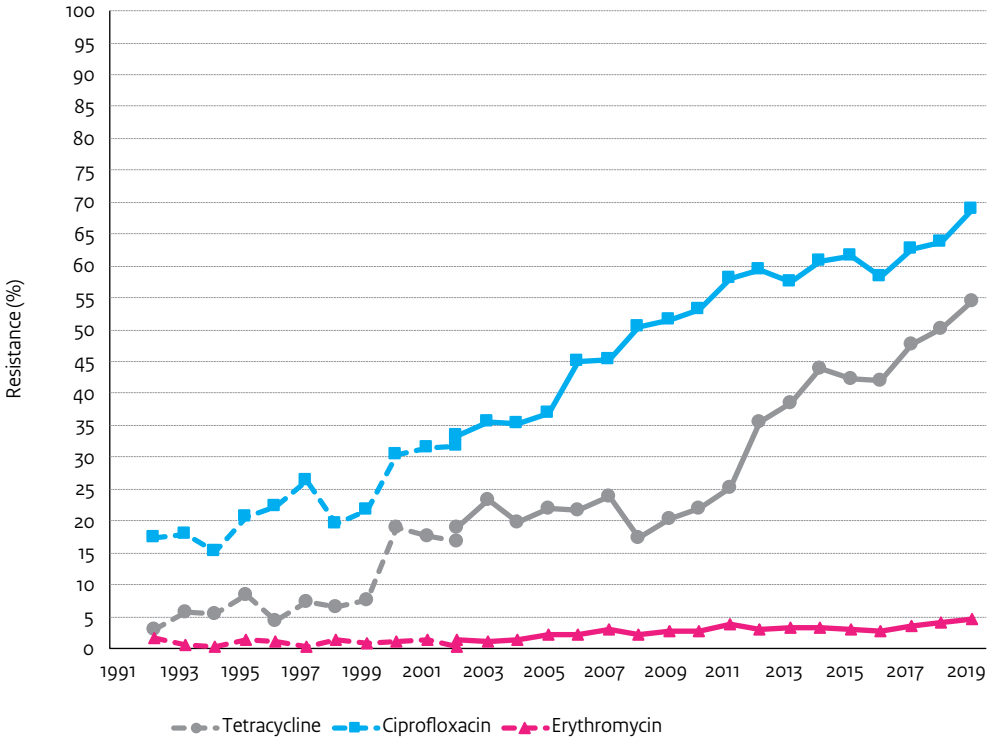
three antimicrobials show an increasing trend since 2013. Resistance proportions were higher for *C. coli* isolates than *C. jejuni* isolates.

Table C03 Resistance in *C. jejuni* and *C. coli* isolated from humans from 2009 - 2019.

	2014-2019				2009-2013			
	C. jejuni		*C. coli*		*C. jejuni*		*C. coli*	
	N	R%	N	R%	N	R%	N	R%
Fluoroquinolone	9037	60.9	782	69.1	8703	55.4	717	56.5
Tetracycline	5425	45.0	590	65.6	2009	24.3	323	41.3
Erythromycin	333	2.2	202	18.0	438	2.9	181	14.6

	Campylobacter spp. (R%)					
	2019	2018	2017	2016	2015	2014
Fluoroquinolone	68.9	63.6	62.6	58.3	61.4	60.6
Tetracycline	54.4	50.2	47.6	42.0	42.3	43.9
Erythromycin	4.7	4.0	3.5	2.6	2.9	3.2

Figure C03 Trends in resistance (%) of *Campylobacter* spp. isolated from humans between 1992 and 2019. 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)

Highlights

1. The increasing tendency for resistance against ampicillin, sulfamethoxazole, tetracycline and trimethoprim in human STEC O157 isolates since 2009 did not continue in 2018 and 2019.
2. Resistance to the quinolones (ciprofloxacin and nalidixic acid) was not detected in human STEC O157 isolates in 2019.
3. No ESBL-producing isolates were detected in 2019.

Human STEC O157 isolates

STEC is a bacterial zoonotic agent associated with human disease with varying clinical manifestations, including diarrhea, haemorrhagic colitis and (occasionally fatal) haemolytic 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 sheep. Although, therapeutic treatment of STEC infections with antimicrobials is not 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. Shiga-toxin producing *E. coli* O157 (STEC O157) isolates from human clinical cases (N = 64) were tested for susceptibility. Isolates were obtained from regional public health laboratories within the RIVM national laboratory surveillance of STEC. Table STEC01 shows the MIC results for all *E. coli* O157 isolates from humans; Figure STEC01 presents the trends over time.

Figure STEC01 Trends in resistance (in %) of *E. coli* STEC O157 isolated from humans in the Netherlands from 1999 - 2019.

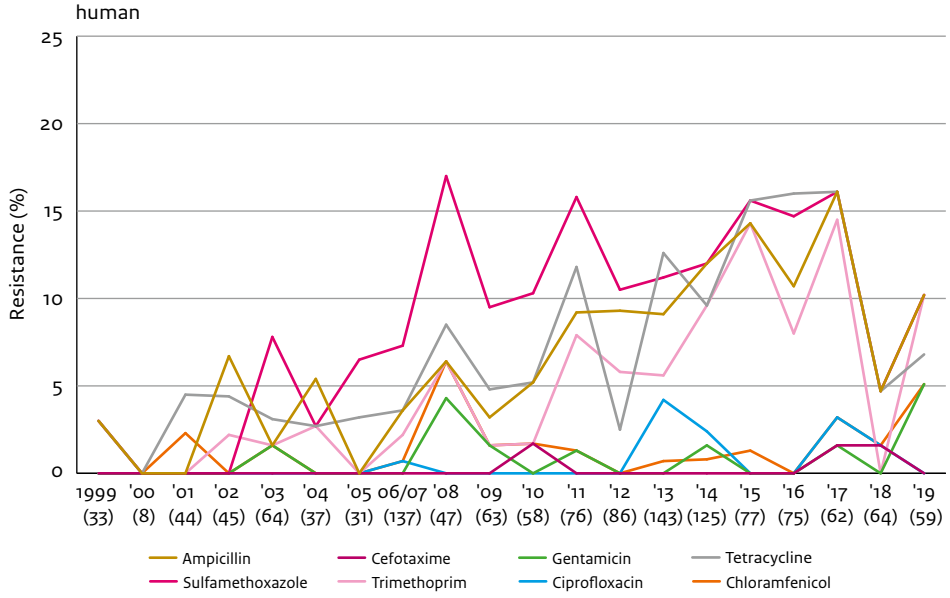


Table STEC01 MIC distribution (in %) and resistance percentages (R%) for *E. coli* STEC O157 (N=59) isolated from humans the Netherlands in 2019.

E. coli N = 1880	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									88.1	1.7				10.2					10.2	3.8 - 20.1
Cefotaxime					100.0														0.0	0.0 - 6.1
Ceftazidime						100.0													0.0	0.0 - 6.1
Gentamicin						71.2	23.7				1.7	1.7	1.7						5.1	1.1 - 14.2
Tetracycline								81.4	11.9			1.		6.8					6.8	1.9 - 16.5
Sulfamethoxazole										88.1	1.7						1.7	8.5	10.2	3.8 - 20.8
Trimethoprim					84.7	3.4	1.7						10.2						10.2	3.8 - 20.8
Ciprofloxacin	72.9	27.1																	0.0	0.0 - 6.1
Nalidixic acid									96.6	3.4									0.0	0.0 - 6.1
Chloramphenicol										88.1	6.8				5.1				5.1	1.1 - 14.2
Azithromycin*								6.8	86.4	6.8									0.0	0.0 - 6.1
Colistin**							98.3	1.7											0.0	0.0 - 6.1
Meropenem		100.0																	0.0	0.0 - 6.1
Tigecycline***					98.3	1.7													0.0	0.0 - 6.1

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.

After a substantial decrease of resistance proportions of human isolates for most antibiotics in 2018, in 2019 an increase of resistance proportions was found for the majority of antibiotics. Since approximately 2009, resistance proportions for ampicillin, tetracycline and trimethoprim showed a tendency to increase until 2017, then showed a decrease in 2018, but increased again in 2019, although not to the levels of 2017 (Figure STEC01). Resistance against sulfamethoxazole was high, but fluctuating since 2008, and also decreased in 2018 and increased in 2019. Resistance for ciprofloxacin and nalidixic acid was not detected in 2015 and 2016, was very low in 2017 and 2018, and was not detected in 2019. No ESBL-producing isolates were detected in 2019.

3.2 Commensal indicator organisms

This chapter describes the susceptibility profiles of commensal bacteria from the gastro-intestinal tract of food-producing animals and meat and vegetables. The level of antimicrobial resistance in bacteria inhabiting the intestinal tract directly reflects the selection pressure as a result of the use of antibiotics in animals, especially over time. *E. coli* is therefore included as indicator organism for the Gram-negative flora. As a result of less priority for including enterococci representing the Gram-positive flora in the surveillance, no enterococci are reported since 2017.

EFSA prescribes the sampling strategy and isolation methodology of bacteria from caeca of randomly picked 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 in the Netherlands.

EU legislation on monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria (2013/652/EU) was implemented in 2014. This includes susceptibility testing by broth microdilution according to ISO 20776-1:2006 with mandatory panels of antimicrobials. 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 for some antibiotics not be clinically resistant.

Highlights 2019

1. Among indicator *E. coli* from animals and meat, resistance levels to ampicillin, tetracycline, sulfamethoxazole and trimethoprim were still relatively high in broilers, pigs, (white) veal calves and chicken and turkey meat.
2. Resistance in indicator *E. coli* from caecal samples showed a tendency to stabilise in broilers and pigs but showed a slight decrease in veal calves. In dairy cattle it fluctuates at a low level.
3. For the first time in twenty years no *E. coli* isolates resistant to extended spectrum cephalosporins were detected in faecal samples from broilers, pigs, dairy cattle and veal calves.
4. Resistance to fluoroquinolones was at the same level as in 2018, and was still commonly present in indicator *E. coli* from caecal samples of broilers and meat thereof.

Resistance levels

Table Eco01 shows resistance levels, presented as MIC-distributions, of 1209 *E. coli* isolates obtained from caecal samples from broilers, pigs, veal calves and faecal samples of dairy cows. Table Eco02 presents resistance percentages per animal species. Trends in resistance levels from 1998 to 2019 are shown in Figure Eco01 and information on trends in multidrug resistance is shown in Figure Eco02.

Table Eco03 presents resistance percentages of 395 *E. coli* isolates collected from raw chicken meat, turkey meat, beef, pork and vegetables. Figure Eco03 shows trends in resistance of *E. coli* in the Netherlands from 2002 to 2019 isolated from raw meat of chicken, turkey, cattle and pig.

For most drugs or drug classes, resistance levels varied substantially between the different animal species (Table Eco02). 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. This pattern was also observed in previous years. Overall, the highest resistance levels were seen for ampicillin, tetracycline, sulfamethoxazole and trimethoprim. These drug classes are the most frequently used classes in veterinary medicine in The Netherlands.

Table Eco01 MIC distribution (in %) and resistance percentages (R%) for all *E. coli* (N=1209) isolated as indicator organism from intestines of food producing animals in the Netherlands in 2019.

E. coli	MIC (%) distribution mg/L																		R%	95% CI
N = 1209	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048		95% CI
Ampicillin							1.4	20.3	51.6	6.0	0.1	0.1	0.1	20.4					20.7	18.4 - 23.1
Cefotaxime					100.0														0.0	0 - 0.3
Ceftazidime						100.0													0.0	0 - 0.3
Gentamicin						46.3	45.5	5.9	0.4		0.7	0.7	0.6						2.3	1.5 - 3.3
Tetracycline								62.2	8.1	0.5	0.5	0.6	10.7	17.5					29.2	26.7 - 31.9
Sulfamethoxazole										76.4	0.1	0.1	0.2		0.2		0.2	22.8	23.2	20.9 - 25.7
Trimethoprim					41.9	37.6	2.3	0.2			0.1	0.1	17.8						17.9	15.8 - 20.2
Ciprofloxacin	78.4	11.2	0.3	0.4	6.6	1.5	0.7	0.2	0.1	0.4	0.2								10.1	8.4 - 11.9
Nalidixic acid									88.1	2.4	1.1	0.2	0.8	3.0	4.5				9.5	7.9 - 11.3
Chloramphenicol										85.9	6.3	1.2	1.5	2.2	3.0				7.8	6.3 - 9.4
Azithromycin*								5.2	55.4	36.8	2.2	0.2	0.1	0.2					0.4	0.1 - 1.0
Colistin							92.6	7.4											0.0	0 - 0.3
Meropenem		99.8	0.2																0.0	0 - 0.3
Tigecycline					90.9	9.1													0.0	0 - 0.3

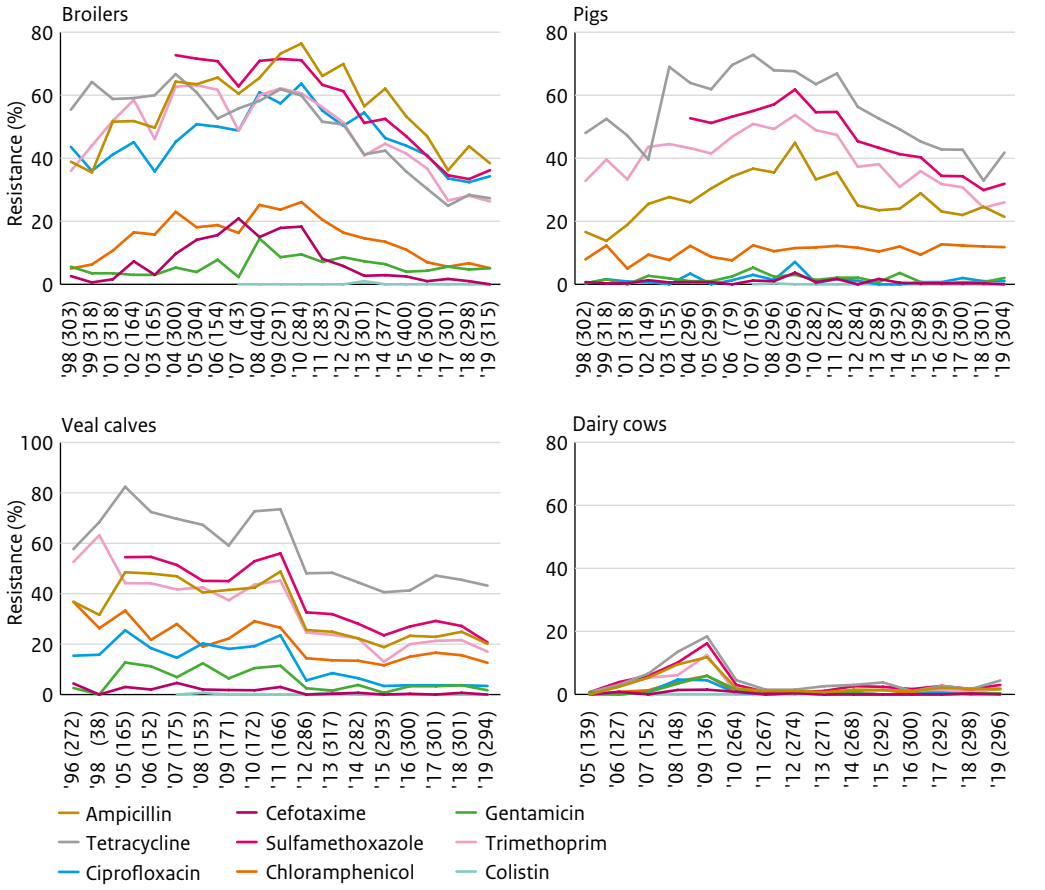
The white areas indicate the dilution range tested for each antimicrobial agent. Values above this range indicate MIC values > the highest concentration in the range. Values at the lowest concentration tested indicate MIC-values ≤ the lowest concentration in the range. Vertical bars indicate the epidemiological cut-off values (ECOFF), used as breakpoints. If available, dashed bars indicate the clinical breakpoints. For ampicillin, chloramphenicol and colistin the ECOFF and clinical breakpoint are identical.

** tentative ECOFF set by EURL established by EFSA data*

Fluoroquinolone resistance

Highest resistance levels for fluoroquinolones were found in *E. coli* from broilers: 34.3% resistance to both ciprofloxacin and nalidixic acid in isolates from Dutch broilers. This level of resistance is similar to the previous two years and seems to stabilise after a decreasing trend from 2013 until 2017. Resistance to ciprofloxacin was 4.8% in *E. coli* isolates from white veal calves, 1.0% in pigs, 0.3% in dairy cattle and could not be detected in isolates from rosé veal calves.

Figure Eco01 Trends in proportion of resistance (%) of *E. coli* isolated from faecal samples of broilers, slaughter pigs, veal calves and dairy cattle in the Netherlands from 1998 - 2019.



Resistance to fluoroquinolones in *E. coli* from meat was tested for chicken and turkey meat samples, beef, pork and vegetable samples from retail in The Netherlands (Table Eco03). No samples from meat imported from outside the EU were analysed for indicator *E. coli* in 2019. Figure Eco03 shows that resistance in chicken products at retail was approximately at the same level as in 2018: the percentage of *E. coli* with resistance to ciprofloxacin and nalidixic acid was 25.7% (27.4% in 2018) and 24.0% (25.0 % in 2018), respectively. Resistance percentages in isolates from turkey products were after a steep increase in 2018

back to the levels in 2017 with 26,7% of resistance for both ciprofloxacin and nalidixic acid. These fluctuations in time are most probably due to the low number of samples per year for turkey. Therefore, these results should be interpreted carefully. Resistance percentages in isolates from beef, pigs and vegetables were low compared to poultry: with respectively 5.5%, 2.5% and 1.2% of the isolates showing identical resistance to both ciprofloxacin and nalidixic acid. Because the ECOFF for nalidixic acid was lowered from 16 mg/L to 8 mg/L the difference in resistance levels between ciprofloxacin and nalidixic acid declined for isolates with plasmid mediated quinolone resistance (PMQR). As a consequence the monitoring system becomes less sensitive to detect this specific type of quinolone resistance.

Cefotaxime resistance

For the first time in twenty years of monitoring livestock, resistance to third generation cephalosporins (ESC-resistant), indicative of ESBL/pAmpC production, was absent amongst randomly isolated commensal indicator *E. coli* in all animals species tested (broilers, slaughter pigs, veal calves and dairy cattle). This indicates a further decrease of ESBL/AmpC producing *E. coli* at a level below the detection limit for all animal species (Figure Eco01).

Despite of the above ESC-resistant *E. coli* are still detected in caecal samples of different animal species when using a selective isolation method. Importantly, the prevalence of broilers carrying ESC-resistant *E. coli* further decreased from 50.3% of the animals sampled in 2016 and 32.6% in 2017 to 23.0 % in 2018 and 17.9% in 2019 (see chapter 4). The ongoing decrease in prevalence and concentrations of ESC-resistant *E. coli* in broilers and on poultry meat is an important finding because it suggests that the exposure of humans to ESC-resistant *E. coli* through contaminated meat is also decreasing. After a period of increasing prevalence in white veal calves, the proportion of animals tested with ESC-resistant *E. coli* in the GI tract decreased from 47.6% to 39.8%, which is similar to the level in 2017 (40.5%). In rosé veal calves it steeply declined from 26.9% to 14.0% comparable to the relative low levels in 2014 (11.3%) en 2015 (10.0%). The prevalence in 2019 of animals positive for ESC-resistance in pigs and dairy cattle were alike previous years with 9.9% and 8.3% respectively.

In chicken meat samples, one cefotaxime resistant isolate (0.6%) was detected. The low proportion of cefotaxime resistance is comparable to 2018 with 1.1% resistance. No cefotaxime resistance was detected in indicator *E. coli* isolates from turkey, beef, pork and vegetables.

The small proportion of cefotaxime resistant *E. coli* from chicken meat samples, in randomly isolated strains cultured on non-selective media, suggests that the prevalence of ESC-resistant *E. coli* on meat is reducing. This is confirmed by the decreasing proportion of fresh chicken meat samples in which ESC-resistant *E. coli* were found using selective media from 31.4% in 2017 to 14.4% in 2018 and 11.0% in 2019 (see chapter 4). One has to consider the fact that part of the retail meat included in the sampling originates from EU countries outside the Netherlands where resistance prevalences might be higher.

Broiler chickens

Proportion of resistance in commensal *E. coli* isolated from caecal samples of broiler chickens stabilised for most antimicrobial classes (Figure Eco01 and Table Eco02). Resistance remained high for ampicillin (38.4), tetracycline (27.3%), trimethoprim (26.3%), sulfamethoxazole (36.2%) and ciprofloxacin (34.3%). For the first time in 20 years cefotaxime resistance was not detected amongst commensal indicator *E. coli* from broilers (Figure ESBL01).

Slaughter pigs

Overall resistance proportion stabilised in slaughter pigs (Figure Eco01). Resistance proportions for tetracycline, sulfamethoxazole and trimethoprim in *E. coli* isolates from pigs, sampled in 2019, were higher than in 2018 (tetracycline from 32.9% in 2018 to 41.8% in 2019, sulfamethoxazole from 29.9% to 31.9% and trimethoprim from 24.3% to 26.0%). The resistance percentage for ampicillin decreased from 24.6% in 2018 to 21.4% in 2019. Resistance to the 3rd generation cephalosporins was not detected.

Veal calves

Resistance data on white and rosé veal calves are reported separately, because of the difference in production systems. White veal calves are fattened on a milk diet with a required minimal uptake of roughage, while rosé veal calves are also fed corn silage, straw or pelleted feed. Most antibiotics are administered during the starting period in both production systems. On average, in white veal calves more antibiotics are used than in rosé calves and rosé calves are slaughtered at an older age, which results in a longer time period with relatively low antibiotic exposure. This results in a difference in resistance levels at slaughter between the two husbandry types. 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 slaughter-house. 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. This explains part, but not all of the increase in resistant rates of *E. coli* in veal calves in 2016 and 2017 compared to 2015. After 2017 a tendency of decreasing resistances is observed for most antimicrobial classes.

In 2019, a slight decreasing tendency was observed in veal calves for most antimicrobials tested. Highest resistance levels in veal calves were observed for tetracycline (54.1% and 16.5% for white and rosé respectively), sulfamethoxazole (24.4% and 9.4%), trimethoprim (22.0% and 3.5%) and chloramphenicol (14.8% and 7.1%). *E. coli* isolates resistant to the 3rd generation cephalosporins were not detected in caecal samples of white and rosé veal calves (TableEco02).

Table Eco02 Resistance percentages (R%) of *E. coli* isolated from faecal samples of broilers, pigs, dairy cows, white veal calves and rosé veal calves in the Netherlands in 2019.

Faecal samples	Broilers	Pigs	Dairy	Veal calves	
	N = 315	N = 304	N = 296	White, N = 209	Rosé, N = 85
Ampicillin	38.4	21.4	1.7	24.4	9.4
Cefotaxime	0.0	0.0	0.0	0.0	0.0
Ceftazidime	0.0	0.0	0.0	0.0	0.0
Gentamicin	5.1	2.0	0.3	1.9	1.2
Tetracycline	27.3	41.8	4.4	54.1	16.5
Sulfamethoxazole	36.2	31.9	3.0	25.4	9.4
Trimethoprim	26.3	26.0	2.0	22.0	3.5
Ciprofloxacin	34.3	1.0	0.3	4.8	0.0
Nalidixic acid	34.3	0.7	0.0	2.4	0.0
Chloramphenicol	5.1	11.8	1.7	14.8	7.1
Azithromycin	0.6	0.7	0.3	0.0	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	0.0	0.0

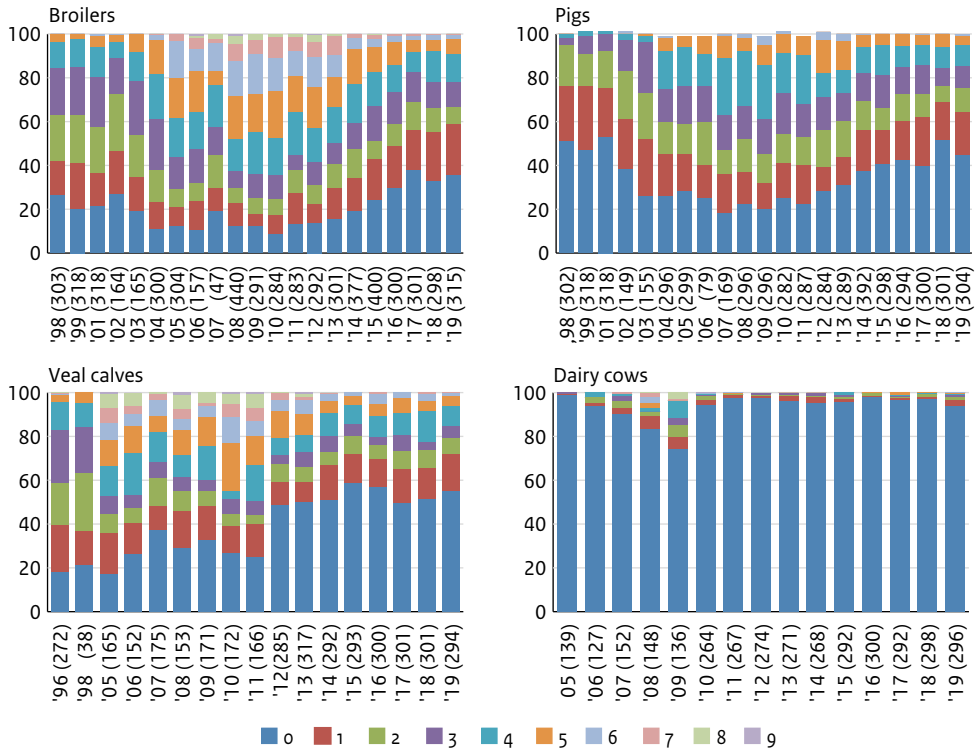
Dairy cattle

Resistance in *E. coli* isolated from dairy cattle was, as always, very low compared to resistance proportions observed in pigs, broilers and veal calves (Table Eco02), reflecting the low use of antibiotics in this husbandry system. However, in 2019 a slight increase in resistance was observed for tetracycline (from 1.7% to 4.4%), sulfamethoxazole (1.7% to 3.0%), trimethoprim (0.7% to 2.0%) and chloramphenicol (1.0% to 1.7%). As in previous years resistance to the 3rd generation cephalosporins was not detected.

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 Resistance percentages (R%) of *E. coli* isolated from faecal samples of broilers, pigs, dairy cows, white veal calves and rosé veal calves in the Netherlands in 2019.



In general, the level of multidrug resistance (showing resistance to three or more classes of antimicrobials) stabilised in the last 3 years. In broilers, the proportion of multidrug resistance isolates was relatively high with 33.3%, but similar to previous years (33.9% in 2018, 31.4% in 2017). The proportion of multidrug resistance stabilised in pigs (25.0% in 2019, 24.1% in 2018), but decreased in veal calves from 26.7% in 2018 to 20.7% in 2019. In dairy cattle multidrug resistance in *E. coli* slightly increased to 2.0% of the isolates, but is still at a low level compared to the other animals species.

During the last decade, proportions of complete susceptibility have considerably increased in all animals species. Compared to 2018, the percentage of completely susceptible *E. coli* isolates increased for broiler and calf isolates, but decreased for pigs (Figure Eco02).

E. coli in raw-meat and vegetables

Table Eco03 presents resistance percentages of *E. coli* isolated from raw meat of chicken, turkey, pigs and cattle as well as vegetables, sampled at retail by the Dutch Food and Consumer Product Safety Authority (NVWA). Meat from retail can include meat produced in The Netherlands, but also other EU countries. Meat products imported from outside the EU were not analysed for indicator *E. coli* in 2019. All vegetables were sampled as fresh products at retail and originated from within EU.

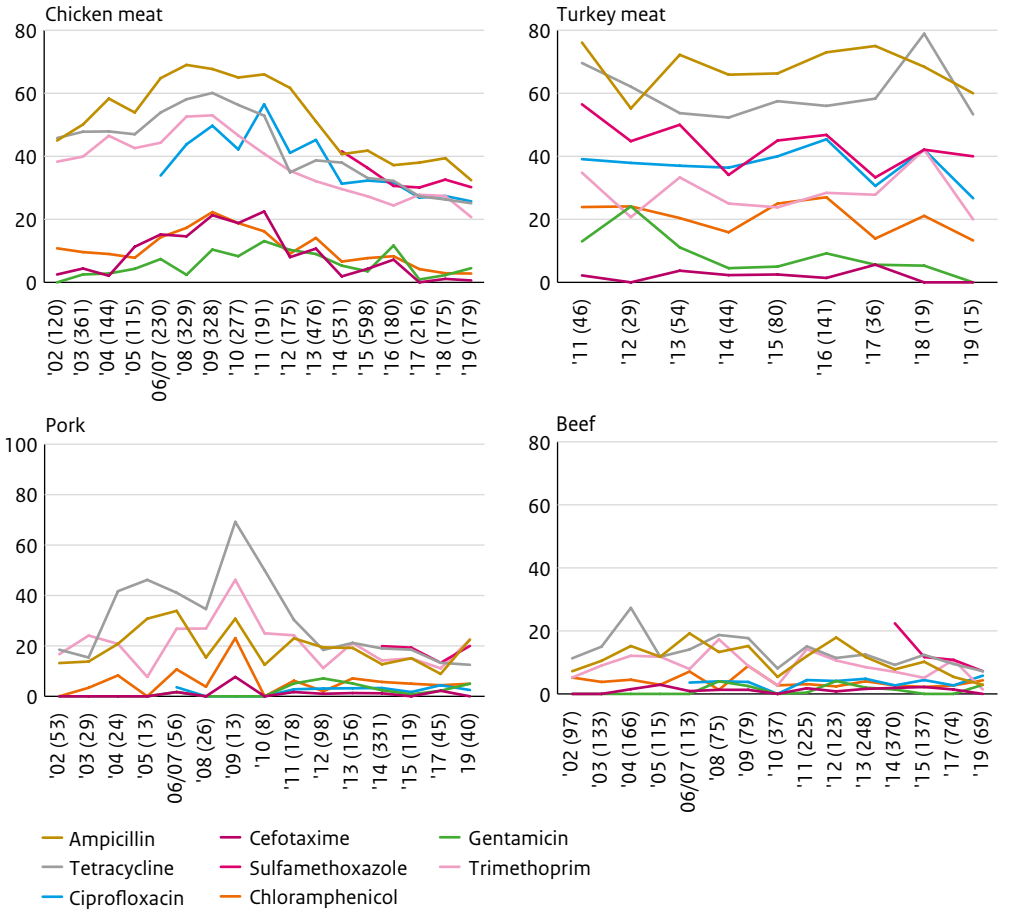
Table Eco03 Resistance percentages (R%) of *E. coli* isolated from raw chicken meat, turkey meat and vegetables at retail in the Netherlands in 2019.

Products	Chicken	Turkey	Bovine	Pig	Vegetables
	N = 179	N = 15	N = 69	N = 40	N = 92
Ampicillin	32.4	60.0	2.9	22.5	8.4
Cefotaxime	0.6	0.0	0.0	0.0	0.0
Ceftazidime	0.6	0.0	0.0	0.0	0.0
Gentamicin	4.5	0.0	2.9	5.0	0.0
Tetracycline	25.1	53.3	7.2	12.5	6.0
Sulfamethoxazole	30.2	40.0	7.2	20.0	3.6
Trimethoprim	20.7	20.0	1.4	22.5	2.4
Ciprofloxacin	25.7	26.7	5.8	2.5	1.2
Nalidixic acid	24.0	26.7	4.3	2.5	1.2
Chloramphenicol	2.8	13.3	4.3	5.0	2.4
Azithromycin	0.0	0.0	0.0	0.0	0.0
Colistin	0.0	13.3	0.0	0.0	0.0
Meropenem	0.0	0.0	0.0	0.0	0.0
Tigecycline	0.0	0.0	0.0	0.0	0.0

Fig Eco03 shows the trends in resistance in the meat samples. Resistance percentages in chicken meat show a tendency to decrease from 2010 onward, and seems to stabilise with some fluctuations since 2015. In turkey meat, resistance rates have been at a constant high level since 2011. The relative high degree of variation is due to the low number of turkey meat samples analysed in 2018 and in previous years. Therefore results must be interpreted with care. Cefotaxime resistance was not be detected in *E. coli* isolates from turkey meat in 2019, and was at a very low level in chicken meat samples (0.6%).

Fluctuations in resistance rates of meat samples might be caused by a year-to-year variation in the proportion of retail poultry meat produced outside of the Netherlands included in the survey.
In vegetables, resistance levels of *E. coli* isolates were very low. No resistance was detected to 3rd generation cephalosporins (cefotaxime and ceftazidime) and gentamicin. Percentages of resistance to ampicillin, chloramphenicol, quinolones, tetracycline, trimethoprim and sulfamethoxazole were all below 10%. This was the second consecutive year that vegetable samples were tested, so trends in results could not be determined.

Figure Eco03 Trends in proportion of resistance (%) of *E. coli* isolated from raw chicken meat, turkey meat, pork and beef in the Netherlands from 1998 - 2019.



4
Screening for ESBL, AmpC, carbapenemase-producing and colistin-resistant Enterobacteriaceae in food-producing animals and meat in the Netherlands in 2019

This chapter the describes the data specifically for resistance against antibiotics of specific interest because of their importance for human medicine, a previous increasing resistance in livestock in the Netherlands or a rise of resistance in livestock abroad. The chapter describes the data for extended-spectrum beta-lactamases (ESBL) and AmpC producing *E. coli* and *Salmonella* which are resistant to extended-spectrum cefalosporins, carbapenemase producing *E. coli* and *Salmonella* which are resistant to carbapenems and MCR producing *E. coli* which are resistant to colistin.

Highlights

1. For the first time since the start of the monitoring program, ESBL/AmpC were not detected in randomly selected *E. coli*.
2. In 2019, a reduction in prevalence of animals carrying ESBL/AmpC producing *E. coli* was observed in all livestock species compared to 2018.
3. The largest reduction in the prevalence of ESBL/AmpC-producing *E. coli* has been achieved in broilers decreasing from 66.0% in 2014 to 17.9% in 2019.
4. The overall prevalence of ESBL/AmpC-producing *E. coli* stabilised in retail meat. This was also the case in broilers with 13.7% of the meat being positive for ESBL/AmpC-producing *E. coli*.
5. No ESBL/AmpC producing *Salmonella* were found in livestock and retail meat.
6. No carbapenemase-producing *Enterobacteriaceae* were detected in livestock and companion animals.
7. In 2019, *mcr*-1 was identified at very low level (< 1%) in caecal samples from slaughter pigs and white veal calves. For the second year in row *mcr*-4 was detected in white veal calves at low level (2%).
8. No *mcr* genes were identified in *E. coli* isolated from broilers and in chicken meat indicative for a further reduction of *mcr*-1 in the broiler sector.
9. The first results of a comparative study suggest an overall low genetic relatedness between LA-MRSA isolates from livestock (pigs and poultry) and humans. Moreover, the emergence of a more virulent (PVL-positive) LA-MRSA subclade is probably transmitted independent of livestock exposure.

4.1 ESBL/AmpC-producing Enterobacteriaceae

The monitoring for extended-spectrum cefalosporins (ESC) resistant Enterobacteriaceae occurs at two levels in parallel, consisting of monitoring the proportion of ESC resistance in randomly isolated *E. coli* as described in chapter 3 and an in-depth analysis of selectively isolated Enterobacteriaceae in this chapter. These combined results provide information about the prevalence of resistance in the entire population of Enterobacteriaceae but also at the level of individual animals or meat samples.

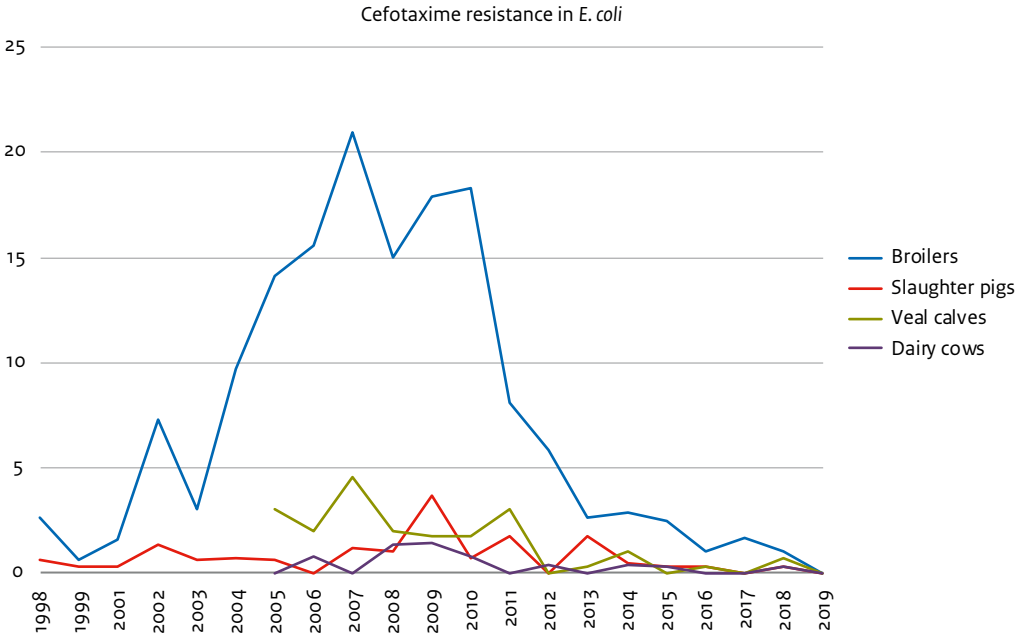
4.1.1 Randomly isolated ESBL/AmpC-producing bacteria from livestock in 2019

To determine the proportion of resistance against ESC in the population of *E. coli* from food-producing livestock, EFSA guidelines describe the surveillance of non-selectively isolated *E. coli* (European Food Safety Authority, 2012). For this analysis, 1209 caecal samples were collected at slaughter of broilers, veal calves and pigs, while samples of dairy cattle are collected at farms. A minimum number of 170 per species is prescribed where dairy cows and veal calves are analysed as separate categories. Isolates are considered ESBL/AmpC-suspected when phenotypic measurement indicates a reduced susceptibility against ESC cefotaxime and/or ceftazidime compared to epidemiological cut-off values as determined by EUCAST, see also chapter 3.

The graphs in Figure ESBL01 display the trends over time of cefotaxime resistance from randomly isolated *E. coli.* Interestingly, 2019 was the first year in which resistance against cefotaxime was not detected in randomly isolated *E. coli* from any of the livestock categories that were included in the sampling. For slaughter pigs, veal calves and dairy cows, prevalence of cefotaxime resistance has never exceeded 5% of

the population of *E. coli*. In all three of these categories, previous years have also incidentally reported no cefotaxime resistance in randomly isolated *E. coli.*

Figure ESBL01 Trends in cefotaxime resistance (%) of *E. coli* randomly isolated from faecal samples of broilers, slaughter pigs, veal calves and dairy cows.



Since the start of the monitoring program in 1998, the greatest fluctuations have been observed in broilers. A peak prevalence of cefotaxime resistance was reported in 2007 of 20.9%. Since 2011, a steep decrease was witnessed which resulted in a prevalence below 5% since 2013.

Any ESBL/AmpC suspected isolates are characterised using molecular techniques to determine the gene responsible for resistance. These data are used to determine trends in resistance genes over time as visible in Table ESBL01. While no ESBL/AmpC genes were detected in the randomly isolated *E. coli* in 2019, these data can serve for comparison to the selectively isolated *E. coli* in 2019 as presented in Table ESBL03.

Table ESBL01 ESBL-genes found in E. coli isolates with reduced susceptibility to cefotaxime randomly isolated from faecal samples of broilers, veal calves, slaughter pigs, dairy cows and turkey (only 2011 and 2012) during 2007-2019.

	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	bSHV-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	1026	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	1002	5.9
2011	23	5	5	0	6	39	9			8		9	2	3	3	5	1096	3.6
2012	26	2	0	1	n.t.	29	8			4		8		5		4	1328	2.2
2013	13	1	4	0	n.t.	18	7			4		3		3	1		1371	1.3
2014	11	3	2	0	n.t.	16	8			1		4			1	2	1519	1.1
2015	10	0	1	1	n.t.	12	3		2	1		1		2	3		1283	0.9
2016	3	1	1	0	n.t.	5	2			1				1	1		1492	0.3
2017	5	0	0	0	n.t.	5	2			1			2				1194	0.4
2018	3	2	0	0	n.t.	7	2					3			2		1198	0.6
2019e	0	0	0	0	n.t.	0											1209	0.0
Total	274	29	31	8	6	350	137	19	3	39	2	45	11	44	22	28		

[a] *All were blaCTX-M-1, only in 2011 one blaCTX-M-3 gene was found in an isolate from a veal calf.*

[b] *One combination of blaSHV-12 together with blaTEM-52 occured in 2012 in one broiler isolate.*

[c] *In broilers, three combinations were found: in 2008: blaCTX-M-1 with blaCTX-M-2; in 2009: blaCTX-M-1 with blaSHV-12 and blaCTX-M-1 with blaSHV-12 and blaCMY-2.*

[d] *In dairy cows, one combination of blaCMY-42 with blaTEM-190.*

[e] *no ESBL-suspected isolates found in 2019*

n.t.: *not tested*

4.1.2 Selective isolation of ESBL/AmpC producing Enterobacteriaceae in 2019

While the randomly isolated *E. coli* described in chapter 3 and in 4.1.1 aim to provide an insight in the total community of Enterobacteriaceae in livestock, the selectively isolated *E. coli* that are discussed in this paragraph aim to determine precisely in what percentage of the animals and meat products ESBL/AmpC producing Enterobacteriaceae are present. Isolation is performed as described by the European Union Reference Laboratory for Antimicrobial Resistance (https://www.eurl-ar.eu/protocols.aspx).
Selective isolation of caecal content occurs through sampling a unique herd of broilers, pigs and veal calves at slaughter houses while faecal samples of dairy cattle are collected at farms. 1 gram of faecal material is mixed in 9 ml of Buffered Peptone Water (BPW) and incubated overnight at 37 °C, followed by selective isolation on MacConkey agar with 1 mg/L cefotaxime. Selective isolation from meat is performed by mixing 25 gram of meat with 225 ml of BPW and incubating overnight at 37 °C, followed by selective isolation on MacConkey agar with 1 mg/L cefotaxime and on Brilliance ESBL Agar. Species identification was performed using MALDI-TOF (Bruker Biotyper). MIC analysis, as described in Chapter 2, is carried out to confirm the ESC-resistant phenotype. Molecular analysis is performed by PCR and microarray analysis using Check-points CT101. These are followed by more specific PCR and DNA sequencing to determine the ESBL/AmpC at allele level.

Results of selective isolation of ESBL/AmpC-producing *E. coli* in faeces
In 2019, a total of 1209 faecal samples from livestock animals were analysed by selective isolation resulting in an overall prevalence of 17.0%, Table ESBL02. Comparing this data to previous years shows that at the start of the selective isolation ESBL/AmpC-producing *E. coli* in faeces in 2014, a reduction in the prevalence was seen for all livestock species that were collected, Figure ESBL02. Between 2016-2018, some small to moderate increases in prevalence were observed in several livestock species. In 2019, a reduction in prevalence was seen in all livestock species compared to 2018 although some are still higher than the prevalence observed in 2014-2015.

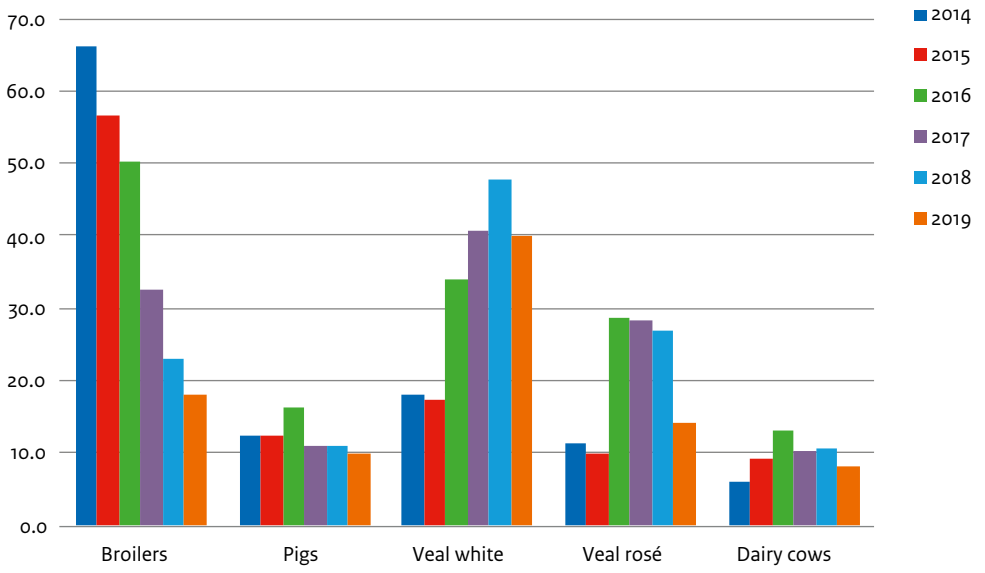
The largest reduction in the prevalence of ESBL/AmpC-producing *E. coli* has been achieved in broilers. While in 2014 66.0% of broiler samples contained ESBL/AmpC-producing *E. coli*, this steadily decreased to 17.9% in 2019. Both in slaughter pigs and dairy cows, the prevalence of ESBL/AmpC producing *E. coli* has been relatively low compared to other categories, both fluctuating at approximately 10% over time.

For both rose and white veal calves an increase in prevalence was observed in 2016. For the rosé veal calves, this rise went from 10% in 2015 to 28.7% in 2016. In 2017 and 2018, the prevalence was stable (respectively 28.3% and 26.9%) but now in 2019, the prevalence was reduced again to 14%. In white veal calves, a further increase was observed between 2016 to 2018 (respectively 33.9%, 40.5%, 47.6%) but in 2019 this decreased to 39.8%.

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 2019.

	N samples	N suspected ESBL	N confirmed ESBL	Prevalence(%) ESBL confirmed
Broilers	308	58	55	17.9
Pigs	304	51	30	9.9
Veal calves:				
white	211	90	84	39.8
rosé	86	13	12	14.0
Dairy cows	300	31	25	8.3
Total	1209	243	206	17.0

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 2014-2019 determined by using selective isolation.



Results of the molecular analysis to determine the resistance genes responsible for the ESC phenotype are presented in Table ESBL03. As seen in previous years, $bla_{CTX\text{-}M\text{-}1}$ is present in all livestock in the Netherlands (also discussed by Cecceralli et al, 2019). Certain variants of low-prevalent genes were not detected in 2019, possibly due to the reduction in ESBL prevalence, but overall there is still a high number of different ESBL/AmpC variants circulating in the Dutch livestock. Fluctuations of these ESBL/AmpC genes occur over time in all livestock species. Nonetheless, the relative abundance of certain resistance genes has changed more strongly over time in the different livestock species and further in-depth analysis is needed to determine if these trends are statistically significant or if these may be caused by the reduction in prevalence (MARAN 2002-2018).

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 2019.

		Broilers	Slaughter pigs	Veal calves		Dairy cows	Total
				White	Rose		
CTX-M-1 group	CTX-M-1	15	18	28	4	4	69
	CTX-M-15	1	1	40	3	10	55
	CTX-M-32			4			4
	CTX-M-55			2		1	3
CTX-M-2 group	CTX-M-2			1	2	1	4
CTX-M-8/25 group	CTX-M-8		1				1
CTX-M-9 group	CTX-M-9				2		2
	CTX-M-14		2	4	1	3	10
	CTX-M-27	1		1		1	3
	CTX-M-65			1			1
	CTX-M-235					1	1
TEM	TEM-52c	5	4	2		1	12
	TEM-52cVar	2	1				3
SHV	SHV-2a	1					1
	SHV-12	21		1			22
CMY	CMY-2	9	3			3	15
Chromosomal *amp*C	*amp*C-type-3	3	21	6	1	6	37
Total		58	51	90	13	31	243

Over the period from 2014 to 2019, in broilers the relative proportion of bla_{SHV-12} has increased from 15% to 36% while $bla_{CTX-M-1}$ and bla_{CMY-2} have decreased respectively from 42% to 26% and from 28% to 16%. During this same period, both in veal calves and dairy cows, the relative proportion of $bla_{CTX-M-1}$ decreased respectively from 31 to 13% and from 46 to 31% while in both populations $bla_{CTX-M-15}$ went up from 9 to 32% and from 14 to 44%. Interestingly, in rosé veal calves both proportions were relative stable ($bla_{CTX-M-1}$ from 35% to 31% and $bla_{CTX-M-15}$ from 20% to 23%). The reduction in antimicrobial usage in livestock has significantly reduced the prevalence of ESBL/AmpC producing *E. coli* (Hesp et al. 2019).[5] Changes in the relative proportion of resistance genes could be caused by the genetic carriers, i.e. plasmids, that encode the resistance gene as well as other gene products on which selective pressure may still exist. It is unclear why persistence of certain resistance genes may be stronger than others.

Currently, the cause of the increase in prevalence in veal calves is unknown and is the topic of ongoing investigation in a longitudinal study at several dairy and veal farms throughout the Netherlands to determine the transmission of ESBL producing bacteria within the chain and on farms. This study is part of the policy supporting research (beleidsondersteunend onderzoek) and is performed in collaboration with the Public Private Partnership 'Vitaal en Gezond Kalf.' So far, the study has shown that 24.8% of the animals are colonised at the moment they are transported from the dairy farm. Subsequently, ESBL

prevalence increases early in the production cycle and decreases closer to slaughter age, as previously reported (Hordijk et al. 2013). While the prevalence in the study at the last sampling moment before slaughter is 27.7% over all eight veal farms, the prevalence per farm varied from 0.9% to 90.4%. Molecular characterisation and identification of risk factors is part of the ongoing study.

Results of selective isolation of ESBL/AmpC-producing E. coli in raw meat

The prevalence of ESBL/AmpC-producing *E. coli* in raw meat was determined as described above. A total of 1947 samples of fresh meat produced in the EU were analysed with of which 55 contained ESBL/AmpC-producing *E. coli*. This overall prevalence of 2.8% is the same as the prevalence reported in 2018, see Table ESBL04.

Table ESBL04 Prevalence of ESBL/*Amp*C-positive *E. coli* isolates from raw meat products in the Netherlands in 2019.

Animal source	N screened	N ESBL/AmpC suspected	% ESBL/AmpC positive
Beef	573	4	0.7
Veal	209	5	2.4
Pork	296	1	0.3
Chicken	262	36	13.7
Turkey	14	3	21.4
Lamb	238	2	0.8
Goat	2	0	0.0
Fish and shrimps	304	8	2.6
Exotic meat	49	0	0.0
Total	1947	55	2.8

The prevalence on poultry meat has always been relatively high and also in 2019, turkey and chicken meat are the only categories in which > 10% of the meat was observed positive. When comparing the results of separate categories, a rise in the prevalence in turkey meat (from 9.5% to 21.4%) is observed but with the low number of samples that are analysed in this category, this is not a significant change.

In 2018 a reduction was reported in the prevalence of ESBL/AmpC-producing *E. coli* on chicken meat from 31.6% to 13.7% which appears permanent as the prevalence in 2019 was also 13.7%. Similarly for veal, a reduction from 2017 to 2019 was maintained with respectively 7.5%, 3.4% and 2.4% prevalence and for fish and shrimps, 12.5%, 2.6% and 2.6% and for pork 1.5%, 0%, 0.3%. Exotic meat such as frog and crocodile are reported combined and although the number of sampled products has increased to 49, no ESBL/AmpC-producing *E. coli* were observed in 2019.

The molecular analysis of the genes that are responsible for the ESC resistant phenotype in *E. coli* from fresh meat was performed on 51 of 55 isolates, see Table ESBL05.

Table ESBL05 Beta-lactamases identified in *E. coli* from raw meat products in the Netherlands in 2019.

	ESBL gene	Chicken	Turkey	Beef	Veal	Lamb	Fish and shrimps	Total
CTX-M-1group	CTX-M-1	11	1	1	2			15
	CTX-M-15	1	1				3	5
	CTX-M-55			1	1			2
CTX-M-9 group	CTX-M-27		1					1
TEM	TEM-52c	1						1
	TEM-52cVar	2				1		3
	SHV-12	11						11
CMY	CMY-2	8					2	10
Chromosomal ampC	*amp*C-type-3	1		1	1			3
not tested		1		1	1	1		
Total		36	3	4	5	2	5	55

Due to the reduction in the prevalence of ESBL/AmpC-producing *E. coli*, the variation in genes is also lower than observed in previous years. While $bla_{CTX\text{-}M\text{-}1}$ and $bla_{CMY\text{-}2}$ have generally been the predominant genes, some fluctuations in the relative proportion of the genes are always seen over time. It is of note to mention that since 2016 to 2019, the relative proportion of $bla_{SHV\text{-}12}$ has been rising from 5,0% to 20%. This rise is presumably linked to a similar rise in the relative proportion of this gene in the selective isolations from chicken faecal samples taken at slaughter but, as described above, it is currently unknown what mechanism is responsible for the slower reduction of this gene from the population compared to other ESBL/AmpC genes.

ESBL/AmpC-producing *Salmonella*

In the Netherlands, surveillance takes place for ESBL/AmpC-producing *Salmonella* from both humans and meat. 2019 is the second consecutive year in which no ESBL/AmpC-producing *Salmonella* were isolated from fresh meat produced in the EU.

A total of 1880 *Salmonella* isolates from various serovars were tested for resistance to cefotaxime and ceftazidime of which 19 were resistant and suspected ESBL/AmpC producers. These isolates represent 8 different serovars, see Table ESBL06, of which *S. Typhimurium*, *S. Kentucky* and *S. Infantis* are most commonly found, all containing a different allele from the CTX-M-9 group respectively $bla_{CTX\text{-}M\text{-}9}$, $bla_{CTX\text{-}M\text{-}14b}$ and $bla_{CTX\text{-}M\text{-}65}$. The proportion of *Salmonella* that produce ESBL/AmpC was 1.0% in 2019, which is comparable to previous years, see Table ESBL07. When comparing the ESBL/AmpC genes that are detected in the *Salmonella*, it is interesting to notice that between 2010-2015, genes of the CTX-M-1 group were first replaced in the population by $bla_{CMY\text{-}2.}$ However, since 2015 genes of the CTX-M-9 group have become the most frequent ESBL/AmpC genes in the population of *Salmonella*. The precise cause for these variations over time are unknown but are possibly the result of changes in selective pressure by antibiotic usage and

other selective compounds. In addition, yearly changes in the sampling strategy lead to decreasing proportions of imported meat from outside EU might also have influenced the outcomes.

In summary, in the past decade the prevalence of ESBL/AmpC producing bacteria has decreased in livestock animals and meat, as determined by several methods discussed above. In 2009, cefotaxime resistance proportion varied in randomly *E. coli* varied between 1.5% and 17.9% in different animals species while in 2019, for the first time ESBL/AmpC producing *E. coli* could not be detected. Since the start of selectively isolation of ESC resistant *E. coli* in 2014, the prevalence in broilers has decreased greatly from 66.0% to 17.9%. Using this method, in 2016 an increase in both rosé and white veals calves was measured which could not be observed in the randomly isolated *E. coli*. A decrease for both of these categories was now witnessed in 2019. Finally both ESBL/AmpC producing *E. coli* and *Salmonella* have decreased on fresh meat of which the latter has not been detected in 2018 and 2019.

Table ESBL06 Beta-lactamases identified in *Salmonella* in 2019 (18 human isolates and 1 isolate of unknow origin).

		CTX-M-1 group		CTX-M-9 group						
Serovar	**CTX-M-1**	**CTX-M-15**	**CTX-M-55**	**CTX-M-9**	**CTX-M-14**	**CTX-M-14b**	**CTX-M-65**	**SHV-12**	**CMY-2**	**Total**
1,4,5,12:i:-			1						2	3
[a]Heidelberg									1	1
Infantis							3			3
Kentucky						3				3
Panama					1					1
Rissen								1		1
Typhi	1	1								2
Typhimurium		1		3						4
Uganda			1							1
Total	**1**	**2**	**2**	**3**	**1**	**3**	**3**	**1**	**3**	**19**

[a] *origin unknown (non-human)*

Table ESBL07 Beta-lactamases identified in *Salmonella* isolates collected in 2007-2019

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	1514	3.1
2008	25	12		1	1	13	1		6	2		61	2149	2.8
2009	12	4			2	3		1	9			31	2232	1.4
2010	8	3			1	2		3	4			21	1715	1.2
2011	5	3			1	1		2	13			25	1444	1.7
2012	14	5			2	2			10	1		34	1795	1.9
2013	1	3		5	4	5	1		36			55	1369	4.0
2014	6			2	3	1			21			33	1688	2.0
2015	13	2			6	1			12			34	1761	1.9
[f]2016	7				15	2			10		1	36	2117	1.7
[g]2017	3				23			1	3		1	31	1697	1.8
[h]2018	2		1	1	8				2			14	1718	0.8
2019	4				11			1	3			19	1880	1.0
Total	109	45	1	9	77	47	4	12	131	3	2	422	23079	1.8

[a] *contains blaCTX-M-1, blaCTX-M-55, blaCTX-M-15, blaCTX-M-3 and a combination with blaCMY-2 (n=2, 2014, 2015).*

[b] *In 2008 one combination of blaCTX-M-2 with blaTEM-52 was found in S. Paratyphi B var Java.*

[c] *contains blaCTX-M-9, blaCTX-M-14 and blaCTX-M-65.*

[d] *In 2007 three S. Concord were found containing both blaSHV-12 and blaCTX-M-15.*

[e] *In 2015 a combination of blaCMY-2 and blaTEM-52 was found in S. Oranienburg and a combination of blaCMY-2 with blaCTX-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 only human isolates were molecularly characterised.*

[h] *In 2018 only human isolates were molecularly characterised.*

4.2 Carbapenemase producing Enterobacteriaceae

4.2.1 Monitoring in livestock

In 2015, a sensitive molecular method was applied to screen for carbapenemase producers, extended spectrum beta-lactamases that can also hydrolyse carbapenems (MARAN 2016 for method details). This is important in an environment with a very low anticipated prevalence of carbapenem resistance. All faecal samples sent by NVWA to WBVR for antimicrobial resistance surveillance were screened with this method. Samples were grown overnight in BPW and after incubation five individual samples were pooled, centrifuged and DNA isolated from the pellet. A commercial RT-PCR (Check-Points, CarbaCheck MDR RT) that can detect the most important carbapenemase gene families (bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP} and bla_{OXA-48}) was used according to manufacturer's instructions. If RT-PCR gave suspicious or positive results, a step-wise analysis was performed to confirm the results:

1. Five conventional PCR were performed on purified DNA of the 5 individual samples of the pool;
2. If PCR was positive, genes were identified with Sanger sequencing;
3. 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).

Carbapenemase screening in 2019 (n=1209) resulted in seven bla_{OXA-48}-like positive faecal samples in the RT-PCR (three dairy cows, two broilers, one veal calf and one slaughter pig). In all seven samples the presence of bla_{OXA-48}-carrying *Shewanella* was confirmed by bacterial culturing followed by PCR and sequencing: $bla_{OXA-48b}$ (n=3), $bla_{OXA-48b-like}$ (n=2), $bla_{OXA-252}$ (n=1), and $bla_{OXA-416}$ (n=1). These results confirm the findings of previous years (MARAN reports 2013 – 2018) 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 isolated from livestock in the Netherlands in 2019. Screening for carbapenemase-producing isolates in faecal samples of food-producing animals will continue in 2020.

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 (González-Torralba *et al*, 2016), France (Melo, *et al*, 2017) and the UK (Reynolds *et al*, 2019). 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 have not been detected in the Netherlands (MARAN 2017). In 2017, the first case of a bla_{OXA-48} producing *E. coli,* isolated from a fecal dog sample, was reported (MARAN 2018). The fecal 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 $bla_{OXA-181}$ respectively. Both samples originated from different parts of the Netherlands and were sent to the VMDC for parasitology diagnostics. The monitoring was continued in 2019.

Fecal samples of cats and dogs were obtained through the VMDC. Because the expected prevalence of CPE remains low and reported CPE are frequently multi-resistant, the inclusion criterion for dog fecal samples was antimicrobial treatment of the animal. Since cats are not frequently treated with antimicrobials, no inclusion criterion was defined and available fecal samples from cats submitted to VMDC were included. In 2019, 138 fecal samples from cats and 114 fecal samples from dogs 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 again inoculated 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), blaVIM (Ellington et al, 2007), *bla*OXA-group-23, -24, -51, -58 (Voets et al, 2011) and *bla*OXA-group-48 (Poirel et al, 2004).

No CPE were detected in the screened fecal samples from dogs and cats in 2019.

4.2.3 Monitoring in imported seafood

In 2019, 304 batches of frozen fish and shrimps originating from fish farms in South-East Asia were screened for the presence of CPE. The samples consisted of 102 batches of Pangasius, 99 batches of Tilapia and 103 batches of shrimps. As in previous years, a small number of carbapenemase-producing *Enterobacter cloacae* (*E. cloacae*) complex isolates were detected in batches of frozen shrimps. Two isolates were cultured from frozen shrimps (*Penaeus monodon*) from Vietnam and one isolate was obtained in a batch of frozen shrimps (*Penaeus monodon*) from Bangladesh. Molecular analysis of the isolates revealed chromosomally located bla_{IMI-1} embedded in an insertion element (EcloIMEX) genetically closely related to the earlier described *E. cloacae* complex isolate obtained from Vietnamese shrimps in 2017 (Brouwer *et al*, 2018). In 2018 another bla_{IMI-1} harbouring *E. cloacae* isolate was also found in a batch of Vietnamese shrimps (MARAN 2019).

For the third year in a row, carbapenemase-producing *Enterobacteriaceae* were detected in batches of imported frozen shrimps from South-East Asia. Our findings demonstrate the undesired side effect of the high consumption of antimicrobials in South-East Asia both in humans and in animals, specifically in aquaculture as an environment with a high selective pressure for resistant bacteria, including CPE, and potential for faecal contamination.

4.3 Colistin resistance

In 2019, 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 1209 faecal samples of Dutch livestock were tested with for the presence of *mcr*-1, *mcr*-2, *mcr*-3, *mcr*-4 and *mcr*-5 using in 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*. As a result, *mcr*-1 positive *E. coli* were identified in four faecal samples (0.4%) in veal calves (n=2, 0.7%) and slaughter pigs (n=2, 0.7%).

Noticeably, the presence of *mcr*-4 was identified with PCR in four white veal calf samples. Additional bacterial culturing confirmed the presence of *mcr*-4.6 in *E. coli* in one sample and *mcr*-4.6 coinciding with *mcr*-1 in *Hafnia alvei* in another sample. For the first time since the start of the active screening *mcr* genes were not detected in caecal samples of broilers. Finally, no colistin resistant isolates were identified amongst the randomly selected indicator *E. coli* isolated from faecal samples of livestock.

Colistin resistance was present amongst indicator *E. coli* from turkey meat (13.3%), but for the first time since the start of the monitoring of retail meat, this type of resistance was completely absent amongst indicator *E. coli* from chicken meat. These results indicate a further decline of the prevalence of *mcr* in livestock, particularly in broilers and boiler meat.

4.4 MRSA surveillance in pigs, poultry and humans using Whole genome sequencing

Introduction

Worldwide, MRSA causes hospital- and community-associated infections and asymptomatic carriage in humans. During the last decade, 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, veal calves and poultry. Recently, however, the number of persons colonized or infected with LA-MRSA in The Netherlands who did not have direct contact with livestock, seems to be increasing. A Dutch study found that 15% of persons carrying or infected with LA-MRSA did not report direct contact with pigs, broilers or veal calves (Lekkerkerk *et al.*. 2012). The origin and transmission route of these cases remains unknown. Prolonged carriage of LA-MRSA, can be demonstrated after more than 30 months in persons with and without professional livestock contact (Bosch *et al.* 2015; Meijs *et al.* 2020). Transmission of LA-MRSA between pig veterinarians to their household members occurs frequently (Bosch *et al.* 2015).In addition, Panton–Valentine leukocidin (PVL) positive LA-MRSA is also increasing in humans. PVL is a cytotoxin associated with increased virulence of certain strains of *S. aureus*. Because of these changing features of LA-MRSA found in the human surveillance of MRSA a study was conducted by joined forces of NVWA, RIVM, WFSR and WBVR to investigate LA-MRSA isolates from livestock using Whole Genome Sequencing and compare those strains to human LA-MRSA isolates as an example of a One Health approach in studying AMR. The surveillance started using poultry and pig LA-MRSA isolates originating from nasal/throat swabs from animals, dust samples collected at farms or at the slaughterhouse and from meat at retail, but will be extended to isolates from other livestock in the future.

Methods

A total of 212 animal-derived MRSA isolates were sequenced using Illumina Hiseq (see table 1).

Table MRSA01 Numbers of animal-derived MRSA isolates included in the study

Source	Number included
Pigs	112
Poultry, dust from broiler houses and broiler slaughterhouses	55
Poultry meat	29
Poultry farmers and their family members	16
Total	212

Whole genome MLST and the available wgMLST *S. aureus* scheme comprising 2,567 genes was used. The results were compared to wgMLST of 915 human ST398 MRSA from the national surveillance.

Results and conclusions

All pig isolates belonged to the livestock-associated ST398. Most poultry related isolates (n=87) also belonged to ST398 and related STs, while 13 poultry-related isolates belonged to ST9 (n=11), ST1 (n=1) and ST72 (n=1). Three pig-human LA-MRSA isolate pairs differed by less than 15 genes indicating that these might be epidemiologically related. In one of these pairs the human isolate originated from a pig farmer. The other pig isolates and all poultry-associated ST398 isolates differed by more than 15 genes from the human isolates. There were different clusters of LA-MRSA isolates and one cluster, all PVL-positive isolates, contained human LA-MRSA isolates only. None of the animal isolates carried the *lukF* and *lukS* genes encoding for the PVL-toxin.

References

European Food Safety Authority; Technical specifications on the harmonised monitoring and reporting of antimicrobial resistance in *Salmonella*, *Campylobacter* and indicator *Escherichia coli* and *Enterococcus* spp. bacteria transmitted through food. EFSA Journal 2012; 10(6):2742.

Ceccarelli et al. Diversity of Plasmids and Genes Encoding Resistance to Extended Spectrum Cephalosporins in Commensal Escherichia coli From Dutch Livestock in 2007-2017. Front Microbiol. 2019 Feb 4;10:76. doi: 10.3389/fmicb.2019.00076.

Hesp et al. Monitoring antimicrobial resistance trends in commensal Escherichia coli from livestock, the Netherlands, 1998 to 2016. Euro Surveill. 2019 Jun;24(25). doi: 10.2807/1560-7917.ES.2019.24.25.1800438.

Hordijk et al. Within-farm dynamics of ESBL/AmpC-producing Escherichia coli in veal calves: a longitudinal approach. J Antimicrob Chemother. 2013 Nov;68(11):2468-76. doi: 10.1093/jac/dkt219.

Bradford *et al*; Emergence of Carbapenem-Resistant Klebsiella Species Possessing the Class A Carbapenem-Hydrolyzing KPC-2 and Inhibitor-Resistant TEM-30 β-Lactamases in New York City. Clin Infect Dis. 2004 Jul 1;39(1):55-60

Ellington *et al*; Multiplex PCR for rapid detection of genes encoding acquired metallo-beta-lactamases. J Antimicrob Chemother. 2007 Feb;59(2):321-2

González-Torralba *et al*; Survey of Carbapenemase-Producing *Enterobacteriaceae* in Companion Dogs in Madrid, Spain. Antimicrob Agents Chemother, 2016: 60(4):2499-2501

Brouwer *et al*; *Enterobacter cloacae* Complex Isolated from Shrimps from Vietnam Carrying *bla* $_{IMI-1}$ Resistant to Carbapenems but Not Cephalosporins. Antimicrob Agents Chemother. 2018 Jun 26;62(7). pii: e00398-18. doi: 10.1128/AAC.00398-18

Manchanda *et al*; 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. 2011 Jul-Sep;29(3):249-53

MARAN Reports (2002-2019): https://www.wur.nl/nl/Expertises-Dienstverlening/Onderzoeksinstituten/Bioveterinary-Research/Publicaties/MARAN-Rapporten.htm

Melo *et al*; OXA-48-producing ST372 *Escherichia coli* in a French dog. Antimicrob Chemother. 2017; 72(4):1256-1258

Poirel *et al*; Emergence of oxacillinase-mediated resistance to imipenem in Klebsiella pneumoniae. Antimicrob Agents Chemother. 2004 Jan;48(1):15-22.

Pulss et al; Multispecies and Clonal Dissemination of OXA- 48 Carbapenemase in Enterobacteriaceae from companion animals in Germany, 2009-2016. Front Microbiol. (2018); Jun 14;9:1265.

Reynolds et al, Occurrence and characterization of *Escherichia coli* ST410 co-harbouring *bla*NDM-5, *bla*C-MY-42 and *bla*TEM-190 in a dog from the UK. J Antimicrob Chemother J Antimicrob Chemother (2019). Feb 6. doi: 10.1093/jac/dkz017.

Stolle *et al*; Emergence of OXA-48 carbapenemase-producing *Escherichia coli* and *Klebsiella pneumoniae* in dogs. J Antimicrob Chemother, 2013; 68(12);2802-2808

Voets *et al*; A set of multiplex PCRs for genotypic detection of extended-spectrum β-lactamases, carbapenemases, plasmid-mediated AmpC β-lactamases and OXA β-lactamases. Int J Antimicrob Agents. 2011 Apr;37(4):356-9

Lekkerkerk WS, van de Sande-Bruinsma N, van der Sande MA *et al*. Emergence of MRSA of unknown origin in the Netherlands. *Clin Microbiol Infect* 2012; 18: 656-61.

Bosch T, Verkade E, van Luit M *et al*. Transmission and persistence of livestock-associated methicillin-resistant *Staphylococcus aureus* among veterinarians and their household members. *Appl Environ Microbiol* 2015; **81**: 124-9.

Meijs A, Hengeveld P, Dierikx CM, Maassen CM, de Greeff S, de Haan A, Bosch T, van Duijkeren E. Prolonged carriage of (livestock-associated) MRSA in individuals without professional livestock contact. J Antimicrob Chemother 2020 Mar 3.