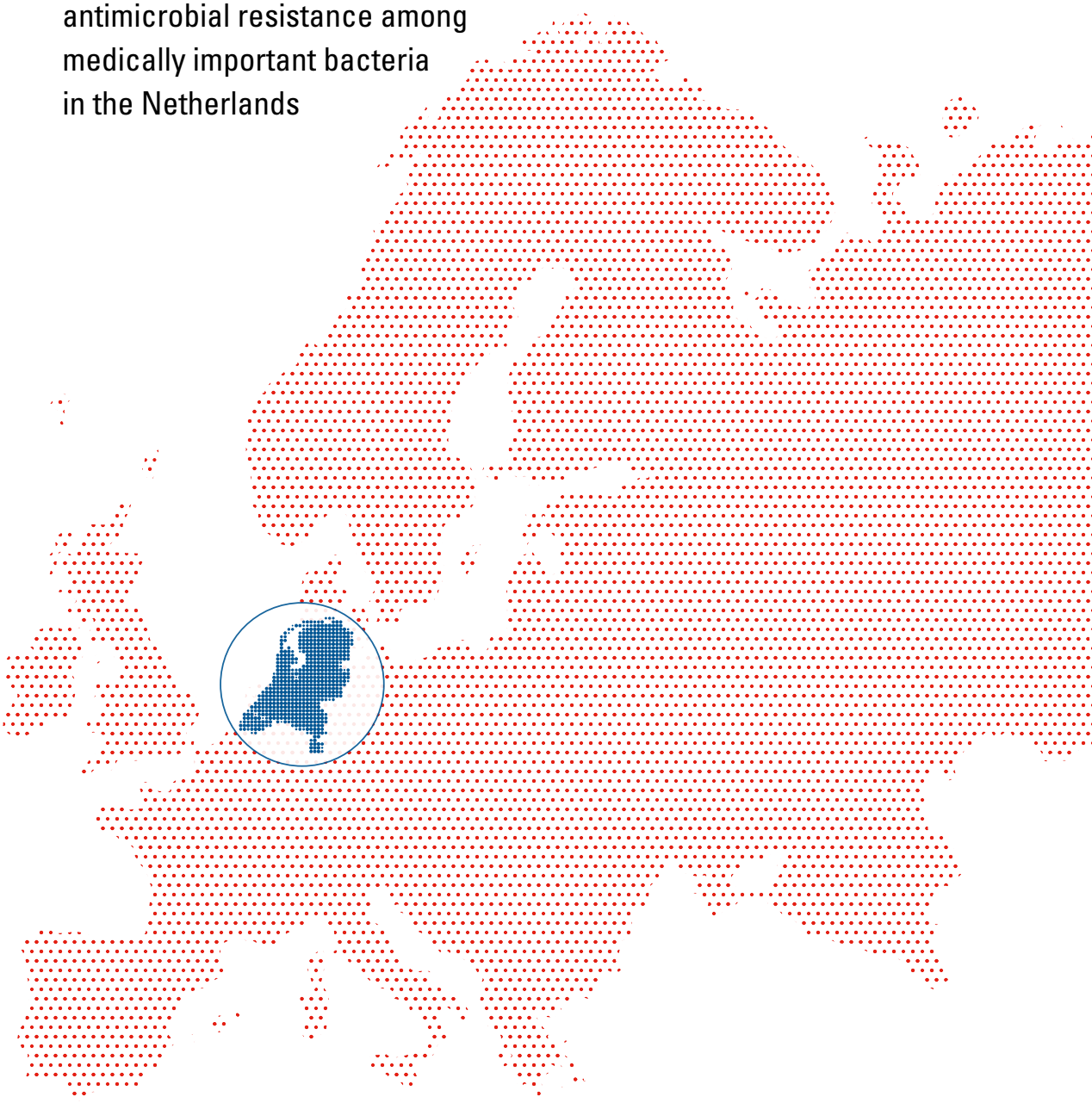
NethMap 2021

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 2021

Consumption of antimicrobial agents and
antimicrobial resistance
among medically important bacteria
in the Netherlands
in 2020

June 2021

Synopsis

NethMap/MARAN-report

The outbreak of Covid 19 (the coronavirus) has put the health care sector in the Netherlands under extreme pressure. Many more people were admitted to intensive care, and the regular care activities were down-scaled. Nevertheless, it does not seem that more bacteria developed resistance to antibiotics in 2020. For some types of bacteria, resistance even seems to have diminished in comparison to previous years. In addition, the number of bacteria that are resistant to various antibiotics at the same time, making it more difficult to treat them, remained the same. The long-term effects of the corona outbreak on antibiotic resistance are not yet clear.

Over the entire world, we are seeing increasing numbers of infections caused by bacteria with resistance to antibiotics. This problem is less severe in the Netherlands than in many other countries. However, due to the global situation, it remains important to be on the alert in the Netherlands. If the problem of resistance does increase, it will then be easier to detect it in time.

To prevent antibiotic resistance from developing, it is important to use antibiotics properly and only when necessary. General practitioners prescribed approximately 10% fewer courses of antibiotics in the past year compared to previous years. Due to the Covid 19 measures, such as social distancing and working from home, many infectious diseases that are spread by social contacts occurred less frequently. In addition, fewer people visited their general practitioner. The total quantity of antibiotics used in hospitals in 2019 remained fairly stable. The data on their use in hospitals in 2020 is not yet available.

The scope of the measures implemented in the Netherlands to combat antibiotic resistance extend further than the health care sector. After all, resistant bacteria also occur in animals, food and in the environment (One Health approach).

Over the last decade, the intestinal bacteria in pigs, cows, and chickens kept for food production (farm animals) have become less resistant. The level of antibiotic resistance in the various animal sectors remained approximately the same in comparison to 2019. ESBL producing intestinal bacteria in broiler chickens and on chicken meat were less prevalent in 2020. In the other animal sectors, the prevalence of these resistant bacteria was the same as in 2019. ESBLs are enzymes that can break down commonly used antibiotics such as penicillins. The quantity of antibiotics sold in 2020 for farm animals increased somewhat compared to 2019. In comparison to 2009, the reference year, the sale of antibiotics decreased by almost 70%. Almost no antibiotics that are crucial for treating infections in humans have been used for farm animals in recent years.

This is shown in the annual report NethMap/MARAN 2021, in which various organisations jointly present data on antibiotic use and resistance in the Netherlands, for both humans and animals.

Keywords:
Antibiotic resistance, bacteria, antibiotic use, infection

Publiekssamenvatting

NethMap/MARAN-rapport

De uitbraak van SARS-CoV-2 (het coronavirus) heeft de gezondheidszorg in Nederland erg belast. Er hebben meer mensen op de IC gelegen en de reguliere zorg is afgeschaald. Toch lijkt het er niet op dat er in 2020 meer bacteriën resistent zijn geworden tegen antibiotica. Bij sommige bacteriesoorten is de resistentie zelfs afgenomen ten opzichte van de jaren ervoor. Ook is het aantal bacteriën dat resistent is tegen verschillende antibiotica tegelijk, waardoor ze moeilijker te behandelen zijn, gelijk gebleven. De effecten van de corona-uitbraak op de antibioticaresistentie op de langere termijn zijn nog niet duidelijk.

Wereldwijd komt het steeds vaker voor dat infecties worden veroorzaakt door bacteriën die resistent zijn tegen antibiotica. In Nederland is dat probleem minder groot dan in veel andere landen. Vanwege de situatie in de wereld blijft het belangrijk om in Nederland waakzaam te blijven. Dan kan het op tijd worden opgemerkt als het resistentieprobleem toeneemt.

Om antibioticaresistentie te voorkomen is het belangrijk om antibiotica op de juiste manier te gebruiken en alleen als het nodig is. Huisartsen schreven het afgelopen jaar in Nederland ongeveer 10 procent minder antibioticakuren voor dan de jaren daarvoor. Door de maatregelen tegen het coronavirus, zoals afstand houden en thuis werken, kwamen veel infectieziekten die van mens op mens overdraagbaar zijn minder vaak voor. Ook gingen er minder mensen naar een huisarts. In ziekenhuizen bleef de totale hoeveelheid gebruikte antibiotica in 2019 ongeveer stabiel. De gegevens over het gebruik in ziekenhuizen in 2020 zijn nog niet bekend.

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

De laatste tien jaar zijn bij varkens, koeien en kippen die voor de voedselproductie worden gehouden (landbouwhuisdieren) de aanwezige darmbacteriën steeds minder resistent geworden. Ten opzichte van 2019 is de antibioticaresistentie in de verschillende diersectoren ongeveer gelijk gebleven. ESBL-producerende darmbacteriën in vleeskuikens en op kippenvlees kwamen in 2020 minder vaak voor. In de andere diersectoren zijn deze resistente bacteriën ongeveer even vaak aangetroffen als in 2019. ESBL zijn enzymen die veelgebruikte antibiotica kunnen afbreken, zoals penicillines. In 2020 zijn voor landbouw-huisdieren iets meer antibiotica verkocht dan in 2019. 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 van cruciaal belang zijn om infecties bij de mens te behandelen.

Dit blijkt uit de jaarlijkse rapportage NethMap/MARAN 2021. Hierin presenteren diverse organisaties samen de gegevens over het antibioticagebruik en -resistentie in Nederland, voor mensen en dieren.

Kernwoorden:
Antibioticaresistentie, bacteriën, antibioticagebruik, infectie

Colophon

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

NethMap can be ordered from the SWAB secretariat, c/o Secretariaat SWAB p/a
Leids Universitair Medisch Centrum (LUMC), afdeling Infectieziekten C5-P t.a.v. SWAB, Postbus 9600 2300 RC Leiden or by email to secretariaat@swab.nl.
NethMap 2021 and earlier versions are also available from the website of the SWAB: www.swab.nl and www.rivm.nl.

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Centres contributing to the surveillance of antibiotic consumption

Alkmaar, NWZ; Almelo en Hengelo, ZGT; Almere, Flevoziekenhuis; Amersfoort, Meander MC; Amsterdam, AMC; Amsterdam, OLVG-oost; Amsterdam, OLVG-west; Amsterdam, VUMC; Apeldoorn en Zutphen, Gelre ziekenhuizen; Arnhem, Rijnstate ziekenhuis; Assen, Wilhelminaziekenhuis; Bergen op Zoom en Roosendaal, Bravis ziekenhuis; Boxmeer, Maasziekenhuis Pantein; Breda, Amphia; Delft, Reinier de Graaf Gasthuis; Den Bosch, JBZ; Den Haag, HAGA ziekenhuis; Den Haag, HMC; Den Helder, NWZ; Deventer, Deventerziekenhuis; Dordrecht, Albert Schweizerziekenhuis; Ede, Gelderse Vallei; Eindhoven, Maxima MC; Emmen, TREANT; Geldrop, St Anna; Gouda, Groene Hart ziekenhuis; Groningen, Martini; Groningen, Ommelander; Groningen, UMCG; Haarlem, Spaarne Gasthuis; Hardenberg, Ropcke Zweers; Harderwijk, St Jansdal; Heerenveen, Tjongerschans; Heerlen en Sittard, Zuyderland ziekenhuis; Hilversum, Tergooi; Leeuwarden, MCL; Leiden, LUMC; Maastricht, MUMC; Meppel, Isala klinieken; Nieuwegein, St Antonius ziekenhuis; Nijmegen, CWZ; Nijmegen, Radboudumc; Roermond, Laurentius ziekenhuis; Rotterdam, Erasmus MC; Rotterdam, Franciscus Vlietland; Rotterdam, Ikazia ziekenhuis; Rotterdam, Maasstad ziekenhuis; Schiedam, Franciscus Vlietland; Terneuzen, ZorgSaam; Tilburg, ETZ; Utrecht, Diakonessenhuis; Utrecht, UMCU; Weert, St Jans Gasthuis; Winterswijk, SKB; Zeeland, ADRZ; Zoetermeer, Lange Land ziekenhuis; Zwolle, Isala klinieken.

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

Alkmaar, Noordwest Ziekenhuisgroep; Amersfoort, Meander MC; Amsterdam, Amsterdam UMC; Amsterdam, Atalmedial; Amsterdam, OLVG / Almere; Amsterdam, Streeklaboratorium Amsterdam – GGD; Apeldoorn, Gelre Ziekenhuizen; 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; 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 2021, the SWAB/RIVM report on the use of antibiotics, trends in antimicrobial resistance and antimicrobial stewardship programmes in the Netherlands in 2020 and previous years. NethMap is a cooperative effort of the Dutch Working Group on Antibiotic Policy (SWAB; Stichting Werkgroep Antibiotica Beleid) and the Centre for Infectious Disease Control Netherlands (CIb) at the National Institute for Public Health and the Environment (RIVM). NethMap is issued back-to-back together with MARAN, reporting on trends in antimicrobial resistance and antimicrobial use in animal husbandry.

In 1996, SWAB was founded as an initiative of The Netherlands Society for Infectious Diseases, The Netherlands Society of Hospital Pharmacists and The Netherlands Society for Medical Microbiology. SWAB is fully funded by a structural grant from the CIb, on behalf of the Ministry of Health, Welfare and Sports. The major aim of the SWAB is to contribute to the containment of the development of antimicrobial resistance and provide guidelines for optimal use of antibiotics, taking into account resistance surveillance data. Based on the national AMR surveillance system (ISIS-AR) performed by the CIb-RIVM, trends in antimicrobial resistance are monitored using routine antibiotic susceptibility testing data from microbiology laboratories in the Netherlands. Furthermore, the CIb subsidizes 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 2021 extends and updates the information of the annual reports since 2003. Each year, we try to further improve and highlight the most important trends. The appearance of highly resistant microorganisms (HRMOs) receives attention in 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 sixth consecutive year.

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

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

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

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2
Extensive summary

This chapter provides a summary of the findings described in this report and relevant conclusions with respect to antimicrobial use, policy and resistance surveillance in both humans (NethMap 2021) and the veterinary sector (MARAN 2021).
Without any doubt, the COVID-19 epidemic has had an enormous impact on the Dutch healthcare system. As a consequence, also many of the data presented in this edition of NethMap will be influenced by this epidemic.

2.1 Most important trends in antimicrobial use

In outpatients

- In 2020 COVID-19 has had a major impact on antibiotic use in outpatients.
- Total systemic antibiotic use in outpatients decreased by 10.5% from 8.68 DDD/1,000 inhabitant days (DID) in 2019 to 7.77 DID in 2020.
- There was a major decrease in use of penicillins with an extended spectrum from 1.26 DID to 0.98 DID, mainly driven by decreased amoxicillin use.

In hospitals

- The inpatient use of antibiotics in 2019 decreased from 81.0 to 79.3 when expressed as DDD/100 patient-days and increased from 303.2 to 318.5 when expressed as DDD/100 admissions. Total use of antibiotics for systemic use, calculated as DDD/1,000 inhabitant-days, decreased with 4.4%, from 0.84 in 2018 to 0.80 in 2019.
- The use of combinations of penicillins (co-amoxiclav, piperacillin/tazobactam) decreased markedly, from 12.0 to 10.1 DDD/100 patient-days.
- The use of fluoroquinolones further decreased from 7.7 to 7.0 DDD/100 patient-days, mainly due to an ongoing reduction in the use of ciprofloxacin.
- The use of third-generation cephalosporins increased with 0.8 DDD/100 patient-days, to 7.7 DDD/100 patient-days.
- Vancomycin use increased from 1.6 to 1.8 DDD/100 patient-days.

- There are large differences in total antibiotic drug use between Dutch hospitals (range 50-111 DDD/100 patient-days). General hospitals used the least antibiotics (71.9 DDD/100 patient-days), whereas large teaching hospitals reported the highest overall antibiotics use (82.6 DDD/100 patient-days).
- Since 2017, the use of antimycobacterials was higher than in the 7 years before, but in 2019 the use decreased from 5.2 to 4.5 DDD/100 patient-days.
- The use of antimycotics for systemic use has decreased from 13.3 in 2018 to 11.4 DDD/100 patient-days in 2019.
- Antibiotic use expressed as days of therapy (DOT)/100 patient-days informs on patient level exposure to antibiotics. Total inpatient use of antibiotics decreased from 64.4 in 2018 to 58.6 DOT/100 patient-days (-8.9%) in 2019. The use for all groups of antibiotics decreased, except for the third generation cephalosporins and the glycopeptides.

In long-term care facilities

- The mean use of antibiotics in long-term care facilities (LTCF) varies from year to year. In 2019, the mean of total systemic antibiotic use decreased from 53.9 in 2018 to 50.4 DDD/1,000 residents/day (range 25.5-142.5 DDD/1,000 residents/day) in 2019.
- Antimicrobial use varied highly between the different LTCF with a minimum of 21.4 and a maximum of 109.3 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 2021.

Table 2.2.1 Overview of antimicrobial resistance surveillance programs included in NethMap 2021

Surveillance program	Origin of isolates	Availability	Sources 2020	Central or decentral susceptibility testing	Method of susceptibility testing
Surveillance program aimed at resistance surveillance in a wide range of pathogens					
ISIS-AR	GP, Hospital, LTCF	2008-	46 laboratories	Decentral testing	Various methods used in routine susceptibility testing
Surveillance programs aimed at resistance surveillance in specific pathogens					
Neisseria meningitidis	Hospital	1994-	Nationwide	Central testing	Gradient testing
Neisseria gonorrhoeae	SHC	2006-	14 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, LTCF, hospital	2005-	NIVEL GP sentinels, SNIV LTCF sentinels, hospital/regional laboratories	central testing (RIVM, NIC-ErasmusMC, WHO-CC London)	Sanger sequencing, whole genome NGS, or site-specific PCR; Neuraminidase enzyme inhibition assay
Resistance among anaerobic pathogens	Hospital	2010-	1 lab	Central testing	Gradient testing
Clostridium difficile	Hospital, LTCF	2005-	21 hospitals	(de)central testing	Agar dilution testing and PCR
Azole resistance in *Aspergillus fumigatus*	Hospital	2011-	5 University hospitals + 5 teaching hospitals	Central testing	EUCAST microbroth dilution methodology
MRSA	GP, hospital, LTCF	2008-	Nationwide	Central testing	MLVA, NGS
CPE	GP, hospital, LTCF	2011-	Nationwide	Central testing	Gradient testing, Carba-PCR, NGS
CPPA	GP, hospital, LTCF	2016-	Nationwide	Central testing	Gradient testing, multiplex PCR

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

In GPs

- For most antimicrobials, there are no statistically significant and clinically relevant shifts in resistance levels since 2016.
- For isolates from urine cultures a distinction is made for patients aged below and above 12 years of age in accordance with age categories used in the urinary tract infection guidelines of the Dutch College of General Practitioners (NHG). In general, resistance 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 2016, 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 to 2018, there was no further increase in resistance to co-amoxiclav.
- Compared to 2016, the increase in ceftazidime resistance was statistically significant and clinically relevant in *K. pneumoniae* from patients aged ≤12 years (from 0% to 5%).
- Extended spectrum beta-lactamase (ESBL) production percentages in *E. coli* and *K. pneumoniae* are stable and low: 3% for *E. coli* and 4% for *K. pneumoniae* in 2020.
- Ciprofloxacin resistance percentages in *E. coli*, *P. mirabilis* and *K. pneumoniae* for both age groups were stable or even decreasing in both age groups, around 5% for patients aged ≤12 years for the 3 pathogens, and around 10% for the 3 pathogens in patient aged >12 years. For *K. pneumoniae* in patients aged >12 years, there is a decreasing trend from 14% in 2017 to 12% in 2020.
- Resistance to trimethoprim and co-trimoxazole in *E. coli* and *K. pneumoniae* show a tendency to decrease in the last 5 years. Compared to 2016, the decrease was significant and clinically relevant for *K. pneumoniae* in patients aged >12 years. In 2020, it is below 20% for co-trimoxazole in *E. coli*, and is around 7% for *K. pneumoniae* in both age-groups. In *P. mirabilis* resistance to trimethoprim and co-trimoxazole is higher, above 25% for trimethoprim, and above 20% for co-trimoxazole.
- The percentage of HRMO and multidrug resistance was ≤5% in all *Enterobacterales*, with the exception of *K. pneumoniae* in patients ≤12 years, most likely caused by the high level of resistance to co-amoxiclav.
- In patients above 12 years of age, resistance of *P. aeruginosa* to ciprofloxacin is stable and around 10%.
- In *S. aureus* resistance is generally low, with the exception of resistance to fusidic acid (18%), and (inducible) resistance to clindamycin (11%) and macrolides (13%).
- For *E. coli*, *K. pneumoniae*, and *S. aureus* resistance percentages are shown per region, these indicate that there are only minor differences in susceptibility between regions in the Netherlands.
- For both β-haemolytic *Streptococcus* spp. group A and group B, resistance for doxycycline was 20% or higher. There was a statistically significant and clinically relevant increase in resistance to clindamycin including inducible resistance (from 5% in 2016 to 8% in 2020) in β-haemolytic *Streptococcus* spp. group A.

In hospitals

- Compared to 2016, overall resistance rates for almost all antimicrobials in *Enterobacterales* were similar.
- In *P. aeruginosa* resistance rates are stable for the last 5 years.
- In all hospital departments, compared to 2016 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 to 2018, there was no further increase in resistance to co-amoxiclav.
- The overall rise of resistance in *K. pneumoniae* appears to have stopped. Resistance to all antimicrobials decreased in 2020, with the exception of piperacillin-tazobactam, which has risen increased significantly and clinically relevant, in all hospital departments, in intensive care units this resistance to piperacillin-

tazobactam has reached 13% in 2020.
- When compared to other hospital departments, *Enterobacterales* isolates of urology patients (inpatient and outpatient) have higher levels of ciprofloxacin resistance. The highest level of resistance to ciprofloxacin is in *E. coli* of urology inpatients (25%).
- In 2020, ESBL production in *E. coli* and *K. pneumoniae* was stable, when compared to the previous years. For *K. pneumoniae* in patients on the intensive care unit, ESBL production increased to 15% in 2020, compared to 12% in 2019. The reason for this unexpected rise is unknown, and the number of strains involved are low.
- HRMO and multidrug resistance in *Enterobacterales* and *P. aeruginosa* are stable over the last years and are below 10%. Only in *K. pneumoniae* of inpatients it is slightly higher (~10%).
- Resistance to empiric therapy combinations was ≤10% 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.
- Antimicrobial resistance in the anaerobic pathogens *B. fragilis* complex and *C. perfringens* is low, with the exception of clindamycin resistance in *B. fragilis* complex of 15%. Clindamycin resistance in *C. perfringens* is decreasing over the past 5 years from 16% (2016) to 9% in 2020.
- In intensive care units resistance in all Gram-negative species is stable, with the exception of co-amoxiclav and piperacillin-tazobactam (as mentioned before).
- The percentage HRMO in intensive care units was 10% for *E. coli* and 16% for *K. pneumoniae*.
- In intensive care units, resistance of *P. aeruginosa* was stable. Ciprofloxacin resistance decreased in 2020 to 10% (2019: 14%)
- Resistance levels of 20% or higher were observed for doxycycline in β-haemolytic *Streptococcus* spp. groups B and G (≥31%).

Specific pathogens and situations

- Vancomycin resistance (VRE) in infection related isolates of *E. faecium* remains very low, around 0.4%. In addition, the number of outbreaks with VRE reported to SO-ZI/AMR was low, with only 5 outbreaks reported in 2020, compared to 19 in 2019.
- In *S. pneumoniae*, the percentages of R and I+R results for (benzyl)penicillin are low, ≤8% in GP patients and hospital patients.
- In *H. influenzae* isolates a statistically significant and clinically relevant increase in resistance was observed for amoxicillin/ampicillin in hospital patients (from 31% in 2016 to 36% in 2020), and for co-amoxiclav in both GP patients (from 12% to 17%) and hospital patients (from 10% to 12%).
- MRSA prevalence in diagnostic samples is 2% and remained stable over the past 5 years. The MRSA prevalence in blood culture isolates remained low, at 2%.
- PVL positivity in MRSA increased over the years and is now 28%. In diagnostic isolates it is 42%, in screening isolates it is 21%.
- Remarkably: in 2020, PVL-genes were present in 8% of LA-MRSA isolates (MC0398), 75% (33/44) of these PVL-positive isolates had MLVA-type MT0569.
- In gonococci, no resistance to ceftriaxone, the current first-line treatment was found. However, MIC values of ceftriaxone, when compared to previous years, are higher in 2019 and 2020. This trend needs close surveillance. Resistance to ciprofloxacin more than doubled since 2016, to 57.1% in 2020. Resistance to azithromycin is stabilizing the last years and is 10% in 2020
- Data on antimicrobial susceptibility of anaerobic bacteria (with the exception of *B. fragilis* and *C. perfringens*)

is limited. To gain more insight in resistance in anaerobic bacteria a more extensive surveillance program is being initiated.

- In 2020, we added data for *H. pylori* to NethMap. Resistance is high for levofloxacin (29%), clarithromycin (53%), and metronidazole (48%), and low for amoxicillin (6%) and tetracycline (2%). Over the years, an increasing trend in resistance is seen for most antimicrobial agents.
- Azole resistance frequency in 2020 was 11.8% in UMCs and 4.7% in teaching hospitals, which was lower than in 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 2020, and has remained stable over the past five years. The overall percentage of confirmed non-susceptible *E. coli* and *K. pneumoniae* is still very low (0.05% in *E. coli* and 0.33% in *K. pneumoniae*).
- In 2020, 204 unique carbapenemase-producing *Enterobacterales* isolates were obtained from 180 persons (mean age 61 years and 56% male) This sharp decrease in submitted strains is due to the COVID-19 epidemic. In 2019, these numbers were 363 unique carbapenemase-producing *Enterobacterales* isolates from 316 persons.
- In 2020, 49/204 (24%) of carbapenemase-producing *Enterobacterales* had an MIC for meropenem above the cut-off of 8 mg/L.
- Targeted or routine screening is the reason for sampling in 71% of CPE-positive persons. In 33% there is a relation with hospitalization abroad for more than 24 hours during the last two months, making this the main risk factor for CPE in the Netherlands.
- In 2020, 720/14 348 (5%) of *P. aeruginosa* in diagnostic isolates were phenotypically resistant to carbapenems. Only 1% of the *P. aeruginosa* isolates was MDR and 56% of these MDR isolates were carbapenem-resistant.
- In 36 of 182 (20%) submitted *P. aeruginosa* isolates a carbapenemase was produced.
- The most predominant (50%) carbapenemase-encoding allele in carbapenemase-producing *P. aeruginosa* was *bla*VIM-2.
- In 2020, 34 outbreaks, including 7 by SARS-COV-2, were reported to the Early warning and response meeting of Healthcare associated Infections and AntiMicrobial Resistance (SO-ZI/AMR). Most outbreaks (18; 11 in LTCF) were caused by MRSA. There were no outbreaks with carbapenemase-producing bacteria reported in 2020. The risk of an outbreak for public health was estimated as low for all outbreaks in 2020, since all outbreaks were classified as phase 1.

2.3 Antibiotic use and resistance in animals and resistance in human foodborne pathogens

Antimicrobial use

- In 2020 in total 154 tonnes of antimicrobial veterinary medicinal products (AVMPs) were sold, which is an increase of 2% compared to 2019 and which resulted in a slight bounce back in attaining the governmental 70% reduction goal. A decrease in sales by 69.0 % over the years 2009-2020 is attained (with 2009 considered a reference year by the Dutch Government).
- Antimicrobial use (AMU) based on prescription data stabilised in most animal sectors in 2020 except in veal calves in which the use continued to decrease. In rabbits in 2020 an increase in use was observed, while in turkeys in 2020 use decreased substantially.
- 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 2020, while sales decreased.

Antimicrobial resistance

- Overall, the highest resistance proportions in *Salmonella* were again observed for (in decreasing order) sulfamethoxazole (24.4% in 2019 to 26.3% in 2020), tetracycline (25.5% in 2019 to 25.4 in 2020), ampicillin (24.8% in 2019 to 21.7% in 2020), nalidixic acid (16,7% in 2019 to 16.4% in 2020), ciprofloxacin (17.0% in 2019 to 16.0% in 2020), trimethoprim (10.7% in 2019 to 12% in 2020) and chloramphenicol (7.1% in 2019 to 6.7% in 2020).
- Among the most frequently isolated *Salmonella* serovars, those showing the highest resistance levels, were *S.* Infantis, *S.* Paratyphi B var. Java, the (monophasic) *S.* Typhimurium variants 4,12:i:- and 1,4,[5],12:i:-, and *S.* Typhimurium.
- Resistance to fluoroquinolone increased significantly among *S.* Infantis (to 63%) but decreased for *S.* Typhimurium and *S.* Enteritidis.
- In total, 6 (0.5%) ESBL suspected *Salmonella* isolates were detected among six different serovars, with 4 isolates from humans and 2 non-human isolates of unknown origin.
- Resistance proportions in *Campylobacter jejuni* isolates from caecal samples of broilers and meat thereof stabilized at a high level for quinolones and tetracycline. Resistance to macrolides was not detected in *C. jejuni* isolates from broilers and poultry meat, and was at low levels in *C. coli* isolates from broilers and poultry meat.
- In humans, resistance proportions were higher in *C. coli* than in *C. jejuni* isolates, but were overall lower in 2020 compared to previous years. This is most likely due to a substantial reduction of travel-related campylobacteriosis as a result of the COVID-19 lockdown, which is associated with higher resistance proportions than domestically acquired campylobacteriosis.
- Ciprofloxacin resistance in *Campylobacter* isolates from humans was high again in 2020, which is a concern for public health. It was, however, lower compared to 2017-2019.
- Resistance to erythromycin, representative for macrolides being first choice antibiotics in human medicine for campylobacteriosis, remained low.
- In Shiga toxin-producing *E. coli* (STEC) O157 a tendency of increasing resistance was observed until 2017 and fluctuates on a lower level since 2018.
- Proportions of resistance were higher in human STEC/aEPEC non-O157 than in STEC O157.
- Resistance to the quinolones (ciprofloxacin and nalidixic acid) was very low in both STEC O157 and

STEC/aEPEC non-O157 human isolates in 2020.

- No ESBL-producing isolates were detected in STEC O157, but one O104 isolate was confirmed as ESBL-producer carrying $bla_{CTX\text{-}M\text{-}15}$.
- Almost all STEC O146 isolates- associated with small ruminants as main reservoir- were pan-susceptible.
- 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.
- In slaughter pigs, resistance in indicator *E. coli* decreased to the lowest levels in fifteen years. In contrast, in broilers and veal calves increasing resistance was observed compared to 2019. In dairy cattle resistance fluctuates at a traditional low level. However, over the last decade decreasing trends in resistance were observed for all animal sectors involved in the monitoring.
- In meat, resistance proportions were highest in *E. coli* from turkey meat followed by broiler meat. Lower resistance proportions were found in *E. coli* from pork and beef.
- Resistance to fluoroquinolones was still commonly present in indicator *E. coli* from caecal samples of broilers and meat thereof.
- In 2020, the sample prevalence of ESBL/AmpC-producing *E. coli* (determined with a selective method) remains highest in veal calves contrary to the continuous low levels observed in pigs and dairy cattle. The prevalence of ESBL/AmpC-producing *E. coli* from broilers and chicken meat further reduced below 10% which can be considered a great success of the measures on reducing antimicrobial use initiated since 2011.
- As in former years, prevalence of mcr-1 was low in livestock and meat and no carbapenemase-producing *Enterobacteriaceae* were detected in livestock and companion animals.
- Prevalence of LA-MRSA was high in dust samples from pig farms (76%), but could not be detected in dust samples from broiler farms. At retail, MRSA was detected in < 10% of the pork and bovine meat, but in almost 20% of the poultry meat (both chicken and turkey).
- The first *cfr*-positive LA-MRSA isolates were detected in dust samples from one pig farm obtained in 2019 as well as in five human LA-MRSA isolates in 2018 – 2020. The first findings of this multi-resistance encoding gene in MRSA from humans and pigs demonstrated the importance of AMR monitoring from a One Health perspective.

2.4 Implications for therapy

Over the last years, resistance rates in the Netherlands are stable. As in 2019, the resistance rates in 2020 did not increase for most antibiotics and for many antibiotics there has even been a further 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 caution.
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 LTCF 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 is stable and low (≤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* is high, and its usefulness in the treatment of urinary tract infection in some patient categories is becoming more and more limited.
- Treatment of complicated urinary tract infection in general practice with oral antibiotics is complicated by the relatively high resistance rates to co-amoxiclav, co-trimoxazole and ciprofloxacin. Urinary culture is often necessary to guide therapy.
- Ciprofloxacin resistance in patients >12 years of age is stable in *Enterobacterales* and is around 10% for *E. coli* and *P. mirabilis*, and 10-15% for *Klebsiella pneumoniae.* In *P. aeruginosa* it is 10%. 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 for those infections possibly caused by *S. aureus*, such as skin and soft tissue infections. In ß-haemolytic *Streptococcus* spp. group A clindamycin (inducible) resistance is 8% now and rising.
- Resistance percentages are available per region, and 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

- 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.
- Therapy for patients with positive blood cultures can be optimized in shorter time due to direct and rapid susceptibility testing of positive blood cultures (results available after 4 to 8 hours of incubation), which has now been approved by EUCAST.
- Local resistance levels in hospitals and even hospital wards vary significantly, including from time to time. Tailored therapy and culture remain the mainstay of therapy.

Outpatient departments

- The levels of resistance in *Enterobacterales* limit the chance of success of empirical treatment with oral agents for complicated UTI; culture, antibiograms and tailored therapy are mostly necessary for successful treatment.
- Resistance levels are stable in all Gram-negative species. The rise in resistance of *K. pneumoniae* to many antimicrobial agents seen in the previous years has stopped in 2019 and 2020, with the exception of piperacillin-tazobactam, for which a worrying increase in resistance is seen.
- HRMO and multidrug resistance in *Enterobacterales* has stabilised in 2020.
- Clindamycin (inducible) resistance and resistance to macrolides in *S. aureus* rises every year, and is now almost 15%, which limits its usefulness in empiric therapy for e.g. skin and soft tissue infections.

Unselected hospital patient departments

- The rise of resistance in *K. pneumoniae* appears to have stopped. Nevertheless, patients with an infection with *K. pneumoniae* have a considerate risk of non-adequate empiric treatment, especially in those infections for which antibiotic monotherapy is prescribed.
- 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 percentage resistance in 2020 for *E. coli* is 34% and in *K. pneumoniae* it is 22%. This renders the drug unsuitable for empiric therapy for any infection potentially caused by Gram-negative bacteria, unless it is combined with a second drug, for instance an aminoglycoside.
- For *P. aeruginosa* resistance is relatively low and stable for all antibiotics.
- 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* complex and *C. perfringens* is low, with the exception of clindamycin resistance in *B. fragilis* complex of 14%, limiting its use as part of empiric therapy in infections of the gastro-intestinal tract.

Intensive care units

- Resistance in all species of *Enterobacterales* is stable for most antimicrobial agents in 2020.
- 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 percentage HRMO in *K. pneumoniae* was 16% in 2020 and

the percentage of ESBL in diagnostic cultures from ICU is 15% which should be taken into account when prescribing empiric therapy in this setting.

Specific pathogens and situations

- Carbapenemase-production in *Enterobacterales* and in *P. aeruginosa* isolates is rare, and risk of infection caused by or carriage of these specific pathogens is closely monitored.
- ESBL-producing *Enterobacterales* are of special concern, and treatment is often difficult, with few options remaining. More research and a national molecular surveillance could be helpful to monitor the situation in Dutch patients.
- The increase in resistance to amoxicillin or co-amoxiclav of *H. influenzae* isolates (mostly isolates from the respiratory tract) makes these antimicrobials less useful for therapy.
- Resistance in *H. pylori* is high, and treatment failures are expected to be more common. Therapy after failure should be guided by culture and susceptibility testing.

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:

- Nurses are increasingly involved in antimicrobial stewardship in hospitals.
- Analysis of data on the quality of antibiotic use and its determinants shows room for improvement.
- Barriers lie at the level of data acquisition and analysis. In terms of solutions, A-teams ask not only more IT support, benchmarked feedback data, but also for quality indicators for the functioning of A-teams.
- The management of community-acquired pneumonia can be improved. The empirical therapy is too broad, iv-oral switch is probably too infrequent and the treatment duration too long.

2.6 Implications for public health and health policy

In 2020, the world has faced the start of the outbreak of COVID-19, which was declared a Public Health Emergency of International Concern on 30 January 2020 by the World Health Organization, and a pandemic from 11 March 2020 onwards.
The COVID-19 pandemic has led to a huge amount of hospitalizations and intensive care admissions. The treatment of and care for these patients, and the downscaling of regular care, may have affected trends in antibiotic use and the occurrence of healthcare associated infections. Moreover, the increased hygiene precautions and control measurements may have affected transmission of micro-organisms in general. The effects of the COVID-19 pandemic can be noticed in the various AMR surveillance systems. Still, the interpretation of the data is complicated by the wide variety of changes that took place during the pandemic. For example, the number of HRMO outbreaks in healthcare institutes almost halved in 2020 compared to previous years, which probably results from increased infection prevention measures. Although the numbers of medical microbiological laboratories reporting their data to the national surveillance system of antimicrobial resistance (ISIS-AR) did not change compared to previous years, the absolute number of isolates per month was obviously lower during the first COVID-19 wave compared to the period before and after, as a result of the alterations in the patient population in hospitals and at the GPs. The absolute numbers of carbapenem-resistant *Enterobacterales* submitted to the national surveillance system Type-Ned decreased with almost 50% compared to 2019, which is most likely the result of reduced travel and a reduction in regular health care. The outpatient use of systemic antibiotics decreased by 10%, which is encouraging, but possibly changes in healthcare delivery due to COVID-19 have played a role here as well.

In the meanwhile, antibiotic resistance continues to be a serious threat to public health worldwide and in Europe, leading to increased healthcare costs, prolonged hospital stays, treatment failures and sometimes death. Data from the European Antimicrobial Resistance Surveillance Network (EARS-Net) show that in Europe in 2019 wide variations in the occurrence of antimicrobial resistance across the EU/EEA exist. Although in many countries in Europe MRSA percentages among *S. aureus* isolates decline, MRSA remains an important pathogen in the EU/EEA, as the levels of MRSA were still high in several countries, and combined resistance to other antimicrobial groups was common.
The global rise of carbapenem-resistant *Enterobacterales* (CRE) is alarming and represents an increasing threat to healthcare delivery and patient safety. Carbapenem resistance in *E. coli* slightly increased, but remained rare (0.3%) in 2019. 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. The distribution of colistin resistance is difficult to assess through EARS-Net, since colistin susceptibility testing is methodologically challenging, and in many countries, colistin susceptibility testing is generally not part of the initial routine susceptibility testing for *Enterobacterales*. Combined resistance to different antimicrobial groups is also high for *K. pneumoniae*, with 28.6% of the isolates reported to EARS-Net for 2019 being resistant to at least two of the surveyed antimicrobial groups (fluoroquinolones, aminoglycosides, third-generation cephalosporines, carbapenems). Combined resistance to fluoroquinolones, third-generation cephalosporins and aminoglycosides was 19.3%. In *E. coli*, combined resistance to these three antibiotic groups was lower with a percentage of 5.9%

in 2019, although resistance to third-generation cephalosporins was still high at 15.1% and to fluoroquinolones 23.8%.
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* in 2020 was low (0.05% and 0.33%) 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 February 2021, the Minister provided an update on the progress made and decided to continue the current program and policy.[2] Five goals for the coming years were defined:
1) Promoting and improving a high quality of antimicrobial use both for humans and animals, 2) To slow down the emergence of new resistant microorganisms, by investing in dedicated research, 3) To prevent transmission of highly resistant microorganisms between patients within and outside healthcare centres, and the environment and livestock sectors, 4) To decrease the number of healthcare-associated infections caused by HRMO and to decrease the number of outbreaks in healthcare institutes by surveillance and adequate infection prevention, 5) To intensify international cooperation on this subject. The scope of the program was broadened from antibiotic resistance to antimicrobial resistance which includes resistance against antifungal therapy and against antiviral therapy as well.
In 2019, the ten Regional Cooperative Networks concerning antimicrobial resistance became fully operative. 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. The project "Eenheid van Taal – Antimicrobial Resistance" aims to implement standardized communication of microbiological, clinical and epidemiological data between stakeholders. 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 and discussion

The data presented in NethMap/MARAN 2021 demonstrate that ongoing attention is needed to combat antibiotic resistance and optimize antimicrobial use in humans and animals. In 2020, the COVID-19 pandemic has had a major impact on healthcare systems and its effects can be noticed in the various AMR surveillance systems. It is very positive to see that, in spite of the ongoing crisis, all surveillance systems continued to work properly and that data were available for the indicators described in this report, comparable to earlier years. Still, the interpretation of the data is complicated by the wide variety of changes that took place during the pandemic. It remains to be seen what will be the long-term impact of COVID-19 on the prevalence of AMR in the Netherlands and worldwide. Extra vigilance and analyses of data are needed in the coming period when the COVID-19 pandemic is declining.

[1] https://www.rijksoverheid.nl/documenten/kamerstukken/2015/06/24/kamerbrief-over-aanpak-antibioticaresistentie
[2] https://www.rijksoverheid.nl/documenten/kamerstukken/2021/02/09/kamerbrief-over-voortgang-aanpak-antibioticaresistentie

For now, it is encouraging to see that use of antimicrobials in humans is stable and antimicrobial resistance is not rising and sometimes even going down in many important species. The total use of antimicrobials in animals has decreased with almost 70% compared to 2009 and this was reflected in the reduction of the level of resistance in 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 still missing at the moment, and surveillance of resistance in anaerobic bacteria is limited, although an intensified survey in 8 laboratories on 2 anaerobic bacterial species will be performed in 2021.

3 Use of antimicrobials

3.1 Outpatient antibiotic use

Methods

Data on outpatient antibiotic use in the Netherlands over 2020 was obtained from the SFK (Foundation for Pharmaceutical Statistics, the Hague) and is expressed in Defined Daily Doses (DDD) for each ATC-5 code. The SFK collects dispensing data from 90% of the Dutch community pharmacies (serving 93% of the Dutch population) and extrapolates the data to 100%. These data include prescriptions from general practitioners, as well as prescriptions from outpatient clinics and dentists. Data is presented as DDD per 1,000 inhabitants per day (DID). In 2019, two major changes in DDD were implemented by the World Health Organisation (WHO): for penicillins with extended spectrum and penicillins with beta-lactamase inhibitors.[1] The data from 2019 and 2020 were processed using these new DDD definitions. To enable comparison of the 2020 and 2019 data with 2018, the data from 2018 are presented as they were in 2018, as well as using the 2019 DDD definitions.

Results

Total outpatient use of systemic antibiotics decreased by 10.5%, from 8.68 DID in 2019 to 7.77 DID in 2020 (table 3.1.1). Decreases in antibiotic use were particularly seen in antibiotics used for respiratory tract infections: penicillins with extended spectrum, tetracyclines and macrolides. The decrease in penicillins with extended spectrum with 0.28 DID to 0.98 DID was mainly 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 0.64 DID (figure 3.1.1), which was mainly driven by a decrease in ciprofloxacin use (figure 3.1.2C). The use of nitrofurantoin started decreasing in 2019 and an even larger decrease was seen in 2020.

Discussion

Total outpatient antibiotic use in the Netherlands substantially decreased in 2020. The year 2020 is characterized by the COVID-19 pandemic, which started in March. Antibiotic use was heavily influenced by the context of the pandemic. This pandemic of a viral illness with measures as social distancing, school closure and working from home has decreased other respiratory tract infections and altered help seeking behavior for infectious diseases at GP practices. Despite initial messages of a potential benefit of macrolides for COVID-19, a decrease in macrolides use was seen.

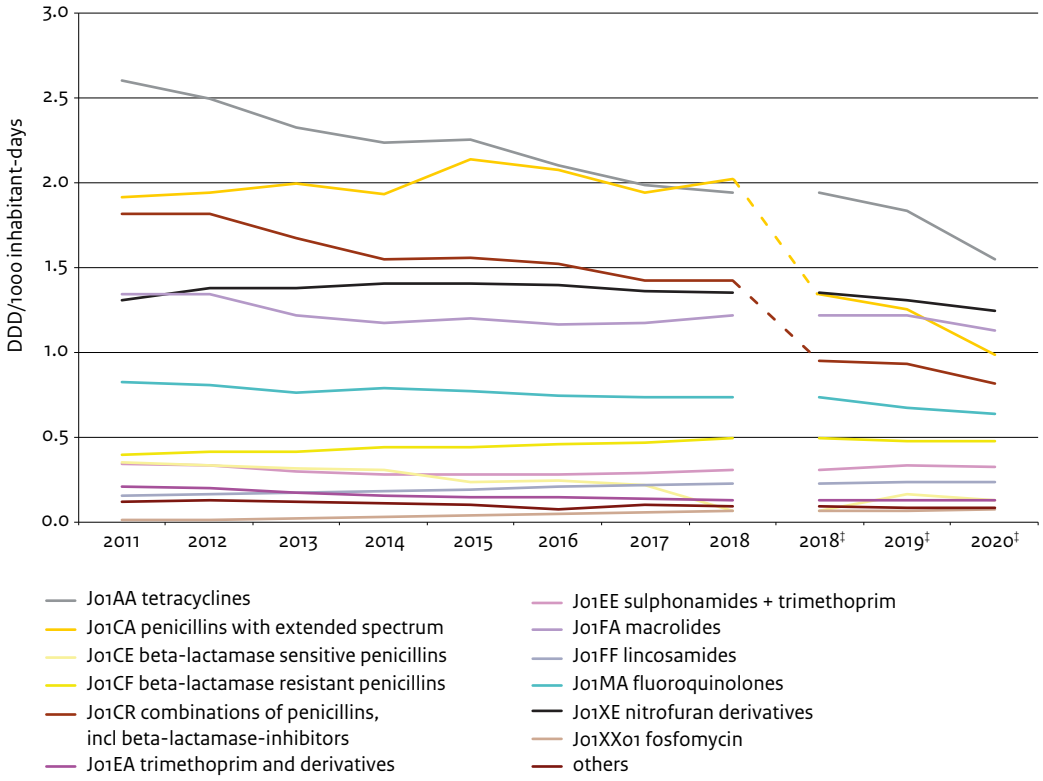
Table 3.1.1. Ten years data on the use of antibiotics for systemic use (J01) in outpatients (DDD/1,000 inhabitant-days), 2011-2020 (source: SFK)

ATC Group*	Therapeutic group	2011	2012	2013	2014	2015	2016	2017	2018	2018‡	2019‡	2020‡
J01AA	Tetracyclines	2.60	2.49	2.33	2.23	2.25	2.10	1.98	1.94	1.94	1.83	1.54
J01CA	Penicillins with extended spectrum	1.91	1.94	1.99	1.94	2.13	2.08	1.94	2.02	1.35	1.26	0.98
J01CE	Beta-lactamase sensitive penicillins	0.35	0.33	0.31	0.30	0.23	0.24	0.22	0.07	0.07	0.16	0.12
J01CF	Beta-lactamase resistant penicillins	0.39	0.41	0.41	0.44	0.43	0.46	0.46	0.49	0.49	0.48	0.47
J01CR	Penicillins + beta-lactamase-inhibitors	1.82	1.82	1.67	1.55	1.56	1.52	1.42	1.42	0.95	0.93	0.81
J01D	Cephalosporins & carbapenems	0.04	0.04	0.04	0.04	0.04	0.03	0.03	0.03	0.03	0.03	0.03
J01EA	Trimethoprim and derivatives	0.20	0.19	0.17	0.16	0.14	0.14	0.13	0.13	0.13	0.12	0.12
J01EE	Sulphonamides + trimethoprim	0.34	0.33	0.29	0.28	0.28	0.28	0.29	0.30	0.30	0.33	0.33
J01FA	Macrolides	1.34	1.34	1.22	1.18	1.20	1.17	1.17	1.22	1.22	1.22	1.13
J01FF	Lincosamides	0.15	0.16	0.17	0.18	0.19	0.20	0.21	0.23	0.23	0.23	0.23
J01GB	Aminoglycosides	0.03	0.04	0.03	0.03	0.03	0.02	0.02	0.02	0.02	0.02	0.02
J01MA	Fluoroquinolones	0.82	0.80	0.76	0.79	0.77	0.75	0.73	0.73	0.73	0.67	0.64
J01XE	Nitrofuran derivatives	1.31	1.38	1.37	1.40	1.40	1.39	1.36	1.35	1.35	1.30	1.24
J01XX01	Fosfomycin	0.01	0.01	0.02	0.03	0.04	0.05	0.05	0.07	0.06	0.06	0.07
	others	0.05	0.05	0.04	0.04	0.04	0.02	0.05	0.04	0.04	0.03	0.03
J01	**Antibiotics for systemic use (total)**	**11.37**	**11.34**	**10.83**	**10.58**	**10.72**	**10.44**	**10.06**	**10.06**	**8.90**	**8.68**	**7.77**

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

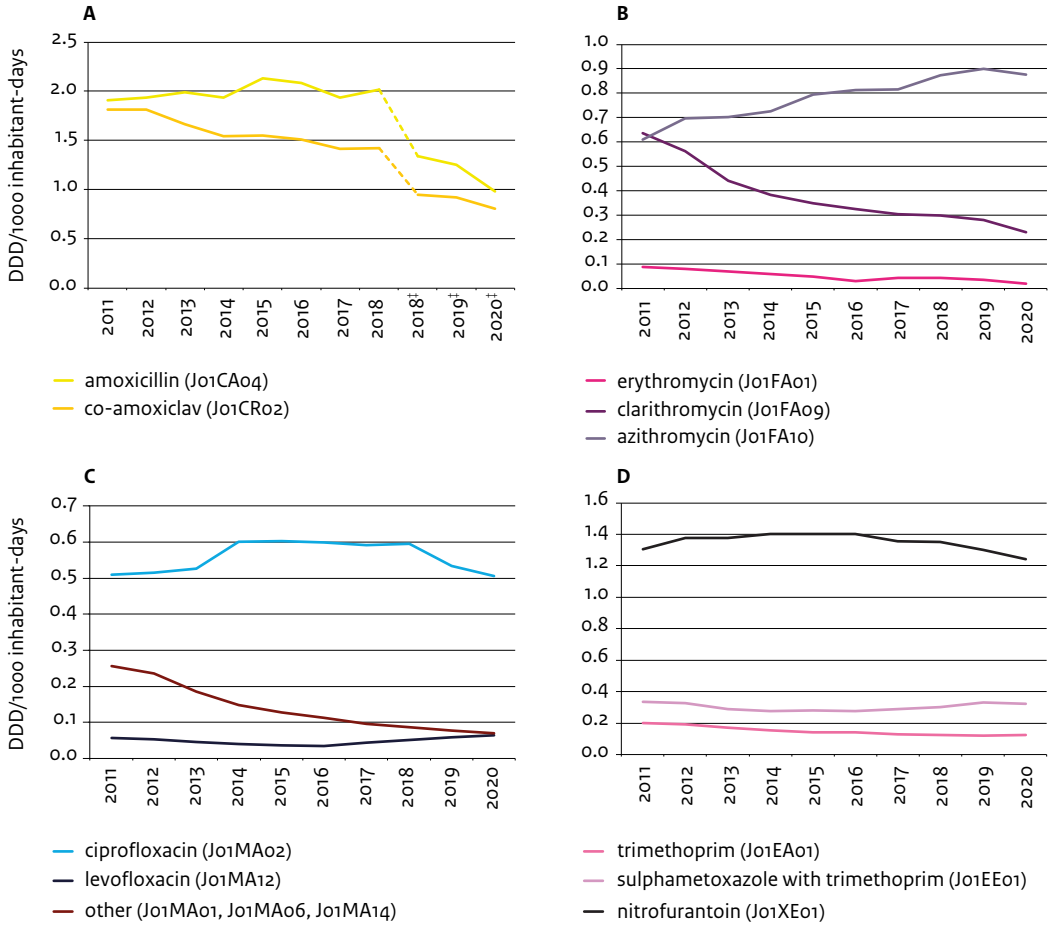
‡ DDD including changes as of 2019 (source: WHO)

Figure 3.1.1 Use of antibiotics for systemic use (J01) in outpatients at ATC-4 level, 2011-2020 (source: SFK)



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

Figure 3.1.2 A-D Use of antibiotics for systemic use (J01) in outpatients at ATC-5 level, 2011-2020 (source: SFK)



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

3.2 Hospital care

3.2.1 Hospital antibiotic use in DDD

Methods

Data on the use of antibiotics in Dutch hospitals in 2019 was collected by means of a questionnaire distributed to all Dutch hospital pharmacies. DDDs per ATC-code and route of administration, according to the WHO in 2019[2] were extracted from the Dutch drug database (Z-index) on unit and product level, and used to calculate total antibiotic use. Several changes in DDD definitions were implemented by the WHO in 2019.[1] For these antibiotic groups, both DDDs calculated with the previous (until 2018) and new WHO definitions (starting from 2019) DDDs are depicted for the year 2018 in the tables and figures (as a dashed line), to enable comparison of surveillance data from 2018 and 2019.

Use of antibiotics is expressed as DDD/100 patient-days and DDD/100 admissions. The number of patient-days was estimated by subtracting the number of admissions from the number of bed-days to compensate for the fact that in bed-days statistics, both the day of admission and the day of discharge are counted as full days. Hospital consumption data and corresponding hospital statistics were used to estimate total hospital consumption in the Netherlands. Methods are further described by Kwint et al.[3]

Hospital extrapolated data are expressed in DDD/1,000 inhabitants per day (DID), as is used in the international antibiotic consumption surveillance of the European Centre for Disease Prevention (ECDC). Data on the annual number of inhabitants in the Netherlands were obtained from Statistics Netherlands (CBS).

Unfortunately, due to COVID-19, the PREZIES (PREventie van ZIEkenhuisinfecties door Surveillance) point prevalence study 2020[4] only consisted of data from four hospitals, in comparison to 21 hospitals in 2019. It was decided to not report these data, since this is a too low number of included hospitals to obtain representative data.

Results

Data over 2019 were received from 57 hospital locations (representing 51 hospital organizations), together with the annual number of bed-days and admissions. The inpatient use of systemic antibiotics slightly decreased (-2.1%) to 79.29 DDD/100 patient-days in 2019 (table 3.2.1.1). Expressed as DDD/100 admissions, total inpatient use of systemic antibiotics increased with 15.39 to 318.5 in 2018 (+5.1%; table 3.2.1.1), although this remained below mean use of the last 5 years.

Total use of antibiotics for systemic use, calculated as DDD/1,000 inhabitant-days, decreased from 0.836 in 2018 to 0.799 in 2019 (-4.4%) (table 3.2.1.2).

Although overall total antibiotic consumption in hospitals slightly decreased, increases in antibiotic use were observed for third generation cephalosporins, sulfonamides and trimethoprim, glycopeptides and polymyxins. The use of glycopeptides has been increasing since 2011, reaching a level of 1.99 DDD/100 patient-days in 2019; an increase of 15.1% compared to 2018. This increase is mainly driven by vancomycin use (figure 3.2.1.2).

Notable decreases were observed in the use of combinations of penicillins (including beta-lactamase inhibitors), trimethoprim and derivatives, aminoglycosides and nitrofuran derivatives, which decreased with 15.4%, 16.1%, 11.3% and 13.9%, respectively.

Figure 3.2.1.1 shows that fluoroquinolone use is decreasing since 2016, which is mainly driven by a decreased ciprofloxacin use (figure 3.2.1.2).

After many years of stable aminoglycoside use, the DDD/100 patient-days declined in 2019.

This was mainly caused by a decrease in gentamicin and tobramycin use (figure 3.2.1.2). A large variation in systemic antibiotic drug use is seen between Dutch hospitals (figure 3.2.1.3, 3.2.1.4 and 3.2.1.5). Considering site of care, in 2019, general hospitals had the lowest systemic antibiotic use (median 71.9 DDD/100 patient-days), whereas large teaching hospitals reported the highest overall systemic antibiotic use (median 82.6 DDD/100 patient-days) as shown in figure 3.2.1.4.
Carbapenems, third generation cephalosporins, fluoroquinolones, macrolides, glycopeptides and polymyxins are used to a larger extent in university hospitals, whereas most of the use of combinations of penicillins and beta-lactamase inhibitors, penicillins with extended spectrum and lincosamides originates from general hospitals (figure 3.2.1.6). Figure 3.2.1.6 also shows a large decrease in fluoroquinolone use for all types of hospitals. After a long time of increasing meropenem use, this stabilized in all types of hospitals in 2019. The use of aminoglycosides decreased in all types of hospitals.
In general, cephalosporin use increased in 2019, although this increase was less than observed in previous years (table 3.2.1.1, figure 3.2.1.6, figure 3.2.1.7). The use of third generation cephalosporins is increasing since 2011, resulting in a level of 7.73 DDD/100 patient-days in 2019 (+12.4% compared to 2018, table 3.2.1.1).There was an increase in the use of first-generation cephalosporins in university and general hospitals (figure 3.2.1.6A), with 7.3% and 7.6% respectively. The use of first generation cephalosporins in large teaching hospitals slightly decreased (-3.7%). The use of second generation cephalosporins is the highest in large teaching hospitals, compared to other types of hospitals, as was also the case in previous years. Figure 3.2.1.7 shows the use of the different cephalosporins per hospital type, where it is shown that cefuroxime remains the most frequently used cephalosporin in general and large teaching hospitals, but not in university hospitals. For university hospitals, both ceftriaxone and cefazolin remain the most frequently used cephalosporins.

The use of antimycotics for systemic use further decreased in 2019, resulting in a use of 11.35 DDD/100 patient-days (-14.3%, table 3.2.1.3).
For the first time, the use of antimycobacterials decreased in 2019, after a previous increase for 10 years, resulting in 4.53 DDD/100 patient-days in 2019 (-13.5%). This included rifampicin used for treatment of tuberculosis or as combination therapy for *S. aureus* infections. After a large increase in 2018, the use of neuraminidase inhibitors decreased with 40.9% to 0.416 DDD/100-patient-days in 2019. Since 2010, the use of nucleosides (without reverse transcriptase inhibitors) has been increasing every year. This increase was halted in 2019; when the use of 2.79 DDD/100 patient-days was back at the level of 2015 (table 3.2.1.3).

Discussion

In 2019, antibiotic use in hospitals decreased slightly when expressed as DDD/100 patient-days and increased when expressed as DDD/100 admissions. Both parameters were below the mean use of the last 5 years. Shifts are observed from one subgroup of antibiotics to another, e.g. penicillin and fluoroquinolone use decreased, but the use of cephalosporins and glycopeptides continued to rise in 2019. 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 decrease in fluoroquinolone use, especially ciprofloxacin, might be explained by the safety warning of the European Medicines Agency (EMA) for these drugs concerning side effects involving muscles, tendons or joints and the nervous system, which was published in March 2019[5].

Table 3.2.1.1 Ten years use of antibiotics for systemic use (J01) in hospitals (DDD/100 patient-days), 2010-2019 (source: SWAB)

ATC group*	Therapeutic group	2010	2011	2012	2013	2014	2015	2016	2017	2018	2018‡	2019‡
J01AA	Tetracyclines	1.67	1.84	1.74	1.75	1.90	1.89	1.96	1.97	2.05	2.05	2.10
J01CA	Penicillins with extended spectrum	7.25	7.31	7.62	7.95	8.42	9.24	10.88	10.22	11.08	5.26	4.92
J01CE	Beta-lactamase sensitive penicillins	1.54	1.52	1.74	1.86	2.40	2.39	2.55	2.50	2.26	2.26	2.49
J01CF	Beta-lactamase resistant penicillins	6.80	6.73	7.14	8.09	8.67	7.74	8.73	9.59	10.76	10.76	10.64
J01CR	Combinations of penicillins, incl. beta-lactamase-inhibitors	15.97	15.85	14.96	14.84	14.48	14.31	14.62	14.73	14.48	11.98	10.13
J01DB	First-generation cephalosporins	3.04	3.49	3.64	3.71	4.35	4.59	4.63	5.29	6.43	6.43	6.68
J01DC	Second-generation cephalosporins	3.42	3.68	4.09	4.68	4.98	5.33	5.75	5.87	7.99	7.99	7.99
J01DD	Third-generation cephalosporins	3.73	3.90	4.37	5.04	5.67	5.49	5.95	6.39	6.88	6.88	7.73
J01DH	Carbapenems	1.20	1.38	1.48	1.65	1.65	1.74	1.83	1.98	1.93	1.32	1.41
J01EA	Trimethoprim and derivatives	0.53	0.39	0.31	0.30	0.26	0.26	0.25	0.27	0.23	0.23	0.20
J01EE	Combinations of sulfonamides and trimethoprim, incl. derivatives	2.02	1.89	1.77	1.92	1.89	1.76	2.13	2.38	2.15	2.15	2.41
J01FA	Macrolides	2.66	2.86	2.81	2.64	2.88	2.74	2.97	2.82	2.66	2.66	2.75
J01FF	Lincosamides	2.34	2.29	2.21	2.30	2.30	2.35	2.45	2.43	2.54	2.54	2.36
J01GB	Aminoglycosides	4.06	3.95	3.26	3.55	3.57	3.66	3.70	3.62	3.76	3.76	3.34
J01MA	Fluoroquinolones	9.03	9.16	8.90	8.65	9.02	8.39	9.15	8.65	8.45	7.67	6.99
J01XA	Glycopeptides	1.25	1.28	1.36	1.49	1.59	1.60	1.62	1.72	1.73	1.73	1.99
J01XB	Polymyxins	0.39	0.22	0.16	0.23	0.19	0.23	0.23	0.24	0.14	0.11	0.15
J01XD	Imidazole derivatives	1.95	2.16	2.33	2.55	2.60	2.58	2.80	3.00	3.20	3.20	3.21
J01XE	Nitrofuran derivatives	1.19	1.24	1.22	1.30	1.55	1.42	1.67	1.73	1.63	1.63	1.40
J01XX	Other antibacterials **	0.13	0.09	0.10	0.10	0.09	0.12	0.13	0.28	0.24	0.24	0.28
	Others***	0.12	0.07	0.10	0.08	0.07	0.07	0.07	0.08	0.10	0.10	0.13
J01	**Antibiotics for systemic use (total)**	**70.29**	**71.31**	**71.31**	**74.68**	**78.55**	**77.89**	**84.05**	**85.68**	**90.71**	**80.98**	**79.29**
	expressed in DDD/100 admissions:											
J01	**Antibiotics for systemic use (total)**	**315.9**	**306.4**	**295.7**	**307.8**	**326.0**	**330.1**	**326.1**	**340.2**	**339.7**	**303.2**	**318.5**

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

** *fosfomycin, methenamine, linezolid, daptomycin*

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

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

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), 2010-2019 (source: SWAB)

ATC Group*	Therapeutic group	2010	2011	2012	2013	2014	2015	2016	2017	2018	2018‡	2019‡
J01AA	Tetracyclines	0.027	0.026	0.024	0.022	0.023	0.025	0.022	0.021	0.023	0.023	0.021
J01CA	Penicillins with extended spectrum	0.110	0.103	0.100	0.099	0.101	0.118	0.125	0.117	0.110	0.052	0.063
J01CE	Beta-lactamase sensitive penicillins	0.023	0.020	0.023	0.023	0.028	0.028	0.029	0.029	0.033	0.033	0.024
J01CF	Beta-lactamase resistant penicillins	0.097	0.089	0.093	0.100	0.105	0.097	0.102	0.103	0.105	0.105	0.104
J01CR	Combinations of penicillins, incl. beta-lactamase-inhibitors	0.256	0.223	0.211	0.199	0.187	0.186	0.171	0.159	0.153	0.128	0.109
J01DB	First-generation cephalosporins	0.042	0.045	0.049	0.047	0.052	0.055	0.053	0.065	0.070	0.070	0.066
J01DC	Second-generation cephalosporins	0.055	0.050	0.052	0.055	0.058	0.065	0.066	0.067	0.070	0.070	0.077
J01DD	Third-generation cephalosporins	0.050	0.050	0.057	0.062	0.066	0.067	0.068	0.067	0.072	0.072	0.074
J01DH	Carbapenems	0.015	0.018	0.019	0.020	0.019	0.021	0.020	0.021	0.020	0.014	0.014
J01EA	Trimethoprim and derivatives	0.009	0.006	0.005	0.004	0.003	0.003	0.003	0.003	0.003	0.003	0.002
J01EE	Combinations of sulfonamides and trimethoprim, incl. derivatives	0.030	0.026	0.024	0.024	0.022	0.021	0.024	0.023	0.022	0.022	0.022
J01FA	Macrolides	0.041	0.037	0.038	0.034	0.034	0.034	0.034	0.030	0.030	0.030	0.026
J01FF	Lincosamides	0.035	0.032	0.031	0.032	0.028	0.030	0.028	0.027	0.026	0.026	0.024
J01GB	Aminoglycosides	0.058	0.054	0.044	0.045	0.044	0.046	0.043	0.037	0.037	0.037	0.033
J01MA	Fluoroquinolones	0.138	0.127	0.124	0.116	0.112	0.112	0.106	0.097	0.087	0.079	0.071
J01XA	Glycopeptides	0.016	0.017	0.017	0.018	0.018	0.019	0.019	0.019	0.018	0.018	0.018
J01XB	Polymyxins	0.006	0.003	0.002	0.003	0.002	0.003	0.002	0.001	0.002	0.001	0.001
J01XD	Imidazole derivatives	0.030	0.027	0.029	0.030	0.030	0.032	0.032	0.034	0.033	0.033	0.033
J01XE	Nitrofuran derivatives	0.018	0.015	0.018	0.016	0.018	0.018	0.018	0.019	0.017	0.017	0.015
J01XX	Other antibacterials**	0.002	0.001	0.002	0.002	0.001	0.002	0.002	0.003	0.003	0.003	0.003
	Others***	0.002	0.001	0.002	0.000	0.000	0.001	0.000	0.001	0.001	0.001	0.001
J01	**Antibiotics for systemic use (total)**	**1.061**	**0.971**	**0.963**	**0.950**	**0.953**	**0.982**	**0.968**	**0.942**	**0.934**	**0.836**	**0.799**

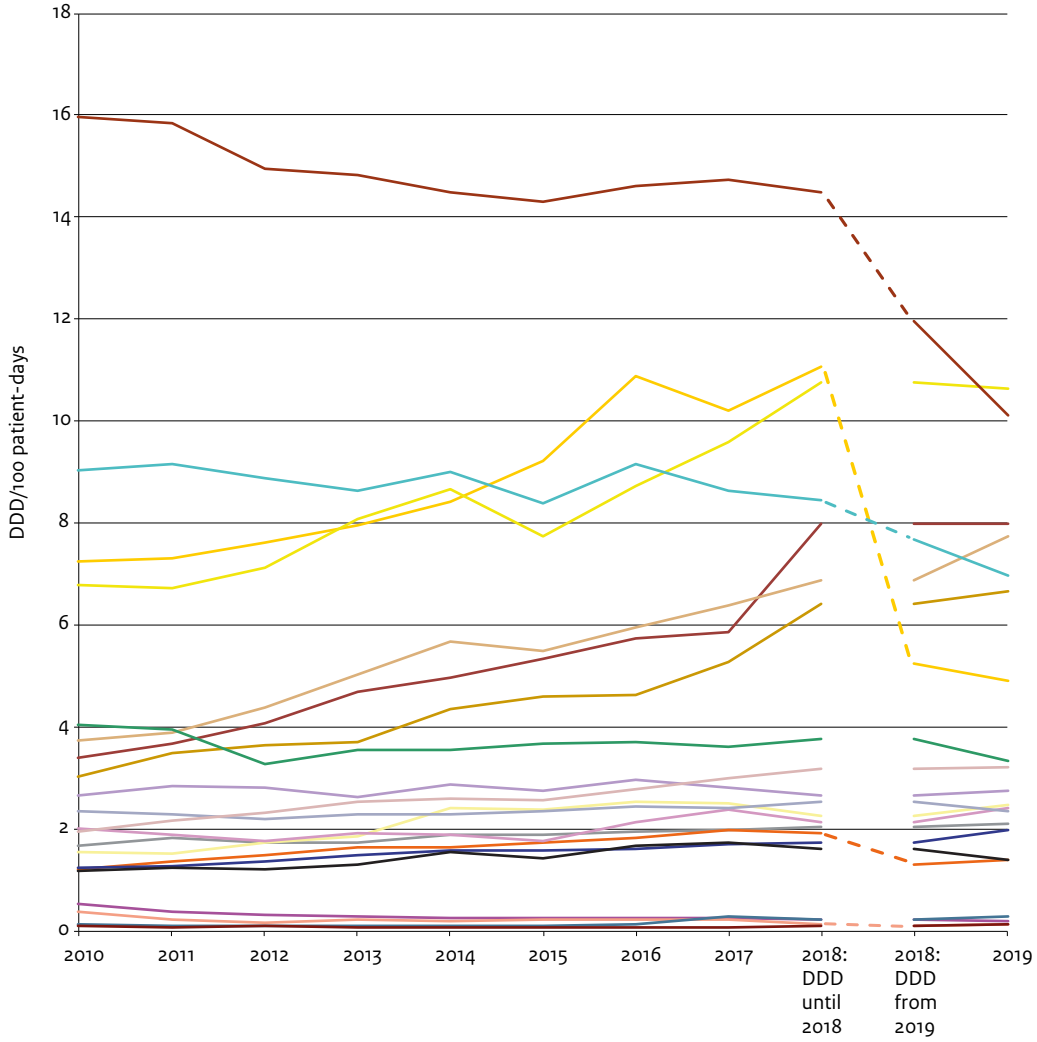
** From the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system*

*** fosfomycin, methenamine, linezolid, daptomycin*

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

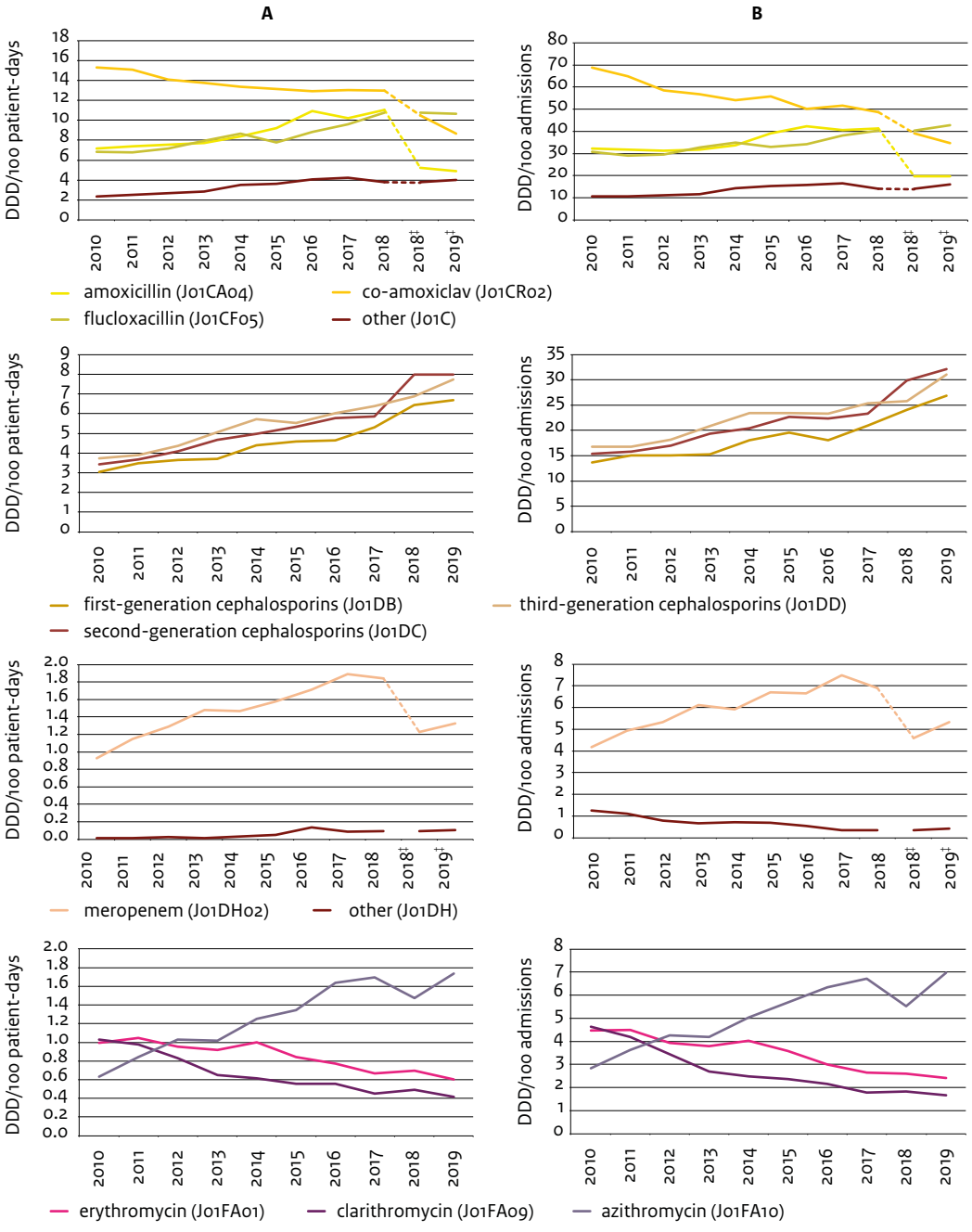
‡ DDD including changes as of 2019 (source: WHO)

Figure 3.2.1.1 Use of antibiotics for systemic use (J01) in hospitals (DDD/100 patient-days) at ATC-4 level, 2010-2019 (source: SWAB)



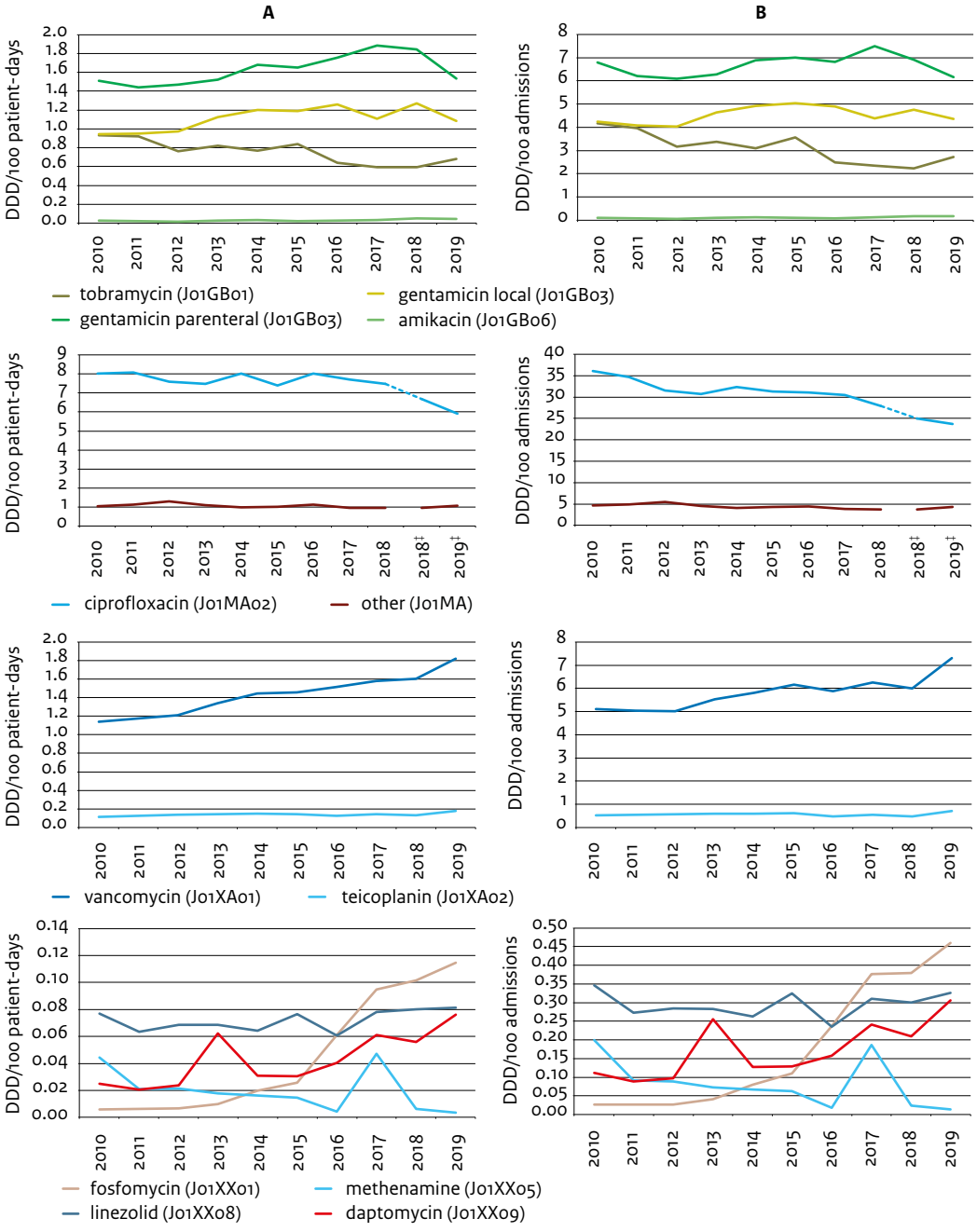
- J01AA tetracyclines
- J01CA penicillins with extended spectrum
- J01CE beta-lactamase sensitive penicillins
- J01CF beta-lactamase resistant penicillins
- J01CR combinations of penicillins, incl. beta-lactamase-inhibitors
- J01DB first-generation cephalosporins
- J01DC second-generation cephalosporins
- J01 DD third-generation cephalosporins
- J01DH carbapenems
- J01EA trimethoprim and derivatives
- J01EE combinations of sulfonamides and trimethoprim, incl. derivatives
- J01FA macrolides
- J01FF lincosamides
- J01GB aminoglycosides
- J01MA fluoroquinolones
- J01XA glycopeptides
- J01XB polymyxins
- J01XD imidazole derivatives
- J01XE nitrofuran derivatives
- J01XX other antibacterials
- others (J01DF, J01DI, J01EC and J01XC)

Figure 3.2.1.2 Use of beta-lactams, macrolides, aminoglycosides, fluoroquinolones, glycopeptides and other antibiotics in hospitals expressed as DDD/100 patient-days (A) and DDD/100 admissions (B) 2010-2019 (source: SWAB)



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

Figure 3.2.1.2 (continued) Use of beta-lactams, macrolides, aminoglycosides, fluoroquinolones, glycopeptides and other antibiotics in hospitals expressed as DDD/100 patient-days (A) and DDD/100 admissions (B) 2010-2019 (source: SWAB)



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

Figure 3.2.1.3 Comparison of the total systemic antibiotic drug use (J01) across Dutch hospitals in 2019 (source: SWAB)

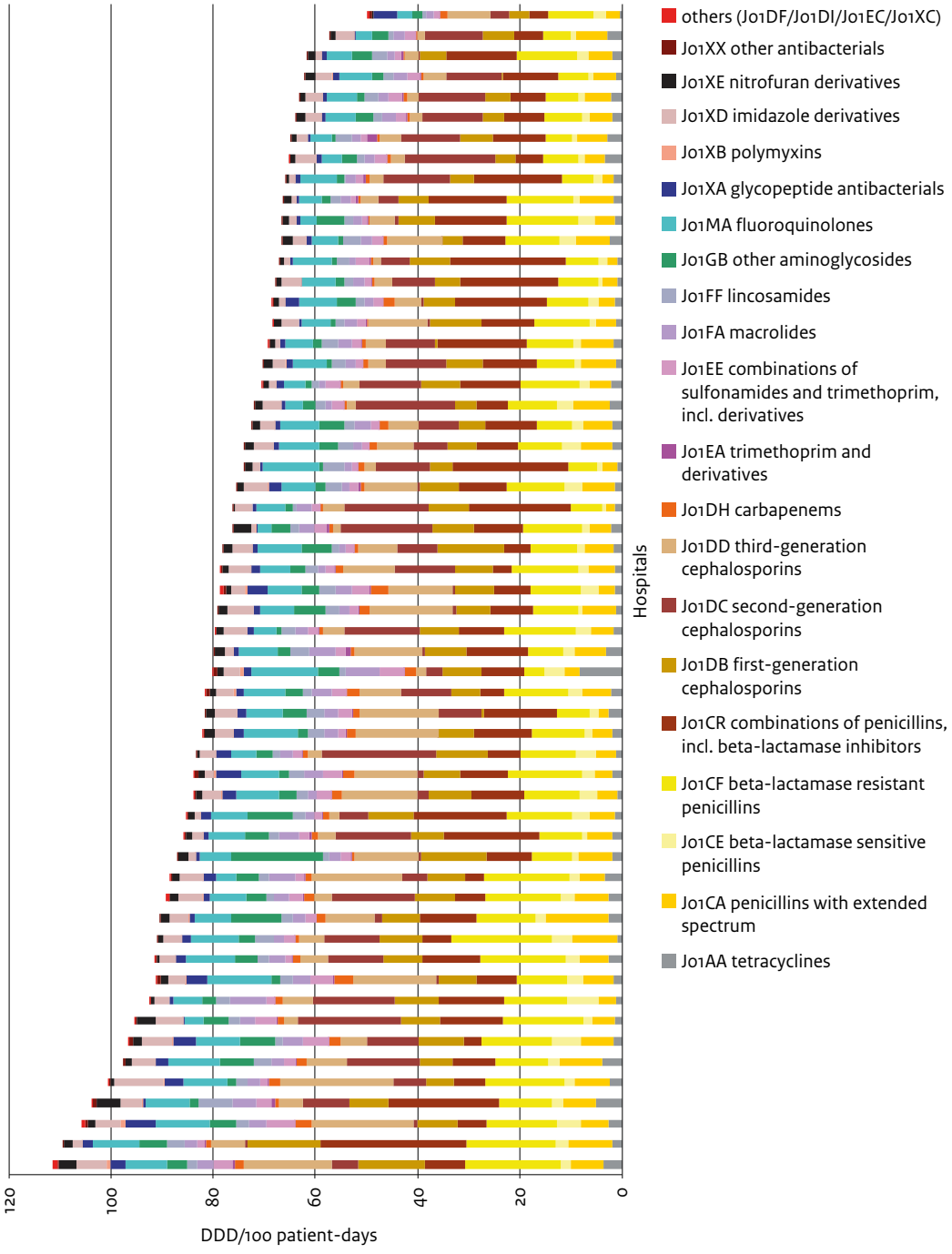


Figure 3.2.1.4 Total systemic antibiotic use (J01) and comparison across university, large teaching and general hospitals in 2019 (source: SWAB)

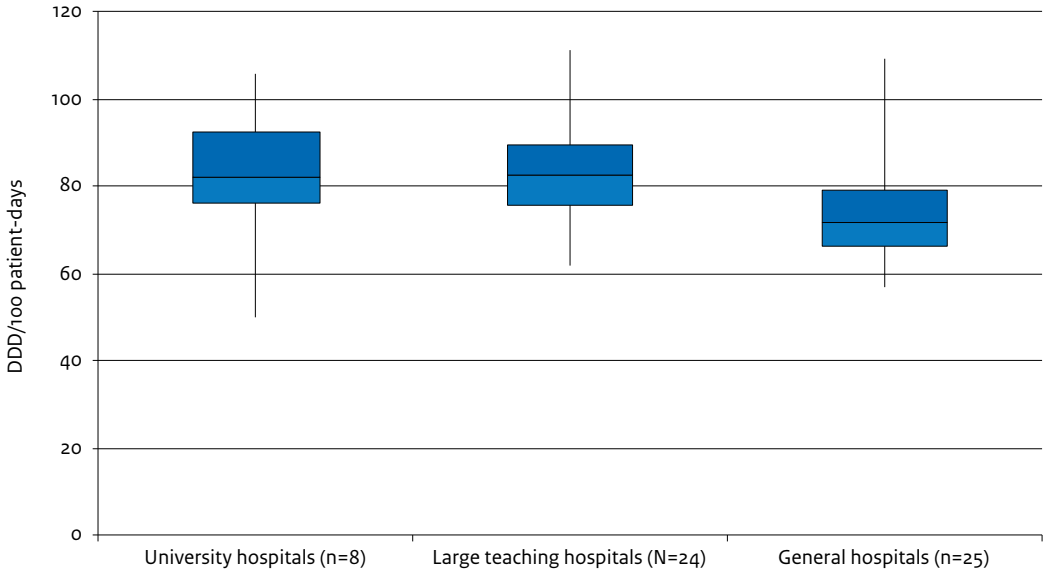


Figure 3.2.1.5 Distribution (%) of the use of antibiotics for systemic use (J01) in hospitals, 2019 (source: SWAB)

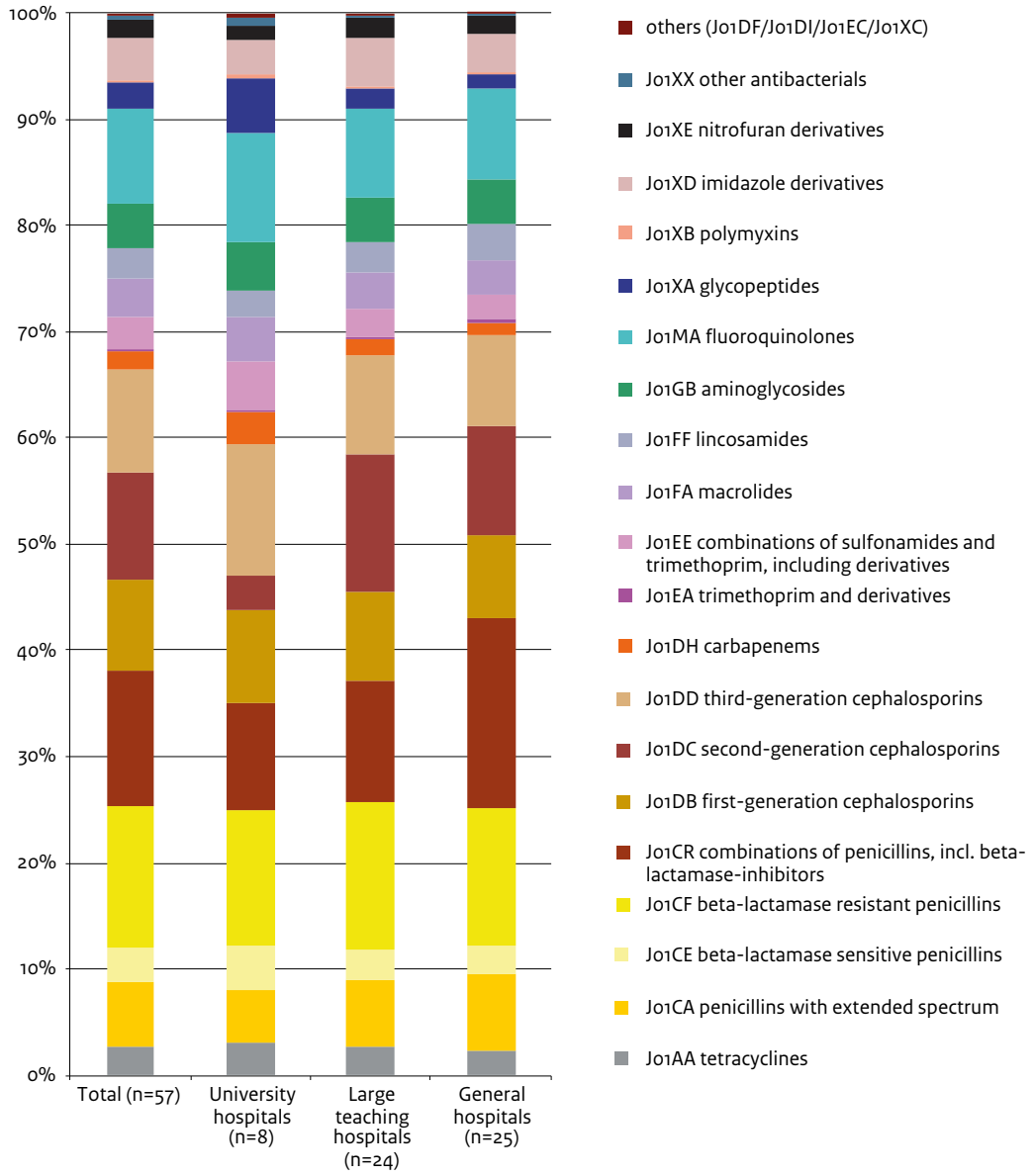
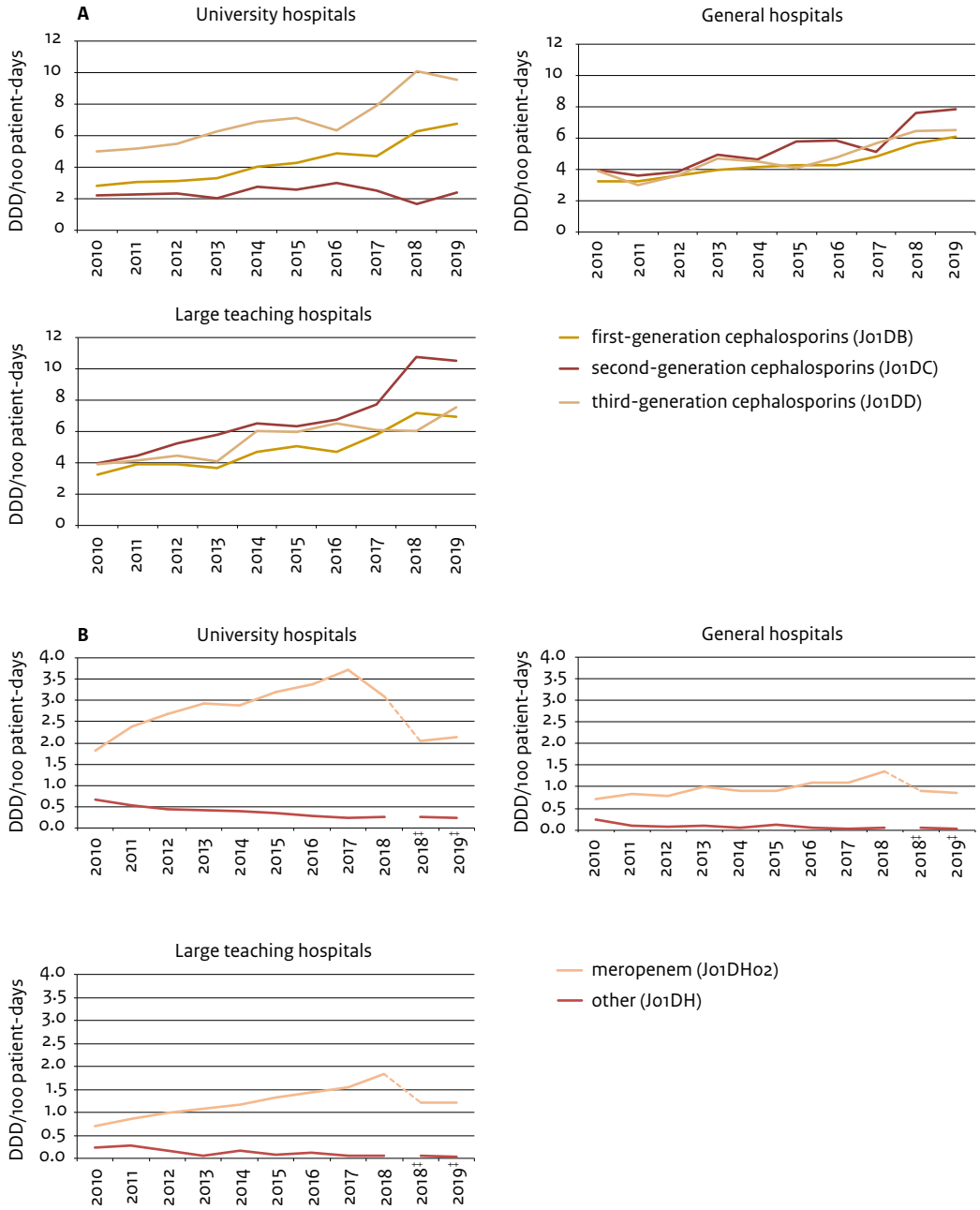


Figure 3.2.1.6 Use of cephalosporins (A), carbapenems (B), aminoglycosides (C), glycopeptides (D) and fluoroquinolones (E) in hospitals broken down by type of hospital, expressed as DDD/100 patient-days, 2010-2019 (source: SWAB)



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

Figure 3.2.1.6 (continued) Use of cephalosporins (A), carbapenems (B), aminoglycosides (C), glycopeptides (D) and fluoroquinolones (E) in hospitals broken down by type of hospital, expressed as DDD/100 patient-days, 2010-2019 (source: SWAB)

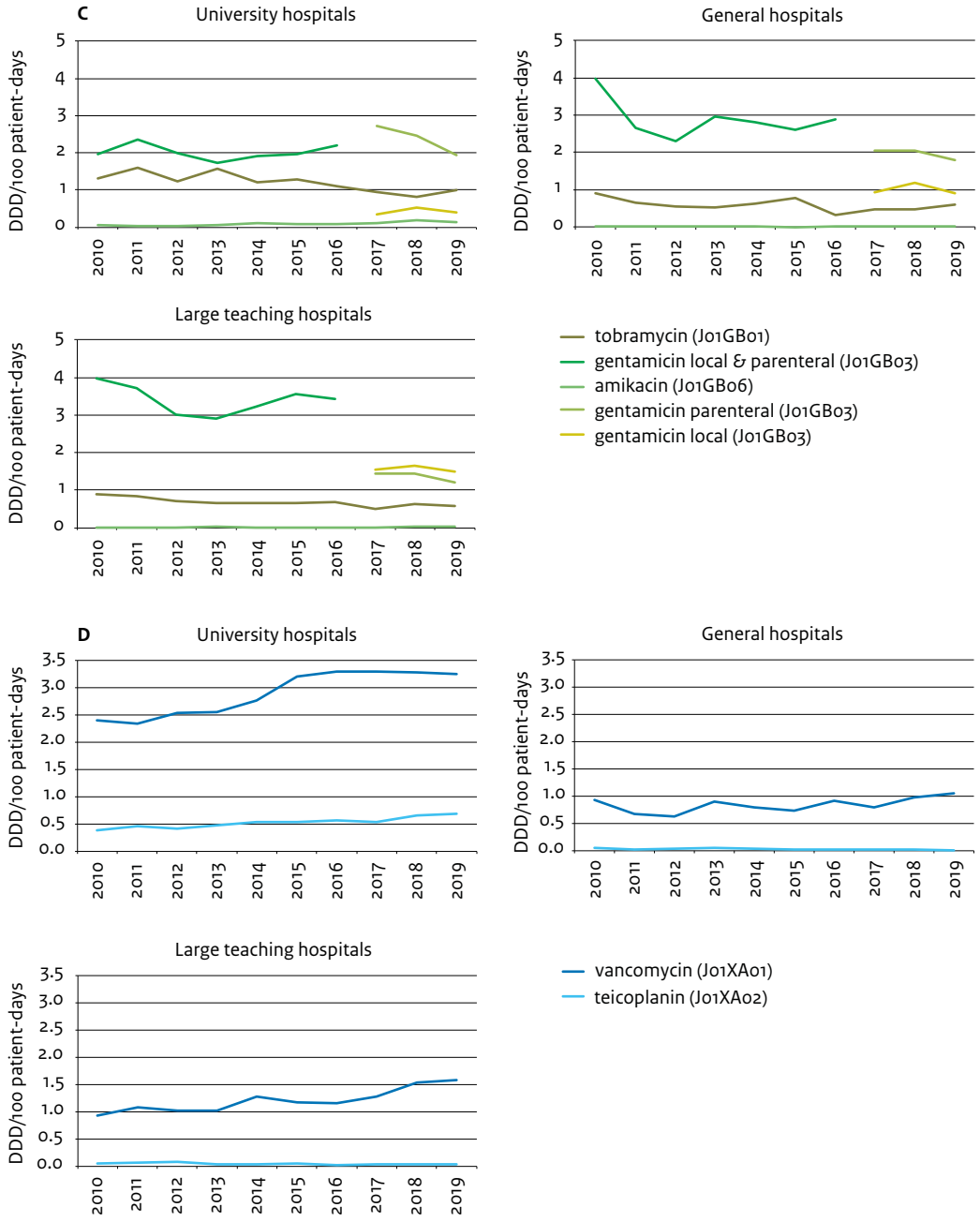
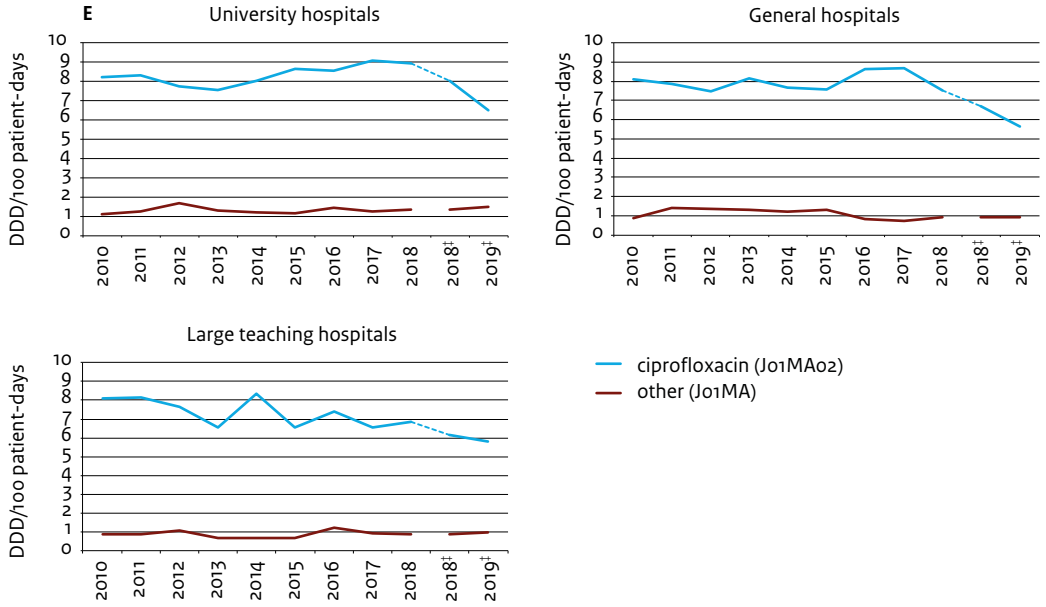


Figure 3.2.1.6 (continued) Use of cephalosporins (A), carbapenems (B), aminoglycosides (C), glycopeptides (D) and fluoroquinolones (E) in hospitals broken down by type of hospital, expressed as DDD/100 patient-days, 2010-2019 (source: SWAB)



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

Figure 3.2.1.7 Use of 1st, 2nd and 3th generation cephalosporins in university, large teaching and general hospitals at ATC-5 level in 2019 (source: SWAB)

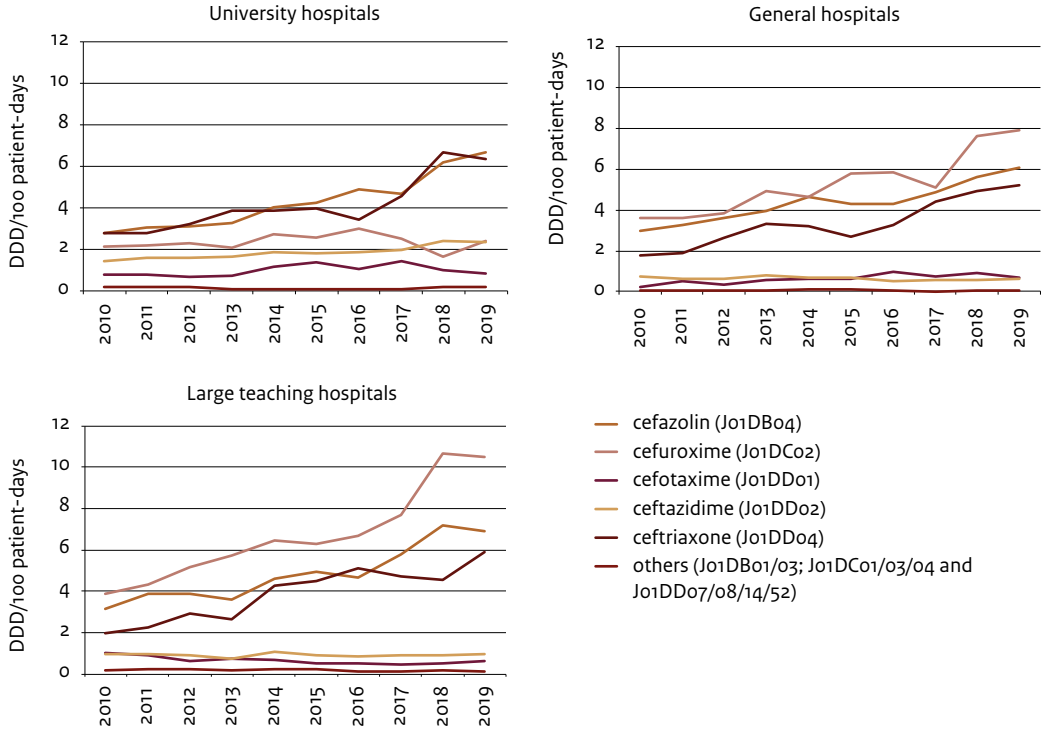


Table 3.2.1.3 Use of antimycotics, antimycobacterials and antivirals for systemic use (J02, J04, J05) in university hospitals (DDD/100 patient-days), 2010-2019 (source: SWAB)

ATC group *	Therapeutic group	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019
J02AA01	Antibiotics (amphotericin B)	1.65	1.77	2.43	3.01	3.46	4.17	4.34	4.80	4.36	3.10
J02AB02	Imidazole derivatives (ketoconazole)	0.15	0.09	0.10	0.06	0.24	0.34	0.04	0.08	0.02	0.06
J02AC	Triazole derivatives	6.31	5.83	6.25	6.29	7.15	7.55	9.22	7.80	7.84	7.12
J02AX	Other antimycotics for systemic use (mainly echinocandines)	0.56	0.57	0.55	0.71	0.61	0.64	0.64	0.96	1.03	1.08
J02	**Antimycotics for systemic use (total)**	**8.66**	**8.26**	**9.33**	**10.06**	**11.47**	**12.70**	**14.23**	**13.63**	**13.25**	**11.35**
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)	1.41	1.56	1.24	1.43	1.39	1.33	1.13	1.69	1.89	1.60
J04AC	Hydrazides (mainly isoniazide)	0.34	0.30	0.40	0.57	0.56	0.35	0.30	0.67	0.98	0.59
J04AD	Thiocarbamide derivatives	0.00	0.01	0.00	0.00	0.00	0.12	0.14	0.01	0.02	0.00
J04AK	Other drugs for treatment of tuberculosis (pyrazinamide, ethambutol)	0.37	0.26	0.31	0.16	0.28	0.19	0.15	0.66	0.95	0.67
J04AM	Combinations of drugs for tuberculosis	0.00	0.00	0.01	0.02	0.04	0.07	0.11	0.15	0.22	0.26
J04BA	Drug for treatment of lepra (dapson)	0.45	0.49	0.62	0.70	0.60	0.70	0.71	1.13	1.18	1.40
J04	**Antimycobacterials for systemic use (total)**	**2.58**	**2.62**	**2.57**	**2.88**	**2.87**	**2.76**	**2.55**	**4.31**	**5.24**	**4.53**
J05AB	Nucleosides an nucleotides excl. Reverse transcriptase inhibitors	2.02	2.18	2.24	2.33	2.71	2.76	2.97	2.99	4.37	2.79
J05AD	Phosphonic acid derivatives	0.10	0.10	0.15	0.12	0.16	0.14	0.20	0.20	0.31	0.29
J05AE	Protease inhibitors	0.78	0.55	0.81	0.63	0.40	0.33	0.30	0.31	0.25	0.19
J05AF	Nucleoside reverse transcriptase inhibitors	0.67	0.63	0.69	0.54	0.59	0.71	0.52	0.70	0.78	0.85
J05AG	Non-nucleoside reverse transcriptase inhibitors	0.22	0.14	0.18	0.16	0.18	0.23	0.22	0.26	0.23	0.12
J05AH	Neuraminidase inhibitors	0.21	0.42	0.19	0.49	0.16	0.30	0.43	0.31	0.70	0.42
J05AP	Antivirals for treatment of HCV infections								0.08	0.03	0.07
J05AR	Antivirals for the treatment of HIV, combinations	0.76	0.69	0.91	0.89	0.94	0.95	0.99	1.12	1.96	1.43
J05AX	Other antivirals	0.15	0.17	0.24	0.29	0.22	0.33	0.46	0.72	1.18	0.79
J05	**Antivirals for systemic use (total)**	**4.91**	**4.89**	**5.41**	**5.47**	**5.37**	**5.75**	**6.09**	**6.68**	**9.82**	**6.94**

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

3.2.2 Hospital antibiotic use in days of therapy (DOT)

One day of therapy (DOT) represents the administration of a single agent on a given day regardless of the doses administered. DOTs track the duration of treatment with an antibiotic. This is different from DDDs, which represent the assumed average maintenance dose per day for a drug used for its main indication in adults. The DDD is annually determined by the WHO.
The ratio DDD/DOT gives information on whether the prescribed daily doses match the DDD as determined by the WHO. A ratio >1 indicates that doses higher than the DDD are prescribed, a ratio <1 indicates that doses lower than the DDD are prescribed.

Methods
Electronic prescriptions for antibiotics on patient level were extracted from Dutch hospital electronic prescribing systems over 2020. 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.

Results
Data over 2019 was evaluated for 26 hospitals (3 university hospitals, 7 large teaching hospitals and 16 general hospitals) compared to 31 hospitals in 2018. 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, decreased from 64.37 in 2018 to 58.61 DOT/100 patient-days (-8.9%) in 2019.
The use for all groups of antibiotics decreased, except for the third generation cephalosporins and the glycopeptides.
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 and tetracyclines.
The DDD/DOT-ratio was highest (>1.5) for the use of tetracyclines, penicillins with extended spectrum, beta-lactamase sensitive penicillins, beta-lactamase resistant penicillins, macrolides, aminoglycosides and polymyxins. The highest increase in DDD/DOT ratio compared to 2018 was observed for beta-lactamase sensitive penicillins (+0.47), macrolides (+0.53) and polymyxins (+0.60). Overall, the DDD/DOT ratio for imidazole derivates was the lowest (0.80) and highest for beta-lactamase resistant penicillins (3.95).

Discussion
For the majority of the antibiotic groups, the DOTs were decreased in 2019, compared to 2018. DOTs for third generation cephalosporins increased, probably explained by the changed sepsis protocol in different hospitals, where second generation cephalosporins were replaced by third generation cephalosporins. The large decrease in DOTs for the aminoglycoside might also be caused by a change in sepsis protocols, where aminoglycosides are less frequently advised.

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. The increase in the DDD/DOT ratio for tetracyclines might be due to a movement from intramural use of doxycycline for respiratory tract infections (lower dose) to long-term treatments using higher doses for indications as Q fever and Lyme disease. The increase in DDD/DOT ratio for beta-lactamase resistant penicillins might be due to a change of indication, where especially flucloxacillin is mainly used for indications that require high doses. Furthermore, increasing knowledge on PKPD and the large therapeutic window of these agents may result in the prescription of higher daily dosages. The increase in the DDD/DOT ratio for first generation cephalosporins between 2018 and 2019 might reflect the changed perioperative prophylaxis guideline, where the standard dose of cefazolin was changed from 1g to 2g.[6]

Table 3.2.2.1 Antibiotic use in hospitals expressed as days of therapy (DOT)/100 patient-days, defined daily dose (DDD)**/100 patient-days and ratio DDD/DOT at ATC-4 level in 2018 (n=31) and 2019 (n=26)

ATC Group*	Therapeutic group	2018: DDD/100 patient-days	2019: DDD/100 patient-days	2018: DOT/100 patient-days	2019: DOT/100 patient-days	2018: Ratio DDD/DOT	2019: Ratio DDD/DOT
J01AA	Tetracyclines	2.05	2.10	1.13	0.93	1.82	2.26
J01CA	Penicillins with extended spectrum	5.26	4.92	3.58	2.99	1.47	1.65
J01CE	Beta-lactamase sensitive penicillins	2.26	2.49	1.72	1.39	1.32	1.79
J01CF	Beta-lactamase resistant penicillins	10.76	10.64	3.16	2.69	3.40	3.95
J01CR	Combinations of penicillins, incl. beta-lactamase-inhibitors	11.98	10.13	10.97	10.33	1.09	0.98
J01DB	First-generation cephalosporins	6.43	6.68	7.53	6.98	0.85	0.96
J01DC	Second-generation cephalosporins	7.99	7.99	6.65	5.32	1.20	1.50
J01DD	Third-generation cephalosporins	6.88	7.73	5.78	6.91	1.19	1.12
J01DH	Carbapenems	1.32	1.41	1.32	1.30	1.00	1.08
J01EA	Trimethoprim and derivatives	0.23	0.20	0.28	0.22	0.84	0.89
J01EE	Combinations of sulfonamides and trimethoprim, including derivatives	2.15	2.41	2.53	2.35	0.85	1.02
J01FA	Macrolides	2.66	2.75	2.12	1.54	1.26	1.79
J01FF	Lincosamides	2.54	2.36	1.84	1.64	1.38	1.44
J01GB	Aminoglycosides	3.76	3.34	1.85	1.40	2.04	2.38
J01MA	Fluoroquinolones	7.67	6.99	6.58	5.67	1.17	1.23
J01XA	Glycopeptides	1.73	1.99	1.35	1.42	1.28	1.41
J01XB	Polymyxins	0.11	0.15	0.10	0.09	1.09	1.69
J01XD	Imidazole derivatives	3.20	3.21	4.17	4.02	0.77	0.80
J01XE	Nitrofuran derivatives	1.63	1.40	1.46	1.25	1.11	1.12
J01XX	Other antibacterials	0.24	0.28	0.24	0.17	1.00	1.66
	Total	**80.88**	**79.16**	**64.37**	**58.61**	**1.26**	**1.35**

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

*** DDDs as defined by the WHO in 2019*

3.3 Long-term care facilities

Methods

Due to the COVID-19 pandemic, no point prevalence study was executed by the SNIV (Surveillance Netwerk Infectieziekten Verpleeghuizen) network of the RIVM in 2020. Therefore, only data on antibiotic use obtained from hospital pharmacies was used in this chapter. All hospital pharmacies 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 2019. 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.

Results

The antibiotic use of 9707 residents of long-term facilities was included in the data analysis for 2019, originating from 15 long-term care facilities or organizations. The size of long-term facilities varied from 61-1963 residents per home or organization, with a mean of 647 residents.
Compared to 2018, the mean antibiotic use in long-term care facilities decreased by 3.5 DDD/1,000 residents/day to 50.4 DDD/1,000 residents/day. The use varied highly between the different long-term care facilities with a minimum of 21.4 and a maximum of 109.3 DDD/1,000 residents/day. The use of tetracyclines, trimethoprim and derivatives, and nitrofurantoin decreased compared to 2018; the use of carbapenems, combinations of sulfonamides and trimethoprim, including derivatives, and glycopeptides increased (table 3.3.1).

Discussion

Although the total antibiotic use in long-term care facilities decreased in 2019 compared to 2018, it is still within the range that was observed in the past years. One should be aware that the WHO implemented some important changes in DDDs from 2019 (see also table 3.3.1), which can be misleading when comparing this years' data to the data obtained in previous years. The data from 2018 were therefore recalculated with the 'new' DDDs, to facilitate comparison. The pattern of use is similar to 2018, with amoxicillin with clavulanic acid (the main agent of the group 'combinations of penicillins, including beta-lactamase-inhibitors'), 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 antibiotic use, it is a worrisome development that the top 3 increases all have a substantial broader spectrum than the top 3 decreases.

Table 3.3.1 Distribution of the use of antibiotics for systemic use (J01) in long-term care facilities, (expressed as weighted mean) DDD/1,000 residents/day, 2011-2019 (source: SWAB)

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

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

** *fosfomycin, methenamine, linezolid, daptomycin*

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

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

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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 2020, 46 laboratories were connected to ISIS-AR, all performing antimicrobial susceptibility testing (AST) according to EUCAST guidelines. Out of these 46 laboratories, 37 provided complete data on the last five years (2016 to 2020). Five of these 37 laboratories exclusively served university hospitals; 30 laboratories served non-university hospitals, general practices, and long-term care facilities; and two laboratories exclusively served general practices and long-term care facilities. For the analyses in sections 4.2, 4.3, and 4.5 we selected only data from these 37 laboratories to avoid bias in time trends due to incomplete data.

Because no time trends were calculated for resistance by regional network in section 4.2 and resistance percentages in section 4.5, we used for those analyses data from 36 non-university laboratories for which at least complete data on 2020 were available (34 serving non-university hospitals, general practices, and long-term care facilities; and two serving general practices and long-term care facilities only).

All data provided to ISIS-AR are carefully validated[1]. Data with confirmed or probable technical errors are, after consultation with the laboratory that provided the data, corrected or excluded from the analyses in this report.

Selection of isolates

We calculated resistance levels and, if applicable, time trends by setting of care, i.e. general practices (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 general practices (section 4.2) and long-term care facilities (section 4.4), we selected urine isolates for analysis of resistance in *Enterobacterales* and *Pseudomonas aeruginosa*, wound or pus isolates for analysis of resistance in *Staphylococcus aureus / Staphylococcus argenteus*, respiratory and wound or pus isolates for analysis of resistance in β-haemolytic *Streptococcus* group A, and urinary and genital isolates for analysis of resistance in β-haemolytic *Streptococcus* group B. For analyses on data from outpatient departments (section 4.3.1), inpatient departments (excl. intensive care units, section 4.3.2), and intensive care units (section 4.3.3), we selected isolates from blood, cerebrospinal fluid, urine, lower respiratory tract, and wound or pus. Additionally, we conducted a separate analysis for blood isolates from inpatients (incl. patients from intensive care units, section 4.3.4). For urology departments (section 4.3.5), we selected only urine isolates. Finally, in section 4.5, we performed a separate analysis on respiratory pathogens (*Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis*), separately for general practitioners' patients and hospital patients. We selected isolates from the upper and lower respiratory tract for the analysis on general practitioners' patients. For the analysis on hospital patients, we additionally selected isolates from blood and cerebrospinal fluid.

Since the number of *S. argenteus* isolates was too small for 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 sections 4.2 through 4.4 *S. argenteus* comprised 0.0 to 0.03% of the isolates from this complex. *Staphylococcus schweitzeri*, the third member of the *S. aureus* complex, was not found in the ISIS-AR database.

The category wound or pus isolates comprises isolates from deep and superficial wounds, pus (including pus from abscesses), but also skin (excluding perineal swabs), normally sterile sites or taken using a sterile procedure (i.e. biopsy, aspiration), synovial fluid, peritoneal cavity fluid and fluid for continuous ambulatory peritoneal dialysis (CAPD), eyes (both normally sterile and non-sterile sites), amniotic fluid, and samples of / related to medical implants.

For each analysis, we selected the first isolate per species per patient per year to avoid repeated sampling causing bias in the calculation of resistance levels and time trends. We included only data on diagnostic samples, and only calculated resistance levels for pathogens for which at least 100 isolates in each year were available for analysis. Furthermore, to avoid bias due to selective testing of 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 a pathogen-agent combination if the data from at least 50% of the selected laboratories could be included.

Calculation of resistance levels

We calculated the percentage of resistant isolates ('R'). To avoid bias due to differences in breakpoint guidelines and expert rules used in the participating laboratories, we first reinterpreted all crude test values according to EUCAST breakpoints version 10.0. We included data from all laboratories for which at least 80% of testvalues could be reinterpreted each year. Where reinterpretation was not possible, this was due to missing crude data or test values that were not compatible with EUCAST breakpoints. 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 the Netherlands (88% of all 41 included 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 R-breakpoint of >32 mg/L. Therefore, in sections 4.2 through 4.4, we only present resistance to co-amoxiclav and all combinations of agents that include co-amoxiclav according to the breakpoint for non-uncomplicated urinary tract infections.
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 reinterpretation of oxacillin test values, or, if the result for oxacillin was I or R, on reinterpretation of test values for (benzyl)penicillin. However, available gradient tests (Etest™ and MTS™) systematically underestimate (benzyl)penicillin MIC values in *S. pneumoniae*[2]. Therefore, resistance percentages for (benzyl)penicillin in *S. pneumoniae* may be biased towards a lower level.
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* and *P. mirabilis*), and doxycycline/tetracycline, and often the laboratories report results for either one. For these combinations, we calculated the percentage of isolates that was resistant to at least one of both agents. Additionally, for Gram-negative bacteria except *E. cloacae* complex and *Acinetobacter* spp., we calculated resistance to specific combinations of agents that are frequently used for empiric therapy (for *Enterobacterales*: gentamicin + co-amoxiclav, gentamicin + cefuroxime, gentamicin + cefotaxime/ceftriaxone, ciprofloxacin + co-amoxiclav, ciprofloxacin + cefuroxime, and ciprofloxacin + cefotaxime/ceftriaxone; for *P. aeruginosa*: tobramycin + ceftazidime and tobramycin + ciprofloxacin). For these combinations, we defined resistance as resistance to both agents.
For *S. aureus* and coagulase-negative *Staphylococcus* spp., we calculated resistance to ciprofloxacin as a class indicator for resistance to fluoroquinolones. However, ciprofloxacin should not be considered as a first choice for treatment of infections with these microorganisms.
To calculate the percentage of highly resistant microorganisms (HRMO), we used the definitions of the

Working Group on Infection Prevention (WIP)[3]. We considered *E. coli*, *K. pneumoniae*, and *P. mirabilis* to be an HRMO if they were 1) extended-spectrum β-lactamase (ESBL)-producing, estimated by ESBL confirmatory tests, or, if no data on confirmatory tests were available, by resistance to cefotaxime/ceftriaxone and/or ceftazidime, 2) resistant to both fluoroquinolones and aminoglycosides, or 3) carbapenemase producing (CPE), estimated by confirmatory tests of carbapenemase production (either phenotypical or molecular), or, if no data on confirmatory tests were available, by resistance to meropenem or imipenem (for *P. mirabilis*: meropenem only). We considered *E. cloacae* complex to be an HRMO if at least one of the situations 2 and 3, as described for the other *Enterobacterales*, was true. We considered *P. aeruginosa* to be an HRMO if it was resistant to ≥3 antimicrobial groups among fluoroquinolones, aminoglycosides, carbapenems (or, if a confirmatory test for carbapenemase production, either phenotypical or molecular, was available, we prioritized this), ceftazidime, and piperacillin-tazobactam. Finally, for *Acinetobacter* spp., we defined HRMO as at least one of the following: 1) carbapenemase producing, estimated by confirmatory tests of carbapenemase production, or, if no data on confirmatory tests were available, by resistance to imipenem or meropenem, or 2) resistant to both fluoroquinolones and aminoglycosides.
In addition, for *Enterobacterales* isolates, we calculated the percentage of isolates that was multidrug resistant, which we defined as resistance to the oral agents co-amoxiclav, ciprofloxacin, and co-trimoxazole combined.
For *E. coli*, *K. pneumoniae*, and *S. aureus / S. argenteus* isolates from general practitioners' patients, we conducted an extra analysis to calculate resistance to a selection of antibiotics in 2020 by regional cooperative network[4]. We compared resistance levels in general practitioners' patients within the regional cooperative networks with the resistance percentage in all regions combined, with a two-sided p-value of <0.05 being statistically significant and a difference that was larger than the square root of the national resistance percentage being clinically relevant. In the corresponding figures, differences in resistance percentages that were both statistically significant and clinically relevant are indicated by an asterisk.

Calculation of time trends

In addition to resistance levels in 2020, we calculated for sections 4.2 and 4.3 time trends over the last five years (2016 to 2020) using logistic regression models, except when data in one or more years before 2020 did not meet criteria for calculation of resistance levels. Because adoption of new guidelines or changes in breakpoints can have a substantial effect on resistance levels, we only analysed trends for resistance levels that were based on reinterpretation of crude test values. We made an exception for trends in resistance for flucloxacillin and clindamycin including inducible resistance in *S. aureus*, which we based on laboratory S/I/R interpretation. However, we do not expect spurious time trends in resistance for these two pathogen-agent combinations because EUCAST breakpoints for these combinations were not changed between 2016 and 2020. 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 for trend <0.05 to be statistically significant. When the absolute difference in predicted resistance from the logistic regression model between 2016 and 2020 was larger than the square root of the predicted resistance in 2016, we considered the trend to be clinically relevant. Statistically significant increasing trends that are considered to be clinically relevant are indicated in a red font, whereas decreasing trends that meet the same criteria are indicated in green.

In addition, the resistance levels from 2016 to 2020 are shown in bar charts for each pathogen-agent combination for which the resistance levels were higher than 0.5% for at least one year and lower than 30% for at least three years.

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

[2] EUCAST 2019, Warning against the use of gradient tests for benzylpenicillin MIC in Streptococcus pneumoniae, accessed 29 March 2021, http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Warnings/Warnings_docs/Warning_-_gradient_for_benzyl_and_pnc_21nov2019.pdf.

[3] Werkgroep Infectiepreventie 2017, Bijzonder resistente micro-organismen (BRMO), Rijksinstituut voor volksgezondheid en milieu (RIVM), accessed 29 March 2021, https://www.rivm.nl/wip-richtlijn-brmo-bijzonder-resistente-micro-organismen-zkh.

[4] Rijksinstituut voor volksgezondheid en milieu (RIVM) 2019, Regionale aanpak, accessed 29 March 2021, https://www.rivm.nl/antibioticaresistentie/nationale-aanpak-antibioticaresistentie/zorgnetwerken.

4.1.2 Description of the ISIS-AR data

In this section, 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 sections 4.2 through 4.5, is shown by 4-digit postal code area. Furthermore, in the same figure the geographical distribution of laboratories is presented by status of connection to ISIS-AR and inclusion in the analyses in sections 4.2 through 4.5 (see section 4.1.1 for inclusion criteria). In table 4.1.2.1, characteristics of included isolates are listed by pathogen.

Figure 4.1.2.1 Geographical distribution of laboratories, by status of connection to ISIS-AR and inclusion in the analyses in sections 4.2 to 4.5, together with smoothed geographical distribution of isolates, based on the percentage of inhabitants for whom at least one isolate was included in those analyses, by 4-digit postal code area and with regional cooperative network borders

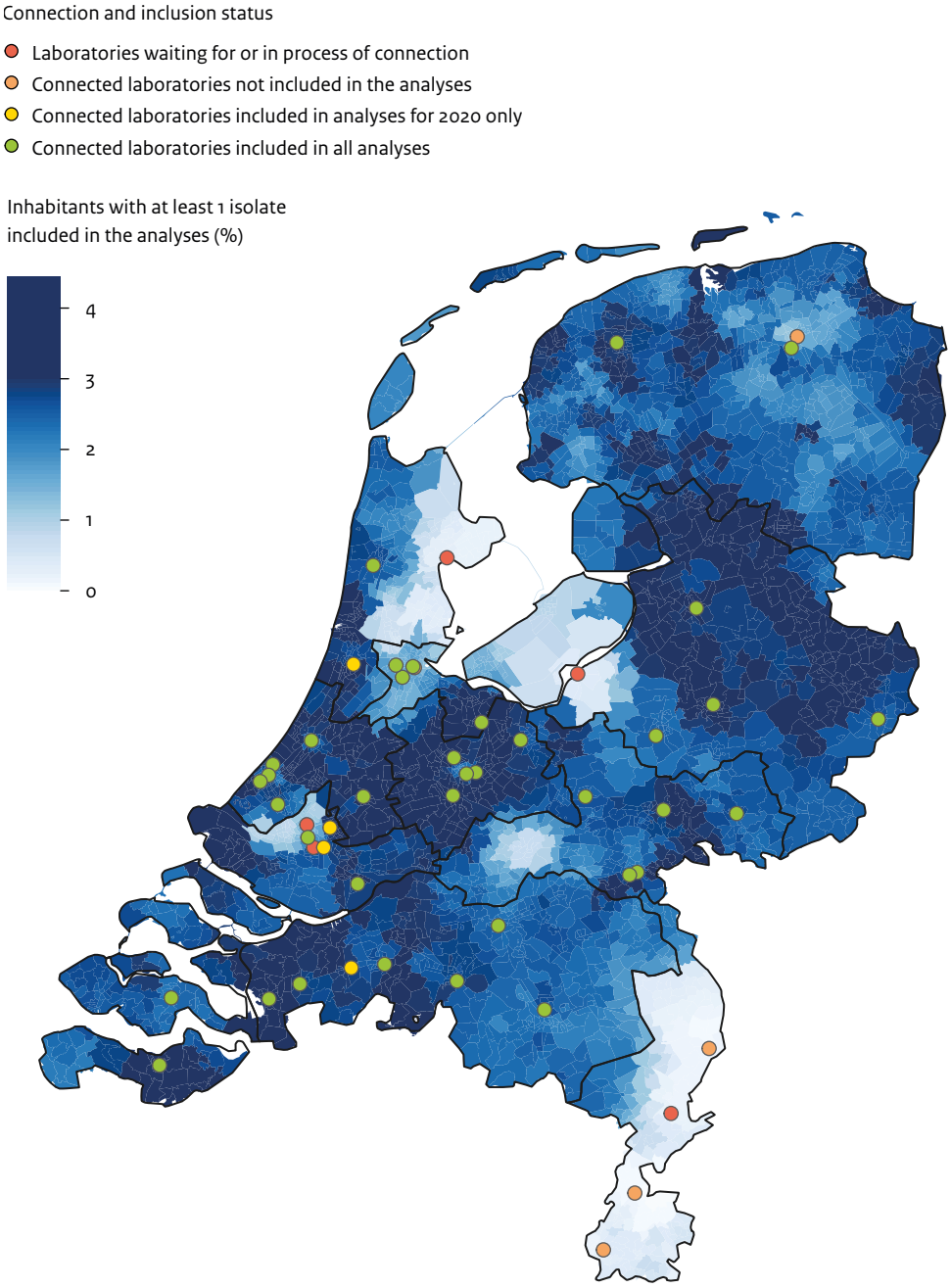


Table 4.1.2.1 Characteristics of 394,853 isolates included in the analyses in sections 4.2 through 4.5, by pathogen

	E. coli	*K. pneumoniae*	*P. mirabilis*	*E. cloacae* complex	*P. aeruginosa*	*Acinetobacter* spp.	*E. faecalis*	*E. faecium*	*S. aureus*	CNS
Total number of isolates	167,200	28,300	20,472	10,106	22,135	3,863	26,699	6,088	49,044	21,844
Sex of patient (%)										
Male	28	34	41	55	54	52	53	54	54	60
Female	72	66	59	45	46	48	47	46	46	40
Setting of care (%)										
General practices	61	50	49	34	33	45	45	8	27	9
Outpatient departments	14	18	17	22	29	24	20	11	38	13
Inpatient departments (excl. Intensive Care Units)	19	23	21	34	29	24	28	56	28	58
Intensive Care Units	1	2	1	5	4	4	3	21	4	19
Long-term care facilities	5	7	11	4	5	2	5	3	3	1
Age category of patient in years (%)										
0-4	3	1	3	4	2	4	3	1	4	5
5-18	5	2	2	2	6	5	2	1	7	3
19-64	34	27	21	29	30	32	27	32	44	40
>65	57	70	74	65	62	59	67	66	44	53
Isolate source (%)										
Blood	3	4	2	4	3	4	4	17	5	51
Lower respiratory tract	1	3	2	9	16	10	0	3	16	0
Urine	90	86	83	59	44	61	84	49	14	14
Wound/Pus	4	5	12	24	34	23	10	26	57	27
Genital	1	0	0	0	1	0	0	0	3	0
Other	1	1	1	4	3	3	1	5	4	8
Type of hospital (hospital isolates only, %)										
General	41	36	39	31	32	27	35	26	32	29
Top clinical	46	48	48	46	46	47	49	49	48	43
University hospital	13	17	13	23	22	26	16	25	21	29

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

Table 4.1.2.1 (Continued) Characteristics of 394,853 isolates included in the analyses in sections 4.2 through 4.5, by pathogen (Continued)

	β-haemolytic Streptococcus spp. group A	β-haemolytic Streptococcus spp. group B	β-haemolytic Streptococcus spp. group C	β-haemolytic Streptococcus spp. group G	*S. anginosus*	*S. mitis/S. oralis*	*B. fragilis* complex	*C. perfringens*	*S. pneumoniae*	*H. influenzae*	*M. catarrhalis*
Total number of isolates	2,496	18,570	1,222	1,861	1,874	921	1,379	332	2,870	6,157	1,420
Sex of patient (%)											
Male	41	22	51	51	49	57	55	55	55	53	51
Female	59	78	49	49	51	43	45	45	45	47	49
Setting of care (%)											
General practices	40	51	36	28	10	15	3	5	6	11	11
Outpatient departments	30	24	28	33	25	21	20	14	29	46	41
Inpatient departments (excl. Intensive Care Units)	27	22	32	36	58	58	71	74	55	36	40
Intensive Care Units	2	1	1	1	6	5	6	7	9	6	5
Long-term care facilities	1	2	3	2	1	1	1	1	0	1	2
Age category of patient in years (%)											
0-4	16	1	1	1	1	5	2	1	7	9	11
5-18	14	3	4	4	6	6	6	1	3	5	3
19-64	51	66	56	51	51	46	40	31	37	36	30
>65	19	30	39	45	41	43	53	67	54	51	56
Isolate source (%)											
Blood	7	2	7	9	12	38	24	30	29	2	1
Lower respiratory tract	11	2	6	5	2	0	1	0	56	86	89
Urine	9	54	20	18	20	27	1	2	1	0	0
Wound/Pus	50	12	49	53	61	32	70	62	11	9	10
Genital	20	28	14	14	4	0	2	2	0	1	0
Other	4	2	4	2	2	3	2	3	2	1	0
Type of hospital (hospital isolates only, %)											
General	34	35	39	37	31	19	37	31	35	30	32
Top clinical	51	49	48	45	54	45	42	50	50	49	52
University hospital	15	15	13	19	15	37	21	19	15	21	17

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

Key results

Coverage

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

Isolate characteristics

- *E. coli* (72%), *K. pneumoniae* (66%), *P. mirabilis* (59%), and β-haemolytic *Streptococcus* spp. groups A (59%) and B (78%) were more often isolated from female patients than from male patients. Coagulase-negative *Staphylococcus* spp. (60%) and *S. mitis/S. oralis* (57%) were more often isolated from males. 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*, β-haemolytic *Streptococcus* spp. groups A, B, C, G, *H. influenzae*, and *M. catarrhalis* were most often isolated from patients receiving outpatient care (General practices, outpatient hospital departments, and long term care facilities, combined 54%-80%, depending on the pathogen), whereas a large part of *E. faecium*, coagulase-negative *Staphylococcus* spp., *S. anginosus*, *S. mitis/S. oralis*, *B. fragilis* complex, *C. perfringens,* and *S. pneumoniae* was isolated from inpatients (combined 63%-81%, depending on the pathogen).
- For most pathogens, the majority of isolates originated from patients of 65 years and older (51-74%, depending on the pathogen). For *S. aureus* 44% of the isolates was from patients aged between 19 and 65 years and 44% from those aged >65 years. For β-haemolytic *Streptococcus* spp. groups A, B, C, G, and for *S. anginosus*, 51-66% of the isolates originated from patients aged 19-64 years.
- *E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, *P. aeruginosa*, *Acinetobacter* spp., *E. faecalis*, *E. faecium*, and β-haemolytic *Streptococcus* spp. group B were mainly isolated from urine (44-90%, depending on the pathogen), whereas *S. aureus*, β-haemolytic *Streptococcus* spp. groups A and G, *S. anginosus*, *B. fragilis* complex, and *C. perfringens* were mainly isolated from wound or pus (53-70%, depending on the pathogen). Coagulase-negative *Staphylococcus* spp. were mainly isolated from blood (51%), and *H. influenzae*, *S. pneumoniae*, and *M. catarrhalis* from the lower respiratory tract (56-89%).
- Depending on the pathogen, 13 to 29% of the isolates originated from university hospital patients.

4.2 Primary care

The distribution of pathogens in diagnostic urine, wound or pus, respiratory, and genital samples from general practitioners' (GP) patients in 2020 is presented in table 4.2.1. The resistance levels in 2020 for *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* isolates from urine samples are presented in table 4.2.2. In accordance with age categories used in the guidelines of the Dutch College of General Practitioners (NHG) for urinary tract infections, resistance levels and five-year trends for urine isolates are calculated separately for patients aged ≤12 years and patients aged >12 years. For *S. aureus* isolates from wound or pus samples resistance levels in 2020 are presented in table 4.2.3, and for β-haemolytic *Streptococcus* spp. group A isolates from wound/pus, respiratory, or genital samples as well as for β-haemolytic *Streptococcus* spp. group B isolates from urine or genital samples in table 4.2.4. Five-year trends in resistance are shown in figure 4.2.1 (*E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa*), figure 4.2.4 (*S. aureus*) and figure 4.2.6 (β-haemolytic *Streptococcus* spp. group A and group B). Finally, the smoothed geographical distribution of diagnostic isolates, and resistance levels for a selection of antibiotics in *E. coli*, *K. pneumoniae*, and *S. aureus* are shown by regional cooperative network in figures 4.2.2a and 4.2.2b (*E. coli*), 4.2.3a and 4.2.3b (*K. pneumoniae*), and 4.2.5a and 4.2.5b (*S. aureus*).

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

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

Table 4.2.1 Distribution of isolated pathogens in diagnostic urine samples (by patient age category) and diagnostic wound or pus, respiratory, and genital samples from selected general practitioners' patients, ISIS-AR 2020

Pathogen	Urine		Wound or pus	Respiratory tract	Genital
	Age≤12 N (%)	Age>12 N (%)	N (%)	N (%)	N (%)
E. coli	9,357 (73)	94,032 (54)	660 (4)	103 (3)	386 (8)
K. pneumoniae	240 (2)	13,932 (8)	195 (1)	70 (2)	56 (1)
P. mirabilis	644 (5)	9,119 (5)	518 (3)	36 (1)	49 (1)
Other *Enterobacterales*[1]	624 (5)	18,688 (11)	1,746 (10)	306 (10)	108 (2)
P. aeruginosa	181 (1)	4,416 (3)	2,837 (16)	223 (7)	81 (2)
Other non-fermenters[2]	141 (1)	2,658 (2)	601 (3)	244 (8)	13 (0)
Other Gram-negatives[3]	3 (0)	11 (0)	257 (1)	529 (18)	61 (1)
S. aureus	125 (1)	3,337 (2)	8,251 (47)	1,173 (39)	920 (18)
β-haemolytic *Streptococcus* spp. group A	84 (1)	85 (0)	412 (2)	79 (3)	491 (10)
β-haemolytic *Streptococcus* spp. group B	110 (1)	6,622 (4)	491 (3)	38 (1)	2,461 (49)
Other Gram-positives[4]	1,333 (10)	20,520 (12)	1,642 (9)	174 (6)	427 (8)

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

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

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

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

Table 4.2.2 Resistance levels (%) among diagnostic urine isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* from selected general practitioners' patients, by age category, ISIS-AR 2020

	E. coli		*K. pneumoniae*		*P. mirabilis*		*P. aeruginosa*	
	age≤12	age>12	age≤12	age>12	age≤12	age>12	age≤12	age>12
median age	6	68	4	74	3	76	4	79
Antibiotic								
amoxicillin/ampicillin	32	36	-	-	16	20	-	-
co-amoxiclav[1] - non-uuti	26	29	29	18	4	6	-	-
piperacillin-tazobactam	-	-	-	-	-	-	2	4
cefuroxime	4	8	7	13	0	1	-	-
cefotaxime/ceftriaxone	2	4	3	4	0	1	-	-
ceftazidime	2	3	5	3	0	0	1	1
meropenem	-	-	-	-	-	-	1	1
imipenem	-	-	-	-	-	-	3	5
ciprofloxacin	5	10	5	12	6	10	1	10
gentamicin	3	4	1	2	5	6	-	-
tobramycin	3	4	1	2	2	3	0	1
fosfomycin	1	1	16	31	6	16	-	-
trimethoprim	19	21	10	18	25	31	-	-
co-trimoxazole	17	19	6	7	20	23	-	-
nitrofurantoin	0	2	-	-	-	-	-	-
Multidrug resistance								
HRMO[2]	3	5	6	5	3	4	1	0
multidrug resistance[3] - non-uuti	1	3	2	2	0	1	-	-

10	Significant and clinically relevant increasing trend since 2016
10	Significant and clinically relevant decreasing trend since 2016
10	No significant and clinically relevant time trend

(For the definition of a clinically relevant trend see section 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 section 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 β-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 (either phenotypical or molecular), or, if no data on confirmatory tests were available, by resistance to meropenem or imipenem (for P. mirabilis: meropenem only); for P. aeruginosa as resistant to ≥3 antimicrobial groups among fluoroquinolones, aminoglycosides, carbapenems (or, if a confirmatory test for carbapenemase production, either phenotypical or molecular, was available, we prioritized this), 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.*

Figure 4.2.1 Trends in antibiotic resistance (from left to right 2016 to 2020) 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

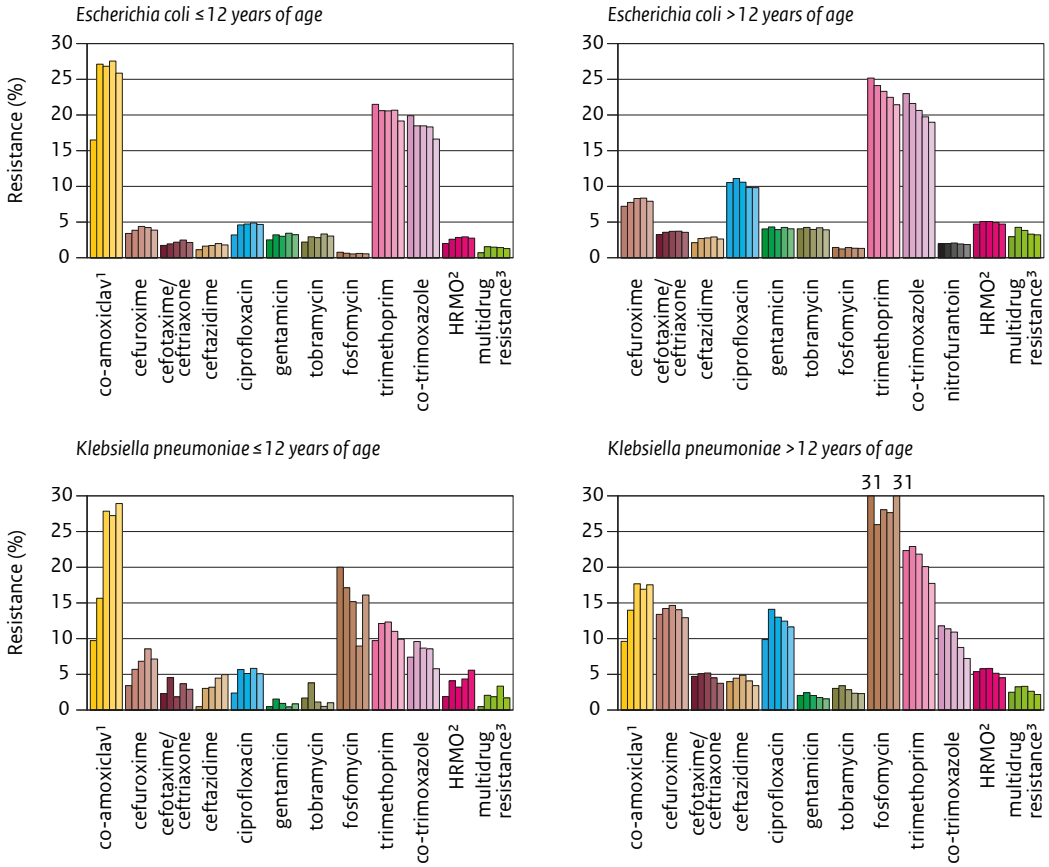
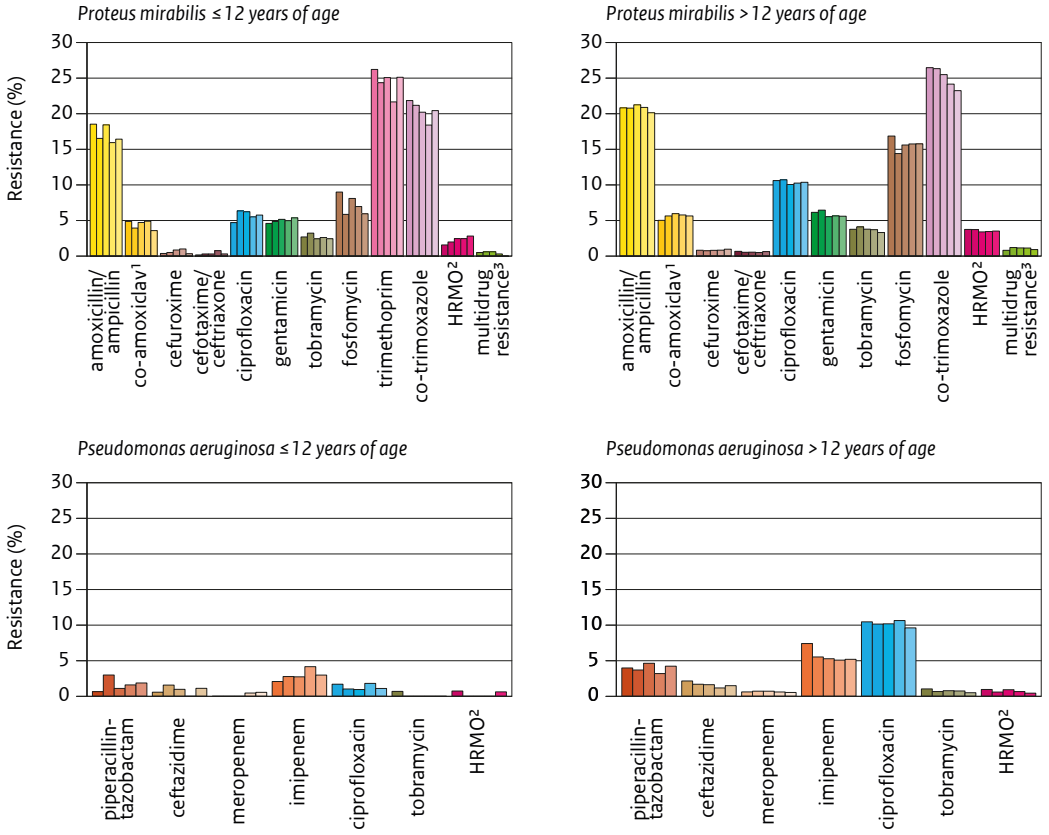


Figure 4.2.1 (Continued) Trends in antibiotic resistance (from left to right 2016 to 2020) among diagnostic urine isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* from selected general practitioners' patients in ISIS-AR, by age category

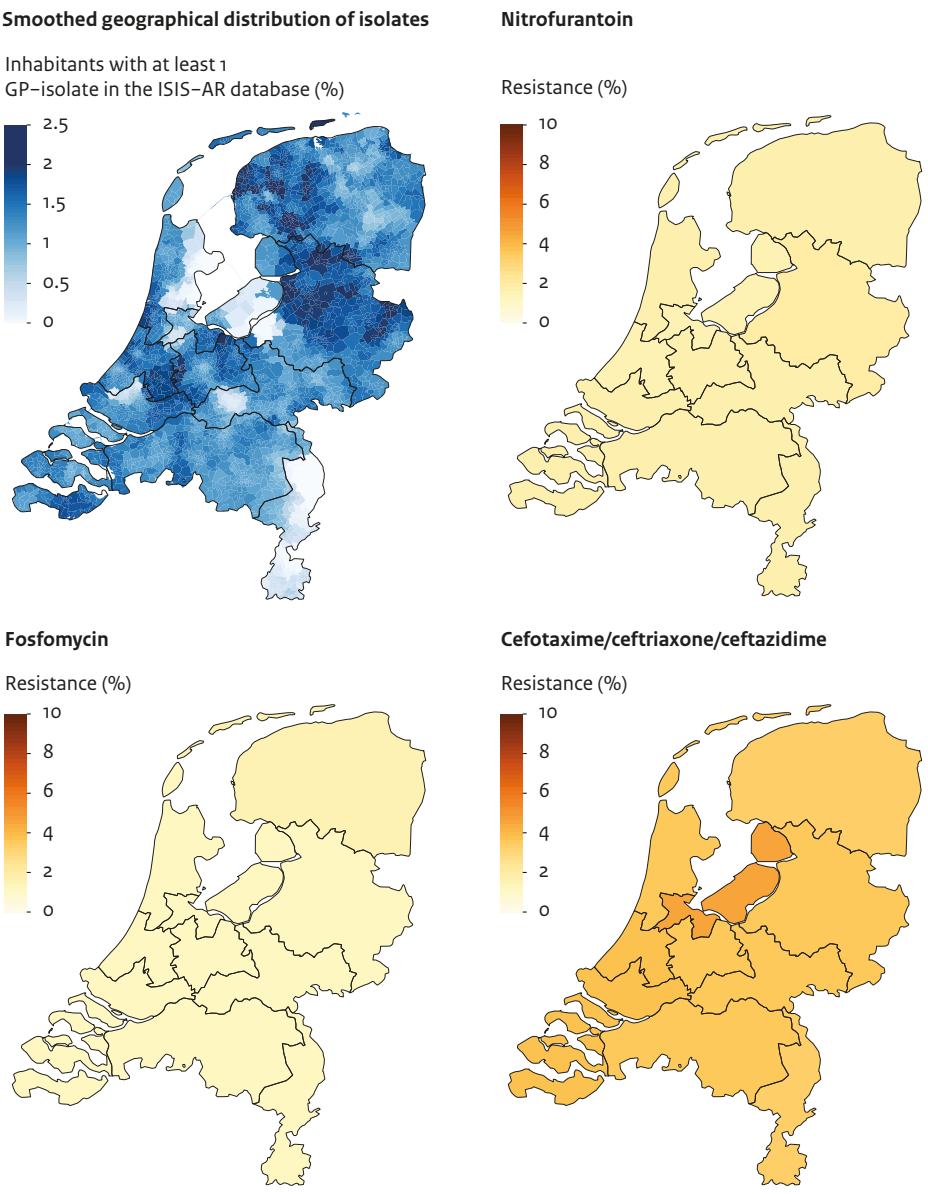


[1] *Resistance to co-amoxiclav was calculated according to the breakpoint for non-uncomplicated urinary tract infection. During 2016, a new testpanel for Gram-negative bacteria, with co-amoxiclav concentrations being adapted to EUCAST testing guidelines, was introduced for the VITEK2 automated system. This results in higher MIC values for co-amoxiclav, which subsequently influence resistance from 2016 onward to higher levels than before (see section 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 β-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 (either phenotypical or molecular), or, if no data on confirmatory tests were available, by resistance to meropenem or imipenem (for P. mirabilis: meropenem only); for P. aeruginosa as resistant to ≥3 antimicrobial groups among fluoroquinolones, aminoglycosides, carbapenems (or, if a confirmatory test for carbapenemase production, either phenotypical or molecular, was available, we prioritized this), ceftazidime, and piperacillin-tazobactam.*

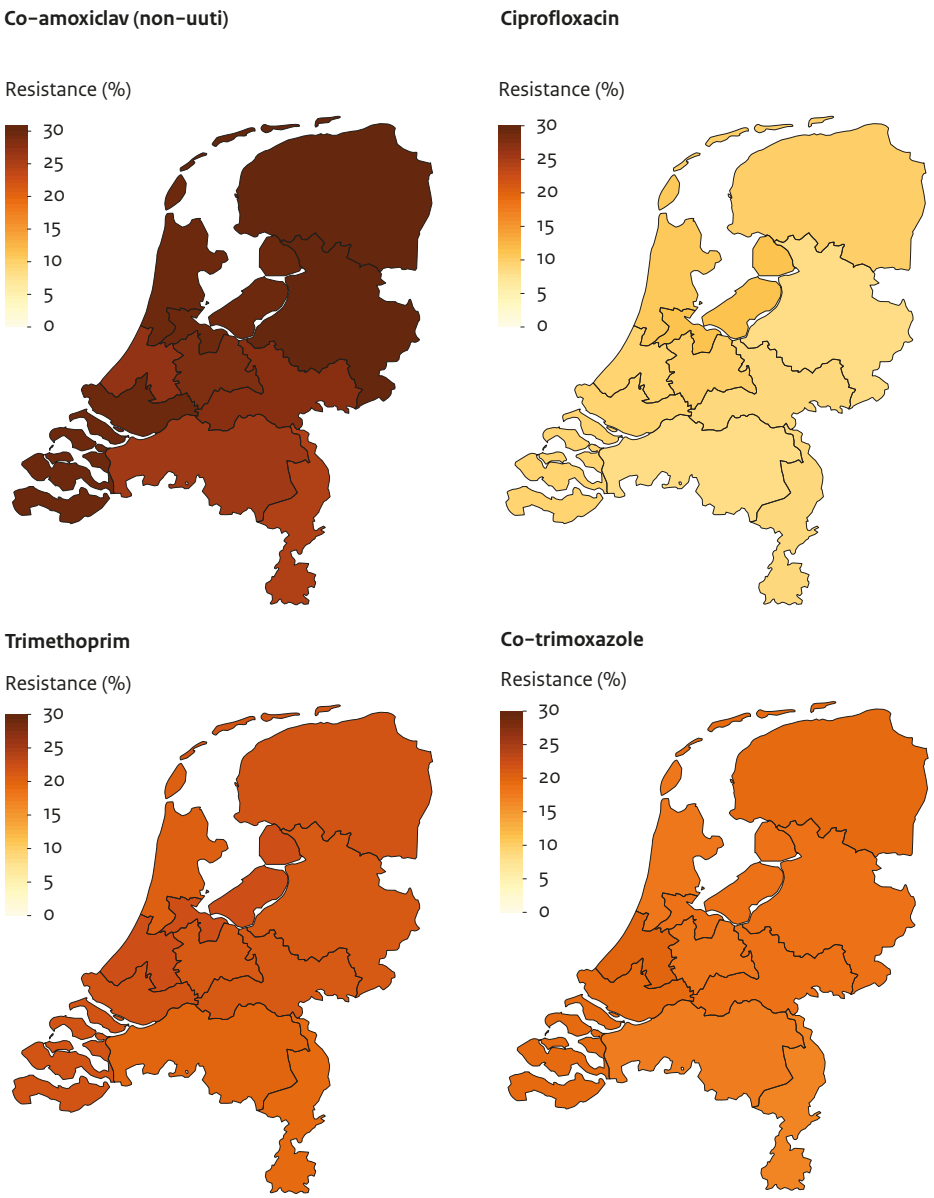
[3] *Defined as resistance to all of the following oral agents: co-amoxiclav (according to breakpoint for non-uncomplicated urinary tract infection), ciprofloxacin, and co-trimoxazole.*

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 2020



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

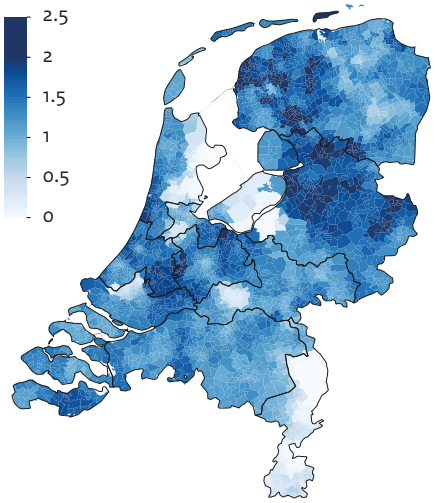
Figure 4.2.2b Resistance levels in diagnostic urinary *E. coli* isolates on a gradient scale between 0 and 30% for co-amoxiclav, ciprofloxacin, trimethoprim, and co-trimoxazole from selected general practitioners' patients, by regional cooperative network, ISIS-AR 2020



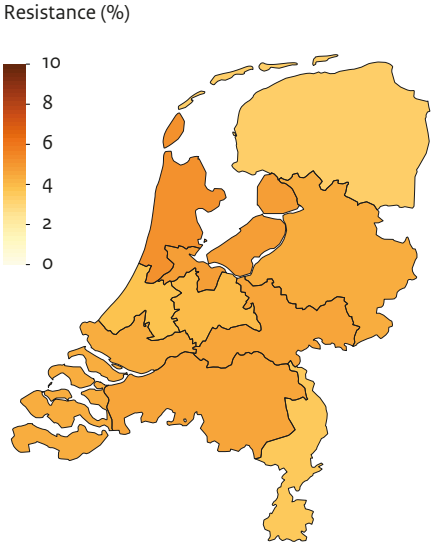
Note: No statistically significant and clinically relevant differences of regional resistance levels were found for the selected antibiotics in comparison to all regions combined (for details see section 4.1.1).
non-uuti = according to breakpoint for non-uncomplicated urinary tract infection.

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 2020

Smoothed geographical distribution of isolates



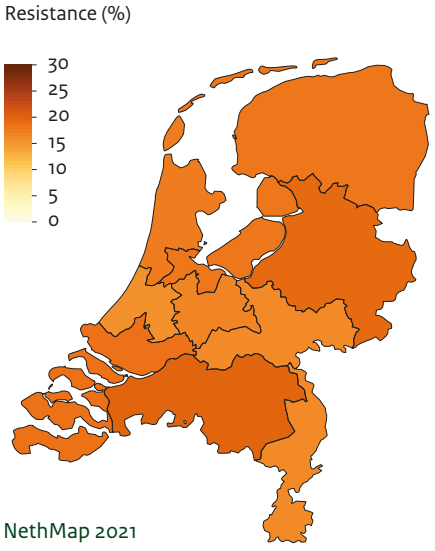
Cefotaxime/ceftriaxone/ceftazidime



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

Figure 4.2.3b Resistance levels in diagnostic urinary *K. pneumoniae* isolates on a gradient scale between 0 and 30% for co-amoxiclav, ciprofloxacin, fosfomycin, trimethoprim, and co-trimoxazole from selected general practitioners' patients, by regional cooperative network, ISIS-AR 2020

Co–amoxiclav (non–uuti)



Ciprofloxacin

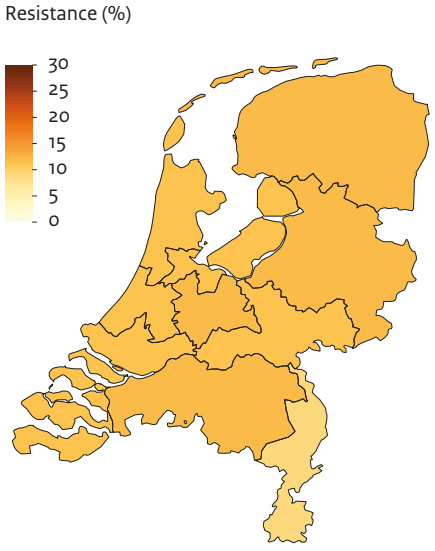
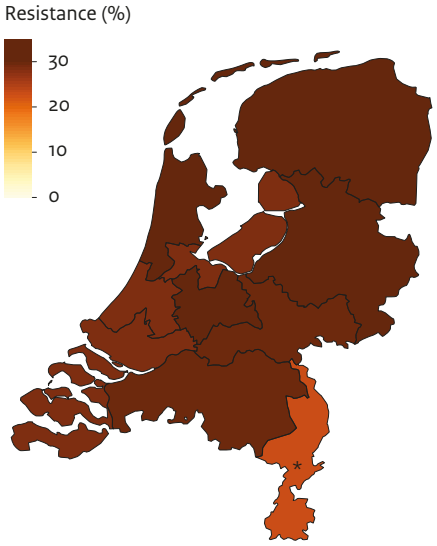
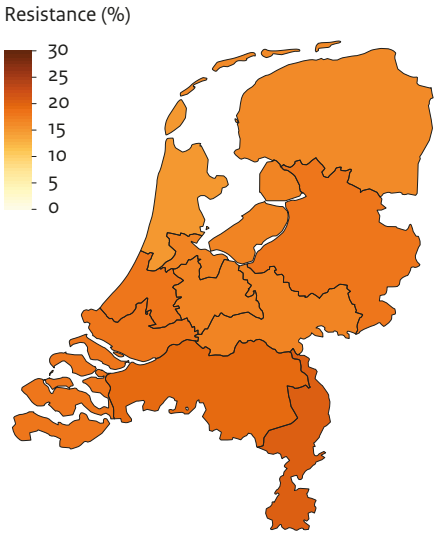


Figure 4.2.3b (continued) Resistance levels in diagnostic urinary *K. pneumoniae* isolates on a gradient scale between 0 and 30% for co-amoxiclav, ciprofloxacin, fosfomycin, trimethoprim, and co-trimoxazole from selected general practitioners' patients, by regional cooperative network, ISIS-AR 2020

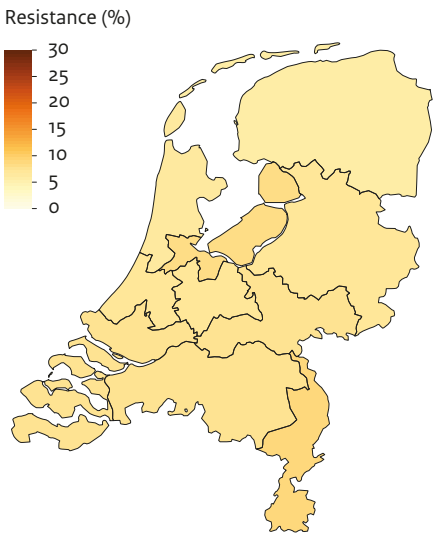
Fosfomycin



Trimethoprim



Co-trimoxazole



* *Statistically significant and clinically relevant difference of resistance in the regional cooperative network compared with all regions combined (for details see section 4.1.1).*
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 2020

	S. aureus
Antibiotic	
flucloxacillin[1]	3
ciprofloxacin[2]	4
erythromycin	13
clindamycin including inducible resistance[3]	11
doxycycline/tetracycline	4
fusidic acid	18
co-trimoxazole	3

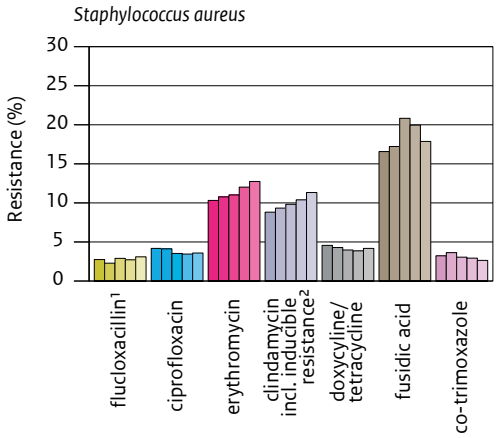
10	Significant and clinically relevant increasing trend since 2016
10	Significant and clinically relevant decreasing trend since 2016
10	No significant and clinically relevant time trend
	(For the definition of a clinically relevant trend see section 4.1.1)

[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 section 4.1.1 for more detailed information).*

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

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

Figure 4.2.4 Trends in antibiotic resistance (from left to right 2016 to 2020) among diagnostic wound or pus isolates of *S. aureus* from selected general practitioners' patients in ISIS-AR

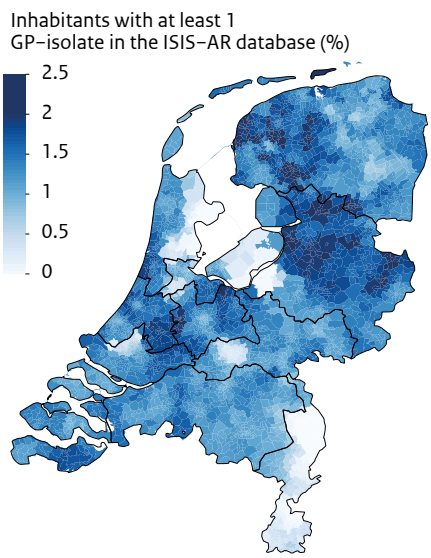


[1] *Resistance to flucloxacillin was estimated based on laboratory S/I/R interpretation for cefoxitin, or, if no cefoxitin test was available, for oxacillin/flucloxacillin (see section 4.1.1 for more detailed information).*

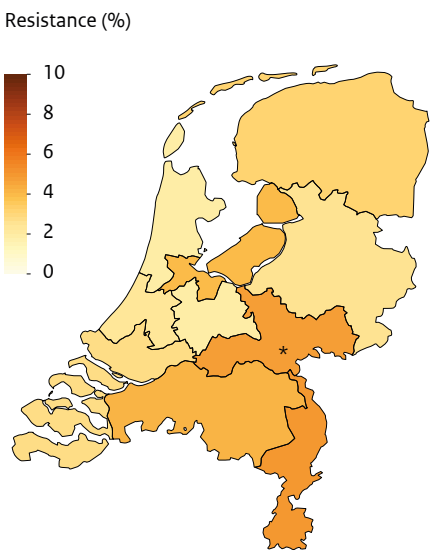
[2] *To estimate clindamycin resistance including inducible resistance, the laboratory S/I/R interpretation was used (see section 4.1.1 for more detailed information).*

Figure 4.2.5a Smoothed geographical distribution of isolates from selected general practitioners' patients, based on percentage of inhabitants for whom at least one isolate was included in the analyses, and the resistance levels in diagnostic wound or pus *S. aureus* isolates on a gradient scale between 0 and 10% for flucloxacillin and clindamycin including inducible resistance by regional cooperative network, ISIS-AR 2020

Smoothed geographical distribution of isolates



Flucloxacillin[1]

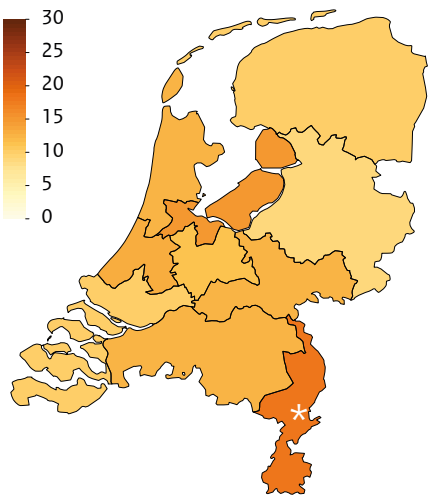


* *Statistically significant and clinically relevant difference of resistance in the regional cooperative network compared with all regions combined (for details see section 4.1.1).*

[1] *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 section 4.1.1 for more detailed information).*

Figure 4.2.5b Resistance levels in diagnostic wound or pus *S. aureus* isolates on a gradient scale between 0 and 30% for clindamycin including inducible resistance by regional cooperative network, ISIS-AR 2020

Clindamycin incl. inducible resistance[1]



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

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

Table 4.2.4 Resistance levels (%) among diagnostic wound/pus, respiratory or genital isolates of β-haemolytic *Streptococcus* spp. group A and diagnostic urine or genital isolates of β-haemolytic *Streptococcus* spp. group B from selected general practitioners' patients, ISIS-AR 2020

	β-haemolytic *Streptococcus* spp. group A	β-haemolytic *Streptococcus* spp. group B
Antibiotic		
erythromycin	8	19*
clindamycin including inducible resistance[1]	8	16
doxycycline/tetracycline	21	77
co-trimoxazole	4	2

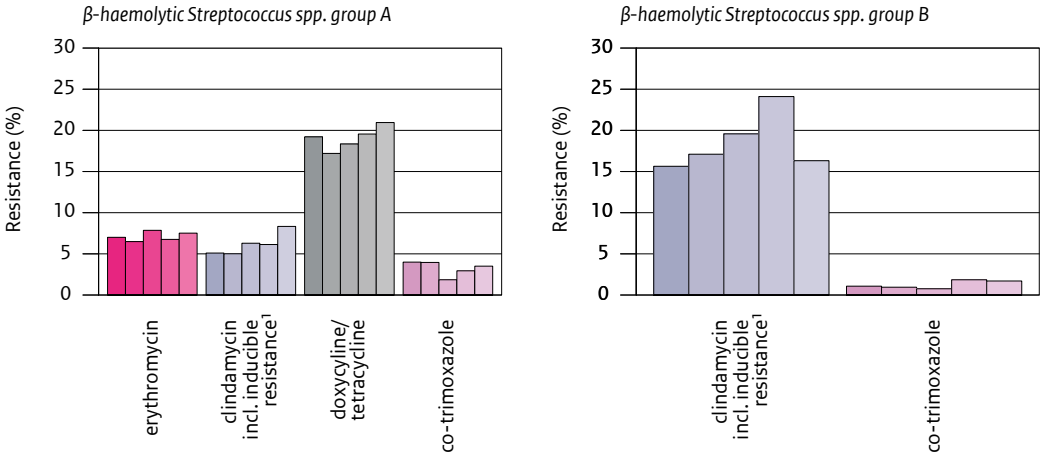
10	Significant and clinically relevant increasing trend since 2016
10	Significant and clinically relevant decreasing trend since 2016
10	No significant and clinically relevant time trend

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

* *Trend not calculated because data from the years before 2020 did not meet the criteria for trend analysis (see section 4.1.1).*

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

Figure 4.2.6 Trends in antibiotic resistance (from left to right 2016 to 2020) among diagnostic wound/pus, respiratory or genital isolates of β-haemolytic *Streptococcus* spp. group A and diagnostic urine or genital isolates of β-haemolytic *Streptococcus* spp. group B from selected general practitioners' patients in ISIS-AR



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

Key results

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

Enterobacterales

- Resistance levels in selected GP patients aged >12 years were generally higher than in patients aged ≤12 years, except for co-amoxiclav (29% in patients ≤12 years old and 18% in patients >12 years old) and ceftazidime (5% in patients ≤12 years old and 3% in patients >12 years old) in *K. pneumoniae*.
- For all *Enterobacterales*, resistance levels of 10% or lower were observed for cefuroxime (≤8%, except for *K. pneumoniae* in patients aged >12 years, 13%), cefotaxime/ceftriaxone (≤4%), ceftazidime (≤5%), ciprofloxacin (≤10%, except for *K. pneumoniae* in patients aged >12 years, 12%), gentamicin (≤6%), and tobramycin (≤4%). Additionally, resistance levels ≤10% were found for fosfomycin (1%) and nitrofurantoin (≤2%) in *E. coli*, for trimethoprim (patients aged ≤ 12 years only, 10%) and co-trimoxazole (≤7%) in *K. pneumoniae*, and for co-amoxiclav (≤6%) and fosfomycin (patients aged ≤12 years only, 6%) in *P. mirabilis*.
- Resistance levels ≥20% were found for amoxicillin/ampicillin (≥32%), co-amoxiclav (≥26%), and trimethoprim (patients aged >12 years only, 21%) in *E. coli;* for co-amoxiclav (patients aged ≤12 years only, 29%), and fosfomycin (patients aged>12 years only, 31%) in *K. pneumoniae*; and for amoxicillin/ampicillin (patients >12 years only, 20%), trimethoprim (≥25%), and co-trimoxazole (≥20%) 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 17% in 2016 to 26% in 2020 for patients aged ≤12 years and from 20% to 29% for patients aged >12 years; in *K. pneumoniae* from 10% to 29% and from 10% to 18% 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 section 4.1.1). Notably, in the last four years, resistance for this agent in *E. coli* remained rather stable. A statistically significant and clinically relevant increase in resistance was also found for ceftazidime in *K. pneumoniae* from patients aged ≤12 years (from 0% in 2016 to 5% in 2020). Furthermore, there was a statistically significant and clinically relevant decrease in resistance to trimethoprim (from 22% in 2016 to 18% in 2020) and co-trimoxazole (from 12% to 7%) in *K. pneumoniae* from patients aged >12 years.
- The percentage of HRMO and multidrug resistance was ≤6% in all *Enterobacterales*.
- 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 lower resistance percentage was found for fosfomycin in the regional cooperative network 'Limburgs infectiepreventie en antibioticaresistentie netwerk (LINK)' (23% in the region versus 31% in all regions combined).

P. aeruginosa

- Resistance levels ≤10% were found for each of the selected agents in both age groups.
- The percentage of HRMO was ≤1% in both age groups.

S. aureus

- Resistance levels of 10% or lower were observed for flucloxacillin (3%), ciprofloxacin (4%), doxycycline/tetracycline (4%), and co-trimoxazole (3%).
- For *S. aureus*, a statistically significant and clinically relevant higher resistance percentage was found for flucloxacillin in the regional cooperative network 'Gelders Antibioticaresistentie & Infectiepreventie Netwerk (GAIN)' (5% in the region versus 3% in all regions combined) and for clindamycin incl. inducible resistance in the regional cooperative network 'Limburgs infectiepreventie en antibioticaresistentie netwerk (LINK)' (18% in the region versus 12% in all regions combined).

β-haemolytic *Streptococcus* spp. group A and group B

- For both β-haemolytic *Streptococcus* spp. group A and group B, a resistance level of 10% or lower was observed for co-trimoxazole (≤4%). Resistance levels ≤10% were also found for erythromycin (8%), and clindamycin including inducible resistance (8%) in β-haemolytic *Streptococcus* spp. group A.
- For both β-haemolytic *Streptococcus* spp. group A and group B, a resistance level of 20% or higher was observed for doxycycline/tetracycline (≥21%).
- There was a statistically significant and clinically relevant increase in resistance to clindamycin including inducible resistance (from 5% in 2016 to 8% in 2020) in β-haemolytic *Streptococcus* spp. group A.

4.3 Hospital departments

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

4.3.1 Outpatient departments

The distribution of pathogens isolated from diagnostic samples (lower respiratory tract, urine, and wound or pus) from patients attending outpatient departments in 2020 is presented in table 4.3.1.1. The resistance levels for a selection of pathogens isolated from these patients in 2020 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 2020

	Lower respiratory tract	Urine	Wound or pus
Pathogen	**N (%)**	**N (%)**	**N (%)**
E. coli	392 (4)	20,810 (42)	1,931 (6)
K. pneumoniae	183 (2)	4,420 (9)	465 (1)
P. mirabilis	105 (1)	2,346 (5)	1,093 (3)
Other *Enterobacterales*[1]	701 (8)	6,872 (14)	3,382 (10)
P. aeruginosa	1,490 (17)	1,822 (4)	3,182 (10)
Other non-fermenters[2]	972 (11)	822 (2)	862 (3)
Other Gram-negatives[3]	2,686 (30)	26 (0)	793 (2)
S. aureus	1,609 (18)	1,780 (4)	13,539 (42)
Other Gram-positives[4]	761 (9)	11,179 (22)	6,990 (22)

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

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

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

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

Table 4.3.1.2 Resistance levels (%) among diagnostic isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* from patients attending outpatient departments, ISIS-AR 2020

	E. coli	*K. pneumoniae*	*P. mirabilis*	*P. aeruginosa*
Antibiotic				
amoxicillin/ampicillin	42	-	22	-
co-amoxiclav[1] - non-uuti	34	20	7	-
piperacillin-tazobactam	4	9	0	6
cefuroxime	12	14	1	-
cefotaxime/ceftriaxone	6	7	1	-
ceftazidime	4	6	0	3
meropenem/imipenem	0	0	-	-
meropenem	-	-	0	2
imipenem	-	-	-	5
ciprofloxacin	16	13	12	14
gentamicin	6	3	7	-
tobramycin	6	5	4	3
fosfomycin	2	29	14	-
trimethoprim	26	21	31	-
co-trimoxazole	23	12	25	-
nitrofurantoin	3	-	-	-
Empiric therapy combinations				
gentamicin + co-amoxiclav - non-uuti	4	3	2	-
gentamicin + cefuroxime	2	3	0	-
gentamicin + cefotaxime/ceftriaxone	1	3	0	-
ciprofloxacin + co-amoxiclav - non-uuti	10	6	2	-
ciprofloxacin + cefuroxime	6	8	0	-
ciprofloxacin + cefotaxime/ceftriaxone	4	5	0	-
Multidrug resistance				
HRMO[2]	8	8	4	1
multidrug resistance[3]- non-uuti	6	4	1	-

10	Significant and clinically relevant increasing trend since 2016
10	Significant and clinically relevant decreasing trend since 2016
10	No significant and clinically relevant time trend

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

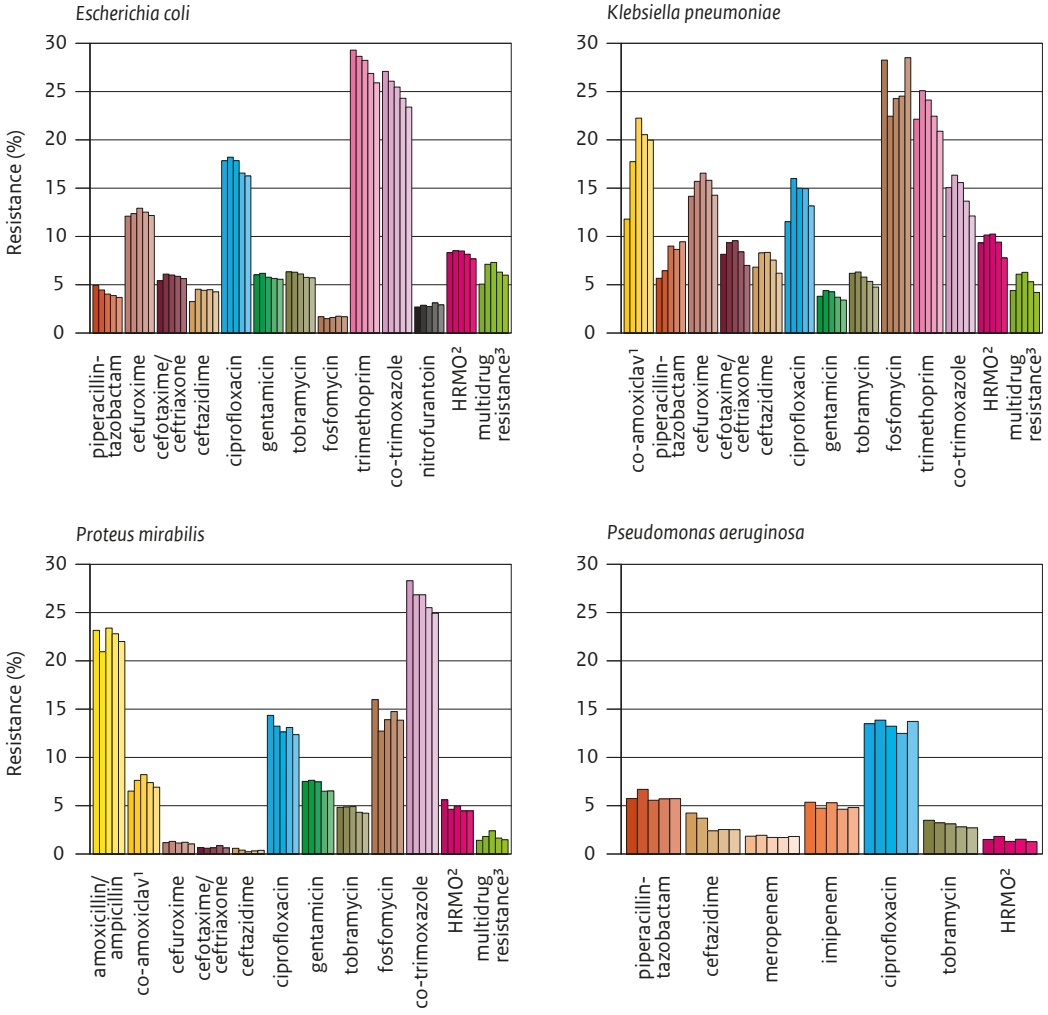
- = Resistance not calculated.
non-uuti = according to breakpoint for non-uncomplicated urinary tract infection.

[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 section 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 ß-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 (either phenotypical or molecular), or, if no data on confirmatory tests were available, by resistance to meropenem or imipenem (for P. mirabilis: meropenem only); for P. aeruginosa as resistant to ≥3 antimicrobial groups among fluoroquinolones, aminoglycosides, carbapenems (or, if a confirmatory test for carbapenemase production, either phenotypical or molecular, was available, we prioritized this), 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.*

Figure 4.3.1.1 Trends in antibiotic resistance (from left to right 2016 to 2020) 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 section 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 β-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 (either phenotypical or molecular), or, if no data on confirmatory tests were available, by resistance to meropenem or imipenem (for P. mirabilis: meropenem only); for P. aeruginosa as resistant to ≥3 antimicrobial groups among fluoroquinolones, aminoglycosides, carbapenems (or, if a confirmatory test for carbapenemase production, either phenotypical or molecular, was available, we prioritized this), 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 2020

	S. aureus
Antibiotic	
flucloxacillin[1]	2
ciprofloxacin[2]	5
gentamicin	1
erythromycin	16
clindamycin including inducible resistance[3]	14
doxycycline/tetracycline	4
fusidic acid	8
linezolid	0
co-trimoxazole	2
rifampicin	0

10	Significant and clinically relevant increasing trend since 2016
10	Significant and clinically relevant decreasing trend since 2016
10	No significant and clinically relevant time trend

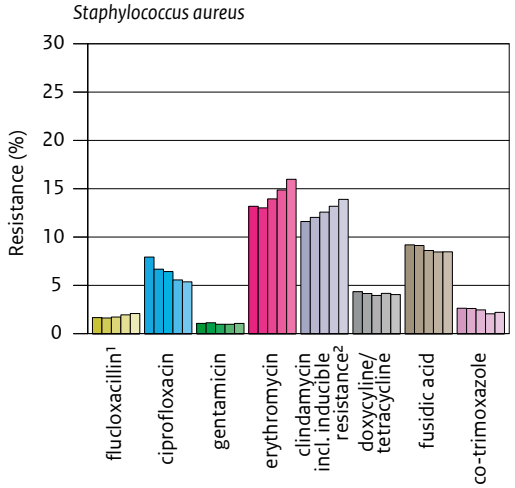
(For the definition of a clinically relevant trend see section 4.1.1)

[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 section 4.1.1 for more detailed information).*

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

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

Figure 4.3.1.2. Trends in antibiotic resistance (from left to right 2016 to 2020) 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 section 4.1.1 for more detailed information).*

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

Key results

Enterobacterales

- For all *Enterobacterales*, resistance levels of 10% or lower were found for piperacillin-tazobactam (≤9%), cefotaxime/ceftriaxone (≤7%), ceftazidime (≤6%), gentamicin (≤7%), and tobramycin (≤6%). Resistance levels ≤10% were also found for meropenem/imipenem in *E. coli* and *K. pneumoniae* (0%); fosfomycin (2%) and nitrofurantoin (3%) in *E. coli*; and co-amoxiclav (7%), cefuroxime (1%), and meropenem (0%) in *P. mirabilis*.
- Resistance of 20% or higher was found for trimethoprim in all *Enterobacterales* (≥21%), for co-amoxiclav in *E. coli* and *K. pneumoniae* (≥20%), for amoxicillin/ampicillin (≥22%) and co-trimoxazole (≥23%) in *E. coli* and *P. mirabilis*, and for fosfomycin in *K. pneumoniae* (29%).
- A statistically significant and clinically relevant increase in resistance was observed for co-amoxiclav in *E. coli* (from 23% in 2016 to 34% in 2020) and in *K. pneumoniae* (from 12% to 20%), which may be partly due to the introduction of a new testpanel for the VITEK2 automated system in 2016 (for details see section 4.1.1). Notably, in the last four years, resistance for this agent in *E. coli* remained rather stable. Additionally, in *K. pneumoniae*, a statistically significant and clinically relevant increasing trend was observed for piperacillin-tazobactam in the last five years (from 6% to 9%).
  For all *Enterobacterales* resistance levels of 10% or lower were observed for all selected empiric therapy combinations.

- For all *Enterobacterales*, the percentage HRMO was ≤8% and the percentage of multidrug resistance was ≤6%.

P. aeruginosa

- Resistance levels of 10% or lower were observed for each of the selected agents (≤6%), except for ciprofloxacin (14%).

S. aureus

- Resistance levels of 10% or lower were observed for each of the selected agents (≤8%), except for erythromycin (16%) 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) in 2020 is presented in table 4.3.2.1. The resistance levels for a selection of pathogens isolated from these patients in 2020 are presented in tables 4.3.2.2 (*E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, *P. aeruginosa*, and *Acinetobacter* spp.), 4.3.2.3 (*E. faecalis* and *E. faecium*), 4.3.2.4 (*S. aureus* and coagulase-negative *Staphylococcus* spp.), 4.3.2.5 (β-haemolytic *Streptococcus* spp. groups A, B, C, G, *S. anginosus,* and *S. mitis/S. oralis*), and 4.3.2.6 (*B. fragilis* complex and *C. perfringens*). Five-year trends in resistance are shown in figures 4.3.2.1 (*E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, *P. aeruginosa*, and *Acinetobacter* spp.), 4.3.2.2 (*E. faecalis* and *E. faecium*), 4.3.2.3 (*S. aureus* and coagulase-negative *Staphylococcus* spp.), 4.3.2.4 (β-haemolytic *Streptococcus* spp. groups A, B, C, G, *S. anginosus,* and *S. mitis/S. oralis*), and 4.3.2.5 (*B. fragilis* complex and *C. perfringens*).

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

Table 4.3.2.1 Distribution of isolated pathogens in diagnostic samples from patients admitted to inpatient departments (excl. intensive care units), ISIS-AR 2020

	Blood or cerebrospinal fluid	Lower respiratory tract	Urine	Wound or pus
Pathogen	**N (%)**	**N (%)**	**N (%)**	**N (%)**
E. coli	4,813 (21)	871 (8)	21,532 (42)	3,828 (12)
K. pneumoniae	890 (4)	463 (4)	4,342 (8)	894 (3)
P. mirabilis	307 (1)	159 (1)	3,018 (6)	850 (3)
E. cloacae complex	370 (2)	417 (4)	1,423 (3)	1,291 (4)
Other *Enterobacterales*[1]	1,179 (5)	1,261 (12)	5,315 (10)	3,010 (9)
P. aeruginosa	483 (2)	1,386 (13)	2,741 (5)	1,860 (6)
Acinetobacter spp.	131 (1)	92 (1)	319 (1)	303 (1)
Other non-fermenters[2]	99 (0)	1,029 (10)	267 (1)	375 (1)
B. fragilis complex	298 (1)	0 (0)	17 (0)	693 (2)
Other Gram-negatives[3]	193 (1)	2,386 (22)	7 (0)	206 (1)
E. faecalis	693 (3)	33 (0)	5,485 (11)	1,786 (6)
E. faecium	518 (2)	16 (0)	1,807 (3)	1,224 (4)
S. aureus	2,304 (10)	1,606 (15)	1,504 (3)	8,098 (25)
CNS	8,261 (36)	9 (0)	583 (1)	3,656 (11)
β-haemolytic *Streptococcus* spp. group A	144 (1)	30 (0)	38 (0)	365 (1)
β-haemolytic *Streptococcus* spp. group B	399 (2)	80 (1)	1,359 (3)	724 (2)
β-haemolytic *Streptococcus* spp. group C	78 (0)	16 (0)	28 (0)	226 (1)
β-haemolytic *Streptococcus* spp. group G	145 (1)	18 (0)	63 (0)	358 (1)
S. anginosus	179 (1)	5 (0)	72 (0)	805 (3)
S. mitis/S. oralis	308 (1)	3 (0)	37 (0)	188 (1)
C. perfringens	88 (0)	0 (0)	3 (0)	163 (1)
Other Gram-positives[4]	1,347 (6)	758 (7)	1,906 (4)	997 (3)

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

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

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

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

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

Table 4.3.2.2 Resistance levels (%) among diagnostic isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, *P. aeruginosa*, and *Acinetobacter* spp. from patients admitted to inpatient departments (excl. intensive care units), ISIS-AR 2020

	E. coli	*K. pneumoniae*	*P. mirabilis*	*E. cloacae* complex	*P. aeruginosa*	*Acinetobacter* spp.
Antibiotic						
amoxicillin/ampicillin	42	-	23	-	-	-
co-amoxiclav[1] - non-uuti	34	22	7	-	-	-
piperacillin-tazobactam	4	11	0	-	7	-
cefuroxime	13	15	1	-	-	-
cefotaxime/ceftriaxone	6	9	1	-	-	-
ceftazidime	5	8	0	-	4	-
meropenem/imipenem	0	0	-	0	-	1
meropenem	-	-	0	-	2	-
imipenem	-	-	-	-	5	-
ciprofloxacin	13	12	11	4	10	4
gentamicin	5	4	7	3	-	3
tobramycin	5	6	4	3	1	2
fosfomycin	1	22	13	45	-	-
trimethoprim	24	17	31	6	-	-
co-trimoxazole	21	11	25	5	-	3
nitrofurantoin	2	-	-	-	-	-
Empiric therapy combinations						
gentamicin + co-amoxiclav - non-uuti	4	3	2	-	-	-
gentamicin + cefuroxime	2	3	0	-	-	-
gentamicin + cefotaxime/ceftriaxone	1	3	0	-	-	-
tobramycin + ceftazidime	-	-	-	-	1	-
tobramycin + ciprofloxacin	-	-	-	-	1	-
ciprofloxacin + co-amoxiclav - non-uuti	8	7	2	-	-	-
ciprofloxacin + cefuroxime	6	8	0	-	-	-
ciprofloxacin + cefotaxime/ceftriaxone	4	6	0	-	-	-
Multidrug resistance						
HRMO[2]	8	10	5	2	2	2
multidrug resistance[3] - non-uuti	5	5	2	-	-	-

10	Significant and clinically relevant increasing trend since 2016
10	Significant and clinically relevant decreasing trend since 2016
10	No significant and clinically relevant time trend

(For the definition of a clinically relevant trend see section 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 section 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 β-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 (either phenotypical or molecular), or, if no data on confirmatory tests were available, by resistance to meropenem or imipenem (for P. mirabilis: meropenem only); 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 (or, if a confirmatory test for carbapenemase production, either phenotypical or molecular, was available, we prioritized this), 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.

[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 4.3.2.1 Trends in antibiotic resistance (from left to right 2016 to 2020) 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

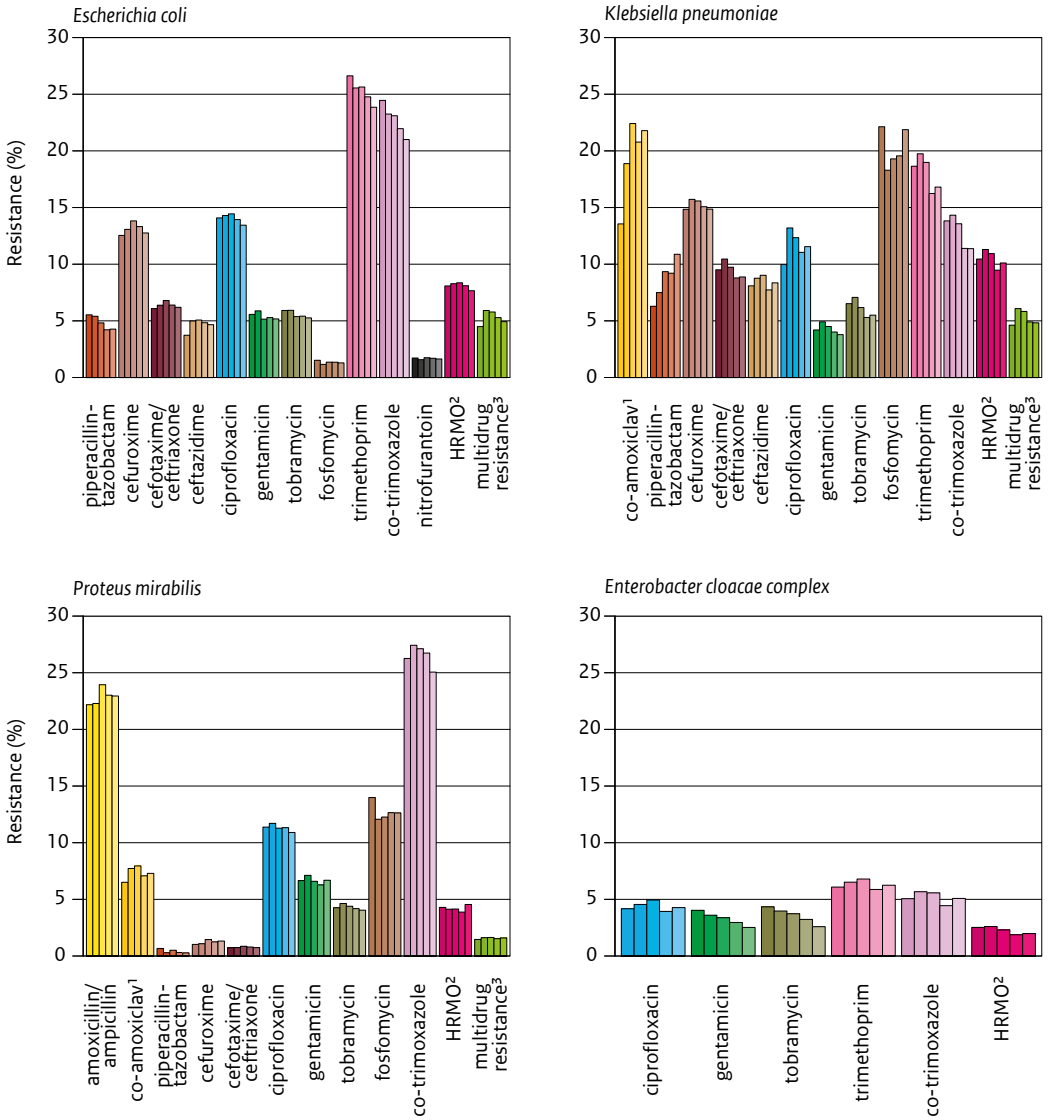
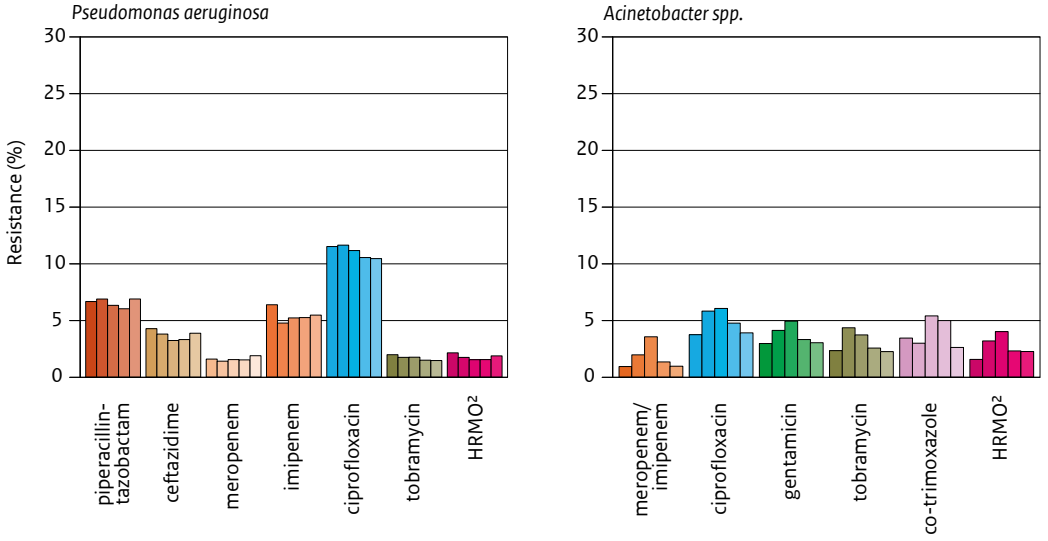


Figure 4.3.2.1 (continued) Trends in antibiotic resistance (from left to right 2016 to 2020) among diagnostic isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, *P. aeruginosa*, and *Acinetobacter* spp. from patients admitted to inpatient departments (excl. intensive care units) 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 section 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 β-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 (either phenotypical or molecular), or, if no data on confirmatory tests were available, by resistance to meropenem or imipenem (for P. mirabilis: meropenem only); 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 (or, if a confirmatory test for carbapenemase production, either phenotypical or molecular, was available, we prioritized this), 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.*

[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 4.3.2.3 Resistance levels (%) among diagnostic isolates of *E. faecalis* and *E. faecium* from patients admitted to inpatient departments (excl. intensive care units), ISIS-AR 2020

	E. faecalis	*E. faecium*
Antibiotic		
amoxicillin/ampicillin	-	86
vancomycin	0	0
nitrofurantoin	1	-

10	Significant and clinically relevant increasing trend since 2016
10	Significant and clinically relevant decreasing trend since 2016
10	No significant and clinically relevant time trend
	(For the definition of a clinically relevant trend see section 4.1.1)

- = Resistance not calculated.

Figure 4.3.2.2 Trends in antibiotic resistance (from left to right 2016 to 2020) among diagnostic isolates of *E. faecalis* and *E. faecium* from patients admitted to inpatient departments (excl. intensive care units) in ISIS-AR

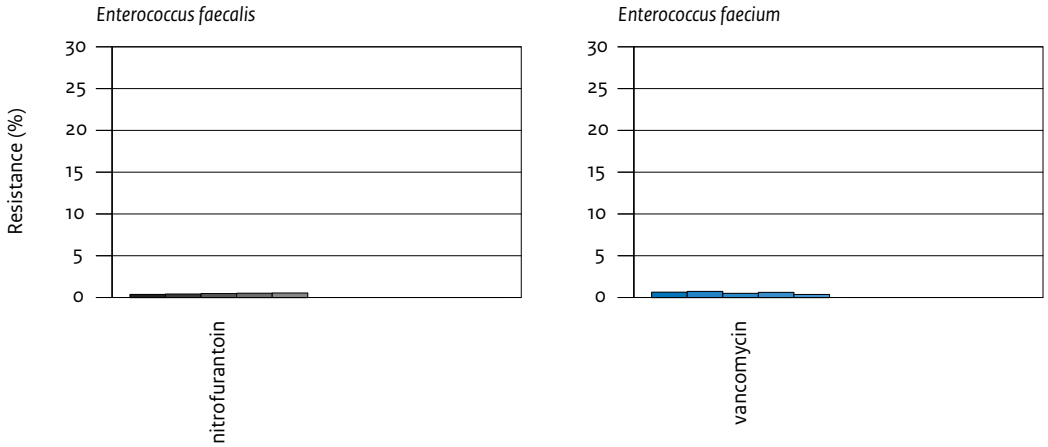


Table 4.3.2.4 Resistance levels (%) among diagnostic isolates of *S. aureus* and coagulase-negative *Staphylococcus* spp. from patients admitted to inpatient departments (excl. intensive care units), ISIS-AR 2020

	S. aureus	CNS
Antibiotic		
flucloxacillin[1]	2	41
ciprofloxacin[2]	5	29
gentamicin	1	26
erythromycin	15	43
clindamycin including inducible resistance[3]	13	30
doxycycline/tetracycline	3	16
fusidic acid	6	42
linezolid	0	0
co-trimoxazole	2	17
rifampicin	0	5

10	Significant and clinically relevant increasing trend since 2016
10	Significant and clinically relevant decreasing trend since 2016
10	No significant and clinically relevant time trend

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

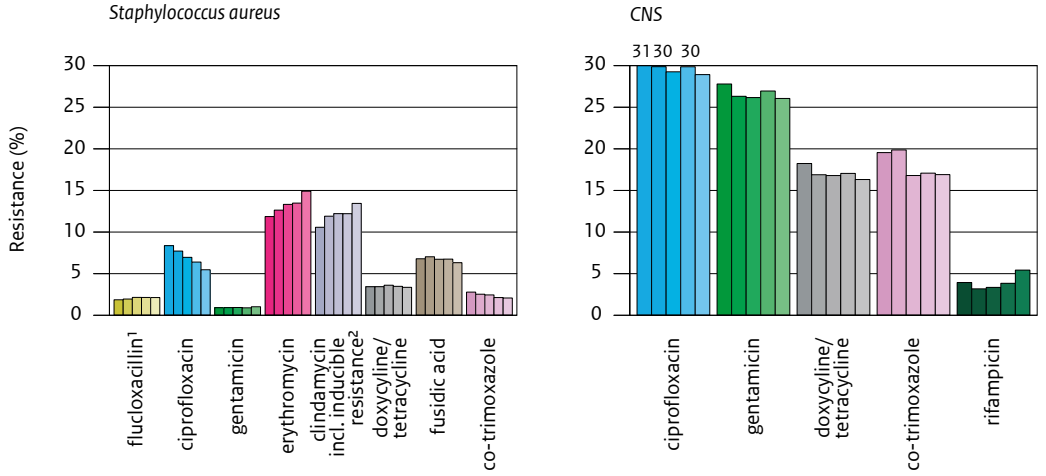
CNS = Coagulase-negative Staphylococcus spp., including S. epidermidis.

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

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

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

Figure 4.3.2.3 Trends in antibiotic resistance (from left to right 2016 to 2020) 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 section 4.1.1 for more detailed information).*

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

Table 4.3.2.5 Resistance levels (%) among diagnostic isolates of β-haemolytic *Streptococcus* spp. groups A, B, C, G, *S. anginosus*, and *S. mitis/S. oralis* from patients admitted to inpatient departments (excl. intensive care units), ISIS-AR 2020

	β-haemolytic *Streptococcus* spp. group A	β-haemolytic *Streptococcus* spp. group B	β-haemolytic *Streptococcus* spp. group C	β-haemolytic *Streptococcus* spp. group G	*S. anginosus*	*S. mitis/ S. oralis*
Antibiotic						
(benzyl)penicillin	-	-	-	-	0	5
(benzyl)penicillin (I)[1]	-	-	-	-	0	7
amoxicillin/ampicillin	-	-	-	-	-	2*
erythromycin	7	19*	5	15	-	-
clindamycin including inducible resistance[2]	7	16	8	14	8	6
doxycycline/tetracycline	19*	74*	11*	31*	-	-
co-trimoxazole	4*	1	1	0	-	-

10	Significant and clinically relevant increasing trend since 2016
10	Significant and clinically relevant decreasing trend since 2016
10	No significant and clinically relevant time trend

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

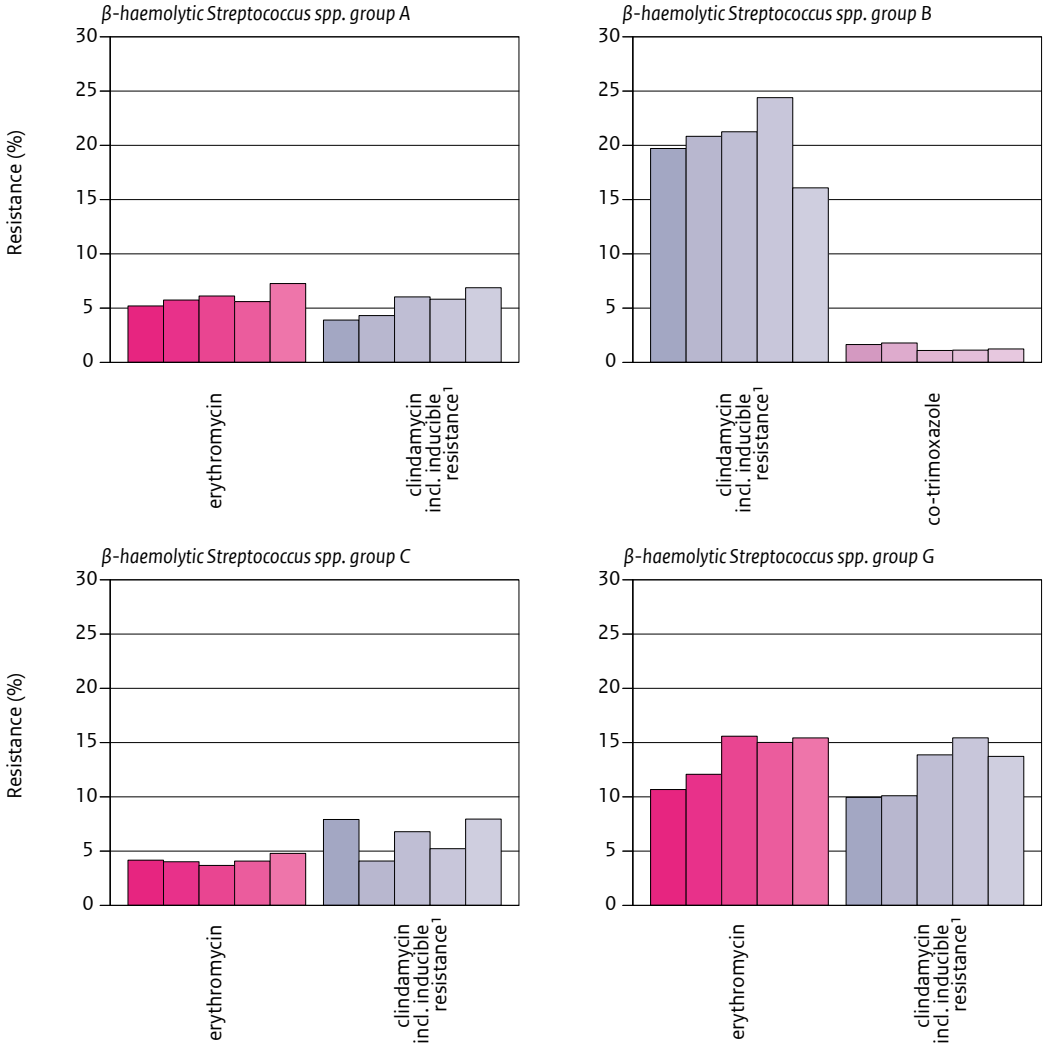
- = Resistance not calculated.

* *Trend not calculated because data from the years before 2020 did not meet the criteria for trend analysis (see section 4.1.1).*

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

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

Figure 4.3.2.4 Trends in antibiotic resistance (from left to right 2016 to 2020) among diagnostic isolates of β-haemolytic *Streptococcus* spp. groups A, B, C, G, *S. anginosus*, and *S. mitis/S. oralis* from patients admitted to inpatient departments (excl. intensive care units) in ISIS-AR



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

Figure 4.3.2.4 (continued) Trends in antibiotic resistance (from left to right 2016 to 2020) among diagnostic isolates of β-haemolytic *Streptococcus* spp. groups A, B, C, G, *S. anginosus*, and *S. mitis/S. oralis* from patients admitted to inpatient departments (excl. intensive care units) in ISIS-AR

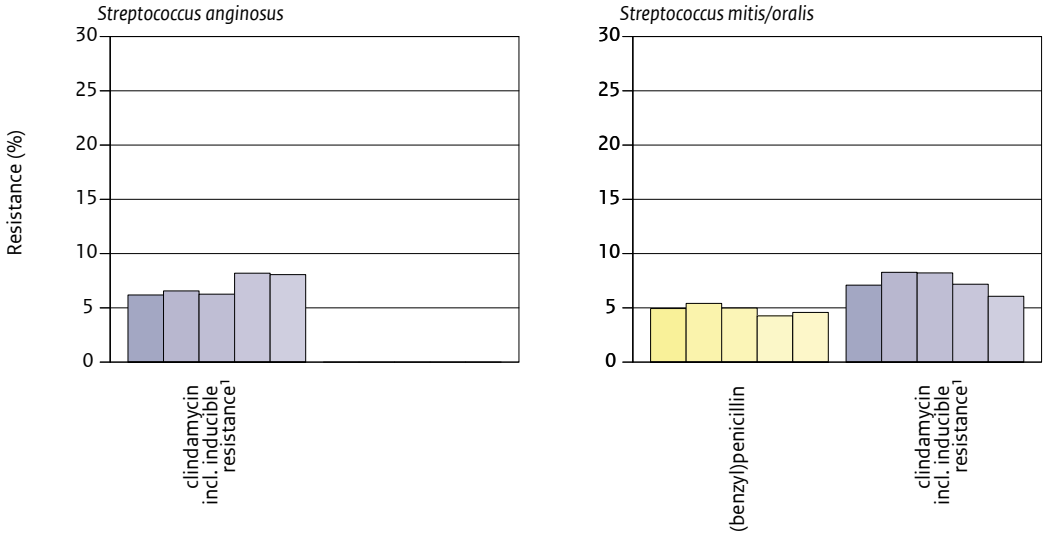


Table 4.3.2.6 Resistance levels (%) among diagnostic isolates of *B. fragilis* complex and *C. perfringens* from patients admitted to inpatient departments (excl. intensive care units), ISIS-AR 2020

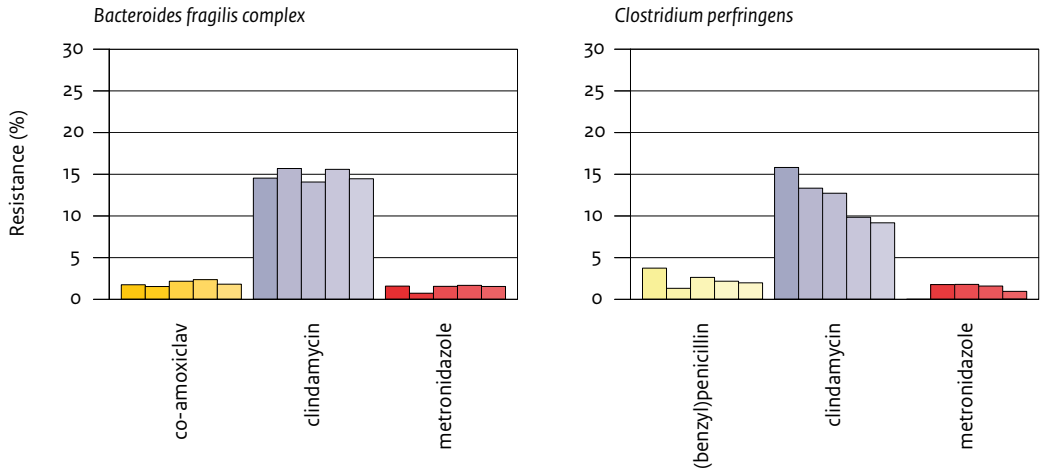
	B. fragilis complex	*C. perfringens*
Antibiotic		
(benzyl)penicillin	-	2
co-amoxiclav	2	0
clindamycin	14	9
metronidazole	2	1

10	Significant and clinically relevant increasing trend since 2016
10	Significant and clinically relevant decreasing trend since 2016
10	No significant and clinically relevant time trend

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

- = Resistance not calculated.

Figure 4.3.2.5 Trends in antibiotic resistance (from left to right 2016 to 2020) 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 cefotaxime/ceftriaxone (≤9%), ceftazidime (≤8%), gentamicin (≤7%), and tobramycin (≤6%). Resistance was also ≤10% for meropenem/imipenem (0%) in *E. coli*, *K. pneumoniae*, and *E. cloacae* complex; piperacillin-tazobactam in *E. coli* and *P. mirabilis* (≤4%); fosfomycin (1%) and nitrofurantoin (2%) in *E. coli*; co-amoxiclav (7%), cefuroxime (1%), and meropenem (0%) in *P. mirabilis*; and ciprofloxacin (4%), trimethoprim (6%), and co-trimoxazole (5%) in *E. cloacae* complex.
- Resistance was ≥20% for amoxicillin/ampicillin (≥23%), trimethoprim (≥24%), and co-trimoxazole (≥21%) in *E. coli* and *P. mirabilis*; for co-amoxiclav in *E. coli* and *K. pneumoniae* (≥22%); and for fosfomycin in *K. pneumoniae* and *E. cloacae* complex (≥22%).
- A statistically significant and clinically relevant increase in resistance was observed for co-amoxiclav in *E. coli* (from 24% in 2016 to 34% in 2020) and *K. pneumoniae* (from 14% to 22%), which may be partly due to the introduction of a new testpanel for the VITEK2 automated system in 2016 (for details see section 4.1.1). Notably, in the last four years, resistance for this agent in *E. coli* remained rather stable. In *K. pneumoniae*, a statistically significant and clinically relevant increase in resistance was observed for piperacillin-tazobactam (from 6% in 2016 to 11% in 2020).
- Resistance was ≤8% for empiric therapy combinations in all *Enterobacterales*.
- The percentage HRMO and multidrug resistance was ≤10% in all *Enterobacterales*.

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 (≤4%).
- The percentage HRMO was 2%.

E. faecalis and E. faecium

- Resistance was ≤10% for vancomycin (0% in both pathogens) and nitrofurantoin in *E. faecalis* (1%).
- Resistance was ≥20% for amoxicillin/ampicillin in *E. faecium* (86%).

S. aureus

- Resistance was ≤10% for each of the selected agents (≤6%), except for erythromycin (15%) and clindamycin including inducible resistance (13%).

Coagulase-negative Staphylococcus spp.

- Resistance was ≥20% for each of the selected agents (≥26%), except for doxycycline/tetracycline (16%), linezolid (0%), co-trimoxazole (17%), and rifampicin (5%).

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

- In all β-haemolytic *Streptococcus* spp., resistance levels ≤10% were found for co-trimoxazole (≤4%). In addition, resistance was ≤10% for erythromycin (≤7%) and clindamycin including inducible resistance (≤8%) in β-haemolytic *Streptococcus* spp. groups A and C.
- Resistance levels ≥20% were observed for doxycycline/tetracycline in β-haemolytic *Streptococcus* spp. groups B and G (≥31%).
- A statistically significant and clinically relevant increase in resistance was observed for clindamycin including inducible resistance in β-haemolytic *Streptococcus* spp. group A (from 4% in 2016 to 7% in 2020) and group G (from 10% to 14%); and for erythromycin in β-haemolytic *Streptococcus* spp. group G (from 11% to 15%).

S. anginosus and S. mitis/S. oralis

- Resistance levels ≤10% were observed for each of the selected agents (≤8%). The percentage I for (benzyl)penicillin was 0% in *S. anginosus* and 7% in *S. mitis/S. oralis*.

B. fragilis complex and C. perfringens

- Resistance was ≤10% for each of the selected agents (≤9%), except for clindamycin in *B. fragilis* complex (14%).
- A statistically significant and clinically relevant decrease in resistance was observed for clindamycin in *C. perfringens* (from 16% in 2016 to 9% in 2020).

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 in 2020 is presented in table 4.3.3.1. The resistance levels for a selection of pathogens isolated from these patients in 2020 are presented in tables 4.3.3.2 (*E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, *P. aeruginosa*, and *Acinetobacter* spp.), 4.3.3.3 (*E. faecalis and E. faecium*), and 4.3.3.4 (*S. aureus* and coagulase-negative *Staphylococcus* spp.). Five-year trends in resistance are shown in figures 4.3.3.1 (*E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, *P. aeruginosa*, and *Acinetobacter* spp.), 4.3.3.2 (*E. faecium*), and 4.3.3.3 (*S. aureus* and coagulase-negative *Staphylococcus* spp.). For β-haemolytic *Streptococcus* spp. groups A, B, C, G, *S. anginosus, S. mitis/S. oralis*, *B. fragilis* complex, and *C. perfringens*, resistance levels and trends were not calculated because in 2020 results for the majority of antibiotics were available for less than 100 isolates.

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

Table 4.3.3.1 Distribution of isolated pathogens in diagnostic samples from patients admitted to intensive care units, ISIS-AR 2020

	Blood or cerebrospinal fluid	Lower respiratory tract	Urine	Wound or pus
Pathogen	**N (%)**	**N (%)**	**N (%)**	**N (%)**
E. coli	229 (4)	473 (9)	579 (36)	351 (14)
K. pneumoniae	70 (1)	250 (5)	111 (7)	90 (4)
P. mirabilis	17 (0)	87 (2)	76 (5)	52 (2)
E. cloacae complex	50 (1)	278 (5)	44 (3)	115 (5)
Other Enterobacterales[1]	127 (2)	943 (18)	154 (10)	232 (9)
P. aeruginosa	66 (1)	436 (8)	109 (7)	158 (6)
Acinetobacter spp.	14 (0)	92 (2)	14 (1)	13 (1)
Other non-fermenters[2]	17 (0)	390 (8)	7 (0)	29 (1)
Other Gram-negatives[3]	27 (1)	438 (9)	0 (0)	74 (3)
E. faecalis	256 (5)	70 (1)	201 (12)	229 (9)
E. faecium	607 (12)	102 (2)	189 (12)	324 (13)
S. aureus	217 (4)	1,199 (23)	34 (2)	265 (10)
CNS	3,388 (64)	36 (1)	31 (2)	351 (14)
β-haemolytic *Streptococcus* spp. group B	17 (0)	55 (1)	23 (1)	35 (1)
S. anginosus	25 (0)	18 (0)	2 (0)	68 (3)
Other Gram-positives[4]	132 (3)	283 (5)	35 (2)	149 (6)

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

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

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

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

4 *In order of frequency: β-haemolytic Streptococcus spp. group G, β-haemolytic Streptococcus spp. group A, S. pneumoniae, S. dysgalactiae subsp. equisimilis, β-haemolytic Streptococcus spp. group C, S. dysgalactiae n.n.g., S. mitis/S. oralis, Enterococcus spp. (non-faecalis, non-faecium), A. urinae, C. perfringens, Staphylococcus spp. (non-aureus, 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*, and *Acinetobacter* spp. from patients admitted to intensive care units, ISIS-AR 2020

	E. coli	*K. pneumoniae*	*P. mirabilis*	*E. cloacae* complex	*P. aeruginosa*	*Acinetobacter* spp.
Antibiotic						
amoxicillin/ampicillin	45	-	23	-	-	-
co-amoxiclav[1] - non-uuti	37	25	7	-	-	-
piperacillin-tazobactam	5	13	0	-	12	-
cefuroxime	17	21	0	-	-	-
cefotaxime/ceftriaxone	9	14	1	-	-	-
ceftazidime	7	12	0	-	7	-
meropenem/imipenem	0	0	-	0	-	5
meropenem	-	-	0	-	3	-
imipenem	-	-	-	-	6	-
ciprofloxacin	14	14	13	6	10	6
gentamicin	5	7	6	7	-	5
tobramycin	6	9	5	6	3	8
co-trimoxazole	21	13	25	5	-	6
Empiric therapy combinations						
gentamicin + co-amoxiclav - non-uuti	5	6	3	-	-	-
gentamicin + cefuroxime	3	6	1	-	-	-
gentamicin + cefotaxime/ceftriaxone	2	6	0	-	-	-
tobramycin + ceftazidime	-	-	-	-	1	-
tobramycin + ciprofloxacin	-	-	-	-	2	-
ciprofloxacin + co-amoxiclav - non-uuti	10	11	3	-	-	-
ciprofloxacin + cefuroxime	7	12	0	-	-	-
ciprofloxacin + cefotaxime/ceftriaxone	6	10	0	-	-	-
Multidrug resistance						
HRMO[2]	10	16	5	4	4	5
multidrug resistance[3] - non-uuti	6	8	2	-	-	-

10	Significant and clinically relevant increasing trend since 2016
10	Significant and clinically relevant decreasing trend since 2016
10	No significant and clinically relevant time trend

(For the definition of a clinically relevant trend see section 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 section 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 ß-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 (either phenotypical or molecular), or, if no data on confirmatory tests were available, by resistance to meropenem or imipenem (for P. mirabilis: meropenem only); 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 (or, if a confirmatory test for carbapenemase production, either phenotypical or molecular, was available, we prioritized this), 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.*

[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 4.3.3.1 Trends in antibiotic resistance (from left to right 2016 to 2020) among diagnostic isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, *P. aeruginosa*, and *Acinetobacter* spp. from patients admitted to intensive care units in ISIS-AR

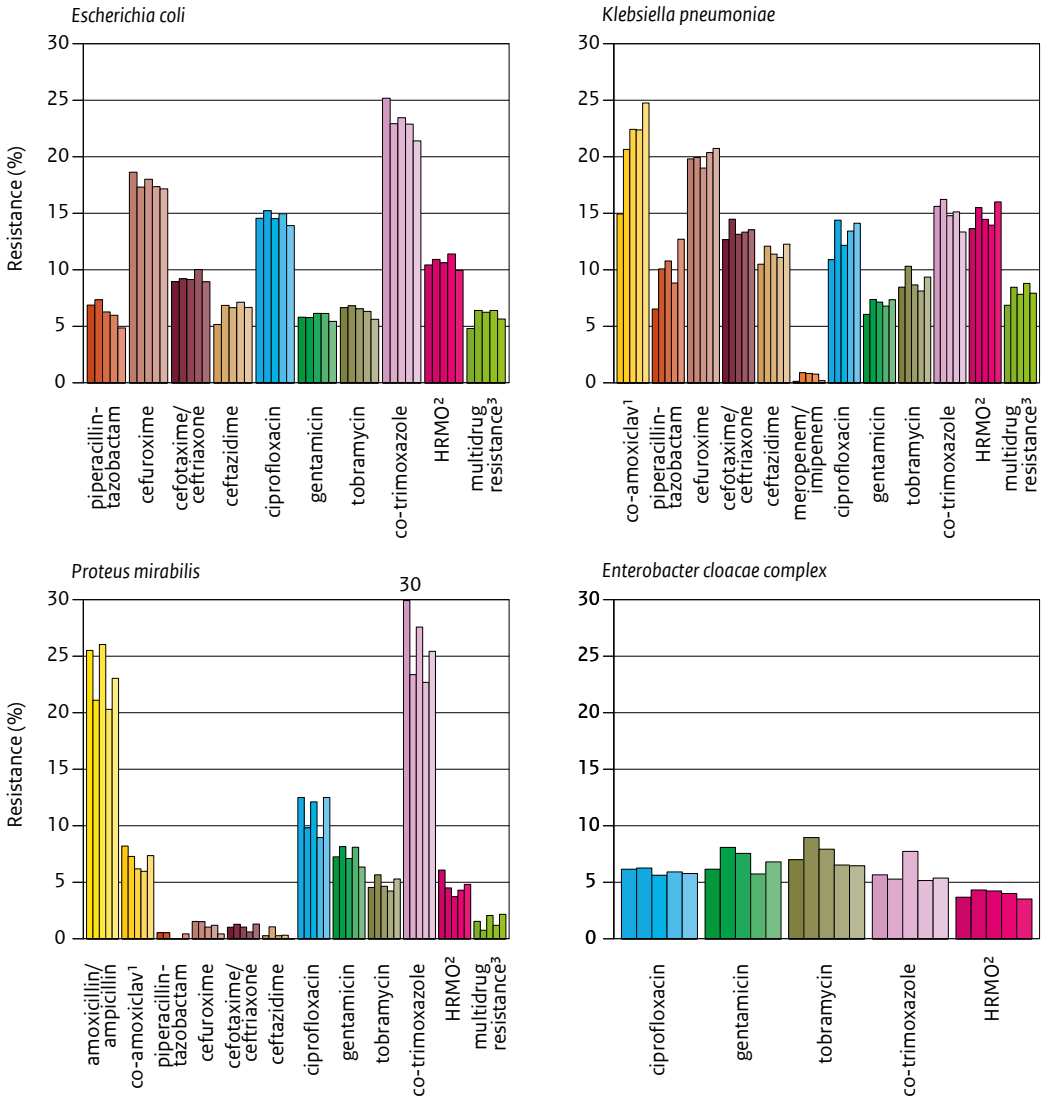
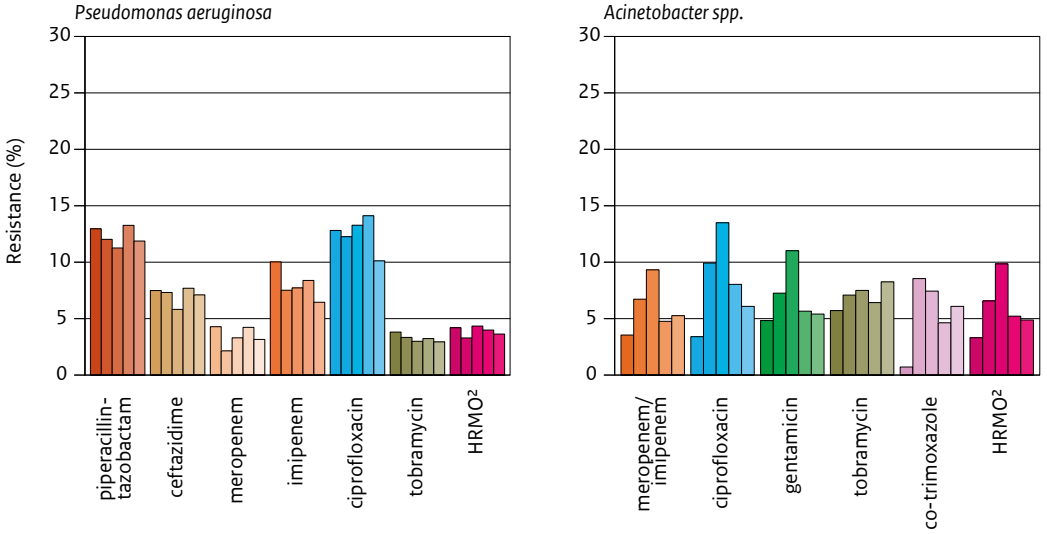


Figure 4.3.3.1 (continued) Trends in antibiotic resistance (from left to right 2016 to 2020) among diagnostic isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, *P. aeruginosa*, and *Acinetobacter* spp. from patients admitted to intensive care units in ISIS-AR



[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 section 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 β-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 (either phenotypical or molecular), or, if no data on confirmatory tests were available, by resistance to meropenem or imipenem (for P. mirabilis: meropenem only); 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 (or, if a confirmatory test for carbapenemase production, either phenotypical or molecular, was available, we prioritized this), 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.*

[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 4.3.3.3 Resistance levels (%) among diagnostic isolates of *E. faecalis* and *E. faecium* from patients admitted to intensive care units, ISIS-AR 2020

	E. faecalis	*E. faecium*
Antibiotic		
amoxicillin/ampicillin	-	90
vancomycin	0	0

10	Significant and clinically relevant increasing trend since 2016
10	Significant and clinically relevant decreasing trend since 2016
10	No significant and clinically relevant time trend

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

- = Resistance not calculated.

Figure 4.3.3.2 Trends in antibiotic resistance (from left to right 2016 to 2020) among diagnostic isolates of E. faecium from patients admitted to intensive care units in ISIS-AR

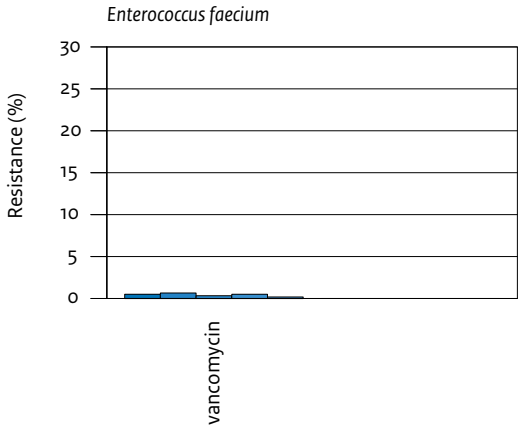


Table 4.3.3.4 Resistance levels (%) among diagnostic isolates of *S. aureus* and coagulase-negative *Staphylococcus* spp. from patients admitted to intensive care units, ISIS-AR 2020

	S. aureus	CNS
Antibiotic		
flucloxacillin[1]	3	81
ciprofloxacin[2]	3	72
gentamicin	1	64
erythromycin	14	73
clindamycin including inducible resistance[3]	13	64
doxycycline/tetracycline	4	27
fusidic acid	4	48
linezolid	0	0
co-trimoxazole	2	30
rifampicin	0	15

10	Significant and clinically relevant increasing trend since 2016
10	Significant and clinically relevant decreasing trend since 2016
10	No significant and clinically relevant time trend
	(For the definition of a clinically relevant trend see section 4.1.1)

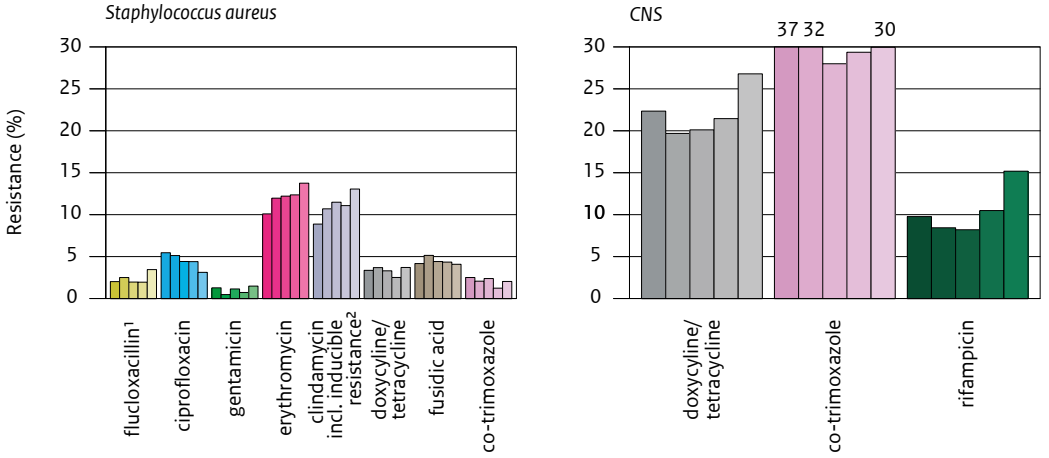
CNS = Coagulase-negative Staphylococcus spp., including S. epidermidis.

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

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

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

Figure 4.3.3.3 Trends in antibiotic resistance (from left to right 2016 to 2020) 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 section 4.1.1 for more detailed information).*

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

Key results

Enterobacterales

- In all *Enterobacterales*, resistance was ≤10% for gentamicin (≤7%), and tobramycin (≤9%). Resistance was also ≤10% for meropenem/imipenem (0%) in *E. coli*, *K. pneumoniae*, and *E. cloacae* complex; piperacillin-tazobactam (≤5%), cefotaxime/ceftriaxone (≤9%), and ceftazidime (≤7%) in *E. coli* and *P. mirabilis*; co-amoxiclav (7%), cefuroxime (0%), and meropenem (0%) in *P. mirabilis*; and ciprofloxacin (6%) and co-trimoxazole (5%) in *E. cloacae* complex.
- Resistance was ≥20% for co-amoxiclav (≥25%) in *E. coli* and *K. pneumoniae*, for cefuroxime (21%) in *K. pneumoniae*, and for amoxicillin/ampicillin (≥23%) and co-trimoxazole (≥21%) in *E. coli* and *P. mirabilis*.
- A statistically significant and clinically relevant increase in resistance was observed for co-amoxiclav in *E. coli* (from 29% in 2016 to 37% in 2020) and *K. pneumoniae* (from 15% to 25%), which may be partly due to the introduction of a new testpanel for the VITEK2 automated system in 2016 (for details see section 4.1.1). Notably, in the last four years, resistance for this agent in *E. coli* remained rather stable. In *K. pneumoniae*, resistance to piperacillin-tazobactam also increased to a statistically significant and clinically relevant extent in the last five years (from 7% to 13%).
- Resistance was ≤10% for all selected empiric therapy combinations in all *Enterobacterales*, except for ciprofloxacin + co-amoxiclav (11%) and ciprofloxacin + cefuroxime (12%) in *K. pneumoniae*.
- The percentage HRMO and multidrug resistance was ≤10%, except for HRMO in *K. pneumoniae* (16%).

P. aeruginosa

- Resistance was ≤10% for each of the selected agents, except for piperacillin-tazobactam (12%).
- Resistance was ≤2% for empiric therapy combinations.
- The percentage HRMO was 4%.

Acinetobacter spp.

- Resistance was ≤10% for each of the selected agents (≤8%).
- The percentage HRMO was 5%.

E. faecalis and E. faecium

- Resistance was ≤10% for vancomycin (0% in both pathogens).
- Resistance was ≥20% for amoxicillin/ampicillin in *E. faecium* (90%).

S. aureus

- Resistance was ≤10% for each of the selected agents (≤4%), except for erythromycin (14%) and clindamycin including inducible resistance (13%).
- A statistically significant and clinically relevant increase in resistance was observed for clindamycin including inducible resistance (from 9% in 2016 to 13% in 2020).

Coagulase-negative Staphylococcus spp.

- Resistance was ≥20% for each of the selected agents (≥27%), except for linezolid (0%) and rifampicin (15%).
- Statistically significant and clinically relevant increasing trends in resistance were found for ciprofloxacin (from 58% in 2016 to 72% in 2020), gentamicin (from 53% to 64%), clindamycin including inducible resistance (from 58% to 64%), doxycycline/tetracycline (from 22% to 27%), and rifampicin (from 10% to 15%). A decreasing trend in resistance was observed for co-trimoxazole (from 37% in 2016 to 30% in 2020).

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

The distribution of pathogens isolated from blood of patients admitted to non-intensive care inpatient departments (non-ICU) and intensive care units (ICU) in 2020 is presented in table 4.3.4.1. Resistance levels for a selection of pathogens isolated from these patients in 2020 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.), 4.3.4.5 (β-haemolytic *Streptococcus* spp. groups A, B, C, G, *S. anginosus,* and *S. mitis/S. oralis*), and 4.3.4.6 (*B. fragilis* complex). Five-year trends in resistance are presented in figures 4.3.4.1 (*E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, and *P. aeruginosa*), 4.3.4.2 (*E. faecium*), 4.3.4.3 (*S. aureus* and coagulase-negative *Staphylococcus* spp.), 4.3.4.4 (β-haemolytic *Streptococcus* spp. groups A, B, C, G, *S. anginosus,* and *S. mitis/S. oralis*), and 4.3.4.5 (*B. fragilis* complex). For *Acinetobacter* spp. and *C. perfringens* resistance levels and trends were not calculated because in 2020 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 2020

	Non-ICU	ICU
Pathogen	**N (%)**	**N (%)**
E. coli	6,955 (24)	226 (4)
K. pneumoniae	1,259 (4)	75 (1)
P. mirabilis	465 (2)	20 (0)
E. cloacae complex	452 (2)	62 (1)
Other *Enterobacterales*[1]	1,537 (5)	155 (3)
P. aeruginosa	652 (2)	73 (1)
Other non-fermenters[2]	250 (1)	32 (1)
B. fragilis complex	346 (1)	17 (0)
Other Gram-negatives[3]	234 (1)	11 (0)
E. faecalis	877 (3)	268 (5)
E. faecium	601 (2)	650 (12)
S. aureus	2,926 (10)	213 (4)
CNS	9,279 (32)	3,281 (63)
β-haemolytic *Streptococcus* spp. group A	192 (1)	8 (0)
β-haemolytic *Streptococcus* spp. group B	470 (2)	16 (0)
β-haemolytic *Streptococcus* spp. group C	96 (0)	2 (0)
β-haemolytic *Streptococcus* spp. group G	184 (1)	3 (0)
S. anginosus	220 (1)	24 (0)
S. mitis/S. oralis	353 (1)	29 (1)
Other Gram-positives[4]	1,677 (6)	83 (2)

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

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

[2] *In order of frequency: Acinetobacter spp., Pseudomonas spp. (non-aeruginosa), S. maltophilia, M. catarrhalis, B. cepacia.*
[3] *In order of frequency: H. influenzae, H. parainfluenzae, C. coli, C. jejuni, N. meningitidis.*
[4] *In order of frequency: S. pneumoniae, S. dysgalactiae n.n.g., S. dysgalactiae subsp. equisimilis, Enterococcus spp. (non-faecalis, non-faecium), C. perfringens, A. urinae, Staphylococcus spp. (non-aureus, non-CNS), 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 2020

	E. coli	*K. pneumoniae*	*P. mirabilis*	*E. cloacae* complex	*P. aeruginosa*
Antibiotic					
amoxicillin/ampicillin	42	-	21	-	-
co-amoxiclav[1] - non-uuti	34	19	6	-	-
piperacillin-tazobactam	4	8	0	-	6
cefuroxime	12	15	1	-	-
cefotaxime/ceftriaxone	6	10	0	-	-
ceftazidime	5	9	0	-	3
meropenem/imipenem	0	0	-	0	-
meropenem	-	-	0	-	1
imipenem	-	-	-	-	4
ciprofloxacin	13	13	13	4	9
gentamicin	6	5	6	4	-
tobramycin	6	8	3	4	1
co-trimoxazole	22	14	21	5	-
Empiric therapy combinations					
gentamicin + co-amoxiclav - non-uuti	5	4	2	-	-
gentamicin + cefuroxime	2	4	1	-	-
gentamicin + cefotaxime/ceftriaxone	2	4	0	-	-
tobramycin + ceftazidime	-	-	-	-	0
tobramycin + ciprofloxacin	-	-	-	-	1
ciprofloxacin + co-amoxiclav - non-uuti	8	8	1	-	-
ciprofloxacin + cefuroxime	6	9	0	-	-
ciprofloxacin + cefotaxime/ceftriaxone	4	7	0	-	-
Multidrug resistance					
HRMO[2]	8	12	3	3	1
multidrug resistance[3] - non-uuti	5	6	1	-	-

10	Significant and clinically relevant increasing trend since 2016
10	Significant and clinically relevant decreasing trend since 2016
10	No significant and clinically relevant time trend
	(For the definition of a clinically relevant trend see section 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 section 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 ß-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 (either phenotypical or molecular), or, if no data on confirmatory tests were available, by resistance to meropenem or imipenem (for P. mirabilis: meropenem only); 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 (or, if a confirmatory test for carbapenemase production, either phenotypical or molecular, was available, we prioritized this), 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.*

Figure 4.3.4.1 Trends in antibiotic resistance (from left to right 2016 to 2020) 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

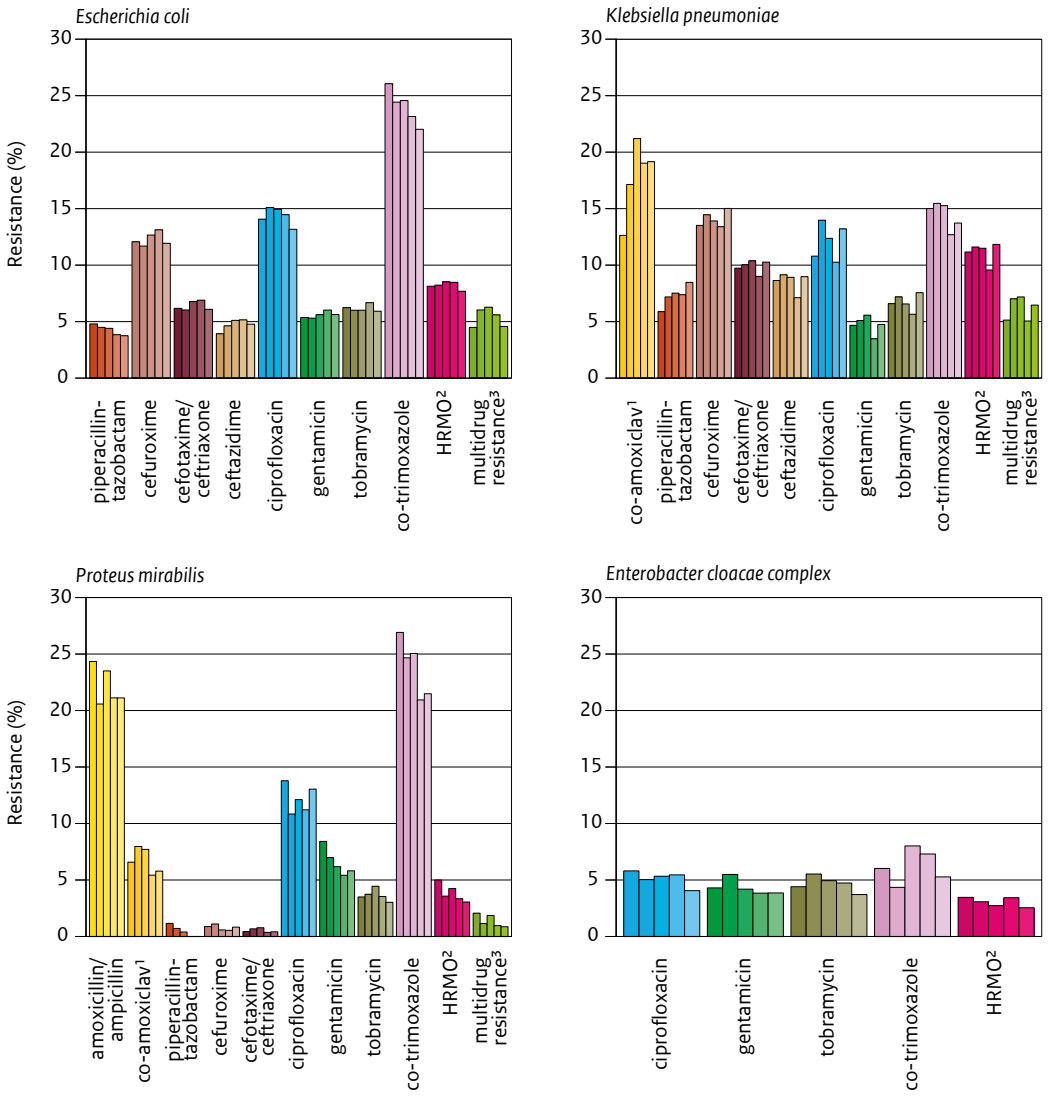
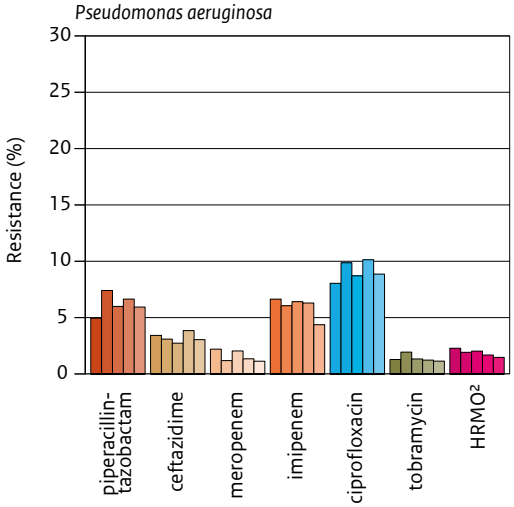


Figure 4.3.4.1 (continued) Trends in antibiotic resistance (from left to right 2016 to 2020) among diagnostic blood isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex, and *P. aeruginosa* from patients admitted to inpatient departments (incl. intensive care units) in ISIS-AR



[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 section 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 β-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 (either phenotypical or molecular), or, if no data on confirmatory tests were available, by resistance to meropenem or imipenem (for P. mirabilis: meropenem only); 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 (or, if a confirmatory test for carbapenemase production, either phenotypical or molecular, was available, we prioritized this), 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 4.3.4.3 Resistance levels (%) among diagnostic blood isolates of *E. faecalis* and *E. faecium* from patients admitted to inpatient departments (incl. intensive care units), ISIS-AR 2020

	E. faecalis	*E. faecium*
Antibiotic		
amoxicillin/ampicillin	-	89
vancomycin	0	0

10	Significant and clinically relevant increasing trend since 2016
10	Significant and clinically relevant decreasing trend since 2016
10	No significant and clinically relevant time trend
	(For the definition of a clinically relevant trend see section 4.1.1)

- = Resistance not calculated.

Figure 4.3.4.2 Trends in antibiotic resistance (from left to right 2016 to 2020) among diagnostic blood isolates of *E. faecium* from patients admitted to inpatient departments (incl. intensive care units) in ISIS-AR

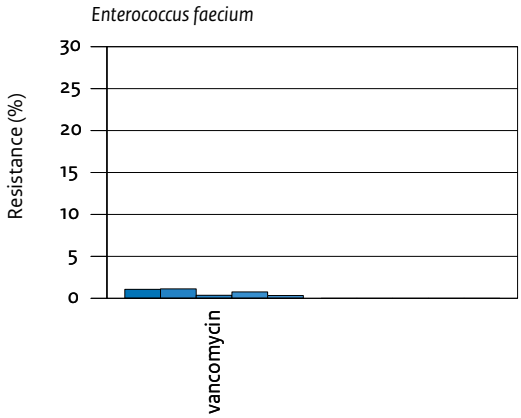


Table 4.3.4.4 Resistance levels (%) among diagnostic blood isolates of *S. aureus* and coagulase-negative *Staphylococcus* spp. from patients admitted to inpatient departments (incl. intensive care units), ISIS-AR 2020

	S. aureus	CNS
Antibiotic		
flucloxacillin[1]	2	50
ciprofloxacin[2]	4	36
gentamicin	1	34
erythromycin	12	50
clindamycin including inducible resistance[3]	11	37
doxycycline/tetracycline	3	20
linezolid	0	0
co-trimoxazole	1	18
rifampicin	0	6

10	Significant and clinically relevant increasing trend since 2016
10	Significant and clinically relevant decreasing trend since 2016
10	No significant and clinically relevant time trend
	(For the definition of a clinically relevant trend see section 4.1.1)

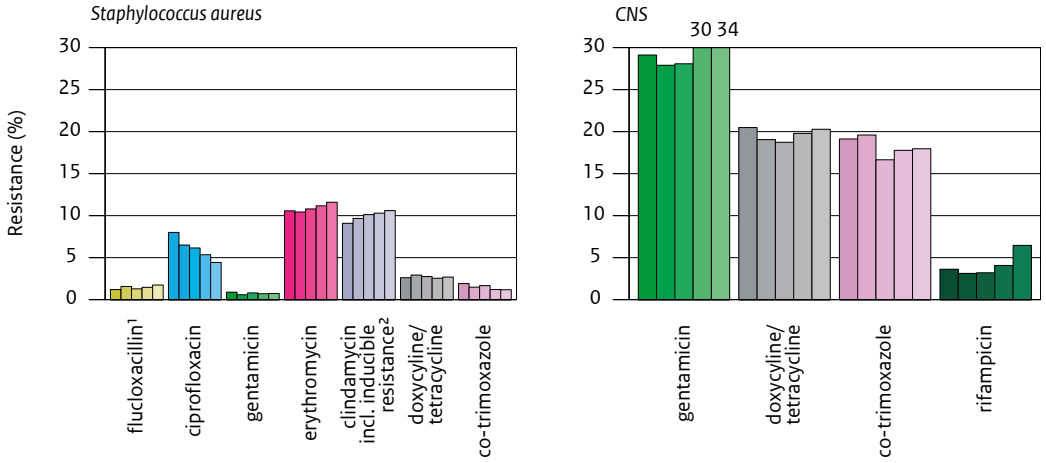
CNS = Coagulase-negative Staphylococcus spp., including S. epidermidis.

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

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

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

Figure 4.3.4.3 Trends in antibiotic resistance (from left to right 2016 to 2020) 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 section 4.1.1 for more detailed information).*

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

Table 4.3.4.5 Resistance levels (%) among diagnostic blood isolates of β-haemolytic *Streptococcus* spp. groups A, B, C, G, *S. anginosus*, and *S. mitis/S. oralis* from patients admitted to inpatient departments (incl. intensive care units), ISIS-AR 2020

	β-haemolytic *Streptococcus* spp. group A	β-haemolytic *Streptococcus* spp. group B	β-haemolytic *Streptococcus* spp. group C	β-haemolytic *Streptococcus* spp. group G	*S. anginosus*	*S. mitis/ S. oralis*
Antibiotic						
(benzyl)penicillin	-	-	-	-	0	6
(benzyl)penicillin (I)[1]	-	-	-	-	0	10
amoxicillin/ampicillin	-	-	-	-	-	2*
erythromycin	6	23	4	13	-	-
clindamycin including inducible resistance[2]	5	19	11	15	7	5
doxycycline/tetracycline	15	78	15*	31	-	-
co-trimoxazole	4*	1*	0*	1*	-	-

10	Significant and clinically relevant increasing trend since 2016
10	Significant and clinically relevant decreasing trend since 2016
10	No significant and clinically relevant time trend

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

- = *Resistance not calculated*

* *Trend not calculated because data from the years before 2020 did not meet the criteria for trend analysis (see section 4.1.1.).*

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

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

Figure 4.3.4.4 Trends in antibiotic resistance (from left to right 2016 to 2020) among diagnostic blood isolates of β-haemolytic *Streptococcus* spp. groups A, B, C, G, *S. anginosus*, and *S. mitis/S. oralis* from patients admitted to inpatient departments (incl. intensive care units) in ISIS-AR

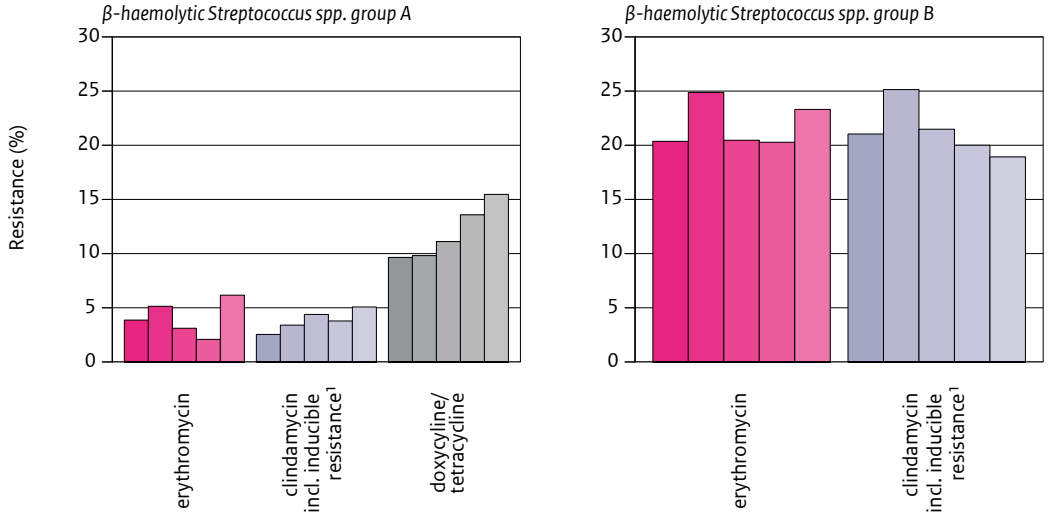
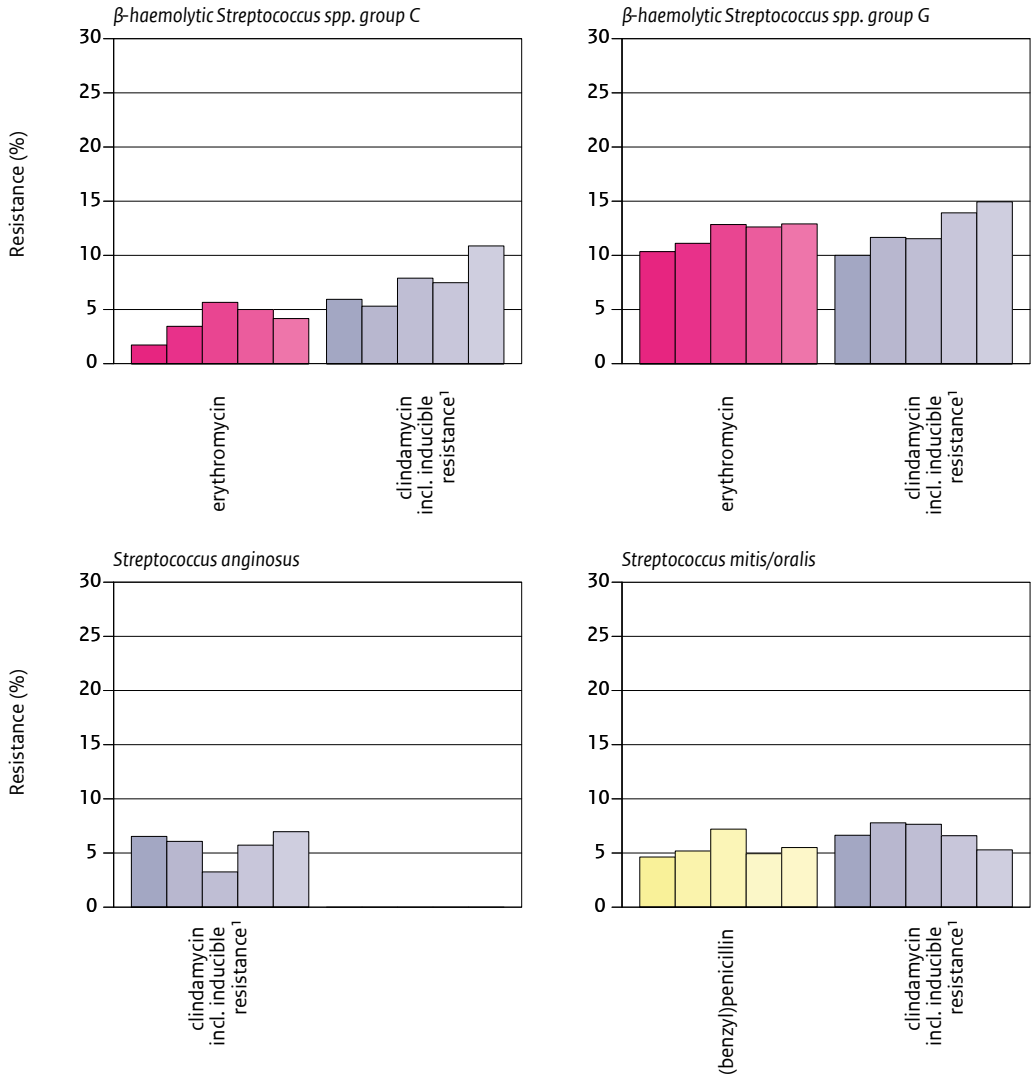


Figure 4.3.4.4 (continued) Trends in antibiotic resistance (from left to right 2016 to 2020) among diagnostic blood isolates of β-haemolytic *Streptococcus* spp. groups A, B, C, G, *S. anginosus*, and *S. mitis/S. oralis* from patients admitted to inpatient departments (incl. intensive care units) in ISIS-AR



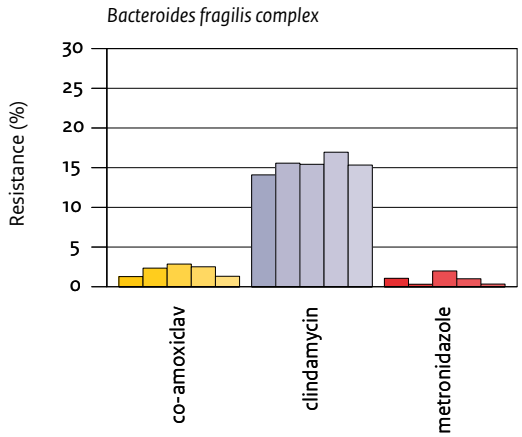
[1] *To estimate clindamycin resistance including inducible resistance, the laboratory S/I/R interpretation was used (see section 4.1.1 for more detailed information).*

Table 4.3.4.6 Resistance levels (%) among diagnostic blood isolates of *B. fragilis* complex from patients admitted to inpatient departments (incl. intensive care units), ISIS-AR 2020

	B. fragilis complex
Antibiotic	
co-amoxiclav	1
clindamycin	15
metronidazole	0

10	Significant and clinically relevant increasing trend since 2016
10	Significant and clinically relevant decreasing trend since 2016
10	No significant and clinically relevant time trend
	(For the definition of a clinically relevant trend see section 4.1.1)

Figure 4.3.4.5 Trends in antibiotic resistance (from left to right 2016 to 2020) 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 (85%) 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 (≤8%), cefotaxime/ceftriaxone (≤10%), ceftazidime (≤9%), gentamicin (≤6%), and tobramycin (≤8%). In addition, resistance was ≤10% for meropenem/imipenem (0%) in *E. coli*, *K. pneumoniae*, and *E. cloacae* complex; co-amoxiclav (6%), cefuroxime (1%), and meropenem (0%) in *P. mirabilis*; and for ciprofloxacin (4%) and co-trimoxazole (5%) in *E. cloacae* complex.

- Resistance was ≥20% for amoxicillin/ampicillin (≥21%) and co-trimoxazole (≥21%) in *E. coli* and *P. mirabilis*; and for co-amoxiclav in *E. coli* (34%).
- A statistically significant and clinically relevant increase in resistance was observed for co-amoxiclav in *E. coli* (from 24% in 2016 to 34% in 2020) and *K. pneumoniae* (from 13% to 19%), which may be partly due to the introduction of a new testpanel for the VITEK2 automated system in 2016 (for details see section 4.1.1). Notably, in the last four years, resistance for this agent in *E. coli* remained rather stable. In *P. mirabilis*, resistance to piperacillin-tazobactam (from 1% to 0%) and co-trimoxazole (from 27% to 21%) decreased to a statistically significant and clinically relevant extent in the last five years.
- Resistance was ≤9% for all selected empiric therapy combinations in all *Enterobacterales*.
- The percentage HRMO and multidrug resistance was ≤8% in all *Enterobacterales*, except for HRMO in *K. pneumoniae* (12%).

P. aeruginosa
- Resistance levels ≤10% were observed for each of the selected agents (≤9%).
- Resistance to empiric therapy combinations was ≤1%.
- The percentage HRMO was 1%.

E. faecalis and E. faecium
- Resistance levels ≤10% were found for vancomycin (0% in both pathogens).
- Resistance ≥20% was observed for amoxicillin/ampicillin in *E. faecium* (89%).

S. aureus
- Resistance levels ≤10% were observed for each of the selected agents (≤3%), except for erythromycin (12%) and clindamycin including inducible resistance (11%).
- A statistically significant and clinically relevant decrease in resistance was observed for ciprofloxacin (from 8% in 2016 to 4% in 2020).

Coagulase-negative Staphylococcus spp.
- Resistance levels ≥20% were observed for each of the selected agents, except for linezolid (0%), co-trimoxazole (18%), and rifampicin (6%).
- A statistically significant and clinically relevant increase in resistance was observed for rifampicin (from 4% in 2016 to 6% in 2020).

β-haemolytic Streptococcus spp. groups A, B, C, G
- In all β-haemolytic *Streptococcus* spp., resistance levels ≤10% were found for co-trimoxazole (≤4%). In addition, resistance was ≤10% for erythromycin in β-haemolytic *Streptococcus* spp. groups A and C (≤6%), and for clindamycin including inducible resistance in β-haemolytic *Streptococcus* spp. group A (5%).
- Resistance levels ≥20% were observed for erythromycin in β-haemolytic *Streptococcus* spp. group B (23%), and for doxycycline/tetracycline in β-haemolytic *Streptococcus* spp. groups B and G (≥31%).

S. anginosus and S. mitis/S. oralis
- Resistance levels ≤10% were observed for each of the selected agents (≤7%). The percentage I for (benzyl)penicillin was 0% in *S. anginosus* and 10% in *S. mitis/S. oralis.*

B. fragilis complex
- Resistance levels ≤10% were observed for co-amoxiclav (1%) and metronidazole (0%).

4.3.5 Urology services

The distribution of pathogens in urine samples from patients attending urology outpatient departments (OPD) and patients admitted to urology inpatient departments (IPD) in 2020 is presented in table 4.3.5.1. Resistance levels for a selection of pathogens isolated from these patients in 2020 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 2020

	OPD	IPD
Pathogen	**N (%)**	**N (%)**
E. coli	11,284 (38)	1,965 (31)
K. pneumoniae	2,719 (9)	510 (8)
P. mirabilis	1,415 (5)	284 (5)
Other *Enterobacterales*[1]	4,670 (16)	1,058 (17)
P. aeruginosa	1,137 (4)	412 (7)
Other non-fermenters[2]	586 (2)	158 (3)
Other Gram-negatives[3]	16 (0)	6 (0)
E. faecalis	3,296 (11)	848 (13)
E. faecium	226 (1)	199 (3)
Other Gram-positives[4]	4,729 (16)	842 (13)

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

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

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

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

Table 4.3.5.2 Resistance levels (%) among diagnostic urine isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* from patients attending urology outpatient departments (OPD) and patients admitted to urology inpatient departments (IPD), ISIS-AR 2020

	E. coli		*K. pneumoniae*		*P. mirabilis*		*P. aeruginosa*	
	OPD	IPD	OPD	IPD	OPD	IPD	OPD	IPD
Antibiotic								
amoxicillin/ampicillin	43	48	-	-	22	18	-	-
co-amoxiclav[1] - non-uuti	35	40	19	25	6	5	-	-
piperacillin-tazobactam	4	4	9	13	0	0	5	7
cefuroxime	13	17	14	17	1	2	-	-
cefotaxime/ceftriaxone	6	9	6	11	1	2	-	-
ceftazidime	5	7	5	10	1	0	2	2
meropenem/imipenem	0	0	0	0	-	-	-	-
meropenem	-	-	-	-	0	0	1	1
imipenem	-	-	-	-	-	-	4	6
ciprofloxacin	20	25	15	16	17	13	14	12
gentamicin	6	8	3	4	8	5	-	-
tobramycin	6	9	4	7	5	3	1	1
fosfomycin	2	2	32	30	17	21	-	-
trimethoprim	28	29	23	18	35	31	-	-
co-trimoxazole	26	27	12	14	28	22	-	-
nitrofurantoin	4	3	-	-	-	-	-	-
Empiric therapy combinations								
gentamicin + co-amoxiclav - non-uuti	5	7	2	3	2	1	-	-
gentamicin + cefuroxime	2	3	2	4	1	1	-	-
gentamicin + cefotaxime/ceftriaxone	1	2	2	3	0	1	-	-
tobramycin + ceftazidime	-	-	-	-	-	-	0	0
tobramycin + ciprofloxacin	-	-	-	-	-	-	1	0
ciprofloxacin + co-amoxiclav - non-uuti	12	15	6	9	3	1	-	-
ciprofloxacin + cefuroxime	7	10	8	12	1	1	-	-
ciprofloxacin + cefotaxime/ceftriaxone	4	7	4	9	0	1	-	-
Multidrug resistance								
HRMO[2]	9	12	7	13	6	5	1	1
multidrug resistance[3] - non-uuti	7	10	4	8	2	0	-	-

10	Significant and clinically relevant increasing trend since 2016
10	Significant and clinically relevant decreasing trend since 2016
10	No significant and clinically relevant time trend

(For the definition of a clinically relevant trend see section 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 section 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 ß-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 (either phenotypical or molecular), or, if no data on confirmatory tests were available, by resistance to meropenem or imipenem (for P. mirabilis: meropenem only); for P. aeruginosa as resistant to ≥3 antimicrobial groups among fluoroquinolones, aminoglycosides, carbapenems (or, if a confirmatory test for carbapenemase production, either phenotypical or molecular, was available, we prioritized this), 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.*

Figure 4.3.5.1 Trends in antibiotic resistance (from left to right 2016 to 2020) 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

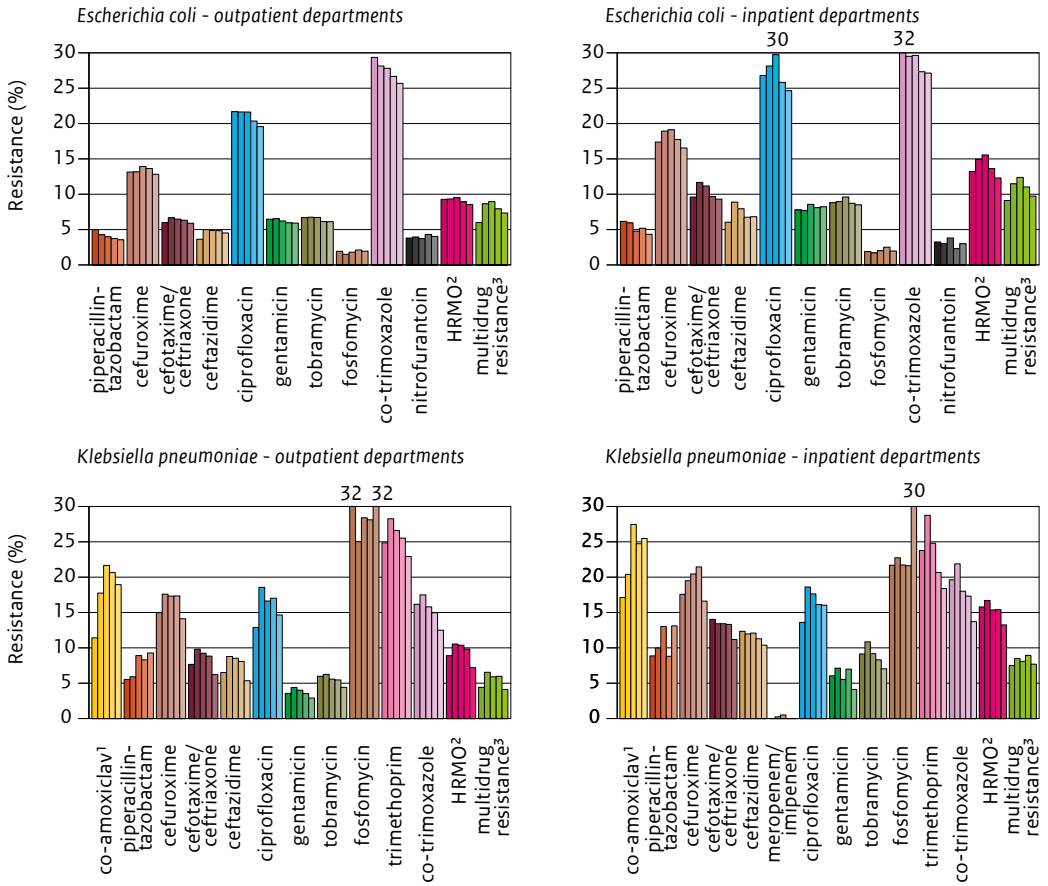
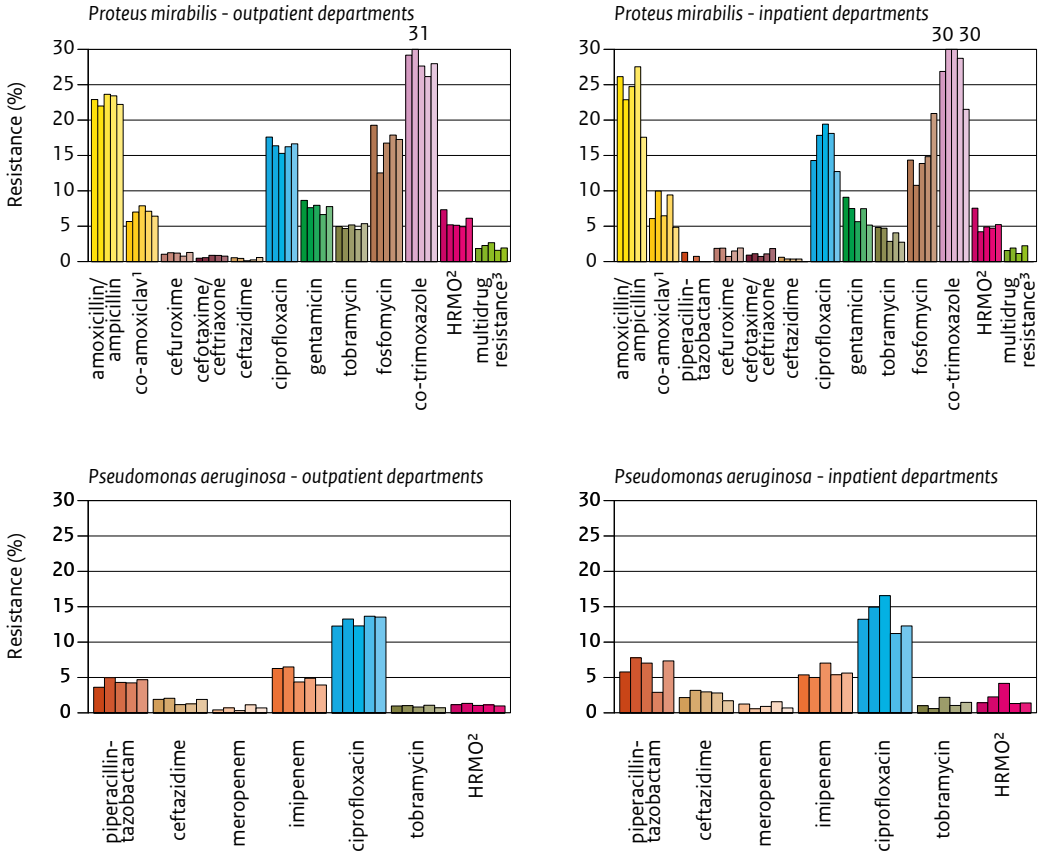


Figure 4.3.5.1 (continued) Trends in antibiotic resistance (from left to right 2016 to 2020) among diagnostic urine isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* from patients attending urology outpatient departments and patients admitted to urology inpatient departments 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 section 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 β-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 (either phenotypical or molecular), or, if no data on confirmatory tests were available, by resistance to meropenem or imipenem (for P. mirabilis: meropenem only); for P. aeruginosa as resistant to ≥3 antimicrobial groups among fluoroquinolones, aminoglycosides, carbapenems (or, if a confirmatory test for carbapenemase production, either phenotypical or molecular, was available, we prioritized this), 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.*

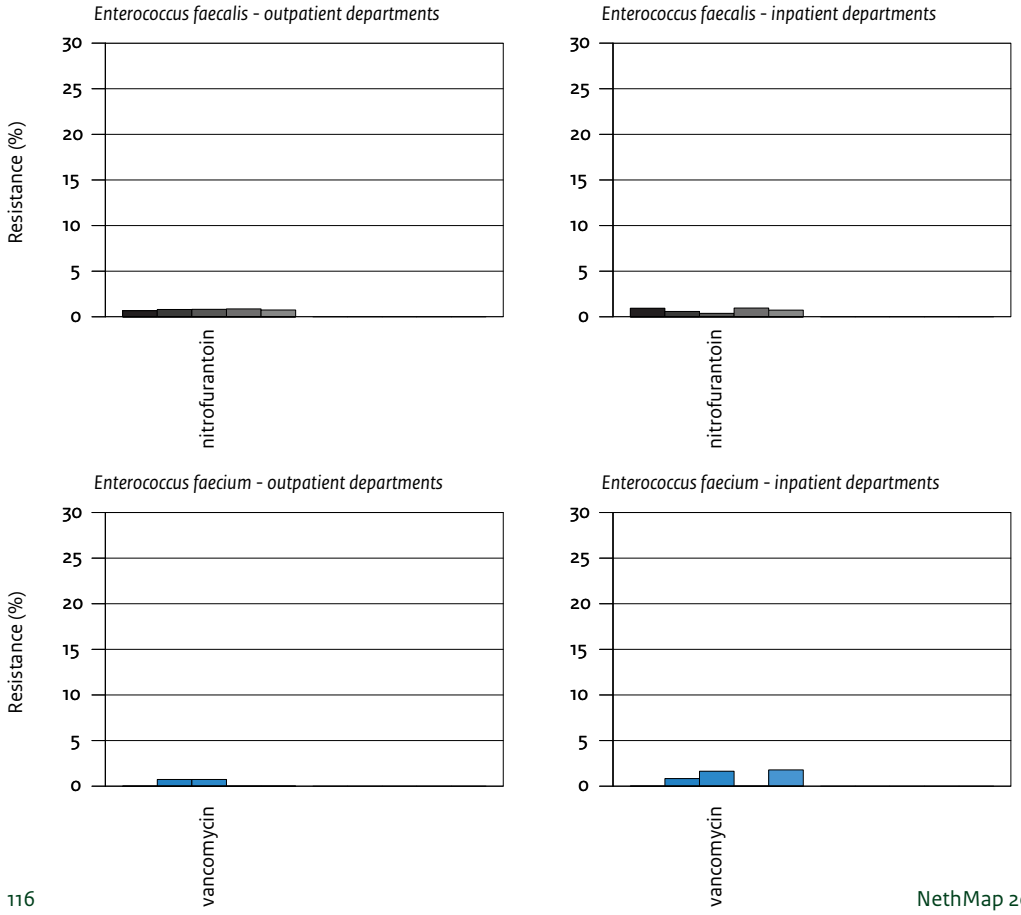
Table 4.3.5.3 Resistance levels (%) among diagnostic urine isolates of *E. faecalis* and *E. faecium* from patients attending urology outpatient departments (OPD) and patients admitted to urology inpatient departments (IPD), ISIS-AR 2020

	E. faecalis		*E. faecium*	
	OPD	IPD	OPD	IPD
Antibiotic				
amoxicillin/ampicillin	-	-	78	95
vancomycin	0	0	0	2
nitrofurantoin	1	1	-	-

10	Significant and clinically relevant increasing trend since 2016
10	Significant and clinically relevant decreasing trend since 2016
10	No significant and clinically relevant time trend
	(For the definition of a clinically relevant trend see section 4.1.1)

- = *Resistance not calculated.*

Figure 4.3.5.2 Trends in antibiotic resistance (from left to right 2016 to 2020) 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%, except in *K. pneumoniae* from IPD patients: 13%), cefotaxime/ceftriaxone (≤9%, except in *K. pneumoniae* from IPD patients: 11%), ceftazidime (≤10%), gentamicin (≤8%), and tobramycin (≤9%). 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 (≤6%), cefuroxime (≤2%), and meropenem (0%) in *P. mirabilis*.
- In all *Enterobacterales*, resistance of 20% or higher was observed for trimethoprim (≥23%, except in *K. pneumoniae* from IPD patients: 18%). Furthermore, resistance of 20% or higher was found for co-amoxiclav in *E. coli* (≥35%) and *K. pneumoniae* from IPD patients (25%), for amoxicillin/ampicillin in *E. coli* (≥43%) and *P. mirabilis* from OPD patients (22%), for co-trimoxazole (≥22%) in *E. coli* and *P. mirabilis*, for fosfomycin in *K. pneumoniae* (≥30%) and *P. mirabilis* from IPD patients (21%), and for ciprofloxacin (≥20%) in *E. coli*.
- A statistically significant and clinically relevant increase in resistance was observed for co-amoxiclav in *E. coli* (from 23% in 2016 to 35% in 2020 in OPD, from 28% to 40% in IPD) and *K. pneumoniae* (from 11% to 19% in OPD, from 17% to 25% in IPD), which may be partly due to the introduction of a new testpanel for the VITEK2 automated system in 2016 (for details see section 4.1.1). Notably, in the last four years, resistance for this agent in *E. coli* remained rather stable. In addition, in *K. pneumoniae*, resistance increased to a statistically significant and clinically relevant extent for piperacillin-tazobactam in OPD (from 6% in 2016 to 9% in 2020). In *K. pneumoniae* from IPD patients, statistically significant and clinically relevant decreases in resistance were observed for trimethoprim (from 24% in 2016 to 18% in 2020), and co-trimoxazole (from 20% to 14%).
- Resistance was ≤10% for empiric therapy combinations in all *Enterobacterales*, except for ciprofloxacin + co-amoxiclav (≥12%) in *E. coli*, and ciprofloxacin + cefuroxime (12%) in *K. pneumoniae* from IPD patients. In *E. coli*, resistance to gentamicin + co-amoxiclav in IPD increased to a statistically significant and clinically relevant extent (from 5% in 2016 to 7% in 2020).
- The percentage of HRMO and multidrug resistance was ≤10% in all *Enterobacterales*, except for HRMO in *E. coli* (12%) and *K. pneumoniae* (13%) from IPD patients.

P. aeruginosa

- Resistance levels of 10% or lower were found for each of the selected agents (≤7%), except for ciprofloxacin (≤14%).
- Resistance to empiric therapy combinations was ≤1%.
- The percentage HRMO was ≤1%.

E. faecalis and E. faecium

- Resistance levels of 10% or lower were observed for vancomycin (≤2%) and nitrofurantoin (1%, presented for *E. faecalis* only).
- Resistance levels of 20% or higher were observed for amoxicillin/ampicillin in *E. faecium* (≥78%).

4.4 Long-term care facilities

The distribution of pathogens in diagnostic urine and wound or pus samples from residents of long-term care facilities (LTCF) in 2020 is presented in table 4.4.1. The resistance levels in 2020 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 2020

	Urine	Wound or pus
Pathogen	**N (%)**	**N (%)**
E. coli	9,911 (41)	170 (8)
K. pneumoniae	2,421 (10)	56 (3)
P. mirabilis	2,745 (11)	200 (9)
Other *Enterobacterales*[1]	2,448 (10)	179 (8)
P. aeruginosa	1,216 (5)	284 (13)
Other non-fermenters[2]	163 (1)	40 (2)
Other Gram-negatives[3]	0 (0)	17 (1)
S. aureus	918 (4)	949 (44)
Other Gram-positives[4]	4,281 (18)	273 (13)

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

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

[3] *In order of frequency: B. fragilis complex.*

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

Table 4.4.2 Resistance levels (%) among diagnostic urine isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* from selected residents of long-term care facilities, ISIS-AR 2020

	E. coli	*K. pneumoniae*	*P. mirabilis*	*P. aeruginosa*
Antibiotic				
amoxicillin/ampicillin	44	-	21	-
co-amoxiclav[1] - non-uuti	36	23	7	-
piperacillin-tazobactam	5	14	0	5
cefuroxime	15	13	1	-
cefotaxime/ceftriaxone	6	5	1	-
ceftazidime	5	5	0	2
meropenem/imipenem	0	0	-	-
meropenem	-	-	0	1
imipenem	-	-	-	5
ciprofloxacin	18	12	16	10
gentamicin	7	3	6	-
tobramycin	7	3	4	1
fosfomycin	2	31	18	-
trimethoprim	23	17	34	-
co-trimoxazole	21	8	26	-
nitrofurantoin	3	-	-	-
Multidrug resistance				
HRMO[2]	10	7	4	1
multidrug resistance[3] - non-uuti	5	3	2	-

- = 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 section 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 β-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 (either phenotypical or molecular), or, if no data on confirmatory tests were available, by resistance to meropenem or imipenem (for P. mirabilis: meropenem only); for P. aeruginosa as resistant to ≥3 antimicrobial groups among fluoroquinolones, aminoglycosides, carbapenems (or, if a confirmatory test for carbapenemase production, either phenotypical or molecular, was available, we prioritized this), 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 4.4.3 Resistance levels (%) among diagnostic wound or pus isolates of *S. aureus* from selected residents of long-term care facilities, ISIS-AR 2020

	S. aureus
Antibiotic	
flucloxacillin[1]	2
ciprofloxacin[2]	19
erythromycin	14
clindamycin including inducible resistance[3]	14
doxycycline/tetracycline	3
fusidic acid	8
co-trimoxazole	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 section 4.1.1 for more detailed information).*

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

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

Key results

Enterobacterales

- For all *Enterobacterales*, resistance levels of 10% or lower were found for cefotaxime/ceftriaxone (≤6%), ceftazidime (≤5%), gentamicin (≤7%), and tobramycin (≤7%). In addition, resistance levels of 10% or lower were also found for piperacillin-tazobactam (5%), meropenem/imipenem (0%), fosfomycin (2%), and nitrofurantoin (3%) in *E. coli*; for meropenem/imipenem (0%) and co-trimoxazole (8%) 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 (≥23%). Additionally, resistance levels were ≥20% for amoxicillin/ampicillin (≥21%), trimethoprim (≥23%), and co-trimoxazole (≥21%) in *E. coli* and *P. mirabilis*; and for fosfomycin in *K. pneumoniae* (31%).
- The percentage of HRMO and multidrug resistance was ≤10% in all *Enterobacterales*.

P. aeruginosa

- Resistance levels for each of the selected agents were ≤10%.

S. aureus

- Resistance lower than 10% was found for flucloxacillin (2%), doxycycline/tetracycline (3%), fusidic acid (8%), and co-trimoxazole (1%).

4.5 Respiratory pathogens

The distribution of pathogens isolated from diagnostic lower and upper respiratory tract samples from general practitioners' (GP) patients and hospital patients (outpatients and inpatients, including intensive care patients) in 2020 is presented in table 4.5.1. Resistance levels for respiratory pathogens (*S. pneumoniae*, *H. influenzae*, and *M. catarrhalis*) in 2020 are presented by patient group in table 4.5.2. Five-year trends in resistance are shown in figure 4.5.1.

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

In hospitals in the Netherlands, a sample is taken for routine diagnostic purposes when 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 previous treatment failure, chronic obstructive pulmonary diseases (COPD), and cystic fibrosis (CF) may be overrepresented.

Table 4.5.1 Distribution of isolated pathogens in diagnostic respiratory samples from general practitioners' patients (GP) and in diagnostic blood or cerebrospinal fluid and respiratory samples from hospital patients (outpatient and inpatient departments, incl. intensive care units), ISIS-AR 2020

	GP		Hospital departments		
	Lower respiratory tract	Upper respiratory tract	Blood or cerebrospinal fluid	Lower respiratory tract	Upper respiratory tract
Pathogen	N (%)	N (%)	N (%)	N (%)	N (%)
S. pneumoniae	101 (6)	7 (1)	943 (3)	1,565 (7)	66 (1)
Other Gram-positives[1]	243 (15)	1,113 (83)	20,653 (60)	4,791 (20)	3,730 (65)
H. influenzae	465 (28)	27 (2)	147 (0)	4,909 (21)	257 (4)
M. catarrhalis	114 (7)	11 (1)	13 (0)	1,152 (5)	79 (1)
Other non-fermenters[2]	311 (19)	31 (2)	966 (3)	4,337 (18)	430 (7)
Enterobacterales[3]	366 (22)	149 (11)	11,108 (32)	6,447 (27)	1,120 (20)
Other Gram-negatives[4]	32 (2)	5 (0)	466 (1)	445 (2)	54 (1)

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

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

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

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

Table 4.5.2 Resistance levels (%) among diagnostic isolates of *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* from general practitioners' patients, patients attending outpatient departments and patients admitted to inpatient departments (incl. intensive care units), ISIS-AR 2020

	S. pneumoniae		*H. influenzae*		*M. catarrhalis*	
	GP	Hospital	GP	Hospital	GP	Hospital
Antibiotic						
(benzyl)penicillin[1] - nonmen	0	0	-	-	-	-
(benzyl)penicillin[1] - men	8	6	-	-	-	-
amoxicillin/ampicillin	-	-	37	36	-	-
co-amoxiclav	-	-	17	12	2	3
erythromycin	18	11	-	-	3	4
doxycycline/tetracycline	10	10	1	2	1	0
co-trimoxazole	9	9	23	25	5	4

- = Resistance not calculated.
nonmen = according to the breakpoint for indications other than meningitis, men = according to the breakpoint for meningitis.
[1] *Available gradient strip tests (Etest™ and MTS™) systematically underestimate (benzyl)penicillin MIC values in S. pneumoniae (for details see section 4.1.1). Resistance percentages may therefore be biased toward a lower level.*

Figure 4.5.1 Trends in antibiotic resistance (from left to right 2016 to 2020) among diagnostic isolates of *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* from general practitioners' patients and hospital patients (outpatient and inpatient departments, incl. intensive care units), ISIS-AR 2020

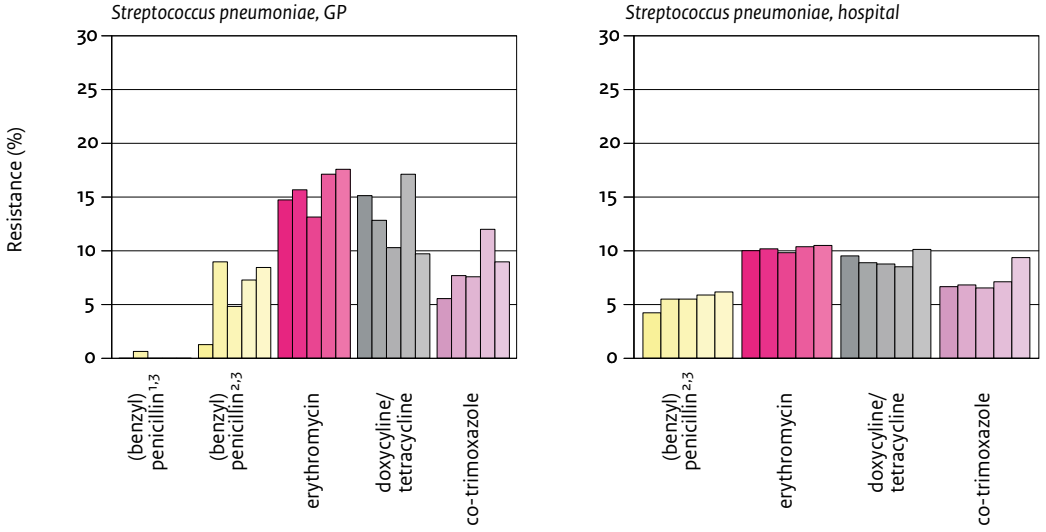
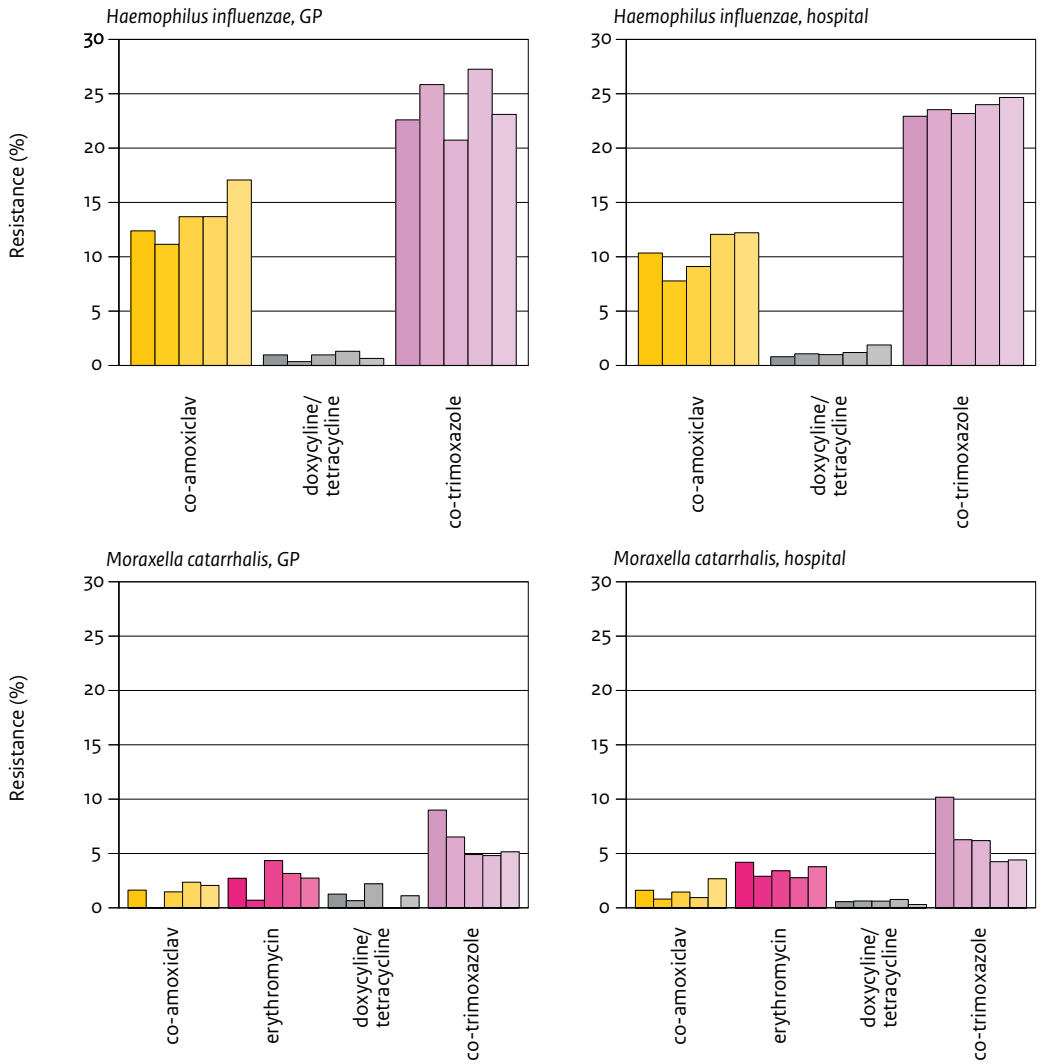


Figure 4.5.1 (continued) Trends in antibiotic resistance (from left to right 2016 to 2020) among diagnostic isolates of *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* from general practitioners' patients and hospital patients (outpatient and inpatient departments, incl. intensive care units), ISIS-AR 2020



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

[2] *According to breakpoint for meningitis.*

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

Key results

S. pneumoniae

- Resistance levels ≤10% were observed for (benzyl)penicillin (0% according to the breakpoint for indications other than meningitis and ≤8% according to the breakpoint for meningitis), doxycycline/tetracycline (10%), and co-trimoxazole (9%).

H. influenzae

- Resistance of 10% or lower was found for doxycycline/tetracycline (≤2%).
- Resistance levels of 20% or higher were found for amoxicillin/ampicillin (≥36%) and co-trimoxazole (≥23%).
- A statistically significant and clinically relevant increase in resistance was observed for amoxicillin/ampicillin in hospital patients (from 31% in 2016 to 36% in 2020), and for co-amoxiclav in both GP patients (from 12% to 17%) and hospital patients (from 10% to 12%).

M. catarrhalis

- Resistance of 10% or lower was observed for each of the selected agents (≤5%).
- A statistically significant and clinically relevant decrease in resistance was observed for co-trimoxazole in hospital patients (from 10% in 2016 to 4% in 2020).

4.6 Antimicrobial resistance in *Helicobacter pylori* infections

Introduction

Helicobacter pylori is a Gram-negative curved bacterium that resides only on the gastric epithelium. Primary colonization often occurs during childhood and can last a lifetime. The global prevalence of *H. pylori* carriage is estimated to range between 20%-30% in Northern and Central European countries, to over 70% in parts of Asia, Africa and Southern Europe.[1] *H. pylori* has been found an important factor in the etiology of a wide range of gastric disorders including peptic ulcer disease, chronic gastritis, Mucosa-Associated Lymphoid Tissue (MALT) lymphoma, and gastric cancer.[2] In the past decades, this highly prevalent infection has been treated with various antimicrobial regimens, and concerns about antimicrobial resistance in this pathogen are rising, also in the Netherlands.[3] In this section we describe (trends in) antimicrobial resistance to a selection of agents frequently used, in *H. pylori* in the Netherlands during the period 2016-2020.

Methods

Data from 37 laboratories for which continuous data from 2016 to 2020 were available in the ISIS-AR database, were considered for analysis. We included isolates of *H. pylori* from all specimen types (as we could not distinguish gastric specimens specifically) and their antimicrobial susceptibility test (AST) data for amoxicillin/ampicillin, levofloxacin, clarithromycin, doxycycline/tetracycline, and metronidazole in the years 2016-2020. If multiple isolates per patient per year were available, we selected the first, to avoid repeated sampling causing bias in the results. To avoid bias due to selective testing of antibiotics, for each agent we included only data from laboratories that tested at least 50% of isolates for that specific agent in each year. To avoid bias due to differences in breakpoint guidelines and expert rules used in the participating laboratories, we reinterpreted MIC values according to EUCAST clinical breakpoints version 10.0, 2020. Laboratories for which less than 80% of MIC values could be reinterpreted in one or more years were excluded from analysis. Using logistic regression models on the resulting antimicrobial susceptibility categories (S/I/R), we calculated resistance ('R') percentages and linear time trends for the selected antibiotics, and for combined resistance to clarithromycin and metronidazole. Statistical significance and clinical relevance of trends were assessed using the criteria described in section 4.1.1.

Results

In total, 2 610 isolates from 34 laboratories were found in the database for the selected time period. After the exclusion criteria were applied, data from 11-27 laboratories could be included for the analysis of the selected antimicrobial agents and combined resistance. Resistance to amoxicillin/ampicillin (6%) and doxycycline/tetracycline (2%) were lower than 10%, but resistance to levofloxacin (29%), clarithromycin (53%), and metronidazole (48%) were higher than 20% in 2020 (Table 4.6.1). Combined resistance to clarithromycin and metronidazole was 33%. Between 2016 and 2020, resistance increased to a statistically significant and clinically relevant extent for clarithromycin (from 40% to 53%), doxycycline/tetracycline (from 0% to 2%), metronidazole (from 35% to 48%), and clarithromycin + metronidazole (from 21% to 33%, Figure 4.6.1).

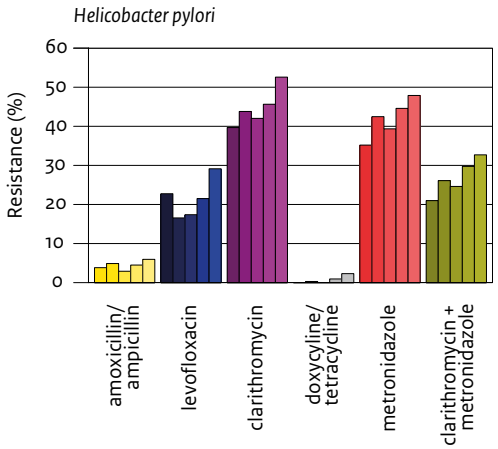
Table 4.6.1 Resistance levels (%) among isolates of *Helicobacter pylori*, ISIS-AR 2020.

	Helicobacter pylori
Antibiotic	
amoxicillin/ampicillin	6
levofloxacin	29
clarithromycin	53
doxycycline/tetracycline	2
metronidazole	48
clarithromycin + metronidazole	33

10	Significant and clinically relevant increasing trend since 2016
10	Significant and clinically relevant decreasing trend since 2016
10	No significant and clinically relevant time trend

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

Figure 4.6.1 Trends in antibiotic resistance (from left to right 2016 to 2020) among isolates of *Helicobacter pylori* in ISIS-AR.



Discussion

In *H. pylori*, substantial and increasing resistance levels were observed for clarithromycin, metronidazole, and for the combination of both agents. This finding is consistent with reports from other countries and challenges the international and national treatment guidelines.[4-6] However, the resistance percentages presented in this section should be interpreted with caution. For phenotypical antimicrobial susceptibility testing a biopsy from the gastric epithelium is required. However, in general *H. pylori* infection is diagnosed using non-invasive methods such as a stool antigen test or a urea breath test, and a biopsy is likely only performed when empirical treatment was unsuccessful. The resistance percentages presented in this section are therefore expected to be an overestimation of resistance in the general population. Nonetheless, the results are considered to provide a valid estimate of resistance in patients presenting with *H. pylori* infections in hospitals. In addition, the increasing time trend is expected to be valid for both populations and is alarming. Consequently, several initiatives are ongoing to get more insight in the clinical relevance of increased resistance for treatment of patients in primary healthcare and to consider alternative treatment options for multidrug resistant *H. pylori.*

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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 Healthcare 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

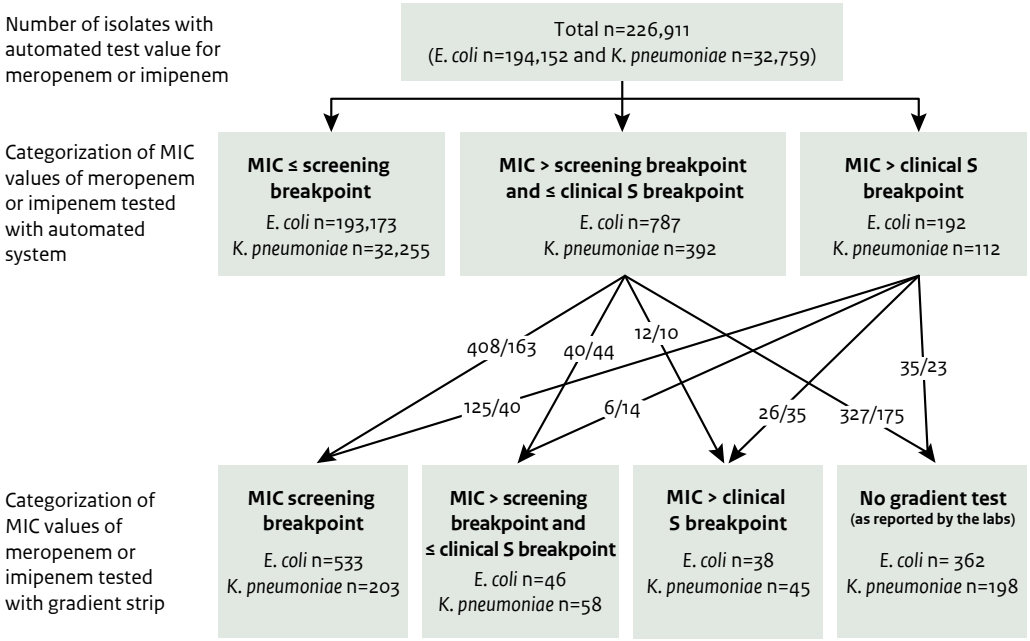
These analyses focus on *E. coli* and *K. pneumoniae*, as the most prevalent *Enterobacterales* species. We searched the ISIS-AR database (years 2016-2020) for diagnostic and non-diagnostic isolates that were tested for meropenem and/or imipenem by automated system. Based on the crude automated test values, we categorized them as having either i) MIC ≤ the screening breakpoint as defined by the Dutch national guideline[5] (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, for isolates with elevated automated MIC (i.e. > the screening breakpoint), we searched the ISIS-AR and Type-Ned database for data on confirmatory tests (i.e. gradient strip tests and tests for carbapenemase production (phenotypic) or carbapenemase genes (genotypic)). We included only one isolate per patient per species per year: an isolate with a gradient strip test was prioritized over an isolate with an automated test only. If, subsequently, multiple isolates were eligible for inclusion, we prioritized the most resistant isolate. Based on data of isolates from 41 laboratories, we calculated numbers of isolates with automated MIC in the respective categories in 2020. Subsequently, isolates with elevated automated MIC were categorized into the same categories as previously mentioned, according to gradient strip test results. Based on data from 36 laboratories that continuously submitted data to ISIS-AR from 2016 to 2020, we assessed the percentage of isolates with i) elevated MIC, in automated testing and gradient strip test confirmed separately, and ii) elevated automated MIC that underwent further testing, by year.

Results

Absolute numbers of isolates and categorization according to automated and gradient strip test MICs in 2020 are presented in Figure 4.7.1.1. Of a total number of 226,911 isolates with an automated test value for meropenem or imipenem (194,152 *E. coli* and 32,759 *K. pneumoniae*), an elevated MIC on automated testing

was found in 0.7% of isolates (1,483). The gradient strip method (performed in 62.2% of isolates with elevated MIC) confirmed elevated carbapenem MIC values in 20% (187/923) of tested isolates (14% (84/617) of *E. coli* and 34% (103/306) of *K. pneumoniae*). Among 1,483 isolates with an elevated MIC on automated testing, 83 (5.6%) had an MIC > the clinical S breakpoint on gradient strip testing (38/979 in *E. coli* (3.9%) and 45/504 in *K. pneumoniae* (8.9%)).

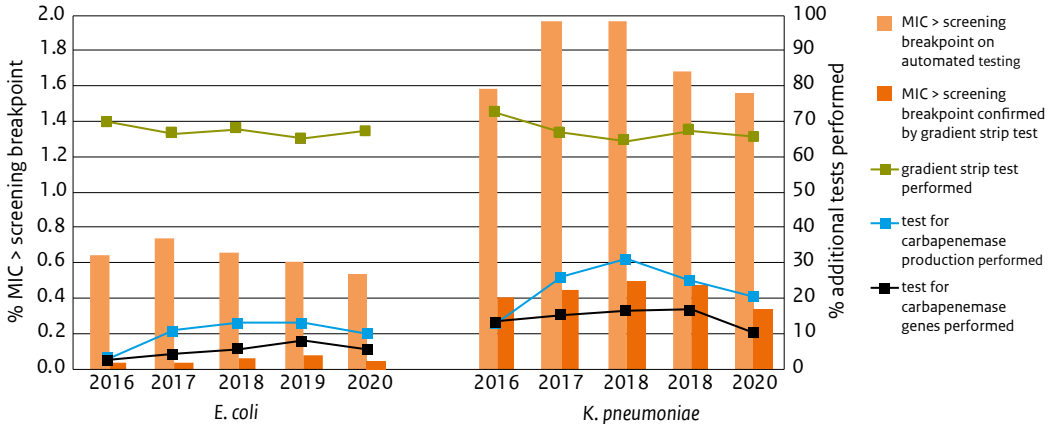
Figure 4.7.1.1 Results of automated and gradient strip testing of carbapenem susceptibility in *E. coli* and *K. pneumoniae* in 2020, according to NVMM guideline Laboratory detection of highly resistant microorganisms (version 2.0, 2012) in 41 laboratories participating in ISIS AR



Screening breakpoint: meropenem 0.25 mg/L, imipenem 1 mg/L.
Clinical S breakpoint: meropenem 2 mg/L, imipenem 2 mg/L.
One isolate per patient per species was selected: the most completely tested and most resistant isolate (refer to Methods section).

The overall prevalence of *E. coli* and *K. pneumoniae* strains with gradient strip test-confirmed MIC > the screening breakpoint has gradually increased between 2016 and 2019 (from 0.04% to 0.07% in *E. coli*, and from 0.40% to 0.49% in *K. pneumoniae*), but was lower in 2020 (0.05% in *E. coli* and 0.33% in *K. pneumoniae*, Figure 4.7.1.2). The use of gradient strip tests to confirm elevated automated carbapenem MIC values decreased from 70% in 2016 to 68% in 2020 in *E. coli* (not statistically significant), and from 72% to 65% in *K. pneumoniae*. In isolates with elevated MIC on automated testing, the percentage of phenotypic tests for carbapenemase production increased between 2016 and 2018 in both species (from 4% to 13% in *E. coli* and from 14% to 29% in *K. pneumoniae*) but decreased since then (to 10% and 19% for the respective species in 2020). The percentage of tests for carbapenemase genes increased until 2019 (from 3% to 9% in *E. coli* and from 15% to 20% in *K. pneumoniae*) but was lower in 2020 (6% and 10%, respectively).

Figure 4.7.1.2 (Additional testing of) elevated carbapenem MIC (%) in *E. coli* and *K. pneumoniae* by year, in 36 laboratories, ISIS-AR 2016-2020



Screening breakpoint: meropenem 0.25 mg/L, imipenem 1 mg/L.
The percentages of gradient tests and tests for carbapenemase production and carbapenemase genes performed were calculated for isolates with MIC > screening breakpoint on automated testing.
One isolate per patient per species was selected: the most completely tested and most resistant isolate (refer to Methods section).

Discussion

An elevated carbapenem MIC on automated testing was found in an overall 0.7% of *E. coli* and *K. pneumoniae* isolates in 2020. 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 decreased since 2016, especially in *K. pneumoniae*. Of note, the proportion of isolates with elevated automated MIC that underwent phenotypic or genotypic testing for carbapenemase production or genes, was lower in 2020 compared to 2019. Potentially this relates to the COVID-19 pandemic, which caused patient populations to shift and possibly led to increased routine screening in ICU's. Whether this continues to decrease further after 2020, needs to be monitored in the coming years.

Molecular epidemiology

Methods

For the enhanced surveillance of CPE, Dutch laboratories are requested to submit isolates to the RIVM with an MIC for meropenem of >0.25 mg/L and/or an MIC for imipenem >1 mg/L and/or producing carbapenemase and/or a detected carbapenemase encoding gene. For the surveillance, the Type-Ned system is used, with the restriction that the laboratory can only send the first isolate from a person within a year. The RIVM allows consecutive isolates from the same person if these are other *Enterobacterales* species/carbapenemase-encoding gene combinations. The RIVM confirms the species by MALDI-ToF, the MIC for meropenem, carbapenemase production by the carbapenemase inactivation method (CIM),[6] assesses the presence of carbapenemase-encoding genes by PCR (carba-PCR), and performs next-generation sequencing (NGS) for all isolates that are CIM positive.[7] 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 2017-2020 (based on sampling date

and allele based on NGS). Samples without a person ID (n=23) were excluded from further analysis. Up to 30 June 2019, epidemiological data on CPE isolates was collected using a questionnaire in Type-Ned. Based on whole-genome multi-locus sequence typing (wgMLST), closely genetically related (20-25 allelic distance) *E. coli*, *K. pneumoniae*, *E. cloacae* complex and *C. freundii* complex isolates are grouped in genetic clusters and assigned consecutive cluster numbers. A genetic cluster is defined per bacterial species and includes ≥2 isolates that differ typically ≤20 alleles (25 for *E. coli*). Assigning clusters started in 2018, but includes all sequenced isolates available from the national surveillance. Clusters of multiple isolates all from the same patient, including over different years and/or submitted by different laboratories, were not counted.
From 1 July 2019 onwards, CPE is mandatory notifiable[8] 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 a sampling date between 1 January and 31 December 2020 with status 'definite' are included in this chapter. Incomplete or unapproved notifications, and notifications that do not meet the notification criteria were excluded from this chapter. Questionnaire data was analysed on person level and not on isolate level.
Finally, we searched the SO-ZI/AMR database for CPE outbreaks that were reported in 2020.

Results

A total of 212 *Enterobacterales* isolates obtained in 2020, were submitted to the RIVM by 41 of the 55 Dutch medical microbiology laboratories. Among these were 204 unique carbapenemase-producing *Enterobacterales* isolates (species/carbapenemase allele combination), obtained from 180 persons (mean age 61 years and 56% male). Of the 204 isolates, 91 (44.6%) were *Escherichia coli*, 53 (25.9%) *Klebsiella pneumoniae*, 23 (11.3%) *Enterobacter cloacae* complex and the remaining 37 (18.1%) isolates belonged to other species. When the EUCAST clinical breakpoints were applied, 49/204 (24%) had an MIC for meropenem above the cut-off of 8 mg/L. The number of unique carbapenemase-producing isolates submitted to the RIVM increased from 234 in 2017, to 310 in 2018, 363 in 2019 and decreased to 204 in 2020. This decrease can most likely be attributed to the COVID-19 pandemic. Despite the decrease, neither the fraction of carbapenemase-producing isolates nor the fraction of meropenem resistant isolates significantly changed over this three-year time period (Fig 4.7.1.3 and Fig 4.7.1.4).

Figure 4.7.1.3 Carbapenemase production of *Enterobacterales* isolates submitted with a sampling date in 2017-2020

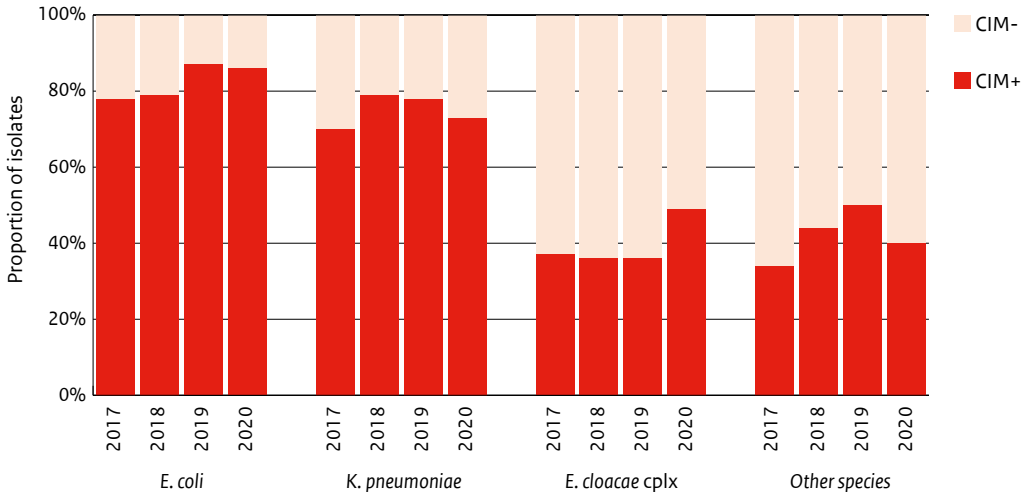
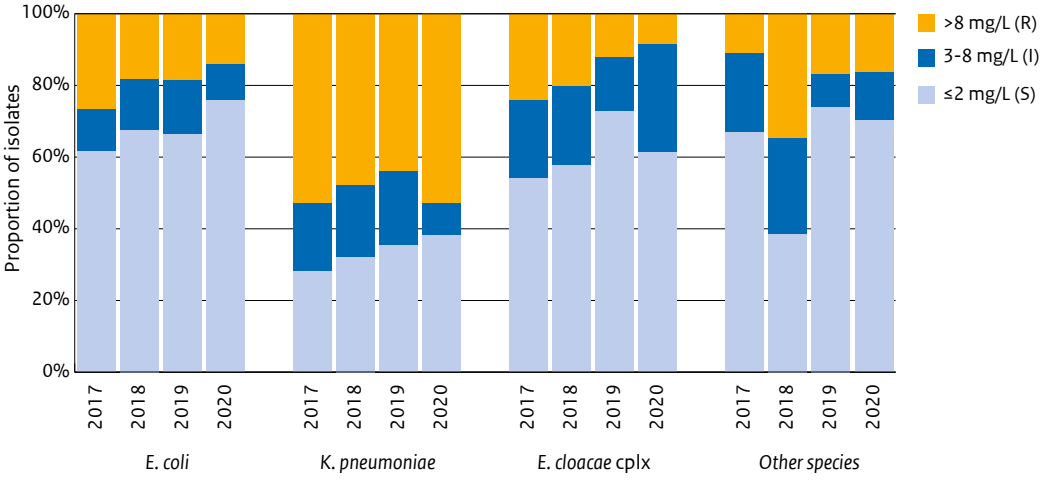


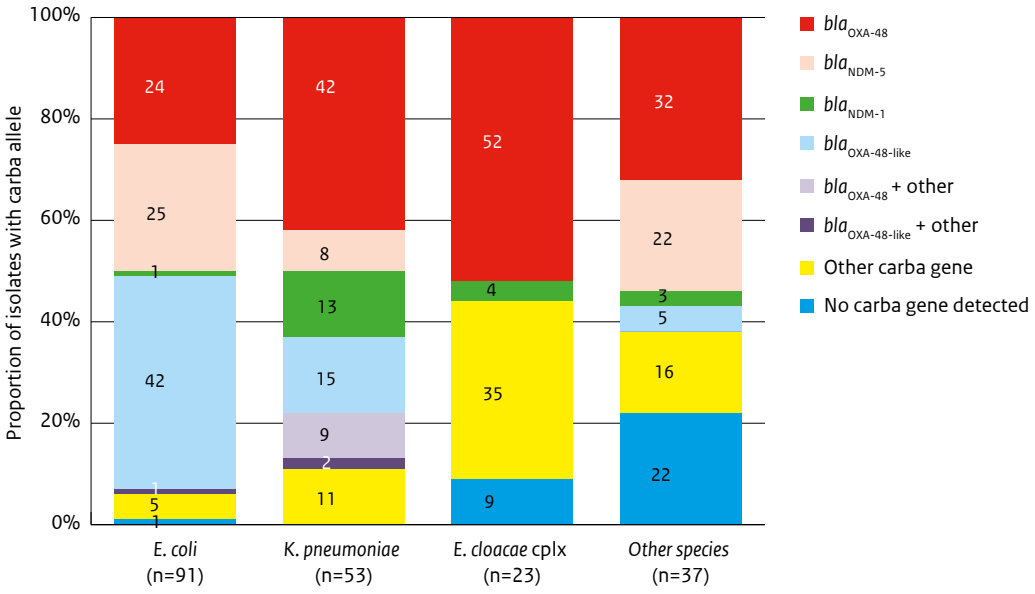
Figure 4.7.1.4 Distribution of meropenem susceptibility of CIM+ isolates of *Enterobacterales* isolates submitted with a sampling date in 2017-2020



As in previous years, the bla_{OXA-48} gene was the most frequently identified carbapenemase-encoding gene in CPE isolates cultured and submitted in 2020. The bla_{OXA-48} allele, either alone or in combination with another carbapenemase-encoding gene, was present in 24%, 42% and 52% of the *E. coli*, *K. pneumoniae* and *E. cloacae* complex, respectively (Figure 4.7.1.5). In *E. coli*, 25% of the isolates carried bla_{NDM-5} and the gene was found in 8% of the *K. pneumoniae* isolates. Conversely, bla_{NDM-1} was found predominantly in *K. pneumoniae* isolates (13%) and only in 1% of the *E. coli* isolates. The $bla_{OXA-48-like}$ alleles ($bla_{OXA-181}$, $bla_{OXA-232}$, $bla_{OXA-244}$ and $bla_{OXA-245}$)

were found in 42% and 15% of the *E. coli* and *K. pneumoniae* isolates, respectively. In all isolates, 60% (123/204) of the CPE analysed in 2020 carried a bla_{OXA-48} or $bla_{OXA-48\text{-}like}$ gene. In 2020, there was a substantial 26% increase of submitted isolates carrying the $bla_{OXA-244}$ allele. This $bla_{OXA-48\text{-}like}$ allele was found in *E. coli* only, comprising 30/91 (33%) of all carbapenemase-producing *E. coli* isolates submitted in 2020. In 2019, only 7% of the *E. coli* isolates carried $bla_{OXA-244}$. All $bla_{OXA-244}$ *E. coli* isolates submitted in 2020 had MICs for meropenem ≤2 mg/L. Some of these isolates were genetically highly related, but there was no indication of outbreaks or large-scale transmission, as the isolates were spread over six different genetic clusters. Four percent (8/204) of the CPE carried two carbapenemase-encoding genes. In 11 (5%) of the 204 CPE isolates cultured from patients in 2020 no carbapenemase-encoding gene was detected. Of these isolates, 7 (64%) were *Enterobacter* spp. and 2 (18%) *Klebsiella aerogenes*, formerly classified as *Enterobacter aerogenes*. The nature of the apparent carbapenemase production in *Enterobacter* spp. is still under investigation in the RIVM, but carbapenemase activity of an AmpC enzyme seems to be the likely explanation.

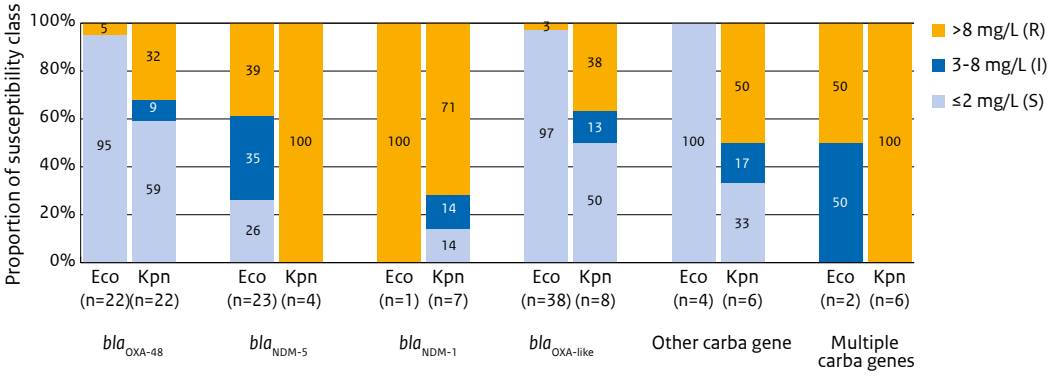
Figure 4.7.1.5 Distribution of carbapenemase-encoding genes in carbapenemase producing isolates submitted with a sampling date in 2020



$bla_{OXA-48\text{-}like}$ denotes the bla_{OXA-48} gene variants $bla_{OXA-181}$, $bla_{OXA-232}$, $bla_{OXA-244}$ and $bla_{OXA-245}$
Carba gene is short for carbapenemase-encoding gene

There was a strong correlation between the MIC for meropenem and the presence of particular species/carbapenemase-encoding allele combinations. Only a single *E. coli* isolate carrying bla_{OXA-48} had an MIC above the clinical breakpoint for meropenem resistance (MIC >8 mg/L; Figure 4.7.1.6). In contrast, 32% of the *K. pneumoniae* carrying bla_{OXA-48} were meropenem resistant. In general, a larger proportion of the *K. pneumoniae* isolates (53%, 28/53) were meropenem resistant compared to the *E. coli* isolates (14%, 13/91), irrespective of the carbapenemase-encoding genes present.

Figure 4.7.1.6 Relationship between the MIC for meropenem and the carbapenemase-coding genes in *E. coli* and *K. pneumoniae* isolates submitted with a sampling date in 2020



Since the end of 2019, genetic cluster numbers for CPE are reported in the Type-Ned. This has thus far resulted in 41 genetic clusters with *E. coli*, 55 with *K. pneumoniae*, 12 with *E. cloacae* complex and 7 with *C. freundii* complex. Of the 187 isolates from 2020 of these four species, 76 of these fell in one of 38 genetic clusters. Fourteen new genetic clusters arose in 2020, 9 by addition of an isolate from 2020 to an earlier isolate and 5 with isolates from 2020 alone. All new genetic clusters comprise two or three isolates only. MMLs are notified by email that isolates they submitted within a period of one year fall in a genetic cluster. Of the new clusters in 2020, 11 concerned multi-institutional genetic clusters, i.e. isolates were submitted by more than one lab. In 7 clusters, the previous isolates from another lab were detected up to twelve months ago and the MMLs involved were contacted by the RIVM to consent to share their name to each other to enable collaboration in potential transmission control.
Additional epidemiological questionnaire data was available in OSIRIS for 153 CPE positive persons with a sampling date in between 1 January 2020 and 31 December 2020 (Table 4.7.1.1). For 141 of the 153 definite notifications (92%) one or more isolates were identified in the Type-Ned database. For 51 persons in Type-Ned no corresponding notification could be identified in OSIRIS.
Screening was the reason for taking the sample in 71% of the persons in 2020, compared to 69% in 2019 and 72% in 2018. Hospitalization abroad for at least 24 hours within the previous two months was the most common risk factor for the presence of CPE (n= 51, 33% of all notifications), with Turkey (n= 8) and Egypt (n= 7) leading the list of countries reported. This was 40% in 2019 and 50% in 2018. No risk factor was identified in 48% of persons, which was higher compared to 38% in 2019 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 (10%) and the majority had no risk factor (67%). Among persons with an obtained screening isolate, 44% had been hospitalized abroad for at least 24 hours during the previous two months. Thirty-nine percent had no risk factor, but were screened as part of routine screening (e.g. on admission, because of prolonged hospital stay or as part of selective decontamination regimens) or targeted screening because of suspected CPE carriage.

In 2020, no new outbreaks with carbapenemase-producing *Enterobacterales* were reported to SO-ZI/AMR. See chapter 4.7.6 for more details about SO-ZI/AMR.

Table 4.7.1.1 Epidemiological data of notifications of persons carrying CPE (data from OSIRIS with sampling date 1 January – 31 December 2020)[1]

Characteristic	CPE positive persons n (%)
Any questionnaire data available	153
Reason for culturing	
Diagnostic	42 (27)
Screening	108 (71)
Other/unknown	3 (2)
Colonisation with CPE or infection caused by CPE	
Colonisation	98 (64)
Urinary tract infection	21 (14)
Respiratory tract infection	0 (0)
Sepsis/bacteraemia	3 (2)
Other infection	6 (4)
Unknown	25 (16)
Residence	
Living independently	132 (86)
Nursing or elderly home	4 (3)
Facilities for small-scale housing for elderly	1 (1)
Asylum seekers centre	3 (2)
Rehabilitation centre	1 (1)
Other/unknown	12 (8)
Invasive medical procedure/diagnostics	
No	53 (35)
Surgery	32 (21)
Other (including invasive procedure like endoscopy, cystoscopy, urinary catheter, renal dialysis)	32 (21)
Unknown	36 (24)
Risk factors	
No risk factor known/unknown	73 (48)
Hospitalization abroad >24 hours during the previous two months	51 (33)

Table 4.7.1.1 (continued) Epidemiological data of notifications of persons carrying CPE (data from OSIRIS with sampling date 1 January – 31 December 2020)[1]

Characteristic	CPE positive persons n (%)
Hospitalized in a country in:	
North Africa	10/51 (20)
West Asia (including Turkey)	8/51 (16)
South Asia	5/51 (10)
South Europe	9/51 (18)
West Europe	6/51 (12)
Other region of the world/unknown	13/51 (25)
Already known carrier of CPE	9 (6)
Received care in a department of another healthcare facility with an ongoing outbreak of CPE in the previous two months	3 (2)
Contact with a hospital abroad in the last year in a different way than >24 hours during the previous two months	8 (5)
Travelling abroad in the past six months (Type-Ned)/twelve months (OSIRIS) without hospitalization or visiting a hospital	9 (6)

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

Discussion

In 2020, the number of carbapenemase-producing *Enterobacterales* isolates that were submitted to the RIVM was considerably lower than in previous years. This decrease most likely is the result of the COVID-19 pandemic which has led to reduced travel and a reduction in regular healthcare. 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 healthcare centers.

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

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: 92% of the definite notifications have a corresponding isolate in Type-Ned. Remarkably, a substantial number of CPE isolates of positive persons are submitted to Type-Ned without a corresponding notification, which may be the result of several causes: the notification 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. The majority of CPE cases were identified upon routine or targeted screening and were colonized without signs of infection due to CPE. One third of all notified CPE cases were hospitalized abroad >24 hours during the previous two months.

Conclusions

- The overall percentage of *E. coli* and *K. pneumoniae* isolates with elevated carbapenem MIC values (i.e. > the screening breakpoint) on automated testing was 0.7% in 2020. Among isolates with an elevated MIC on automated testing, 5.6% had an MIC > the clinical S breakpoint on gradient strip testing.
- The percentage of *E. coli* and *K. pneumoniae* isolates with elevated carbapenem MIC values (i.e. > the screening breakpoint) on automated testing decreased between 2017 and 2020. However, the percentage of isolates with a gradient strip test-confirmed elevated MIC has increased between 2016 and 2019, but was lower in 2020 compared to 2019.
- Confirmatory testing of elevated MIC values with a gradient strip method has decreased since 2016, especially in *K. pneumoniae*.
- The use of tests for carbapenemase production (phenotypic) or carbapenemase genes has increased since 2016, but was lower in 2020 compared to 2019.
- Due to the COVID-19 pandemic the number of CPE submitted to the RIVM in 2020 has decreased with 46% compared to 2019, which is most likely the result of reduced travel and a reduction in regular healthcare.
- The most frequently identified carbapenemase encoding genes in *Enterobacterales* were $bla_{\text{OXA-48}}$, $bla_{\text{OXA-48-like}}$ genes, $bla_{\text{NDM-1}}$ and $bla_{\text{NDM-5}}$.
- There was a remarkable increase of $bla_{\text{OXA-244}}$ carrying *E. coli* submitted in 2020.
- The predominant carbapenemase-producing *Enterobacterales* species were *E. coli*, *K. pneumoniae* and species belonging to the *E. cloacae* complex.
- The MIC for meropenem was generally higher for *K. pneumoniae* than for *E. coli* isolates harbouring $bla_{\text{OXA-048}}$ or $bla_{\text{OXA-48-like}}$ genes. Still, these isolates were more sensitive for meropenem than isolates carrying other carbapenemase-encoding genes.
- Of the *K. pneumoniae*, *E. coli*, *E. cloacae* complex and *C. freundii* complex isolates, 76 (41%) fell in one of 38 genetic clusters.
- Fourteen new genetic clusters arose in 2020, 9 by addition of an isolate from 2020 to an earlier isolate and 5 with isolates from 2020 alone. All new genetic clusters comprise two or three isolates only.
- Seventy-one percent of CPE cases were identified upon routine screening or targeted screening because of suspected CPE carriage.
- In 33% 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 Egypt are the countries that are most often reported.
- In 48% of the CPE positive persons no known risk factor is present. In 56% of these cases, cultures were taken for screening purposes and 38% because of a diagnostic reason.

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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_{fm}). 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_{fm} are no longer available.

Methods

VRE_{fm} 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 vancomycin resistance 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 37 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

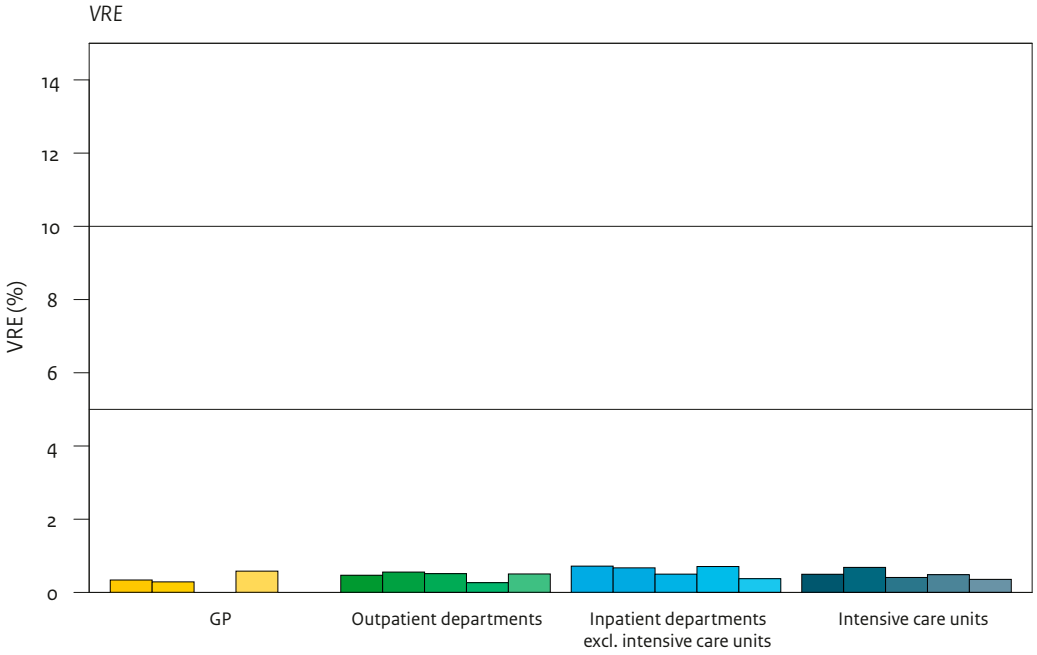
In 2020, five outbreaks with VRE_{fm} have been reported in the Netherlands in SO-ZI/AMR, all of them in hospitals, with a median reported number of 20 patients involved (range 4 – 61 patients). This number was much lower than the 19 outbreaks reported in 2019, and around 10 – 15 in the years before. In total, since the start of SO-ZI/AMR in April 2012, 111 outbreaks with VRE_{fm} have been reported in the Netherlands. The contribution of VRE_{fm} outbreaks was substantial in the previous years, with a proportion varying between 20 and 32% of all reported outbreaks in SO-ZI/AMR yearly. In 2020, this proportion was much lower, which was influenced by the number of COVID-19 outbreaks having been reported to the SO-ZI/AMR.
The percentage of VRE_{fm} isolates in general practitioner patients and outpatient and inpatient hospital departments in 2020 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_{fm} was continuously low over the years.

Table 4.7.2.1 Vancomycin-resistant *E. faecium* (VRE) in the Netherlands in 2020, based on ISIS-AR data

Type of department	Tested isolates, N	VRE, N (%)
GP	243	0 (0)
Outpatient departments	399	2 (1)
Inpatient departments excluding intensive care units	2,422	9 (0)
Intensive care units	850	3 (0)
Total	**4,014**	**14 (0)**

Numbers are based on a selection of 37 laboratories.
The first diagnostic E. faecium isolate per patient was selected.
The prevalence of VRE_{fm} isolates was based on positivity of confirmation tests, or, if these tests were lacking, on re-interpretation of test-values for amoxicillin/ampicillin and vancomycin according to EUCAST 2020, with VRE_{fm} being defined as resistant to amoxicillin/ampicillin and vancomycin.

Figure 4.7.2.1 Trends in VRE_{fm} in the Netherlands (from left to right 2016 to 2020), based on ISIS-AR data



Numbers are based on a selection of 37 laboratories.
The first diagnostic E. faecium isolate per patient per year was selected.
The prevalence of VRE_{fm} isolates was based on positivity of confirmation tests, or, if these tests were lacking, on re-interpretation of test-values for amoxicillin/ampicillin and vancomycin according to EUCAST 2020, with VRE_{fm} being defined as resistant to amoxicillin/ampicillin and vancomycin.

Discussion

Currently, there are no centrally collected data on molecular typing of VRE_{fm} isolates or acquisition of novel resistance determinants by VRE_{fm} in the Netherlands, even though the WHO marked VRE_{fm} as a "high priority antibiotic resistant organism". Thus, there are no longer reliable data available on the molecular epidemiology of VRE_{fm} in Dutch hospitals since 2018. The number of reported VRE_{fm} outbreaks in 2020 was remarkably lower compared to the previous years. This is most likely the result of the COVID-19 pandemic which led to a downscale of provided regular healthcare in hospitals and a change in infection prevention measures. The proportion of VRE_{fm} outbreaks compared to the total number of reported outbreaks was also lower in 2020, as a result of the substantial number of reported COVID-19 outbreaks in hospitals. Notably, this is in contrast to the majority of European countries, where the number of VRE_{fm} isolates is considerably increasing in the past years.[1,2] In 2015 in the EU/EEA, the population-weighted mean percentage of invasive VRE_{fm} was 10.5% and increased significantly to 18.3% in 2019.[1] Likewise, a recent retrospective observational study on vancomycin resistance in *E. faecium* and *E. faecalis* isolates from patients with bloodstream infections in the EU/EEA using data from the European Antimicrobial Resistance Surveillance Network (EARS-Net) database from 2012 to 2018, revealed that proportions of VRE_{fm} increased from 8.1% (95%CI 6.7–9.7%) in 2012 to 19.0% (95% CI 16.8–21.5%) in 2018.[3] Rising VRE_{fm} proportions were observed across all European regions. In contrast, *E. faecalis* remained generally susceptible to vancomycin as the

mean proportion of vancomycin-resistant *E. faecalis* in Europe was low (1.1% [95%CI 0.9–1.4%]). Furthermore, enterococci are capable to develop resistance towards last resort antibiotics such as daptomycin, linezolid and/or tigecycline.[4] Without a national surveillance to monitor the emergence of VRE_{fm} with these resistance mechanisms, novel resistances will be missed and may disseminate.[4, 5]

Conclusions

- The number of reported hospital outbreaks with VREfm was remarkably lower in 2020 compared to previous years due to the COVID-19 pandemic.
- The proportion of VRE_{fm} in infection-related isolates with *E. faecium* in various healthcare settings varies marginally below 1% and has not changed in the previous five years.
- There are no longer reliable data available on the molecular epidemiology of VRE_{fm} in Dutch hospitals, which is a cause for great concern.

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

Methods

Prevalence

From the ISIS-AR database, *S. aureus* isolates, including MRSA, that were sampled during the five most recent years (2016 to 2020) were identified. Numbers are based on data from 37 laboratories that continuously reported complete data to the ISIS-AR database during the selected period. The first diagnostic *S. aureus* isolate per patient per year from blood, cerebrospinal fluid, urine, lower respiratory tract, or wound/pus was selected. Prevalence of MRSA was calculated as the percentage of *S. aureus* isolates for which the MRSA confirmation test (presence of *mecA* gene or *pbp2*) was positive, or, if these tests were lacking, laboratory S/I/R interpretation for cefoxitin was R, or, if no data on a cefoxitin test was available, the laboratory S/I/R interpretation of flucloxacillin/oxacillin was R. An additional analysis was conducted for *S. aureus* isolates from blood only.

Molecular results

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). Isolates that could not be classified in diagnostic or screening based on material were excluded from the analysis. 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 selected isolates only.
The data from the molecular surveillance were based on the first MRSA isolate per person per year sampled in the period 2008 to 2020 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 *mecA* or *mecC* gene, samples that could not be typed by MLVA, and isolates without a person ID were also excluded from further analysis.

Epidemiology

Since 2017, as part of the enhanced surveillance, an epidemiological questionnaire on patient characteristics is requested to be completed by the general practitioner, microbiologist, or infection control practitioner, depending on the location of sampling. Late November 2018, a new version of the epidemiological questionnaire was launched. For the epidemiological analyses the same inclusion criteria were used as for the molecular analyses except that the isolates that could not be classified in diagnostic or screening based on material only were not excluded. Epidemiological data in this section are described for 2020 and compared with previous years, for all isolates combined and by reason for sampling.

Results

Prevalence

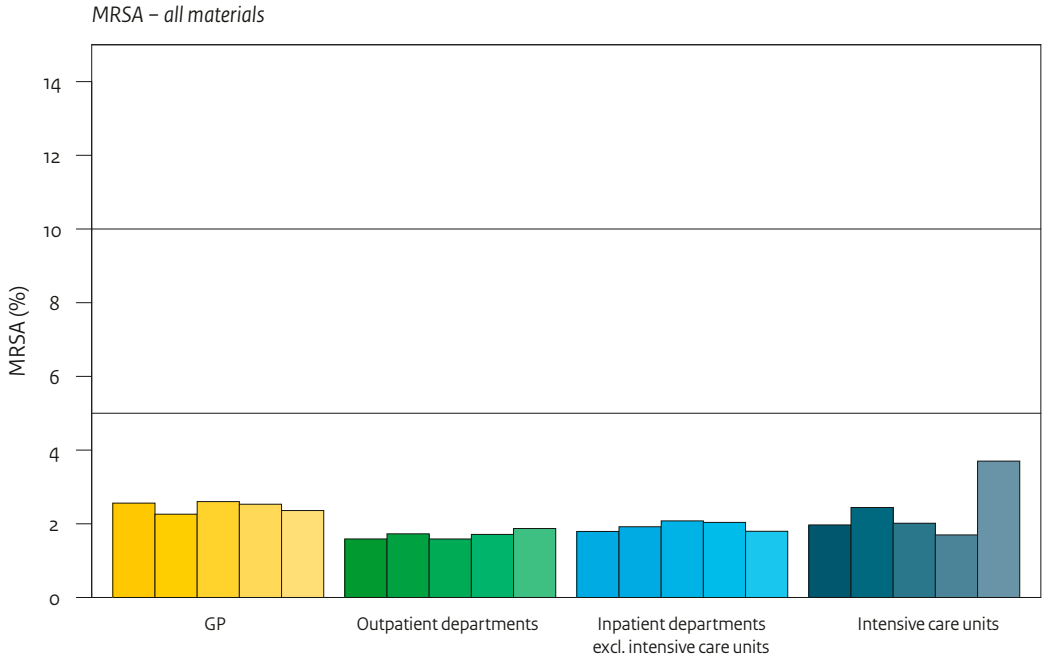
In ISIS-AR, the proportion of diagnostic isolates of *S. aureus* in 2020 that was identified as MRSA was 2% (n=604/29,450). The percentages were similar among the various types of departments, except for intensive care units in which the prevalence was 4% (Table 4.7.3.1). In blood, the prevalence of MRSA in 2020 was 2% (n=46/2,939). Figure 4.7.3.1 shows the trends in MRSA from 2016 to 2020 in all diagnostic isolates, which were quite stable, except in intensive care units in which the prevalence increased from ~2% in the first four years (n=31/1574 in 2016, 36/1474 in 2017, 32/1588 in 2018, and 24/1413 in 2019) to 4% in 2020 (n=52/1405).

Table 4.7.3.1 Methicillin-resistant *S. aureus* (MRSA) in the Netherlands in 2020, based on ISIS-AR data

Type of department	Tested isolates, N	MRSA, N(%)
GP	7,119	168 (2)
Outpatient departments	10,630	199 (2)
Inpatient departments excluding intensive care units	10,296	185 (2)
Intensive care units	1,405	52 (4)
Total	**29,450**	**604 (2)**

Numbers are based on a selection of 37 laboratories.
The first diagnostic S. aureus isolate per patient was selected.
The prevalence of MRSA isolates was based on positivity of confirmation tests (presence of mecA gene or pbp2), or, if these tests were lacking, on laboratory S/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 2016 to 2020), based on ISIS-AR data



Numbers are based on a selection of 37 laboratories.
The first diagnostic S. aureus isolate per patient per year was selected.
The prevalence of MRSA isolates was based on positivity of confirmation tests (presence of mecA gene or pbp2), or, if these tests were lacking, on laboratory S/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

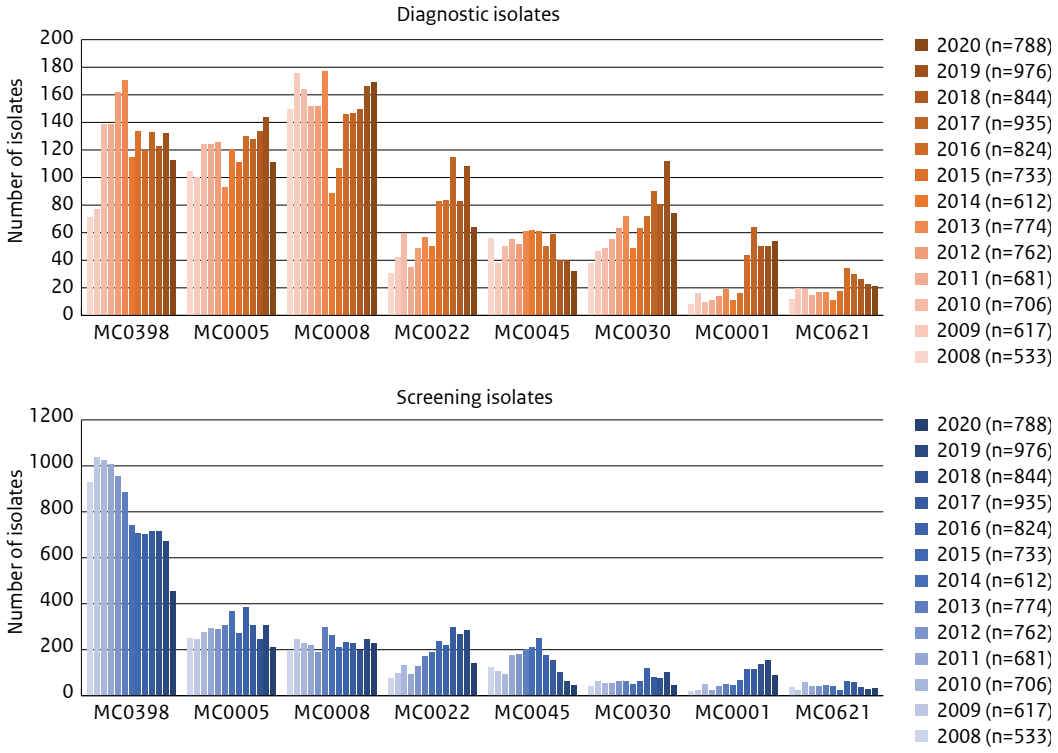
The RIVM received 2,749 isolates that fulfilled the inclusion criteria, submitted by 53 medical microbiology laboratories (MML) . As only the first isolate per person per year was used, 2,376 isolates from 2,376 persons were included for further analyses. The persons from whom MRSA were cultured had a mean age of 44 years (SD 25 years) and 1,252 (52%) were male.

As in previous years, the majority of the isolates were cultured from samples submitted to the MML from hospitals (n= 1,452; 61%), followed by GPs (n= 722; 30%) and nursing or elderly homes (n= 103; 4%). Based on culture methods and origin of the samples, 67% (n= 1,588) of the isolates were submitted as screening samples representing mainly swabs from nose, throat and perineum (Figure 4.7.3.2). A total of 788 isolates (33%) were submitted as diagnostic isolates with the majority being cultured from wound material or pus (599/788; 71%), and 32 isolates from blood cultures (1%). The distribution of materials from which the MRSA were isolated is similar to that of previous years.

The number of MRSA isolates submitted to the RIVM decreased from 3,306 in 2019 to 2,376 in 2020. This 28% decrease can most likely be attributed to the COVID-19 pandemic. For 2020, the MRSA population could be divided into 661 MLVA-types, which were grouped into 23 MLVA-complexes (MCs; 2,173 isolates). For 81 MLVA-types no MLVA-complex (132 isolates) could be assigned. The most frequently identified

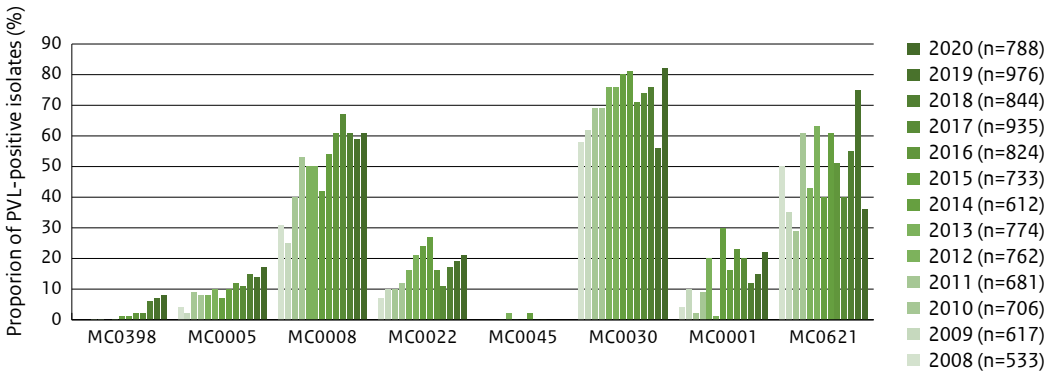
MLVA-complex in 2020 was MC0398, also known as LA-MRSA, which was detected in 569/2,376 (24%) of the isolates.
During the 2008-2020 surveillance period, there has been a considerable increase in the number of submitted MC0022, MC0001 and MC0030 isolates, whereas numbers for MC0398 and MC0045 isolates have dropped (Figure 4.7.3.2). For MC0398 the number of submitted isolates cultured from diagnostic samples increased from 71 in 2008 to 171 in 2013 to drop thereafter and remain relatively stable with 132 isolates in 2019. The number of isolates obtained from screening samples remained stable at approximately 1,000 isolates until 2012 after which the number dropped to around 700 isolates submitted annually. As a result the proportion of LA-MRSA from diagnostic samples increased from 7% in 2008 to 16% in 2015-2019. In 2020 the number of submitted LA-MRSA obtained from diagnostic samples dropped with 14%, whereas the number of isolates from screening samples dropped with 32%, resulting in a higher proportion of isolates from diagnostic samples. The proportion increased from 15% in Q1 of 2020 to reach 24% in Q4 of 2020 reflecting decreased screening practices due to an overload of the healthcare system as a result of the COVID-19 pandemic. In 2020 MC0398 ranked second in absolute numbers of all diagnostic isolates among all MLVA complexes. The MC0030 complex had the highest proportion isolates classified as diagnostic (62%, 74/120), but ranked fourth in absolute numbers among MLVA-complexes.
Panton-Valentine Leukocidin (PVL) positivity among all submitted MRSA isolates increased from 12% in 2008 to 18% in 2014 reaching 28% in 2020. In 2020, 42% (329/788) of the diagnostic isolates carried the PVL-encoding genes, whereas 21% (328/1,588) of the screening isolates were PVL positive. In 2020, MC0030 isolates had the highest proportion of PVL-positivity (82%, 98/120) (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% (44/569) in 2020. Within MC0398, 75% (33/44) 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 2020) 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 2020), 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.

Epidemiology

Additional epidemiological questionnaire data for 2020 was available for 75% (n=1,925/2,552) of persons, which was lower than in previous years, in which the percentage ranged from 82-85%. This might be explained by lack of time of those who had to fill in the questionnaire during the COVID-19 pandemic. For 112 (6%) persons it was recorded in the questionnaire that they were employees in a healthcare facility that were tested as part of a local screening programme. After we excluded this group, data from 1813 patients were left for the current analysis. Based on the information in the epidemiological questionnaire, in 43% (n=782/1,813) of patients a sample was taken for diagnostic reasons. For 55% (n=995) of patients the reason of sampling was screening/active surveillance, and for 2% (n=36) of patients the reason was unknown.
In Table 4.7.3.2 a selection of epidemiological data on included patients is summarized. Approximately two-third of patients (67%) were sampled in the hospital, with the majority being sampled in outpatient departments. For 383 of the patients the COVID-19 infection status at time of sampling was available.
The proportion of patients with a proven COVID-19 infection was lower in the group of patients that were sampled for diagnostic reasons (4%) than in patients that were sampled for screening/active surveillance (7%).
In the group of patients that were sampled for screening/active surveillance, the large majority met the WIP risk category 1, 2, or 3[1] (91%), whereas in diagnostic isolates this proportion was much lower but increased from 14% in 2017 and 16% in 2018 to 33% in 2019 and 39% in 2020. Work-related exposure to livestock animals was reported for 5% of patients with diagnostic samples and 26% of patients with samples that were taken for screening/active surveillance. The main group of livestock animals to which this group was exposed were pigs (70%), and from 92% of patients with a livestock related profession a LA-MRSA was sampled. Out of all patients with LA-MRSA, 24% (n=22/90) of patients with diagnostic samples, and 71% (n=164/231) of patients that were sampled for screening/active surveillance, were patients with work related exposure to livestock animals. Hospitalization abroad for at least 24 hours during the previous two months was recorded for 1% of patients with diagnostic isolates and 8% of patients that were sampled for screening/active surveillance. The number of patients for whom hospitalisation abroad was recorded was much lower in 2020 than in the years before (n=152/2702 in 2017, 146/2403 in 2018, 165/1821 in 2019, 52/1083 in 2020), probably because of the travel restrictions during the

COVID-19 pandemic. The main continents of hospitalisation in 2020 were Western Europe (29%) and Western Asia (including Turkey, 21%), whereas in other years the proportion of hospitalisation in Western Europe was lower (~18%). From patients with a diagnostic sample, 2% was living in an asylum centre, whereas this percentage was 10% in patients that were sampled for screening/active surveillance.

Table 4.7.3.2. Epidemiological data of 1813 MRSA positive persons (excluding employees) with a genotyped isolate in the enhanced MRSA surveillance system, with a sampling date in 2020

	Diagnostic and screening combined		Diagnostic		Screening/active surveillance	
Characteristic	**Data available (N)**	**n (%)**	**Data available (N)**	**n (%)**	**Data available (N)**	**n (%)**
Sample taking location (hospital only)						
Outpatient departments	1,215	541 (45)	547	240 (44)	648	294 (45)
Inpatient departments (excluding Intensive Care Units)	1,215	316 (26)	547	155 (28)	648	158 (24)
Intensive Care Units	1,215	73 (6)	547	31 (6)	648	41 (6)
Other/unknown	1,215	285 (23)	547	121 (22)	648	155 (24)
Proven COVID-19 infection						
Proven COVID-19 infection[a]	383	22 (6)	182	8 (4)	198	14 (7)
Risk factors						
Meeting WIP[1] risk category 1,2, or 3[a,b]	1,555	1,074 (69)	647	250 (39)	889	811 (91)
Work-related exposure to livestock animals	1,219	204 (17)	524	25 (5)	683	178 (26)
Pigs	204	143 (70)	25	16 (64)	178	127 (71)
Cattle	204	40 (20)	25	8 (32)	178	31 (17)
Other/unknown	204	21 (10)	25	1 (4)	178	20 (11)
Hospitalization abroad >24 hours during the previous two months	1,083	52 (5)	475	6 (1)	595	45 (8)
Western Asia (including Turkey)	52	11 (21)	6	2 (33)	45	9 (20)
Southern Europe	52	6 (12)	6	0 (0)	45	6 (13)
Western Europe	52	15 (29)	6	2 (33)	45	13 (29)
Other	52	20 (38)	6	2 (33)	45	17 (38)
Living in asylum centre	1,656	103 (6)	708	13 (2)	922	90 (10)

WIP: Working Party in Infection Control.

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

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

Discussion

Prevalence

Within the ISIS-AR database all routine cultures from medical microbiological laboratories are collected. However, general practitioners usually send samples for culture and susceptibility testing in case of antimicrobial therapy failure. As a result, the presented resistance levels are likely to be higher than those for all patients with infections caused by *S. aureus* presenting at the general practice, although it is not known to what extent. Among patients attending hospital departments, the rate of sampling is higher than among general practitioner patients. Therefore, bias due to selective sampling will be lower than in general practitioner patients and resistance percentages in this section are considered more representative of resistance in hospital departments. Blood isolates are taken routinely in case of suspected bloodstream infection or meningitis, with case definitions based on uniform guidelines, and are, therefore, considered to be the least biased.

Within ISIS-AR an increase was found in the proportion MRSA in ICU's in 2020. The explanation of this finding is currently unclear. Probably the changes in population characteristics during the COVID-19 pandemic play a role. Increased transmission is not a likely explanation since no large clusters were found in the molecular data enhanced MRSA surveillance.

Molecular results

Within the enhanced MRSA surveillance database, 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 2020 still missing for 15% to 19% of the isolates. Therefore, distinction between screening and diagnostic isolates within the analyses on molecular results, is solely based on the material and origin of the samples and 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*. 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).

Epidemiology

From November 2016 on epidemiological questionnaire data are available for persons from whom an MRSA was sampled. Late November 2018, a new version of the epidemiological questionnaire was launched. This may have caused a change in the trend for some of the investigated characteristics. The increase in 2019 in the proportion of diagnostic samples that met the WIP risk category 1, 2, or 3,[1] might therefore be a registration artefact.

Conclusions

Prevalence

- Within ISIS-AR, the proportion of *S. aureus* that was MRSA positive was 2%, with an increase in resistance in ICU, probably as an additional effect of the COVID-19 pandemic.

Molecular results

- Due to the COVID-19 pandemic the number of MRSA submitted to the RIVM in 2020 has decreased with 28% compared to 2019.
- LA-MRSA is still the predominant MRSA clade in the Dutch enhanced MRSA surveillance, ranking second in the most frequently identified MLVA complex in MRSA cultured from diagnostic samples.
- During the 2008-2020 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 28% in 2020. In 2019, 42% of the diagnostic isolates carried the PVL-encoding genes, whereas 21% of the screening isolates were PVL positive. MC0030 isolates had the highest proportion of PVL-positivity in 2020 (82%). In recent years the proportion of PVL-positive isolates found among LA-MRSA (MC0398) has been increasing, reaching 8% in 2020.

Epidemiology

- In 43% of MRSA positive patients, the samples were taken for diagnostic reasons and this proportion is increasing over the years.
- The majority of patients with samples that were taken for screening/active surveillance, met WIP-category 1,2, or 3[1] (91%), with the main risk factor being work-related exposure to livestock animals (26%).

References

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

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

Introduction

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

Methods

Data on carbapenem-resistant and carbapenemase producing *P. aeruginosa* were obtained from ISIS-AR and the national surveillance on carbapenemase-producing *P. aeruginosa* (CPPA) which started in 2020. From the ISIS-AR database for each patient the first *P. aeruginosa* isolate per year was extracted. To avoid overestimation of the percentage CRPA caused by active screening for this highly resistant microorganism, only data on diagnostic cultures (as categorized by the reporting laboratory) from blood, cerebrospinal fluid, urine, lower respiratory tract, and wound or pus were included in the analysis.
First, the number of phenotypical carbapenem-resistant isolates was determined (based on re-interpretation according to EUCAST 2020). 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 37 laboratories (out of a total of 51 laboratories in the Netherlands) which provided complete data on the last five years (2016 to 2020).
In 2020 the national surveillance for carbapenemase-producing *P. aeruginosa* (CPPA) was started, and medical microbiology laboratories (MMLs) sent *P. aeruginosa* isolates to the RIVM via Type-Ned CPE for additional analyses if the isolates had an MIC for meropenem >2 mg/L or an MIC for imipenem >4 mg/L, or produced carbapenemase or carried a gene encoding carbapenemase. 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. All carbapenemase-producing isolates were subjected to next-generation sequencing (NGS) and the NGS data was used to assess the genetic relationship between isolates and the presence of antibiotic resistance genes.

Results

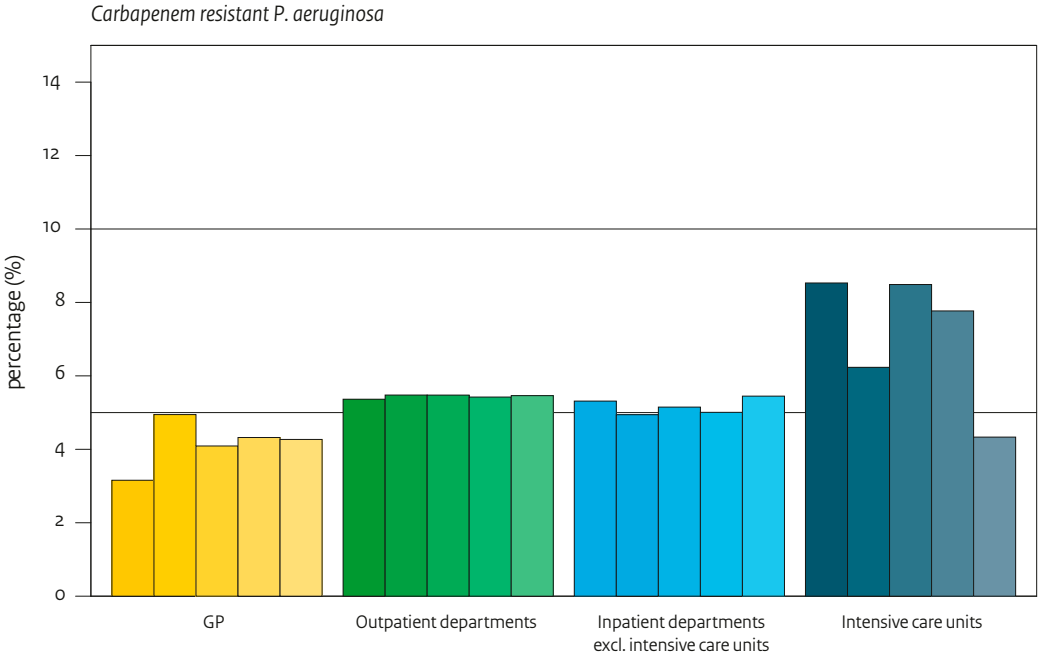
A search in the 2020 ISIS-AR database revealed that 5% (720/14,348) of the diagnostic *P. aeruginosa* isolates were phenotypically resistant to carbapenems (MIC >8 mg/L) (Table 4.7.4.1). The observed proportion of resistance appears to be relatively stable over the 2016-2020 time period, except for a sharp decrease in carbapenem-resistant *P. aeruginosa* in isolates from ICUs in 2020 compared to the previous years (Figure 4.7.4.1). Of the total number of 720 carbapenem-resistant *P. aeruginosa* isolates, for 61 (8%), obtained from 8 laboratories, data on tests for carbapenemase production was available, of which 7 (11%) showed a positive result.
Additional analyses in the 2020 ISIS-AR database showed that approximately 1% (186/13,047) of the diagnostic *P. aeruginosa* isolates were MDR (Table 4.7.4.2). Approximately 56% (104/186) 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 2020, based on ISIS-AR data

Type of department	*P. aeruginosa*, N	Phenotypical carbapenem resistant *P. aeruginosa*, N(%)
GP	4,732	202 (4)
Outpatient departments	3,955	216 (5)
Inpatient departments excluding intensive care units	5,084	277 (5)
Intensive care units	577	25 (4)
Total	**14,348**	**720 (5)**

Numbers are based on a selection of 37 laboratories.
The first diagnostic P. aeruginosa isolate per patient was selected.
Based on re-interpretation according to EUCAST 2020.

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 2016 to 2020), based on ISIS-AR data



Numbers are based on a selection of 37 laboratories.
The first diagnostic P. aeruginosa isolate per patient per year was selected.
Based on re-interpretation according to EUCAST 2020.

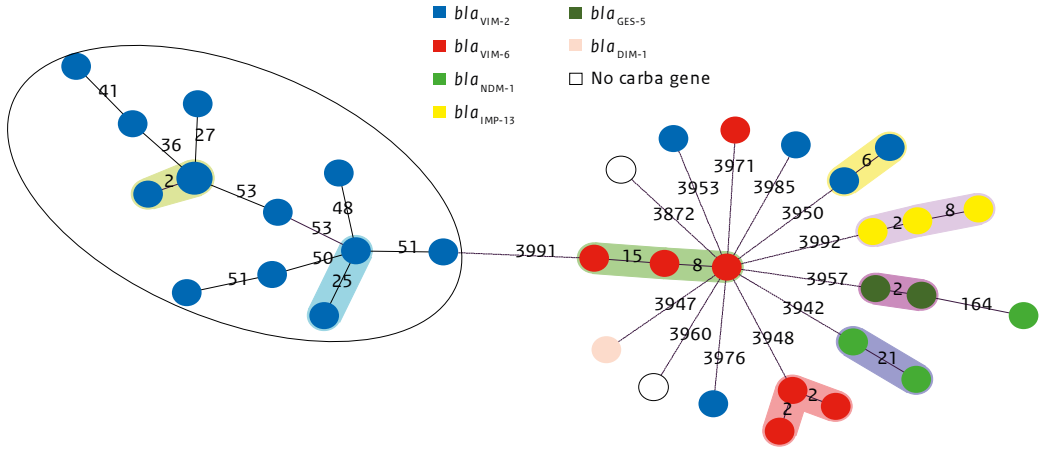
Table 4.7.4.2 Multidrug resistant (MDR) *P. aeruginosa* in the Netherlands in 2020, and phenotypical carbapenem resistant *P. aeruginosa* in relation to MDR *P. aeruginosa*, based on ISIS-AR data

Type of department	*P. aeruginosa*, N	MDR *P. aeruginosa*, N(%)	Phenotypical carbapenem resistant *P. aeruginosa* in relation to MDR *P. aeruginosa*, N(%)
GP	4,480	26 (1)	11 (42)
Outpatient departments	3,504	69 (2)	42 (61)
Inpatient departments excluding intensive care units	4,580	80 (2)	46 (57)
Intensive care units	483	11 (2)	5 (45)
Total	**13,047**	**186 (1)**	**104 (56)**

Numbers are based on a selection of 37 laboratories.
The first diagnostic P. aeruginosa isolate per patient was selected.
Based on re-interpretation according to EUCAST 2020.
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 186 *P. aeruginosa* isolates via Type-Ned CPE/CPPA from 182 patients which were sampled in 2020 and submitted by 36 MMLs. Of these isolates, 36 (20%, 36/182, one isolate per person) produced carbapenemase and were submitted by 16 MMLs. Isolates not producing carbapenemase as determined by the CIM test, did not yield a PCR product. Analysis of NGS data of all carbapenemase-producing isolates revealed that half of the isolates (18/36; 50%) carried a bla_{VIM-2} allele. In the remaining isolates seven carried bla_{VIM-6} (19%), three bla_{IMP-13} (8%), two bla_{GES-5} (2%), a single isolate carried bla_{DIM-1} and in five isolates (14%) no carbapenemase-encoding gene could be identified. The genetic relations were assessed by performing whole-genome multiple-locus sequence typing (wgMLST) (Figure 4.7.4.2). This revealed that most of the bla_{VIM-2} isolates resided in a group of genetically closely related bla_{VIM-2} carrying isolates, designated as Group 1. There also were several genetic clusters, representing possible transmission events. Of the CPPA isolates 78% (28/36) had MICs for meropenem above the clinical breakpoint (Table 4.7.4.3), whereas 36% (53/146) of the *P. aeruginosa* not producing carbapenemase had MICs above the clinical breakpoint. Although it is unknown which subsequent isolate of the patient was submitted via Type-Ned CPA/CPPA, the following sample materials were reported: Eight CPPA were isolated from wounds, a single isolate from blood, five from sputum and six from urine samples. The majority (34/36) of the CCPA were obtained from materials submitted by hospitals. For 2020 no epidemiological questionnaires were available.

Figure 4.7.4.2 Minimum spanning tree of wgMLST analysis of CPPA isolates from patients sampled in 2020



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

Table 4.7.4.3 Distribution of carbapenemase-encoding genes based on PCR in carbapenemase-producing *P. aeruginosa* isolates received via Type-Ned CPE/CPPA by the RIVM in 2020

	Carbapenemase encoding alleles							
MIC meropenem	**bla$_{VIM-2}$**	**bla$_{VIM-6}$**	**bla$_{VIM}$**	**bla$_{NDM-1}$**	**bla$_{GES-5}$**	**bla$_{DIM-1}$**	**No carba allele found**	**Total (%)**
≤ 2 mg/L (S)	3						1	4 (11%)
3-8 mg/L (I)	3	1						4 (11%)
>8 mg/L (R)	12	6	3	3	2	1	1	28 (78%)
Total	**18**	**7**	**3**	**3**	**2**	**1**	**2**	**36**

Discussion

In 2020, in ISIS-AR, 5% of *P. aeruginosa* in diagnostic isolates were phenotypically resistant to carbapenems. For only 8% of these isolates, data on carbapenemase tests (phenotypically or genotypically) were available in the ISIS-AR database. Of the 61 phenotypical carbapenem-resistant isolates with test results, 7 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, 1% of *P. aeruginosa* in diagnostic isolates were MDR, of which approximately 56% were phenotypically resistant to carbapenems. The proportion of phenotypical carbapenem-resistant *P. aeruginosa* in ICUs was remarkably lower in 2020

compared to previous years, while these proportions in the other types of departments were not different than before. Due to the COVID-19 pandemic in 2020, patient characteristics and infection prevention measures especially in ICUs were different than in the years before, which might have resulted in, for example, lower transmission of and lower numbers of infections with carbapenem-resistant *P. aeruginosa*. Further research to explore these findings will be performed.
Half of the CPPA submitted via Type-Ned carried the bla_{VIM-2} allele. Only 78% of the CPPA isolates had MICs for meropenem above the clinical breakpoint. The 2020 results were similar to those of the 2014-2019 period. Of the submitted isolates not producing carbapenemase, 36% had MICs for meropenem above the clinical breakpoint. It is likely this resistance is caused by other mechanisms than carbapenemase production such as reduced cell wall permeability, increased efflux pump activity, AmpC activity etc.
Unfortunately, it is not yet possible to get a complete overview of carbapenem-resistant and carbapenemase-producing *P. aeruginosa* in the Netherlands, since not all laboratories submitted complete data to one or both of the surveillance systems ISIS-AR and Type-Ned CPE/CPPA in 2020. Therefore, the data as shown here are an underestimation of the number present in the Netherlands.

Conclusions

- In 2020, 5% of the Dutch *P. aeruginosa* in diagnostic isolates were phenotypically resistant to carbapenems. Only for 8% of the phenotypically resistant *P. aeruginosa* isolates, information was available on carbapenemase production, and of these only 11% produced carbapenemase. In contrast to earlier years, the prevalence of carbapenem-resistant *P. aeruginosa* compared to the total number of diagnostic *P. aeruginosa* isolates in the ICU department was not higher compared to other departments and lower compared to previous years, probably due to the COVID-19 pandemic. 1% of the total number of *P. aeruginosa* isolates was MDR and 56% of these MDR isolates were carbapenem-resistant.
- The most predominant (50%) carbapenemase-encoding allele in carbapenemase-producing *P. aeruginosa* was bla_{VIM-2}.
- Only 78% of the carbapenemase-producing *P. aeruginosa* had MICs for meropenem above the EUCAST defined clinical breakpoints.
- Data from both ISIS-AR and Type-Ned CPE/CPPA could not give a complete overview of carbapenem-resistant and carbapenemase-producing *P. aeruginosa* in the Netherlands, since not all laboratories submitted data to one or both of the surveillance systems.

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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 2020 clinical breakpoints.

Results

In table 4.7.5.1 and 4.7.5.2 the estimated percentages of ESBL carrying *E. coli* and *K. pneumoniae* are shown by site, i.e. general practice (GP), outpatient departments, inpatient departments and intensive care units (ICUs), in 2020. Trends in ESBL percentages (from left to right 2016 to 2020) among clinical isolates of *E. coli* and *K. pneumoniae* by site are shown in figure 4.7.5.1. The percentages of ESBL have slightly increased for *E. coli* over the past years with stabilizing ESBL percentages between 3 and 8% depending on type of department in 2019 and 2020. From 2019-2020 there is a notably sharp increase in ESBL percentage for *K. pneumoniae* from 12 to 15% in ICUs. The data show an increasing trend correlated with the complexity of care with highest ESBL percentages in the ICUs. Despite the increase in ESBL *K. Pneumoniae* in 2020 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 2020, based on ISIS-AR data

Type of department	Tested isolates, N	ESBL
GP	92,122	2,958 (3)
Outpatient departments	17,397	856 (5)
Inpatient departments excluding intensive care units	25,383	1,290 (5)
Intensive care units	1,107	92 (8)
Total	**136,009**	**5,196 (4)**

Numbers are based on a selection of 37 laboratories.
The first diagnostic E. coli isolate per patient was selected.
Based on re-interpretation according to EUCAST 2020.
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 2020, based on ISIS-AR data

Type of department	Tested isolates, N	ESBL
GP	12,662	472 (4)
Outpatient departments	3,855	237 (6)
Inpatient departments excluding intensive care units	5,320	469 (9)
Intensive care units	388	59 (15)
Total	**22,225**	**1,237 (6)**

Numbers are based on a selection of 37 laboratories.
The first diagnostic K. pneumoniae isolate per microorganism per patient was selected.
Based on re-interpretation according to EUCAST 2020.
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* and *K. pneumoniae* in the Netherlands (from left to right 2016 to 2020), based on ISIS-AR data

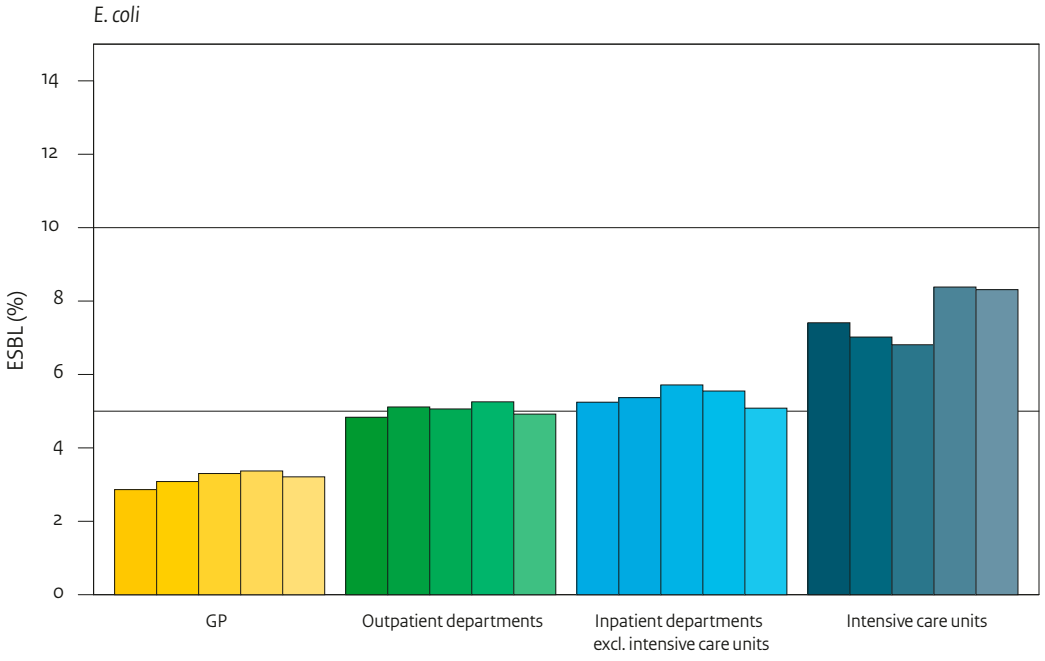
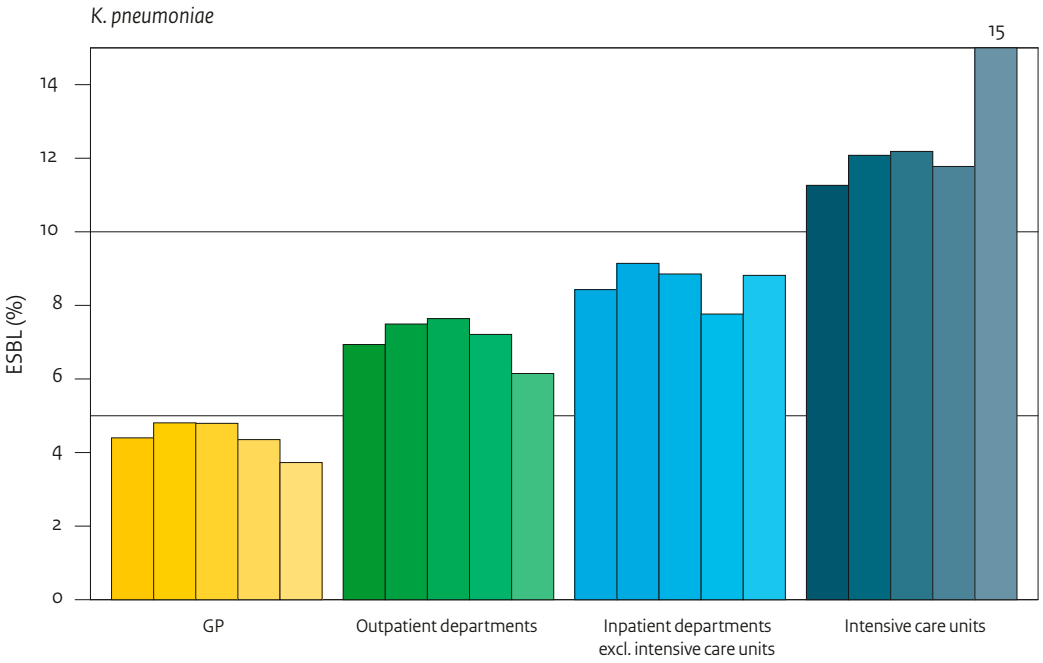


Figure 4.7.5.1 (continued) Trends in extended spectrum beta-lactamase (ESBL) producing *E. coli* and *K. pneumoniae* in the Netherlands (from left to right 2016 to 2020), based on ISIS-AR data



Numbers are based on a selection of 37 laboratories.
The first diagnostic isolate per patient per year was selected.
Based on re-interpretation according to EUCAST 2020.
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]

The sharp increase of the proportion of ESBL-producing *K. pneumoniae* in ICUs in 2020 compared to 2019 might be related to the COVID-19 pandemic. The patient population in hospitals and especially in ICUs was different compared to previous years, with longer lengths-of-stay in ICUs potentially leading to an increase of resistant gram-negative bacteria through selection following antimicrobial treatment. A retrospective cohort study from the UK described a high rate of Gram-negative infections among COVID-19 patients associated with longer ICU stay.[7] On the other hand, the absolute number of isolates in 2020 was relatively low, probably as a result of the downscale of regular healthcare, and the ESBL proportion was therefore

only calculated based on a relatively low number of isolates (n=59/388). Unfortunately, no additional epidemiological data on the patient or genotypical information of these isolates was available, and it is unknown if transmission of resistant strains between patients might have played a role. Although the COVID-19 pandemic restrictions resulted in reduced international travel, there was no decrease of the proportion of ESBL-producing *Enterobacterales* in these selected diagnostic samples.

Conclusions

- In 2020, the percentages of ESBL for *E. coli* stabilized between 3 and 8%, while there was a sharp increase in the percentage of ESBL to 15% for *K. pneumoniae* in the intensive care units for which further analysis will follow

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

Introduction

In 2012, the Early warning and response meeting for Hospital-acquired Infections and AntiMicrobial Resistance (SO-ZI/AMR) was founded. The initial purpose of the SO-ZI/AMR is to mitigate large-scale outbreaks of AMR in hospitals and to prevent spread to other healthcare facilities through early warning and reporting. Since 2015 long-term care facilities (LTCFs) are also invited to report outbreaks of highly-resistant microorganisms (HRMO). Since then, the name of the early warning and response meeting was changed to Healthcare associated Infections and AntiMicrobial Resistance (SO-ZI/AMR).
The SO-ZI/AMR consists of experts in the field of clinical microbiology, infection prevention, elderly care and public health and meets once a month. The SO-ZI/AMR assesses the risk of the outbreak to public health, monitors the course of the outbreak and facilitates – on request of the hospital or LTCF – in the acquisition of external expertise. An overview of active outbreaks is reported to professionals involved in infection prevention on a monthly basis.
Notifications are voluntary, but do not come without obligations. All hospitals have committed themselves to participate in SO-ZI/AMR. In order to benefit from a financial compensation rule introduced in 2017 to compensate for detection and control of all outbreaks in LTCF, these outbreaks have to be reported to the SO-ZI/AMR.[1]

Methods

Healthcare facilities send outbreak notifications using a standardized webbased form to RIVM or NVMM (the Dutch Society of Medical Microbiology), where the information is copied into one database at the RIVM. Monthly updates are provided by institutions until the outbreak is considered ended.

Results

Table 4.7.6.1 provides an overview of the thirty-four outbreaks reported in 2020. These were reported by 26 different healthcare institutions: 21 outbreaks in hospitals and 13 in LTCFs. Most outbreaks (n=27) ended in 2020, 6 ended in 2021 and 1 was retrospectively reported and took place from December 2018 until February 2019. As reported in the table, the most frequent reason for notification of an outbreak in a hospital was the imminent closure of wards (90%); 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 (4) was similar as in 2019, and only slightly higher compared to LTCFs (3), although the maximum number of involved patients was almost three times as high in hospitals (61 vs 20).
Methicillin-resistant *Staphylococcus aureus* (MRSA) outbreaks were most often reported, comparable to previous years. Approximately two third of these MRSA outbreaks were reported by LTCFs. The number of outbreaks with vancomycin-resistant enterococci (VRE) was much lower compared to previous years, which might be a direct result of changed infection prevention policies and altered hospital population due to the COVID-19 pandemic.
No outbreaks of carbapenemase-producing strains were reported, compared to three in 2019 and eight in 2018. Seven outbreaks of COVID-19 were reported to SO-ZI/AMR, all but one in hospitals.
Seven outbreaks included more than 10 patients. No outbreaks were classified as phase 2. Of the data available, the majority of the outbreaks appear to have been reported within a month after detection.
One reported outbreak of COVID-19 included a request for extra advice by the SO-ZI/AMR consultation team, since transmission was ongoing in spite of the infection prevention measures taken. Potential causes

for ongoing transmission were discussed with the reporting medical microbiologist of the hospital, which was considered to be helpful in managing the outbreak.

Table 4.7.6.1 Characteristics of outbreaks reported to the SO-ZI/AMR in 2020

	Hospitals n=21 n (%)	LTCFs n=13 n (%)	Total 2020 n=34 n (%)
Microorganism (resistance mechanism)[1]			
Staphylococcus aureus (MRSA)	7 (33)	11 (85)	18 (53)
COVID-19	6 (29)	1 (8)	7 (21)
Enterococcus faecium (VRE)	5 (24)		5 (15)
Escherichia coli (FAR)		1 (8)	1 (3)
Serratia marcescens	2 (10)		2 (6)
C. difficile	1 (5)		1 (3)
Reason of reporting			
threatening of ward closure	19 (90)	4 (31)	23 (68)
ongoing transmission	2 (10)	1 (8)	3 (9)
combination of both			
HRMO outbreak (not in a hospital)		8 (62)	8 (24)
unknown			
Highest level phase[2]			
phase 1	21 (100)	13 (100)	34 (100)
Median number of patients: (range)	4 (0-61)[3]	3 (1-20)	4 (0-61)[3]
Median duration outbreak in days from reporting date until end of the outbreak: (range)	28 (6-111)	27 (11-89)	28 (6-111)
Request for help	1 (5)	0	1 (3)

[1] *MRSA=methicillin-resistant Staphylococcus aureus; VRE=vancomycin-resistant Enterococcus faecium; FAR=fluoroquinolone- and aminoglycoside-resistant.*

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

[3] *In one outbreak, only healthcare workers were involved and no patients.*

Discussion

The total number of outbreaks was remarkably lower than in 2019, when 59 outbreaks were reported. Most likely, this is due to the COVID-19 pandemic and could be attributed to various factors, such as downscaling of provided regular healthcare in hospitals and a change in infection prevention policy both in hospitals and LTCF. On the other hand, although not very likely, it cannot be ruled out that in fact a higher number of outbreaks did happen in healthcare facilities which have not been reported to SO-ZI/AMR, either because of decreased detection of outbreaks due to changed laboratory protocols and priorities, or diminished capacity for reporting outbreaks.

The number of healthcare-associated (HRMO) outbreaks will be followed up in the coming year, and it remains to be seen if the number will increase again to the levels pre-COVID-19.
Although not being HRMO, 7 healthcare-associated outbreaks of COVID-19 were reported to the SO-ZI/AMR, 6 in hospitals and 1 in a long-term care facility. Since COVID-19 is a mandatory notifiable disease, all individual cases are reported directly to Municipal Health Services and are included in the reporting by the RIVM. In addition, outbreaks of infectious diseases in healthcare institutions including an abnormal number of cases are supposed to be notified to Municipal Health Services as well, according to the public health act 'Wet Publieke Gezondheid Artikel 26', which subsequently can be reported to RIVM. It is known (e.g. through the media) that much more healthcare-associated outbreaks of COVID-19 have taken place in 2020 and that the reported outbreaks to the SO-ZI/AMR are only a fraction of the true number of outbreaks.

Conclusions

- On average three outbreaks a month were reported to the SO-ZI/AMR in 2020, which is much lower as in the previous years, most likely due to the COVID-19 pandemic.
- All outbreaks were classified as phase 1.
- 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).
- Seven outbreaks of COVID-19 were reported, all but one in hospitals.
- The median number of patients involved in an outbreak was 4.

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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 (0.064 mg/L). Since no MIC assay is 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 2011-2020, a total of 370 strains from cerebrospinal fluid (CSF) or CSF and blood and 757 strains from blood were included in the surveillance project of the NRLBM. Over these years, the overall number of isolates ranged between 54 in 2020 and 186 in 2018, with an average of 113 isolates per year. Patients with a blood isolate and PCR positive CSF sample are counted as CSF. The MIC for penicillin was determined by Etest using Mueller Hinton Fastidious Agar (MHF)plates and incubation at 37°C under 5% Co_2 for 18-24 h. EUCAST criteria for resistance were applied (susceptible: MIC ≤0.064 mg/L; moderately susceptible: MIC 0.064-0.25 mg/L; resistant: MIC >0.25 mg/L). In addition, the nucleotide sequence of *penA* coding for penicillin binding protein 2 (PBP2) was sequenced.[1,2] In case of moderate susceptibility or resistance to penicillin, susceptibility to ceftriaxone was also assessed by Etest using MHF plates and incubation at 37°C under 5% Co_2 for 18-24 h.

Results

In 2020, the NRLBM received a total of 54 meningococcal isolates, 15 from CSF and 39 from blood, which represents a decrease of 60% compared to the number of isolates received in 2019. The sharp decrease is likely explained by a combination of factors including the vaccination against menW (switch from MenC to MenACWY vaccine as of 1 May 2018) and the COVID-19 containment measurements, which likely affected transmission of *N. meningitidis*. A similar decrease is also observed in other countries.[3] Of 54 isolates, one (1.9%; CSF isolate, serogroup B) was resistant to penicillin, whereas 26.7% (4/15) of CSF (or CSF and blood) isolates and 17.9% (7/39) of the blood isolates were moderately susceptible to penicillin. The proportion of isolates moderately susceptible to penicillin in 2020 was higher than in 2019 (table 4.8.1.1 and 4.8.1.2). The moderately susceptible isolates were not equally distributed among serogroups. Of those 11 moderately susceptible isolates from blood and/or CSF in 2020, eight belonged to serogroup B (8/30; 26.7%), two to serogroup Y (2/9; 22%) and one to serogroup W (1/10; 10%). Resistance to ceftriaxone or rifampicin was not detected.

Alterations in the *penA* gene, associated with non-susceptibility to penicillin[2], were detected in 7 (13%) of the 54 isolates. Of these isolates, one was phenotypically susceptible, 5 were moderately susceptible and 1 was resistant by Etest (table 4.8.1.3).

penA genotyping yielded more isolates (13%) resistant to penicillin as compared to phenotypic testing with Etest using EUCAST criteria (1.9%) and both methods do not agree completely.

Table 4.8.1.1 Susceptibility of *N. meningitidis* isolated from CSF or CSF and blood to penicillin, 2011-2020

	Penicillin*								
	MIC ≤ 0.064 sensitive		0.064 < MIC ≤ 0.25		0.25 < MIC ≤1.0		MIC >1.0		Total
	n	%	n	%	n	%	n	%	
2011	30	**81.1**	7	**18.9**	0	**0**	0	**0**	**37**
2012	24	**58.5**	16	**39.0**	1	**2.4**	0	**0**	**41**
2013	37	**88.1**	4	**9.5**	1	**2.4**	0	**0**	**42**
2014	27	**81.8**	6	**18.1**	0	**0**	0	**0**	**33**
2015	30	**93.8**	2	**6.2**	0	**0**	0	**0**	**32**
2016	32	**88.9**	4	**11.1**	0	**0**	0	**0**	**36**
2017	37	**80.4**	9	**19.6**	0	**0**	0	**0**	**46**
2018	40	**72.7**	14	**25.5**	1	**1.8**	0	**0**	**55**
2019	30	**90.9**	3	**9.1**	0	**0**	0	**0**	**33**
2020	10	**66.7**	4	**26.7**	1	**6.6**	0	**0**	**15**

* *MIC values in mg/L.*

Table 4.8.1.2 Susceptibility of *N. meningitidis* isolated from blood only to penicillin, 2011-2020

	Penicillin*								
	MIC ≤ 0.064 sensitive		0.064 < MIC ≤ 0.25		0.25 < MIC ≤1.0		MIC >1.0		Total
	n	%	n	%	n	%	n	%	
2011	33	**62.3**	20	**37.7**	0	**0**	0	**0**	**53**
2012	27	**67.5**	13	**32.5**	0	**0**	0	**0**	**40**
2013	52	**74.3**	17	**24.3**	1	**1.4**	0	**0**	**70**
2014	36	**90**	4	**10.0**	0	**0**	0	**0**	**40**
2015	47	**90.4**	5	**9.6**	0	**0**	0	**0**	**52**
2016	89	**88.1**	12	**11.9**	0	**0**	0	**0**	**101**
2017	104	**80.6**	24	**18.6**	1	**0.8**	0	**0**	**129**
2018	99	**75.6**	30	**22.9**	2	**1.5**	0	**0**	**131**
2019	92	**90.2**	10	**9.8**	0	**0**	0	**0**	**102**
2020	32	**82.1**	7	**17.9**	0	**0**	0	**0**	**39**

* *MIC values in mg/L.*

Table 4.8.1.3 Alterations in the *penA* gene and penicillin susceptibility in *N. meningitidis*, 2020

Alterations *penA* gene**	Number (%) of strains with penicillin MIC*: MIC ≤ 0.06 sensitive	0.064< MIC ≤ 0.25	0.25 < MIC ≤ 1.0	MIC >1.0
Yes	1	5	1	0
No	41	6	0	0
Total	**42**	**11**	**1**	**54**

* *MIC values in mg/L.*

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

Discussion

Alterations in *penA* associated with resistance to penicillin are present in 13% of all isolates compared to 1.9% 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) 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

- The number of received meningococcal isolates was 60% lower compared to 2019.
- Phenotypic penicillin resistance is rare; 8/1,127 isolates in 10 years divided across different serogroups.
- In 2020, the proportion of moderately susceptible or resistant strains increased compared to the previous year; from 9.6% (13/136) in 2019 to 20.4% (11/54) in 2020.
- Alterations in penA associated with non-susceptibility to penicillin are present in 13% of all isolates but show weak correlation with phenotypic penicillin susceptibility.
- Resistance to rifampicin and ceftriaxone was not found in 2020.

References

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4.8.2 *Neisseria gonorrhoeae*

Introduction

Neisseria gonorrhoeae is a species of Gram-negative bacteria responsible for the sexually transmitted infection (STI) gonorrhoea. Gonorrhoea is the second most common bacterial STI in the Netherlands. It can result in severe reproductive complications and can increase the transmission of HIV. Third generation cephalosporins, such as ceftriaxone and cefixime, are the current first-line treatment for gonorrhoea in most countries. In the Netherlands, cefotaxime was the first-line therapy for gonorrhoea from 2003-2006, and ceftriaxone from 2006 onwards. However, the susceptibility of gonococci to these cephalosporins has been decreasing and *Neisseria gonorrhoeae* has developed antimicrobial resistance to most drugs used for treatment in the past, including azithromycin, which is used as an alternative treatment in patients allergic to ceftriaxone.

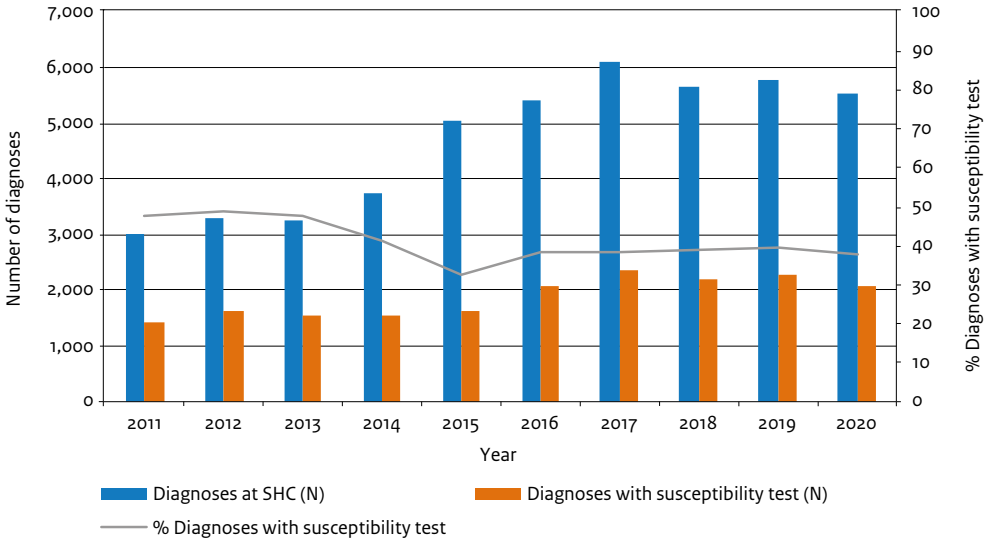
Methods

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. In 2020, 14 out of the 24 SHC participated in GRAS, which together accounted for 81% of SHC gonorrhoea diagnoses. Diagnosis of gonorrhoea is made by PCR on patients' materials. For GRAS, additional culture and susceptibility testing is performed using Etest. Isolates are tested for ciprofloxacin, cefotaxime, ceftriaxone, and azithromycin. Resistance levels are calculated using the EUCAST breakpoints for resistance.[1]

Results

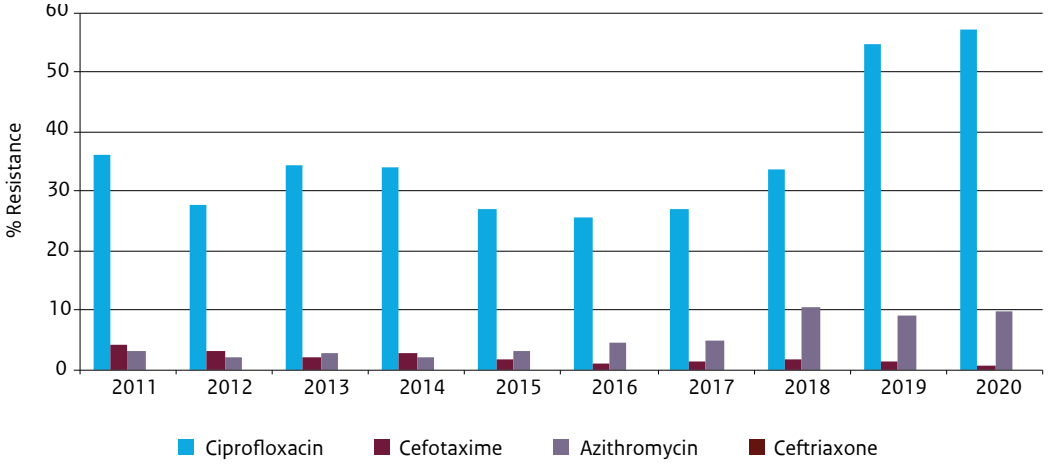
The number of gonorrhoea diagnoses reported by SHC participating in GRAS has been between 5,000 and 6,000 since 2015, with 5,514 diagnoses in 2020. The percentage of diagnoses including a susceptibility test has been stable around 39% since 2016 (37.8% in 2020, Figure 4.8.2.1).

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, 2011-2020



Gonococcal resistance to ciprofloxacin decreased from 36.4% in 2011 to 25.8% in 2016, but increased again in the past few years to 57.1% in 2020. Resistance for cefotaxime has been slowly decreasing and was 0.7% in 2020. For azithromycin, resistance steadily increased from 2.1% in 2012 to 10.8% in 2018 but has since stabilised and was 10.1% in 2020. No resistance was reported to ceftriaxone (Figure 4.8.2.2).

Figure 4.8.2.2 Trends in antimicrobial resistance among *Neisseria gonorrhoeae* (following EUCAST breakpoints) in the Netherlands, 2011-2020

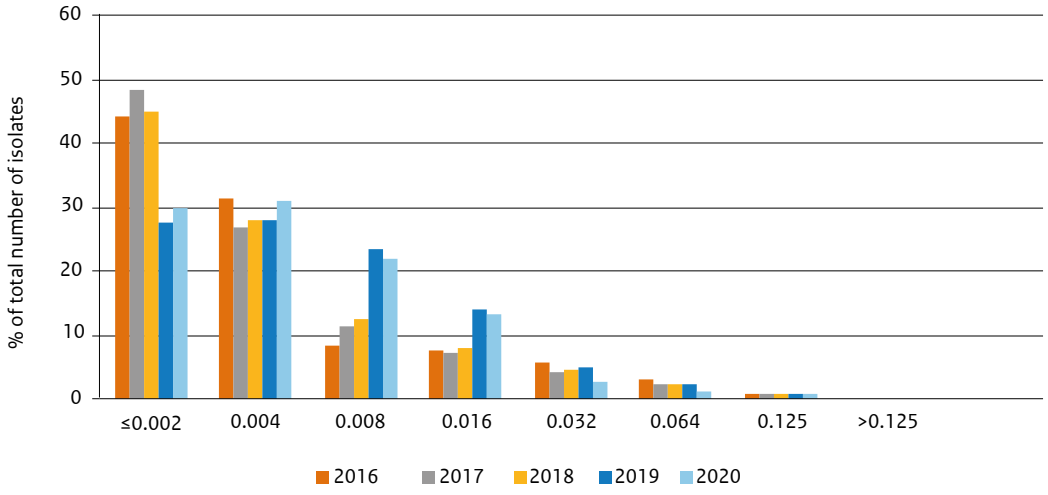


No resistance to ceftriaxone has been reported.

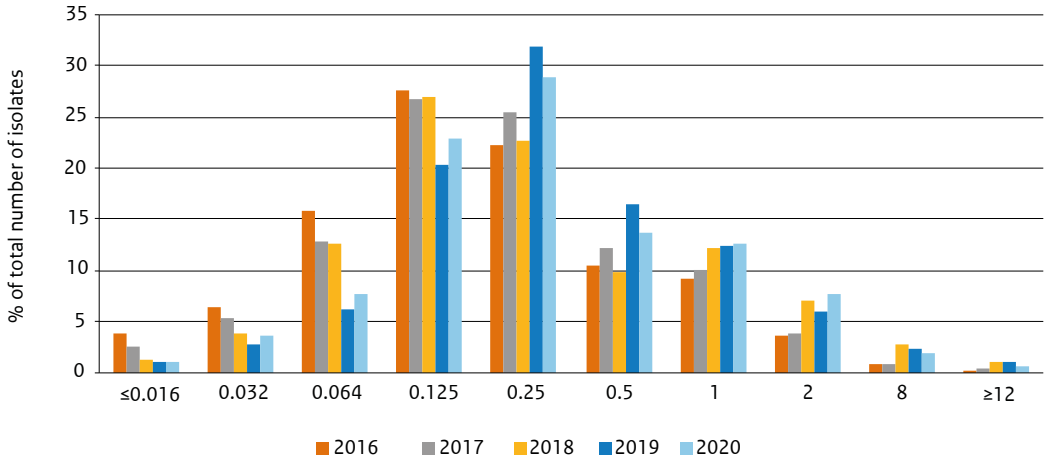
In the MIC distribution of ceftriaxone a shift was observed in 2019 where the proportion of very susceptible isolates (MIC <0.006 mg/L) decreased and the proportion of isolates with slightly reduced susceptibility (MIC 0.006-0.016 mg/L) increased (Figure 4.8.2.3a). The distribution in 2020 was similar to 2019.
For azithromycin a similar pattern was observed with a shift towards reduced susceptibility in 2019 which stabilised in 2020 (Figure 4.8.2.3b).

Figure 4.8.2.3 MIC distributions of ceftriaxone and azithromycin for *Neisseria gonorrhoeae*, 2016-2020

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



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



Discussion

In 2020 in less than half (37.8%) of all gonorrhoea diagnoses at the SHCs participating in GRAS resistance levels were measured by additional susceptibility testing. This low number can partially be explained by a large proportion of diagnoses being culture negative and/or only based on PCR, making susceptibility testing impossible. Due to COVID-19, sexual health care at the SHCs has sometimes been downscaled in 2020, especially during the first wave in April and May. The SHC performed stricter triaging to only allow testing of persons at highest risk of STI. The effect of this downscaling on GRAS has been limited. Cultures were less often performed (68.3% of gonorrhoea patients in 2020 versus 77.5% in 2019), but the percentage of patients with reported susceptibility testing results only slightly decreased (37.8% in 2020 versus 39.8% in 2019).

In the Netherlands, the recommended treatment for gonorrhoea is a single injection with ceftriaxone (500mg). Thus far, no ceftriaxone resistance has been reported. Yet, a few isolates have reached the borderline MIC value of 0.125 mg/L in the past years (1 in 2020). Trends of decreasing susceptibility have been 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

- 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 and 2020 compared with previous years.
- Resistance to ciprofloxacin more than doubled since 2016, to 57.1% in 2020.
- The trends of increasing resistance and reduced susceptibility to azithromycin seem to have stabilised since 2019.

References

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4.8.3 *Mycobacterium tuberculosis*

Introduction

Of all infectious diseases, tuberculosis (TB) has one of the highest mortalities worldwide. Although the incidence is slowly declining worldwide, 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. In 2020, the total number of reported TB cases declined with 17% to 626 cases and this may be related to the COVID-19 pandemic because of reduced immigration, less transmission and delayed diagnosis.

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 TB reference laboratories to monitor the quality of the resistance testing.

Methods

Around 30 laboratories in the Netherlands are involved in the diagnosis of TB and send all *M. tuberculosis* isolates to the RIVM for epidemiological typing to support the investigations on TB transmission by Municipal Health Services. For all these strains also (sub) species identification and (molecular and/or) phenotypic resistance testing are performed. The secondary laboratory diagnosis of TB, involving (species) identification, resistance testing and epidemiological typing is since 2019/2020 mainly based on Whole Genome Sequencing (WGS).

Since 2020, WGS is performed to screen for resistance of *M. tuberculosis* isolates against first line drugs. In case no resistance mutations are observed, no additional phenotypic testing on resistance to first line drugs is performed. Because injectables are no longer part of the TB treatment regimen, we no longer determine the resistance against streptomycin.

Results

The presented data on 2020 is preliminary, because data may not be complete. 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 WGS has been finalized.

In 2020, the number of notified TB cases amounted to 626, of which 421 represented bacteriologically confirmed cases, of which isolates were received at the RIVM. It is expected there are still isolates of 2020 missing that will shortly be received at the RIVM.

In 2020 there was a clear increase in INH resistance to 8.3% compared to 5.0% in 2019 (including combined resistance to different first line antibiotics). In 2018, any rifampicin resistance decreased to 1.2%, but this increased to 1.7% of the cases in 2019. This increase continued in 2020 to 2.8%. In 2019, in 0.8% of the cases ethambutol resistance was detected, and this decreased to 0.7 % in 2020.

In 2019, 7 MDR-TB cases, defined as resistance to at least INH and rifampicin, and one mono rifampicin resistant (RR) case, defined as resistance to only rifampicin, were detected. In addition, one XDR-TB (defined as resistance to INH, rifampicin and a fluoroquinolone) case was diagnosed. Combined MDR, XDR and RR in 2019 amounted to 1.2% of the cases. In 2020, 8 MDR-TB cases and three RR cases were detected. No XDR-TB was diagnosed.

Figure 4.8.3.1 Percentage antibiotic resistance for *M. tuberculosis* isolates 2005-2020

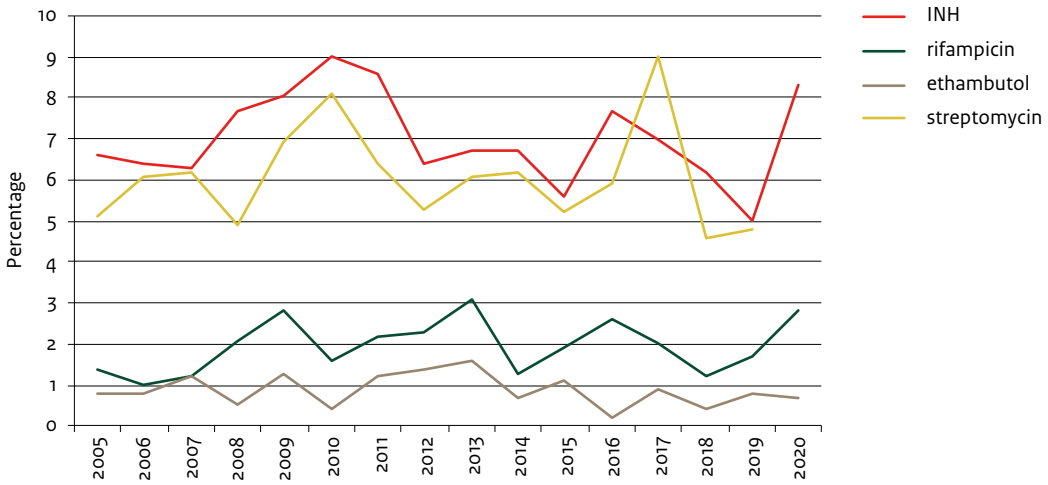
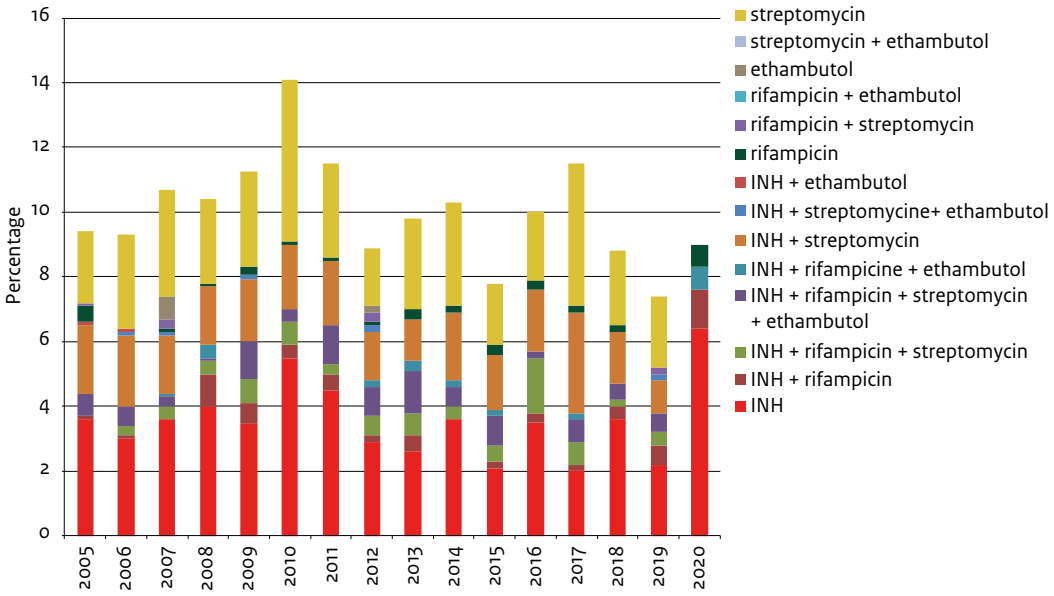


Figure 4.8.3.2 Percentage combined antibiotic resistance for *M. tuberculosis* isolates 2005-2020



Discussion

Worldwide, resistance is an important aspect of TB control. Because the vast majority of TB cases in The Netherlands are diagnosed in patients originating from high prevalence areas, it remains important to continue the structural 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 2019 there was again a minor decrease in the number of TB cases recorded. In 2020, probably as a result of the COVID-19 pandemic, there was a sudden decrease of 17% in TB notifications. It is currently unclear whether this decrease will be long term, or that the number of notifications will rise again next year.
In 2020, 11.4 % (48/421) of the isolates tested in the Netherlands revealed some form of resistance. This seems somewhat higher than the percentage observed in previous years. Although the number of multidrug resistant (including RR) isolates remained low and amounted to 11 cases, due to the extended hospitalization of patients and the complicated treatment this problem continues to deserve special attention. The higher percentage of INH resistant isolates cannot yet be readily explained, but will be monitored. This could be due to changes in the patient population, but could also be related to an improved and more centralized detection of mutations associated with resistance to first line drugs by the introduction of WGS at the RIVM.

Conclusions

- Resistance to the antibiotics to treat tuberculosis remained almost stable over the last 5 years, and showed a slight increase in 2020.
- MDR-TB remained stable in the recent years (average of 10 cases per year).
- There was a remarkable increase of mono INH resistance (from 2.2 to 6.4%) in 2020.
- There was a sharp decline in TB notification in 2020 (626 cases; 17 % less), presumably related to the COVID-19 pandemic.

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4.8.4 Antiviral resistance

The plan of changing "antibacterial resistance (ABR)" surveillance programmes to "antimicrobial resistance (AMR)" will add to the existing influenza antiviral susceptibility surveillance also surveillance of other viruses for reduced susceptibility to antivirals. Antiviral drugs are here defined as chemical compounds that interact with virus replication at the virus or host level. Active and passive immunization using vaccines and antibodies are not subject of this chapter. Resistance to antivirals can be detected using genotypic methods identifying antiviral reduced susceptibility associated amino acid substitutions by sequencing of genes encoding viral proteins directly or indirectly involved in the mechanism of action of antivirals. This only possible if these amino acid substitutions have been characterized fully by phenotypic methods. Phenotypic methods measuring reduced drug susceptibility in cell culture or reduced functional activities of antiviral drug targeted proteins are the gold standard but often much more laborious because the virus isolate is needed.

Currently, the monitoring of **influenza** antiviral susceptibility is embedded in the surveillance of influenza by general practitioner (GP) sentinels, that is coordinated by the Nivel Netherlands Institute for Health Services Research and the National Institute for Public Health and the Environment (RIVM) location of the National Influenza Centre (NIC), and the surveillance of influenza viruses received from mainly hospital laboratories by the Erasmus MC location of the NIC.[1] The GP network offers an opportunity to study other respiratory viruses that potentially have an impact on the public health, such as SARS-CoV-2, and RSV for which antiviral agents are available or will become available. High on the list of agents for treatment of COVID-19 is remdesivir, but susceptibility tests are performed on a very small scale. New treatments are currently being developed and preclinical laboratory tests are necessary to establish antiviral activity. In addition, preparedness for resistance development by the availability of appropriate tests is also required, especially when antivirals with new mode of action become available.

Though the treatment of infections with the **respiratory syncytial virus (RSV)** in infants and elderly Is primarily supportive, new antiviral treatments are in development. RSV is a major cause of severe lower respiratory tract infections and hospitalization in infants under 1 year of age and its burden is similar to influenza in the frail elderly. Since the burden of RSV infection is high), surveillance and susceptibility tests for newly developed antiviral agents are necessary to monitor the epidemiology and strain variation.[2]

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

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

Liver infection with Hepatitis C Virus (HCV) is mainly blood transmissible disease, affecting specific subpopulations with a high, chronic burden of disease. It is a mandatory reporting disease (B2). The treatment options have been considerably expanded and also include monitoring of antiviral resistance for genotypes in various disease stages. Infections with hepatitis B decreased in The Netherlands due to the vaccination programme for specific subpopulations. There is a variety of treatment guidelines.[4] A guideline is available in The Netherlands. FDA approved antiviral agents include lamivudine, adefovir, entecavir, and tenofovir (known as nucleos(t)ide analogues), and interferon-α and (pegylated-) IFN-α therapy. Resistance markers have been reported for these antivirals, of which tenofovir and entecavir have the lowest risk of developing resistance.

Since the introduction of combination antiretroviral therapy (cART) in 1996, there have been substantial changes and improvement in the use of antiretroviral drugs for the treatment and prevention of HIV infection. The current treatment guidelines recommend to initiate cART as soon as possible for all people newly diagnosed with HIV, regardless of CD4 count. In the Netherlands, approximately 23,700 individuals are infected with HIV (see www.hiv-monitoring.nl) than 85% receive antiviral treatment to improve the clinical outcome and prevent transmission of the disease. For HIV treatment, 20 agents belonging to 5 different classes are available and a combination of at least 3 different agents taken daily is advised, also to prevent resistance development. Therefore, monitoring of resistance development and its spread are import pillars of HIV treatment and prevention strategies, such as pre-exposure prophylaxis (PrEP).

Infections with **rubella, measles, mumps** and **poliovirus/enterovirus** are nationally surveyed as part of monitoring of the vaccination programme. There are no treatment options for rubella, measles and mumps, except for the humane hyperimmune globulins treatment for measles. Apart from vaccination against polio (and Enterovirus A71 related hand-foot and mouth disease in Asia), there are also no treatment options available for poliovirus and enteroviruses, such as enterovirus D68, which caused the recent upsurge of paralysis cases worldwide. Currently, for these and other viruses, many new antivirals are being developed, are in clinical trials, or used off-market or experimentally. It is important to asses both the clinical and public health impact of known and new antivirals with regards to antiviral resistance development.

Conclusions

- In collaboration with national (NVMM and Nederlandse Werkgroep voor Klinische Virologie) and international stakeholders, a selection will be made for antiviral susceptibility surveillance programmes of specific viruses.
- Using the existing network of “Kiemsurveillance”, a start will be made with susceptibility testing of SARS-CoV-2.

Table 4.8.4.1 Overview of surveillance of viral pathogens and antiviral resistance in the Netherlands

Virus	Estimated burden of disease	Antiviral treatment	National surveillance in the Netherlands	National monitoring of antiviral resistance
Influenza	High	Amantadine, rimantadine, oseltamivir, zanamivir, baloxavir marboxil	Yes	Yes
COVID-19	Very high	Remdesivir	Yes	In development
RSV	High	In development	No	No
Herpes simplex virus 1 and 2	High	Acyclovir and its derivates, foscarnet, cidofovir	No	No
Cytomegalovirus	High in immunocompromised patients, low in neonates	Acylcovir, ganciclovir, foscarnet, letermovir	No	No
Hepatitis B	Vaccination is recommended for specific populations	Various treatment guidelines with lamivudine, adefovir, entecavir, telbivudine, tenofovir, and (pegylated) interferon-α. See https://www.hbvrichtsnoer.nl/behandeling-met-nucleostide-analogen-nucs/	No	No
Hepatitis C	High in specific subpopulations	See https://hcvrichtsnoer.nl/	No	AMC, Erasmus MC and UMCU.
Mumps, measles, rubella and poliovirus/enterovirus	Low	In development	Part of vaccination programme monotoring	No
HIV	High	20 agents belonging to 5 different classes	Yes, by “HIV monitoring”	Yes

4.8.4.1 Influenza virus antiviral drug resistance

Introduction

When vaccination against influenza is not available or fails due to antigenic mismatch with circulating viruses, influenza antiviral drugs can be used for (post exposure) prophylaxis as well as for treatment of influenza cases with 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.[5] In January 2021 the European Commission base on advice from the European Medicine Agency approved baloxavir marboxil (Xofluza®) (BXM), a cap-dependent acidic endonuclease inhibitor, for treatment of uncomplicated influenza and prophylaxis for patients and individuals aged 12 years and above respectively.[6] Monitoring of reduced susceptibility amino acid substitutions in the polymerase acidic protein (PA) has been added to the surveillance since the 2019/2020 season.

Methods

Monitoring of influenza antiviral susceptibility is embedded in the integrated clinical and virological surveillance of influenza using general practitioner (GP) sentinels, that is carried out by the Nivel Netherlands Institute for Health Services Research and the National Institute for Public Health and the Environment (RIVM) location of the National Influenza Centre (NIC). 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 susceptibility include Sanger sequencing, whole genome Next Generation Sequencing or site-specific polymerase chain reaction (PCR) assays for known reduced inhibition markers for both NAIs and BXM. For a subset of influenza viruses, the susceptibility to NAIs is determined 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.[5,7] Table 4.8.4.2 displays an overview of the antiviral susceptibility of influenza viruses since the 2010/2011 influenza season. Figure 4.8.4.1 shows the utilization of oseltamivir and zanamivir since 2010. No BXM has been utilized in the Netherlands since its EU authorization early 2021. In the 2020/2021 season only one seasonal A(H3N2) was reported, highly likely due to COVID-19 measures. This virus showed no evidence for NAI reduced inhibition. Additionally, one swine influenza A(H1N1)v was detected which showed highly reduced inhibition by oseltamivir following treatment of the patient with oseltamivir. Only few oseltamivir prescriptions were observed during the 2020/2021 season so far, highly likely reflecting the absence of detection of influenza cases due to COVID-19 measures despite continued testing for influenza viruses. Zanamivir has only been prescribed once up to March 2021 during the 2010/2021 season.

Table 4.8.4.2 (Higly) reduced inhibition of influenza viruses by NAIs and BXM in the Netherlands, 2010/2011 - 2020/2021[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
2020/2021[12,13]	0/1	ND	ND	ND	ND	ND

[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.*

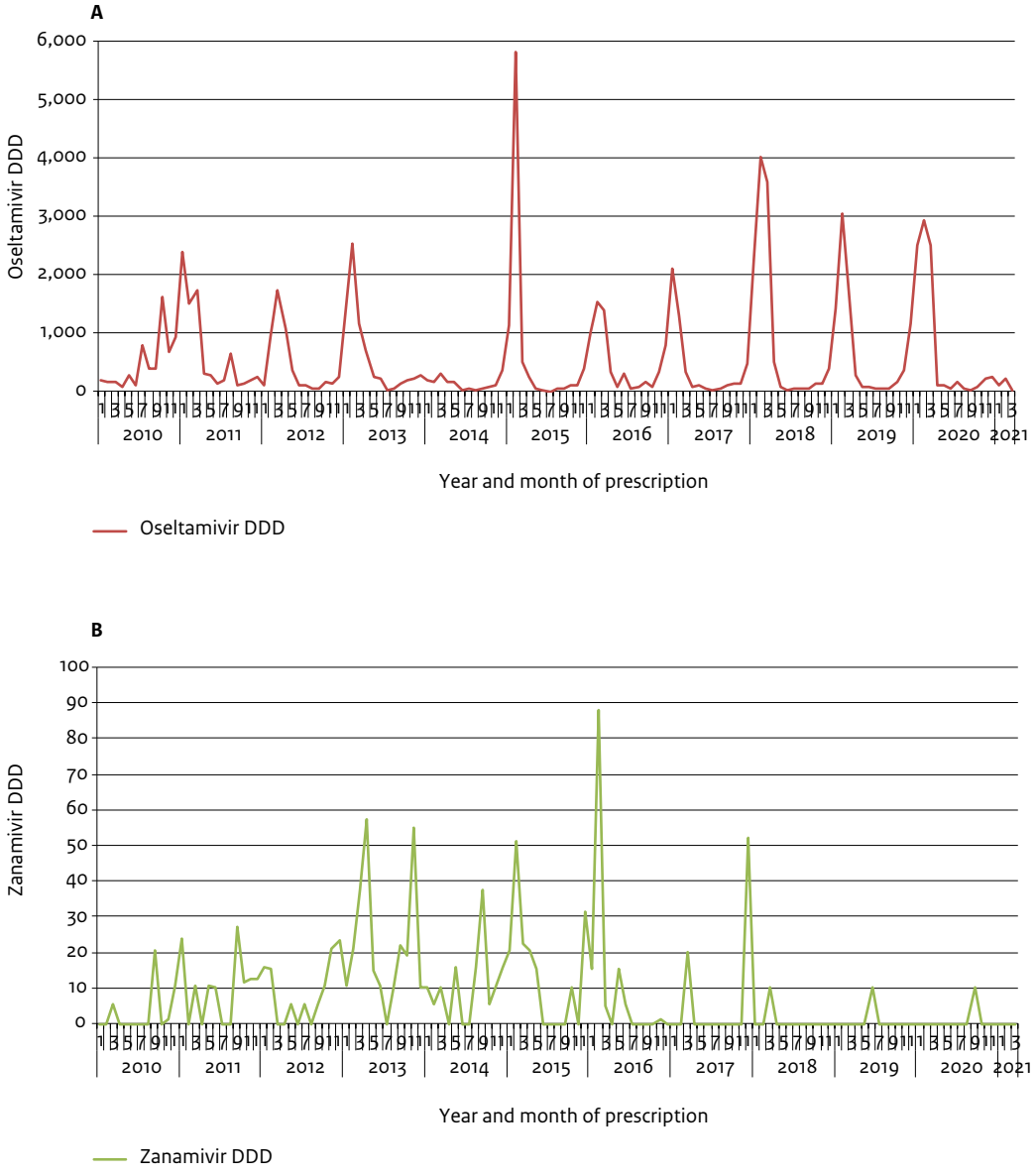
[10] *One virus with highly reduced inhibition by oseltamivir due to mixture 275H/Y amino acid substitution. No patient characteristics or viral exposure data available.*

[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.*

[12] *Early in the season additionally a case of swine influenza A(H1N1)v was detected that showed highly reduced inhibition by oseltamivir due to H275Y amino acid substitution following oseltamivir treatment.*

[13] *Preliminary data up to week 17/2021.*

Figure 4.8.4.1 Prescriptions of oseltamivir (A) and zanamivir (B) in the Netherlands, 2010/2011 - 2020/2021. Shown are the Defined Daily Doses (DDD) cumulated by month. Data kindly provided by Foundation for Pharmaceutical Statistics (SFK), the Netherlands



Discussion

In the Netherlands, and globally, the proportion of NAI reduced susceptible influenza viruses remains very low.[4] 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.[8]

Conclusions

- Over the last 11 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, except during the COVID-19 pandemic similar to the 2013/2014 season.
- Prescriptions of zanamivir remain very low.

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

Introduction

As in previous years, we report on the antibiotic susceptibility profile of several different genera of anaerobic bacteria. In order to determine whether changes in the profile of gram-negative anaerobic bacteria differed from those observed in previous years, we compared the percentage resistance to antibiotics observed in different years with each other. As multi-drug resistant (MDR) *Bacteroides fragilis* isolates, defined as resistant to at least 3 categories of antibiotics, were observed in the last years by several laboratories[1], special attention will be paid to the prevalence of MDR Bacteroidetes isolates.

Methods

Isolates were obtained from clinical specimens at the department of Medical Microbiology and Infection prevention (MMBI) at the University Medical Center in Groningen (UMCG). Isolates were derived from a variety of specimens and no special selection criteria were used. All isolates were identified using MALDI-TOF MS (Bruker Daltonics, Bremen, Germany) and their MIC for amoxicillin, amoxicillin-clavulanic acid (only gram-negative anaerobic bacteria), clindamycin, metronidazole and meropenem (only *Bacteroides* and *Prevotella* isolates) were determined using Etest. Resistance was assessed using EUCAST breakpoints (www.eucast.org).

No data was available for the amoxicillin MICs of *Bacteroides*, *Parabacteroides* and *Prevotella*, since these genera are only tested when no beta-lactamase production was observed, using a cefinase disk. Resistance of isolates belonging to the genera *Actinomyces* or *Cutibacterium* against metronidazole is considered to be intrinsic and is therefore not determined.

Results

A summary of the results is presented in Table 4.8.5.1. A total of 1,076 clinical isolates were included, which is a similar as in previous years. High rates of resistance to amoxicillin was observed among *Bilophila* spp. Amoxicillin-clavulanic acid resistance was only observed among *Bacteroides*, *Bilophila* and *Parabacteroides* isolates, 16.4%, 27.3% and 26.3% respectively.

Parabacteroides isolates showed the highest rate of resistance against clindamycin (52.2%), followed by isolates belonging to the genera *Bacteroides* and *Anaerococcus*, 37.6% and 32.4%, respectively.

For the first time we report on the antibiotic susceptibility profile of *Dialister* species (formerly considered to be part of the genus *Bacteroides*). These isolates showed a high resistance rate against metronidazole (66.7%). A previous study showed that these isolates did not harbor nim-genes, nor did these isolates harbor pCD-METRO (unpublished). Furthermore, resistance was observed among a few *Parabacteroides*, *Prevotella* and *Clostridium* isolates.

Meropenem resistance was only observed among *Bacteroides* isolates (2.7%). These were three *B. fragilis* isolates, a *Bacteroides ovatus* and a *Bacteroides vulgatus* isolate. One *B. fragilis* isolate and the *B. ovatus* isolate were derived from the same patient. All these isolates were susceptible for metronidazole and clindamycin, with the exception of one *B. fragilis* isolate which was resistant for clindamycin. It should be noted that the other two *B. fragilis* isolates had elevated MICs for clindamycin, 3 mg/L and 4 mg/L respectively. Whole genome sequencing is performed to assess the genetic mechanisms present, responsible for the observed antibiotic resistance.

Also 7 *Parabacteroides* isolates were tested for meropenem resistance and were all shown to be susceptible (data not shown). Since 2016, the percentage meropenem resistant *Bacteroides* isolates varies between 0 and 3%.

For most anaerobic genera the percentage of resistance against the different antibiotics varies per year. However, the last two years we see an increase in amoxicillin-clavulanic acid resistance among isolates of *Bacteroides* and *Bilophila* (Fig. 4.8.5.1), using an Etest (BioMerieux, l'Etoile, France) with a fixed ratio of amoxicillin and clavulanic acid. Studies have reported that using a fixed ratio might result in lower MIC's for this drug combination. Rentenaar et al. advise to use a fixed concentration amoxicillin-clavulanic acid.[2] Furthermore, metronidazole resistant *Bacteroides* and/or *Prevotella* isolates are encountered each year (Fig. 4.8.5.2).

In 2020, two MDR Bacteroidetes isolates were observed, a *Parabacteroides distasonis* and a *Prevotella bivia*. The first isolate showed resistance for amoxicillin/clavulanic acid (MIC 12 mg/L), doxycycline (MIC 16 mg/L) and metronidazole (MIC 8 mg/L). Furthermore, the MIC for clindamycin was 4 mg/L. The latter isolate was resistant to amoxicillin (MIC >256 mg/L), clindamycin (MIC >256 mg/L) and metronidazole (MIC 16 mg/L).

Table 4.8.5.1 The MIC_{50}, MIC_{90} and percentage resistance of different anaerobic genera, isolated from human clinical specimens in 2020, for different kind of antibiotics

	amoxicillin			amoxicillin/clavulanic acid			clindamycin			meropenem			metronidazole		
	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
breakpoint	**> 2 mg/L**			**> 8 mg/L**			**> 4 mg/L**			**> 8 mg/L**			**> 4 mg/L**		
Gram-negative (n)															
Bacteroides spp. (183-194)[a]	n.d.[b]	n.d.	n.d.	0.5	3	16.4	2	>256	37.6	0.19	0.5	2.7	0.25	0.75	0
Bilophila spp. (10-11)[a]	96	>256	100	1.5	>256	27.3	0.75	1.5	0	n.d.	n.d.	n.d.	0.047	0.125	0
Dialister spp. (12)	0.125	0.38	8.3	0.064	0.38	0	0.38	>256	16.7	n.d.	n.d.	n.d.	8	>256	66.7
Fusobacterium spp. (31-32)[a]	0.023	0.064	0	0.023	0.064	0	0.064	0.25	0	n.d.	n.d.	n.d.	0.016	0.25	0
Parabacteroides spp. (19-23)[a]	n.d.	n.d.	n.d.	2	16	26.3	6	>256	52.2	n.d.	n.d.	n.d.	0.25	1	4.5
Porphyromonas spp. (22)	0.023	32	13.6	0.023	0.75	0	0.016	>256	27.3	n.d.	n.d.	n.d.	0.094	0.75	0
Prevotella spp. (134-143)[a]	n.d.	n.d.	n.d.	0.094	0.75	0	0.016	>256	21.7	0.032	0.125	0	0.125	0.75	0.7
Veillonella spp. (33-38)[a]	0.75	2	6.1	0.75	2	0	0.125	0.38	0	n.d.	n.d.	n.d.	1	2	0
breakpoint	**> 8 mg/L**						**> 4 mg/L**						**> 4 mg/L**		
Gram-positive (n)															
Actinomyces spp. (132-137)[a]	0.125	0.5	0.8	n.d.	n.d.	n.d.	0.125	6	10.9	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Anaerococcus spp. (33-35)[a]	0.032	0.125	0	n.d.	n.d.	n.d.	0.25	>256	32.4	n.d.	n.d.	n.d.	0.19	0.75	0
Clostridium spp. (47-49)[a]	0.38	1	4.3	n.d.	n.d.	n.d.	2	32	24.5	n.d.	n.d.	n.d.	0.25	0.75	2
Eggerthella lenta (8-9)[a]	2	3	0	n.d.	n.d.	n.d.	0.5	1	0	n.d.	n.d.	n.d.	0.125	0.125	0
Finegoldia magna (64-70)[a]	0.19	0.25	0	n.d.	n.d.	n.d.	0.75	>256	15.7	n.d.	n.d.	n.d.	0.19	0.38	0
Parvimonas micra (37)	0.023	0.064	0	n.d.	n.d.	n.d.	0.19	0.75	8.1	n.d.	n.d.	n.d.	0.064	0.5	0
Peptoniphilus spp. (46-50)[a]	0.023	0.094	0	n.d.	n.d.	n.d.	0.75	>256	16	n.d.	n.d.	n.d.	0.25	0.75	0
Peptostreptococcus spp. (20)	0.25	2	5	n.d.	n.d.	n.d.	0.19	0.5	0	n.d.	n.d.	n.d.	0.064	0.125	0
Cutibacterium spp. (184-194)[a]	0.094	0.25	0	n.d.	n.d.	n.d.	0.047	0.19	2.6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

[a] *Not all isolates were tested for all antibiotics.*
[b] *Not determined.*

Figure 4.8.5.1 Percentage metronidazole resistance among *Bacteroides* and *Prevotella* isolates, per year, 2011-2020

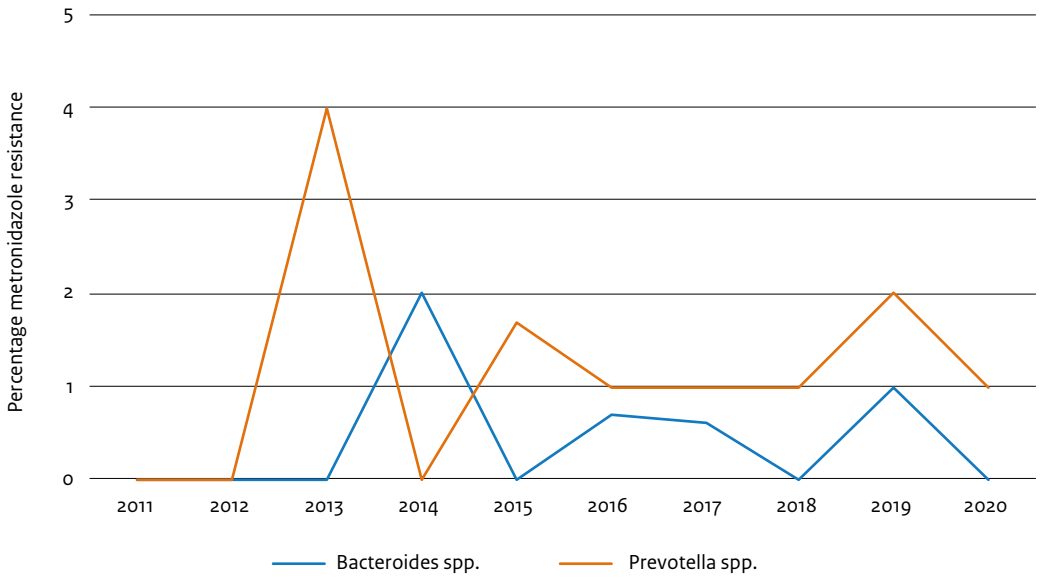
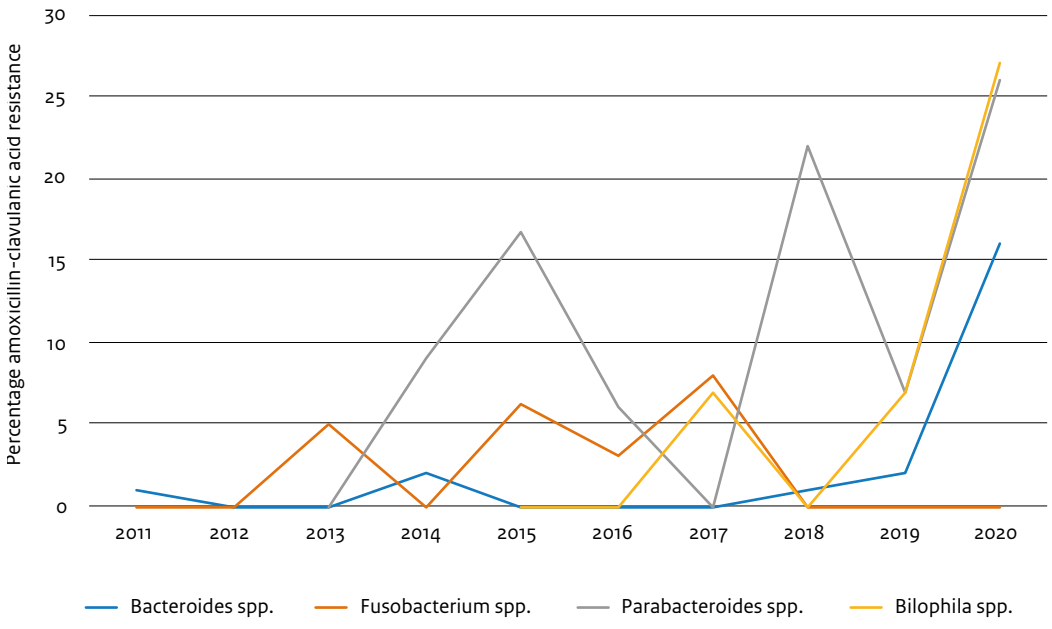


Figure 4.8.5.2 Percentage amoxicillin/clavulanic acid among several gram-negative anaerobic genera, per year, 2011-2020. In genera not shown, no resistance was observed during the years



Discussion

As reported in previous NethMap editions the percentage of antibiotic resistance differs per year. There seems to be a trend of increasing resistance against amoxicillin-clavulanic acid. It should be noted that susceptibility and/or resistance against this combination does not always correspond with resistance/susceptibility for piperacillin-tazobactam, due to loss of porin.[3]

At least two MDR Bacteroidetes isolates were encountered, *P. distasonis* and *P. bivia*. *Parabacteroides* species (former *Bacteroides*) tend to be more resistant to antibiotics than *Bacteroides* species. *P. bivia* is the most encountered and resistant *Prevotella* species, in which MDR has been described before.[4] The fact that regularly MDR Bacteroidetes isolates are encountered in various medical microbiology laboratories is a worrisome development.[5] In 2021, a survey coordinated by UMCG and RIVM with 8 laboratories will be performed to assess the prevalence of (multi) drug resistant *Bacteroides* and *Prevotella* species in clinical relevant patients materials.

Conclusions

- The percentage antibiotic resistance among the different anaerobic genera differs per year.
- There seem to be an increase of resistance to amoxicillin-clavulanic acid among *Bilophila*, *Parabacteroides* and *Bacteroides* isolates.
- As in previous years, metronidazole resistance in clinical isolates was observed.
- Resistance to meropenem was only observed among *Bacteroides* isolates and the percentage of resistance remained stable.
- MDR Bacteroidetes isolates were observed.
- In 2021, a survey with 8 laboratories will be performed to assess the prevalence of (multi) drug resistant *Bacteroides* and *Prevotella* species in clinical relevant patient materials.

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4.8.6 *Clostridioides difficile*

Introduction

The Centre for Infectious Disease Control (CIb) of the National Institute for Public Health and the Environment (RIVM) established a National Reference Laboratory for *Clostridioides difficile* at the Leiden University Medical Center (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 and metronidazole was not detected until 2017. In December 2017, a clinical *C. difficile* isolate with PCR ribotype 020 was found (MIC metronidazole=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. Very recently, a new heme dependent mechanism with a specific hsmA genetic signature has been found resulting in increased MIC values for metronidazole (3). This latter mechanism is not included in routine surveillance yet.

Methods

Patient data of the period 2019-2020 are not available yet and therefore incidence data are provided for the year 2018-2019. In the period May 2018 to May 2019, 24 acute care hospitals participated in the sentinel surveillance programme. In these hospitals, all hospitalized patients 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 recommended agar dilution for 57 randomly selected *C. difficile* sentinel surveillance isolates from 21 different hospitals, collected between May 2019 to May 2020 (4).
Additionally, all submitted *C. difficile* isolates were subjected to a PCR assay to detect plasmid-associated metronidazole resistance (pCD-METRO) (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 in that time period 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 of the randomly selected *C. difficile* sentinel surveillance isolates, collected between May 2019 to May 2020 is depicted per ribotype in table 4.8.6.1.
No resistance to vancomycin was detected using EUCAST ECOFF cut-off levels of 2 mg/L (5), but there was resistance detected to metronidazole using EUCAST ECOFF cut-off levels of 2 mg/L in one isolate with an MIC of >8 mg/L, belonging to ribotype 010.
Applying the PCR for plasmid-mediated metronidazole resistance, 3 of the 1282 tested strains were positive, including the RT 010 isolate with an MIC of >8 mg/L for metronidazole. Of the two other isolates, one also belonged to ribotype 010 and the other one to (toxigenic) 005.

Discussion

The epidemiology of CDI is relatively stable in the past few years, except that *C. difficile* infections due to the hypervirulent PCR ribotype 027 are decreasing significantly compared to May 2009 - May 2014. 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 (2). Between May 2019 and May 2020, among 1282 clinical isolates sent to the Reference Laboratory only 3 (0.2%) were pCD-METRO positive.
The presence of the plasmid always correlated with increased MIC levels to metronidazole.

Table 4.8.6.1. MIC90 and range (mg/L) of 57 *C. difficile* sentinel surveillance isolates collected between May 2019 to May 2020

	MIC_{90}	Range
Ribotype 001 (n = 3)		
Metronidazole	0.125	0.06 – 0.125
Vancomycin	0.125	<0.06 – 0.125
Ribotype 002 (n = 4)		
Metronidazole	0.125	0.06 – 0.125
Vancomycin	0.06	<0.06 – 0.06
Ribotype 010 (n = 4)		
Metronidazole	>8	<0.06 - >8
Vancomycin	<0.06	<0.06 - <0.06
Ribotype 014/020 (n =7)		
Metronidazole	0.125	0.06 – 0.125
Vancomycin	0.06	<0.06 – 0.06
Ribotype 078/126 (n = 4)		
Metronidazole	0.125	0.06 – 0.125
Vancomycin	0.125	<0.06 – 0.125
Other ribotypes (n = 35)		
Metronidazole	0.125	<0.06 – 0.25
Vancomycin	0.125	<0.06 – 1

Conclusions

- No resistance of *C. difficile* to vancomycin was found by agar dilution.
- Phenotypical resistance to metronidazole was found in 1 of 57 tested isolates, which had an MIC of ≥8 mg/L.
- Plasmid-mediated resistance to metronidazole (pCD-METRO) was found in 0.2% of 1282 tested clinical isolates and is still very low. A recently found heme dependent metronidazole resistance has not been included in the routine surveillance yet (6).
- The effects of COVID-19 on the incidence of CDI and resistance of *C. difficile* is currently studied.

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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. Host groups at risk to develop invasive aspergillosis include patients with neutropenia, but in recent decades increasingly invasive aspergillosis has been observed in nonneutropenic hosts. Emerging risk groups include patients with severe influenza, and most recently critically ill patients with coronavirus 2019 disease (COVID-19).[1] Triazoles are first choice antifungals to treat *Aspergillus* diseases, but response rates and survival is affected by acquired triazole resistance.
In *A. fumigatus*, resistance is mainly due to isolates harboring TR_{34}/L98H or TR_{46}/Y121F/T289A mutations in the *Cyp51A*gene, which are associated with environmental resistance selection through exposure to azole fungicides. 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 (VIPcheck™, MediaProducts, Groningen, the Netherlands). Three agars contain medical triazoles, itraconazole, voriconazole and posaconazole, and one well acts as growth control. Growth on one of the triazole containing wells is highly indicative for resistance and these isolates are sent to the reference laboratory for MIC-testing and sequence-analysis of the Cyp51A-gene. MIC testing is performed using the EUCAST microbroth dilution method and using recommended clinical breakpoints. Underlying disease information was collected for patients harboring a triazole-resistant isolate. The resistance frequency based on the number of patients screened was determined for all participating centers and compared with previous years.

Results

In 2020 *A. fumigatus* isolates from 1,521 culture-positive patients were screened for triazole resistance, including 737 (range 83 to 193 per center) patients from UMCs and 784 (range 95 to 193 per center) patients from teaching hospitals. Overall 124 patients (8.2%) harbored a triazole-resistant isolate, with a resistance frequency of 11.8% (87 of 737 patients) in UMCs and 4.7% (37 of 784 patients) in teaching hospitals (Table 4.8.7.1). The resistance frequency in most UMCs was around 10%, while the highest frequency was observed in UMCG. The resistance frequency was lower in teaching hospitals with a range from 2.0% to 7.8%.
In total 135 isolates from 124 patients were analyzed for resistance mutations in the *Cyp51A* gene. Environmental resistance mutations, e.g. TR_{34}/L98H and TR_{46}/Y121F/T289A, were most frequently present in all centers accounting for 60.7% and 25.9% of the detected resistance mutations, respectively. Both TR_{34} and TR_{46} isolates were found to harbor additional short nucleotide polymorphisms (SNPs) or additional *Cyp51A*gene mutations in 5 of 82 (6.1%) TR_{34} isolates, and 14 of 35 (40%) TR_{46} isolates. Of TR_{34} isolates, 19 (23.2%) had a voriconazole MIC of 2 mg/L, which is considered as intermediate susceptibility.
Of 112 patients with triazole-resistant *A. fumigatus* and known underlying disease, 31 (27.7%) suffered from a structural lung disease and 26 (23.2%) from cystic fibrosis. A total of 16 patients (12.9%) with a triazole-resistant *A. fumigatus* was reported to be SARS-CoV-2 positive.

Discussion

The triazole resistance frequency in *A. fumigatus* was lower in 2020 compared with previous years, with an overall resistance rate of 8.2%. Similar to previous years the resistance frequency in teaching hospitals was about half of that found in UMCs and the resistance mutations were dominated by environmental mechanisms, which accounted for nearly 87% of the detected Cyp51A-mutations. Furthermore, 46% of resistant isolates were recovered from patients with structural lung diseases or cystic fibrosis. However, the COVID-19 pandemic has had notable impact on hospital care due to downscaling of regular care and admissions of COVID-19 patients. This has been relevant for our *Aspergillus* resistance surveillance as COVID-19 associated pulmonary aspergillosis (CAPA) has been reported in up to 30% of critically ill COVID-19 patients.[2] This is illustrated by the total number of *A. fumigatus* isolates sent to the mycology reference laboratory in Nijmegen, which shows two distinct peaks that align with the two corona waves in the Netherlands compared with previous years (Figure 4.8.7.1). Our surveillance included 16 COVID-19 patients from whom triazole-resistant *A. fumigatus* was cultured, although it is not known if these patients had CAPA. Thus the observed triazole resistance frequency in 2020 might have been influenced by corona, which complicates comparisons with previous years.

Table 4.8.7.1 Triazole resistance proportion in unselected clinical *A. fumigatus* isolates in 5 University Medical Centers, 2013-2020, and 5 teaching hospitals, 2018-2020

	2013		2014		2015		2016		2017		2018		2019		2020	
	N	AzoleR (%)	N	AzoleR (%)	N	AzoleR (%)	N	AzoleR (%)	N	AzoleR (%)	N	AzoleR (%)	N	AzoleR (%)	N	AzoleR (%)
UMCs																
ErasmusMC	231	10 (4.3)	265	10 (3.8)	22	7 (31.8)[a]	186	24 (12.9)	147	19 (12.9)	129	17 (13.2)	102	18 (17.6)	108	12 (11.1)
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)	83	8 (9.6)
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)	193	20 (10.4)
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)	181	31 (17.1)
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)	172[c]	16 (9.3)
Total UMCs	**760**	**58 (7.6)**	**814**	**59 (7.2)**	**600**	**64 (10.7)[b]**	**784**	**101 (12.9)**	**774**	**114 (14.7)**	**764**	**112 (14.7)**	**703**	**88 (12.5)**	**737**	**87 (11.8)**
Teaching hospitals																
Medisch Spectrum Twente											88	5 (5.7)	90	2 (2.2)	95	2 (2.1)
St Antonius Hospital											265	28 (10.6)	177	10 (5.7)	193	15 (7.8)
PAMM											81	4 (4.9)	147	8 (5.4)	150	3 (2)
CWZ											155	11 (7.1)	90	6 (6.7)	163	7 (4.3)
Isala											195	13 (6.7)	222	18 (8.1)	183	10 (5.5)
Total teaching hospitals											**784**	**50 (7.8)**	**726**	**42 (6.1)**	**784**	**37 (4.7)**

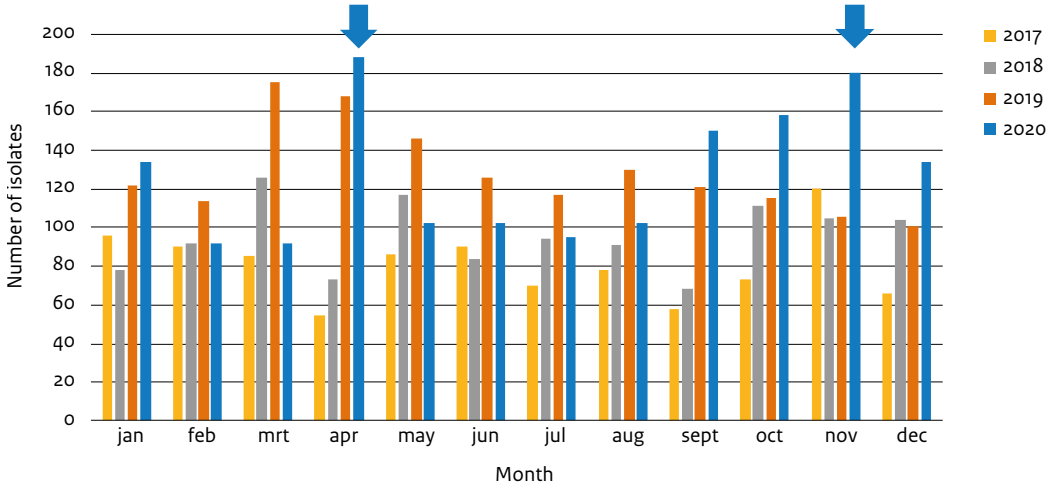
N: number of screened isolates.

[a] *Resistance was screened for in high risk patients only.*

[b] *Resistance frequency was calculated based on the data of four centers.*

[c] *Includes both Vumc and AMC, now AmsterdamUMC.*

Figure 4.8.7.1 Monthly number of *A. fumigatus* isolates sent to the mycology reference laboratory between 2017 and 2020. Compared with previous years, there are two peaks visible in 2020 (blue bars, arrows) that coincide with the first and second COVID-19 wave



Conclusions

- Triazole resistance frequency in 2020 was 11.8% in UMCs and 4.7% in teaching hospitals, which was lower than in the previous years.
- The COVID-19 pandemic might have impacted on *Aspergillus* resistance surveillance, due to changes in patient populations admitted to hospitals.
- Triazole-resistant *A. fumigatus* was recovered from 16 patients with COVID-19.

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5 Antimicrobial stewardship monitor in hospitals

Introduction

Since 2015, every hospital in the Netherlands must have an antimicrobial stewardship team (A-team) that monitors and improves the quality of antibiotic use. The antimicrobial stewardship monitor reports on 1) the stewardship activities employed by A-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 2020, an web-based survey was sent to all 73 acute care hospitals in the Netherlands to assess stewardship activities employed by A-teams in hospitals. The aim was to evaluate the performance and perceived barriers and motivators of A-teams. The survey consisted of 27 questions. Results are presented as percentages of the responding hospitals. Trends were described comparing the data with the previous four years.

Results

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

Thirty-seven A-teams completed the survey (response rate 51%). These hospitals had a mean number of beds of 528 (range 200-1300). Seven (19%) of the hospitals were university hospitals, 14 (38%) were 'top clinical hospitals' and 16 (43%) were general hospitals. An A-team was present in all hospitals. The responding 37 A-teams all included at least one hospital pharmacist and all but one (97%) had at least one medical microbiologist in their A-team. Twenty-eight (76%) included at least one infectious disease specialist. Twelve (32%) of the A-teams also had a nurse employed, five (14%) an infection prevention

specialist, and four (11%) a quality of care officer. Twenty-five A-teams (68%) received financial support from the hospital boards of directors or the cooperation of medical specialists. The median financial support received by these 25-teams was 0.9 FTE A-team staff (range 0.1-2.6). Table 5.1.1 summarizes the A-team characteristics in comparison with previous years.

Stewardship activities

Monitoring, i.e. the assessment and documentation whether stewardship goals are met for relevant aspects of clinical care, and the frequency with which A-teams analyze the data is depicted in Figure 5.1.1. When topics for improvement with regard to antibiotic use were identified, 62% of the A-teams usually provided feedback on this to the hospital departments. All A-teams said to have performed a targeted improvement intervention, although only 46% of the A-teams indicated to perform a determinant analysis in case structural inappropriate antibiotic use was identified. Table 5.1.2 summarizes the performance and monitoring of bedside consultations.

Perceived barriers and solutions

Many A-teams experienced obstacles in carrying out their activities. The top 5 were: lack of IT support (59%), lack of time (51%), lack of financial support (40%), lack of insight into the quality of antibiotic use (27%) and lack of insight into the causes of inappropriate antibiotic use (27%). Figure 5.1.2 indicates what A-teams consider necessary to further improve the functioning of A-teams nationally. On the question "To what extent are indicators desirable to improve the functioning of A teams nationally?" 5% of the A-teams responded "highly desirable", 60% "desirable", 24% A-teams "neutral", and 11% A-teams "undesirable". Suggested indicators are listed in Figure 5.1.3.

Table 5.1.1 Trends in A-team characteristics and monitoring between 2016 and 2020

	2016	2017	2018	2019	2020
Survey response rate, N (%)*	42 (48%)	64 (80%)	35 (45%)	39 (51%)	37 (51%)
A-team characteristics					
Presence of an A-team in responding hospitals	88%	94%	100%	97%	100%
A-team consists of at least:					
≥1 clinical microbiologist	100%	100%	100%	100%	97%
≥1 hospital pharmacist	100%	100%	100%	97%	100%
≥1 infectious disease specialist	70%	68%	86%	71%	76%
≥1 nurse	5%	10%	23%	21%	32%
≥1 infection prevention specialist	10%	14%	14%	16%	14%
Time spent on stewardship per team, mean [hours per week], (range)	15.0 (1-47)	19.8 (3-58)	36.7 (4-134)	36.2 (2-144)	not available
Budget provided by hospital board of directors	39%	41%	79%	55%	54%
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)	0.9 (0.1-2.6)

** total number of hospitals in the Netherlands has changed. Total number of hospitals in 2016: 88, in 2017: 80, in 2018: 78, in 2019: 76, in 2020: 73*

*** percentage of total number of 37 responding hospitals*

Figure 5.1.1a The assessment whether stewardship goals are met for relevant aspects of clinical care, as percentage of the 37 responding A-teams

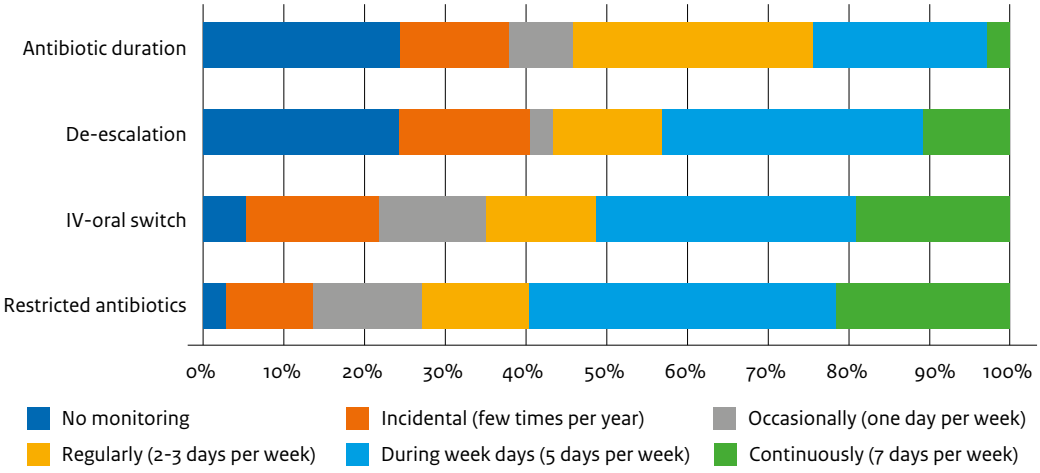
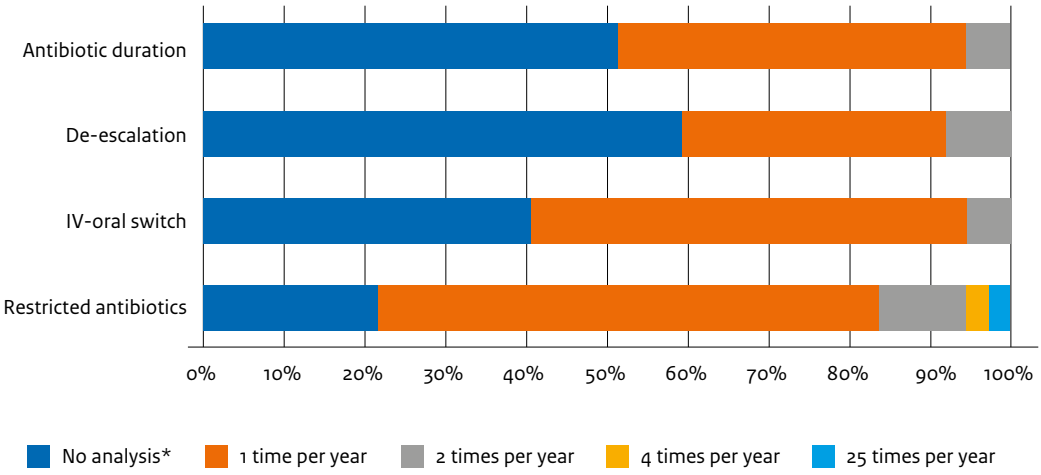


Figure 5.1.1b The frequency with which A-teams analyze the data on quality of antibiotic use, as percentage of total number of the 37 responding A-teams



** includes A-teams that do not perform continous measurement.*

Table 5.1.2 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 37 hospitals)	Monitoring of performance of bedside consultation, N (% of hospitals with indication for consultation)
Staphylococcus aureus bacteremia	37 (100%)	36 (97%)
Infective endocarditis	13 (35%)	2 (15%)
Prosthetic joint infection	5 (14%)	1 (20%)
Vascular prosthesis infection	6 (16%)	1 (17%)
Invasive fungal infection	8 (22%)	3 (38%)

Figure 5.1.2 Actions that A-teams consider necessary to further improve the functioning of A-teams nationally, as percentage of the 37 responding A-teams

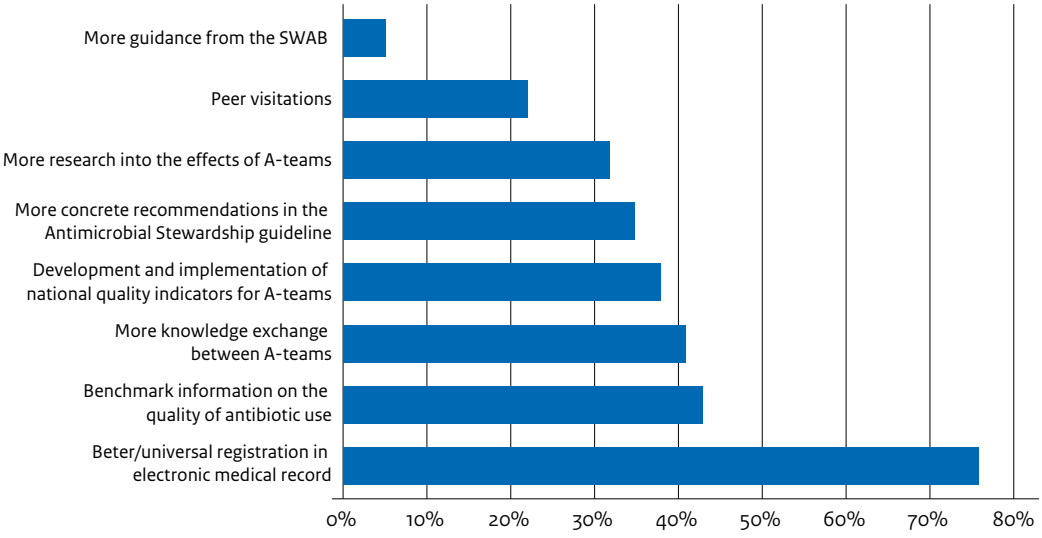
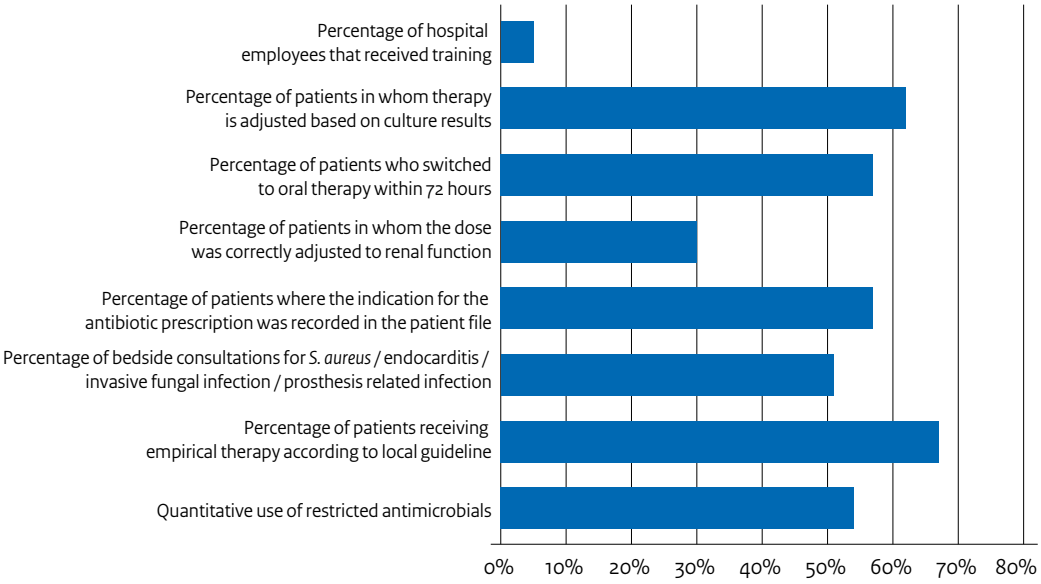


Figure 5.1.3 Suggested indicators to improve the functioning of A-teams, as percentage of the responding 37 A-teams



5.2 Management of adults with community-acquired pneumonia

Methods

In 2020, on the initiative of the National Health Care Institute (Zorginstituut Nederland), in collaboration with the SWAB, 62 acute care hospitals that already participated with LOGEX, a healthcare analytics company, were approached to participate in a study to assess the management of adult patients with CAP in Dutch hospitals. Hospitals were asked to extract antibiotic prescription data from 2015 until 2019 from their electronic medical record and to allow the reuse of reimbursement data ("DBC-diagnosis" codes, care activity, ICD-codes).
The full report can be found on www.zorginstituutnederland.nl/ivm-rapport-pneumonie.

Inclusion criteria

1. An episode with one of the following DBC-diagnosis codes:
   a. 313-401: internal medicine, pneumonia not otherwise specified
   b. 322-1401: pulmonary medicine, pneumonia
   c. 335-273: clinical geriatrics, pneumonia
2. An episode with one of the following ICD-9-codes: ICD-9 480-486 or 487.0, or one of the following ICD-10-codes: J9.0, J10.0, J11.0, J12-J18 or A48.1.
3. A DBC opening date from 1-1-2015.
4. The patient was discharged on or before 31-12-2019
5. Age ≥ 18 years at the start of the DBC
6. ≥1 admission day
7. At least one prescription for an antibiotic, neuraminidase inhibitor, or antifungal agent was started on the day before admission, the day of admission or the day after admission.

Exclusion criteria

1. Patients with a hospital admission in the 30 days before the admission for pneumonia (as defined above).
2. Repeat admissions within the same DBC-episode.
3. Patients receiving tuberculosis treatment as defined by prescriptions with ATC-code J04.

Analysis

We assessed the empiric antibiotic treatment and calculated the number of patients in which intravenous (iv) to oral switch was applied. Furthermore, we calculated length of antibiotic treatment.
Empiric treatment was defined as the prescription(s) that was/were active 12 hours after the start of the first antibiotic prescription. In this analysis, hospitals that did not provide data on the exact timing of the antibiotic prescription were excluded, because in those hospitals it was not possible to distinguish antibiotic therapy that was prescribed one after another from combination therapy.
IV to oral switch was defined as the time when all parenteral antibiotics had been discontinued and the patient continued to be treated with only oral antibiotics. The day iv-oral switch was performed counted for half a day to determine the duration of intravenous treatment prior to oral stepdown.
The length of treatment included both parenteral and oral antibiotic treatment and was calculated in days as: 'stop date of antibiotic use' minus 'start date of antibiotic use' plus 1. This analysis included only the hospitals that also had extracted data on extramural prescriptions, i.e. the antibiotics that were prescribed when a patient was discharged.

Results

Ten hospitals participated and provided data on 14 485 episodes of CAP. For 2018 and 2019 all hospitals provided data for the entire year. For 2017, 8 hospitals provided data for the entire year and 1 hospital for only part of the year (a number of consecutive months). For 2016, complete data were available for 3 hospitals, and partially for 2 hospitals. Three hospitals provided data for 2015, two of which for part of the year. The age distribution of the patients in the episodes was stable over the years. Overall, 24% had an age of 18-64 years, 23% of 65-74 years, and 53% an age of ≥75 years. Fifty-six percent was male.

Empiric antibiotic treatment

Table 5.2.1 summarizes empiric treatment and included the five hospitals that did provide data on the exact timing of the antibiotic prescription. In 2019, monotherapy penicillin or amoxicillin was prescribed in 18% of the episodes, monotherapy with a second or third generation cephalosporin in 33%, and monotherapy co-amoxiclav in 13%, without a clear trend.

IV to oral switch

In 2019, 54% of patients that were not admitted to the intensive care unit switched from iv to oral treatment. There was variation between the hospitals (Figure 5.2.1) but the percentage of patients that were switched was relatively stable over the years as shown in Table 5.2.2 IV to oral switch took place at a median of 3.5 days (IQR 2.5 – 4.5) after admission.

Length of treatment

The median length of treatment was 8 days (IQR 6 - 10) in 2019. This analysis included only the 5 hospitals that also had extracted data on extramural prescriptions, i.e. the antibiotics that were prescribed when a patient was discharged (Table 5.2.3). The overall distribution is shown in Figure 5.2.2a and the distribution between the hospitals in Figure 5.2.2b.

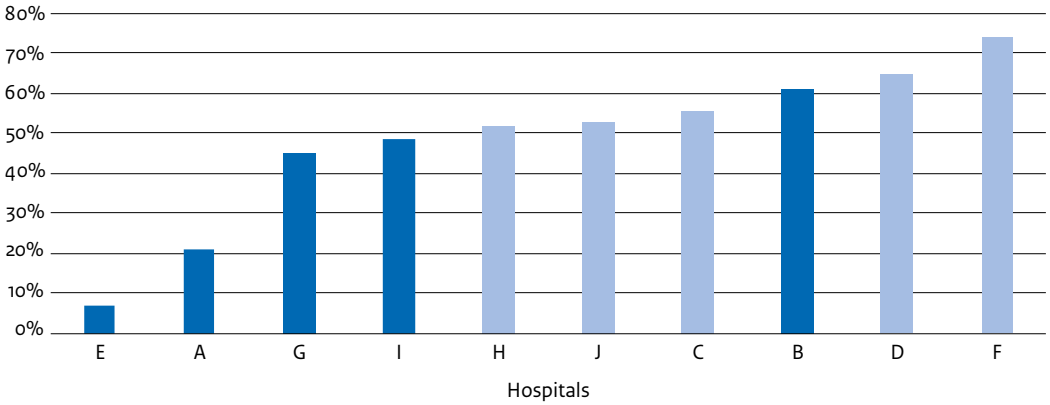
Table 5.2.1 Empiric antibiotic treament for community-acquired pneumonia in hospitals from 2015-2019

	2015	2016	2017	2018	2019	Total
Number of hospitals	2	4	5	5	5	
Antibiotic treatment (number of episodes [%])						
Penicillin or amoxicillin (monotherapy)	47 (18%)	199 (16%)	489 (19%)	365 (14%)	394 (18%)	1,494 (17%)
Penicillin or amoxicillin + ciprofloxacin	39 (15%)	104 (9%)	221 (9%)	71 (3%)	55 (2%)	490 (5%)
Penicillin or amoxicllin + other	3 (1%)	37 (3%)	107 (4%)	91 (3%)	63 (3%)	301 (3%)
Doxycyclin mono- or combination therapy	5 (2%)	28 (2%)	105 (4%)	78 (3%)	51 (2%)	267 (3%)
2nd/3rd generation cephalosporins (monotherapy)	39 (15%)	301 (25%)	558 (22%)	862 (32%)	733 (33%)	2,493 (28%)
2nd/3rd generation cephalosporins + ciprofloxacin	18 (7%)	49 (4%)	133 (5%)	207 (8%)	195 (9%)	602 (7%)
2nd/3rd generation cephalosporins + other	1 (0%)	126 (10%)	250 (10%)	280 (11%)	230 (10%)	887 (10%)
Co-amoxiclav monotherapy	70 (27%)	187 (15%)	393 (15%)	396 (15%)	288 (13%)	1,334 (15%)
Co-amoxiclav combination therapy	20 (8%)	83 (7%)	191 (7%)	172 (6%)	95 (4%)	561 (6%)
Piperacillin-tazobactam mono- or combination therapy	1 (0%)	12 (1%)	36 (1%)	43 (2%)	36 (2%)	128 (1%)
Other*	13 (5%)	84 (7%)	170 (7%)	163 (6%)	146 (7%)	576 (6%)
Total**	**261**	**1,214**	**2,552**	**2,654**	**2,242**	**8,923**

** includes antiviral and antifungal treatment, overall in ~2% of the cases*

*** episodes can occur in multiple rows; not necessarily add up to 100% due to rounding of the percentages*

Figure 5.2.1 Variation in intravenous antibiotic treatment that was switched to oral treatment in community-acquired pneumonia in 2019*



** Light blue columns represent data from hospitals that also provided extramural prescriptions*

Table 5.2.2 Intravenous (iv) to oral switch in patients with community-acquired pneumonia

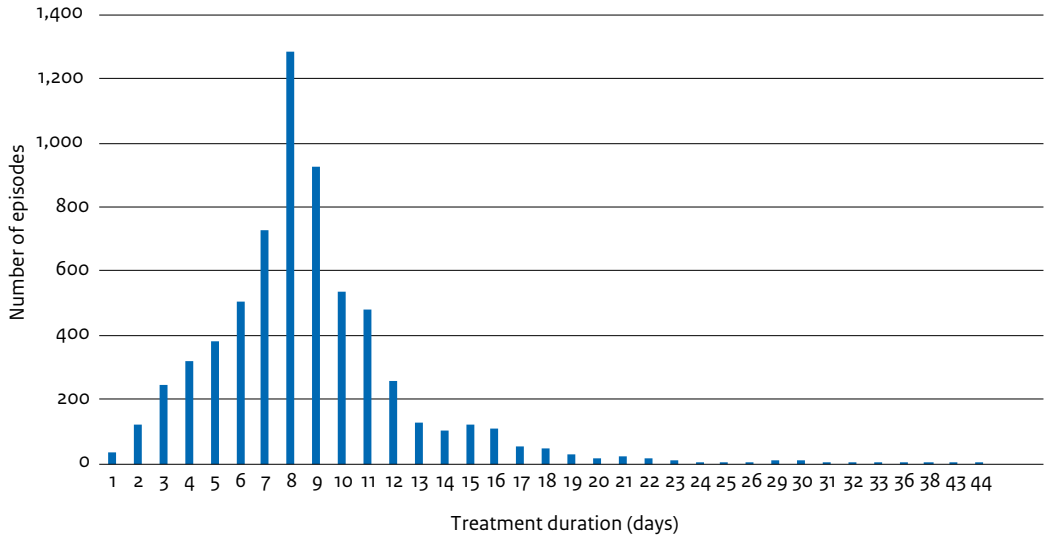
	2015	2016	2017	2018	2019	Total
Number of hospitals	3	5	9	10	10	
Number of episodes with initial iv treatment	330	1,357	2,584	4,021	3,700	11,992
Number of episodes with switch (percentage)	168 (51%)	744 (55%)	1,452 (48%)	2,222 (54%)	2,000 (54%)	6,586 (55%)
Median duration before iv-oral switch, days (25th-75th percentile)	3.5 (2.5-4.5)	3.5 (2.5-4.5)	3.5 (2.5-4.5)	3.5 (2.5-4.5)	3.5 (2.5-4.5)	3.5 (2.5-4.5)

Table 5.2.3 Treatment duration for community-acquired pneumonia in hospitals from 2016-2019*

	2016	2017	2018	2019	Total
Number of hospitals	1	5	5	5	
Number of episodes	530	1,364	2,705	2,493	7,098
Median treatment duration, days (25th-75th percentile)	8.5 (7-11)	9 (7-11)	8 (7-11)	8 (6-10)	8 (7-11)

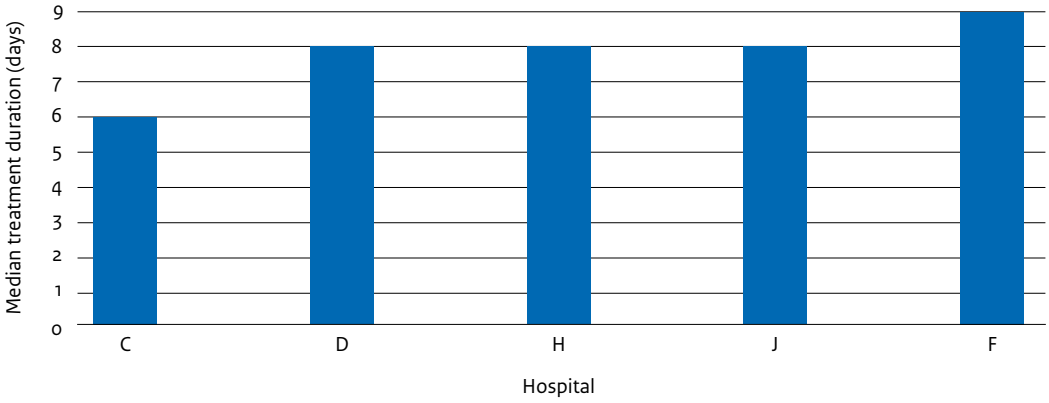
** 2015 contained too few episodes to report.*

Figure 5.2.2a Distribution of treatment duration for community-acquired pneumonia in 2019 - aggregated data of 5 hospitals*



* *To keep the figure clear, treatment durations that occurred less than 5 times are not shown.*

Figure 5.2.2b Variation in median treatment duration for community-acquired pneumonia in 2019



5.3 Discussion

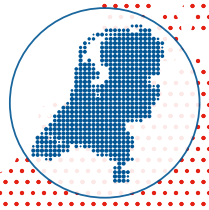
Organization and resources for an antimicrobial stewardship program
In 2020, there was a continuing increase in nurses and other supporting health care workers being part of A-teams, while acknowledging a response rate of 50% with partly different hospitals participating than previous years. Financial support remained on average less than the staffing standard dictates. Almost all of the responding A-teams monitored aspects of antibiotic use to some extent, even though the frequency varied widely. Restricted antibiotics were still the main focus of the A-teams, followed by iv-oral switch. On the other hand, analysis of these data on quality of antibiotics use was often not performed, while this is an essential step for the execution of an efficient and effective improvement strategy.
Even though A-teams put a lot of effort in their antimicrobial stewardship programs, they indicated targets for improvement with regard to their functioning. To improve their functioning, A-teams responded that they would likely benefit from more specific recommendations in the guideline on antimicrobial stewardship and for quality indicators for A-teams. With respect to the latter they agreed with most of the suggested process quality indicators for good antibiotic at patient level. There was also a clear call for better support of A-teams, especially to make data better available by having the opportunity to efficiently and properly record data in the electronic medical records and to extract and analyze this data. Half of the A-teams also indicated that they considered it necessary to have benchmarked data on the quality of antibiotics.

Treatment of community-acquired pneumonia
This year's report shows that, without the use of manual data collection, valuable steering information can be obtained on the management of CAP. In the interpretation, it should be taken into account that the data do not represent all patients with a CAP because patients whose CAP episode was part of an already opened other DBC were not included in the cohort.
Clear points for improvement emerge from this data. First, the empiric therapy was often too broad and not according to the current Dutch guideline. Second and third generation cephalosporins were prescribed to about half of the patients, while in Dutch hospitals only about 13-22% have a severe CAP (Huijts et al. Neth J Med 2013). Prescription of amoxicillin-clavulanic acid was, with 17%, also too high, since amoxicillin-clavulanic acid is only indicated in patients with aspiration pneumonia or healthcare-associated pneumonia. Coverage of so-called atypical pathogens was also too frequent. Second, the treatment duration was too long in most patients. Third, iv-oral switch was often not performed.

Conclusions
- Nurses are increasingly involved in antimicrobial stewardship in hospitals.
- Analysis of data on the quality of antibiotic use and its determinants shows room for improvement.
- Barriers lie at the level of data acquisition and analysis. In terms of solutions, A-teams ask not only more IT support, benchmarked feedback data, but also for quality indicators for the functioning of A-teams.
- The management of community-acquired pneumonia can be improved. The empirical therapy is too broad, iv-oral switch is probably too infrequent and the treatment duration too long.

MARAN 2021

Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2020



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

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Contents

Colophon

This report is published under the acronym MARAN-2021 by Wageningen Bioveterinary Research (WBVR) in collaboration with the Food and Consumer Product Safety Authority (NVWA), Wageningen Food Safety Research (WFSR), the National Institute for Public Health and the Environment (RIVM) and the Netherlands Veterinary Medicines Institute (SDa). The information presented in MARAN-2021 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-2021 is published in a combined back-to-back report with NETHMAP-2021. 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 or on farm level as well as information on differences in prescription patterns between veterinarians is available on the website of the Netherlands Veterinary Medicines Institute (www.autoriteitdiergeneesmiddelen.nl).

MARAN-2021 can be ordered from the secretariat of WBVR, p/a Houtribweg 39, 8221 RA Lelystad, The Netherlands.

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1 Summary

Antibiotic Usage

In 2020 in total 154 tonnes of Antimicrobial Veterinary Medicinal Products (AVMPs) were sold, which is an increase of 2% compared to 2019 and which resulted in a slight relapse in attaining the governmental 70% reduction goal. A decrease in sales by 69.0 % over the years 2009-2020 is attained (with 2009 considered a reference year by the Dutch Government). Antimicrobial use (AMU) based on prescription data stabilised in most animal sectors in 2020 except in veal calves in which the use continued to decrease. In rabbits in 2020 an increase in use was observed, while in turkeys in 2020 use decreased substantially. Finally, use in dairy cattle was traditionally the lowest of all sectors monitored.

The small increase in sales of AVMPs in the Netherlands in 2020 is contradicted by an overall decrease in AMU as observed in the prescription monitoring data. Actual use in animal husbandries can be somewhat different from the quantities sold due to stock piling and cross border use. 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 2020. More efforts to reduce colistin use are warranted, especially in the pig sector and some poultry sectors, not shown here.

Antimicrobial resistance

In 2020, *S.* Enteritidis (25%) followed by *S.* Typhimurium (15%) together with the monophasic variant of *S.* 1,4,[5],12:i:- (9%) and the *S.* Typhimurium variant *S.* 4,12:i:- (11%) were most frequently isolated from humans suffering from clinical salmonellosis. In pigs, the monophasic variant of *S.* Typhimurium (25%) dominated. In cattle, *S.* Typhimurium (32%) and *S.* Dublin (27%) were most commonly isolated, followed by *S.* Enteritidis (21%). In broilers *S.* Infantis dominated (48%, which is an increase from 38% in 2019) while in layers *S.* Enteritidis dominated (63%). Overall, the highest resistance proportions in *Salmonella* were again observed for (in decreasing order) sulfamethoxazole (24.4% in 2019 to 26.3% in 2020), tetracycline (25.5% in 2019 to 25.4 in 2020), ampicillin (24.8% in 2019 to 21.7% in 2020), nalidixic acid (16,7% in 2019 to 16.4% in 2020), ciprofloxacin (17.0% in 2019 to 16.0% in 2020), trimethoprim (10.7% in 2019 to 12% in 2020) and chloramphenicol (7.1% in 2019 to 6.7% in 2020). Among the most frequently isolated serovars,

those showing the highest resistance levels, were *S.* Infantis, *S.* Paratyphi B var. Java, the (monophasic) *S.* Typhimurium variants 4,12:i:- and 1,4,[5],12:i:-, and *S.* Typhimurium. Resistance to fluoroquinolone increased significantly among *S.* Infantis (to 63%) but decreased for *S.* Typhimurium and *S.* Enteritidis.
In total, 6 (0.5%) ESBL suspected isolates were detected among six different serovars, with 4 isolates from humans and 2 non-human isolates of unknown origin. In 2020, no carbapenemase-producing *Salmonella* were found.

In 2020, resistance proportions in *C. jejuni* isolates from caecal samples of broilers and meat thereof stabilized at a high level for quinolones and tetracycline. In laying hens, resistance proportions were much lower than in broilers, especially for *C. jejuni*. Resistance to macrolides was not detected in *C. jejuni* isolates from broilers and poultry meat, and was at low levels in *C. coli* isolates from broilers and poultry meat. In humans, resistance proportions were higher in *C. coli* than in *C. jejuni* isolates, but were overall lower in 2020 compared to previous years. This is most likely due to a substantial reduction of travel-related campylobacteriosis as a result of the COVID-19 lockdown, which is associated with higher resistance proportions than domestically acquired campylobacteriosis. Ciprofloxacin resistance in *Campylobacter* isolates from humans was high again in 2020, which is a concern for public health. It was, however, lower compared to 2017-2019. Resistance to erythromycin, first choice antibiotic in human medicine for campylobacteriosis, remained low.

In STEC O157 a tendency of increasing resistance was observed until 2017 and fluctuates on a lower level since 2018. Resistance to the quinolones (ciprofloxacin and nalidixic acid) was very low in both STEC O157 and STEC/aEPEC non-O157 human isolates in 2020. Proportions of resistance were higher in human STEC/aEPEC non-O157 than in STEC O157. No ESBL-producing isolates were detected in STEC O157, but one O104 isolate was confirmed as ESBL-producer carrying $bla_{\text{CTX-M-15}}$. Almost all STEC O146 isolates - which are primarily associated with small ruminants as reservoir - were pan-susceptible.

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. In slaughter pigs, resistance in indicator *E. coli* decreased to the lowest levels in fifteen years.
In contrast, in broilers and veal calves a tendency of increasing resistance was observed compared to 2019. In dairy cattle resistance fluctuates at a traditional low level. However, over the last decade decreasing trends in resistance were observed for all animal sectors involved in the monitoring. Levels of resistance in indicator *E. coli* increased in laying hens since 2016, but was considerably lower than in broilers reflecting the difference in antibiotic use between these poultry sectors. Resistance proportions in *E. coli* from turkey meat were substantially higher than in *E. coli* from broiler meat, while resistance proportions in *E. coli* from pork and beef were lower than from broiler meat. Resistance to fluoroquinolones was still commonly present in indicator *E. coli* from broilers and meat thereof. For almost all antibiotics tested, levels of resistance in *E. coli* from rosé veal calves were substantially lower than those from white veal calves and differences in resistance between the two veal calf sectors increased in 2020.

Low levels of ESBL/AmpC-production were detected in randomly isolated *E. coli* from pigs, poultry and veal calves in 2020, while all of these populations were negative in 2019. Selective isolation of ESBL/AmpC-producing *E. coli* from broilers and chicken meat shows that prevalence has reduced below 10% in 2020 which reflects the long-term successful effects of the measures on antimicrobials initiated since 2011. Selective isolation of ESBL/AmpC-producing *E. coli* from laying hens also showed a significant reduction of ESBLs since 2016. The prevalence of ESBL/AmpC-producing *E. coli* remains highest in both white and rosé veal calves. In 2020, no carbapenemase-producing Enterobacteriaceae were detected in livestock and companion animals. As in former years, prevalence of *mcr*-1 was low in livestock and meat. Other *mcr* variants were not detected in 2020.

Prevalence of LA-MRSA was high in dust samples from pig farms (76%), but could not be detected in dust samples from broiler farms. At retail, MRSA was detected in < 10% of the pork and bovine meat, but in almost 20% of the poultry meat (both chicken and turkey). The first *cfr*-positive LA- MRSA isolates were detected in dust samples from one pig farm obtained in 2019 as well as in five human LA-MRSA isolates in 2018 – 2020. The first findings of this multi-resistance encoding gene in MRSA from humans and pigs demonstrated the importance of AMR monitoring from a One Health perspective.

It can be concluded that more than ten years of antibiotic reduction policies in the Netherlands has resulted in almost 70 % reduction of sales of AVMPs for veterinary use. Antimicrobial resistance has decreased simultaneously in isolates from most livestock species. In spite of the AMU reduction, prevalence of LA-MRSA is still substantial. ESBL and colistin-resistance remain present at low levels, while no CPE was detected in samples from livestock or meat.

2 Usage of antibiotics in animal husbandry in the Netherlands

Sales and use of antimicrobial veterinary medicinal product (AVMPs) are monitored by the Netherlands Veterinary Medicines Institute (SDa, Diergeneesmiddelenautoriteit). The information described in this part of MARAN is presented in more detail in the annual reports of the SDa (https://www.autoriteitdiergeneesmiddelen.nl/en/publications/general-reports).

2.1 Total sales of veterinary antibiotics in the Netherlands 2020

2.1.1 Analysis of sales data

FIDIN, the federation of the Dutch veterinary pharmaceutical industry, provided sales data for all Antimicrobial Veterinary Medicinal Products (AVMPs) on package level sold in 2020 in the Netherlands, as extracted from the Vetindex and supplemented with AVMPs data of non-FIDIN members. These data are estimated to cover approximately 98% of all sales in the Netherlands. 2.6% of the sold AVMPs is exclusively authorized for companion animals. AVMPs that are marketed in accordance with the legal exemptions such as products for minor species in small packages (article 3.7 Regeling diergeneesmiddelen) and those products that are imported from other EU member states in accordance with cascade legislation are not included. Actual use in animal husbandries can be somewhat different from the quantities sold due to stock piling and cross border use. Monitored mass used in the major livestock farming sectors (pigs, broilers, turkey, other poultry, veal calves, dairy- and other cattle, meat rabbits) covered 93.0% of sales in 2020. AVMPs are reported as active base substance mass (excluding mass of salts and esters), including oral products, injectables, intramammary injectors and topical applications like ointments, eye drops and sprays. The sales data in this report involves total sales, for all animals, not stratified by animal species. Detailed information about antibiotic usage by animal species in the Netherlands is reported in paragraph 2.2.

2.1.2 Trends in total sales

Table 1 shows the trends in the total sales of antibiotics licenced for therapeutic use in animals in the Netherlands. In 2020 in total 154 tonnes of AVMPs were sold, which is an increase of 2% compared to 2019 and which resulted in a slight bounce back in attaining the governmental 70% reduction goal. A decrease in sales by 69.0 % over the years 2009-2020 is attained (with 2009 considered a reference year by the Dutch Government).

Figure 1 shows the trends in sales (mass, black line) in relation to the dynamics of liveweight of Dutch livestock (dashed line) and the total use on farms (mass, bars) of the livestock sectors monitored from 2009 to 2020. 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. Veal calves (light blue) and pigs (green) used almost 80% of the total mass of all antibiotics sold for therapy. Animals treated in these two sectors are large and therefore need more antibiotics per administration than small animals like broiler chickens. This illustrates that sales data provide limited information about exposure of animals at risk. Use data based on mass may result in the suggestion that exposure of broiler chickens to antibiotics is limited based on the small proportion of total mass used in these animals.

The discrepancy in mass in 2020 between sales and usage in monitored sectors was 7.0% as illustrated in Figure 1. The difference between sales and use data fluctuates as described by the difference between the solid black line (mass sold) and bars (mass used in monitored sectors).

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

Tetracyclines
The fraction of doxycycline (not specified in Figure 2) was in 2020 63.2% of the total sales of tetracyclines (68.6% in 2019, fluctuations between 31% and 49% in the years 2011-2018).

Penicillins
Second place in mass, sales of penicillins (including aminopenicillins) show the sharpest increase of all groups in 2020, 12.7% in comparison to 2019. The distribution of broad and narrow spectrum penicillins (in mass sold) is somewhat shifted to broad spectrum, 73%.

(Fluoro)quinolones
The sales of fluoroquinolones decreased with 33kg (18%) in 2020. An overall reduction of 89.9% was realized in comparison with 2011. In 2020, 48% of the sales were 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) were stable compared to 2019 (+ 0.71%); these AVMPs are exclusively applied in food producing animals.

Cephalosporins

Sales of these AVMPs were relatively stable over the period 2015 to 2020. A relatively large increase in sales of 3rd and 4th generation cephalosporins was observed in 2019 (although the total mass sold was still less than 3kg), followed by the lowest sales ever in 2020 (0.63kg).
A reduction of 99.9% of all cephalosporins sales has been achieved since 2011.

Polymyxins

Colistin sales decreased in 2020 with 0.8%, after two years of increasing sales.
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 *Expert Panel of the* Netherlands Veterinary Medicines Institute considers polymyxins as third choice antibiotics, and this antibiotic class is reported as such. This implies that similar as for fluoroquinolones and 3rd/4th generation cephalosporins the Dutch target for use for 2020 onwards will be 0 DDDAF. The ESVAC group introduced in 2016 the colistin desirable-level-benchmark for EU member states of below 1 mg/PCU for sales data, this irrespective of the sectors in which colistin is used. Netherlands is below that unified benchmark, but for some sectors (laying hens) the specific use data show differently. Moreover, many farms have zero colistin usage. Some sectors have a usage above the ESVAC benchmark when only those farms with colistin use are considered in the comparisons with the benchmark. This underpins the limitation of this sector level benchmark.

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

In Figure 3, antimicrobial use (AMU) based on annual prescription data is presented for each livestock sector. Rabbits are monitored since 2016, and the use data in that sector are included in Figure 3 for the first time. Main changes in AMU in the sectors (Figure 3) are seen in turkeys and veal calves.

In Figure 4 is shown that fluoroquinolones (red bar) are almost exclusively used in turkeys, although this use has been reduced by 70% since 2015. In turkeys in 2020 a substantial decrease in total use was observed, and Figure 4 shows that this decrease was specifically due to the reduction in use of second choice antimicrobials (yellow bar) resulting in a shift to more first choice antimicrobials (green and blue bars), 61.1%.
In most sectors, except for broilers, this proportion of first choice AVMP's has attained a stable level, at 70-85%. In veal calves, a large sector with the highest proportion of first choice AVMP's, a steady decrease in use is observed since 2015. This reduction of AMU is attained in all antibiotic classes.
In rabbits, the use of colistin was abandoned in 2020. Total AMU in this sector is still high.

Expressing antibiotic use in number of Defined-Daily Dosage Animal like in Figure 3 and 4 shows that AMU in broilers and in pigs is comparable in number of DDDA, although the distinct differences in applied antibiotic classes is notable.

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

EU regulation 2019/6 (VMP-reg)
The EU Regulation about amongst others the monitoring of veterinary antimicrobial *use* starting from 2023 (reporting in 2024) will be implemented in national legislation for all EU member states, coming into effect January 28th 2022. Sales data will have to be reported to EMA, as is already in place for most EU MS in the ESVAC project. The monitoring of sales and use data will be expanded from anti*bacterial* substances to anti*microbial* substances including antimycotic, antifungal, antiviral and anticoccidial substances. This implies that at first, *sales data* of additional veterinary medicinal products will have to be reported. In the Netherlands 60 authorizations will be added, involving 100 pack sizes. Also, cascade use of products imported from other EU countries will have to be incorporated in sales (and use) data.
In 2023 monitoring *of use* of these (at this moment) 100 products will be implemented in the regular monitoring. For discussion is whether national monitoring will be extended to antiprotozoal (e.g. Leishmaniasis) and antiparasitic veterinary medicinal products as well, for future interpretation of resistance development purposes.
In 2026 the monitoring of use of the indicated products will be extended to sheep, goats, ducks, geese, finfish and horses. Most of these sectors are already preparing the implementation of a monitoring system. In 2029 the use of these products will also be monitored in cats and dogs. For horses and companion animals cascade use of antimicrobial medicinal products for human use will have to be included as well in the *use* monitoring.

Conclusion

Maximal transparency has been created since 2011 through monitoring antibiotics use by veterinarians and farmers. The small increase in sales of AVMPs in the Netherlands in 2020 is contradicted by an overall decrease in AMU as observed in the use monitoring data. The calculation of consumption is based on national conversion factors (DDDAs) of authorized 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 2020, while sales decreased. More efforts to reduce colistin use are warranted, especially in the pig sector and some poultry sectors, not shown here.

Table 1 Antimicrobial veterinary medicinal product sales from 1999-2020 in kg (thousands) (FIDIN, 2020)

year	'99	'00	'01	'02	'03	'04	'05	'06	'07	'08	'09	'10	'11	'12	'13	'14	'15	'16	'17	'18	'19	'20
betalactam antibiotics	35	36	38	38	36	43	51	57	61	70	73	71	66	54	45	48	45	39	42	43	36	40
tetracyclines	162	194	200	214	216	256	292	301	321	257	251	217	157	102	80	69	82	62	68	65	51	49
macrolides & lincosamides	10	15	17	19	17	23	28	42	55	52	46	39	34	26	25	28	23	23	25	25	23	24
aminoglycosides	13	12	11	10	9	9	11	11	12	11	10	8.6	7.3	5.8	3.4	1.8	2.7	2.1	1.9	2.0	1.8	1.7
(fluoro) quinolones	7	7	6	6	5	7	8	7	9	8	8	6.6	5.1	3.1	2.8	3.8	4.2	3.4	3.4	3.9	2.7	2.6
trimethoprim/ sulfonamides	72	80	92	92	88	91	91	93	99	100	92	78	58	48	53	49	42	39	34	33	29	30
otherantibacterials	11	12	11	11	7	6	6	8	8	7	15	13	10	10	8.1	7.8	7.5	7.4	7.2	7.5	7.4	7.2
total sales	310	356	376	390	378	434	487	519	565	506	495	433	338	249	217	207	206	176	181	179	150	154

Figure 1 Mass balance of AVMPs sales data (black line, left y-axis) and use data (colored bars, left x-axis) (kg x 1000), combined with total liveweight of the food animal population (dotted line, right y-axis, kg x 10^6) from 2009-2020

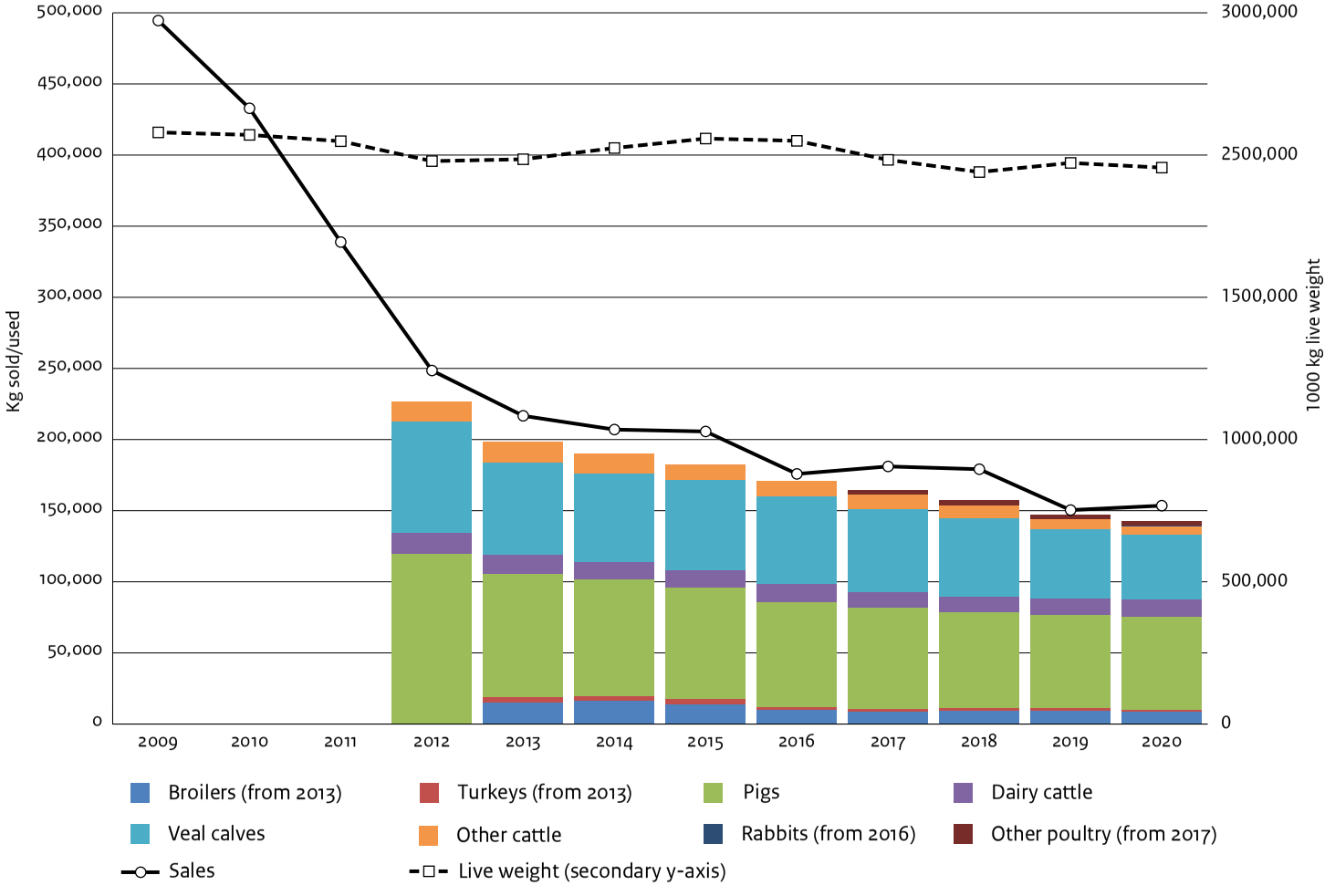


Figure 2 Antimicrobial Veterinary Medicinal Product sales by antibiotic class from 2011-2020 in kg (thousands)

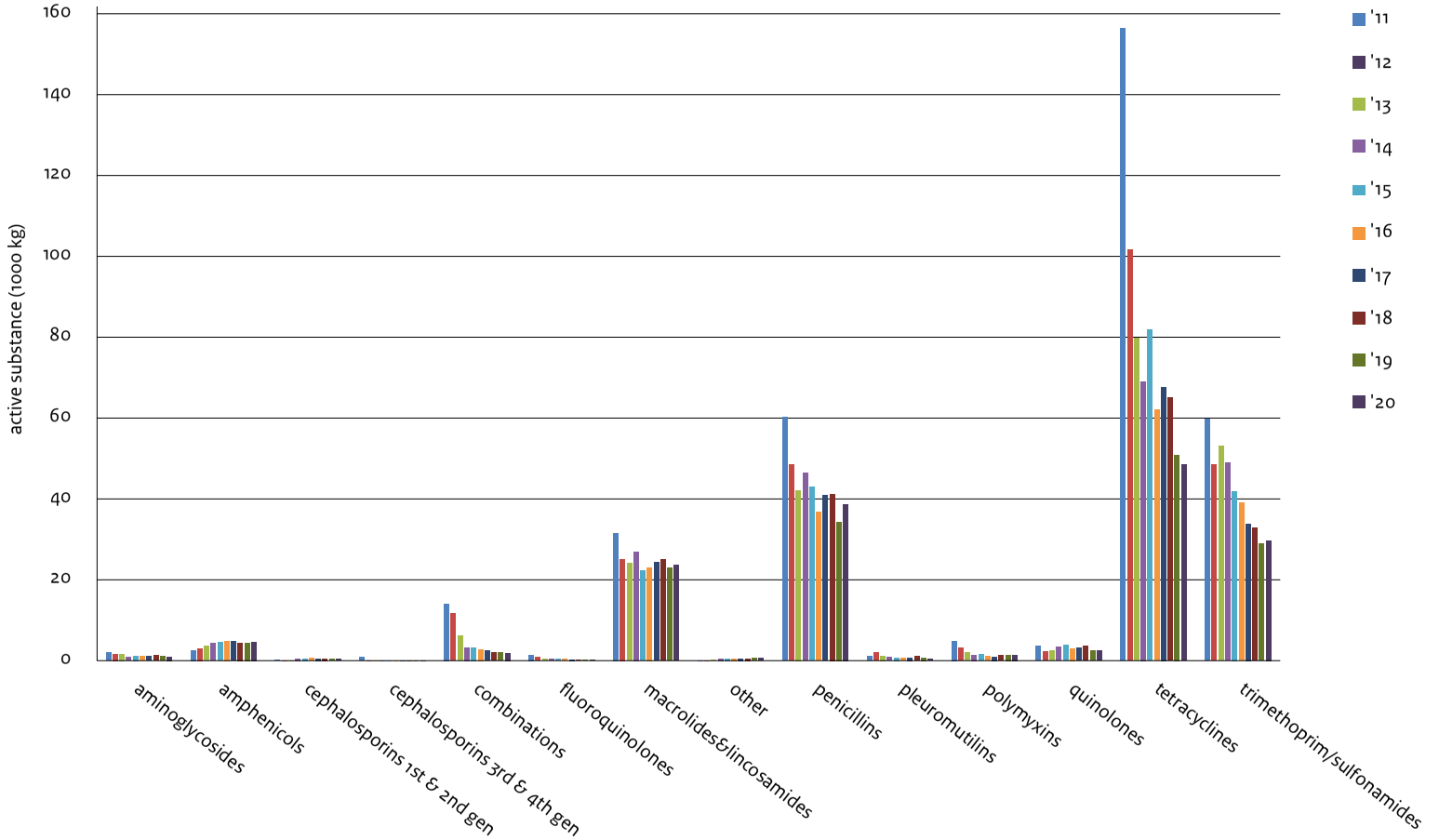


Figure 3 Number of animal-defined daily dosages per animal-year for rabbits (grey), turkeys (purple), veal calves (blue), broilers (orange), pigs (light green) and dairy cattle (dark green) farms as reported by LEI WUR-MARAN (years 2007-2010 as DD/AY) and by SDa (years 2011-2020 as DDDANAT) depicting point estimates (dots), 95% confidence limits (error bars), smoothed trend line (penalized spline) and 95% confidence limits for the spline (shaded area)

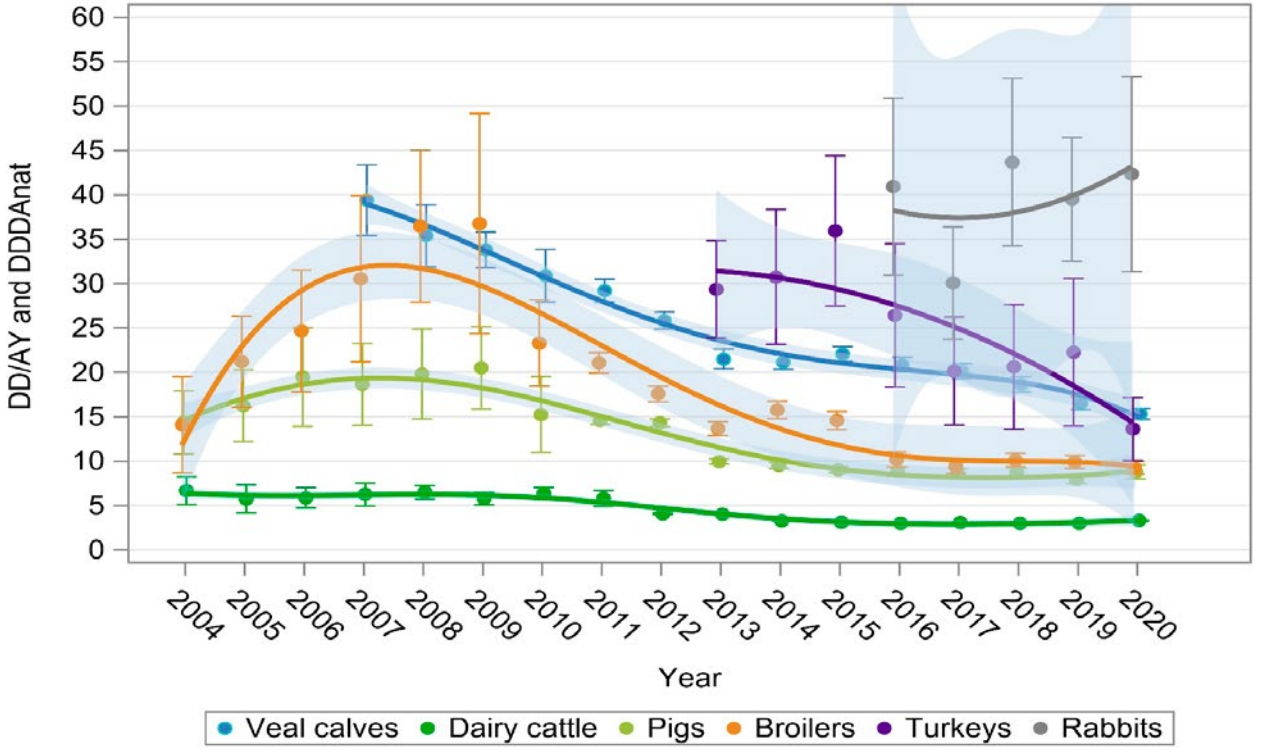
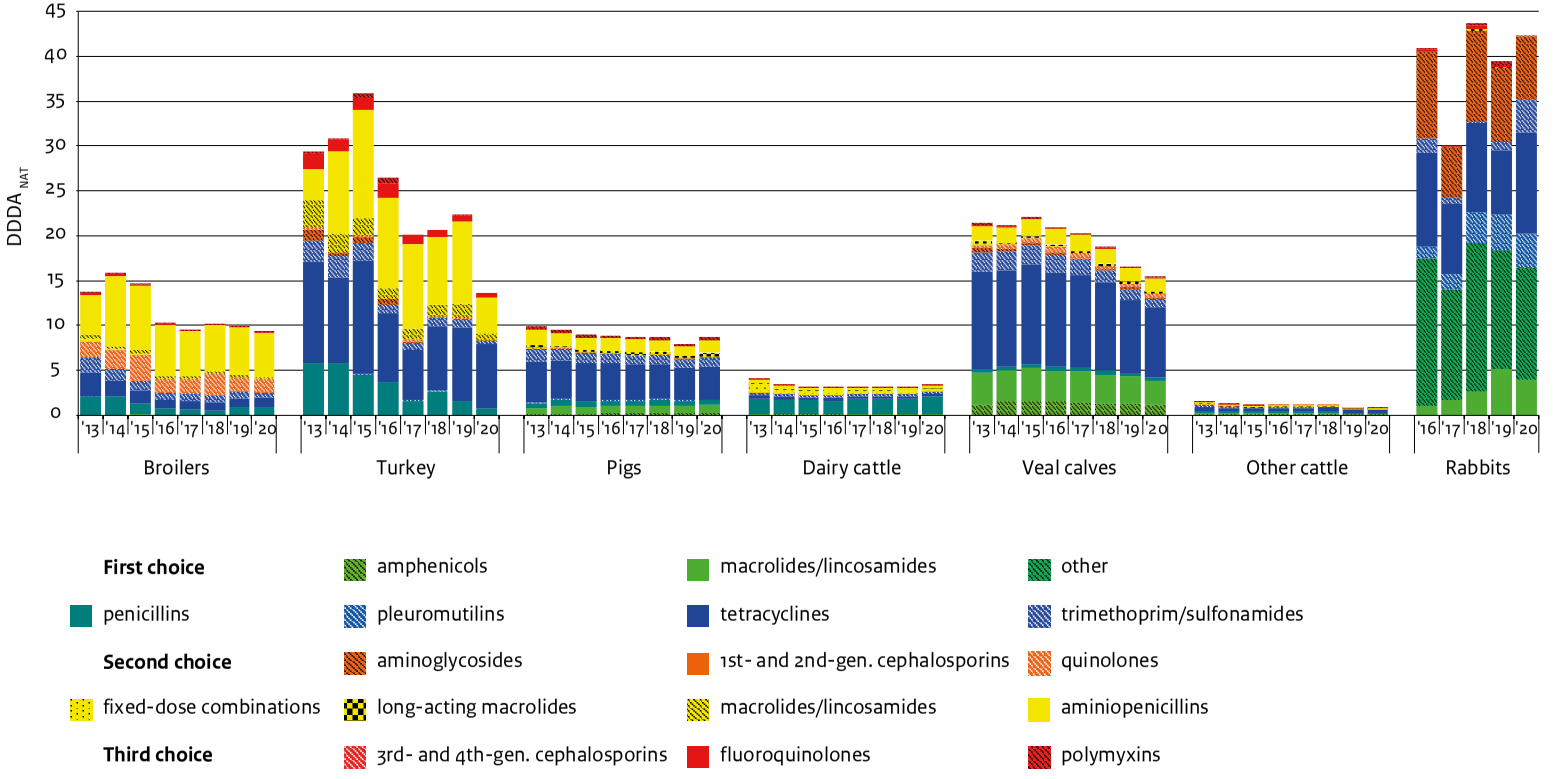


Figure 4 Number of $DDDA_{NAT}$ per animal-year of antimicrobial veterinary medicinal products specified by antibiotic class per animal sector over the years 2013-2020



3 Resistance data

This chapter describes susceptibility test results as determined in 2020 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 and other food products as potential sources of infection for humans via the food chain, and animal feed as potential source of infection for food-producing animals.

Highlights

1. In 2020, *S.* Enteritidis (25%) followed by *S.* Typhimurium (15%) together with the monophasic variant of *S.* 1,4,[5],12:i:- (9%) and the *S.* Typhimurium variant *S.* 4,12:i:- (11%) were most frequently isolated from humans suffering from clinical salmonellosis.
2. In pigs, the monophasic variant of *S.* Typhimurium (25%) dominated. In cattle, *S.* Typhimurium (32%) and *S.* Dublin (27%) were most commonly isolated, followed by *S.* Enteritidis (21%). In broilers *S.* Infantis dominated (48%, which is an increase from 38% in 2019) while in layers *S.* Enteritidis dominated (63%).

3. Overall, the highest resistance proportions in *Salmonella* were again observed for (in decreasing order) sulfamethoxazole (24.4% in 2019 to 26.3% in 2020), tetracycline (25.5% in 2019 to 25.4 in 2020), ampicillin (24.8% in 2019 to 21.7% in 2020), nalidixic acid (16,7% in 2019 to 16.4% in 2020), ciprofloxacin (17.0% in 2019 to 16.0% in 2020), trimethoprim (10.7% in 2019 to 12% in 2020) and chloramphenicol (7.1% in 2019 to 6.7% in 2020).
4. Among the most frequently isolated serovars, those showing the highest resistance levels, were S. Infantis, S. Paratyphi B var. Java, the (monophasic) S. Typhimurium variants 4,12:i:- and 1,4,[5],12:i:-, and S. Typhimurium.
5. Resistance to fluoroquinolone increased significantly among S. Infantis (to 63%) but decreased for S. Typhimurium and S. Enteritidis.
6. In total, 6 (0.5%) ESBL suspected isolates were detected among six different serovars, with 4 isolates from humans and 2 non-human isolates of unknown origin.
7. In 2020, 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 2020 were the same as in previous years: S. Enteritidis (25%), followed by S. Typhimurium (15%) and its (monophasic) variants S. 4,12:i- (9%) and S. 1,4,[5],12:i:- (11%). The most frequent isolated serovars from pigs were S. 1,4,[5],12:i:- (35%), S. 4,12:i- (16%), S. Derby (14%), S. Brandenburg (12%) and S. Typhimurium (10%). For cattle, these were S. Typhimurium (25%), S. Dublin (21%), S. Enteritidis (16%) and S. 1,4,[5],12:i:- (15%). Isolates from broilers were dominated by S. Infantis (49%), followed by S. Paratyphi B var. Java (15%) and S. Enteritidis (9%). Among laying hens, the most frequently isolated serovar were S. Enteritidis (63%) and S. Braenderup (25%).

Table S01 Most prevalent *Salmonella* serotypes isolated in 2020 from humans, pigs (including pork), cattle (including beef), layers (including reproduction animals and eggs)

	Humans	Pigs	Cattle	Broiler	Layer
Total	**568**	**49**	**128**	**194**	**40**
N tested	**509**	**43**	**103**	**146**	**13**
Enteritidis	143		21	17	25
Typhimurium	87	5	32	1	2
4,12:i:-	60	8	2		
1,4,5,12:i:-	52	17	19		
Infantis	30	1	1	94	1
Dublin	19		27		
Virchow	16			8	

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	**568**	**49**	**128**	**194**	**40**
N tested	**509**	**43**	**103**	**146**	**13**
Manhattan	8				
Newport	8		2	1	
Derby	7	7		1	
Goldcoast	6	1	2	2	
Montevideo	6		2		
Bovismorbificans	5	1			
Braenderup	5				10
Bredeney	5				
Kentucky	5				
Paratyphi B var. Java	5			29	
Brandenburg	4	6			
Chester	4				
Coeln	4				
Senftenberg	4				
Typhi	4				
Goettingen	3		1		
Mbandaka	3			2	
Oranienburg	3				
Panama	3			2	
Stanley	3				
OTHER	66	3	19	37	2

Resistance proportions overall.

A selection of all human *Salmonella* isolates received by the RIVM from regional public health and other clinical laboratories (N = 509) was sent to WBVR for susceptibility testing. Moreover, 661 isolates from non-human sources were tested. These included isolates from broilers (N=146), cattle (N=103), pigs (N = 43), and layers (N=13), as well as isolates from a diversity of other sources, including animal feed (N = 225), food products (e.g. seafood, spices) and other animals (e.g. goats, horses) (N = 131). 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 food products sampled 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 plasmid-mediated colistin resistance genes (*mcr-family*) resulted in even more attention for this compound. Therefore, from 2020 onwards the SDa will consider and report it as third choice drugs, comparable to fluoroquinolones and 3rd/4th generation cephalosporins (Chapter 2).

Like in previous years, colistin resistance was not reported in *Salmonella* in 2020 (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 1170 *Salmonella* isolates from different sources tested for susceptibility in 2020 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 (in decreasing order) sulfamethoxazole (24.4% in 2019 to 26.3% in 2020), tetracycline (25.5% in 2019 to 25.4% in 2020), ampicillin (24.8% in 2019 to 21.7% in 2020), nalidixic acid (16,7% in 2019 to 16.4% in 2020), ciprofloxacin (17.0% in 2019 to 16.0% in 2020), trimethoprim (10.7% in 2019 to 12.0% in 2020) and chloramphenicol (7.1% in 2019 to 6.7% in 2020). Similar to previous years, no resistance was detected to the carbapenem antibiotic meropenem. As in previous years, low proportions of resistance were found for tigecycline, azithromycin, cefotaxime, ceftazidime, and gentamicin.

Table So2 MIC distribution (in %) and resistance percentages (R%) for all *Salmonella* isolates (N=1170) tested for antibiotic susceptibility during 2020

Salmonella N = 1170"	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							60.5	15.1	2.5	0.2		0.1	0.3	21.3					21.7	19.3 - 24.0
Cefotaxime					98.0	1.5		0.1		0.5									0.6	0.2 - 1.0
Ceftazidime					95.3	0.5	3.6	0.1	0.2	0.1	0.3								0.6	0.1 - 0.8
Gentamicin						91.0	6.4	0.6	0.2	0.3		0.5	0.9						1.9	1.1 - 2.7
Tetracycline								71.5	2.9	0.2		0.7	2.4	22.3					25.4	22.9 - 27.9
Sulfamethoxazole										36.4	32.1	3.5	1.1	0.6		0.2	0.1	26.0	26.3	23.7 - 28.8
Trimethoprim					78.4	9.2	0.4	0.1				0.2	11.8						12.0	9.9 - 13.6
Ciprofloxacin	42.7	37.8	3.4	0.9	7.6	5.5	1.5	0.1		0.1	0.3								16.0	14.0 - 18.2
Nalidixic acid									80.0	3.6	1.3	1.5	0.3	1.8	11.5				16.4	14.3 - 18.5
Chloramphenicol										88.2	5.1	0.4	0.2	1.8	4.3				6.7	5.2 - 8.0
Azithromycin*								2.7	32.2	59.6	4.6	0.4	0.3	0.2					0.9	0.3 - 1.3
Colistin**							37.2	48.0	10.3	4.4	0.1								-	-
Meropenem		90.8	7.7	1.5															0.0	0.0-0.4§
Tigecycline***					75.0	17.5	6.7	0.8											0.8	0.3 - 1.3

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, 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.

Fluoroquinolone resistance

The class of fluoroquinolones is regarded as the treatment of choice for severe salmonellosis in adults. Currently, EUCAST recommends a clinical breakpoint of 0.06 mg/L for *Salmonella enterica*, based on clinical evidence that there is a poor therapeutic response in systemic infections caused by *Salmonella* spp. with low-level ciprofloxacin resistance (MIC >0.06 mg/L) (www.eucast.org). Using the EUCAST recommended epidemiological cut off value of 0.06 mg/L as breakpoint, 16% of *Salmonella* isolates from 2020 demonstrated an acquired resistance phenotype for ciprofloxacin (Table S02), which is around the same as in 2019 (17%). The highest levels of ciprofloxacin resistance among the most prevalent serovars were observed for *S.* Infantis (63% in 2020 compared to 45% in 2019), and *S.* Paratyphi B var. Java (44% in 2020 compared to 47% in 2019), and S. Enteritidis (18% in 2020 compared to 22% in 2019) (Table S03).

Table S06 shows that the proportion of isolates resistant to ciprofloxacin in chicken meat increased after a decline over the last years (89% in 2017, 69% in 2018, 58% in 2019, and 65.5% in 2020). These isolates (predominantly S. Infantis) were obtained from broiler meat and broiler meat preparations from retail and meat industry. The high proportion of resistance to fluoroquinolones in poultry meat reflects the frequent usage of fluoroquinolones in the poultry production chain within EU.

Table S03 Resistance (%) of the most prevalent (>20 isolates) *Salmonella* serovars isolated in the Netherlands in 2020 (N tested)

	Enteritidis (161)	Typhimurium (117)	4,12:i:- (64)	1,4,[5],12:i:- (96)	Infantis (106)	Dublin (44)	Yoruba (33)	Brandenburg (27)	Paratyphi B var. Java (27)	Livingstone (24)	Virchow (21)	Average over serotypes (N=624
Ampicillin	9.9	44.4	84.4	60.4	15.1	15.9	0.0	7.4	37.0	0.0	4.8	25.4
Cefotaxime	0.0	0.9	0.0	0.0	0.9	0.0	0.0	0.0	3.7	0.0	0.0	0.5
Ceftazidime	0.0	0.9	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.2
Gentamicin	0.0	4.3	3.1	7.3	1.9	2.3	0.0	0.0	0.0	0.0	4.8	2.1
Tetracycline	6.8	33.3	85.9	72.9	62.3	15.9	0.0	7.4	3.7	0.0	4.8	26.6
Sulfamethoxazole	5.6	35.9	82.8	59.4	69.8	18.2	0.0	7.4	59.3	4.2	4.8	31.6
Trimethoprim	1.2	9.4	7.8	11.5	34.0	9.1	0.0	7.4	85.2	4.2	4.8	15.9
Ciprofloxacin	18.0	11.1	12.5	11.5	63.2	0.0	0.0	3.7	44.4	0.0	9.5	15.8
Nalidixic acid	18.0	11.1	12.5	11.5	67.0	0.0	0.0	0.0	44.4	0.0	9.5	15.8
Chloramphenicol	0.6	21.4	7.8	12.5	4.7	18.2	0.0	3.7	3.7	8.3	0.0	7.4
Azithromycin	0.6	0.0	1.6	1.0	1.9	0.0	0.0	3.7	0.0	4.2	0.0	1.2
Meropenem	0.0	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	0.0	0.0	0.0	8.5	0.0	0.0	0.0	0.0	0.0	0.0	0.8

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 2020, the total number of cefotaxime resistant (MIC > 0.5 mg/L) ESBL suspected *Salmonella* isolates was 6/1170 (0.5%) (compared to 24/1880 [1.3%] in 2019), among six different serovars, with 4 isolates from humans (*S.* Derby, *S.* Kentucky, *S.* Infantis, *S.* Typhimurium) and 2 non-human isolates of unknown origin (*S.* Heidelberg, *S.* Agona).

Resistance proportions of the most prevalent serovars.

Table S03 presents resistance percentages for the most prevalent serovars isolated in the Netherlands (all sources together) in 2020. Among these 11 serovars the highest resistance proportions were observed for sulfamethoxazole (31.6%), tetracycline (26.6%) and ampicillin (25.4%). There was considerable variation between the resistance profiles of the different serovars. For all antimicrobials tested, the most resistance serotypes were *S.* Infantis (very high levels of resistance to sulfamethoxazole, ciprofloxacin, nalidixic acid, tetracycline and high levels of resistance to trimethoprim), *S.* Paratyphi B var. Java (very high levels of resistance to trimethoprim and sulfamethoxazole; high levels of resistance to ciprofloxacin, nalidixic acid, and ampicillin), the monophasic *S.* Typhimurium variants 4,12:i:- and 1,4,[5],12:i:- (both very high levels of resistance to ampicillin, sulfamethoxazole, and tetracycline) and *S.* Typhimurium (high levels of resistance to ampicillin, tetracycline, sulfamethoxazole and chloramphenicol). *S.* Infantis and *S.* Paratyphi B var. Java are mainly poultry related while *S.* Typhimurium and its variant are mainly pig and cattle related.

The most prevalent serovars, except for *S.* Yoruba 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 2020 were *S.* Infantis (12/13) and *S.* Typhimurium (10/13). The most common pattern was resistance to ampicillin, sulfamethoxazole and tetracycline (ASuT).

Resistance patterns of *S.* Typhimurium.

The resistance patterns of *S.* Typhimurium are separately depicted in Table S04. Resistance was high for ampicillin, tetracycline, and sulfamethoxazole in human and cattle isolates (only 4 pig isolates were retrieved so not considered in the analysis). Resistance to the clinically important drug cefotaxime was only detected among human isolates (1.2% in 2020 compared to 0.7% in 2019 and 1.7% in 2018). The resistance percentage to fluoroquinolones in human isolates was 14.8% in 2020 (compared to 16.8% in 2019). In 2020, resistance to fluoroquinolones was not found in cattle but in one isolate from a horse.
In contrast to 2019, no resistance to tigecycline was observed for *S.* Typhimurium in 2020. 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 (Fig. S01). Since 2013, resistance proportions seem to fluctuate from year to year.
After a decreasing trend over the last few years the resistance proportions for ampicillin, sulfamethoxazole, and chloramphenicol were higher than in 2019. In contrast, resistance to ciprofloxacin increased over the years but decreased in 2020.

Resistance proportions in *S.* Typhimurium isolates from cattle (Fig. S01) and pig (not shown in Fig. S01 due to low number of isolates [N=5]) varied considerably over the years. This is also related to the relatively small number of isolates per year and trends, and should be interpreted with care. In 2020, the resistance proportions among cattle isolates were generally lower compared to 2019. For pigs too few isolates (n=5) were retrieved for proper inclusion in the trend analysis and were omitted from the trend graph of Fig. S01.

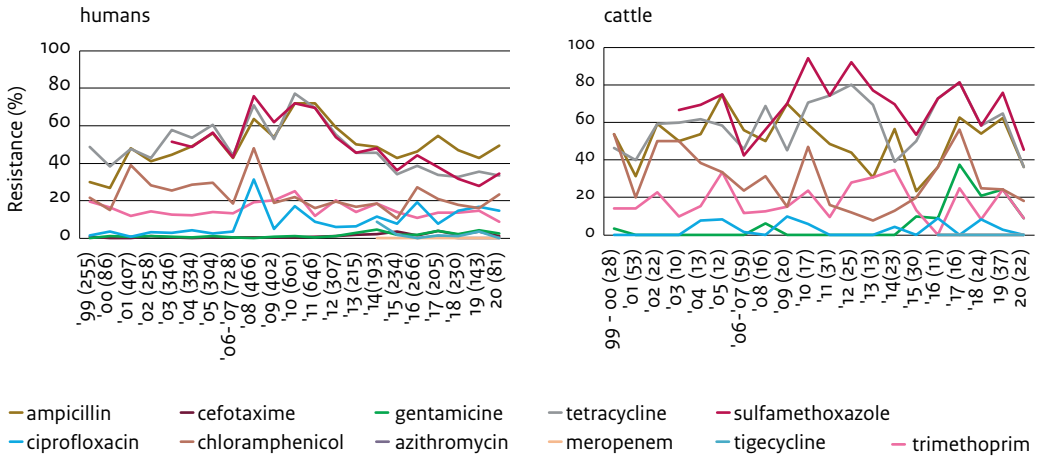
Table S04 Resistance percentages of *S.* Typhimurium (N tested) isolated from humans, cattle, pigs and other sources in 2020

	S. Typhimurium (117)[a]		
	Humans (81)	Cattle (22)	Other sources (14)[b]
Ampicillin	49.4	36.4	28.6
Cefotaxime	1.2	0.0	0.0
Ceftazidime	1.2	0.0	0.0
Gentamicin	2.5	9.1	7.1
Tetracycline	33.3	36.4	28.6
Sulfamethoxazole	34.6	45.5	28.6
Trimethoprim	8.6	9.1	14.3
Ciprofloxacin	14.8	0.0	7.1
Nalidixic acid	14.8	0.0	7.1
Chloramphenicol	23.5	18.2	14.3
Azithromycin	0.0	0.0	0.0
Meropenem	0.0	0.0	0.0
Tigecycline	0.0	0.0	0.0

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

b Other sources include pigs (4), layers (2), horses (3), grains/beans, spices or herbs (2), pigeons (1), seafood (1), and feed (1)

Figure S01 Trends in resistance (%) of *S.* Typhimurium isolated from humans and food-animals in 1999 - 2020



Resistance proportions of *S.* Enteritidis

In the Netherlands, human infections caused by *S.* Enteritidis are mainly related to the consumption of contaminated eggs and, to a lesser extent, of poultry meat products or related to travel abroad.
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, cattle and very few food/feed isolates). Among human isolates, the resistance percentages were relatively high for ciprofloxacin and nalidixic acid (both 20.7% In 2020 compared to 22% in 2019) and to a lesser extent for ampicillin (12.1% in 2020 compared to 13.9% in 2019), tetracycline (7.8% in 2020 compared to 8.3% in 2019) and sulfamethoxazole (6.0% in 2020 compared to 5.4% in 2019). For all other antimicrobials, resistance proportions of human *S.* Enteritidis isolates were very low or not detected. The resistance to ciprofloxacin continued to decrease after two years of increase (2016 and 2017) (Fig. S02). The increasing trend of ampicillin and tetracycline of the last years was reversed in 2020, while resistance to sulfamethoxazole continued to increase. The most important resistance in the isolates from poultry were, alike the human isolates, against ciprofloxacin and nalidixic acid (both 12.5% in 2020 compared to 18% in 2019) (Table S05). Lower resistance percentages were measured for ampicillin, tetracycline, and trimethoprim. In contrast to 2019, resistance to sulfamethoxazole was also observed among non-human isolates from cattle and layers.

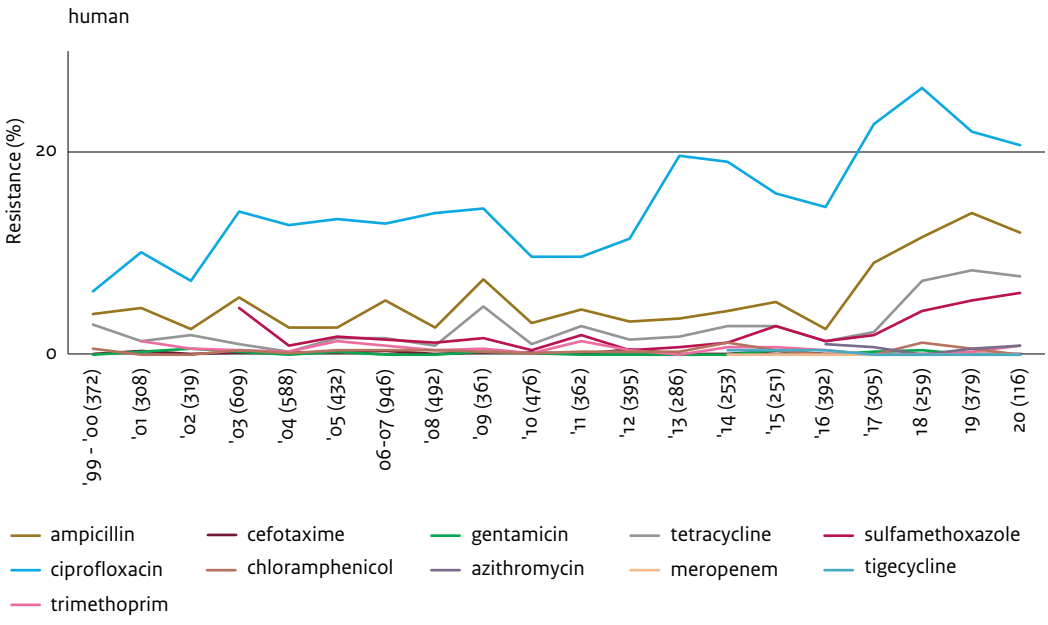
Table S05 Resistance percentages of *S.* Enteritidis (N tested) isolated from humans, poultry and other sources in 2020

	S. Enteritidis (161)		
	Humans (116)	Poultry (24)[a]	Other sources (21)[b]
Ampicillin	12.1	0.0	9.5
Cefotaxime	0.0	0.0	0.0
Ceftazidime	0.0	0.0	0.0
Gentamicin	0.0	0.0	0.0
Tetracycline	7.8	0.0	9.5
Sulfamethoxazole	6.0	4.2	4.8
Trimethoprim	0.9	0.0	4.8
Ciprofloxacin	20.7	12.5	9.5
Nalidixic acid	20.7	12.5	9.5
Chloramphenicol	0.0	0.0	4.8
Azithromycin	0.9	0.0	0.0
Meropenem	0.0	0.0	0.0
Tigecycline	0.0	0.0	0.0

a Includes broilers and layers

b Other sources include cattle (18), pigeons (1), grains/beans, spices or herbs (1), and feed (1).

Fig S02 Trends in resistance (%) of *S.* Enteritidis isolated from humans from 1999 - 2020



Salmonella from chicken meat, other meat sources and spices

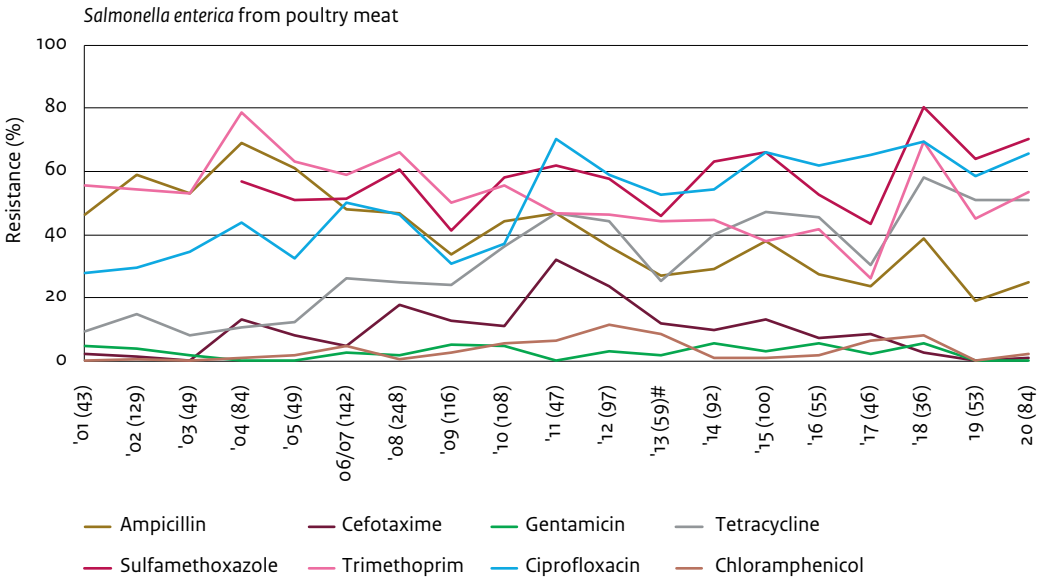
Table S06 shows resistance data of *Salmonella* isolates from chicken meat, other meat, and other products. S. Infantis (74%) was the most prevalent serovar found in chicken meat in 2020 followed by S. Paratyphi B var. Java (14%). Resistance proportions for the quinolones (ciprofloxacin and nalidixic acid) were high (resp. 65.6% and 66.7%) among isolates from chicken meat, and increased relative to 2019 (both 58.5%).
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 (Fig. S03). After an increase in resistance proportions in 2018 for ampicillin, tetracycline, trimethoprim, and sulfamethoxazole, these have decreased in 2019 but increased again in 2020. 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 a 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 2020

	Chicken meat[a]	Other meat[b]	Other products[c]
	N = 84	N = 16	N = 14
Ampicillin	25.0	18.8	7.1
Cefotaxime	1.2	0.0	0.0
Ceftazidime	0.0	0.0	0.0
Gentamicin	0.0	0.0	0.0
Tetracycline	51.2	31.3	7.1
Sulfamethoxazole	70.2	37.5	7.1
Trimethoprim	53.6	18.8	7.1
Ciprofloxacin	65.5	12.5	14.3
Nalidixic acid	66.7	12.5	14.3
Chloramphenicol	2.4	0.0	7.1
Azithromycin	2.4	0.0	0.0
Meropenem	0.0	0.0	0.0
Tigecycline	9.5	0.0	0.0

a Fresh chicken meat sampled at retail and chicken neck skin from verification projects
b Other meat includes pork (n = 5), beef (n = 4), sheep (n = 2), ostrich (1), and meat of unknown origin (n = 4).
c Other products includes seafood (n = 4), sheep (2) and grains/beans, spices or herbs (n = 8).

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 humans. For 2019 and 2020, data on human isolates were obtained from ISIS-AR (see chapter 4), whereas these data were previously obtained from a different laboratory surveillance system (with partly overlapping laboratories). Comparability of resistance proportions between these surveillance systems were assessed in 2019 which revealed negligible differences. As a result of prioritization and changes in legislation, from 2014 onwards the surveillance of antimicrobial resistance in *Campylobacter* focusses mainly on broiler chickens (and poultry meat). In 2020, caecal samples of laying hens were included in the monitoring for the first time in five years. Due to a new legislation the mandatory monitoring of antimicrobial resistance in *Campylobacter* will be extended to *C. coli* from broilers and slaughter pigs, *C. jejuni* and *C. coli* obtained from veal calves (< 1 year) from 2021 onwards and will be reported in 2022.

Table C01 presents the MIC distributions and resistance percentages for all *Campylobacter jejuni* and *C. coli* strains isolated in 2020 from caecal samples of broilers. Resistance percentages of *C. jejuni* and *C. coli* isolated from broilers, laying hens and poultry meat are presented in Table C02. Trends in resistance of *C. jejuni* and *C. coli* from broilers and poultry meat products are presented in Figures C01 and C02. National surveillance data for *Campylobacter* spp. isolated from humans are shown in Figure C03 (from 2002 onwards) and in Table C03 (from 2009 onwards).

Table Co1 MIC distribution (in %) for *Campylobacter jejuni* (N = 167) and C. coli (N = 60) isolated from caecal samples of broilers in 2020

***C. jejuni*, broilers**	**MIC (%) distribution mg/L**												**R%**	**95% CI**
(N = 167)	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**		
Ciprofloxacin	28.7	2.4				1.8	28.1	21.0	18.0				68.9	61.3 - 75.8
Nalidixic acid				0.6	10.2	18.0	4.2			3.6	63.5		67.1	59.4 - 74.1
Erythromycin				74.3	23.4	2.4							0.0	0.0 - 2.2
Gentamicin	55.1	37.1	7.8	0.0		0.0	0.0	0.0					0.0	0.0 - 2.2
Streptomycin		14.4	32.3	28.1	0.6		0.6		24.0				24.6	18.2 - 31.8
Tetracycline			42.5	1.2	1.2			1.8	1.2	8.4	43.7		56.3	48.4 - 63.9
***C. coli*, broilers**	**MIC (%) distribution mg/L**												**R%**	**95% CI**
(N = 60)	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**		
Ciprofloxacin	5.0	3.3			6.7	36.7	26.7	20.0	1.7				91.7	81.6 - 97.2
Nalidixic acid						5.0	3.3			5.0	86.7		91.7	81.6 - 97.2
Erythromycin				85.0	10.0	3.3						1.7	1.7	0.4 - 8.9
Gentamicin	15.0	61.7	23.3	0.0		0.0	0.0	0.0					0.0	0.0 - 6.0
Streptomycin			21.7	56.7	11.7				10.0				10.0	3.8 - 20.5
Tetracycline			33.3	5.0	0.0					13.3	48.3		61.7	48.2 - 73.9

Highlights

1. In 2020, resistance proportions in *C. jejuni* isolates from caecal samples of broilers and meat thereof stabilized at a high level for quinolones and tetracycline.
2. In laying hens, resistance proportions were much lower than in broilers, especially for *C. jejuni*.
3. Resistance to macrolides was not detected in *C. jejuni* isolates from broilers and poultry meat, and was at low levels in *C. coli* isolates from broilers and poultry meat.
4. In humans, resistance proportions were higher in *C. coli* than in *C. jejuni* isolates, but were overall lower in 2020 compared to previous years. This is most likely due to a substantial reduction of travel-related campylobacteriosis as a result of the COVID-19 lockdown, which is associated with higher resistance proportions than domestically acquired campylobacteriosis.
5. Ciprofloxacin resistance in *Campylobacter* isolates from humans was high again in 2020, which is a concern for public health. It was, however, lower compared to 2017-2019.
6. 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.

As in previous years, resistance proportions were higher in *C. coli* than in *C. jejuni* isolates (Table C01 and C02), except for streptomycin. Resistance against gentamicin was not detected in broilers, but infrequently found in *C. coli* isolates from laying hens (Table C02). Resistance to erythromycin was completely absent in *C. jejuni* from broilers and laying hens.

Again, 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 poultry meat and broilers over the last 17 and 21 years, respectively. In general, this figure demonstrates a relatively high similarity in resistance trends between *C. jejuni* obtained from caecal samples at slaughter and those obtained from retail meat. The resistance levels for erythromycin and gentamicin were very low to zero over the last 10 years. A modest increase in streptomycin resistance was observed in 2018 and 2019, followed by a sharp increase in 2020 with 24.6% streptomycin resistance in broilers and 26.3% in poultry meat. Resistance to erythromycin was not detected in isolates from broilers, and poultry meat. Resistance to tetracycline remained high in 2020 in both broilers and poultry meat (56.3% in broilers and 61.3% in poultry meat). Resistance percentages for ciprofloxacin has been high with some fluctuation over the years, stabilizing at a high level in 2020 (68.9% in broilers, 76.3% in poultry meat).

The resistance levels in *C. coli* isolates from broilers and poultry meat are presented in Figure C02. These levels show more fluctuation over years than levels of *C. jejuni*, which is most likely caused by the lower number of isolates in the survey. Resistance in *C. coli* from broilers and poultry meat could not be detected

for gentamicin, which was also seen in the years before. Resistance levels for erythromycin and streptomycin in *C. coli* has been fluctuating substantially over the years. In 2020, resistance level for erythromycin in *C. coli* obtained from broilers (1.7%) was as low as in 2019, but clearly higher in broiler meat (9.4%).
For streptomycin, resistance proportions were higher than in 2019 (10.0% and 15.6% in broilers and broiler meat, respectively). Resistance percentages for ciprofloxacin in broilers and poultry meat have been fluctuating at a high level since 2001, and were still high in 2020. 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 were approximately the same as ciprofloxacin resistance, with a similar trend. However, since 2018 there ciprofloxacin seems to increase and the opposite is observed for tetracycline resistance. resulting in higher differences in resistance levels between these two antimicrobials over time.

Fluoroquinolones

The continuously high proportion of *Campylobacter* spp. isolates from animal origin resistant to fluoroquinolones (Figures C01 and C02) and especially from human patients (Figure C03) is a serious public health concern. The proportion of *C. jejuni* isolates from broilers resistant to quinolones remained at a high level over the last 10 years, and was with 68.9% in 2020 not really different from 2019 (69.7%). The proportion of fluoroquinolone resistance in *C. jejuni* from poultry meat reached the highest level since the beginning of the monitoring with 76.3% in 2020.
In 2020, the *C. coli* isolates from broilers showed an increase of levels of ciprofloxacin resistance to over 90.0% (91.7%), which is the highest ever reported in MARAN since 2001. 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 2020 were similar to 2019 for both ciprofloxacin and nalidixic acid (both at 81.3%).

Macrolides

Erythromycin, or other macrolides (e.g. clarithromycin), are the first-choice drugs for the treatment of campylobacteriosis in humans. As in former years, resistance proportions to macrolides in isolates from animals and humans were low. Table C02 shows that no resistance was detected in *C. jejuni* from caecal samples of broilers, laying hens and from poultry meat. Table C03 shows that 2.3% of human *C. jejuni* isolates was resistant for erythromycin in the period 2016-2020. 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, erythromycin resistance was rarely detected in broilers (1.7%) and laying hens (2.8%), but more often in broiler meat (9.4%) (table C02).

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

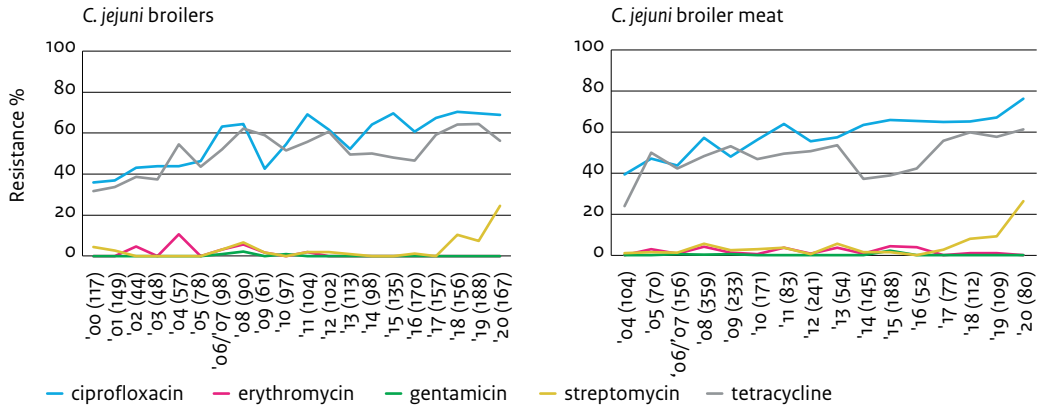
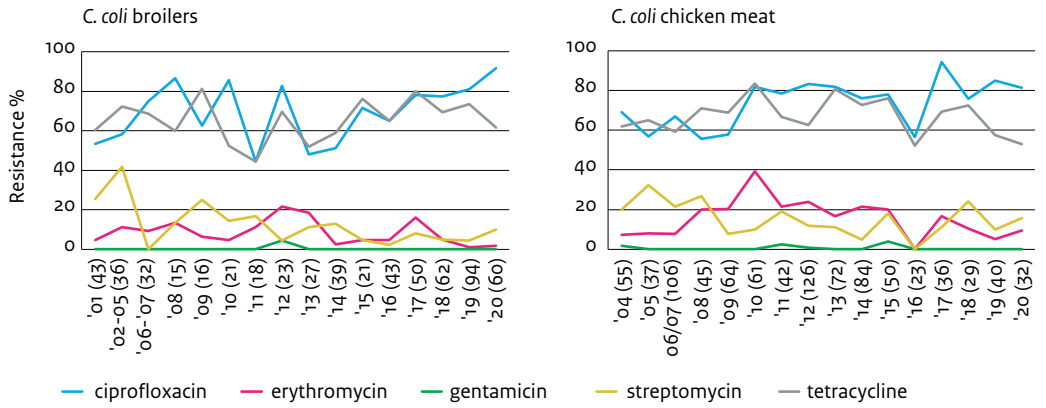


Figure Co2 Trends in resistance of *Campylobacter coli* isolated from broilers and chicken meat in the Netherlands



Broiler chickens, laying hens and poultry meat

In *Campylobacter* from poultry, resistance profiles were determined for isolates recovered from broilers, laying hens as well as from chicken meat samples.

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 isolates from caecal samples of broilers. Resistance levels for *C. coli* isolates from broilers and poultry meat for quinolones were even higher. No resistance to gentamicin was detected in both *C. jejuni* and *C. coli* isolates from broilers and broiler meat. Resistance to erythromycin was absent in *C. jejuni* isolates and rarely found in *C. coli* from broilers (1.7%), but more often in broiler meat (9.4%). A sharp increase in streptomycin resistance was observed in *C. jejuni* with 24.6% resistant isolates in broilers and 26.3% in poultry meat. This increasing trend was less clear in *C. coli* isolates.

Resistance levels in *C. jejuni* isolates obtained from laying hens were lower compared to broilers and broiler meat for quinolones, streptomycin and tetracycline.
Higher resistance proportions 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. Overall, resistance proportions of *C. jejuni* from laying hens were similar to the levels measured in 2016. For *C. coli* resistance substantially increased for ciprofloxacin (56.5% in 2016 and 81.5% in 2020), but this was not observed for the other antimicrobials in the panel.

Table Co2 Resistance percentages of *C. jejuni* and *C. coli* isolated from faecal samples of broilers, layers and from poultry meat in 2020

	C. jejuni			*C. coli*		
	Broilers	Layers	Poultry meat	Broilers	Layers	Poultry meat
N =	167	78	80	60	107	32
Ciprofloxacin	68.9	35.9	76.3	91.7	84.1	81.3
Nalidixic acid	67.1	29.5	75.0	91.7	84.1	81.3
Erythromycin	0.0	0.0	0.0	1.7	2.8	9.4
Gentamicin	0.0	0.0	0.0	0.0	0.9	0.0
Streptomycin	24.6	2.6	26.3	10.0	5.6	15.6
Tetracycline	56.3	17.9	61.3	61.7	52.3	53.1

Campylobacter in humans

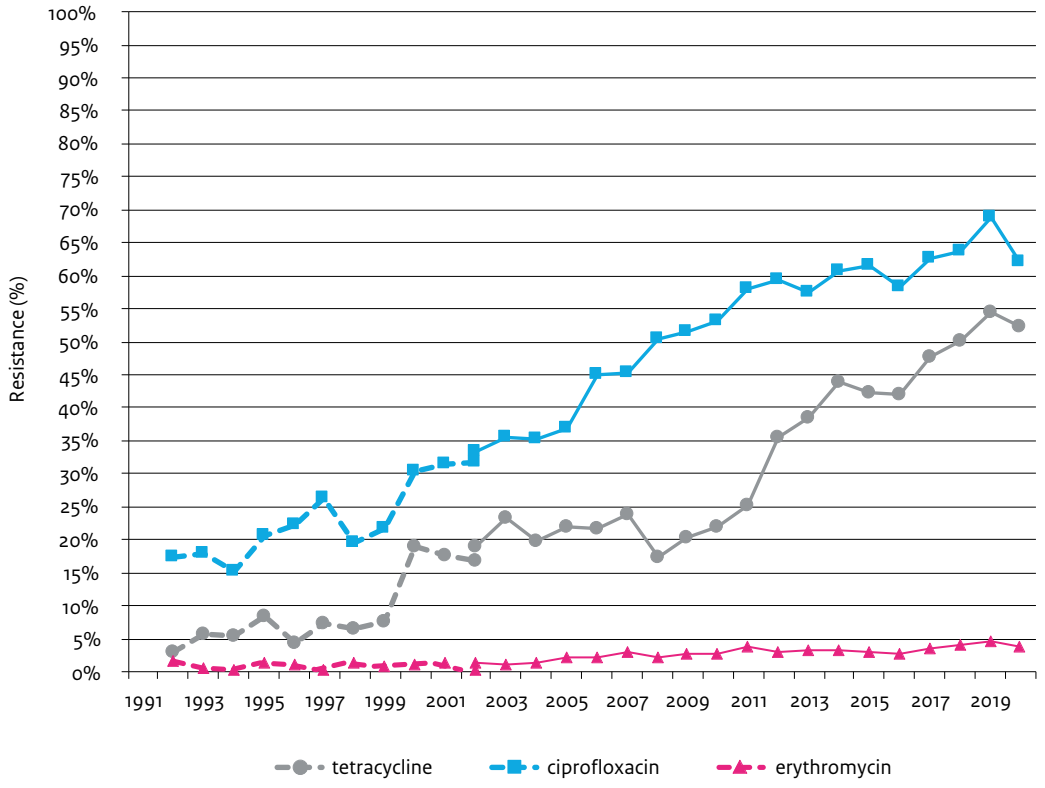
Resistance levels in isolates from human patients were determined for ciprofloxacin, tetracycline and erythromycin, and are shown in Table Co3 and Figure Co3. Figure Co3 shows a continuously increasing trend of ciprofloxacin and tetracycline resistance. In 2020, however, resistance levels for all measured antibiotics dropped. This is most likely due to a substantial reduction in travel-related campylobacteriosis as a result of the COVID-19 lockdown (data on travel history not available), which is associated with higher resistance levels than domestically acquired campylobacteriosis.
In 2020, the resistance levels for ciprofloxacin in human campylobacter isolates were still high with 62.0%, which is a public health concern. As shown in Figure Co3, however, ciprofloxacin resistance was lower than 2017-2019 (range 62.6%-68.9%). Tetracycline resistance also decreased to 52.3% in 2020 compared to 2019 (54.4%), but was still higher than before 2019. Erythromycin resistance was 3.8% in 2020, which is lower than previous years. As said, these reductions are likely the result of the COVID-19 lockdown.
Table Co3 shows the average resistance levels for human *Campylobacter* spp. isolates for the periods 2011-2015 and 2016-2020, and the resistance level per year since 2015. Because since 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 2015, and a reduction in resistance levels in 2020. Resistance proportion were higher for *C. coli* isolates than *C. jejuni* isolates.

Table C03 Resistance in *C. jejuni* and *C. coli* isolated from humans from 2011 - 2020

	2016-2020				2011-2015			
	C. jejuni		*C. coli*		*C. jejuni*		*C. coli*	
	N	R%	N	R%	N	R%	N	R%
Fluoroquinolone	6,754	61.6	673	71.7	8,944	58.5	667	61.5
Tetracycline	4,629	47.4	572	69.4	3,043	35.0	329	51.5
Erythromycin	225	2.3	179	19.4	384	2.5	181	17.0

	Campylobacter spp. (R%)					
	2020	2019	2018	2017	2016	2015
Fluoroquinolone	62.0	68.9	63.6	62.6	58.3	61.4
Tetracycline	52.3	54.4	50.2	47.6	42.0	42.3
Erythromycin	3.8	4.7	4.0	3.5	2.6	2.9

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) and atypical enteropathogenic *E. coli* (aEPEC)

Highlights

1. In STEC O157 a tendency of increasing resistance was observed until 2017 and fluctuates on a lower level since 2018.
2. Resistance to the quinolones (ciprofloxacin and nalidixic acid) was very low in both STEC O157 and STEC/aEPEC non-O157 human isolates in 2020.
3. Proportions of resistance were higher in human STEC/aEPEC non-O157 than in STEC O157.
4. No ESBL-producing isolates were detected in STEC O157, but one O104 isolate was confirmed as ESBL-producer carrying $bla_{CTX\text{-}M\text{-}15}$.
5. Almost all STEC O146 isolates - which are primarily associated with small ruminants as reservoir - were pan-susceptible.

Human STEC and aEPEC[1] isolates

[1] aEPEC = atypical enteropathogenic *E. coli*, which share the LEE-pathogenicity island with STEC but lack stx-genes a well as the EPEC adherence factor plasmid.

Shiga-toxin producing *E. coli* (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 small ruminants. 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.
In contrast to earlier years, in 2020 not only STEC O157 but a larger collection of pathogenic *E. coli* isolates from human clinical cases (N = 251) consisting of multiple STEC/aEPEC non-O157 serotypes were tested for susceptibility. The set consisted of 55 STEC O157 isolates and 196 STEC/aEPEC non-O157 isolates: O26 (n=30), O146 (n=25), O91 (n=13), O103 (n=12), O63 (n=11) and others (N=105). All isolates were obtained from regional public health laboratories within the RIVM national laboratory surveillance of STEC. Table STEC01 shows the MIC results for *E. coli* O157 isolates from humans; Table STEC02 shows resistance proportions of *E. coli* O157 and STEC/aEPEC non-O157 isolates; Figure STEC01 presents the trends over time for STEC O157.

After a tendency of increasing resistance proportions among STEC O157 until 2017, this fluctuated on lower levels in the period 2018 – 2020 (Figure STEC01). In 2020 decrease in resistance was observed for ampicillin, chloramphenicol, sulfamethoxazole, trimethoprim and tetracycline. After one year with no resistance to quinolones low level resistance was observed for ciprofloxacin and nalidixic acid (both 1.8%). No ESBL-producing isolates were detected in 2020 among STEC O157.

Figure STEC01 Trends in resistance (in %) of *E. coli* STEC O157 isolated from humans in the Netherlands from 1999 - 2020

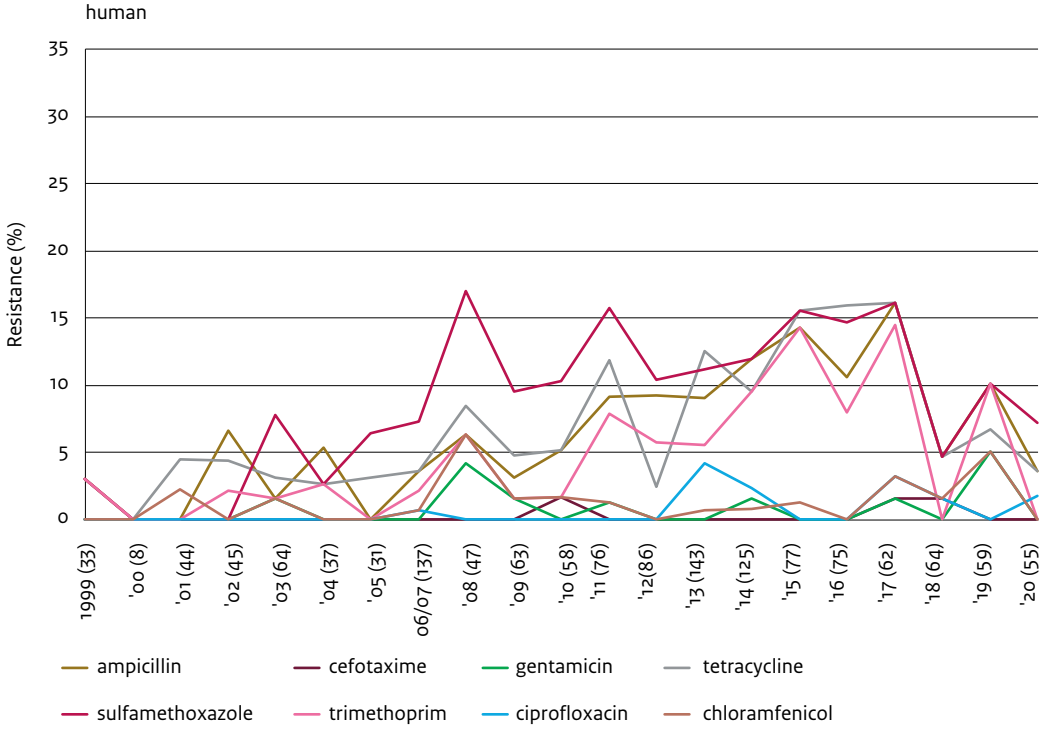


Table STEC01 MIC distribution (in %) and resistance percentages (R%) for *E. coli* STEC O157 (N=55) isolated from humans the Netherlands in 2020

E. coli N = 55"	MIC (%) distribution mg/L																		R%	95% CI
	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048		
Ampicillin								43.6	52.7					3.6					3.6	0.4 - 12.5
Cefotaxime					100.0														0.0	0.0 - 6.5
Ceftazidime						100.0													0.0	0.0 - 6.5
Gentamicin						96.4	3.6												0.0	0.0 - 6.5
Tetracycline								94.5	1.8					3.6					3.6	0.4 - 12.5
Sulfamethoxazole										92.7								7.3	7.3	2.0 - 17.6
Trimethoprim					100.0														0.0	0.0 - 6.5
Ciprofloxacin	94.5	3.6				1.8													1.8	0.1 - 9.7
Nalidixic acid									98.2		1.8								1.8	0.1 - 9.7
Chloramphenicol										96.4	3.6								0.0	0.0 - 6.5
Azithromycin								34.5	65.5										0.0	0.0 - 6.5
Colistine							100.0												0.0	0.0 - 6.5
Meropenem		100.0																	0.0	0.0 - 6.5
Tigecycline					98.2	1.8													0.0	0.0 - 6.5

The white areas indicate the dilution range tested for each antimicrobial agent. Values above this range indicate MIC values > the highest concentration in the range. Values at the lowest concentration tested indicate MIC-values ≤ the lowest concentration in the range. Vertical bars indicate the epidemiological cut-off values, used as breakpoints. Dashed bars indicate the clinical breakpoints.

Table STEC02 shows differences in proportion of resistance between STEC O157 and STEC/aEPEC non-O157 isolates with higher levels of resistance in non-O157 isolates for ampicilline, sulfamethoxazole, ciprofloxacin and nalidixic acid. Moreover, resistance to 3rd generation cephalosporins (cefotaxime and ceftazidime), gentamicin, trimethoprim, chloramphenicol and azithromycin were only detected in the non-O157 isolates tested with relatively low resistance proportions varying from 0.5 to 11.7%. Resistance against azithromycin was detected in one O26 isolate. Most other types of resistance could not be clearly linked to specific serotypes, although multidrug resistance was more frequently observed amongst O26 and O103 isolates. Almost all STEC O146 isolates tested (n=25) - associated with human infections linked to consumption of raw milk products from small ruminants - were pan-susceptible with only one isolate exhibiting resistance against sulfamethoxazole and tetracycline. The ESBL-suspected isolate belonged to serotype O104 and molecular typing confirmed the presence of an ESBL-gene ($bla_{CTX\text{-}M\text{-}15}$).
The higher resistance proportions within the non-O157 group and the detection of resistance against critically important antimicrobials indicates the additional value of monitoring resistance of a larger subset of pathogenic *E. coli*.

Table STEC02 Resistance percentages (R%) of pathogenic *E. coli* in the Netherlands in 2020

E. coli	O157	Other serotypes
	N = 55	N = 196
Ampicillin	3.6	8.2
Cefotaxime	0.0	0.5
Ceftazidime	0.0	0.5
Gentamicin	0.0	1.0
Tetracycline	3.6	16.3
Sulfamethoxazole	7.3	18.9
Trimethoprim	0.0	11.7
Ciprofloxacin	1.8	3.6
Nalidixic acid	1.8	2.6
Chloramphenicol	0.0	5.1
Azithromycin	0.0	0.5
Colistin	0.0	0.0
Meropenem	0.0	0.0
Tigecycline	0.0	0.0

Reference

L. Mughini-Gras, W. van Pelt, M. van der Voort, M. Heck, I. Friesema E. Franz, Attribution of human infections with Shiga toxin-producing *Escherichia coli* (STEC) to livestock sources and identification of source-specific risk factors, The Netherlands (2010–2014), Zoonosis and Public Health, Volume65, Issue1, February 2018 https://doi.org/10.1111/zph.12403

3.2 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[1] 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 2020

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. In slaughter pigs, resistance in indicator *E. coli* from caecal samples decreased to the lowest levels since 2004. In contrast, in broilers and veal calves a tendency of increasing resistance was observed. In dairy cattle resistance fluctuates at a traditional low level. However, over the last decade decreasing trends in resistance were observed for all animal sectors involved in the monitoring.
3. Levels of resistance in indicator *E. coli* increased in laying hens since 2016, but is considerably lower than in broilers reflecting the difference in antibiotic use between these sectors.
4. Resistance proportions in *E. coli* from turkey meat were substantially higher than in chicken meat and resistance proportions in *E. coli* from pig and bovine meat are lower than in broiler meat.
5. Resistance to fluoroquinolones was still commonly present in indicator *E. coli* from caecal samples of broilers and meat thereof.
6. For almost all antibiotics tested, levels of resistance in *E. coli* from caecal samples of rosé veal calves were substantially lower than those from white veal calves and differences in resistance between the two sector increased in 2020.

Resistance levels

Table Ec001 shows resistance levels, presented as MIC-distributions, of 1303 *E. coli* isolates obtained from caecal samples from broilers, layers, pigs, veal calves and faecal samples of dairy cows. Table Ec002 presents resistance percentages per animal species. Trends in resistance levels from 1998 to 2020 are shown in Figure Ec001 and information on trends in multidrug resistance is shown in Figure Ec002.

Table Ec003 presents resistance percentages of 530 *E. coli* isolates collected from raw chicken meat, turkey meat, beef, pork and vegetables. Figure Ec003 shows trends in resistance of *E. coli* in the Netherlands from 2002 to 2020 isolated from raw meat of chicken, turkey, bovine and pig.

For most drugs or drug classes, resistance levels varied substantially between the different animal species (Table Ec002). 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. No resistance was detected for azithromycin, colistin, meropenem and tigecycline.

Table Eco01 MIC distribution (in %) and resistance percentages (R%) for all *E. coli* (N=1303) isolated as indicator organism from intestines of food producing animals in the Netherlands in 2020

"E. coli N = 1303"	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							3.3	25.0	43.4	6.1	0.2	0.1	0.1	21.8					22.1	19.9 - 24.5
Cefotaxime					99.8	0.1		0.1		0.1									0.2	0.1 - 0.7
Ceftazidime						99.9		0.1											0.1	0.0 - 0.4
Gentamicin						66.5	26.0	5.8	0.2	0.2	0.5	0.3	0.6						1.8	1.1 - 2.6
Tetracycline								59.4	13.7	0.6	0.2	0.6	9.3	16.2					26.2	23.9 - 28.7
Sulfamethoxazole										75.8	0.2			0.1				23.9	23.9	21.7 - 26.4
Trimethoprim					40.7	38.1	2.7	0.2	0.1			0.4	18.0						18.4	16.4 - 20.6
Ciprofloxacin	79.6	9.8	0.5	1.2	5.6	1.7	0.4	0.1	0.4	0.4	0.4								10.1	8.5 - 11.8
Nalidixic acid									89.1	1.5	0.9	0.2	1.0	3.5	3.8				9.4	7.8 - 11.1
Chloramphenicol										87.0	6.0	1.4	1.0	1.9	2.8				7.1	5.7 - 8.6
Azithromycin*								3.9	33.8	56.6	5.4	0.1		0.2					0.3	0.1 - 0.8
Colistin							96.2	3.8											0.0	0.0 - 0.0
Meropenem		99.5	0.5																0.0	0.0 - 0.0
Tigecycline					93.7	6.3													0.0	0.0 - 0.0

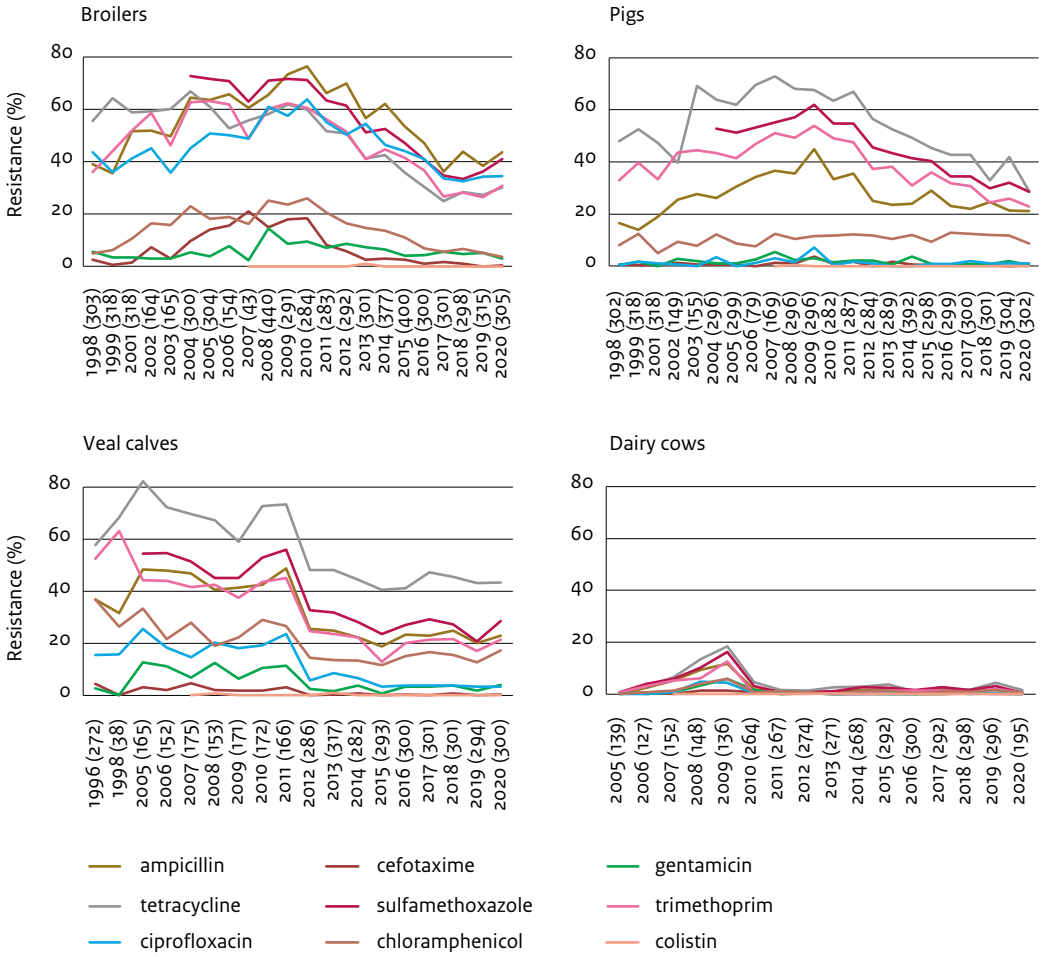
The white areas indicate the dilution range tested for each antimicrobial agent. Values above this range indicate MIC values > the highest concentration in the range. Values at the lowest concentration tested indicate MIC-values ≤ the lowest concentration in the range. Vertical bars indicate the epidemiological cut-off values, used as breakpoints. Dashed bars indicate the clinical breakpoints.

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

Fluoroquinolone resistance

Highest resistance levels for fluoroquinolones were found in *E. coli* from broilers with 34.4% resistance to ciprofloxacin and 32.1% resistance to nalidixic acid (Table Eco02). This level of resistance has stabilised in broilers for the past three years after a decreasing trend from 2013 until 2017 (Figure Eco01). In samples from other animal sectors resistance was low or completely absent: 6.5% in layers, 4.8% in white veal calves, 1.0% in pigs, and undetected in isolates from rosé veal calves and dairy cattle.

Figure Eco01 Trends in proportion of resistance (%) of *E. coli* isolated from broilers, slaughter pigs, veal calves and dairy cattle in the Netherlands from 1998 - 2020



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 2020. Figure Eco03 shows that resistance in

chicken products at retail reduced compared to 2019: the percentage of *E. coli* with resistance to ciprofloxacin and nalidixic acid decreased from 25.7% to 19.3% and from 24.0% to 16.6%, respectively. Resistance percentages in isolates from turkey products also decreased in 2020, but these figures should be interpreted carefully because of the low number of samples. Resistance percentages in isolates from beef, pigs and vegetables were much lower compared to poultry: with respectively 1.6%, 0.0% and 2.2% of the isolates showing resistance to ciprofloxacin.

Resistance against extended-spectrum cephalosporins (cefotaxime and ceftazidime)

After a year of complete absence of *E. coli* being resistant to extended-spectrum cephalosporins (ESC-resistant *E. coli*) amongst randomly isolated commensal indicator *E. coli,* three ESC resistant *E. coli* were detected in 2020. These isolates were obtained in caecal samples of a broiler, a white veal calf and a slaughter pig, respectively. This finding indicates that ESC-R *E. coli* (suspected of ESBL/AmpC production) are still present at low concentrations around the detection limit in different animal species (Figure Eco01).

Notably, the prevalence of broilers carrying ESC-resistant *E. coli* further decreased from 50.3% in 2016, to 32.6% in 2017, 23.0% in 2018, 17.9% in 2019 and 10.2% in 2020 (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 gastro intestinal (GI) tract was similar to 2019 with 40.0%. In rosé veal calves, the percentage positive animals varies over the years and increased from 14.0% in 2019 to 20.0% in 2020. The prevalence in 2020 of pigs positive for ESC resistant *E. coli* was with 16.5% higher than in previous years, but it is important to mention that especially in pigs non-transferable mechanisms like chromosomal promotor mutations are frequently the cause of resistance to ESC. In dairy cattle, the prevalence of animals with ESC-resistant *E. coli* was very similar to former years.

In chicken meat samples, none of the randomly isolated indicator *E. coli* showed resistance to ESC which reflects the ongoing decrease of ESBL/ampC-producing *E. coli* in broilers and on chicken meat. Only one indicator *E. coli* obtained from pork was found resistant to both cefotaxime and ceftazidime. No cefotaxime resistance was detected in indicator *E. coli* isolates from turkey, beef and vegetables.

The small proportion of ESC 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 9.0% in 2020 (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

Proportions of resistance in commensal *E. coli* isolated from caecal samples of broiler chickens increased for four antimicrobial classes (Figure Eco01) and remained high for ampicillin (43.6%), tetracycline (30.2%), trimethoprim (30.8%), sulfamethoxazole (41.0%) and ciprofloxacin (34.4%) (Table Eco02). Resistance to chloramphenicol and gentamicin further decreased to levels below 5%. One *E. coli* isolate was found borderline resistant to cefotaxime (MIC: 0.5 mg/L) without detection of any resistance mechanism.

Layers

Compared to the data of 2016, levels of resistance in layers considerably increased for ampicillin, tetracycline, sulfamethoxazole, trimethoprim and ciprofloxacin. In most cases resistance percentages were more than doubled (see table Eco02 in MARAN 2017).
Still, levels of resistance in layers are substantially lower than in broilers for all antimicrobial classes. This accounts for ampicillin (layer/broiler: 10.5%/43.6%), tetracycline (15.0%/30.2%), sulfamethoxazole (7.0%/41.0%), trimethoprim (6.0%/30.8%) and ciprofloxacin (6.5%/34.4%). This large difference in resistance rates reflects the lower use of antimicrobials in laying hens compared to broiler chicken.

Slaughter pigs

Overall resistance proportion decreased in slaughter pigs (Figure Eco01). This was most clearly observed for tetracycline which demonstrated a steep drop after a peak in 2019. But also for trimethoprim, sulfamethoxazole and chloramphenicol, proportions of resistance further decreased to the lowest resistance levels measured since 2004. For ampicillin, resistance stabilised at around 21% and remained low for ciprofloxacin and gentamicin. 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 slaughterhouse. This has influenced the results from 2012 onwards, because most antibiotic usage is in the younger calves and less in the period before slaughter.
The ratio of sampled white veal calves versus rosé veal calves changed from 50/50% to 60/40% in 2016, and to 70/30% in 2017 onwards, which better reflects the proportions of slaughtered white and rosé calves in The Netherlands. 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. However, in 2020 resistance rates went up specifically in white veal calves which is reflected by an increase in the overall resistance (Figure Eco01) and in larger differences between the two husbandry types (Table Eco02).
In 2020, highest resistance levels in veal calves were observed for tetracycline (57.1% and 11.1% in white and rosé respectively), sulfamethoxazole (37.1% and 8.9%), trimethoprim (28.1% and 5.6%) and chloramphenicol (22.4% and 5.6%). *E. coli* isolates resistant to the 3rd generation cephalosporins were not detected in randomly selected indicator *E. coli* from caecal samples of white and rosé veal calves (TableEco02).

Table Eco02 Resistance percentages (R%) of *E. coli* isolated from faecal samples of broilers, layers, pigs, dairy cows, white veal calves and rosé veal calves in the Netherlands in 2020

Faecal samples	Broilers	Layers	Pigs	Dairy	Veal calves	
	N = 305	N = 200	N = 302	N = 196	White, N = 210	Rosé, N = 90
Ampicillin	43.6	10.5	21.2	0.5	28.6	10.0
Cefotaxime	0.3	0.0	0.3	0.0	0.0	1.1
Ceftazidime	0.0	0.0	0.3	0.0	0.0	0.0
Gentamicin	3.0	0.5	0.3	0.0	5.2	1.1
Tetracycline	30.2	15.0	28.8	1.5	57.1	11.1
Sulfamethoxazole	41.0	7.0	28.5	0.5	37.1	8.9
Trimethoprim	30.8	6.0	22.8	0.5	28.1	5.6
Ciprofloxacin	34.4	6.5	1.0	0.0	4.8	0.0
Nalidixic acid	32.1	6.5	1.0	0.0	3.8	0.0
Chloramphenicol	3.6	1.0	8.6	0.5	22.4	5.6
Azithromycin	0.0	0.0	0.0	0.0	1.9	0.0
Colistin	0.0	0.0	0.0	0.0	0.0	0.0
Meropenem	0.0	0.0	0.0	0.0	0.0	0.0
Tigecycline	0.0	0.0	0.0	0.0	0.0	0.0

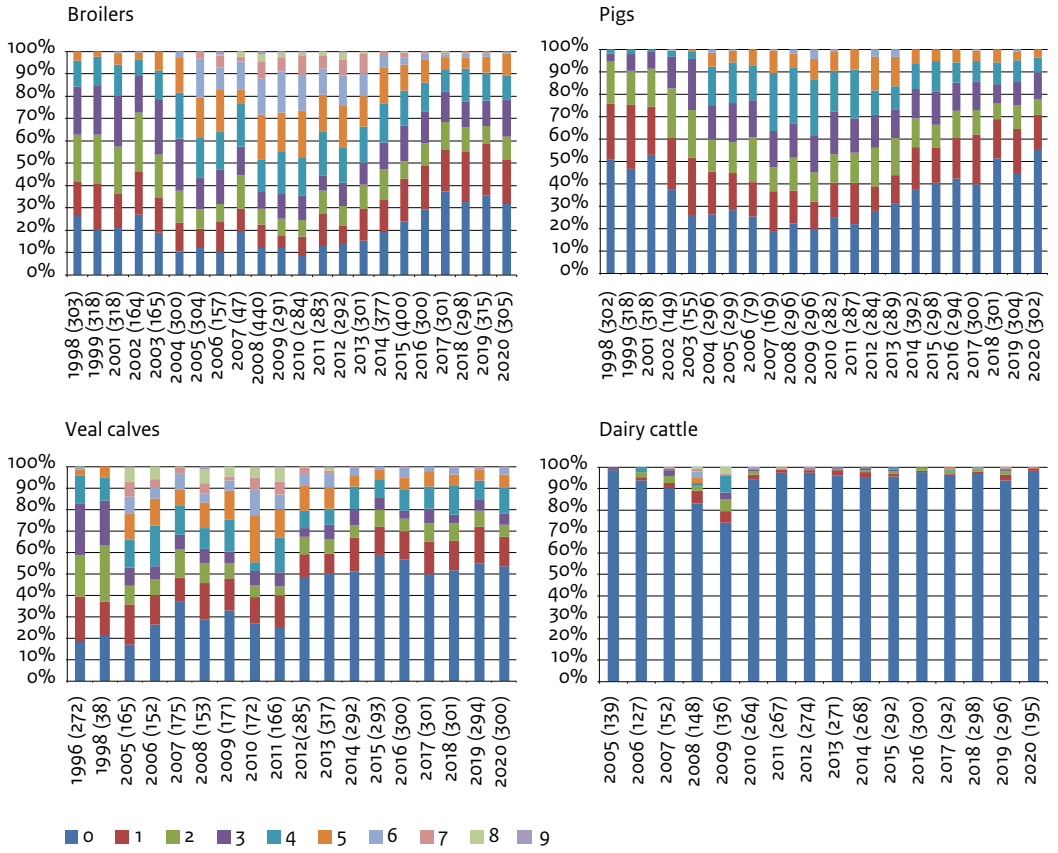
Dairy cattle

Due to COVID-19, less feacal samples were collected for AMR monitoring compared to former years. As a consequence, the number of indicator *E. coli* from dairy cattle included in the monitoring program was substantially lower with 195 isolates instead of the approximately 300 isolates in former years. Nevertheless, the number of *E. coli* isolates is sufficient to measure trends in resistance according to EFSA guidelines (minimum of 170 isolates). 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 dairy farming. After a slight increase in 2019, resistance decreased to one the lowest levels measured since the beginning of the monitoring of dairy cattle in 2005 with resistance rates below 1% for almost all antimicrobials. 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 2020



In general, the level of multidrug resistance (showing resistance to three or more classes of antimicrobials) stabilised in the last four years. In broilers, the proportion of multidrug resistance isolates was relative high with 38.0%, and increased compared to previous years (31.4% - 33.3% in 2017- 2019). The proportion of multidrug resistance stabilised in pigs was slightly lower than in previous years with 22.5% (24.1%-27.3% in 2017-2019). In veal calves the level of multidrug resistance increased to 27.3% compared to 2019 (20.7%) which is similar to the levels measured in 2018 (26.4%) and 2017 (26.7%). In dairy cattle, multidrug resistance in *E. coli* slightly decreased to 0.5% of the isolates which is an extremely 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 2019, the percentage of completely susceptible *E. coli* isolates increased for pig and dairy isolates, but decreased for broiler isolates (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 2020. All vegetables were sampled as fresh products at retail and originated from within EU.

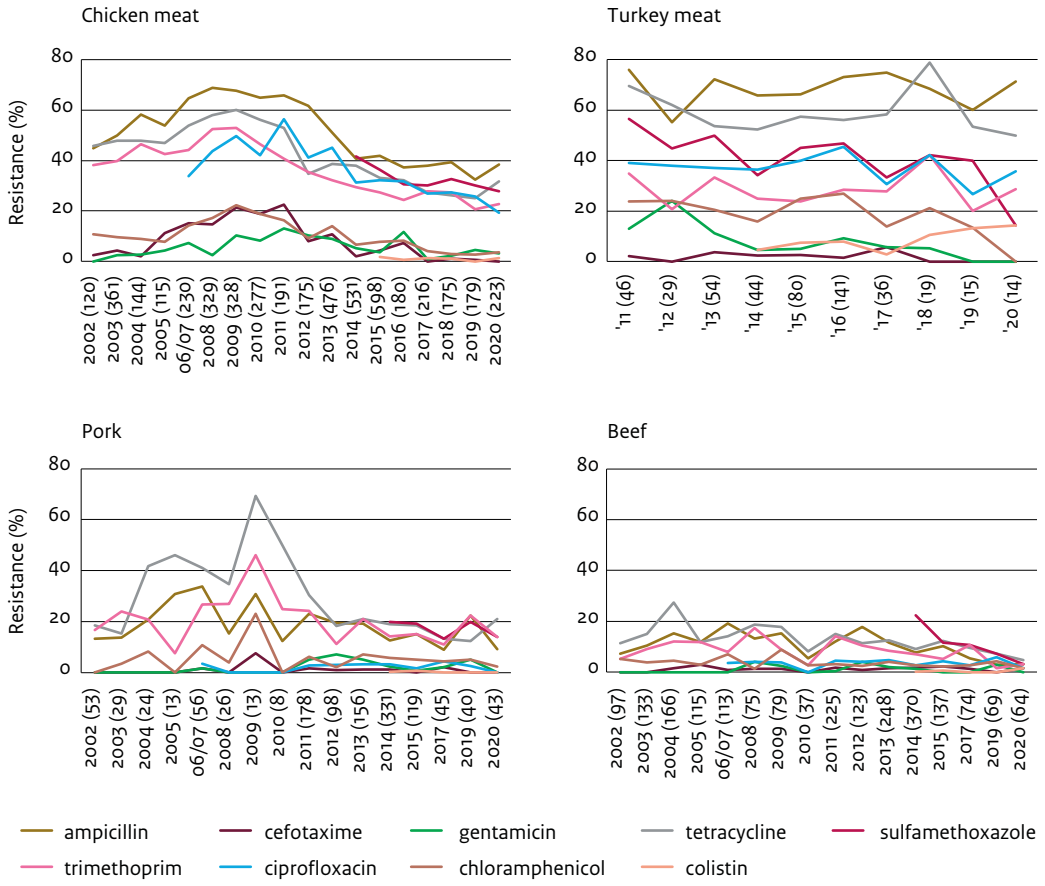
Table Eco03 Resistance percentages (R%) of *E. coli* isolated from raw chicken meat, turkey meat and vegetables at retail in the Netherlands in 2020

Products	Chicken	Turkey	Bovine	Pig	Vegetables
	N = 223	N = 14	N = 64	N = 43	N = 186
Ampicillin	38.6	71.4	3.1	9.3	3.8
Cefotaxime	0.0	0.0	1.6	0.0	0.0
Ceftazidime	0.0	0.0	1.6	2.3	0.0
Gentamicin	3.1	0.0	0.0	0.0	0.5
Tetracycline	31.8	50.0	4.7	20.9	5.4
Sulfamethoxazole	27.8	14.3	3.1	14.0	4.8
Trimethoprim	22.9	28.6	3.1	14.0	2.7
Ciprofloxacin	19.3	35.7	1.6	0.0	2.2
Nalidixic acid	16.6	21.4	1.6	0.0	1.6
Chloramphenicol	3.6	0.0	1.6	2.3	2.2
Azithromycin	1.8	0.0	0.0	0.0	0.0
Colistin	1.3	14.3	1.6	0.0	0.0
Meropenem	0.0	0.0	0.0	0.0	0.0
Tigecycline	0.9	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 seem to stabilise with some fluctuations since 2015. For the first time since 2002, cefotaxime resistance was not detected in indicator *E. coli* from broiler meat. 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 2020 and in previous years. Therefore results must be interpreted with care. Cefotaxime resistance was not detected in *E. coli* isolates from turkey meat for the third year in row. Resistance rates were traditionally low in bovine meat with percentages below 5% for all antimicrobials tested. One isolate showed was suspected of ESBL/ampC-production based on resistance against 3rd generation cephalosporins (cefotaxime and ceftazidime) which was caused by chromosomal mutation in the ampC promotor region. In pork overall resistance rates were higher than in bovine meat but lower than in poultry meat with complete absence of resistance to fluoroquinolones.

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 that was included in the survey. In vegetables, resistance levels of *E. coli* isolates were very low, similar to former years. Percentages of resistance to ampicillin, chloramphenicol, 3rd generation cephalosporins, gentamicin, quinolones, tetracycline, trimethoprim and sulfamethoxazole were all below or equal to 5%.

Figure Eco03 Trends in resistance (%) of *E. coli* isolated from raw chicken meat, turkey meat, pork and beef in the Netherlands from 1998 - 2020



Reference

[1] Report from the Task Force on Zoonoses Data Collection including guidance for harmonized monitoring and reporting of antimicrobial resistance in commensal Escherichia coli and Enterococcus spp. from food animals. http://www.efsa.europa.eu/en/efsajournal/pub/141r.htm.

4
Screening for ESBL, AmpC, carbapenemase-producing and colistin-resistant Enterobacteriaceae and MRSA in food-producing animals and meat in the Netherlands in 2020

This chapter describes the data for the screening of organisms which are resistant to critically important antimicrobials as defined by the World Health Organisation (Critically important antimicrobials for human medicine, 6th revision, 2019), for which resistance is highly prevalent in the Netherlands, or has been in the past, or for which prevalence is high or rising in countries abroad. Results include the non-selective and selective screening for ESBL/AmpC producing Enterobacteriaceae in livestock and meat, carbapenemase producing Enterobacteriaceae in livestock, companion animals and seafood, colistin resistance in *E. coli* in livestock and meat, and MRSA surveillance in livestock.

Highlights

- Low levels of ESBL/AmpC-production were detected in randomly isolated *E. coli* from pigs, poultry and veal calves in 2020, while all of these populations were negative in 2019.
- Selective isolation of ESBL/AmpC-producing *E. coli* from laying hens showed a significant reduction since 2016.
- Selective isolation of ESBL/AmpC-producing *E. coli* from broilers and chicken meat shows that prevalence has reduced below 10% in 2020.
- The prevalence of ESBL/AmpC-producing *E. coli* remains highest in both white and rosé veal calves.
- In 2020, no carbapenemase-producing Enterobacteriaceae were detected in livestock and companion animals.
- As in former years, prevalence of *mcr*-1 was low in livestock and meat.
- Prevalence of LA-MRSA was high in dust samples from pig farms (76%), but could not be detected in dust samples from broiler farms.
- At retail, MRSA was detected in < 10% of the pork and bovine meat, but in almost 20% of the poultry meat (both chicken and turkey).
- The first *cfr*-positive LA- MRSA isolates were detected in dust samples from one pig farm obtained in 2019 as well as in five human LA-MRSA isolates in 2018 – 2020.

4.1 ESBL/AmpC-producing Enterobacteriaceae

The production of ESBL/AmpC by Enterobacteriaceae results in resistance against beta-lactam antibiotics including the medically important extended-spectrum cephalosporins (ESC). Due to the high prevalence of these resistance mechanisms in livestock in the past, resistance is monitored at two levels; molecular analysis is performed for the indicator *E. coli* from livestock described in Chapter 3 and on selectively isolated *E. coli* from livestock and meat. These results provide an indication of the prevalence of ESBL/AmpC producing Enterobacteriaceae as part of the population and of the prevalence of these bacteria in individual animals and meat samples.

4.1.1 Randomly isolated ESBL/AmpC-producing *E. coli* from livestock

The prevalence of ESC resistance in the population of *E. coli* in food-producing animals is determined using non-selectively isolated *E. coli* as described in Chapter 3. The isolation is performed according to guidelines as described by EFSA (European Food Safety Authority, 2012). A minimal number of at least 170 samples per category was met in which dairy cows and veal calves are considered separate categories. Caecal samples of broilers, pigs and veal calves are collected at slaughter while faecal samples of dairy cows are collected at farms. The phenotype of the bacteria is determined by measuring the minimal inhibitory concentration and comparing these to the epidemiological cut-off values as determined by EUCAST. The bacterial isolate is ESBL-suspected when reduced susceptibility is determined for the ESC ceftazidime and/or cefotaxime. Upon confirmation of the phenotype, molecular analysis is performed to determine what mechanism is responsible. Molecular confirmation consists of targeted PCR screening followed by amplicon sequencing. Figure ESBL01 shows the trends over time for the prevalence of resistance against cefotaxime in randomly isolated *E. coli*. While ESC resistance was not detected in this population of *E. coli* in any of the livestock species in 2019, in 2020 for broilers, slaughter pigs and veal calves, a single suspected ESBL-producing *E. coli* was isolated from each livestock species. The isolate from slaughter pigs was confirmed to encode

bla$_{CTX-M-1}$ and *bla*$_{SHV-2}$ was identified in the isolate from broilers, see Table ESBL01. The minor increase is expected to be part of the natural variation and not due to significant changes in the prevalence. No ESC resistance was detected in this *E. coli* population from dairy cows for the second consecutive year, which generally have a low prevalence.

Figure ESBL01 Trends in cefotaxime resistance (%) of *E. coli* randomly isolated from faeces of broilers, slaughter pigs, veal calves and dairy cows

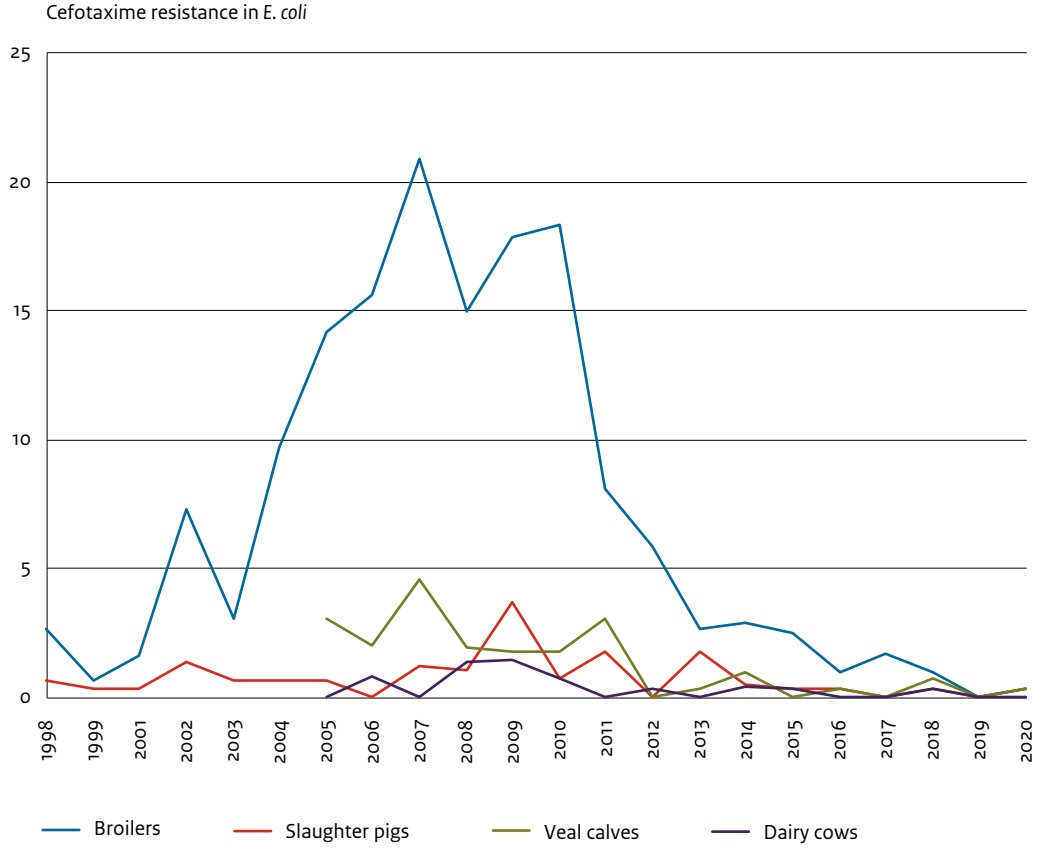


Table ESBL01 ESBL-genes found in *E. coli* isolates with reduced susceptibility to cefotaxime derived from broilers, veal calves, slaughter pigs, dairy cows and turkey (only 2011 and 2012) during 2007-2020

Year	ESBLs isolated from					Total ESBL suspected (n)	ESBL-genes detected										Total *E. coli* (n)	% ESBL of total *E. coli*
	[c]Broilers	Veal calves	Slaughter pigs	[d]Dairy cows	Turkeys		[a]CTX-M-1-group	CTX-M-2	CTX-M-9-group	TEM-52c	TEM-20	[b]SHV-12	SHV-2	CMY-2	chromosomal *ampC*	no gene found		
2007	9	6	2	0	*n.t.*	17	3	1		3				1	2	7	539	3.2
2008	66	4	3	2	*n.t.*	75	38	5	1	9			2	12	3	5	1,026	7.3
2009	53	2	11	2	*n.t.*	68	34	7		2	1	8	1	12	3		894	7.6
2010	52	3	2	2	*n.t.*	59	21	6		5	1	9	4	5	3	5	1,002	5.9
2011	23	5	5	0	6	39	9			8		9	2	3	3	5	1,096	3.6
2012	26	2	0	1	*n.t.*	29	8			4		8		5		4	1,328	2.2
2013	13	1	4	0	*n.t.*	18	7			4		3		3	1		1,371	1.3
2014	11	3	2	0	*n.t.*	16	8			1		4			1	2	1,519	1.1
2015	10	0	1	1	*n.t.*	12	3		2	1		1		2	3		1,283	0.9
2016	3	1	1	0	*n.t.*	5	2			1				1	1		1,492	0.3
2017	5	0	0	0	*n.t.*	5	2			1			2				1,194	0.4
2018	3	2	0	0	*n.t.*	7	2					3			2		1,198	0.6
2019	0	0	0	0	*n.t.*	0											1,209	0.0
2020	0	0	1	0	*n.t.*	3	1						1			1	1,103	0.3
Total	274	29	32	8	6	350	138	19	3	39	2	45	12	44	22	29		

a All were $bla_{CTX\text{-}M\text{-}1}$, only in 2011 one $bla_{CTX\text{-}M\text{-}3}$ gene was found in an isolate from a veal calf.

b One combination of $bla_{SHV\text{-}12}$ together with $bla_{TEM\text{-}52}$ occured in 2012 in one broiler isolate.

c In broilers, three combinations were found: in 2008: $bla_{CTX\text{-}M\text{-}1}$ with $bla_{CTX\text{-}M\text{-}2}$; in 2009: $bla_{CTX\text{-}M\text{-}1}$ with $bla_{SHV\text{-}12}$ and $bla_{CTX\text{-}M\text{-}1}$ with $bla_{SHV\text{-}12}$ and $bla_{CMY\text{-}2}$.

d In dairy cows, one combination of blaCMY-42 with $bla_{TEM\text{-}190}$.

n.t. : not tested

4.1.2 Selectively isolated ESBL/AmpC-producing *E. coli*

The selectively isolated *E. coli* aim to provide the percentage of animals and meat products that contain ESBL/AmpC-producing organisms, in contrast to the randomly isolated *E. coli* which provide a prevalence of the complete population that is present in a set of livestock animals. Isolation of these organisms is performed according to protocols provided by the European Reference Laboratory for Antimicrobial Resistance.

Isolation from faeces and caecal content occurs by measuring 1 gram of material in 9 ml of buffered peptone water which is incubated overnight at 37°C. Subsequently, selective screening is performed on plates of MacConkey agar supplemented with 1 mg/L of cefotaxime. The isolation from meat products is performed by adding 25 from of product to 225 ml of buffered peptone water and incubating overnight at 37 °C. Selective screening is performed on plates of MacConkey agar supplemented with 1 mg/L of cefotaxime and on Brilliance BLE ESBL agar. Putative resistant colonies are subcultured and species identification is performed using MALDI-TOF (Bruker Biotyper). The MIC of isolates is determined as described in Chapter 3 using a panel of antibiotics specifically aimed at beta-lactamase producing Enterobacteriaceae. After confirmation of the phenotype, molecular detection is performed to determine the mechanism that is responsible as described in 4.1.1.

Results of selective isolation of ESBL/AmpC-producing *E. coli* in faeces

A total of 1309 caecal and faecal samples were tested for the presence of ESBL/AmpC-producing *E. coli*. In addition to the annually recurring livestock species, boilers, pigs, veal calves and dairy cows, 200 caecal samples from layers from independent flocks, collected at slaughter, were studied in 2020, Table ESBL02. The prevalence of selectively isolated ESBL/AmpC-producing *E. coli* from **broilers** has greatly reduced since 2014 from 66.0% to 9.8% in 2020, figure ESBL02. The monitoring of **layers** is not performed annually as part of the MARAN program, making the data more difficult to interpret. A study by Blaak *et al*. 2015 comparing 3 broiler and 5 layer farms in the Netherlands in 2011 and 2012 showed a prevalence of 81% and 65% respectively, although different sampling strategies were used here than for MARAN. For MARAN, layers were first monitored in 2016 and the prevalence was 28%, compared to 14% in 2020. This indicates that significant reductions have also been realised in this sector.

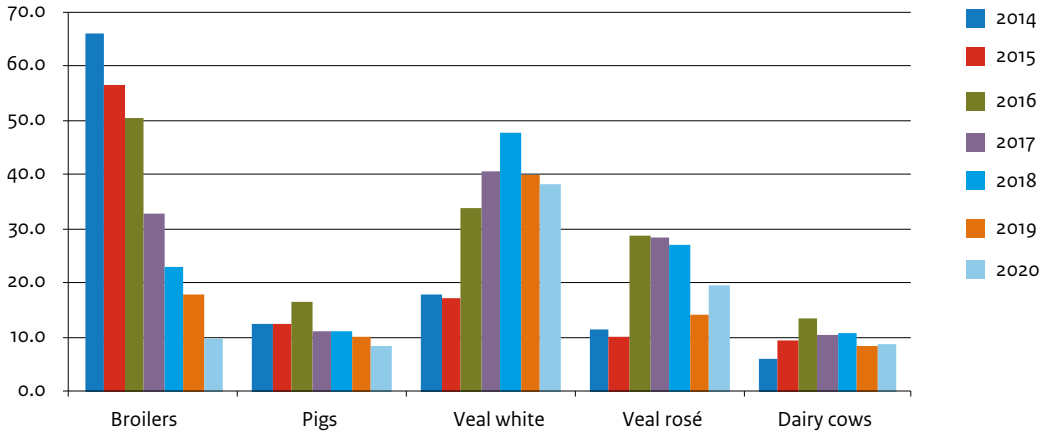
In **slaughter pigs**, small reductions have been observed over the past years from 16.3% in 2016 to 8.3% in 2020. While the prevalence in **dairy cows** has fluctuated over the past years between 6.0% and 13.2%, 2020 is the first year in which the prevalence (8.6%) is slightly higher than for pigs.

For **veal calves**, the samples are divided into two categories based on management differences into white and rosé veal calves. Both categories saw an increase in prevalence in 2016 which continued until 2018 up to 47.6% for the white veal calves, since which small decreases in prevalence were seen over the past two years to 38.1% in 2020. For the rosé veal calves, the highest recorded prevalence was 28.7% in 2016, which reduced to 14.0% in 2019 but showed an increase to 19.4% again in 2020. The mechanisms that contribute to these fluctuations and the transmission of ESBLs between veal calves are currently under investigation as part of the policy supporting research.

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 2020

	N samples	N suspected ESBL	% ESBL suspected	N confirmed ESBL	Prevalence (%) ESBL confirmed
Broilers	305	31	10.2	30	9.8
Layers	200	28	14.0	28	14.0
Pigs	303	50	16.5	25	8.3
Veal calves					
white	210	84	40.0	80	38.1
rosé	93	18	19.4	18	19.4
Dairy cows	198	21	10.6	17	8.6
Total	1,309	232	17.7	198	15.1

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 2014-2020 determined by using selective isolation



Results of molecular typing

Results for the molecular typing to determine the ESBL/AmpC that is responsible for the ESC phenotype are described in Table ESBL03. This molecular typing to describe which variants of ESBL/AmpC are present in Dutch livestock is of importance to determine if transmission occurs between livestock species, the environment and the human population (Mughini-Gras *et al.* 2019).

While the prevalence of ESBL producing *E. coli* in **broilers** went down from 2014-2020, the relative proportion of $bla_{CTX\text{-}M\text{-}1}$ in this population fluctuated little over time and was 35% in 2020. At the same time, the proportion of $bla_{CMY\text{-}2}$ in the population declined from 29% in 2014 to 6% in 2020. Conversely, the proportion of $bla_{SHV\text{-}12}$ went up from 15% in 2014 to 32% in 2020. In **layers**, the proportion of $bla_{CTX\text{-}M\text{-}1}$, $bla_{CMY\text{-}2}$ went down from 55% and 20% in 2016 to 29% and 11% respectively in 2020. While several genes ($bla_{CTX\text{-}M\text{-}15}$, $bla_{CTX\text{-}M\text{-}27}$, $bla_{TEM\text{-}52}$ and $bla_{TEM\text{-}52C}$) were now detected for the first time at low levels, $bla_{CTX\text{-}M\text{-}14}$ has had a significant increase from 5% of the ESC resistant population in 2016 to 43% in 2020.

In **slaughter pigs** $bla_{CTX\text{-}M\text{-}1}$ is also the most prevalent ESBL at 30%, while in 50% of the ESC resistant *E. coli* the phenotype can be attributed to the chromosomal promotor mutation ampC-type-3; this resistance type can only be spread via clonal transmission and not via plasmid transmission. These proportions have been fluctuating only little in slaughter pigs since 2014.

In **dairy cattle**, the chromosomal promotor mutation ampC-type-3 is responsible for 19% of resistant *E. coli*. Since 2014 the proportion of $bla_{CTX\text{-}M\text{-}1}$ has reduced from 41% in 2015 to 14% in 2020 while $bla_{CTX\text{-}M\text{-}15}$ has increased from 0% in 2014 to 38% in 2020, while the total prevalence of ESBL/AmpC-producing *E. coli* has remained quite stable in this population. While the prevalence of ESBL/AmpC-producing *E. coli* increased in both white and rosé veal calves, fluctuations in the mechanisms is limited. In **white veal calves**, the proportion of $bla_{CTX\text{-}M\text{-}1}$ has fluctuated little and was 42% in 2020. $bla_{CTX\text{-}M\text{-}15}$ increased from 14% in 2014 to 44% in 2019 but decreased to 26% in 2020. In **rosé veal calves** $bla_{CTX\text{-}M\text{-}1}$ has fluctuated from 35% in 2014 to 46% in 2017 and since reduced to 22% in 2020. $bla_{CTX\text{-}M\text{-}15}$ increased from 20% in 2014 to 44% in 2018 and reduced now to 33%. While $bla_{CTX\text{-}M\text{-}14}$ was absent in rosé veal calves in 2014, the proportion of prevalence has been inconstant over time and has reached 33% in 2020.

The cause for these changes to the relative proportions of the ESBLs in the different livestock species is probably multi-factorial and likely influenced by direct selection through usage of antimicrobials but is probably also influenced by unknown factors that result in indirect selective pressure of specific *E. coli* clones or plasmids on which the ESBLs are encoded.

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 2020

		Broilers	Layer	Slaughter pigs	Veal calves		Dairy cows	Total
					White	Rose		
CTX-M-1 group	CTX-M-1	11	8	15	35	4	3	76
	CTX-M-15	1	2	2	22	6	8	41
	CTX-M-32	1			4		1	6
	CTX-M-55						1	1
CTX-M-2 group	CTX-M-2				4			4
CTX-M-3 group	CTX-M-3				2			2
CTX-M-8/25 group	CTX-M-8				1			1
CTX-M-9 group	CTX-M-9				1			1
	CTX-M-14	2	12	1	5	6	1	27
	CTX-M-27		1				1	2
TEM	TEM-52		1	1	2			4
	TEM-52c	2	1	1	4	1		9
	TEM-52cVar	1		1				2
SHV	SHV-12	10					1	11
CMY	CMY-2	2	3	4		1	1	11
Chromosomal *ampC*	*ampC*-type-3	1		25	4		4	34
Total		31	28	50	84	18	21	232

Results of selective isolation of ESBL/AmpC-producing *E. coli* in raw meat and vegetables

The screening for the prevalence of ESBL/AmpC producing *E. coli* in food for human consumption was extended in 2020 with raw vegetables and mushrooms, in addition to fresh meat produced in the EU. A total of 1139 meat samples and 1328 vegetable and mushroom samples were analysed as described above, see Table ESBL04. The prevalence in meat was 2.9%, similar to previous years, while the prevalence in vegetables and mushrooms was lower at 0.2% resulting in an overall prevalence of 1.4%.

The biggest reduction was observed in turkey meat from 21.4% in 2019 to 0% in 2020 but the number of samples is always low compared to other types of meat this change is likely not significant. The reduction seen in chicken meat from 13.7% in 2019 to 9% in 2020 is considered more solid as the number of samples was much higher and the prevalence in chicken meat had been reducing for several consecutive years, see Figure ESBL03. The prevalence of ESBL/AmpC producing *E. coli* in beef, pork and lamb was characteristically low in 2020. Due to the low number of samples of veal in 2020, the increase in prevalence from 2.4% in 2019 to 3.8% in 2020 is not considered significant.

The molecular analysis of the genes that causes the ESC resistant phenotype in *E. coli* isolates from food is presented in Table ESBL05. As seen in previous years, the relatively prevalence of ESBL/AmpC producing *E. coli* in chicken meat is caused by a diverse group of mechanisms whereas the reduction in prevalence

over time in the other categories results in small variability. As seen in previous years, $bla_{CTX\text{-}M\text{-}1}$ is the most prevalent ESBL gene on raw meat. While ESBL/AmpC have been reduced over several years in pork, in 2020 the ampC-type-3 was the most abundant mechanism for ESC resistance. Due to the nature of the chromosomal promotor mutation, this mechanism can only be distributed clonally through the population of *E. coli*. The mechanism is found in most livestock species but in slaughter pigs these *E. coli* are most prevalent, making up 50% of the proportion of ESBL/AmpC-producing *E. coli* in 2020 (Table ESBL03).

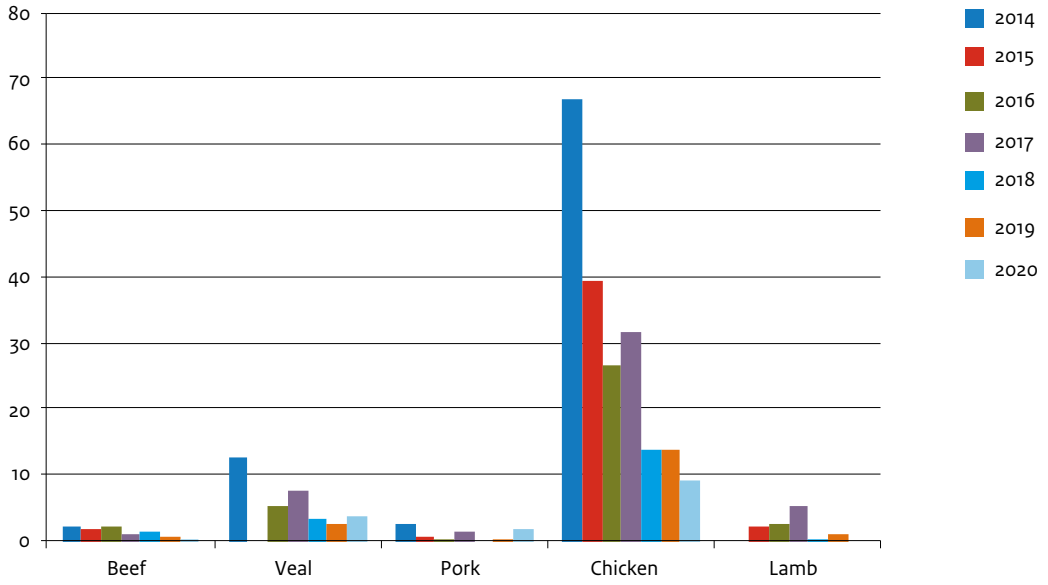
Table ESBL04 Prevalence of ESBL/AmpC-positive *E. coli* isolates from raw meat products in the Netherlands in 2020

Animal source	N screened	N ESBL/AmpC suspected	N ESBL/AmpC confirmed	% ESBL/AmpC positive
Beef	491	2	2	0.4
Veal	52	2	2	3.8
Pork	241	4	4	1.7
Chicken	277	25	25	9.0
Turkey	27	0	0	0.0
Lamb	46	0	0	0.0
Exotic meat	5	0	0	0.0
Vegetables	1,248	2	2	0.2
Mushrooms	80	0	0	0.0
Total	2,467	35	35	1.4

Table ESBL05 Beta-lactamases identified in *E. coli* from raw meat products in the Netherlands in 2020

	ESBL gene	Chicken	Pork	Beef	Veal	Vegetables	Total
CTX-M-1 group"	CTX-M-1	4	1	2			7
	CTX-M-15	1					1
	CTX-M-32	1				1	2
	CTX-M-55	2				1	3
TEM	TEM-52	1					1
	TEM-52c	3					3
	TEM-52cVar	3					3
SHV	SHV-2a	1					1
	SHV-12	4			2		6
	SHV-12; TEM-52c	1					1
CMY	CMY-2	4					4
Chromosomal *ampC*	*ampC*-type-3		3				3
Total		25	4	2	2	2	35

Figure ESBL03 Trends in prevalence of ESBL/AmpC-producing *E. coli* in fresh meat of broilers, pigs, veal calves, dairy cows and lambs from 2015-2020 determined by using selective isolation



ESBL/AmpC-producing *Salmonella*

In 2020, a total of 1310 *Salmonella* isolates from both humans and fresh meat produced in the EU were tested for the production of ESBL/AmpC. Molecular characterisation occurs through the same methods described above for *E. coli*. Isolates from diverse serovars are tested, see Table ESBL06. Contrary to ESBL/AmpC producing *E. coli*, in *Salmonella* the genes of the CTX-M-9 group have been most prevalent since 2016, replacing $bla_{\text{CTX-M-1}}$ and $bla_{\text{CMY-2}}$, see Table ESBL07. Similar to ESBL/AmpC producing *E. coli*, the prevalence of ESBL/AmpC producing *Salmonella* has been decreasing over the past decade. Specifically the reduction of ESBL/AmpC producing *Salmonella* in livestock is at least partially responsible for the shifts in the proportion of the different groups of ESBL/AmpC genes.

Table ESBL06 Beta-lactamases identified in *Salmonella* in 2020 (4 human isolates and 2 non-human isolates of unknow origin)

Serovar	CTX-M-9	CTX-M-14b	CTX-M-65	CMY-2	Total
Heidelberg[a]				1	1
Derby			1		1
Kentucky		1			1
Infantis			1		1
Typhimurium				1	1
Agona[a]	1				1
Total	1	1	2	2	6

[a] *origin unknown (non-human)*

Table ESBL07 Beta-lactamases identified in *Salmonella* isolates collected in 2007-2020

Year	[a]CTX-M-1-group	[b]CTX-M-2	CTX-M-3	CTX-M-8	[c]CTX-M-9-group	TEM-52	TEM-20	[d]SHV-12	[e]CMY-2	ACC-1	DHA-1	Total ESBL	Total *Salmonella* tested	% ESBL of total *Salmonella*
2007	9	13				17	2	4	2			47	1,514	3.1
2008	25	12		1	1	13	1		6	2		61	2,149	2.8
2009	12	4			2	3		1	9			31	2,232	1.4
2010	8	3			1	2		3	4			21	1,715	1.2
2011	5	3			1	1		2	13			25	1,444	1.7
2012	14	5			2	2			10	1		34	1,795	1.9
2013	1	3		5	4	5	1		36			55	1,369	4.0
2014	6			2	3	1			21			33	1,688	2.0
2015	13	2			6	1			12			34	1,761	1.9
[f]2016	7				15	2			10		1	36	2,117	1.7
[g]2017	3				23			1	3		1	31	1,697	1.8
[g]2018	2		1	1	8				2			14	1,718	0.8
2019	4				11			1	3			19	1,880	1.0
2020					4				2			6	1,310	0.5
Total	109	45	1	9	81	47	4	12	133	3	2	422	24,389	1.7

a contains $bla_{CTX-M-1}$, $bla_{CTX-M-55}$, $bla_{CTX-M-15}$, $bla_{CTX-M-3}$ and a combination with bla_{CMY-2} (n=2, 2014, 2015).

b In 2008 one combination of $bla_{CTX-M-2}$ with bla_{TEM-52} was found in S. Paratyphi B var Java.

c contains $bla_{CTX-M-9}$, $bla_{CTX-M-14}$ and $bla_{CTX-M-65}$.

d In 2007 three S. Concord were found containing both blaSHV-12 and $bla_{CTX-M-15}$.

e In 2015 a combination of bla_{CMY-2} and bla_{TEM-52} was found in S. Oranienburg and a combination of bla_{CMY-2} with $bla_{CTX-M-1}$ in S. Molade

f In 2016, one S. Minnesota isolate obtained from poultry meat at NVWA was not included in the molecular analysis.

g In 2017 and 2018 only human isolates were molecularly characterised.

4.2 Carbapenemase producing Enterobacteriaceae

4.2.1 Monitoring in livestock

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 of slaughter pigs, veal calves, dairy cattle and laying hens and a subset of the samples from broilers (n=305) 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-tests 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 2020 (n=1309) resulted in six *bla*$_{OXA-48}$-like positive faecal samples in the RT-PCR (three slaughter pigs, two broilers, one white veal calf). In two samples the presence of *bla*$_{OXA-48}$-carrying *Shewanella* was confirmed by bacterial culturing followed by PCR and sequencing: *bla*$_{OXA-48b}$ (n=1) and *bla*$_{OXA-252}$ (n=1). In the remaining four samples *bla*$_{OXA-48b}$ (n=3), and *bla*$_{OXA-252}$ (n=1) were detected in the enrichment broth with PCR and confirmed by Sanger sequencing, but culturing of *Shewanella* was negative. These results confirm the findings of the previous seven years where *bla*$_{OXA-48-}$like genes have also been found in *Shewanella* obtained in faecal samples from livestock. Given the role of *Shewanella* spp. as natural progenitor of this carbapenemase family (Zong, 2012), these genes were considered of environmental origin and not a public health risk. Most importantly, no carbapenemase-producing Enterobacteriaceae were detected in faecal samples from livestock in the Netherlands in 2020. Screening for carbapenemase-producing isolates in faecal samples of food-producing animals will continue in 2021.

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 had not been detected in the Netherlands (MARAN 2017). In 2017, the first case of a *bla*$_{OXA-48}$ producing *E. coli,* isolated from a faecal dog sample, was reported (MARAN 2018). The faecal sample was submitted to the Veterinary Microbiological Diagnostic Center (VMDC) of Utrecht University for parasitology diagnostics. In 2018, two individual dog samples were found positive for *E. coli,* harboring *bla*$_{OXA-48}$ and *bla*$_{OXA-181}$ respectively. Both samples originated from different parts of the Netherlands and were sent to the VMDC for

parasitology diagnostics. In 2019 all faecal samples from cats (n=138) and dogs (n=114) were tested negative for CPE.

Faecal 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 faecal samples was antimicrobial treatment of the animal. Since cats are not frequently treated with antimicrobials, a randomized stratified subset of faecal samples from cats submitted to VMDC were included. In 2020, 105 faecal samples from cats and 105 faecal 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), bla_{VIM} (Ellington et al, 2007), bla_{OXA}-group-23, -24, -51, -58 (Voets et al, 2011) and bla_{OXA}-group-48 (Poirel et al, 2004).

None of the faecal samples from cats and dogs showed growth on the selective plates (direct and after enrichment). This result indicates a low concentration of CPE present in the samples if any. PCR screening revealed a fragment of bla_{OXA-10} in a faecal sample from a three year old cat treated with metronidazole. This resistance gene could not be linked to a bacterial isolate, because additional culturing experiments were all negative. All other faecal samples were tested negative by PCR. In conclusion: no carbapenemase-producing Enterobacteriaceae were detected in dogs and cats in 2020. Screening for carbapenemase-producing isolates in companion animals will continue in 2021.

4.2.3 Monitoring in imported seafood, seaweed and herbs.

In 2020, 284 batches of frozen fish and shrimps originating from fish farms in South-East Asia were screened for the presence of carbapenemase producing Enterobacteriaceae (CPE) by the WFSR through selective culturing. The samples consisted of 98 batches of Pangasius, 89 batches of Tilapia and 98 batches of shrimps. In addition 198 batches of seaweed and 60 batches of herbs also imported from South-East Asia were screened for the presence of CPE. As in previous years, a small number of carbapenemase-producing *Enterobacter cloacae* (*E. cloacae*) complex isolates were detected. Two isolates were found in tilapia imported from Vietnam and China. One isolate was obtained from seaweed imported from Israel. Molecular analysis of the isolates revealed the presence of bla_{IMI-2} in one *E. cloacae* isolate from Vietnam (Tilapia) and bla_{IMI-3} was identified in two *E. cloacae* isolates from China (Tilapia) and Israel (seaweed). The finding of CPE in Seaweed from Israel was rather unexpected, but might indicate a wider spread of IMI-positive *E. cloacae* in food products.

For the fourth year in a row, carbapenemase-producing *Enterobacteriaceae* were detected in batches of imported frozen seafood and fish from South-East Asia. Our findings reflect the high consumption of antimicrobials in South-East Asia, specifically in aquaculture as an environment with a high selective pressure for resistant bacteria, including CPE, and potential for faecal contamination. The monitoring of imported food will be continued in 2021 and extended to PCR screening in order to increase the sensitivity of the method.

4.3 Colistin resistance

In 2020, 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 1309 faecal samples of Dutch livestock were tested with for the presence of *mcr*-1, *mcr*-2, *mcr*-3, *mcr*-4 and *mcr*-5 using an in house designed multiplex RT-PCR based on the updated EURL-AR protocol (https://www.eurl-ar.eu/CustomerData/Files/Folders/21-protocols/396_mcr-multiplex-pcr-protocol-v3-feb18.pdf). In case of a PCR positive pool, individual samples were tested followed by direct culturing of the original BPW broth on MacConkey agar with 2 mg/L colistin. As a result, *mcr*-1 positive *E. coli* were identified in seven faecal samples (0.5%) in veal calves (n=3, 1.0%) and slaughter pigs (n=4, 1.3%). For the second year 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.

In 2020, colistin resistance was present amongst indicator *E. coli* from turkey meat (14.3%), chicken meat (1.3%) and beef (1.6%). As such, *mcr*-1 was detected in two *E. coli* from turkey meat and one *E. coli* isolate from chicken meat. These results indicate a further decline of the prevalence of *mcr* in livestock, especially in broilers and broiler meat.

4.4 MRSA surveillance in pigs, poultry and humans

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 (Graveland *et al.* 2011). 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 (Lekkerkerk *et al*. 2015). In 2018 a project on surveillance of MRSA in livestock, meat products and humans was started. This project is a collaboration between WVBR, RIVM, NVWA and WFSR. MRSA isolates obtained from animals, meat, dust from livestock farms, farmers and their family members are compared with isolates collected in the Dutch national MRSA surveillance in humans (isolates from asymptomatic carriage as well as clinical isolates) to assess possible changes in the rate or nature of MRSA transmission between animals and humans. Below are the findings obtained within this surveillance project.

4.4.1 Prevalence in poultry, meat and pigs

Poultry
In 2018/2019, 195 broiler farms were investigated and no MRSA was found in the dust samples from the broiler houses. In addition, 133 farmers and persons living or working on the farms volunteered to send in a nasal swab. Four persons were found MRSA-positive (3%) and all were LA-MRSA. This is higher than the prevalence in the population at large (0,1-0,2%).

Meat
In 2018,2019 and 2020 MRSA was detected in 20.2%,17.3% and 15.4% of the chicken meat samples (table MRSA01). This was unexpected high as no MRSA was found on the broiler farms. Possible explanations are that meat sold in Dutch supermarkets is sometimes imported from other countries or meat might be contaminated in the slaughterhouse or retail. Another explanation of the differences found between broilers and chicken meat could be that detection of MRSA was hampered at the broiler farms due to inhibiting substances in the dust of the broiler houses (such as coccidiostats).

MRSA was also found in turkey meat, pork and beef (see table MRSA01). Turkey meat seemed highly contaminated, but because of the low number of samples these results have to be interpreted with care. Generally it is believed that contaminated meat is not an important transmission route for MRSA for the population at large. In some studies, food handling has been implicated as a transmission route for MRSA (Larsen *et al*. 2016).

Table MRSA01 Number of MRSA found on meat (products) from 2018-2020

		2018			2019			2020		
		Positive (n)	Total (n)	Prevalence (%)	Positive (n)	Total (n)	Prevalence (%)	Positive (n)	Total (n)	Prevalence (%)
Pork		8	135	5.9	25	296	8.4	2	57	3.5
Beef		3	140	2.1	11	286	3.8			
Veal								2	52	3.8
Poultry meat total		29	132	22	50	251	19.9	41	248	16.5
	chicken	26	129	20.2	41	237	17.3	36	234	15.4
	turkey	3	3	100	9	14	64.3	5	14	35.7

Pigs

In 2019-2020 dust samples were taken from pig stables at 149 finishing pig farms. Hundred-thirteen farms (76%) were found to be LA-MRSA positive (at least one sample positive). This signifies that pigs are still an important reservoir for LA-MRSA, despite the reduction in antimicrobial use during the last decade. Next-generation sequencing (NGS) data of MRSA isolates were generated. NGS data were used in whole-genome multi-locus sequence typing (wgMLST) to assess the genetic relationship between isolates. ResFinder software was used to determine the presence of acquired antibiotic resistance genes. To reconstruct the complete chromosomes and plasmids in a subset of isolates (see paragraph 4.4.3) both NGS and third-generation sequencing data, obtained by long-read nanopore sequencing, were used in a unicycler hybrid assembly. Broth microdilution was performed to determine minimum inhibitory concentrations (MICs) of the MRSA isolates to a panel of 19 antimicrobial agents (see paragraph 4.4.2 and 4.4.3). Isolates obtained from humans, comprised 1,200 LA-MRSA isolates from 1,150 persons and 3,600 non-LA-MRSA isolates from 3,500 persons collected in 2008-2020, with half of the isolates originating from 2017-2019. In addition, NGS data of 327 MRSA isolates obtained from animals, meat or dust collected in livestock farms during 2008-2020 were used.

When comparing the wgMLST of the animal and human isolates, most pig- and poultry isolates cluster together with isolates from the human surveillance (data not shown), while there were also clusters containing human isolates only. As there were no clusters containing animal isolates only, it is likely that transmission between animals and humans has occurred at some point.

4.4.2 Resistance levels of MRSA from livestock and meat

In 2019 and 2020, susceptibility testing of MRSA isolates was performed at WBVR on a subset of isolates originating from existing strain collections stored at RIVM and WFSR as well as from more recently obtained isolates from meat and dust samples at WFSR. The subset consisted of isolates from chicken meat (n=37; 2018 -2020), bovine meat (n=64; 2007 – 2008 (n=50), and 2018 – 2019 (n=14)), pork (n=32; 2018 - 2020), nasal swabs of slaughter pigs (n=112; 2015) and dust from pig stables (n = 114; 2019- 2020). Bacterial isolates were tested for antimicrobial susceptibility with broth microdilution according to ISO standards in a European antimicrobial test panel intended for Staphylococci as advised by EFSA using

commercially available Sensititre plates (Thermofisher Scientific, panel EUST). The MIC-values were interpreted with ECOFFs as advised by EUCAST. Resistance percentages are depicted for each type of sample in table MRSA02.

Table MRSA02 Resistance percentages (R%) of MRSA isolated from raw chicken meat, bovine meat, pork, nasal swabs from pigs and dust from pigs stables the Netherlands

	Chicken meat	Bovine meat	Pork	Nasal swab pig	Dust pig stable
	N = 37	N = 64	N = 32	N = 112	N = 114
Chloramphenicol	0.0	9.4	9.4	8.0	18.4
Ciprofloxacin	16.2	20.3	15.6	7.1	3.5
Clindamycin	70.3	28.1	21.9	39.3	40.4
Erythromycin	86.5	51.6	43.8	34.8	38.6
Cefoxitin	97.3	100.0	100.0	100.0	100.0
Fusidic acid	0.0	4.7	0.0	0.0	0.0
Gentamicin	0.0	21.9	15.6	39.3	35.1
Kanamycin	0.0	43.8	43.8	21.4	20.2
Linezolid	0.0	0.0	0.0	0.0	0.9
Mupirocin	0.0	0.0	0.0	0.0	0.0
Penicillin	100.0	100.0	100.0	100.0	100.0
Rifampicin	0.0	0.0	0.0	0.0	0.0
Sulfamethoxazole	0.0	1.6	3.1	0.0	0.0
Streptomycine	8.1	37.5	37.5	13.4	2.6
Quinepristin/dalfopristin	59.5	3.1	6.3	10.7	9.6
Tetracycline	100.0	75.0	75.0	100.0	100.0
Tiamulin	59.5	4.7	9.4	12.5	8.8
Trimethoprim	73.0	39.1	25.0	82.1	69.3
Vancomycin	0.0	0.0	0.0	0.0	0.0

As should be expected for MRSA all isolates were tested resistant against (benzyl)penicillin and almost all against cefoxitin. Only one isolate obtained from chicken meat was tested susceptible to cefoxitin. WGS analysis revealed the absence of the *mecA* gene and therefore this isolate should not be considered MRSA. The *mecA* gene was present in all other *S. aureus* isolates tested.

High levels of resistance were observed for tetracycline up to 100% in chicken meat and in samples from pigs (including dust). This is in line with the known high level of tetracycline resistance in LA-MRSA. Also for trimethoprim, high levels of resistance were seen in chicken meat and all types of pig samples (69.3% - 82.1%) where lower levels where observed in bovine meat and pork.

For some antimicrobials resistance levels were clearly higher in chicken meat than in MRSA isolates from other animals species. This was particularly the case for erythromycin, clindamycin, tiamulin and quinepristin/dalfopristin (Synercid). On the contrary, chloramphenicol resistance was observed at relative low levels (<10%) in meat from bovine and pigs and at a higher level in dust from pig stables, but completely absent in chicken meat. Also, resistance against aminoglycosides (gentamicin and kanamycin) was completely absent in chicken meat, but was frequently observed in bovine and pig samples.
No resistance was observed against important human antimicrobials: mupirocin, rifampicin and vancomycin. Resistance against fusidic acid and sulfamethoxazole was only rarely found in meat.

Finally resistance against linezolid was detected in one MRSA isolate from a dust sample of a pig stable obtained in 2019. This resistance confirmed the presence of a *cfr* gene in one of the sampled pig stables. The first finding of *cfr* gene in MRSA in the Netherlands is described in detail in the paragraph 4.4.3.

4.4.3 First identification of the multi-resistance gene *cfr* in livestock-associated methicillin resistant *Staphylococcus aureus* (LA-MRSA) in humans and in pig housing in the Netherlands

During the analyses of the NGS data as described in paragraph 4.4.2, we detected the presence of the multi-resistance gene *cfr* in in two isolates from independent dust samples obtained in one pig farm in 2019. In addition, the *cfr*-gene was also found in five LA-MRSA obtained from humans in 2018 (1), 2019 (2) and 2020 (2). Epidemiological data, only available from three patients, showed that two patients had been in contact with livestock and the other one claimed not to have had animal contact. One isolate was cultured from pus, one from urine and three were obtained from nasal swabs. The *cfr* gene methylates the 23S rRNA resulting in simultaneous resistance against five antibiotic classes: phenicols, lincosamides, oxazolidinones, pleuromutilins and streptogramin A, known as the PhLOPSA phenotype. This phenotype was confirmed by MIC analyses of all but one isolate. The wgMLST showed that the *cfr*-carrying LA-MRSA isolates were genetically unrelated. In all seven isolates the *cfr* gene was located on plasmids. Remarkably, the plasmids differed considerably in size and composition from each other,except the ones found on the same farm. These results show that there is no outbreak with a particular strain or spread of a *cfr* carrying plasmid with these exceptional resistance traits, but suggests multiple introductions of *cfr* in LA-MRSA in the Netherlands. The finding of the multi-resistance gene *cfr* is worrisome and should be closely monitored. Linezolid is not routinely used to treat MRSA infections in humans in the Netherlands, but is important to treat vancomycin-resistant enterococci infections and is classified as critically important antimicrobial by the WHO. In veterinary medicine oxazolidinones (such as linezolid) are not used, but phenicols (florfenicol), pleuromutilins (tiamulin, valnemulin) and lincosamides (lincomycin, pirlimycin, clindamycin) are and could select for LA-MRSA carrying the *cfr* gene. These findings show that it is important to combine and compare data obtained from MRSA surveillance in humans, animals and food from a One Health perspective.

4.4.4 Screening for *lukF* genes in LA-MRSA

In addition, all isolates were screened for the presence of the gene (*lukF* gene) that encodes for Panton–Valentine leukocidin (PVL). PVL positive LA-MRSA is also increasing in humans. PVL is a cytotoxin associated with increased virulence of certain strains of *S. aureus*. PVL can cause lysis of leucocytes. PVL was not found in the isolates originating from animals or meat. It was, however, found in LA-MRSA isolates from

humans. The number of PVL positive LA-MRSA isolates from humans is increasing: until 2014 it were 0-10 isolates per year, in 2017, 24/847 isolates were PVL positive, in 2018 57/805 LA-MRSA isolates and in 2019 60/709 PVL-positive isolates were found. As PVL-positive isolates were only found in humans it seems likely that these are transmitted from humans to humans, without an animal reservoir. This needs to be confirmed, as the number of isolates from animals investigated is still relatively small and not all animal species have been included to date.

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