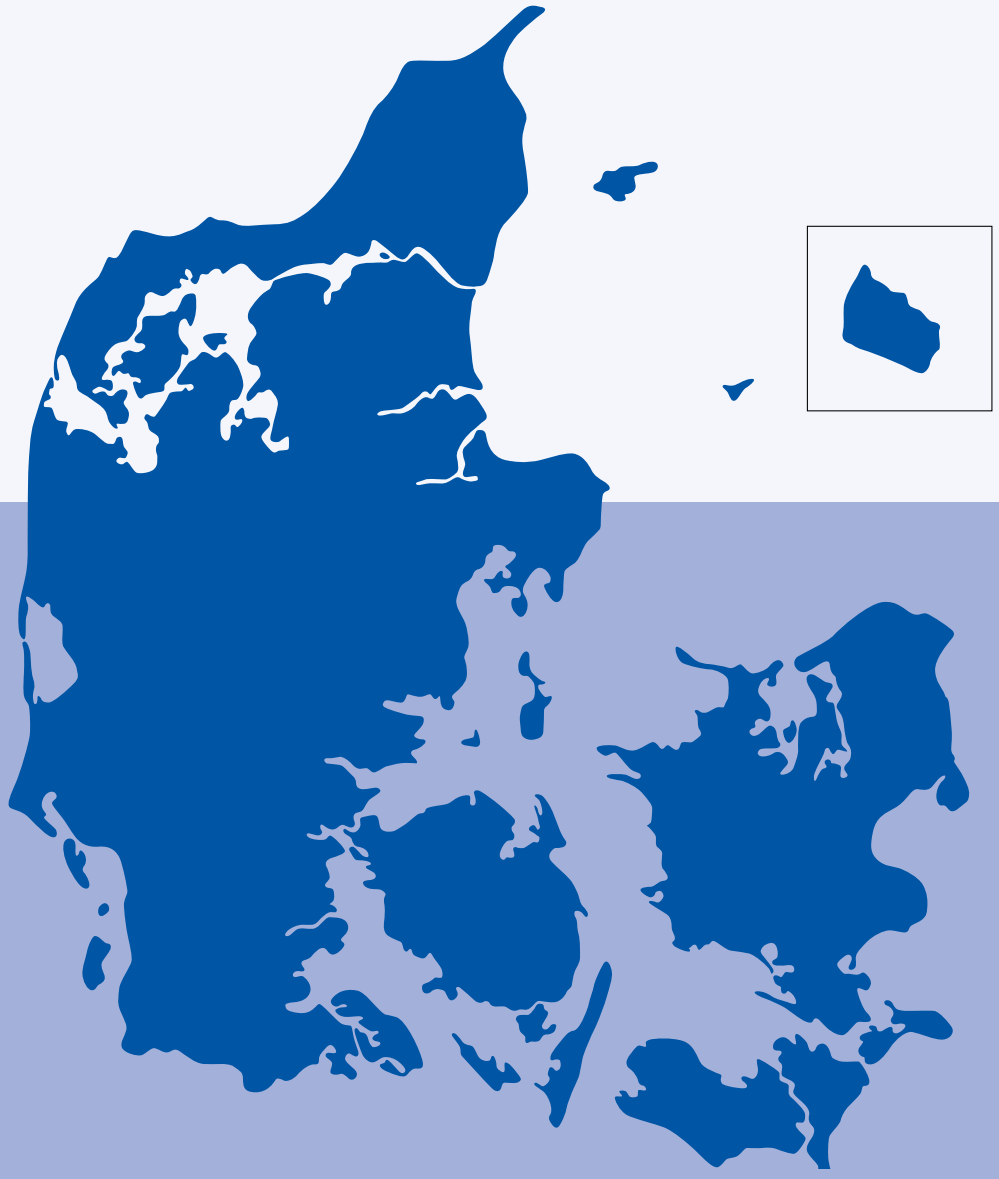
DANMAP 2019

DANMAP 2019 - Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark



Statens Serum Institut
National Food Institute, Technical University of Denmark

DANMAP 2019

Editors:
Helle Korsgaard (hkor@food.dtu.dk)
Johanne Ellis-Iversen (joell@food.dtu.dk)
Ute Wolff Sönksen (uws@ssi.dk)
Sissel Skovgaard (sisk@ssi.dk)

Assisting editors:
Channie Kahl Petersen
Anna Emilie Henius
Majda Attauabi
Lina Maria Cavaco

DANMAP Steering Committee:
National Food Institute: Flemming Bager, Johanne Ellis-Iversen
Statens Serum Institut: Anders Rhod Larsen, Ute Wolff Sönksen

Layout:
Anja Bjarnum, Statens Serum Institut
Photos: Colourbox
Printing: Pekema A/S

Contact:
National Food Institute,
Technical University of Denmark
Kemitorvet, Building 201, DK-2800 Kgs. Lyngby

Infectious Disease Preparedness – Bacteria, Parasites and Fungi,
Statens Serum Institut
Artillerivej 5, DK-2300 Copenhagen

DANMAP 2019 – October 2020 – ISSN 1600-2032

The report is available from www.danmap.org

Authors:

National Food Institute
Helle Korsgaard
Johanne Ellis-Iversen
Rene S. Hendriksen
Birgitte Borck Høg
Troels Ronco

Statens Serum Institut
Majda Attauabi
Jeppe Boel
Tine Dalby
Anette M. Hammerum
Frank Hansen
Henrik Hasman
Anna Emilie Henius
Steen Hoffmann
Mette Bar Ilan
Hülya Kaya
Anne Kjerulf
Brian Kristensen
Jonas Kähler
Anders Rhod Larsen
Eva Møller Nielsen
Stefan Schytte Olsen
Andreas Petersen
Lone Jannok Porsbo
Louise Roer
Sissel Skovgaard
Hans-Christian Slotved
Ute Wolff Sönksen
Mia Torpdahl

Danish Health Data Authority, Register of Medicinal Statistics:
Maja Laursen

Authors of invited textboxes 2019:
Anne-Marie Andersen
Maiken Cavling Arendrup
Lærke Boye Astrup
Peter Damborg
Luca Guardabassi
Rasmus Krøger Hare
Solveig Harksen
Anette Holm
Anne Birgitte Jensen
Vibeke Frøkjær Jensen
Ulrik Stenz Justesen
Diseree Corvera Kløve
Mette Marie Nordestgaard
Peter Poulsen
Malene Risum
Charlotte Mark Salomonsen
Anette Grønkjær Thomsen
Annette Toft

DANMAP 2019

DANMAP 2019 - Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark

Table of contents

1. Editorial 7

2. Summary 9

3. Introduction to DANMAP 15

3.1 The DANMAP surveillance system 16
3.2 Information on demographics and food production 17

4. Antimicrobial consumption in animals 23

Highlights 24
4.1 Introduction 25
4.2 Total antimicrobial consumption in animals 27
4.3 Antimicrobial consumption by animal species 28
Textbox 4.1 Campaign to assess compliance regarding group treatment of piglets 36

5. Antimicrobial consumption in humans 37

Highlights 38
5.1 Introduction 39
5.2 Total consumption (all public healthcare systems in Denmark) 39
5.3 Primary health care 42
5.4 Hospital care 54
Textbox 5.1 National Action Plan on the reduction of antibiotics in humans, 2016-2020 64

6. Resistance in zoonotic bacteria and animal pathogens 67

Highlights 68
6.1 Introduction 69
6.2 *Campylobacter* 69
6.3 *Salmonella* 73
Textbox 6.1 Antimicrobial resistance in clinical isolates from dogs and cats in Denmark 77
Textbox 6.2 MRSA surveys in animals 80
Textbox 6.3 Antimicrobial resistance in mastitis isolates from dairy cows 81
Textbox 6.4 Resistance in bacteria from diagnostic submissions from pigs 83

7. Resistance in indicator bacteria 87

Highlights 88
7.1 Introduction 89
7.2 Indicator *Escherichia coli* 89
7.3 ESBL/AmpC- and carbapenemase-producing *E. coli* 92
7.4 *Enterococcus* 96

8. Resistance in human pathogens 99

Highlights 100
8.1 Introduction 101
8.2 **Surveillance based on MiBa data** 103
8.2.1 *Escherichia coli* 103
8.2.2 *Klebsiella pneumoniae* 108
8.2.3 *Pseudomonas aeruginosa* 113
8.2.4 *Acinetobacter* species 114
8.2.5 Enterococci 115
8.3 **Surveillance based on data from the reference laboratories** 117
8.3.1 Characterisation of ESBL- and pAmpC-producing *Escherichia coli* from bloodstream infections, 2019 Denmark 117

8.3.2 Carbapenemase producing bacteria in Denmark, 2019 119
Textbox 8.1 Investigation of an *Enterobacter cloacae* OXA-436 carbapenemase-outbreak – Shower drains as reservoirs 122
8.3.3 Increase in both *vanA* and *vanB E. faecium* in Denmark 122
8.3.4 Detection of linezolid resistant enterococci and linezolid-vancomycin resistant enterococci 125
8.3.5 *Streptococcus pneumoniae* 126
8.3.6 Beta-haemolytic streptococci 128
8.3.7 *Haemophilus influenzae* 130
8.3.8 *Staphylococcus aureus* 131
8.3.9 *Neisseria gonorrhoeae* 135
Textbox 8.2 Incidence of multiresistant bacteria in Greenland 138
Textbox 8.3 Azole resistance in *Aspergillus fumigatus* – Nationwide surveillance data from the first 18 months 139

9. Materials and methods 143
9.1 General information 144
Animals and meat
9.2 Data on antimicrobial consumption 144
9.3 Collection of bacterial isolates 146
9.4 Microbiological methods 147
9.5 Susceptibility testing 148
9.6 Whole genome sequencing 148
9.7 Data handling 148
Humans
9.8 Data on antimicrobial consumption 150
9.9 *Salmonella* and *Campylobacter* 153
9.10 *E. coli, K. pneumoniae, P. aeruginosa, Acinetobacter* spp., *E. faecium and E. faecalis* 153
9.11 ESBL-producing bacteria 153
9.12 Carbapenemase producing organisms 154
9.13 Vancomycin-resistant enterococci 154
9.14 Invasive *Streptococcus pneumoniae* 154
9.15 Invasive beta-haemolytic streptococci (group A, B, C and G streptococci) 155
9.16 Invasive *Haemophilus influenzae* 155
9.17 *Staphylococcus aureus* including MRSA 156
9.18 Gonococci 156

10. Terminology 157
List of abbreviations 158
Glossary 159

Additional information and supporting data on antimicrobial consumption and antimicrobial resistance is presented in the web annex at www.DANMAP.org.

1. Editorial

The current COVID-19 pandemic caused over 22 million cases and more than 700,000 deaths globally by the end of August 2020. The numbers are expected to increase until an effective vaccine has been developed and made widely available. Preventative measures such as social distancing, use of facemasks and hand sanitization have shown to be effective. However, many countries have struggled with the containment of the virus, and the worldwide socio-economic costs are enormous.

While antimicrobial resistance (AMR) cannot be defined as a pandemic (yet), the increasing levels of AMR nevertheless represent a public health crisis of almost similar magnitude to the COVID-19 crisis, although playing out in slow motion. An increasing number of common infectious agents are now resistant to antimicrobial treatment including the new, broad-spectrum antimicrobials. It is estimated that 700,000 people die annually, as a direct result of infections that no longer respond to antibiotics.

Communicable diseases walk hand in hand. The World Health Organisation (WHO) has voiced concerns that high levels of antibiotic use in COVID-19 patients will exacerbate AMR in common pathogens and included recommendations for prudent antibiotic use in its recently published guidance for the clinical management of COVID-19 patients.

This is in line with former recommendations on proper antibiotic use and national action plans on the containment of AMR issued in recent years. However, even more than COVID-19, AMR is multi-sectorial and the use of antibiotics has knock-on effects on humans and animals with the environment and food production as possible links between these. The EU Commission set targets to reduce the use of antimicrobials in farmed animals and aquaculture by 50 percent by 2030 in its new Farm to Fork strategy.

Simultaneously, the environmental sector has begun working on issues related to development and spread of AMR through water, highlighting the importance of monitoring water as an important source. Both antimicrobials and resistant bacteria may be spread through wastewater and reach drinking water.

In consequence, while overuse of antimicrobials continues to be among the most important factors associated with the rise of AMR and while usage levels must be addressed, other factors are equally important. These factors include improvement of basic sanitation and food hygiene by providing access to clean water as well as ensuring food free from microbiological contaminants.

Interestingly, some of the lessons learnt from the COVID-19 crisis may also help to prevent AMR - preliminary data indicate that the implementation of measures adopted by the population, mainly social distancing and increased focus on hand hygiene, led to a decline of a number of common infectious diseases. Consequently, the use of antimicrobials in the human sector appears to have decreased during the pandemic. These trends will be analysed in more detail and presented in next year's DANMAP report.

It is evident around the world that effective handling of the ongoing COVID-19 outbreak has required bold leadership. A similar bold leadership, not only by national governments but also by all health professionals across all sectors and all countries, will be necessary to effectively combat the spread of AMR and avoid the worst case scenario published in a recent UN report of 10 million deaths due to AMR annually by 2050.

DANMAP Steering Committee

Acknowledgements

DANMAP is based on a strong collaboration between several institutions and on the contributions from highly skilled staff from many specialties and professions. Without their knowledge and engagement, there would be no DANMAP surveillance.

The DTU National Food Institute would like to thank the following:

- The meat inspection staff and the company personnel at the participating slaughterhouses for collecting samples from animals at slaughter. Without their careful recording of the animals' farm of origin, the results would be less useful
- The Laboratory of Swine Diseases, the Danish Agriculture and Food Council, Kjellerup, University of Copenhagen, and DTU Centre for Diagnostics for their contributions on AMR in animal pathogens
- The staff of the Regional Veterinary and Food Control Authorities for collecting food samples and the staff at the Laboratory for isolating and susceptibility testing bacteria
- The Department of Medication Statistics and Research Support at the Danish Health Data Authority (formerly the Danish Medicines Agency and SSI) for collecting and transmitting data on veterinary consumption of antimicrobial agents from the pharmacies
- The Danish Veterinary and Food Administration for collecting and transmitting data on veterinary consumption of antimicrobial agents from VetStat, data on resistance from the national monitoring and control programmes, financing data collection and providing data for interpretation
- The Danish Agriculture and Food Council for cooperation regarding the estimation of live biomass of production animals
- The improvements to chapter 6 were supported by ORION, a One Health EJP project that received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No 773830

Statens Serum Institut would like to thank the following:

- The staff of the Neisseria and Streptococcus Typing Unit at SSI for providing data on samples and resistance in beta-hemolytic streptococci, *H. influenzae* and *Neisseria gonorrhoeae*
- The staff of the Foodborne Pathogens Unit at SSI for providing data on resistance in *Campylobacter* and *Salmonella* from human clinical isolates
- The staff of the Staphylococcus Laboratory at SSI for providing data on invasive staphylococcal infections as well as all MRSA
- The staff of the Antimicrobial Resistance Reference Laboratory and Surveillance Unit at SSI for providing data on resistance in the referred *E. coli, K. pneumoniae, A. baumannii, P. aeruginosa* and vancomycin and linezolid resistant enterococci
- The staff at Unit of Mycology at SSI for providing resistance data for human *Aspergillus*
- Erik Villadsen from the Danish Health Data Authority for providing data on bed-days and admissions
- All departments of clinical microbiology in Denmark and the DANRES/DANRES-M groups - Danish Study Groups for Antimicrobial Resistance Surveillance/-Methods - for providing data on resistance in bacteria from human samples in the Danish Microbiology Database as well as referring samples to the reference laboratories at SSI and for discussing many of the topics included in the report
- Jonas Kähler and Stefan S. Olsen from the department of Data Integration and Analysis (DIAS), SSI, for extractions of data from MiBa and data management support
- Maja Laursen from the Danish Health Data Authority, the Register of Medicinal Products Statistics for providing antimicrobial consumption data: DDD, DID, number of users and prescriptions and for being such a good and comprehensive proofreader
- All Danish hospital pharmacies for providing data on antimicrobials consumed at hospitals through special deliverances

2

SUMMARY

2. Summary

The Danish integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP) was founded in 1995, providing a unique one-health surveillance system for the continuous surveillance and research of antimicrobial consumption and resistance. A key objective of the DANMAP programme is to provide an evidence base for decision-making and to further understand the associations between antimicrobial usage (AMU) and the occurrence of antimicrobial resistance (AMR) across populations.

In Denmark, antibiotics for humans and animals are available by prescription only, and all prescriptions are recorded by the Register of Medicinal Statistics. The data are available for surveillance, control programmes and research through national databases called Medstat and Vetstat. The registration covers the whole nation and dates back to the mid-90s (Medstat) and 2000 (VetStat), respectively.

DANMAP 2019 analyses all records of the types and amounts of antimicrobials prescribed for animal and human treatment in Denmark during 2019 and compares it with historic data. It also analyses susceptibility testing data of isolates obtained from hospitals, general practice, veterinary practice, food-industry laboratories and the Danish Veterinary and Food Administration.

Human isolates cover bacteraemias, urinary tract infections and gastroenteritis caused by the most important pathogenic bacteria. Isolates from animals are collected in accordance with the EU harmonised monitoring of antimicrobial resistance in zoonotic and commensal bacteria [Decision 2013/652/EU].

Statens Serum Institut (SSI) collates and interprets data from the human sectors and the Technical University of Denmark (DTU) collates and interprets data from the food and animals sectors. In the chapter of zoonoses, the data is integrated and interpreted together.

Antimicrobial consumption in animals

Annual consumption of antimicrobials in animals is based on the amount of veterinary prescription medicine registered by pharmacies, private companies, veterinarians and feed mills. In DANMAP, the antimicrobial use in animals is estimated first as kg active compound and further as the number of standardised maintenance doses per day (DADDs). Trends in antimicrobial usage in pigs, cattle, and fur animals are presented in DAPD, which is the "proportion of population in treatment per day" estimated as number of DADD per 1,000 animals per day.

The total use of antimicrobials in animals amounted to approximately 97 tonnes of active ingredients in 2019, 3% less than in 2018. This is the lowest amount since 2002. A consistently decreasing trend has been observed since 2013. In 2019, 30 tonnes less was used than in 2010 (-24%).

The pig sector used approximately 75% of all veterinary-prescribed antimicrobials, equal to 72.6 tonnes active compound. Adjusting for changes in production and export of pigs in 2019, an estimated 2.3% of all pigs received antimicrobial treatment per day (23 DAPD), similar to the level in 2018. In weaner pigs, the annual use in 2019 compared to 2018 was reduced from 91 to 89 DAPD. However, in finishers it increased from 17 to 18 DAPD, while it remained similar to 2018 levels for sows and piglets (19 DAPD).

The use of medical zinc decreased by 7%, from 509 to 475 tonnes from 2018 to 2019, and the industry is still preparing for the EU withdrawal in June 2022.

The types of antimicrobials used in pigs changed notably. The use of tetracycline in pigs has decreased significantly since 2009, mainly from 2016 to 2019 as a response to the differentiated Yellow Card initiative. Similarly, colistin use was phased out by 2017. During the same period, discernible, but smaller, increases in the use of macrolides and aminoglycosides occurred, especially in weaners.

The overall use of antimicrobials in cattle has fluctuated between 12 and 13 tonnes over the past five years. In 2019, more than two thirds of the amount was used to treat older cattle (>1 year) and 4% of this amount was for intramammary treatment. The antimicrobial use for older cattle has decreased from 3.9 to 3.2 DAPD (-18%) over the past decade, while the use for younger cattle (<1 year) has increased from 5.2 to 7.3 DAPD (+39%).

Antimicrobial use in poultry was relatively low. Since 2015, the antimicrobial use in poultry decreased every year, however, in 2019 the use increased from 1,326 kg to 1,612 kg. The use of antimicrobials in the aquaculture industry decreased from 3,557 kg in 2018 to 2,522 kg in 2019, which probably reflects the relatively cold summer in 2019. Increased focus on prudent use combined with low occurrence of disease resulted in an observed 40% reduction in antimicrobial use for fur animals in 2018. In 2019, the use was 3,955 kg, which is 36% lower than in 2017, and is equivalent to a treatment proportion in the mink population of approximately 3% (32 DAPD).

Since 2011, there has been an overall decreasing trend in the use of antimicrobials in dogs and cats, with a marked reduction in the use of cephalosporins. However, critically important antimicrobials are still used in companion animals, and all fluoroquinolones and more than half of the cephalosporins used in animals are prescribed for dogs and cats.

Antimicrobial consumption in humans

Information on consumption in humans is based on total sales from primary pharmacies and hospital pharmacies, reported in volume per package size and transformed into defined daily doses (DDD), the assumed average maintenance dosage per antimicrobial drug as defined by the WHO ATC Center. Data has been reported from primary health care since 1994 and from hospital care since 1997. As per first of January 2019, the WHO ATC Center changed DDD values for several antibiotics, based on recommendations and results from an expert working group. From DANMAP 2018, the new DDD values were applied and all tables and figures were updated ten years back.

For comparison with actual treatment dosages used in Denmark, the DANMAP 2019 also presents consumption in Danish adjusted daily doses (DaDDD) for the primary sector and the hospital sector, respectively. When applying DaDDD, the share of each antibiotic class changed notably and was more correctly resembled in the total, overall showing a smaller use than with the standard DDD. However, to enable comparison with other countries standard DDDs as defined by the WHO were primarily used in the report.

In 2019, **total consumption** of antimicrobials in humans (primary sector and hospital sector combined) was 15.76 DDD per 1000 inhabitants per day (DID), lower than the consumption in 2018 (15.97 DID) and lower than a decade ago in 2010 (18.31 DID). The highest consumption ever reported in Denmark was in 2011 (18.95 DID). In 2010, the consumption of antimicrobial agents in **primary sector** was 16.56 DID, which since decreased to 13.76 DID in 2019 (-17%). The **hospital sector** simultaneously saw an increase from 1.65 DID in 2010 to 1.93 DID in 2019 (17%). When applied and corrected for hospital activity, total antimicrobial consumption at hospitals was 107 DDD per 100 bed-days (DBD) and 306 DDD per 100 admissions (DAD), a rise from 100 DBD and 296 DAD the previous year. From 2010 to 2019, the total consumption at hospitals increased by 49% and 21%, when measured in DBD and DAD, respectively. The proportion of antimicrobial agents prescribed by hospital doctors and redeemed at primary pharmacies increased from constituting 13.1% of the consumption in primary health care in 2015 to constitute 16.7% in 2019 (measured in DID).

Decreases in the past ten years described for the primary sector were observed for all age groups (but less pronounced for the eldest >80 years) and for both genders, regardless of the indicators used. However, the consumption in the 15-19 year olds increased by 3.5% since 2018, primarily driven by an increase in the consumption of tetracyclines. The biggest decrease in consumption the last decade was observed in the youngest (0 to 4 year olds), where the number of treated patients per 1000 inhabitants decreased by 41%. In 2019, there were 262 treated patients per 1000 0-4 year olds corresponding to 435 prescriptions redeemed per 1000 inhabitants.

Comparison of consumption by gender showed that in females, the number of treated patients per 1000 inhabitants decreased by 25% and in males by 28% the last decade. In 2019, the average number of treated patients (regardless of age) was 277 females per 1000 inhabitants and 191 males per 1000 inhabitants. This corresponds to 541 redeemed prescriptions per 1000 inhabitants for females and 347 redeemed prescriptions per 1000 inhabitants for males.

Penicillins remained the most frequently used antimicrobial agents in both primary health care (65%) and hospital care (55%), but the changes in consumption observed within this drug group in the last decade continued. Thus in 2010, beta-lactamase sensitive penicillins constituted 53% of all penicillins consumed in primary health care (5.26 DID of 9.91 DID), while in 2019 this had decreased to 38% (3.44 DID of 8.98 DID). Simultaneously, consumption of combination penicillins increased markedly in both sectors. In 2019, combination penicillins constituted 4.6% of the antimicrobial consumption in primary health care and 16% of the consumption in hospital care. From 2018 to 2019, decreased consumption in primary health care and increased consumption in hospital care were observed for most penicillins.

In Denmark, fluoroquinolones, cephalosporins and carbapenems are defined as **antimicrobials of critical interest** (cephalosporins and carbapenems are only used at hospitals). In 2019, the consumption of the three drug classes constituted altogether 18% of the total consumption at hospitals, a decrease from 20% observed the year before, and a decrease from 31% in 2010. Carbabenems increased from 2.83 DBD in 2018 to 2.99 DBD in 2019 (5.9%) whereas cephalosporins decreased from 10.24 DBD in 2018 to 9.29 DBD in 2019 (-9.2%). Fluoroquinolones continued the decreasing trend observed since 2015. In 2019, fluoroquinolones accounted for a consumption of 6.95 DBD, corresponding to 6.5% of the total consumption at hospitals (7.18 DBD and 7.1% in 2018). In primary care, fluoroquinolones accounted for 0.37 DID equivalent to 2.7% of the total consumption in 2019.

Resistance in zoonotic- and indicator bacteria

Isolates from animals and meat for susceptibility testing are mainly collected by repeated representative, national surveys conducted at Danish slaughterhouses and at retail. Isolates from humans are from clinical investigations, submitted to Statens Serum Institut for further typing.

In Denmark, antimicrobials are generally not recommended for treatment of self-limiting diarrhoea in humans including salmonellosis and campylobacteriosis. In prolonged or severe cases, treatment may be required and in these cases macrolides (azithromycin) and in hospital settings, ciprofloxacin are recommended. Macrolides are used to treat infections in animals in Denmark, whereas flourquinolones and cephalosporins are not used in food-producing animals.

Erythromycin (macrolide) resistance was present in 4% of ***Campylobacter jejuni*** isolates from humans with a known travel history. Erythromycin resistance was not observed in human isolates from domestic infections, Danish broiler meat, broilers and cattle. Resistance to quinolones remained common in *C. jejuni* isolates from humans, broiler meat, broilers and cattle. The levels of ciprofloxacin resistance was 70% in human isolates and 45% and 65% in broilers and meat hereof, respectively. Resistance towards ciprofloxacin were often accompanied by tetracycline resistance and 51% of the human isolates, and 39% and 30% of the broiler and broiler meat isolates, respectively, were tetracycline resistant.

Salmonella isolates from pigs and Danish pork were included in DANMAP 2019, and S. Derby and S. Typhimurium remained the most prevalent serotypes. The level of azithromycin resistance in ***Salmonella* Typhimurium** isolates was 1% in human isolates and 3% in isolates from Danish pork. Among human cases, resistance to fluoroquinolones was observed in 14% of *S.* Typhimurium isolates from travel-related cases and in 5% of the isolates from domestically acquired cases. Fluoroquinolone resistance has not been recorded in *S.* Typhimurium from Danish pigs and pork since 2010 and 2007, respectively. Resistance to 3rd generation cephalosporins and carbapenems was not observed in *S.* Typhimurium isolates from animals, food and domestically acquired human cases. Two percent of *S.* Typhimurium from travel-associated cases were resistant to 3rd generation cephalosporins. Carbapenem resistance was not observed.

Surveillance of resistance in animal pathogens was expanded to include pathogens from small animals and mastitis pathogens in addition to the usual porcine pathogens. Within all populations and pathogens, resistance levels remained fairly stable during the years of analysis.

In 2019, trends and levels of antimicrobial resistance in **indicator *E. coli*** from poultry, pigs and cattle were overall very similar to previous years.

The relative distribution of fully sensitive indicator *E. coli* from broilers (64%) and cattle (87%) was comparable to 2018, whereas a decrease from 53% to 42% was observed in fully sensitive isolates from pigs. Compared to 2018, a statistically significant increase (from 30% to 44%) in sulfonamide resistance and a moderate increase (from 23% to 32%) in occurrence of multidrug-resistance was observed in pig isolates. Among broiler and cattle isolates, the occurrence of multidrug-resistance was comparable to 2018 and only minor fluctuations (1%-2%) in resistance were observed between the years in cattle isolates. However, over the last 5-year period, the occurrence of multidrug-resistant *E. coli* isolates from broilers has decreased significantly (from 23% to 11%).

From broilers, one isolate (<1%) resistant to cefotaxime and ceftazidime was detected by the non-selective method. More phenotypic resistant isolates were detected by the more sensitive selective isolation methods in samples from pigs and cattle, indicating that resistance was present in a small proportion of commensal *E. coli* in pigs and cattle as well. Furthermore, no colistin, meropenem and tigecycline resistance was detected. The slow but steady increase in resistance to ciprofloxacin/nalidixic acid in *E. coli* from broilers, observed over the last ten years, continued.

In 2019, selective isolation methods showed that occurence of **ESBL/AmpC-producing *E. coli*** in animals and meat aligned with trends observed from 2015-2018: lower occurrence in cattle compared to pigs (8% vs. 27%), lower levels in domestically produced broiler meat and pork than in imported (5% and 3% vs. 34% and 42%, respectively), and a continued decreasing occurrence in Danish broiler meat. ESBL transferring enzymes often associated with human infections, such as CTX-M-1, CTX-M-14 and/or CTX-M-15 were detected in low or very low numbers in all sources, and chromosomal AmpC mutations in isolates from pigs and cattle were still dominant.

Importantly, again all samples examined for carbapenemase-producing *E. coli* (including OXA-48) were found negative.

In 2019, **enterococci** from pigs showed no resistance to vancomycin, linezolid, teicoplanin or tigecycline. The *E. faecalis* isolates were resistant to tetracycline (91%), erythromycin (63%) and chloramphenicol (33%), and compared to 2017 increases in tetracycline (13%) chloramphenicol (9%) and erythromycin (8%) resistance were observed. Resistance levels in *E. faecium* were comparable to the latest observations from 2010-2012 and the most commonly observed resistance was to tetracycline (54%), erythromycin (20%) and ampicillin (12%).

Currently, the zoonotic risk linked to transfer of resistance to critically important antimicrobials from animals to humans appears to be very limited in Denmark.

Resistance in human clinical bacteria

The national surveillance of resistance in human clinical bacteria are from two sources. Firstly, data from routine diagnostics performed at the 10 departments of clinical microbiology (DCMs) in Denmark extracted from the Danish Microbiology Database (MiBa). Secondly, isolates submitted to the reference laboratories at SSI due to voluntary extended surveillance of specific species and/or types or as mandatory surveillance of notifiable diseases under the Danish Health Authority.

Surveillance based on MiBa data

Since the beginning of DANMAP and particularly during the past decade the number of human invasive infections under surveillance has increased remarkably with 46% (8,021 cases in 2010 to 11,712 cases in 2019). The number of blood cultures taken as registered in MiBa increased equivalently with 113,986 unique patients' blood cultured in 2010 compared to 169,231 patients in 2019 an 48% increase.

For ***Escherichia coli***, the number of invasive cases increased from approximately 61.8 cases per 100,000 inhabitants in 2010 to 96.7 cases in 2019. A slow but steady increase in resistance to cephalosporins (cefuroxime and/or 3rd generation cephalosporins) was observed for both invasive cases and urinary cases through the decade. Increased resistance to piperacillin-tazobactam was observed from 2018 (3.8% in invasive cases) to 2019 (5.5% in invasive cases). No increases in the numbers of carbapenem resistant clinical *E. coli* cases were observed in 2019.

For ***Klebsiella pneumoniae***, the number of invasive cases increased from approximately 14.4 cases per 100,000 inhabitants in 2010 to 23.4 cases in 2019. The general trends for both invasive and urinary cases were declines in resistance rates to important antimicrobials as cephalosporins, gentamicin and ciprofloxacin through the beginning of the decade, slowing down or stagnating during the past five years. An increase in resistance to piperacillin-tazobactam was observed with 8.7% resistance reported in invasive cases in 2019 compared to 6.1% in 2018.

Regarding invasive ***Pseudomonas aeruginosa*** the situation in Denmark is quite stabile both in the number of cases and resistance profiles, with relatively low overall prevalence of resistance. The highest level of resistance was reported for ciprofloxacin with 5.5%. Meropenem resistance was reported in 3.1% of the cases, and in only 1.7% of the cases, resistance to three or more of the five antimicrobials under surveillance were reported. None of the invasive *P. aeruginosa* isolates were registered as colistin resistant.

Approximately one invasive case of ***Acinetobacter* species** per 100,000 inhabitants was observed in 2019 corresponding to a low number of 72 invasive cases in total. Of these, five isolates were resistant to ciprofloxacin, two were resistant to gentamicin and none was resistant to meropenem or had combined resistance to ciprofloxacin and gentamicin.

The number of invasive cases of ***Enterococcus faecium*** increased from 9.4 cases per 100,000 inhabitants in 2010 to 13.6 cases in 2018 followed by a small decrease to 12.7 cases in 2019. For invasive ***Enterococcus faecalis***, the number remained stable with 10.7 cases per 100,000 inhabitants in 2010 and 10.9 cases in 2019. Over the decade, there has been an alarming increase in invasive cases of vancomycin-resistant and -variable *E. faecium*. While 0.5% of invasive *E. faecium* were reported vancomycin resistant in 2008, this rate increased to 12% in 2018, but with a small decrease to 11% in 2019.

Surveillance based on data from the reference laboratories

Since 2014, the DCMs have voluntarily submitted 3rd generation cephalosporin resistant *E. coli* isolates from bloodstream infections to SSI for characterisation. In 2019, a total of 375 *E. coli* isolates from bloodstream infections were identified with phenotypic test as **ESBL, AmpC or carbapenemase-producing** isolates. As in previous years, CTX-M-15 was the most prevalent resistance-transferring enzyme, although a decrease in prevalence was observed compared to 2018, while the prevalence of CTX-M-14 and CTX-M-55 increased. In 2019, a new *E. coli* ST23 CTX-M-14 -producing clone was reported by seven of the ten DCMs, primarily from emergency departments.

In recent years, the DCMs have voluntarily submitted carbapenem-resistant isolates (both clinical and screening) for verification and genotyping at the National Reference Laboratory for Antimicrobial Resistance at SSI. As of 5 September 2018, the Danish Health Authority made **carbapenemase-producing organisms (CPO)** notifiable. During 2019, 221 CPOs were detected from 187 patients compared with 177 CPOs from 160 patients in 2018, equivalent to a 25% overall increase of submitted CPO isolates compared to 2018. More than one isolate from the same patient were included in DANMAP, if the isolates belonged to different bacterial species and/or if the isolates harboured different carbapenemases. The 221 CPOs consisted of 194 CPEs, 20 *Acinetobacter* spp. and seven *Pseudomonas* spp. New patients were registered in 16 ongoing CPE outbreaks in 2019.

In recent years, *E. faecium* isolates harbouring the *vanA* gene complex, but phenotypically vancomycin susceptible, have been reported from different countries. These enterococci are referred to as **vancomycin-variable enterococci (VVE)**. In 2017, VVE isolates were included in the voluntary **vancomycin-resistant *Enterococcus (VRE)*** surveillance. However, VVE diagnostics differ substantially between the different regions and an underestimation of the prevalence could exist. Furthermore, not all VRE/VVE isolates are received at SSI, and for the 2016 to 2019 reports, the number of submitted isolates was supplemented with the number of VRE/VVE registered in MiBa. This resulted in a total of 660 VRE/VVE isolates from 660 patients in 2019 compared to 603 VRE isolates from 599 patients in 2018. Since 2013, a steep increase in clinical VRE isolates has been observed. Until 2018 the increase has mostly been seen for *vanA E. faecium*, but during 2019, an increase was also detected for *vanB E. faecium*.

Streptococcus pneumoniae from invasive pneumococcal disease (IPD) and resistance towards penicillin and erythromycin have been included in DANMAP since the beginning. Of the 600-800 cases annually, bacteraemia is most common and meningitis only counts for 40-60 of the cases. Antimicrobial susceptibility in *S. pneumoniae* is closely related to serotypes. The distribution of IPD-associated serotypes are again influenced by the PCV vaccines, introduced in the childhood immunisation programme in Denmark in 2007 and enforced in 2010. Some serotypes also have natural cycles over the years. In 2019, 623 cases of IPD from blood or spinal fluid were registered and isolates were received from 606 of the cases. The dominant serotype was serotype 8 (26.7%) and they were

all found susceptible to both penicillin and erythromycin. In total, susceptibility to penicillin was 95.1% susceptible, 4.2% susceptible increased exposure and 0.7% resistant. For erythromycin, 96.6% were susceptible and 3.4% were resistant.

The surveillance of invasive infections caused by **beta-haemolytic streptococci (BHS)** in Denmark is based on voluntary submission of invasive isolates from the DCMs. During the last five years, the number of received isolates of BHS from unique cases of infection has increased from 556 in 2014 to 873 in 2018, followed by a minor decrease to 838 in 2019. The corresponding changes for individual serogroups were: group A, -18%; group B, -2%; group C, +5% and group G, +0.3%. All isolates were susceptible to penicillin. The erythromycin resistance rate as well as the clindamycin resistance rate remained unchanged compared to 2018 for all four serogroups.

Surveillance of invasive ***Haemophilus influenzae*** is mandatory for type b, but isolates of all types are voluntarily submitted to the reference laboratory at SSI. In 2019, 114 cases of invasive *H. influenzae* were identified. This is at the same level as previous years, which ranged from 106 to 128 cases in the period from 2014 to 2018. Of the 114 cases, isolates from 100 cases were received for serotyping and antimicrobial susceptibility testing at SSI and data were supplemented with registered cases in MiBa. The majority of cases were non-capsular (78%), and six isolates were of type b. Resistance to penicillin was observed in 29% of the cases and 24% were resistant to ampicillin, 14% to cefuroxime and 11% to amoxicillin-clavulanic acid, which is similar to data from 2018. Whole-genome sequencing data was available from 93 isolates, and nineteen of these possessed the TEM-1 gene for beta-lactamase. None had the ROB-1 gene.

The number of bloodstream infections with ***Staphylococcus aureus*** was 2,233 cases in 2019 compared to 2,276 cases in 2018; 2.1% of cases were methicillin-resistant (MRSA). Resistance to other antimicrobials remained at the same level as in previous years. The number of new **methicillin-resistant *Staphylococcus aureus* (MRSA)** cases (colonisation or infection) was 3,657 in 2019, close to the number in 2018 (3,669). The number of cases acquired in hospitals (HA-MRSA) remained at a low level (75 cases in 2019); whereas community acquired (CA-) MRSA continued to increase (1,536 in 2019). The number of livestock-associated (LA-) MRSA CC398 decreased to 1,122 cases from 1,215 in 2018 and most of these were found in patients with contact to livestock production (89%). The number of LA-MRSA infected persons without livestock contact was 86 in 2019 and has been stable in recent years, indicating low secondary transmission.

The national surveillance of antimicrobial resistance in ***Neisseria gonorrhoeae*** in Denmark is based on the voluntary submission of gonococcal isolates from the DCMs. From 2011 to 2016, the annual number of received isolates increased, followed by a decrease in 2017 and 2018, and a slight increase in 2019. Concomitantly with these changes, the rate of ciprofloxacin resistance decreased to 18% in 2016 and increased to 41% in 2019 (40% in 2018). Ceftriaxone resistance has not been observed in Denmark, apart from one case in 2017 with a marginally increased MIC (0.25 mg/L).

Future improvements and developments

DANMAP demonstrates that a well-established integrated surveillance programme is important to understand the development of AMR and to point out where prudent use of antimicrobials is necessary.

Antimicrobial use in humans and food animals is relatively low and well regulated in Denmark compared to many other countries in the EU and the rest of the world. This contributes to fairly stable resistance patterns in production animals and in Danish meat compared to the big reductions observed when growth promoters were banned in the 90s.

Over the last decade, we have observed increasing numbers of multi-resistant bacteria in humans and introduction of new critical resistance types such as different ESBLs in food animals and Danish meat. International travel and trade plays an important part in introducing new bacteria and resistance in the Danish populations, where they may propagate and spread. Monitoring critically resistant bacteria such as MRSA, ESBL, CPE and VRE in all relevant reservoirs provides essential information on when and where control measures are needed.

This DANMAP report provides a robust overview of the status on antimicrobial use and antimicrobial resistance in Denmark in 2019 from a One Health perspective. The long history of the report adds certainty to its conclusions, whilst the DANMAP programme continues to evolve and develop as new opportunities and challenges appear.

3

INTRODUCTION TO DANMAP

3. Introduction to DANMAP

3.1 The DANMAP surveillance system

DANMAP is a surveillance system with five key objectives:

- To establish the state-of-nation in regards to the use of antimicrobial agents in food animals and humans
- To carry out surveillance for the occurrence of antimicrobial resistance in bacteria isolated from food animals, food of animal origin (e.g. meat) and humans
- To identify areas for further research e.g. transmission or regarding possible associations between antimicrobial consumption and antimicrobial resistance
- To deliver data to veterinarians, medical doctors and other health professionals for the development of antibiotic guidelines for treatment
- To act as a knowledge base for authorities, academia and politicians when performing risk assessment and management, thus supporting decision making in the prevention and control of resistant bacterial infections

The monitoring programme was initially developed in 1995 by researchers, based on frequent discussions and exchange of knowledge and results from research. Since then, DANMAP has evolved into a governmentally supported organisation. However, much of the design of the programme, including participation of the human laboratories and referral of strains is based on a voluntary principle.

DANMAP surveillance relies on four equally important components: well-established and well-functioning diagnostic systems, well-designed and representative surveys, reliable registers as well as mutual trust and openness between all collaborators.

A positive effect of the regular meetings and exchange between stakeholders is that these prove helpful in other aspects, for example, by contributing to a common knowledge pool regarding laboratory methods. This ensures and contributes to continuous improvements and harmonisation of the laboratory work. Meetings across sectors and between different stakeholders also contribute to a better mutual understanding, facilitating development and work towards mutual goals.

Surveillance is a complex undertaking and DANMAP encompasses many different surveillance components and covers resistance in different populations and contexts.

Three categories of bacteria are always included in DANMAP:

- Human clinical isolates to reflect the antimicrobial resistance levels in the human population that seeks medical care
- Foodborne zoonotic bacteria along the whole farm-to-patient chain to monitor the levels of antimicrobial resistance in shared pathogens
- Indicator bacteria from healthy food-producing animals to monitor status of antimicrobial resistance in the animal reservoirs.

The National Food Institute (situated at Technical University of Denmark, DTU) and Statens Serum Institut (SSI) are responsible for data interpretation and output communication mainly via the annual DANMAP report. Interpretations are independent of policy, risk management and private industries.

The DANMAP programme is funded jointly by the Ministry of Health and the Ministry of Environment and Food. Support from the ministries has also helped build the databases and ensuring the registers, which the current surveillance system relies upon.

For further information on the development and history of DANMAP, please read chapter 2, "DANMAP – A 20 year perspective" in DANMAP 2015.

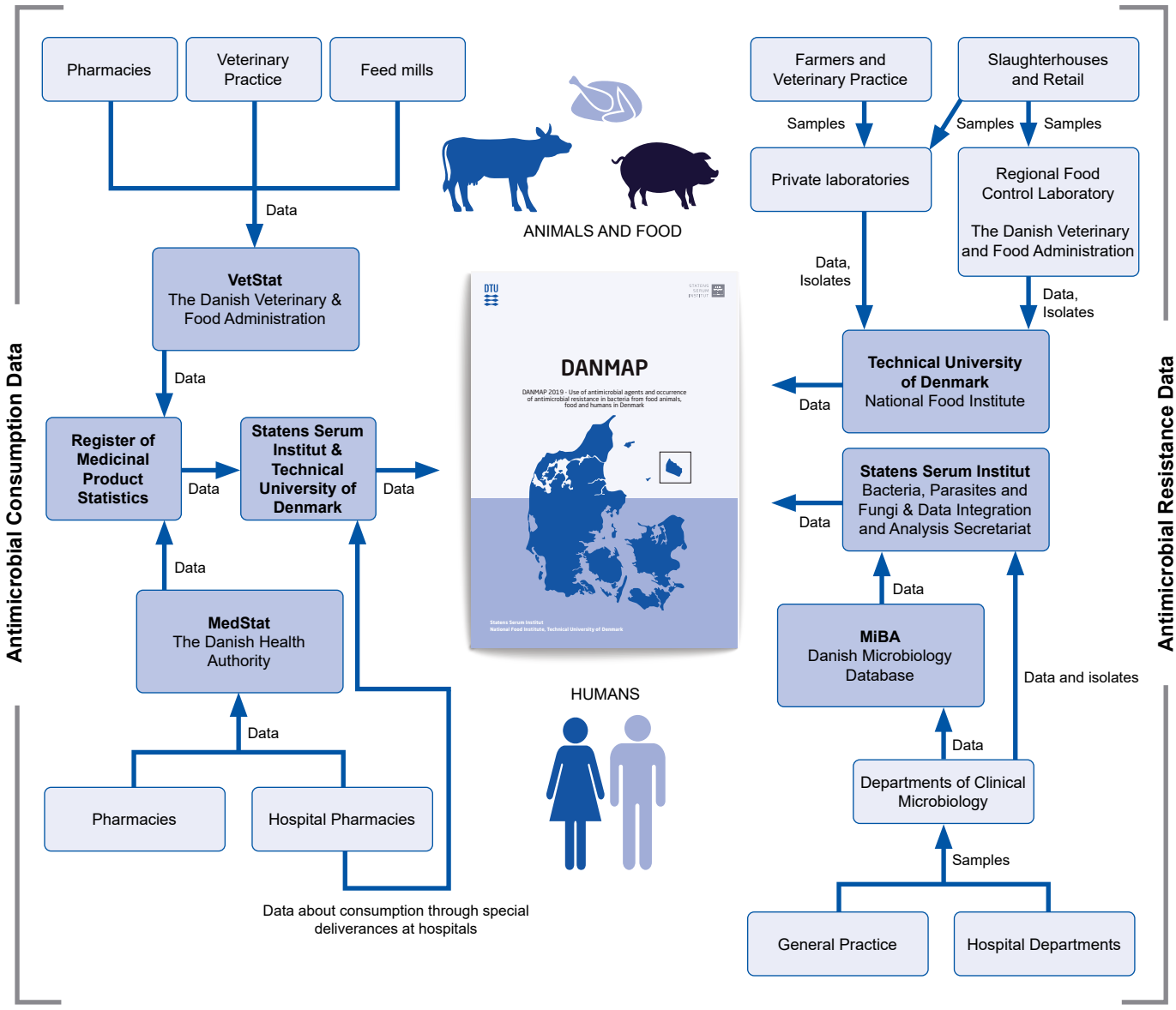
Organisation and data flow

Since 1995, a main purpose of DANMAP has been to monitor the entire chain from farm to fork to patient. The organisation and collection of DANMAP data and the interdisciplinary collaboration between sectors and organisations is presented in Figure 3.1.

The recent introduction of whole genome sequencing (WGS) has been a big step forward for surveillance purposes and in outbreak situations and has become routine standard in most reference laboratories. Whereas, phenotypical testing is still considered relevant, more feasible, cheaper and sometimes faster, especially in a clinical setting. Phenotypical testing also continues being used in combination with WGS to describe and determine which resistance genes are relevant to look for when using molecular analysis. Furthermore, it complies with EU regulations in food and animal testing.

Bacterial isolates from food, food animals and humans are submitted to the Regional Food Control Laboratory or occasionally the Technical University of Denmark and Statens Serum

Figure 3.1 Organisation of the DANMAP collaboration regarding data and data flow DANMAP 2019



Institut, respectively, for further phenotypic and genotypic characterisation (Figure 3.1). In 2019, WGS was extensively performed on selections of single isolates. These isolates were analysed for clonal relationship, as well as antimicrobial resistance genotypes (including ESBL, AmpC and CPO), and the presence of mobile elements such as plasmids. When specific clones carrying the same antimicrobial resistance genes are found in common among both food and human isolates, genomic data analysis such as core genome multilocus sequence typing (cgMLST) and single nucleotide polymorphism (SNP) calling, are used to examine possible transmission between the reservoirs. The choice of the methods in surveying different bacteria and infections is described in more detail in the different chapters and sections of the report.

3.2 Information on demographics and food production

The following sections present some general information for 2019 about the human population in Denmark and the included food production sectors. It also provides an overview of the antimicrobial agents used for therapeutic purposes in humans, and systemic and intramammary administration in animals in 2019.

3.2.1 Populations and productions

Human population and healthcare system

During the past two decades, the human population in Denmark has increased from approximately 5.2 million inhabitants in 1995 to 5.8 million in 2019 [www.dst.dk]. Simultaneously, the average age has increased gradually. In 2019, the national average age was 41.7 years. The population and the respective regional distribution, in 2019, is presented in Figure 3.2, while regional differences and changes in age are presented in Figure 3.3.

In Denmark, microbiological analyses are carried out by ten departments of clinical microbiology (DCMs) altogether, situated at the main regional hospitals in Denmark, also represented in Figure 3.2. The analyses performed cover all samples from public hospitals and most samples from general practitioners (GPs). In addition, some GPs perform culturing of urinary samples from their patients. In the Capital Region of Denmark one private laboratory also performs additional analyses for the GPs.

Figure 3.2 The five Danish healthcare regions and their respective population distributions. In addition, the ten DCMs are marked by black dots. The grey dot indicates the reference laboratories situated at SSI DANMAP 2019

North Denmark Region

No. of inhabitants	589,755
No. of inhabitants/km^2	75
No. of inhabitants/GP	1,934

Central Denmark Region

No. of inhabitants	1,320,678
No. of inhabitants/km^2	102
No. of inhabitants/GP	1,554

Capital Region of Denmark

No. of inhabitants	1,835,562
No. of inhabitants/km^2	717
No. of inhabitants/GP	1,700

Region Zealand

No. of inhabitants	836,738
No. of inhabitants/km^2	116
No. of inhabitants/GP	1,831

Region of Southern Denmark

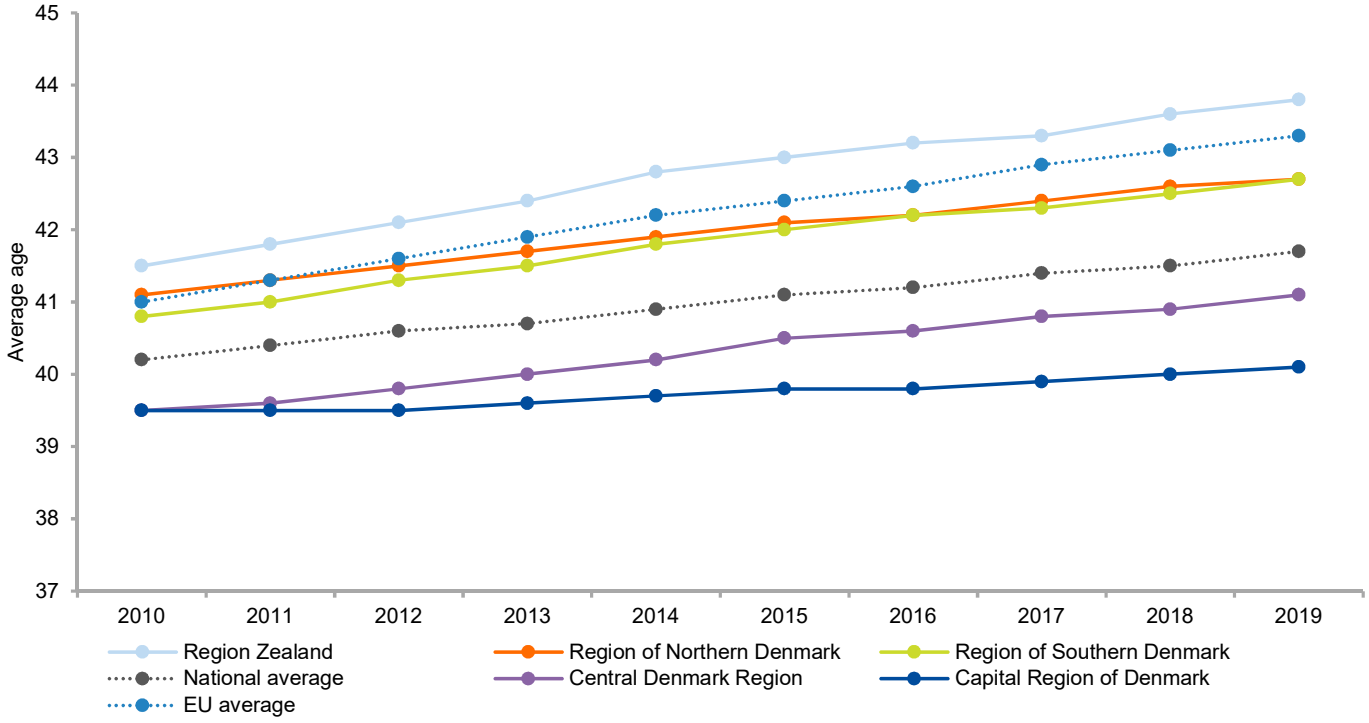
No. of inhabitants	1,223,348
No. of inhabitants/km^2	100
No. of inhabitants/GP	1,494

GP = general practitioner

DCM = department of clinical microbiology

Source: Statistics Denmark [www.dst.dk] and the Danish Medical Association [www.laeger.dk]

Figure 3.3 Changes in regional distribution of average age, Denmark DANMAP 2019



Animal population and food production system

Denmark is an agricultural country, with more than half of its area managed by the agricultural sector. The agricultural sector contributes to employment with around 146,000 jobs in the primary production and processing, and contributes around 24% of the Danish export earnings. Livestock is of great importance and approximately 25% of the agricultural enterprises are specialised in the production of livestock mainly pigs, cattle, chicken and mink [Danish Agriculture and Food Council, 2019].

The production of food animals and the production of meat and milk are presented in Table 3.1 and 3.2. In 2019, the number of pigs produced decreased by approximately 2% compared to 2018, and the number of exported fattening pigs (15-50 kg) continued to increase by approximately 3%.

Since 2004, the total exports of fattening pigs have increased more than seven-fold [Statistics Denmark, Danish Agriculture and Food Council, 2020].

The size of the Danish cattle production has changed slightly between years, and from 2018 to 2019, the number of dairy cows and cattle slaughtered decreased by 2-3%, while the amount of milk produced remained at the same level [Statistics Denmark 2020]. The number of broilers produced increased by 1% and approximately 16% of the broilers produced in Denmark in 2019 were exported for slaughter. The production of turkeys has fluctuated considerably over the past decade. Since 2006, more than 99% of the turkeys produced have been exported for slaughter, thus the majority of turkey meat available for sale in Denmark is listed as imported.

Table 3.1 Production (1000' heads) of food animals and mink, Denmark DANMAP 2019

Year	Pigs		Cattle		Poultry		Fur animals - mink	
	Total	Exported[a]	Slaughter cattle	Dairy cows	Broilers	Turkeys	Females	Kits
2010	28505	7074	519	574	117653	1184	2657	14638
2011	29399	7632	551	575	115454	960	2776	15325
2012	29047	8794	539	580	111080	1103	2936	16147
2013	28996	9318	551	574	117315	692	3143	17634
2014	29926	10517	556	563	115497	595	3296	17963
2015	30874	11563	513	561	114238	598	3387	18798
2016	31660	12771	540	571	120685	834	3161	17260
2017	31662	13679	509	570	117602	601	3410	18378
2018	32558	14028	533	575	122268	642	3385	17634
2019	31758	14542	518	567	123976	661	2495	13224

Source: Statistics Denmark [www.dst.dk] and The Kopenhagen Fur. Export data for poultry from Statistics Denmark (personal communication) and export of 15-50 kg live pigs from the Danish Agriculture and Food Council
a) Export of 15-50 kg live pigs. These are included in total number of heads, but antimicrobial use after export until slaughter is not registered as it takes place outside of Denmark

Table 3.2 Production (mill kg) of meat, milk and fish, Denmark DANMAP 2019

Year	Pork	Beef	Broiler meat[a]	Turkey meat	Milk	Farmed fish[b]	
						Land based	Marine net ponds
2010	1974	142	178	14	4830	42	11
2011	2008	145	175	9	4801	40	11
2012	1902	138	168	12	4928	44	14
2013	1896	140	177	8	5025	48	15
2014	1924	143	174	9	5113	47	14
2015	1954	135	172	9	5278	52	16
2016	1943	142	182	10	5376	49	12
2017	1896	135	178	7	5478	51	14
2018	1967	141	185	10	5615	53	14
2019	1870	137	187	8	5615	53	14

Source: Statistics Denmark [www.dst.dk]. Export data for poultry and average weight after slaughter from Statistics Denmark (personal communication)
a) In 2019, a final slaughtered weight of 1.62 kg per broiler produced and 12.0 kg per turkey produced were assumed
b) The numbers for 2019 are not final. Data now based on accounts statistics for aquaculture. The production of farmed fish includes fish transferred from one production facility to another

3.2.2 Registered antimicrobial agents

Table 3.3 shows the antimicrobial agents registered to treat bacterial infections in humans and animals respectively. Some of these are listed on the highest priority list of critically important antimicrobial agents for the treatment of bacterial infections in humans, according to definitions made by a working group under the World Health Organization [AGISAR, 6.revision, WHO 2019]. In order to be considered critically important, an antimicrobial must meet two criteria; 1) be the only - or one of a limited number of compounds available to treat serious human disease and 2) be used to treat infections caused by bacteria that are either possibly transmitted from non-human sources, or carry resistance genes from non-human sources.

In the newest revision from 2019, five drug classes were considered critically important and of highest priority: fluoroquinolones, 3rd, 4th and 5th generation cephalosporins, macrolides, glycopeptides and polymyxins. In addition, in Europe carbapenems are not allowed to be used in food production. In Denmark, the use of these drug classes in food animals has in general been low or been reduced through either voluntary or legislative restrictions, apart from macrolides, see chapter 4 for more information.

Furthermore, other antimicrobials may also be restricted due to national risk mitigation. For trends and preferred therapeutic choices in the antimicrobial treatment of humans and information on the national action plan from 2017, see chapter 5.

Growth promoters are no longer used for animals in Denmark and are shown in parentheses in Table 3.3. Most of these influenced Gram-positive bacteria. Since 1995, the indicator enterococci from animals and meat have been used as a measure of resistance towards former growth promoters.

Table 3.3 Antimicrobial agents registered for systemic and veterinary intramammary therapeutic use in animals and humans, Denmark DANMAP 2019

ATC / ATCvet codes[a]	Therapeutic group	Antimicrobial agents within the therapeutic groups	
		Animals	Humans
J01AA / QJ01AA, QJ51AA	Tetracyclines	Chlortetracycline, doxycycline, oxytetracycline	Doxycycline, lymecycline, tetracycline, tigecycline
QJ01BA	Amphenicols	Florfenicol	
J01CA / QJ01CA	Penicillins with extended spectrum	Ampicillin, amoxicillin	Ampicillin, pivampicillin, amoxicillin, pivmecillinam, mecillinam
J01CE / QJ01CE	Beta-lactamase sensitive penicillins	Benzylpenicillin, phenoxymethylpenicillin, procaine penicillin, penethamate hydroiodide	Benzylpenicillin, phenoxymethylpenicillin
J01CF / QJ51CF	Beta-lactamase resistant penicillins	Cloxacillin, nafcillin	Dicloxacillin, flucloxacillin
J01CR / QJ01CR	Comb. of penicillins and beta-lactamase inhibitors	Amoxicillin/clavulanate	Amoxicillin/clavulanic acid, piperacillin/tazobactam
J01DB / QJ01DB, QJ51DB	1st generation cephalosporins	Cefalexin, cefadroxil, cefapirin	Cefalexin, cefazolin
J01DC	2nd generation cephalosporins		Cefuroxime
J01DD / QJ01DD, QJ51DD	3rd generation cephalosporins incl. comb. with beta-lactamase inhibitors	Cefoperazone, ceftiofur, cefovecin	Cefotaxime, ceftazidime, ceftriaxone, ceftazidime/avibactam
J01DE / QJ51DE	4th generation cephalosporins	Cefquinome	Cefepime
J01DF	Monobactams		Aztreonam
J01DH	Carbapenems		Meropenem, ertapenem
J01DI	5th generation cephalosporins incl. comb. with beta-lactamase inhibitors		Ceftaroline fasamil, ceftolozan/tazobactam, ceftobiprol
J01EA	Trimethoprim and derivatives		Trimethoprim
J01EB / QJ01EQ	Short-acting sulfonamides	Sulfadimidine	Sulfamethizole
J01EE / QJ01EW	Combinations of sulfonamides and trimethoprim, incl. derivatives	Sulfadiazine/trimethoprim, sulfadoxine/trimethoprim, sulfamethoxasol/trimethoprim	Sulfamethoxazole/trimethoprim
J01FA / QJ01FA	Macrolides	Spiramycin, tylosin, tilmicosin, tylvalosintartrat, tulathromycin, gamithromycin, tildiprocin	Erythromycine, roxithromycine, clarithromycine, azithromycine, telithromycine
J01FF / QJ01FF	Lincosamides	Clindamycin, lincomycin	Clindamycin
QJ01XX[b]	Streptogramins	(Virginiamycin)	
J01GB / QJ01RA, QA07AA	Aminoglycosides	Streptomycin, dihydrostreptomycin, gentamicin, neomycin, apramycin, paromomycin	Tobramycin, gentamicin
J01MA / QJ01MA	Fluoroquinolones	Enrofloxacin, marbofloxacin, difloxacin, ibafloxacin, pradofloxacin	Ciprofloxacin, levofloxacin, moxifloxacin
QJ01MB	Other quinolones	Oxolinic acid	
QJ01MQ[b]	Quinoxalines	(Carbadox, olaquindox)	
J01XA, A07AA / Not in ATCvet[b,c]	Glycopeptides	(Avoparcin)	Vancomycin, teicoplanin, dalbavancin, oritavancin
J01XB / QA07AA[b]	Polypeptides (incl. polymyxins)	Colistin, bacitracin	Colistin
J01XC	Steroid antibacterials		Fusidic acid
J01XD,P01AB[c]	Imidazole derivatives		Metronidazole
J01XE	Nitrofurane derivatives		Nitrofurantoin
J01XX / QJ01FF	Other antibacterials	Spectinomycin	Methenamine, linezolid, daptomycin, tedizolide, fosfomycin
QJ01XQ	Pleuromutilins	Tiamulin, valnemulin	
QP51AG04	Antiprotozoals, sulfonamides	Sulfaclozine	
Not in ATCvet[b]	Oligosaccharides	(Avilamycin)	
Not in ATCvet[b]	Flavofosfolipols	(Flavomycin)	

a) ATCvet codes start with a Q
b) Animal growth promoters used before 1999 are listed in parentheses
c) Intestinal antiinfectives (A07AA) and imidazole derivatives for protozoal diseases (P01AB) were, for the first time, included in DANMAP 2014, since their widespread use in the treatment of *Clostridium difficile* infections makes them belong to the most used antibiotics in human infections in Denmark

4

ANTIMICROBIAL CONSUMPTION IN ANIMALS

4. Antimicrobial consumption in animals

Highlights: The total use of antimicrobials in animals amounted to approximately 97 tonnes of active ingredients in 2019, 3% less than in 2018. This is the lowest amount since 2002. A consistently decreasing trend has been observed since 2013. In 2019, 30 tonnes (-24%) less was used than in 2010.

The pig sector used approximately 75% of all veterinary-prescribed antimicrobials, equal to 72.6 tonnes active compound. Adjusting for changes in production and export of pigs in 2019, an estimated 2.3% of all pigs received antimicrobial treatment per day (23 DAPD), similar to the level in 2018. In weaner pigs, the annual use in 2019 compared to 2018 was reduced from 91 to 89 DAPD. However, in finishers it increased from 17 to 18 DAPD, while it remained similar to 2018 levels for sows and piglets (19 DAPD).

The use of medical zinc decreased by 7%, from 509 to 475 tonnes from 2018 to 2019, and the industry is still preparing for the EU withdrawal in June 2022.

The types of antimicrobials used in pigs has changed notably. The use of tetracyclines in pigs has decreased significantly since 2009, mainly from 2016 to 2019 as a response to the differentiated Yellow Card initiative. Similarly, colistin use was phased out by 2017. During the same period, discernible, but smaller, increases in the use of macrolides and aminoglycosides occurred, especially in weaners.

The overall use of antimicrobials in cattle has fluctuated between 12 and 13 tonnes over the past five years. In 2019, more than two thirds of the amount was used to treat older cattle (>1 year) and 4% of this amount was for intramammary treatment. The antimicrobial use for older cattle has decreased from 3.9 to 3.2 DAPD over the past decade, while the use for younger cattle (<1 year) has increased from 5.2 to 7.3 DAPD.

Antimicrobial use in poultry was relatively low. Since 2015, the antimicrobial use in poultry decreased every year, however, in 2019 the use increased from 1,326 kg to 1,612 kg. The use of antimicrobials in the aquaculture industry decreased from 3,557 kg in 2018 to 2,522 kg in 2019, which probably reflects the relatively cold summer in 2019.

Increased focus on prudent use combined with low occurrence of disease resulted in an observed 40% reduction in antimicrobial use for fur animals in 2018. In 2019, the use was 3,955 kg, which is 36% lower than in 2017, and is equivalent to a treatment proportion in the mink population of approximately 3% (32 DAPD).

Since 2011, there has been an overall decreasing trend in the use of antimicrobials in dogs and cats, with a marked reduction in the use of cephalosporins. However, companion animals still use critically important antimicrobials, and all fluoroquinolones and more than half of the cephalosporins used in animals are prescribed for dogs and cats.

4.1 Introduction

The DANMAP programme began monitoring the national use of antimicrobial agents in humans and animals in 1995. Since the early 1990s, there has been both political and public focus on the consumption of antimicrobial agents in the Danish animal production, which resulted in the discontinued use of antimicrobial agents for growth promotion in the years 1995-1999. The focus on antimicrobial use has continued to increase, more recent initiatives include a voluntary ban on the use of cephalosporins in the pig and cattle production as well as regulatory legislation regarding therapeutic use.

Figure 4.1 shows the total use of antimicrobials in animals and humans since 1990 and 1997, respectively. Changes in the patterns of antimicrobial use in animals can be explained in part by an increase in pig production over the years, but risk management measures to reduce consumption have also contributed to these changes. In addition, the increasing export of pigs at 30-40 kg live weight has also affected the overall use of antimicrobials in animals.

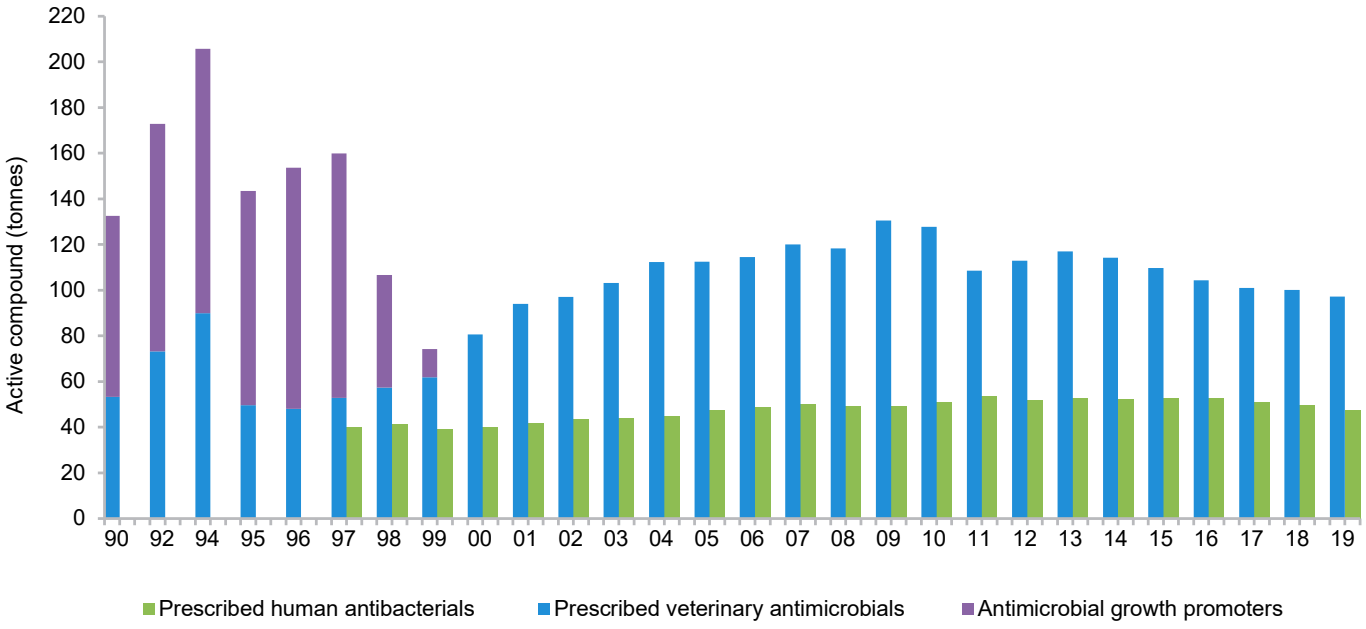
In some periods, the prescription patterns concerning animals were clearly influenced by risk management decisions. For example, the decrease in antimicrobial consumption after 1994 was likely the result of 1) limitation of veterinary practitioners' profit from sales of medicine; 2) implementation of Veterinary Advisory Service contracts (VASCs) with regular visits from the veterinarian in order to promote preventive veterinary strategies and optimize antimicrobial use; and 3) enforcement of the so-called "cascade rule" [Order (DK) 142/1993], limiting the use of (cheaper) extemporaneously produced medicines. To reduce the need for disposal of excess antimicrobials, veterinarians and pharmacies were permitted to split packages of veterinary medicine as from 1 January 2019 [Order (DK) 1655/2018]. This initiative also enhances surveillance by reducing the difference between amounts of antimicrobials prescribed and amounts used.

Other important interventions were the restriction on the use of fluoroquinolones in production animals through legislation implemented in 2002 and 2003, and the voluntary ban on the use of cephalosporins in the pig industry in 2010 followed by a similar initiative in the dairy cattle industry in 2014. From 1 September 2019, the cattle industry implemented a ban on use of 3rd and 4th generation cephalosporins for cattle.

The national action plan against antimicrobial resistance has had several goals throughout time. Initially, a 10% reduction of antimicrobial use in farm animals by 2014 compared to the 2009 level was set as a national target. Furthermore, in 2015 the national action plan to reduce livestock-associated MRSA called for a 15% reduction in antimicrobial use in pigs from 2015 to 2018.

To achieve the action plan goals, the Yellow Card initiative was established in 2010, introducing surveillance at herd level and instating threshold values for antimicrobial use in individual herds to enable legal action on pig farmers with high antimicrobial use per pig [DANMAP 2010]. As a result, a distinct decrease in consumption was observed from 2010 to 2011.

Figure 4.1 Prescribed antimicrobial agents for humans and all animal species, tonnes of active compound, Denmark DANMAP 2019



Sources: Human therapeutics: The Danish Medicines Agency. Antimicrobials for animals: Data are based on reports from the pharmaceutical industry of total annual sales (until 2001), from the Federation of Danish pig producers and slaughterhouses (1994-1995), from the Danish Medicines Agency and Danish Plant Directorate (1996–2000), and since 2001 from VetStat. For DANMAP 2019, consumption data were extracted from VetStat on 3 March 2020 and include all antimicrobial agents registered for use in animals

Effects from other parts of the legislation may be less obvious but are also likely to have affected prescription patterns. As an example, the rules for group medication in pig herds were tightened in 2014 [Order (DK) 534 of 27/05/2014], calling for thorough laboratory diagnoses and frequent veterinary visits before and during prescription of antimicrobials for groups of pigs rather than individuals (See Textbox 4.1).

In 2016, the Yellow Card initiative was revised, adding on multiplication factors to adjust the consumption of certain antimicrobials. Fluoroquinolones, cephalosporins and colistin (added in 2017) were given the highest multiplication factor of 10. Tetracyclines were multiplied by 1.2, and the factor was increased to 1.5 in 2017 [DANMAP 2017].

In 2017, the Ministry of Environment and Food in Denmark and the Ministry of Health in Denmark presented a new One Health strategy against antimicrobial resistance, setting the framework for reducing the development and occurrence of antimicrobial resistance (AMR).

At the same time, two national action plans to reduce AMR were introduced, setting specific targets to further reduce the antimicrobial use for both humans and animals in the coming years. As part of the political agreement on the veterinary strategy 2018-2021 (Veterinærforlig III), an Advisory Committee on Veterinary Medicines was established in 2018.

Official treatment guidelines for pigs and cattle have been available since 1996. The guidelines provide specific recommendations for selection of the appropriate antimicrobial treatment of all common problems in the major production animal species. Since 2005, the Danish Veterinary and Food Administration (DVFA) has updated the guidelines in collaboration with stakeholders and university experts. The guidelines were updated in 2010, when new dynamic evidence-based treatment guidelines for pigs were launched [DANMAP 2010, www.fvst.dk], and a revised version was published in April 2018.

In 2012, to promote prudent use of antimicrobials in dogs and cats the Danish Veterinary Association (DVA) published treatment guidelines developed by clinical specialists and experts from the Faculty of Health and Medical Sciences at the University of Copenhagen and the National Food Institute, Technical University of Denmark.

Revised treatment guidelines for dogs and cats were published in 2018. Similarly, DVA published treatment guidelines for use of antimicrobials in horses in 2017.

4.1.1 Data sources

In Denmark, antimicrobials are available by prescription only, and data on antimicrobial use have been collected in Denmark since 1990.

Since 2001, data on all medicines prescribed for use in animals, including vaccines, antimicrobial growth promoters (no longer permitted) and coccidiostatic agents (non-prescription) have been recorded in the national database VetStat. Since 2010, the VetStat database is hosted and maintained by DVFA. The 2019 data presented in this report were extracted from VetStat on 3 March 2020 and have been analysed and interpreted for DANMAP by the National Food Institute, Technical University of Denmark.

4.1.2 Methods

Metrics of antimicrobial use are numerous, each with its own advantages and limitations. Therefore, the selection of metrics used for monitoring must depend on the monitoring objective and the information available.

The overall amount of antimicrobial agents is measured in kg active compound and is used in section 4.2 for the purpose of an overall crude comparison of antimicrobial use in the veterinary and human sectors (Figure 4.1).

Since 2012, we have further presented "defined animal daily dose" (DADD) and "proportion of population in treatment per day" (DAPD) to monitor trends in antimicrobial consumption. These metrics are defined below, and for additional information on methodology, please refer to chapter 9 and the web annex [www.danmap.org].

DADD - Defined animal daily dose

DADD is the average maintenance dose per day for the main indication of a drug in the appropriate animal species. DADD is not defined at product level but for each antimicrobial agent, administration route, and animal species as mg active compound per kg live animal.

DADDs have been defined specifically for use in DANMAP based on current knowledge and may vary from the prescribed daily dose or the recommended dosage in the summaries of product characteristics (SPC) or in the VetStat database.

DAPD - Proportion of population in treatment per day

Trends in antimicrobial usage in pigs, cattle, and fur animals are presented in DAPD.

DAPD is equal to DADD per 1,000 animals per day, where "animals" are represented by their live biomass and adjusted for lifespan. The estimated live biomass is expressed as the number of standard animals with an estimated average weight on a given day. This may also be referred to as the "standard animals at risk". This metric allows for comparison of antimicrobial use between species with large differences in body mass and lifespan.

DAPD, is a statistical measure that provides a rough estimate of the proportion of animals treated daily with a particular antimicrobial agent. For example, 10 DAPD means that an estimated 1% of the population, on average, receives a certain treatment on a given day (see section 9.2).

In principle, DAPD as a metric is analogous to DID (defined daily dose per 1,000 inhabitants per day), the metric used to measure antimicrobial consumption in the human sector. Please refer to section 9.8 for a description of DID. In DANMAP 2019, we calculated treatment proportions in pigs, cattle, and fur animals.

Export

The large differences in DAPDs between age groups affect the DAPD of the total population, and trends are influenced by changes in population structure. As an example, increased export of live pigs just after weaning could lead to an increase in DAPD in the total pig population since the exported pigs were only in the country when the treatment proportion was highest.

Approximately 45% of the pigs produced in 2019 were exported as live pigs at approximately 30 kg (Table 3.1), in comparison this percentage was approximately 24% in 2010. When estimating DAPD for the total pig production, we account for changes in export of weaners by calculating an adjusted treatment proportion, referred to as DAPDadj, see section 9.2.2.

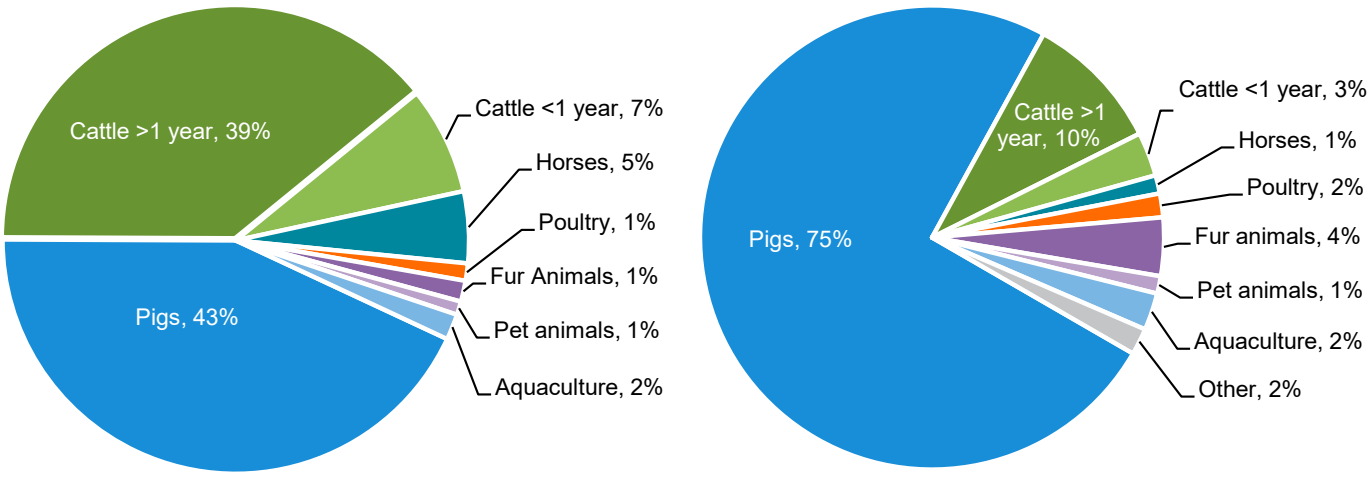
4.2 Total antimicrobial consumption in animals

The total use of antimicrobial agents in all animals amounted to 97.3 tonnes active compound, representing a 3% (-2,788 kg) reduction compared to 2018 (Figure 4.1). Similar to 2018, the 2019 antimicrobial use in pigs, cattle, fur animals, and poultry comprised approximately 75%, 13%, 4% and 2%, respectively, of the total antimicrobial consumption in animals (Figure 4.2). The pig industry is the main driver of antimicrobial usage in animals in Denmark, due to the magnitude of the production. Cattle and pigs comprise almost equal proportions of the total live biomass. However, the vast proportion of cattle biomass consists of dairy cows, which have very low consumption of antimicrobial agents compared with growing animals.

Historically, the overall use of kg active compound was 53% lower in 2019 compared to 1994. A major part of this reduction can be explained by the discontinued use of growth promoters from 1994 to 1999.

Between 2000 (start of VetStat) and 2009, the amount of kg active compound used in animals increased by 62% (Figure 4.1). During this period, the number of pigs produced also increased as did the proportion of exported live pigs at approximately 30 kg. Since then, the proportion of exported live pigs has continued to increase, while there has been an overall gradual decrease in the use of antimicrobials in animals. In 2019, the antimicrobial use was approximately 24% lower than in 2010.

Figure 4.2 Distribution of live biomass and antimicrobial consumption in main animal species, tonnes, Denmark DANMAP 2019



Note: The live biomass is estimated from census data (pigs, cattle, and pet animals) and production data (poultry, fur animals, and aquaculture). For poultry, the figures comprise only the biomass for the main production types (turkeys and broilers). The live biomass estimates for poultry, aquaculture, horses, and pet animals are based on 2012 data and may be underestimated. The estimation procedures are described in section 9.2

Table 4.1 Antimicrobial use by animal species and age group, kg active compound, Denmark DANMAP 2019

Therapeutic group	Aminoglycosides	Amphenicols	Cephalosporins[a]	Fluoroquinolones	Lincosamides	Macrolides	Other AB	Other quinolones	Penicillins, b-lactamase sensitive	Penicillins, others[b]	Pleuromutilins	Sulfonamides and trim-ethoprim	Tetracyclines	Total 2019	Total 2018
Pigs	*8253*	*436*	*<1*	*0*	*1873*	*11874*	*<1*	*0*	*16973*	*8485*	*7203*	*5790*	*11745*	*72632*	*74658*
Sows and piglets	1895	283	0	0	434	504	<1	0	8702	3404	845	4407	1087	21560	22331
Finishers	161	11	<1	0	619	3322	0	0	6059	752	3668	207	2990	17790	18479
Weaners	6197	142	<1	0	820	8048	0	0	2211	4329	2690	1177	7668	33282	33848
Cattle	*844*	*886*	*88*	*<1*	*15*	*190*	*<1*	*0*	*7097*	*828*	*0*	*808*	*1592*	*12350*	*12865*
Intramammaries	9	0	81	0	13	0	<1	0	163	257	0	<1	0	523	534
Cows and bulls	212	12	7	<1	<1	74	<1	0	6308	447	0	693	854	8607	9093
Calves <12 months	590	853	<1	0	1	113	<1	0	501	116	0	110	699	2985	2978
Heifers and steers	32	21	<1	0	<1	3	0	0	125	9	0	6	39	236	261
Poultry	*55*	*<1*	*0*	*<1*	*28*	*228*	*0*	*0*	*333*	*215*	*<1*	*64*	*689*	*1612*	*1326*
All poultry excl. turkeys	55	<1	0	<1	28	228	0	0	333	215	<1	64	689	1612	1326
Other production animals	*274*	*342*	*<1*	*<1*	*92*	*483*	*<1*	*447*	*14*	*2085*	*<1*	*2308*	*463*	*6510*	*7274*
Aquaculture	0	293	0	0	0	0	0	447	0	39	0	1721	22	2522	3557
Fur animals	270	50	0	<1	91	482	<1	0	7	2039	0	580	436	3955	3689
Other[c]	5	<1	<1	<1	1	1	<1	0	7	7	<1	7	5	33	28
Companion animals	*6*	*<1*	*93*	*14*	*63*	*9*	*38*	*0*	*28*	*662*	*<1*	*1473*	*36*	*2423*	*2418*
Pets[d]	5	<1	93	14	63	9	38	0	20	662	<1	246	32	1183	1224
Horses	<1	<1	<1	<1	0	<1	<1	0	8	<1	0	1227	4	1240	1194
Unspecified[e]	*361*	*5*	*3*	*1*	*10*	*36*	*1*	*-6*	*922*	*131*	*4*	*143*	*154*	*1766*	*1541*
Total	9793	1671	185	16	2081	12820	40	441	25367	12406	7208	10587	14679	97293	100082

Note: Data for 2019 were extracted from VetStat on 3 March 2020. Combination products are split into active compounds
a) In 2019, the use of 3rd and 4th generation cephalosporins in cattle, pets, pigs, and horses was 7.5 kg, 1.1 kg, 0.2 kg, and 0.1 kg, respectively
b) Penicillins with extended spectrum and combination penicillins, incl. beta-lactamase inhibitors
c) Mainly sheep and goats
d) Where no animal species are given, antimicrobial agents were allocated to pets based on relevant type of preparation (e.g. tablets, capsules, eye - and eardrops, etc.) or registration (3rd generation cephalosporins and fluoroquinolones only). Approximately 220 kg of the sulfonamides and trimethoprim registered for pets are products (oral paste) typically used for horses. Other AB comprise mainly metronidazole
e) Includes data where the animal species were not registered or where the age group do not apply to the designated animal species

4.3 Antimicrobial consumption by animal species

4.3.1 Antimicrobial consumption in pigs

The majority of antimicrobials in animals are used in the pig production. The total antimicrobial consumption in pigs was 72.6 tonnes of active compound, which was 2,026 kg less than in 2018, yielding a 3% annual reduction (Table 4.1).

The national MRSA action plan aimed to reduce the antimicrobial use in pigs by 15% in 2018 compared to 2014. In 2018, the overall use in the pig production was reduced by approximately 13% when measured in kg active compound, and in 2019 a 16% reduction was achieved compared to 2014.

Treatment proportion

The treatment proportion (DAPD) of the total population reflects the trends in selection pressure within the population. DAPD is much higher in weaners than in finishers and sows. DAPDs in the pig population overall and by age group are presented in Figures 4.3 and 4.4, and DADDs are shown in the web annex (Table A4.1 and in the DADD description). Historically, DAPD increased from 2004 to 2009, followed by a clear decrease in 2010 and 2011 with introduction of the Yellow Card initiative. Since 2013, there has been a gradual decrease in treatment proportion for all age groups (Figure 4.3).

When adjusted for export, the total antimicrobial consumption in pigs was 23 DAPDadj. In 2019, similar to the treatment proportion calculated in 2018 (Figure 4.3). Over a 10 year period, the total antimicrobial use in pigs decreased by 30% (in DAPDadj, Figure 4.3).

While there was a clear reduction in the antimicrobial use in pigs when inspecting crude consumption data (Table 4.1), the changes to the overall treatment proportion are more subtle and vary between age groups and antimicrobial classes. The treatment proportion decreased from 91 to 89 DAPD in weaners, while it remained at almost the same level for sows and piglets and finishers as in 2018 (Figure 4.3, Table A4.1 in the web annex). Thus, on a given day in 2019, approximately 1-2% of sows and piglets and of finisher pigs and approximately 9% of weaner pigs were treated with antimicrobials.

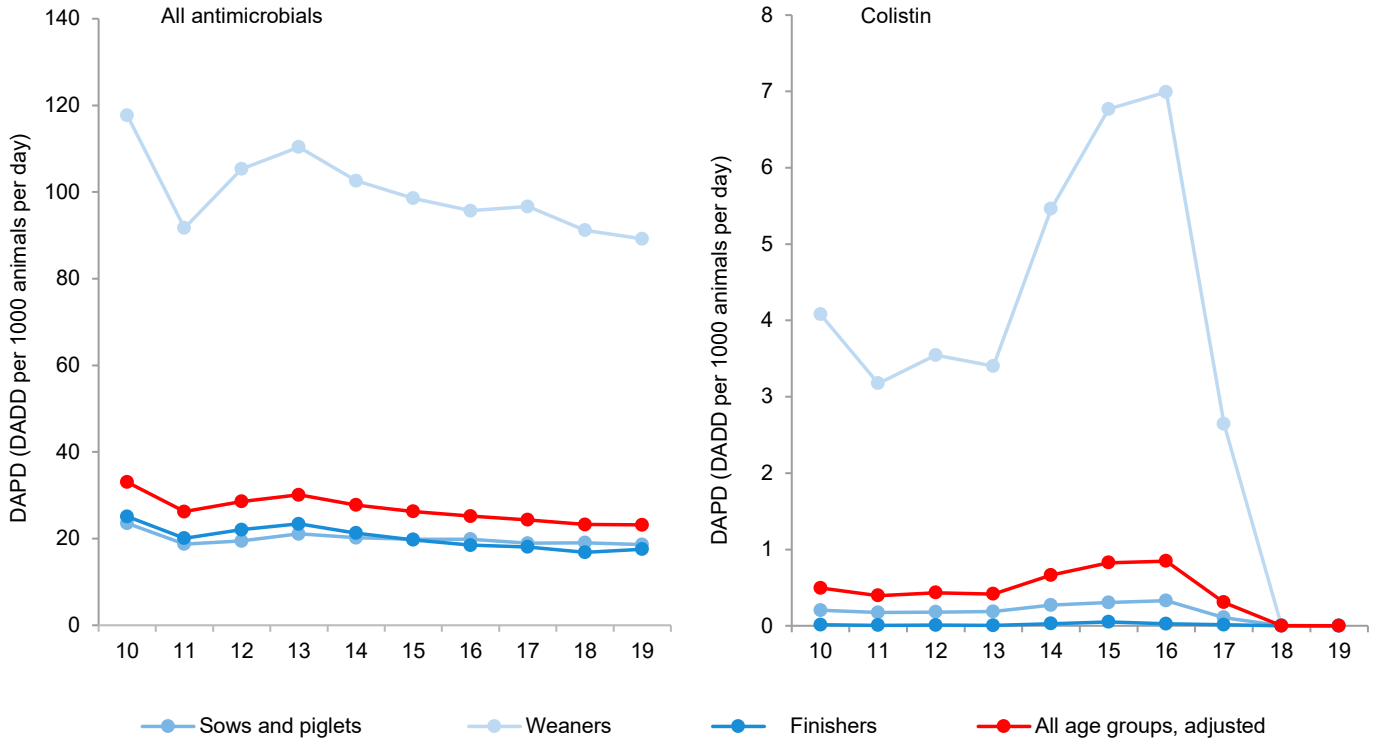
Tetracyclines are some of the most commonly used antimicrobials in the Danish pig production, especially for treatment of gastrointestinal disease in weaners and finishers, and are usually administered orally. The overall use of tetracyclines has decreased since 2013, and in 2019 the treatment proportion was at the lowest level registered in the last 15 years, with the most marked changes following the recent adjustments to the Yellow Card initiative (Figure 4.4). The use of tetracyclines was reduced by 51% from 2015 to 2019 and by 62% since 2010 (in DAPDadj). The proportion of weaners treated with tetracycline on any given day has decreased from approximately 4% (42 DAPD) in 2010 to less than 2% (18 DAPD) in 2019. In contrast, the use of other antimicrobial agents has increased, particularly the use of aminoglycosides (mainly neomycin), macrolides, and beta-lactamase sensitive penicillins (Figures 4.3 and 4.4).

Use of the critically important antimicrobial agents fluoroquinolones and 3rd and 4th generation cephalosporins was close to zero in 2019 (Figure 4.5 and Table 4.1).

Use of medical zinc in pigs

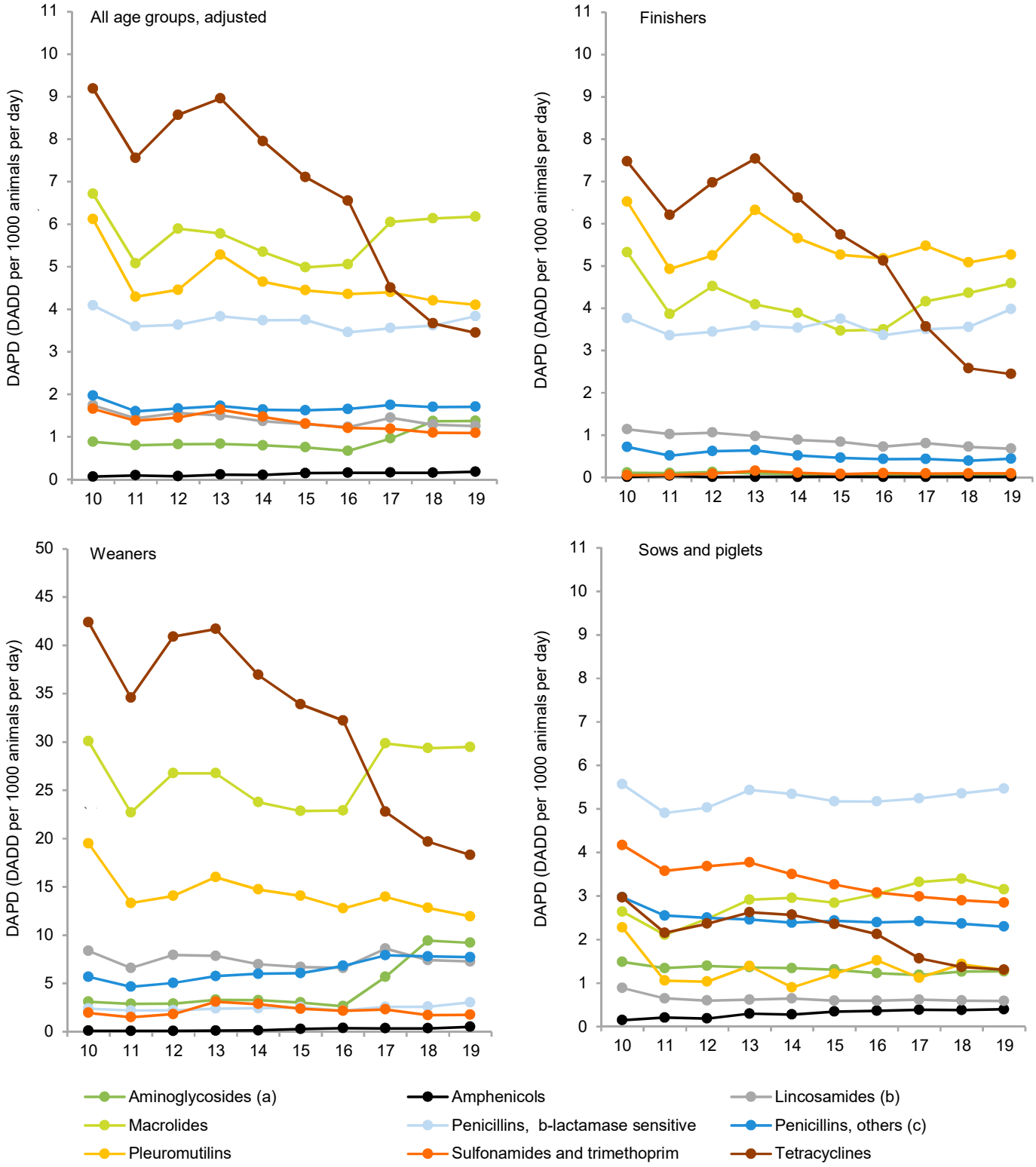
Monitoring the use of medical zink is relevant because its use may select for antimicrobial resistance in some bacteria, including MRSA. Medical zinc in the form of zinc oxide is prescribed to piglets after weaning to prevent or treat diarrhoea. Following a steady increase, the use of zinc oxide for pigs peaked at 548 tonnes in 2015 (Figure 4.6). In 2017, the European Commission announced an EU-wide withdrawal of medical zinc for pigs effective from June 2022. Already in 2016, the Danish pig industry launched an action plan to help the pig producers reduce the use of zinc. This was followed up by an updated action plan in 2018. The use of medical zinc oxide was reduced by 7%, from 509 to 475 tonnes between 2018 and 2019, and has been reduced by 13% since 2015.

Figure 4.3 Total antimicrobial use and use of colistin in the pig production, DAPD, Denmark DANMAP 2019



Note: "Sows and piglets" includes treatment in boars. DAPDs are calculated as the number of standard doses for one kg animal divided by the estimated live biomass in the age group of the total population (in tonnes). DAPDs for "all age groups" are adjusted for export of pigs at 30 kg (see text)

Figure 4.4 Antimicrobial use in the total pig production and in each age group, DAPD, Denmark DANMAP 2019



Note: Intramammaries, gynaecologicals, and topical drugs are not included (approximately 100 kg in 2019). The age group “sows and piglets” includes treatment in boars, where boars constitute 4-5% of the estimated live biomass for the age group. DAPDs are calculated as the number of standard doses for one kg animal divided by the estimated live biomass in the age group or the total population (in tonnes). DAPDs for "all age groups" are adjusted for export of pigs at 30 kg (see text)
a) Aminoglycosides/benzylpenicillinprocain combinations comprise 60% of this group in 2019
b) Lincosamides/spectinomycin combinations comprise 57% of this group in 2019
c) Penicillins with extended spectrum and combination penicillins, incl. beta-lactamase inhibitors, mainly amoxicillin and amoxicillin/clavulanic acid combinations

Figure 4.5 Use of 3rd and 4th generation cephalosporins in pigs and cattle, kg active compound, Denmark DANMAP 2019

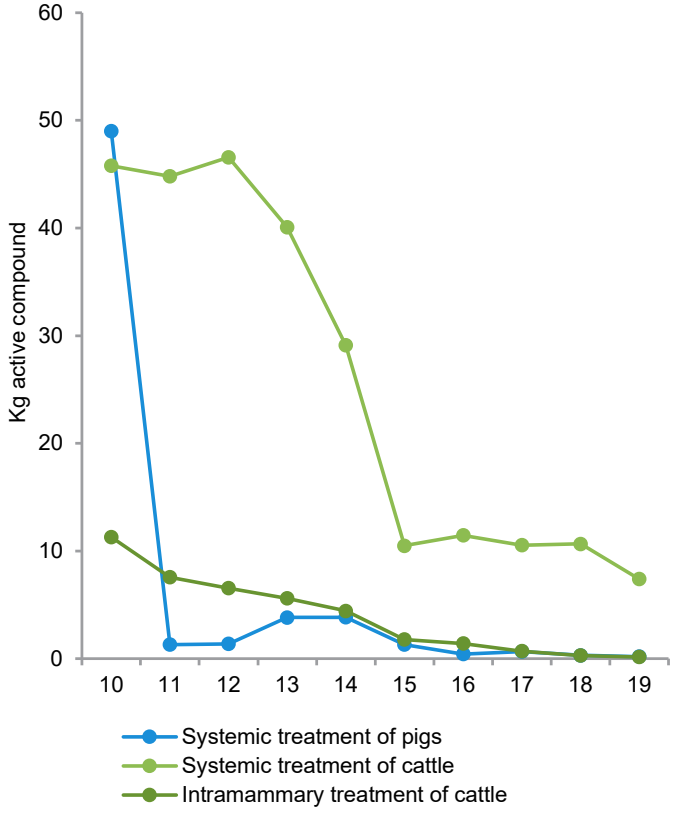
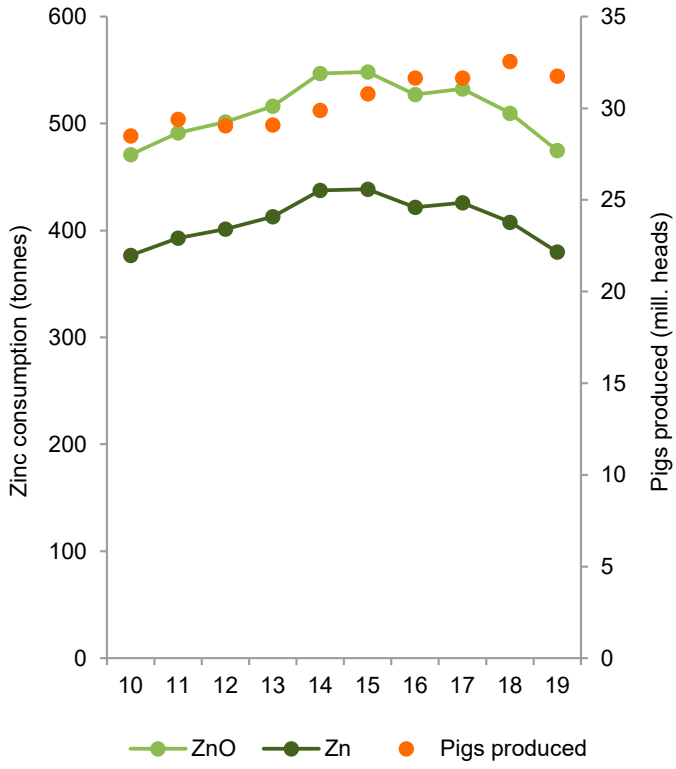


Figure 4.6 Use of medical zinc oxide (ZnO) and zinc (Zn) in the pig production, tonnes, Denmark DANMAP 2019



Note: The most commonly used product is zinc oxide (ZnO), which contains 80% zinc and is largely insoluble in water

4.3.2 Antimicrobial consumption in cattle

Legislation-supported thresholds for antimicrobial use in cattle have been in place since 2011. The overall consumption of antimicrobials in cattle has fluctuated between 12 and 13 tonnes for the past 5 years. In 2019, approximately 12 tonnes were recorded for use in cattle, approximately 500 kg of which were used for intramammary therapeutic- or dry-cow treatment.

Around 75% of the antimicrobials (kg active compound) used for cattle were used to treat adult cattle (>12 months) (Table 4.1).The production of veal and beef has remained relatively stable over the past 5-10 years, while the production of milk has increased (Table 3.1).

Since 2010, there has been an overall decrease in systemic treatment for adult cattle of 18% measured in DAPD, from 3.9 DAPD in 2010 to 3.2 DAPD in 2019.

The main indication for systemic treatment in adult cattle was mastitis, and beta-lactamase sensitive penicillin accounted for approximately two thirds of the antimicrobials used in this age group followed by tetracycline (17%). The use of macrolides constituted 2% in 2019 (Figures 4.7 and 4.8, Table A4.2 in the web annex).

In contrast, the antimicrobial use in calves and young cattle has increased over the past decade from 5.2 DAPD in 2010 to 7.3 DAPD in 2019, equivalent to an increase of 39%. The main indication for systemic treatment in calves is respiratory disease followed by joint/limb infections and gastrointestinal diseases (Figure A4.2 in the web annex).

In calves and young cattle, treatment (DAPD) with amphenicols (florfenicol) has increased steadily over the past decade, and amphenicols have become the most frequently prescribed antimicrobial class (29%), followed by tetracyclines and macrolides (28% and 18%, respectively).

The use of fluoroquinolones in cattle has been close to zero for the last decade. Fluoroquinolones are only prescribed in food-producing animals as a last-line drug, based on microbiological analysis and susceptibility testing in an accredited laboratory. Use of fluoroquinolones in food-producing animals is also notifiable to the DVFA.

In 2014, the cattle industry began to phase out the use of 3rd and 4th generation cephalosporins used for systemic treatment (orally and parenterally), resulting in a significant drop in 2015, and the annual usage stabilised at approximately 10 kg (Figure 4.5). On 1 September 2019, the cattle industry implemented a ban on use of 3rd and 4th generation cephalosporins in all cattle, and the annual use dropped to approximately 7 kg in 2019, mostly ceftiofur used in cows and bulls.

The board of Danish dairy and beef producers has recently renewed its strategy for good udder health. The goals are a 20% reduction in use of antimicrobials for treatment of mastitis and other cattle diseases as well as a lowering of geometric mean bulk tank cell counts to 150,000 by the year 2020. In addition, the dairy industry will promote use of simple penicillins (beta-lactamase sensitive penicillins) when dry-cow therapy or mastitis treatment is required.

The majority of antimicrobials administered parenterally in cattle are used in dairy cows, primarily to treat mastitis. Further, approximately 500 kg are administered as intramammary treatments, either as therapeutic- or dry-cow treatment. The use of intramammary treatment is shown in Figure 4.8.

The number of treatments per cow has remained stable for the past decade, but the usage pattern has changed. The relative proportion of dry-cow treatment has shifted markedly from 27% in 2012 to 55% in 2019. Dry-cow treatment is only permitted following diagnostic testing, where the presence of bacteria causing mastitis has been confirmed.

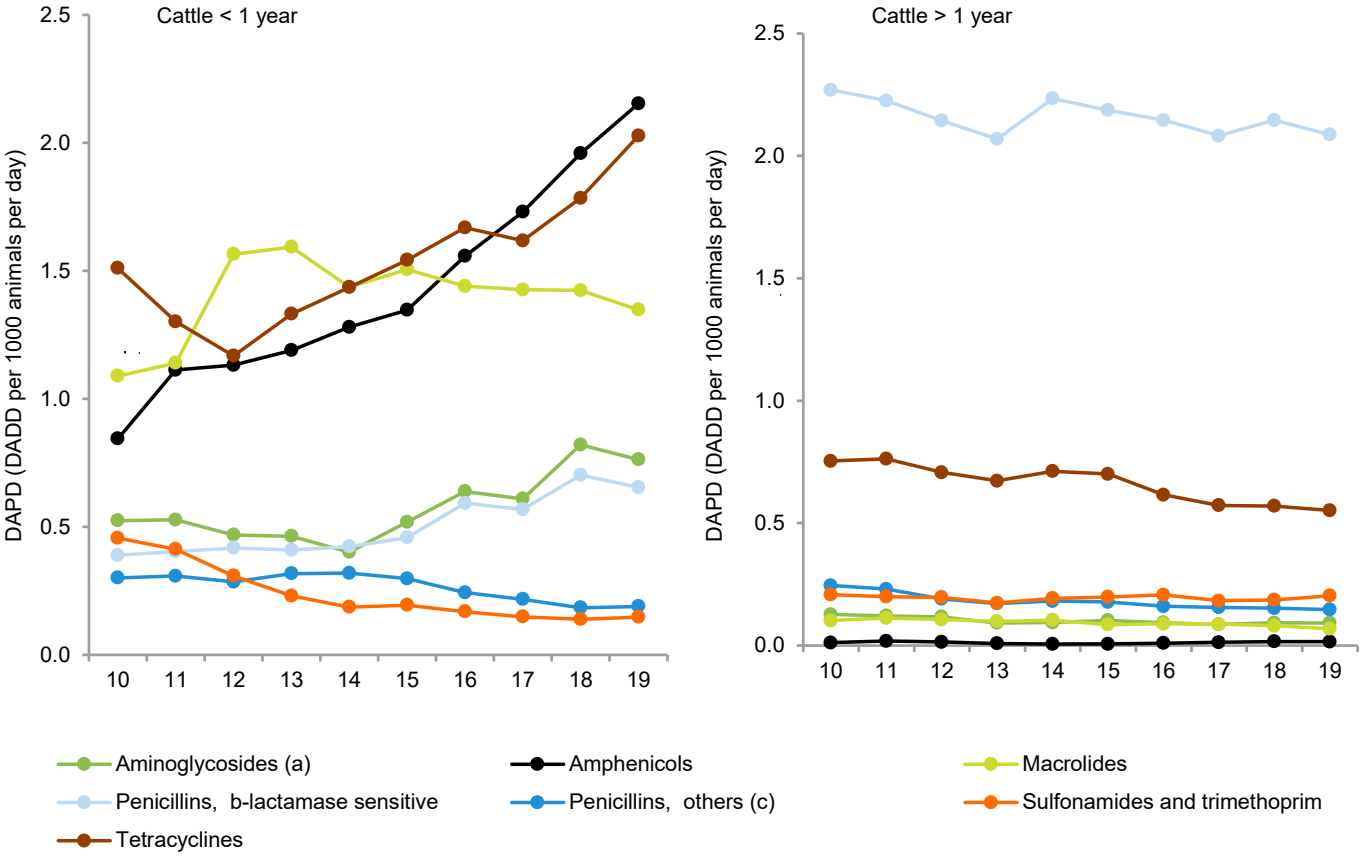
In line with the strategy, the use of beta-lactamase sensitive penicillins increased for dry-cow treatments, whereas use of 1st generation cefalosporins and extended spectrum penicillins decreased from 2012 to 2017.

However, in 2019, there was a remarkable shift in the dry-cow treatments and the use of the beta-lactamase sensitive benzylpenicillin for this purpose almost ceased, while the use of the extended spectrum penicillins, especially cloxacillin, increased. This was due to product shortage, where, the only beta-lactamase sensitive benzylpenicillin product for dry-cow treatment was unavailable for longer periods during 2019, and extended spectrum penicillins, especially product containing cloxacillin, had to be used instead [Personal communication; Michael Farre, Danish Agriculture and Food Council].

Among the therapeutic treatments, beta-lactamase sensitive penicillins (benzylpenicillinprocain) remained the most commonly used antimicrobial.

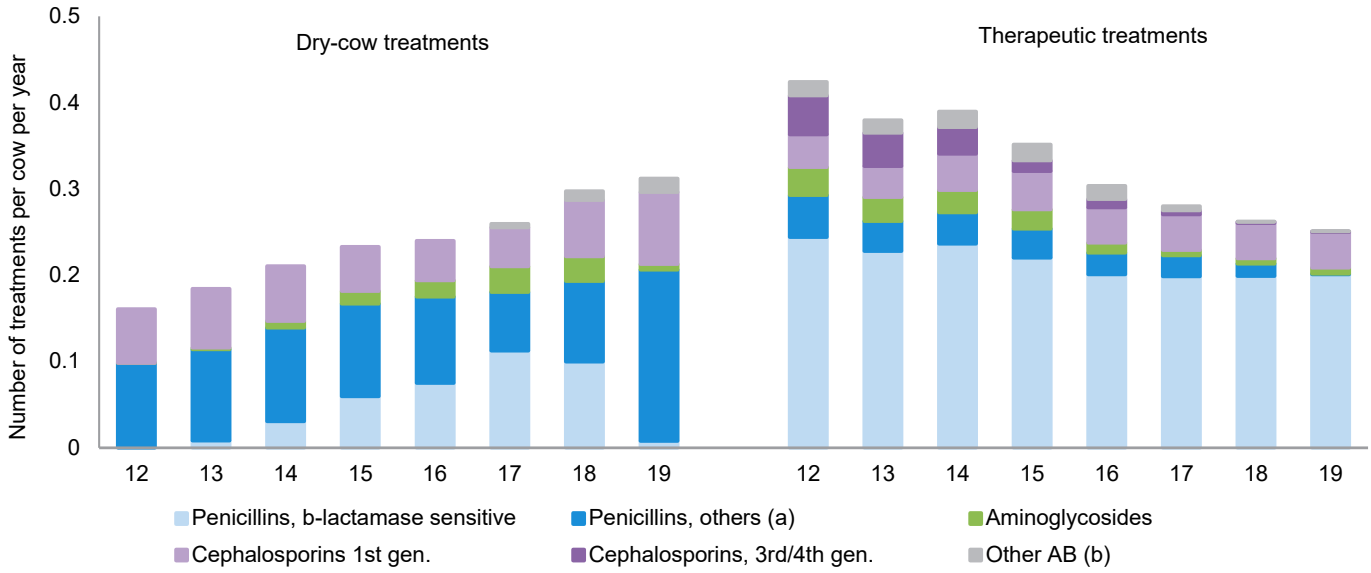
Figure 4.7 Use of antimicrobial agents in cattle, DAPD, Denmark

DANMAP 2019



Note: Intramammaries, gynaecologicals and topical drugs not included (approximately 1 tonne in 2019). DAPDs are calculated as the number of standard doses for one kg animal divided by the estimated live biomass in the age group (in tonnes)
a) Aminoglycosides/benzylpenicillinprocain combinations comprise 67% of this group in 2019
b) Lincosamides/spectinomycin combinations comprise 76% of this group in 2019
c) Penicillins with extended spectrum and combination penicillins, incl. beta-lactamase inhibitors, mainly amoxicillin and ampicillin

Figure 4.8 Use of antimicrobial agents for intramammary application in cattle, treatments per cow per year, Denmark DANMAP 2019



Note: For intramammary treatment, the animal daily doses (ADD) listed in VetStat were used to estimate the number of treatments. For products used for dry-cow treatment, the ADD was primarily 4 tubes (89%), whereas for products used for therapeutic treatments the ADD varied from 1 to 5 tubes, primarily 2 or 3 tubes (86%). Number of cows per year from Statistics Denmark (Table HDYR07)
a) Penicillins with extended spectrum and combination penicillins, incl. beta-lactamase inhibitors, mainly cloxacillin
b) Includes lincomycin for dry-cow treatments. For therapeutic treatment, manily sulfonamides/trimethoprim, but also lincomycin and bacitracin

4.3.3 Antimicrobial consumption in poultry

The poultry production comprises broiler production, egg layers, and turkey production. In addition, there is a small production of ducks, geese, and game birds. Conventional Danish broiler farms have a very high level of biosecurity, and the antimicrobial consumption in broiler production is generally low compared with other species. Accordingly, disease outbreaks in just a few farms can markedly affect the national statistics on antimicrobial usage in the poultry sector (Table 4.2).

VetStat does not allow easy differentiation of the use of antimicrobials between the different types of poultry production. This year's DANMAP presents the total reported use in all poultry species.

In late 2014 and throughout 2015, several outbreaks increased the total use in broilers. In 2016, use of antimicrobials in poultry decreased again. In 2019, the usage was increased by 22% to 1,612 kg (Table 4.2). Approximately 25% were used in the production of broilers and 50% for turkeys for slaughter [personal communication, Mie Nielsen Blom, Danish Agriculture and Food Council]. Increases were seen mainly for tetracyclines prescribed for respiratory disease and macrolides prescribed for enteritis. For the past decade, cephalosporins have not been used in the poultry industry, and the use of fluoroquinolones has been close to zero. Colistin has not been used since 2016.

Table 4.2 Use of antimicrobial agents in poultry, kg active compound, Denmark DANMAP 2019

Therapeutic group	Aminoglycosides	Amphenicols	Fluoroquinolones	Lincosamides	Macrolides	Other AB	Other quinolones	Penicillins, b-lacta-mase sensitive	Penicillins, others[a]	Pleuromutilins	Sulfonamides and trimethoprim	Tetracyclines	Total
2015	258	4	1	129	114	7	0	184	500	<1	446	796	2441
2016	60	5	<1	24	153	6	0	239	225	<1	111	749	1571
2017	65	5	<1	32	206	0	1	321	293	<1	85	483	1491
2018	51	0	<1	26	162	0	0	323	212	<1	37	516	1326
2019	55	<1	<1	28	228	0	0	333	215	<1	64	689	1612

Note: Data for 2019 were extracted from VetStat on 3 March 2020. VetStat does not differentiate between use in the different sectors of poultry production. Combination drugs are divided into active compounds
a) Penicillins with extended spectrum and combination penicillins, incl. beta-lactamase inhibitors, mainly amoxicillin

4.3.4 Antimicrobial consumption in aquaculture, fur animals and companion animals

Aquaculture

Antimicrobial consumption in aquaculture is mainly driven by the summer temperatures, because bacterial diseases are more likely to occur when temperatures are high. In recent years, the aquaculture industry has developed new and better vaccines and improved vaccination strategies to reduce the risk of diseases that may require antibiotic treatment. The summer of 2019 was not as warm as in 2018, and this was reflected in a 29% decrease in antimicrobial use from 3,557 kg in 2018 to 2,522 kg in 2019 (Table 4.3).

Mainly three compounds are used to treat bacterial infections in aquaculture: sulfonamide/trimethoprim (68%), 1st generation quinolones (18%), and amphenicols (12%) (Table 4.3).

Fur animals (mink)

The Danish production of mink has increased over the last decade from 13 million animals in 2004 to 18 million in 2017 (Table 3.1).

During that period the use of antimicrobial agents increased gradually from less than 2 tonnes in 2004 to more than 6 tonnes in 2017. As a response, the industry increased focus on reducing the antimicrobial use and developed an antimicrobial action plan in cooperation with DVFA, DVA, and the veterinary practitioners [Textbox 4.4, DANMAP 2018].

Remarkably, already in 2018 the use was reduced by 40% (from 6,156 kg to 3,689 kg), which was likely a result of the increased focus on reducing antimicrobial usage and a year with fewer disease outbreaks than the previous year.

In 2019, the total use remained at the low end at 3,955 kg, yielding a 7% increase from 2018, but the annual production was reduced from almost 18 million animals to 13 million (Table 3.1). In 2019, the treatment proportion was approximately 3% (32 DAPD) compared to 2% in 2018 (23 DAPD).

The use of tetracyclines, penicillins with extended spectrum, combination penicillins, and macrolides has fluctuated over the past five years (Figure 4.9), and the overall increase in 2019 was equally distributed among these antimicrobial agents. The use of fluoroquinolones and cephalosporins in the fur animal production has been close to zero for more than a decade (Table A4.3 in the web annex).

Companion animals - horses and pets

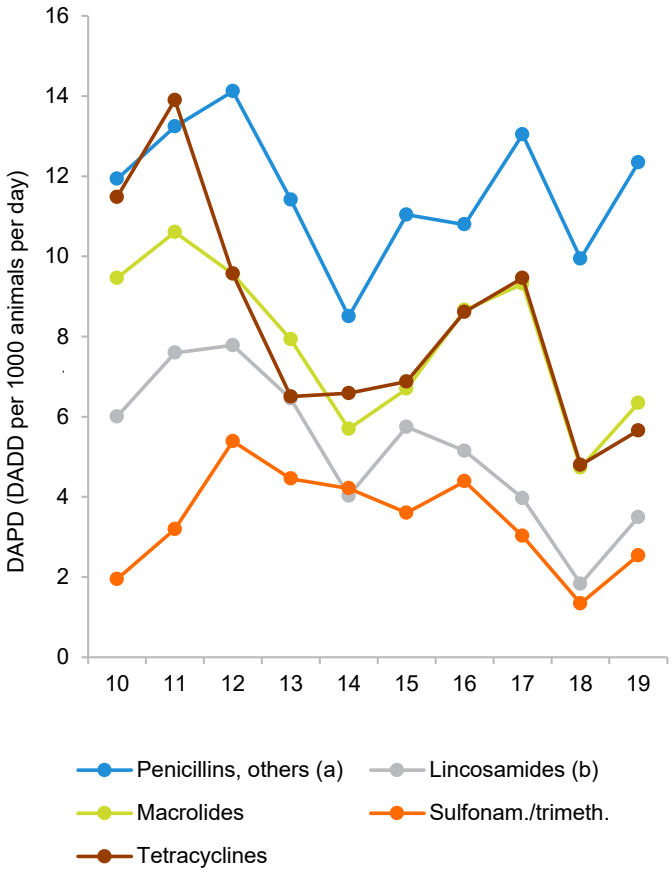
The information available on antimicrobial consumption in companion animals is not as accurate as for production animals, because VetStat allows registration of antimicrobials for companion animals without defining animal species. In DANMAP, the methods used for estimating the consumption for companion animals are described in DANMAP 2016.

Table 4.3 Use of antimicrobial agents for aquaculture, kg active compound, Denmark DANMAP 2019

Therapeutic group	Amphenicols	Other quinolones[a]	Sulfonamides and trimethoprim	Other AB[b]	Total
2015	311	1005	1650	4	2970
2016	315	893	1086	13	2307
2017	350	637	679	31	1697
2018	323	896	2293	45	3557
2019	293	447	1721	61	2522

Note: Data for 2019 were extracted from VetStat on 3 March 2020
a) Oxolonic acid
b) Other antibiotics include mainly amoxicillin (64%) and tetracyclines (36%)

Figure 4.9 Use of antimicrobial agents in fur animals, DAPD, Denmark DANMAP 2019



Note: DAPD is calculated as the number of standard doses for one kg animal divided by the estimated live biomass in the total population (in tonnes)
a) Penicillins with extended spectrum and combination penicillins, incl. beta-lactamase inhibitors, mainly amoxicillin/clavulanic acid
b) Lincosamides/spectinomycin combinations comprise 99.9% of this group in 2019

Table 4.4 Estimated use of antimicrobial agents for horses, kg active compound, Denmark DANMAP 2019

Therapeutic group	Sulfonamides and trimethoprim[a]	Penicillins, b-lacta-mase sensitive	Tetracyclines	Aminoglycosides	Other AB	Total
2015	1049	10	4	3	1	1067
2016	1117	8	5	<1	1	1131
2017	1172	9	3	<1	0	1184
2018	1179	10	4	<1	1	1194
2019	1227	8	4	<1	1	1240

Note: Data were extracted from VetStat on 3 March 2020. The estimates include all antimicrobial agents registered by either pharmacies or veterinarians for use in horses. Where no animal species are given, antimicrobial agents were allocated to horses based on relevant type of preparation (e.g. oral paste) or registration. Antimicrobials administered parenterally - with no information on animal species - are not included
a) Sulfonamides and trimethoprim products in the form of oral paste are typically used for horses, however, some of these products have been registered for use in pets over the past years; 220 kg in 2019 and 242 kg in 2018. These data are included in Table 4.5

The total amount of antimicrobials estimated for use in horses was 1,240 kg and 1,183 kg in pets (Tables 4.4 and 4.5). As in previous years, a substantial amount of sulfonamide/trimethoprim registered as used for dogs and cats appears to be products (oral paste) normally administered to horses. Thus, a substantial amount of sulfonamide/trimethoprim included in Table 4.5 is likely to have been used for horses (220 kg in 2019 and 242 kg in 2018).

A large proportion of antimicrobials used for dogs and cats are prescribed for the treatment of chronic or recurrent disease, mainly dermatitis. Due to the close contact between owners and their pets, the repeated use of critically important antimicrobials may pose a risk to the owners, and the use of these antimicrobials is monitored carefully. Since the treatment guidelines by DVA were published in November 2012 (revised in 2018), the use of cephalosporins has been reduced by 66% (from 272 kg in 2012).

The use of fluoroquinolones in pets, mainly dogs and cats, was 14 kg and represented the majority (89%) of fluoroquinolones used in all animals in 2019. Similarly, the pets accounted for half (93 kg, 50%) of all the cephalosporins used in animals (Table 4.5). In 2019, the use of 3rd and 4th generation cephalosporins in pets represented 12% of the total use in animals (1.2 kg of 9.8 kg active compound).

Birgitte Borck Høg and Helle Korsgaard
For further information: Birgitte Borck Høg, bibo@food.dtu.dk

Table 4.5 Estimated use of antimicrobial agents for dogs and cats, kg active compound, Denmark DANMAP 2019

Therapeutic group	Aminoglycosides	Amphenicols	Cephalosporins[a]	Fluoroquinolones[b]	Lincosamides	Macrolides	Other AB	Other quinolones	Penicillins, b-lacta-mase sensitive	Penicillins, others[c]	Pleuromutilins	Sulfonamides and trimethoprim[d]	Tetracyclines	Total
2015	7	<1	157	14	68	5	33	0	25	655	1	235	39	1240
2016	6	<1	137	15	69	3	31	<1	20	718	<1	275	40	1317
2017	6	1	111	14	67	2	31	0	19	718	<1	280	38	1287
2018	6	<1	97	15	62	2	41	1	20	681	<1	261	37	1224
2019	5	<1	93	14	63	9	38	0	20	662	<1	246	32	1183

Note: Data from 2019 were extracted from VetStat on 3 March 2020. Data include all antimicrobial agents registered by either pharmacies or veterinarians for use in pets. Furthermore, where no animal species are given antimicrobial agents were allocated to pets based on relevant type of preparation (e.g. tablets, capsules, eye- and eardrops, etc.) or registration (3rd generation cephalosporins and fluoroquinolones only)
a) Include use of 3rd generation cephalosporin product CONVENIA (1.1 kg in 2019) where no animal species are given
b) Include use of low concentration fluoroquinolones (maximum of 50 mg/g) dispensed parenterally or orally (4.8 kg in 2019) where no animal species are given
c) Penicillins with extended spectrum and combination penicillins, incl. beta-lactamase inhibitors, mainly amoxicillin/clavulanc acid. Include use of tablets (1.1 kg in 2019) where no animal species are given
d) Sulfonamides and trimethoprim products in the form of oral paste are typically used for horses, but some of these products have been registered for use in pets over the past years, 220 kg in 2019 and 242 kg in 2018

Textbox 4.1

Campaign to assess compliance regarding group treatment of piglets

Background: In 2019, DVFA carried out a "control campaign" to assess compliance with legislative requirements in regard to antimicrobial group treatment of piglets according to the 2014 Danish legislation [Applicable order (DK) 1243 of 26/11/2019]. The definition of group treatment is treatment of two or more pigs with antibiotics in feed or water. The treatment method is widely used, and because of the quantities it is important to safeguard that legislative requirements are met to ensure as prudent use as possible.

The aims of the campaign were to:
- enhance prudent use of group treatment of piglets in Denmark
- evaluate whether the prescribed antibiotics for group treatments were based on diagnostics and susceptibility testing
- reduce the use of antibiotics for group treatment of piglets in Denmark

Compliance with legislation was previously assessed during a campaign in 2015 [DANMAP 2015].

Methods: The 2019 campaign especially focused on the written documentation required by farmers and veterinarians. This included whether the veterinarian complied with the rules about sampling and diagnostics, and prepared action plans to reduce group treatment. The farmers compliance to the treatment instructions and the action plan was also evaluated.

The campaign ran from May 1st to December 31st and included visits to 200 herds of piglets and 35 administrative controls of the veterinarians associated to the herds. The herds and veterinarians included in the campaign was distributed among all regions of Denmark.

Results and conclusion: The results showed that around one third of the visited herds (36%) and veterinarians (31%) did not comply with the legislative requirements within the focus of the campaign. Farmers were sanctioned mainly due to inadequate records (28%) and for inadequate compliance with the veterinarians' written treatment instructions (13%). Sanctions given to the veterinarians were primarily due to substantive deficiencies in herd diagnosis and/or treatment instructions. Despite the high number of non-compliances, none of them were serious enough to require sanctioning by the police. The campaign also revealed a need to clarify the legal requirements in the rules for the action plans to reduce group treatment. The full report (in Danish) is available at www.FVST.dk.

Anette Grønkjær Thomsen, DVFA
For further information: Anette Grønkjær Thomsen, agxt@fvst.dk

5

ANTIMICROBIAL CONSUMPTION IN HUMANS

5. Antimicrobial consumption in humans

Highlights

Total consumption. In 2019, total consumption of antimicrobial agents in humans in Denmark was 15.76 defined daily doses per 1000 inhabitants per day (DID). Within the last decade the consumption decreased with altogether 13.9% (18.31 DID in 2010); since the highest peak of 18.95 DID in 2011 the decreases have been continuous. From 2018 to 2019 decreases were 1.3% (15.97 DID in 2018). Decreases owe to reduced consumption in the primary sector and were observed for all municipalities and within all medical specialties that prescribe in primary health care. In 2019, primary health care accounted for 87% of all prescribed antimicrobials.

Consumption in primary health care. In 2019, the consumption in primary health care was 13.76 DID, a 17% reduction since 2010 (16.56 DID) and a 1.5% reduction since 2018. Most marked decreases within the decade were observed from 2011 to 2012 and from 2016 to 2018 and were due to a reduced number of prescriptions issued, measurable in total reductions as well as in reduced numbers of patients treated. Decreases were observed within all age groups and for both genders. Due to changes in the health system over time, the proportion of antimicrobial agents prescribed by hospital doctors but redeemed at community pharmacies increased from constituting 13.1% of the consumption in primary health care in 2015 to constituting 16.7% in 2019 (measured in DID).

Consumption in hospital care. In 2019, the consumption of antimicrobials at Danish hospitals was 1.93 DID. This corresponds to 107 DDD per 100 occupied bed-days (DBD), a 49% increase since 2010 (71.6 DBD) and a 6.2% increase since 2018 (100.7 DBD). Measured in DDD per 100 admissions (DAD), the consumption in 2019 (306 DAD) was 21.0% higher than in 2010 (253 DAD) and 3.4% higher than in 2018 (296 DAD).

Consumption of penicillins. In 2019, penicillins accounted for 65% of the consumption in primary health care and 54% of the consumption in hospital care. However, due to shifting trends in the usage of different penicillin drug classes, the consumption of beta-lactamase sensitive penicillins decreased from constituting 53% of all penicillins in primary health care in 2010 to constituting 38% in 2019. In hospital care, the consumption of all penicillins increased over the last decade. Most marked increases were in the consumption of beta-lactamase resistant penicillins (+112%) and combination of penicillins incl. beta-lactamase inhibitors (+259%).

National Action Plan. In primary health care, the number of redeemed prescriptions decreased from 462 to 382 prescriptions per 1000 inhabitants per year from 2016 to 2019 (goal 1: 350 presc/1000 inh/year by 2020). However, the proportion of beta-lactamase sensitive penicillins remained unchanged at 31% of total consumption (goal 2: 36% by 2020). The consumption of antimicrobials of special critical interest at hospitals decreased by 7.4% (from 20.77 DBD in 2016 to 19.24 DBD in 2019), (goal 3: 10% reduction by 2020).

5.1 Introduction

In Denmark, all consumption of human medicine is recorded through the Register of Medicinal Product Statistics at the Danish Health Data Authority. This includes sales data from all public and private healthcare providers. Antimicrobial sales data have been submitted from the primary sector since 1994, whereas the hospital sector has submitted data since 1997.

In Denmark, only medical doctors, veterinarians and dentists can prescribe antibiotics and sale is through publicly registered and approved pharmacies. All Danish medical doctors and dentists have the right to prescribe freely what they find appropriate for their patients. There are no restrictions regarding specific antimicrobial classes but prescribing is guided by national and/or local guidelines on prudent use issued through different medical specialties and through recommendations issued by the Danish Health Authority. Recording of the consumption in the primary sector is based on total sales from pharmacies to individuals or private clinics. For all sales, data contains information on the ATC code, formulation, package size and number of packages sold. For sales to individuals, additional information is available from the prescription registry; this includes an identifier of the prescriber and information on the age, gender and address of the patient. Since 2004, it also includes the indication for prescribing the medication. No over-the-counter sale takes place. This enables an almost complete surveillance of all systemic antimicrobials used in Denmark in the primary sector.

For the hospital sector, primarily data from public somatic hospitals with acute care function (referred to as somatic hospitals) is included in the report. Data from psychiatric hospitals, private hospitals and hospices have been excluded, since consumption at these facilities is minor. Furthermore, no reliable denominator for measuring the consumption in these patient populations exists. Figure 5.1a is the only figure in the report, that presents complete Danish consumption, collating data from all healthcare providers, both public and private sectors. The recent upgrade of The National Patient Register, implemented during 2019, has had a major impact on the calculations of the consumption of antibiotics at hospitals, partly because data has been frozen. For more detailed information on data reporting and registration, including hospital activity and bed-day definitions, please see chapter 9.8, materials and methods.

A political plan for the Danish health system from 2018 focuses on enforcement of the primary sector by moving time consuming functions from hospital ambulatory care to smaller health units, rehabilitation centers and GPs in the municipalities. This demands a restructuring and strengthening of collaboration between all sectors. It may affect monitoring systems, since bed-days become more difficult to measure when it comes to describing the actual activity at different parts of the health sector. Boundaries between primary and hospital sector become more fluent and hospital activity becomes harder to determine. It also challenges the comparison of consumption over time.

In January 2019, the WHO Collaborating Centre for Drug Statistics Methodology in Oslo introduced new DDD values for some of the commonly used antimicrobials. These changes were implemented in last years report (DANMAP 2018) and all numbers in the figures and tables were updated 10 years back. Due to new DDD values, figures in the present report are thus comparable only to figures and numbers in DANMAP 2018.

In 2017, Danish adjusted DDDs (DaDDD) were developed for monitoring hospital consumption data. For the 2018 report, new values were also developed for the primary sector. In 2019, consumption in both DDD and DaDDD were applied in two figures, for the primary sector and the hospital sector, respectively (Figure 5.5 and Figure 5.14). For more information regarding DaDDD, see Table 9.5 and 9.6 in chapter 9.8, materials and methods.

In this chapter, the term 'antimicrobial agents' covers all systemic antibacterial agents for human use listed in the Anatomical Therapeutic Chemical (ATC) Classification under the code J01. Additional antimicrobials included are metronidazole (ATC code P01AB01) and for hospitals vancomycin (ATC code A07AA09). Their consumption has been included in DANMAP since 2014. Consumption of tuberculostica, antiviral and antifungal drugs is not included in this chapter.

5.2 Total consumption (all public healthcare systems in Denmark)

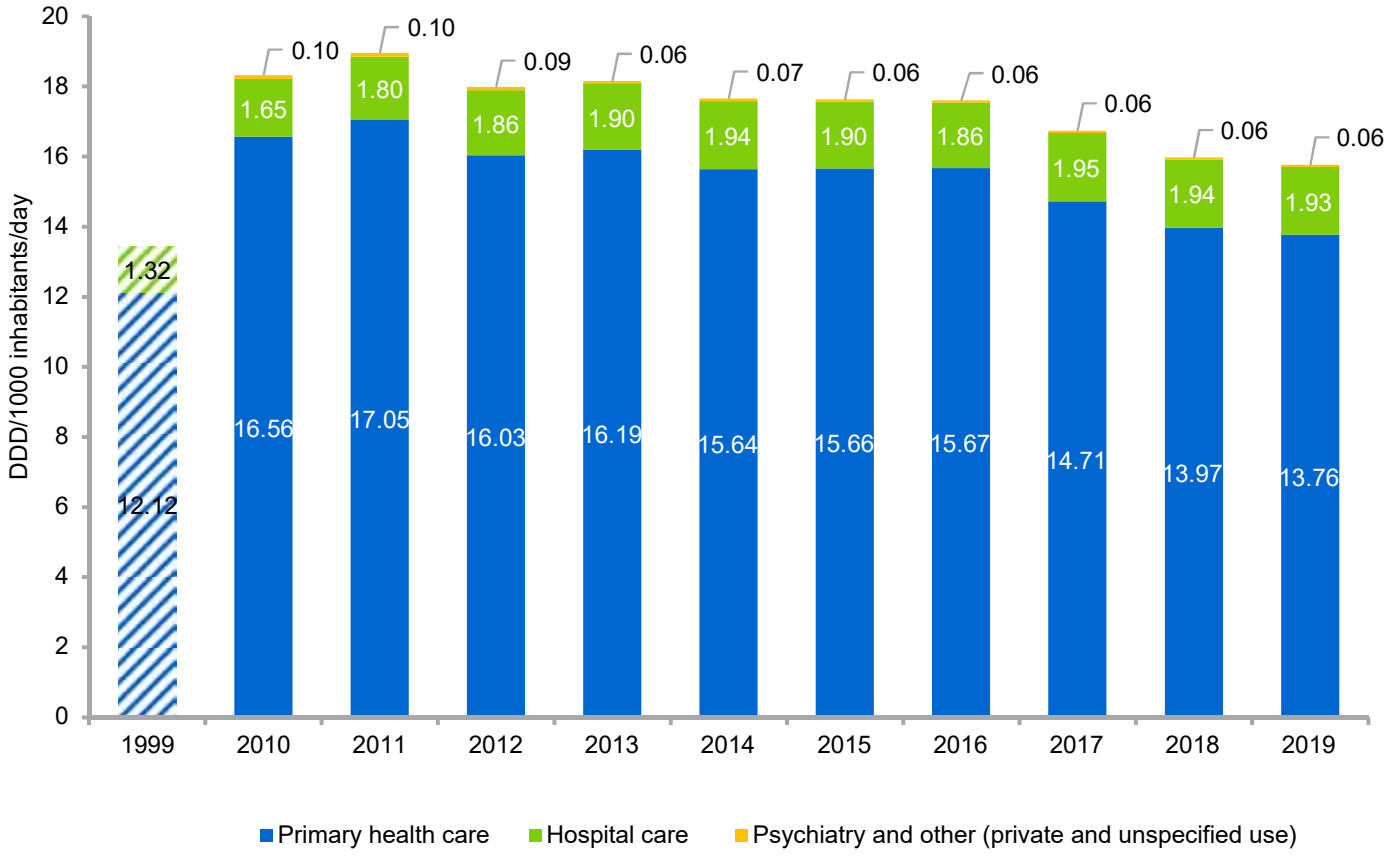
Historically, the consumption of antimicrobials in Denmark showed no significant trends during the first five years of systematic registration from 1996 to 2000, where consumption was estimated to be between 13.40 and 13.63 DDD per 1000 inhabitants per day (DID; based on former WHO DDD values and therefore not directly comparable to newer calculations). After these stabile years, steady increases were observed until 2011. Since then, the consumption has first levelled off and since decreased markedly.

In 2019, consumption of systemic used antimicrobials was 15.76 DID in total (all public and private healthcare systems), which is 1.3% less than the consumption in 2018 (15.97 DID) and 13.9% less than the consumption a decade ago in 2010 (18.31 DID), (Figure 5.1a). The primary sector accounted for 13.76 DID, the somatic hospital sector for 1.93 DID and psychiatry and private or unspecified use ("other") for 0.06 DID. The total consumption in 2019 corresponds to 49.183 kg active compound consumed (Table A5.1 in web annex).

Since 2011, a marked decrease was observed for the total consumption in Denmark. The decrease is driven by reduced prescribing in primary health care, which in 2019 accounted for 87% of all antimicrobials used in humans in Denmark. At hospitals, consumption did not change correspondingly, thus the proportion of antimicrobials used at hospitals has increased during the decade, (Figure 5.1b).

Figure 5.1a Total consumption of systematic antimicrobial agents in humans, DDD per 1000 inhabitants per day, Denmark

DANMAP 2019



Data for this figure is based on the total sales in Denmark
ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system

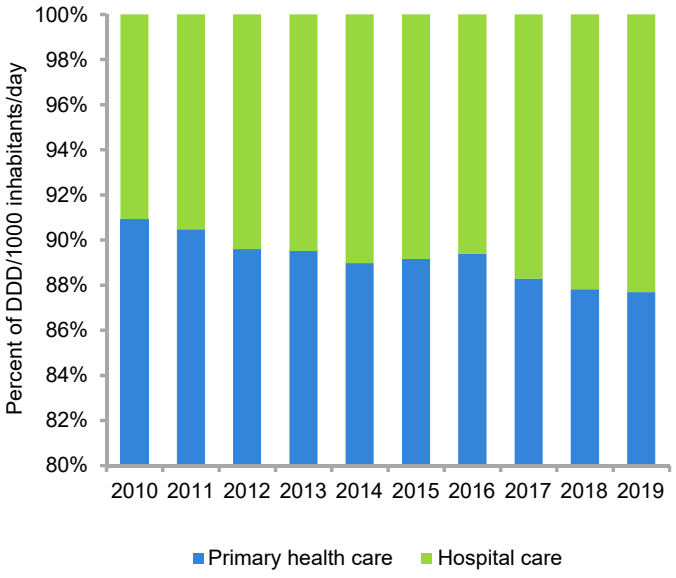
Consumption of the main antimicrobial drug classes by the primary sector and hospital sector, respectively, is presented in Figure 5.1c. Most notable are high use of beta-lactams in both health sectors and exclusive use of cephalosporines at hospitals.

Consumption of antimicrobials in primary care and somatic hospitals in the five regions is presented in Figure 5.2. Although consumption per inhabitant differs between the regions, for all five regions marked decreases were observed in the primary sector since 2016, more pronounced in the first years and less marked in 2019. The two neighbouring regions, the Capital Region and the Region Zealand showed highest total consumptions of 15.77 DID and 16.20 DID, respectively, the Capital Region due to a relatively high consumption at hospitals, and the Region Zealand due to a comparably high consumption in primary care. The Central and Northern Region had the lowest total consumption with 14.02 DID and 14.71 DID, respectively. They were similar in an overall low consumption in both primary and hospital care.

For more information on population size and hospital activity in the five health regions, see Figure 3.2 and Table 5.7.

Figure 5.1b Changes in distribution of total consumption of antimicrobials, % of DDD per 1000 inhabitants per day, Denmark

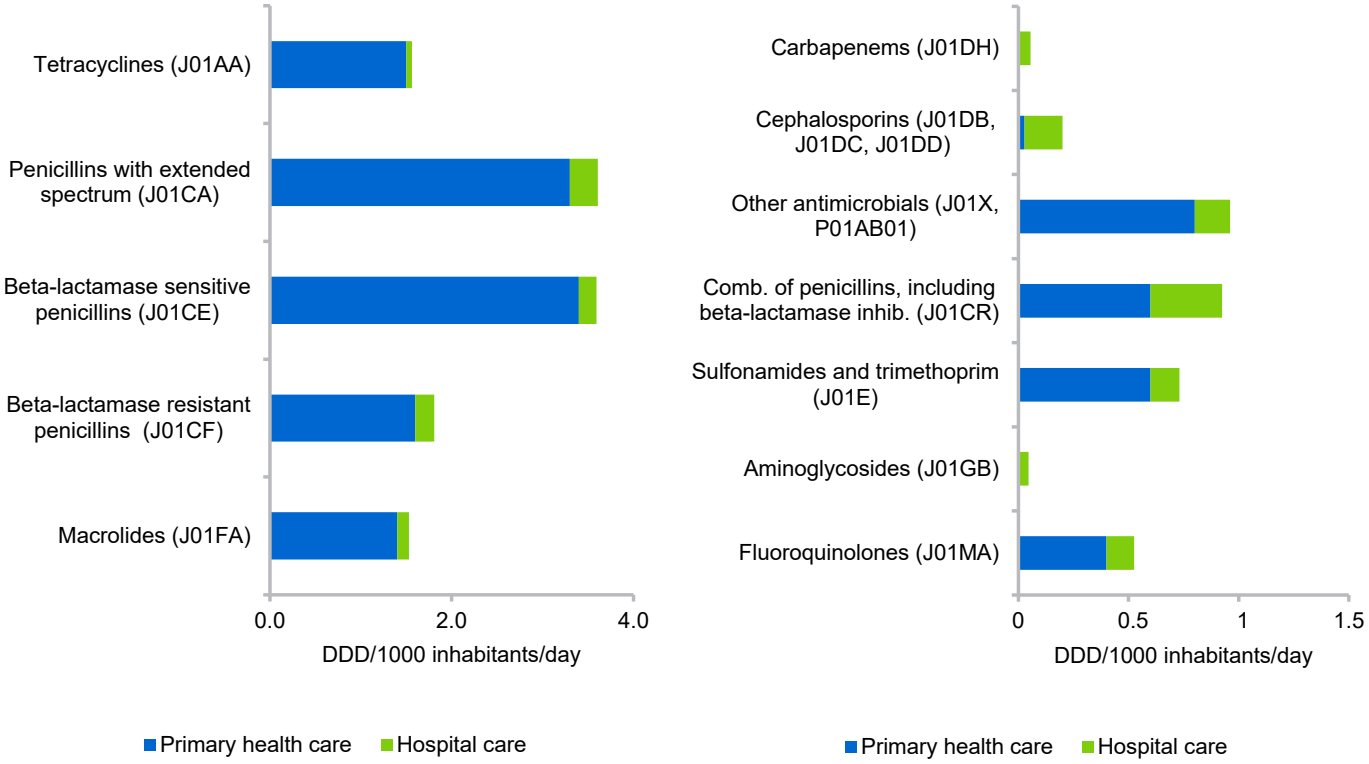
DANMAP 2019



Note: y-axis starts at 80%
Data for this figure is based on the total sales in Denmark
ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 5.1c Distribution of main antimicrobial classes used for humans in primary and hospital care, DDD per 1000 inhabitants per day, Denmark

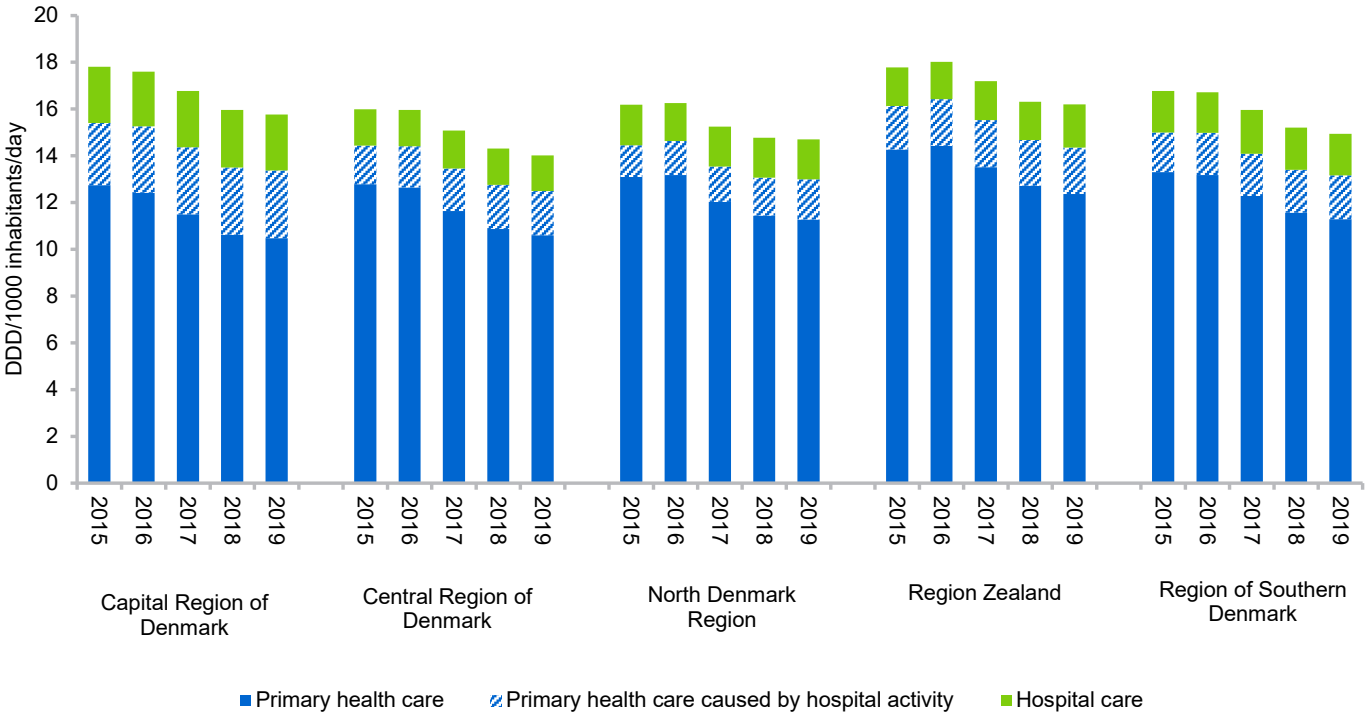
DANMAP 2019



Data used in this figure is based on registered sales to individuals and consumption at somatic hospitals
ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 5.2 Consumption of systemic antimicrobial agents in Danish regions (primary care and at somatic hospitals), DDD per 1000 inhabitants per day, Denmark

DANMAP 2019



Data used in this figure is based on registered sales to individuals and consumption at somatic hospitals
ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system

5.3 Primary health care

5.3.1 Total consumption in primary health care in DID

In 2019, the consumption of antimicrobials in primary health care based on total sales from pharmacies was 13.76 DID, a decline of 1.5% from 2018 (13.97 DID), levelling off from the significant declines observed in previous years (-6.1% in 2017 and -5.1% in 2018). Compared to the peak of 17.05 DID in 2011, consumption in 2019 had changed by -19% (Figure 5.1a). Within the decade, decreases were overall 17% from 16.56 DID in 2010.

Beta-lactamase sensitive penicillins continued to be the biggest group consumed with 3.44 DID (accounting for 25% of the total consumption in primary care, Figure 5.3). Penicillins with extended spectrum accounted for 3.28 DID (24%), beta-lactamase resistant penicillins for 1.63 DID (12%), tetracyclines for 1.48 DID (11%) and macrolides for 1.41 DID (10%), (Figure 5.3 and Table 5.1). A decade ago, in 2010, beta-lactamase sensitive penicillins accounted for 32%, penicillins with extended spectrum for 18%, beta-lactamase resistant penicillins for only 7%, while macrolides accounted for 15% (not shown). For most other antimicrobial groups, their share of the total consumption did not change notably.

5.3.2 Trends in consumption of the leading antimicrobials in DID

Antimicrobial consumption in Denmark increased almost continuously from the beginning of registration in the 90ties until the peak of consumption measured for primary care in 2011. The decreases observed since 2011 were primarily driven by decreased consumption of beta-lactamase sensitive penicillins, the dominating antimicrobial class in Denmark, and macrolides, for many years the third biggest antimicrobial class in Denmark (Figure 5.4). Continued decreases since 2011 were also observed for fluoroquinolones. Simultaneously, the consumption of beta-lactamase resistant penicillins increased since 2010.

In 2019, beta-lactamase sensitive penicillins had decreased by -35% (from 5.29 DID in 2011), macrolides by -46% (from 2.60 DID in 2011) and fluoroquinolones by -35% (0.57 DID in 2011), while beta-lactamase resistant penicillins had increased by 39% (1.17 DID in 2010).

For the penicillins with extended spectrum consumption increased during the first years of the decade, from 3.02 DID in 2010 to 3.36 DID in 2017 (11%), but since then levelled off.

Combination penicillins increased continuously from their introduction to the Danish market in 2009 until 2015 (0.95 DID), showed no changes for 2016 and since declined, in 2019 accounting for 0.63 DID (-34% since 2015 and -4.2% from 0.66 DID in 2018).

Table 5.1 Consumption of antimicrobial agents for systemic use in primary health care (DDD per 1000 inhabitants per day), Denmark DANMAP 2019

ATC group	Therapeutic group	Year										
		2000	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019
J01AA	Tetracyclines	0.99	1.70	1.74	1.76	1.96	1.66	1.60	1.62	1.42	1.40	1.48
J01CA	Penicillins with extended spectrum	1.98	3.02	3.11	3.03	3.12	3.20	3.28	3.33	3.36	3.35	3.28
J01CE	Beta-lactamase sensitive penicillins	4.76	5.26	5.29	4.68	4.65	4.38	4.33	4.16	3.88	3.61	3.44
J01CF	Beta-lactamase resistant penicillins	0.53	1.17	1.22	1.21	1.30	1.36	1.38	1.48	1.56	1.60	1.63
J01CR	Combinations of penicillins, including beta-lactamase inhibitors	0.02	0.45	0.60	0.70	0.81	0.87	0.95	0.95	0.79	0.66	0.63
J01D	Cephalosporins and other betalactam antibiotics	0.03	0.04	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
J01EA	Trimethoprim and derivates	0.33	0.51	0.50	0.52	0.53	0.55	0.56	0.56	0.56	0.53	0.45
J01EB	Short-acting sulfonamides	0.37	0.26	0.24	0.22	0.22	0.21	0.18	0.16	0.15	0.14	0.13
J01EE	Combination of sulfonamides and trimethoprim, including derivates	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
J01FA	Macrolides	2.04	2.44	2.60	2.20	1.94	1.79	1.77	1.82	1.62	1.46	1.41
J01FF	Lincosamides	0.01	0.04	0.04	0.04	0.05	0.05	0.05	0.06	0.06	0.06	0.06
J01GB	Aminoglycosides	0.00	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01
J01MA	Fluroquinolones	0.15	0.57	0.57	0.55	0.52	0.50	0.49	0.48	0.44	0.41	0.37
J01XC	Steroid antibacterials (combination fusidic acid)	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00
J01XE	Nitrofuran derivates (nitrofurantoin)	0.38	0.51	0.50	0.50	0.49	0.48	0.45	0.43	0.26	0.15	0.27
J01XX	Other antibacerials (metheamine >99%)	0.37	0.27	0.26	0.25	0.24	0.24	0.25	0.27	0.28	0.29	0.32
J01XD and P01AB01	Nitroimidazole derivates (metronidazole)	0.16	0.27	0.28	0.28	0.28	0.28	0.28	0.28	0.25	0.24	0.24
J01 and P01AB01	Antibacterial agents for systemic use (total)	12.18	16.56	17.05	16.03	16.19	15.64	15.66	15.67	14.71	13.97	13.76

ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system
Data used for this table is based on total sales in Denmark (individuals and clinics)

Fluoroquinolones represent the smallest antimicrobial drug class among the leading antimicrobial groups in Denmark. Together with the cephalosporines and carbapenems, they belong to the three antibiotic classes in focus in the guidelines on antibiotic use by the Danish Health Authorities from 2012 [www.sst.dk]. They are solely to be used for treatment of very few specific infections, where they are considered the drug of choice. They are also recommended in the case of infection with multidrug-resistant bacteria, where microbiological results point towards a fluoroquinolone to be the best or only choice. The decreasing trends observed for the consumption of fluoroquinolones during the decade follow the overall decreasing trends. Thus, in 2019, fluoroquinolones continued to account for approximately 3% of all antimicrobials consumed in primary care (Figure 5.3 and Figure 5.4). For more details about use of Fluoroquinolones in primary health care, see Figure A5.6 and A5.7 in web annex.

Changes in consumption within the last decade clearly follow different initiatives on a more prudent use of antibiotics, which have been implemented since 2011 due to concerns regarding the continuing increases over the former decades. Mentioned should be the establishment of the National Antibiotic Council in 2012, `happy audit´ and other initiatives on better diagnostics undertaken by general practitioners in recent years, evaluation and harmonization of antibiotic guidelines issued by the different medical associations as well as antibiotic campaigns aimed at the public launched since 2013.

Penicillins

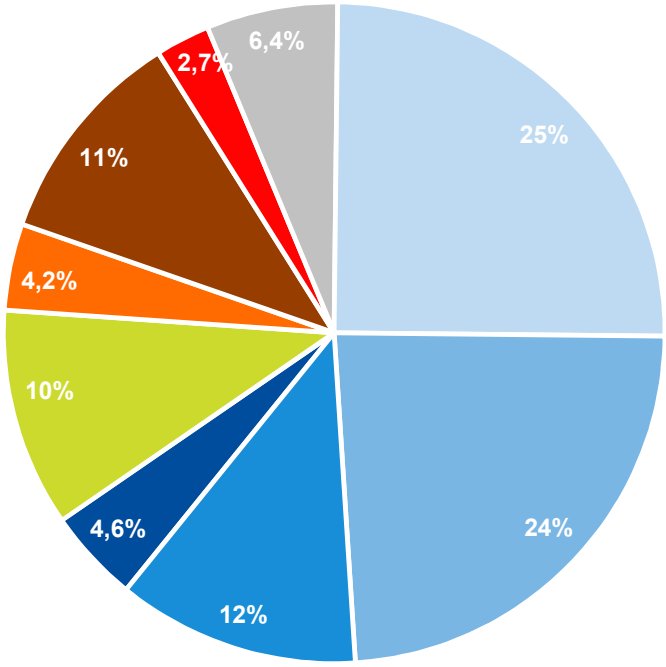
In Denmark, penicillins are the only beta-lactams used in primary health care; other beta-lactams such as cephalosporins, monobactams and carbapenems are solely used in hospital care and primarily at somatic hospitals with surgical or acute care functions.

In 2019, the four groups of penicillins accounted for altogether 8.98 DID, 65% of all antimicrobials consumed; a decade ago in 2010, they accounted for 9.91 DID, 60% of the total antimicrobials consumed that year. However, due to the described continuous decreases in the use of beta-lactamase sensitive penicillins and simultaneous increases in the penicillins with extended spectrum and the beta-lactamase resistant penicillins, in 2019 beta-lactamase sensitive penicillins constituted 38% of all penicillins used, while ten years back, in 2010, the share was 53% (Figure A5.5 in web annex).

The increases described for the penicillins with extended spectrum are primarily due to increases in the consumption of pivmecillinam, in 2019 accounting for approximately 75% of this antimicrobial class, (Figure 5.5a). Over the decade pivmecillinam increased with 45% from 1.67 DID in 2010 to 2.43 DID in 2019. Pivampicillin decreased simultaneously with 67% from 0.40 DID to 0.13 DID and amoxicillin decreased with 25% from 0.92 DID to 0.69 DID, respectively. Consumption of amoxicillin fluctuated within the decade, decreasing from 2009 to 2016 (0.61 DID) and increasing the recent two years, 9% (from 0.63 DID in 2017). Increases in the use of pivmecillinam were related to changed recommendations for the treatment of urinary tract infections (see 5.3.4), while the decreased use of pivampicillin followed increased resistance towards ampicillin in *E. coli* (see 8.2.1.) and use of amoxicillin followed recommendations regarding use of less antimicrobials in young children.

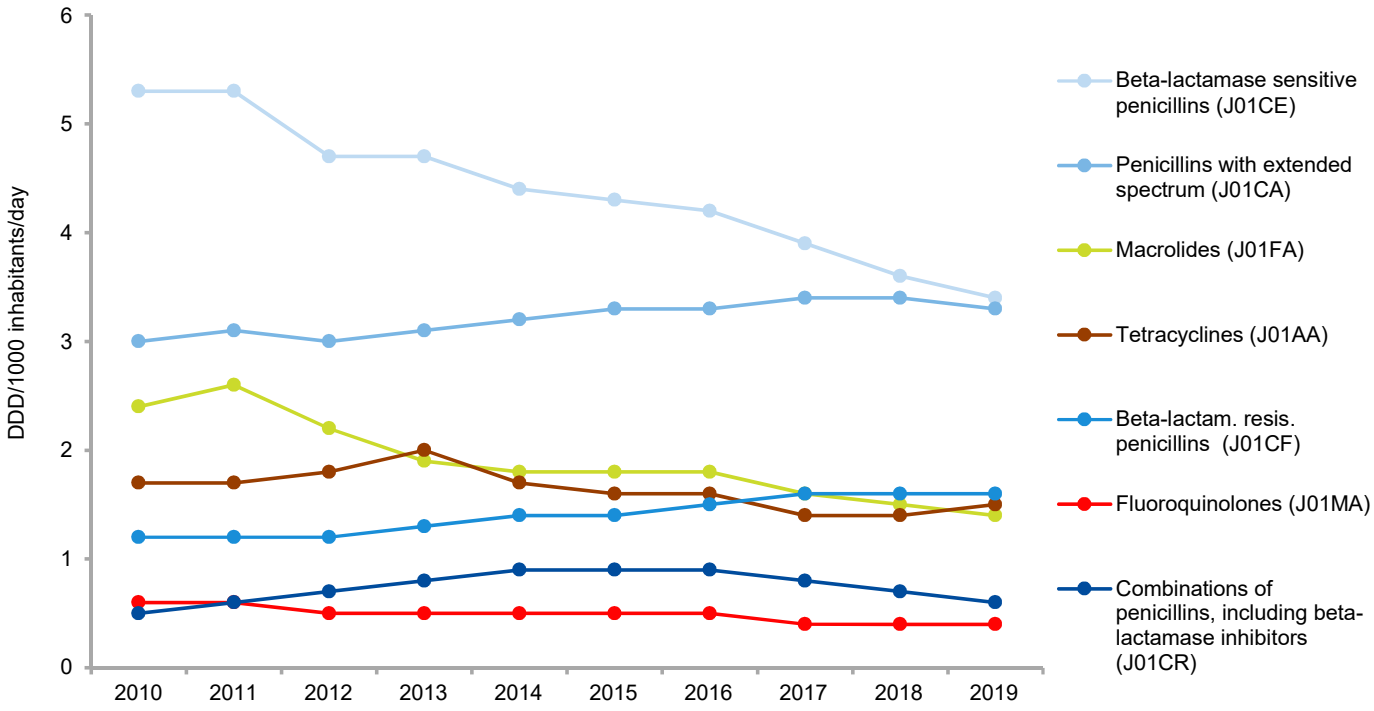
The increased consumption of beta-lactamase resistant penicillins was paralleled by an increased use at hospitals as well and followed the increased occurrence of staphylococcal infections observed in recent years (see section 8.1.3 and 8.3.8).

Figure 5.3 Distribution of antimicrobial groups within the total consumption in primary healthcare based on DDD, Denmark
DANMAP 2019



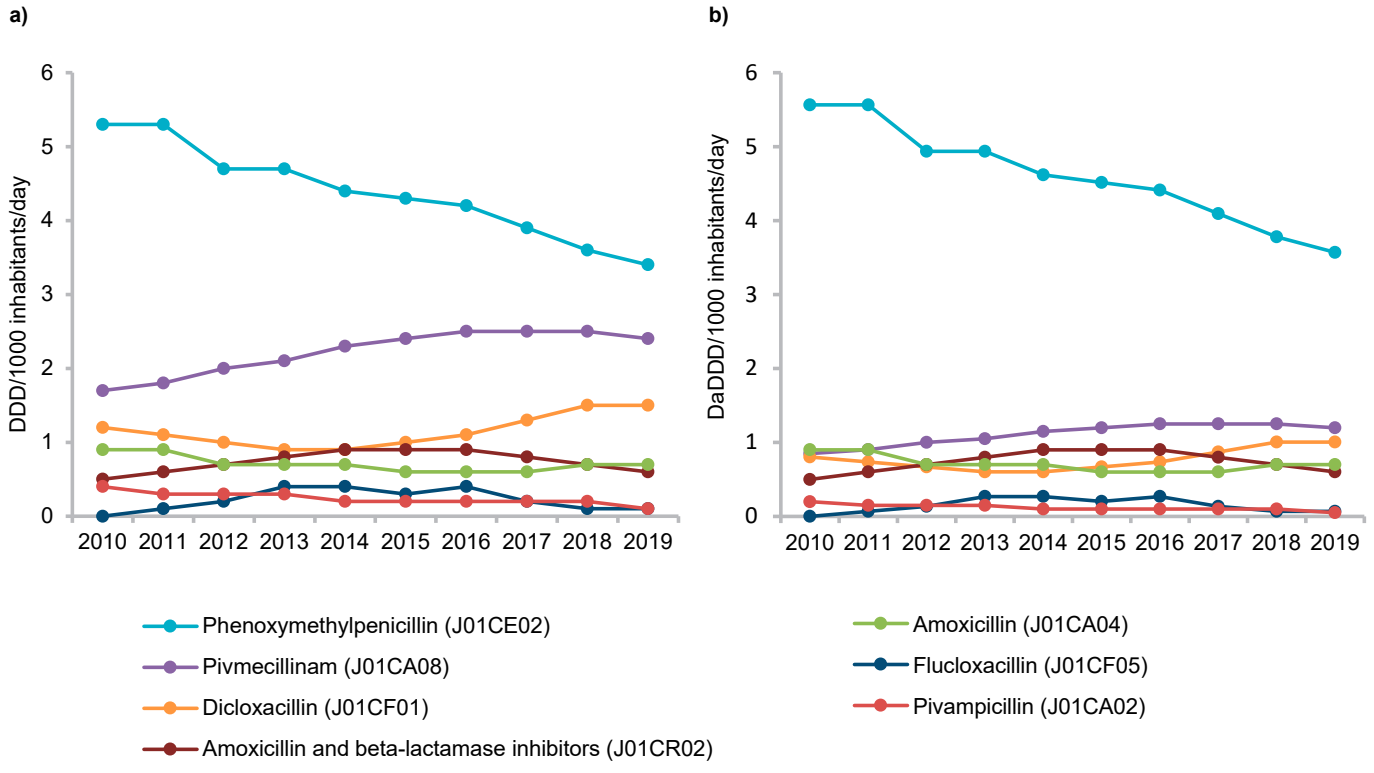
Data used for this figure is based on total sales in Denmark (individuals and clinics)
ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 5.4 Consumption of leading antimicrobial groups for systemic use in primary health care, DDD per 1000 inhabitants per day, Denmark
DANMAP 2019



Data used for this figure is based on total sales in Denmark (individuals and clinics)
ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 5.5 Consumption of main penicillins in the primary health care: a) DDD per 1000 inhabitants per day and b) DaDDD per 1000 inhabitants per day, Denmark
DANMAP 2019



Data used for this figure is based on total sales in Denmark (individuals and clinics)
ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system

The new WHO DDD values from January 2019 applied to amoxicillin and amoxicillin with clavulanic acid changed the records of these notably and their share of the total consumption, when calculated in DID. Since there were no changes in DDD values for the other main penicillins, their charts did not change correspondingly. This initiated the development of the Danish adjusted DDD (DaDDD) for all main penicillins, (Figure 5.5b), based on dosage recommendations from Danish treatment guidelines. Recommended dosages were then compared and adjusted in relation to the average doses actually given per treated patient, an information that was available through the data reported from the pharmacies each year. It is advisable to continue discussing the necessity of changing the DDD for the remaining penicillins not included in the revised DDD values, for a more true and fair reporting of antimicrobial consumption in the future. Consumption based on standard WHO DDD and DaDDD is presented in Figure 5.5a and b, respectively.

5.3.3 Measures at user level

In this and the following sections, the consumption of antimicrobials is described at user level by using either the number of prescriptions per 1000 inhabitants or the number of treated patients per 1000 inhabitants. The measures are thus based on all information available through the sales to individuals and do not include the approximately 4% of antibiotics, mainly penicillins, sold to clinics, dentists and doctors on call.

In 2019, the total number of prescriptions was 445 per 1000 inhabitants, a 3.2% reduction from the 459 prescriptions per 1000 inhabitants in 2018 and a 29% reduction compared to the 629 prescriptions per 1000 inhabitants in 2010 (Table 5.2). Decreases were observed for all antimicrobial drug classes apart from the tetracyclines and the group of "Other antibacterials", which increased from 2018 to 2019.

In 2019, the average number of prescriptions redeemed per patient was 1.90 and the total number of patients treated was 234 per 1000 inhabitants, (Table 5.3). In 2010, the number was 1.97 prescriptions per patient and 319 treated patients per 1000 inhabitants (not shown).

Trends in the number of prescriptions redeemed and the number of treated patients per 1000 inhabitants followed mainly the trends already described for the consumed DIDs. Within the last decade the most pronounced increase in the number of prescriptions per 1000 inhabitants was observed for the combination penicillins, including betalactamase inhibitors (40%). Most pronounced decreases in the number of prescriptions per 1000 inhabitants were observed for the folllowing: macrolides (-48%), beta-lactamase sensitive penicillins (-39%), sulphonamides and trimethoprim (-41%), tetracyclines (-33%) and fluoroquinolones (-41%), (Table 5.2).

Similar decreases were noted for the decade, when measured in the number of patients treated per 1000 inhabitants: macrolides (-47%), beta-lactamase sensitive penicillins (-36%), sulphonamides and trimethoprim (-43%), tetracyclines (-25%) and fluoroquinolones (-42%), (Table 5.3).

A comparison of the different indicators of consumption is presented in Figure 5.6. In 2019, the average DDD/prescription was 10.9, an increase of 2% compared to the 10.7 DDD/prescription in 2018, and an increase of 17% compared to the 9.3 in 2010.

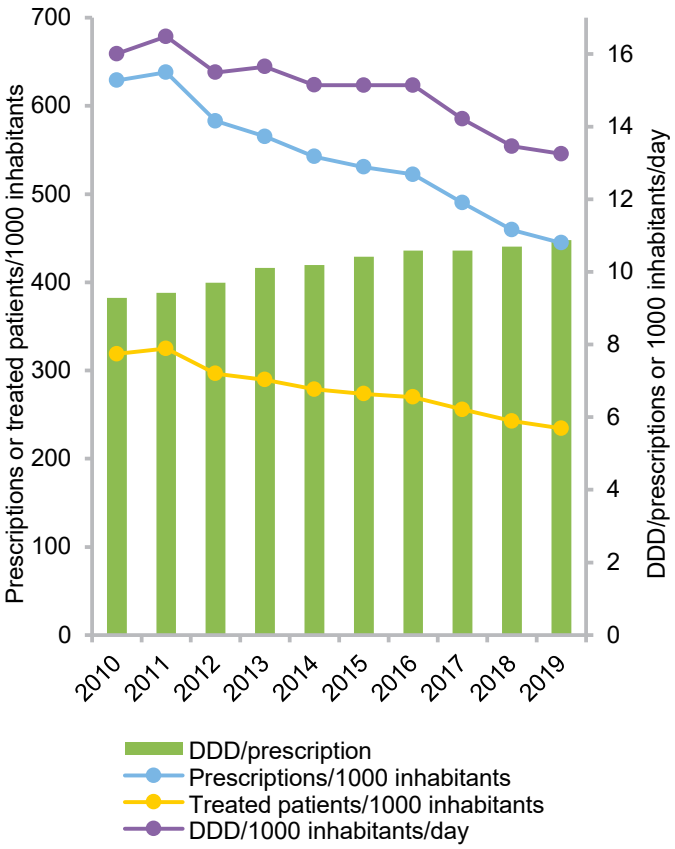
Table 5.2 Number of prescriptions per 1000 inhabitants for leading antimicrobial agents in primary health care, Denmark DANMAP 2019

ATC group	Therapeutic group	Year										
		2000	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019
J01AA	Tetracyclines	19.37	22.49	22.70	22.56	22.89	20.00	17.90	17.18	15.89	14.63	15.11
J01CA	Penicillins with extended spectrum	93.45	127.23	125.17	115.91	114.30	113.83	113.53	113.16	114.37	114.31	112.19
J01CE	Beta-lactamase sensitive penicillins	222.09	212.19	213.32	186.91	180.55	170.70	163.09	157.13	148.52	136.81	128.77
J01CF	Beta-lactamase resistant penicillins	22.13	42.32	42.75	40.42	41.25	41.04	40.81	41.87	41.87	43.35	43.16
J01CR	Combinations of penicillins, including beta-lactamase inhibitors	0.91	16.53	21.11	24.71	28.01	29.02	30.73	31.13	27.09	23.71	23.07
J01E	Sulphonamides and trimethoprim	53.20	47.35	45.05	43.86	43.53	41.51	38.39	36.41	34.29	31.74	28.14
J01FA	Macrolides	88.04	97.34	104.22	85.89	74.51	68.01	68.00	68.85	60.00	52.64	50.71
J01MA	Fluoroquinolones	8.89	23.69	23.15	22.14	20.65	19.67	19.50	18.74	17.37	15.97	13.99
J01X	Other antibacterials (methenamine >99%)	13.02	17.49	18.24	18.03	17.41	16.73	16.28	15.82	10.18	6.76	10.29
P01AB01	Nitroimidazole derivatives (metronidazole)	12.89	19.67	19.69	19.68	19.26	19.06	19.15	18.63	17.26	16.31	15.78
J01 and P01AB01	Antibacterial agents for systemic use (total)	534.87	628.78	638.08	582.80	565.26	542.53	530.56	522.19	490.08	459.39	444.54

ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system
Data used in this table is based on registered sales to individuals

Figure 5.6 Different indicators of antimicrobial consumption (J01 and P01AB01), in primary health care, Denmark

DANMAP 2019



Data used for this figure is based on registred sales to individuals
ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system

5.3.4 Prescribing activity in primary healthcare

Although Denmark has a very homogenous population with relatively small geographic and socioeconomic variations compared to other countries, considerable differences in the prescribing habits among medical doctors are frequently observed. In 2019, the Central Denmark Region had the lowest prescribing activity when compared to the other four regions, with 12.50 DID and 417 prescriptions per 1000 inhabitants per year, (Table 5.4). The Region Zealand had the highest prescribing activity with 14.36 DID and 482 prescriptions per 1000 inhabitants per year. For all regions, significant decreases in the DIDs and number of prescriptions redeemed were observed for the five years presented (on average 12% in DID and 16% in the number of prescriptions per 1000 inhabitants per year).

There may be several reasons to the differences in the number of prescriptions redeemed, e.g. variations in the density of the population and number of general practitioners as well as the proportion of elderly or chronically ill in a given geographic area. Due to differing organisation of general practitioners and clinical practices across the country, comparison of prescribing habits based on the individual clinical praxis is difficult. A clinical praxis can be based on a single physician but can also be a collaboration of up to seven physicians sharing facilities and staff. In addition, due to the lack of general practitioners in some areas, several new models of "health houses" served by physicians and other health staff are being established these years. General practitioners can follow their own prescription habits through the website www.ordiprax.dk, a closed IT system that collects all data on prescriptions and enables comparison with other praxis´ on a regional level.

Table 5.3 Number of treated patients per 1000 inhabitants for leading antimicrobial agents in primary health care, Denmark DANMAP 2019

ATC group	Therapeutic group	Year										
		2000	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019
J01AA	Tetracyclines	12.04	13.44	13.66	13.53	13.86	12.20	11.32	11.04	10.35	9.69	10.10
J01CA	Penicillins with extended spectrum	65.78	85.04	84.19	77.31	76.10	75.32	74.87	74.05	74.04	73.56	71.97
J01CE	Beta-lactamase sensitive penicillins	169.32	162.81	164.34	145.53	142.19	134.79	130.06	125.69	119.32	110.90	104.70
J01CF	Beta-lactamase resistant penicillins	15.65	30.02	30.34	28.51	29.07	29.24	28.85	29.70	29.96	31.10	31.06
J01CR	Combinations of penicillins, including beta-lactamase inhibitors	0.63	11.70	14.95	17.32	19.71	20.52	22.03	22.17	19.89	17.73	17.33
J01E	Sulphonamides and trimethoprim	36.51	29.31	27.63	26.48	26.16	24.65	22.45	21.17	19.87	18.42	16.63
J01FA	Macrolides	65.72	72.67	78.75	64.73	56.16	51.38	51.75	53.21	46.01	40.11	38.45
J01MA	Fluoroquinolones	7.00	18.45	18.10	17.25	16.04	15.30	15.04	14.37	13.36	12.26	10.74
J01X	Other antibacterials (methenamine >99%)	6.84	7.53	7.74	7.54	7.48	7.16	7.35	7.47	5.01	3.62	5.66
P01AB01	Nitroimidazole derivatives (metronidazole)	11.21	16.73	16.90	16.86	16.51	16.31	16.47	16.03	14.84	14.05	13.57
J01 and P01AB01	Antibacterial agents for systemic use (total)	295.78	318.69	324.91	296.40	289.54	278.62	273.49	269.72	255.72	242.55	234.34

ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system
Data used in this table is based on registered sales to individuals

Support of the general practitioners regarding their prescribing habits is in general provided through regional medicine consultants, who also have access to Ordiprax on clinic level, thus being able to monitor consumption and give individual advice. From 2018, the general practitioners in defined geographical areas have been joined in "quality clusters" for mutual support.

In Figure 5.7a and b, the number of prescriptions on municipality level are shown for years 2016 and 2019, respectively. For 2019, the numbers span from 366 to 603 prescriptions per 1000 inhabitants, most municipalities laying within the range of 400 to 500 prescriptions per 1000 inhabitants. Three years earlier, in 2016, the corresponding interval was 434-727 prescriptions per 1000 inhabitants. From the 98 municipalities in Denmark, four were excluded from the figure due to very small populations (typically islands).

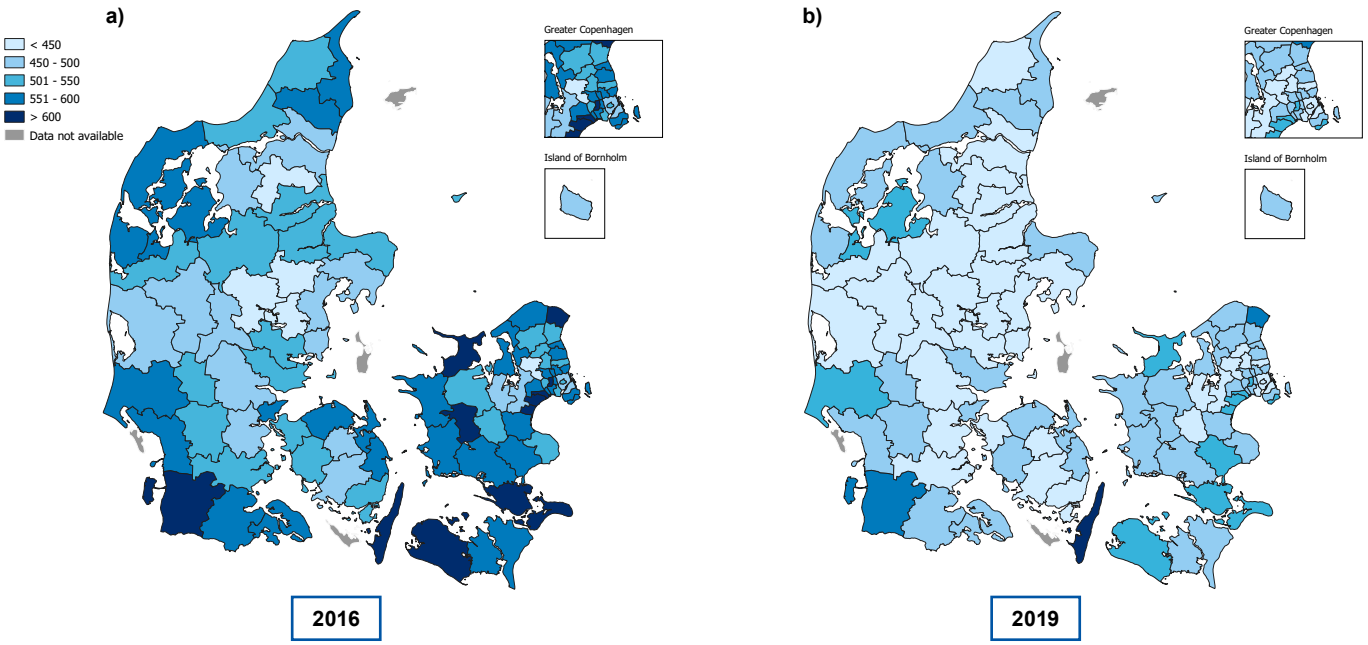
Prescribing habits of doctors with different specialties differ, e.g. in 2019, 63% of antimicrobial prescriptions from specialists in dermato-venerology were for tetracyclines, which is used to treat severe acne, while 58% of prescriptions issued by dentists were for beta-lactamase sensitive penicillins. An overview of the numbers of prescriptions issued by the different specialties can be found in Table 5.5 and Figure 5.8.

Table 5.4 Consumption of antimicrobial agents for systemic use in primary health care at regional level, Denmark DANMAP 2019

Region	Indicator	Year				
		2015	2016	2017	2018	2019
Capital Region	DDD/1000 inhabitants/day	15.40	15.26	14.36	13.50	13.38
	Prescriptions/1000 inhabitants	533.22	519.10	489.47	453.39	441.36
Region Zealand	DDD/1000 inhabitants/day	16.13	16.43	15.53	14.68	14.36
	Prescriptions/1000 inhabitants	575.14	574.86	539.02	501.32	481.81
Region of Southern Denmark	DDD/1000 inhabitants/day	14.99	14.98	14.09	13.40	13.16
	Prescriptions/1000 inhabitants	539.98	530.16	496.75	470.50	454.94
Central Denmark Region	DDD/1000 inhabitants/day	14.44	14.41	13.46	12.76	12.50
	Prescriptions/1000 inhabitants	494.17	487.24	458.31	430.90	417.31
North Denmark Region	DDD/1000 inhabitants/day	14.45	14.64	13.54	13.07	13.00
	Prescriptions/1000 inhabitants	510.13	509.05	472.15	451.55	435.96
Denmark (total)	DDD/1000 inhabitants/day	15.14	15.14	14.21	13.46	13.25
	Prescriptions/1000 inhabitants	530.56	522.19	490.08	459.39	444.54

Data used in this table is based on registered sales to individuals
ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 5.7 Number of prescriptions from primary healthcare per 1000 inhabitants in Danish municipalities in a) 2016 and b) 2019
DANMAP 2019



Data used in this figure is based on registered sales to individuals
ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system

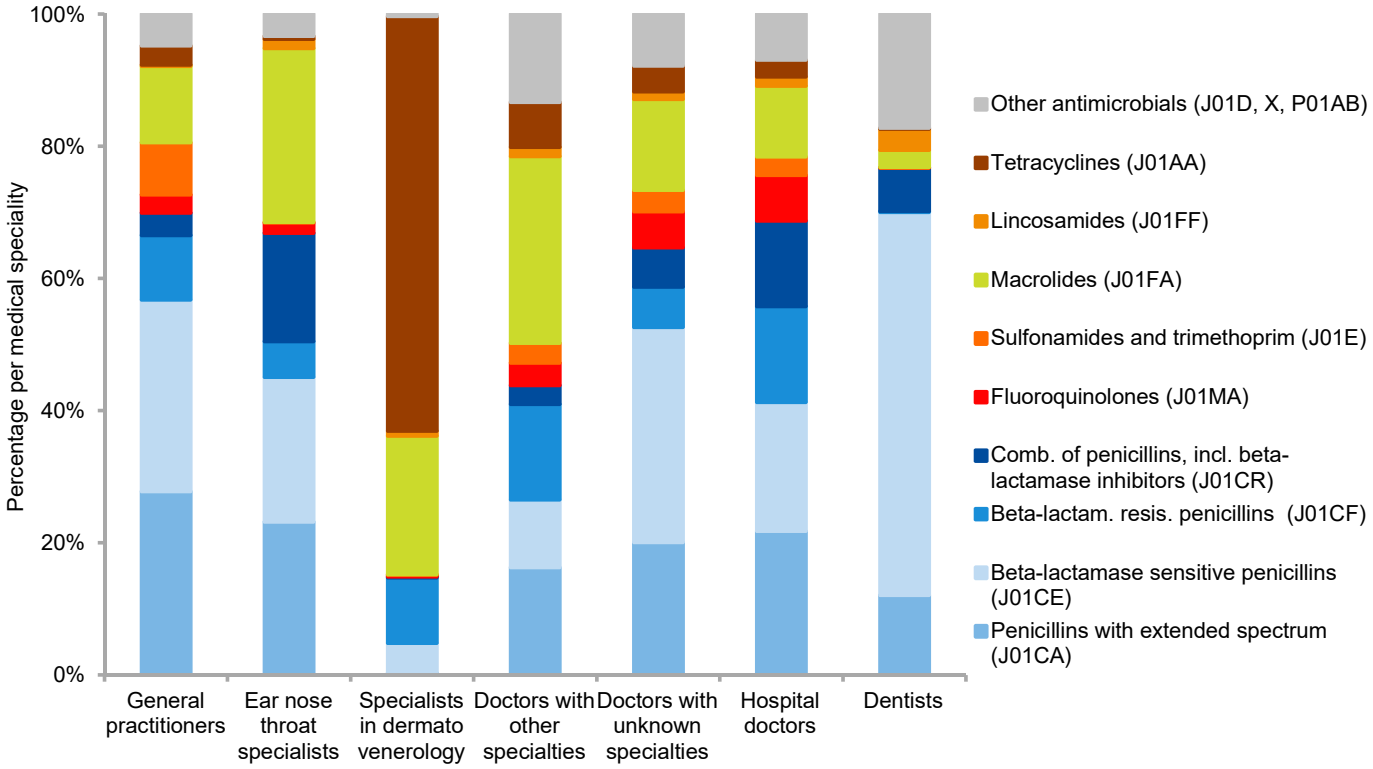
Consumption in primary sector includes prescriptions issued from hospital doctors upon discharge of a patient. In the past decade, the number of prescriptions issued through hospital doctors increased notably, probably due to changes in hospital work flow with shortening of bed-days and increasing activity in ambulatory care. In 2019, hospital doctors accounted for 63 prescriptions per 1000 inhabitants (14% of the antimicrobials sold at pharmacies), (Table 5.5). In 2008, it was 38 prescriptions per 1000 inhabitants (corresponding to 6% of sales), (not shown).

Table 5.5 Number of prescriptions per 1000 inhabitants for different doctor types DANMAP 2019

Doctor type	Year		
	2017	2018	2019
General practitioners	368.6	341.5	326.8
Ear nose throat specialists	8.9	8.4	7.8
Specialists in dermato venerology	5.9	5.2	5.4
Doctors with other specialties	4.3	4.2	4.2
Doctors with unknown specialties	10.9	9.7	8.7
Hospital doctors	62.6	62.8	63.0
Dentists	29.1	27.8	28.8

Data used for this table are based on registered sales to individuals

Figure 5.8 Prescribing habits presented as percentage of prescriptions by main medical specialties, primary sector, Denmark
DANMAP 2019



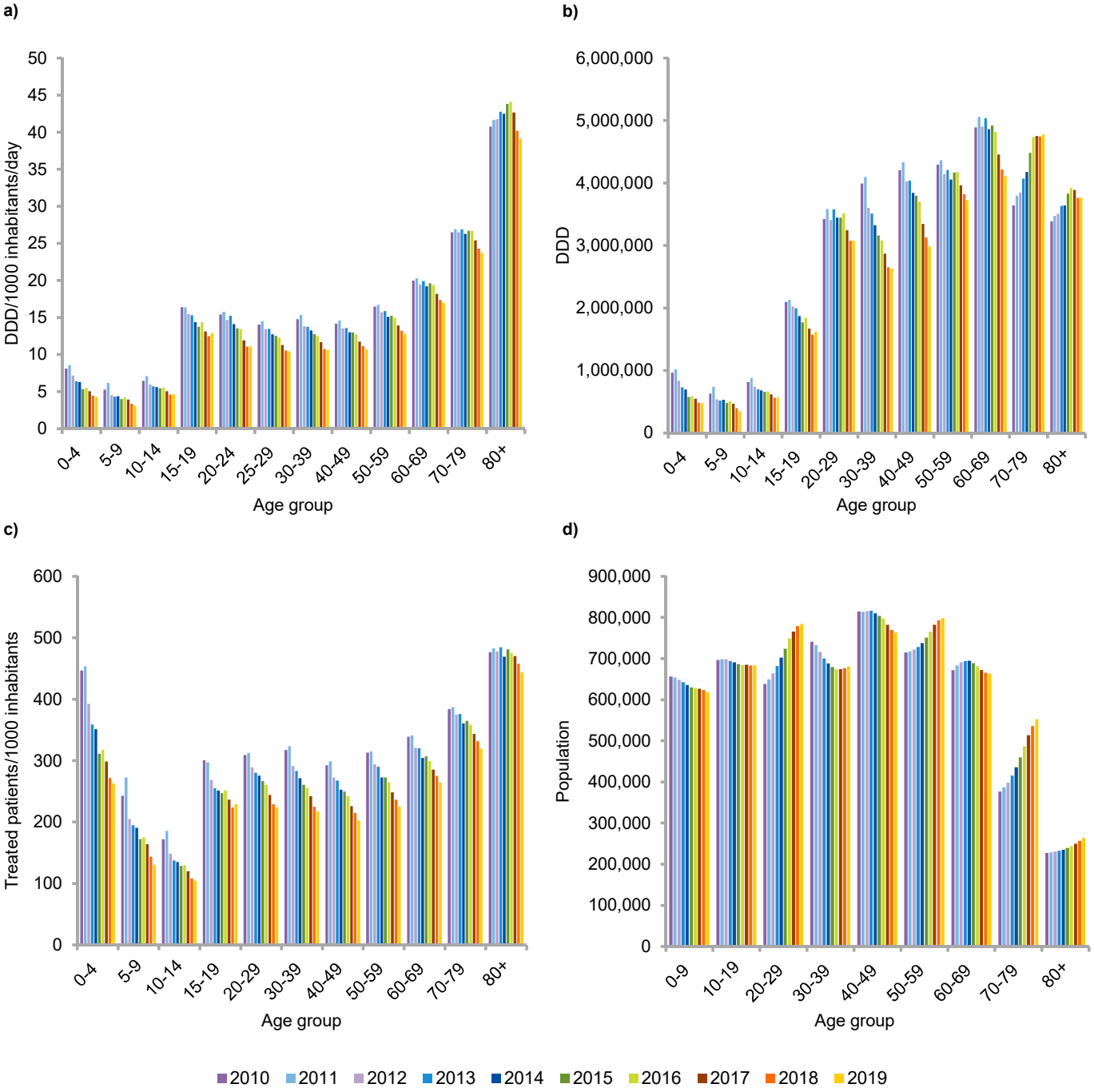
Data used in this figure is based on registered sales to individuals
ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system

5.3.5 Consumption by age group

Initiatives aiming at reducing overuse and misuse of antibiotics often focus on consumption in the youngest and the elderly. But inappropriate use is not restricted to overtreating children with fever conditions or elderly with unspecific urinary symptoms, also other age groups may be over- or undertreated.

Figure 5.9 presents consumption in the different age groups based on different denominators: Figure 5.9a presents consumption in DDD per 1000 inhabitants per day, Figure 5.9b in crude DDD, i.e. not corrected for population size. Figure 5.9c presents the number of patients treated per 1000 inhabitants and 5.6d presents the actual population sizes. All figures show data from 2010 to 2019. For children, WHO DDD values were used, although dosages given to children are based on body weight and therefore not directly comparable to adults. Children and adolescents are also presented in age groups of five years, while all others are clustered in 10-years age groups.

Figure 5.9a to d Consumption of antimicrobials and population per age group, DDD per 1000 inhabitants per day, DDD, number of treated patients per 1000 inhabitants and population size, clustered in five and ten years intervals DANMAP 2019



Data used in this figure is based on registered sales to individuals
ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system
Population size in Figur 5.9d is based on data from Statistics Denmark at www.dst.dk

5.3.6 Consumption of antimicrobials in children

Measuring the consumption of antimicrobial agents in children in defined daily doses (DDD) is problematic, since the DDD is defined as "maintenance dose per day for its main indication in adults". The maintenance dose per day for children may differ from the one for adults due to different pharmacodynamics and -kinetics. Furthermore, in the youngest age groups children of the same age might be treated with different doses, since the dose is calculated based on body weight. Other parameters for measurement of consumption might be more suitable for children, e.g. number of treated patients per 1000 inhabitants and number of prescriptions per 1000 inhabitants. However, assuming that dosage regimens did not change considerably within the last decade, it is possible to compare the consumption in DDD within each age group over time.

In 2019, the overall consumption in children of 0-19 years was 181 treated patients per 1000 inhabitants receiving in total 287 prescriptions per 1000 inhabitants. Since 2010, the consumption decreased from 289 treated patients per 1000 inhabitants (-37%) and 510 prescriptions per 1000 inhabitants (-44%). In 2018, the corresponding numbers were 185 treated patients and 293 prescriptions per 1000 inhabitants (-2.4% and -1.9%, respectively).

Consumption in the 0-4 year olds. Consumption of antimicrobial agents in the youngest age group decreased with 41% from 2010 (447 treated patients per 1000 inhabitants) to 2019 (262 treated patients per 1000 inhabitants). Since 2018, the consumption decreased with 3.4% (272 treated patients per 1000 inhabitants), (Figure 5.10). On average, each treated patient received 2.00 prescriptions in 2010, which decreased to 1.66 prescriptions in 2019, (not shown). In 2019, the total consumption corresponded to 4.27 DID, 47% less than in 2010 (8.10 DID), (Figure 5.9a). The prescription tendency changed also during the last decade. In 2010, penicillins with extended spectrum were the main antimicrobial agents used to treat children between 0-4 years (274 patients per 1000 inhabitants, 61% of total consumption). In 2019, beta-lactamase sensitive penicillins were the most prescribed (141 patients per 1000 inhabitants, 54% of total consumption), (Figure 5.10).

Consumption in the 5-9 year olds. In 2019, 130 patients per 1000 inhabitants of 5-9 years were treated with antimicrobial agents, (Figure 5.9c). This is 46% lower than 2010 (243 patients per 1000 inhabitants) and 9.0% lower than 2018 (143 patients per 1000 inhabitants). On average, each treated patient received 1.53 prescriptions in 2010, which decreased to 1.43 prescriptions in 2019, (not shown). In 2019, the total consumption corresponded to 3.1 DID, 41% less than in 2010 (5.2 DID), (Figure 5.9a). The distribution of the antimicrobials used to treat the 5-9 year olds did not change markedly over the last decade, and beta-lactamase sensitive penicillins remained the main antimicrobial agent used (85 patients per 1000 inhabitants, 66% in 2019).

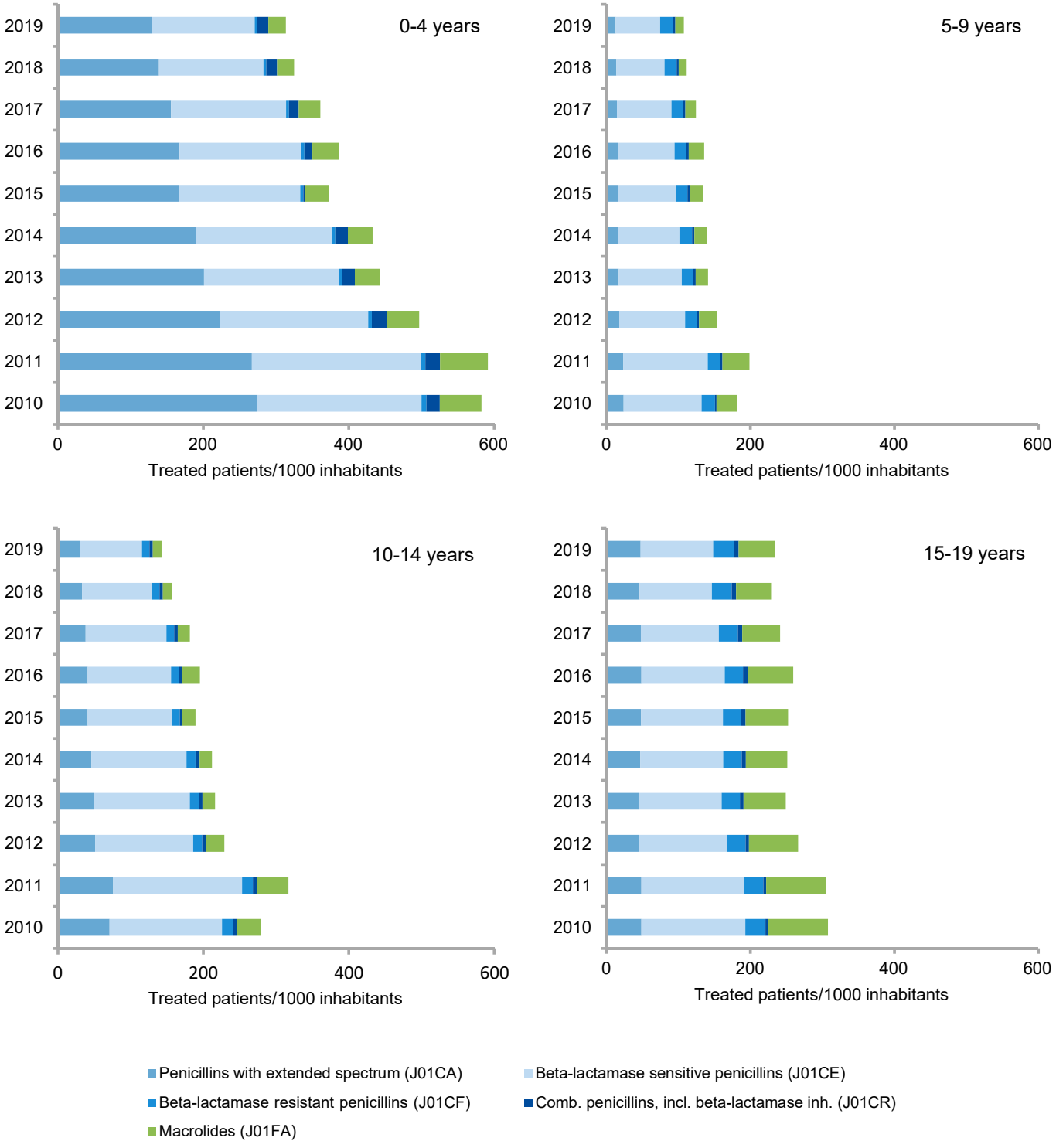
Consumption in the 10-14 year olds. In 2019, the total consumption of antimicrobial agents (105 patients per 1000 inhabitants) was 39% lower than a decade ago (172 patients per 1000 inhabitants) and 3.3% lower than 2018 (108 patients per 1000 inhabitants), (Figure 5.9c). On average, each treated patient received 1.49 prescriptions in 2010, which decreased to 1.44 prescriptions in 2019, (not shown). In 2019, the total consumption corresponded to 4.6 DID, 29% less than in 2010 (6.5 DID), (Figure 5.9a). The main change in the distribution of the consumption was increased share of beta-lactamase resistant penicillins (from 10.8% of total consumption in 2010 to 16.9% in 2019). Beta-lactamase sensitive penicillins remained the main antimicrobial agent (59% in 2019), (Figure 5.10).

Consumption in the 15-19 year olds. Consumption of antimicrobial agents in the oldest children increased in 2019 after several years with decreased consumption (Figure 5.9a-c). In 2019, 229 patients per 1000 inhabitants were treated with antimicrobial agents, 2.3% higher than in 2018 (224 patients per 1000 inhabitants). The increase was caused by increases in several main antimicrobial agents, (Figure 5.10): tetracyclines (+6.2%, not shown), beta-lactamase resistant penicillins (+5.2%), macrolides (+4.9%) and penicillins with extended spectrum (+2.9%). In 2019, the consumption corresponded to 382 prescriptions per 1000 inhabitants and 12.9 DID which is 2.7% and 3.5% higher than 2018, respectively.

Macrolides play an important role in the treatment of infections in children and the young, being the drug of choice for respiratory tract infections with *Mycoplasma pneumoniae* and in pertussis. Macrolides are also used in the adolescents for the treatment of sexually acquired infections, e.g. *Chlamydia*. This, combined with treatment of acute pharyngitis may be the reason for the relatively high consumption of macrolides in the 15-19 year olds, which in 2019 counted 51 patients per 1000 inhabitants. For the 0-4 year olds, the 5-9 year olds and the 10-14 year olds the corresponding numbers were 24, 12 and 12 patients per 1000 inhabitants per year, respectively, (Figure 5.10). However, as for the total population penicillins are the most used antimicrobial agents among children regardless of age (constituting between 44% and 66% per age group), Figure 5.10.

Comparison of consumption among girls and boys showed different tendencies in different age groups (not shown). The youngest boys (0-4 year olds) received 11% more prescriptions per 1000 inhabitants than the girls (457 versus 411). The opposite was observed among older children: girls aged 5-9 years received 24% more prescriptions per 1000 inhabitants than boys (207 versus 167), girls aged 10-14 years received 27% more prescriptions per 1000 inhabitants than boys (134 versus 169) and girls aged 15-19 years received 102% more prescriptions per 1000 inhabitants than boys (514 versus 255).

Figure 5.10 Consumption of five antimicrobial agents by children/adolecents age 0-19, Denmark DANMAP 2019



Data used in Figure 5.10a-c is based on registered sales to individuals
ATC numbers used in Figure 5.10a-b stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system

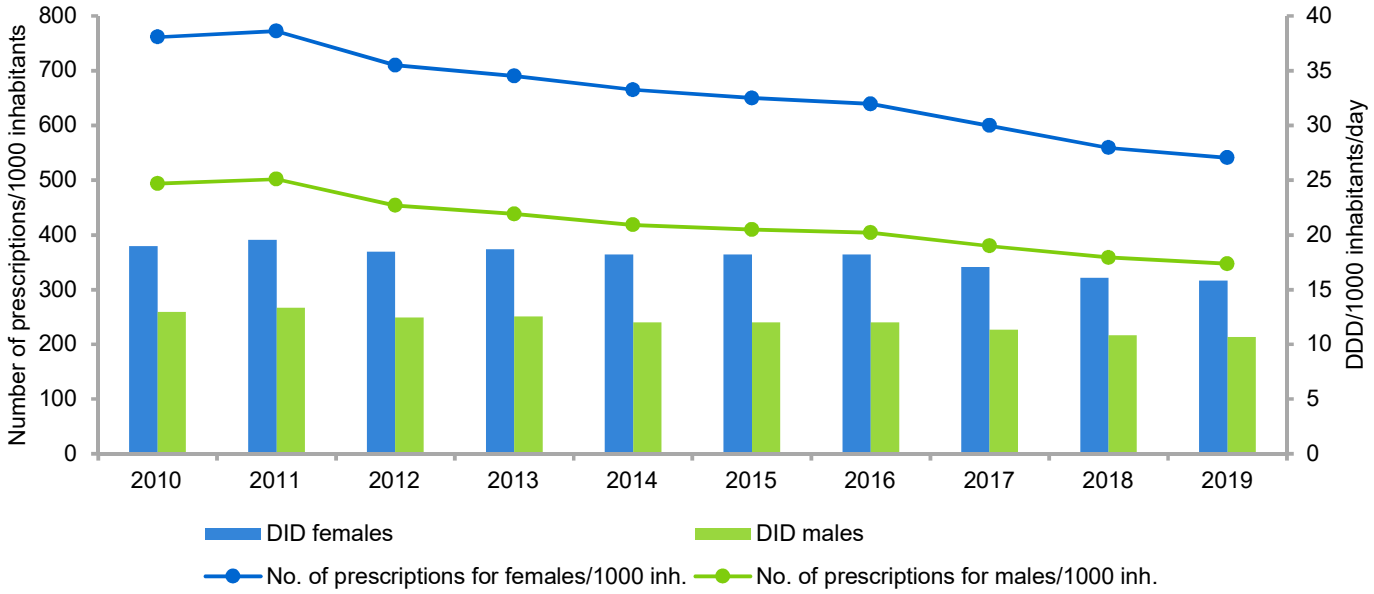
5.3.7 Consumption of antimicrobials according to gender

Differences between genders regarding consumption of antimicrobials are well known. In general, females receive more treatment – a trend driven by a much higher incidence of urinary tract infections in females. Thus, the consumption of pivmecillinam, sulphonamides, trimethoprim and nitrofurantoin is approximately three times higher for females than for males.

Also for beta-lactamase sensitive penicillins and macrolides the differences in consumption, especially when measured in DID, are substantial, (Figure 5.11a and b). From 2010 to 2019, the number of treated females (all age group) decreased from 369 to 277 per 1000 inhabitants (-25%) and the number of treated males per 1000 from 267 to 191 inhabitants (-28%).

Figure 5.11a Consumption of antimicrobial agents (J01 and P01AB01) per gender, prescriptions per 1000 inhabitants per year and DDD per 1000 inhabitants per day, Denmark

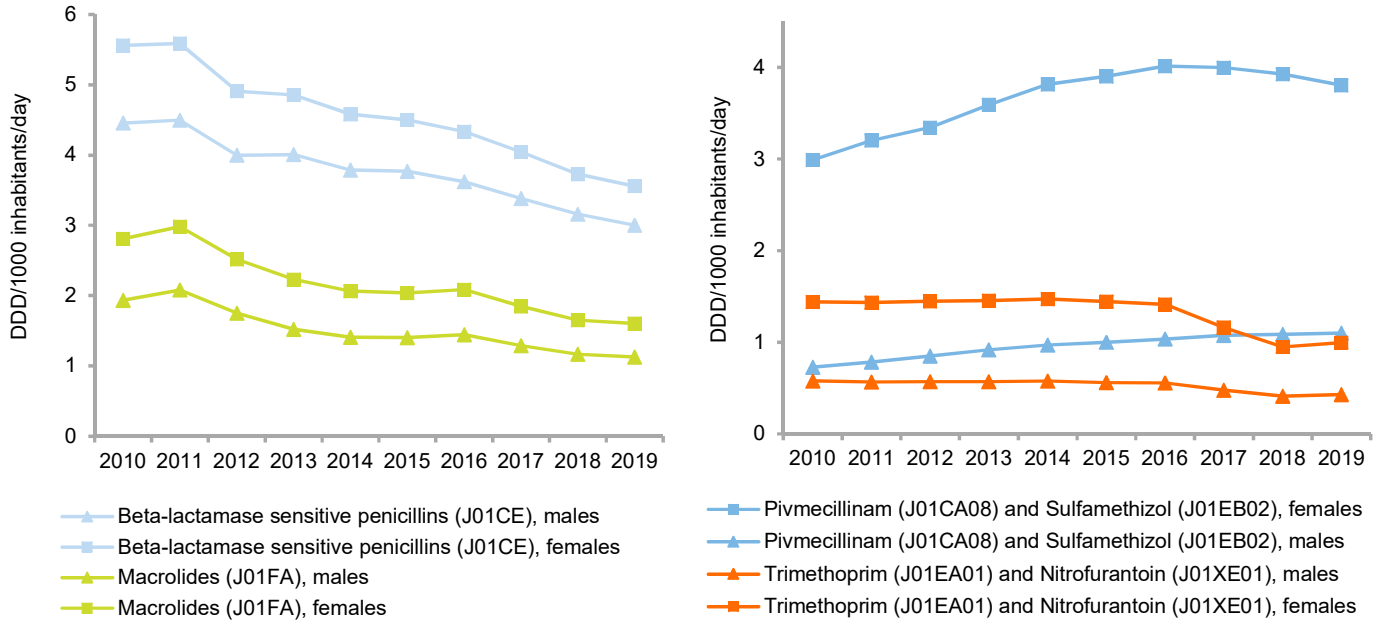
DANMAP 2019



Data used in this figure is based on registered sales to individuals
ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 5.11b Consumption of most used antimicrobials for respiratory tract infections and urinary tract infections, DDD per 1000 inhabitants per day, Denmark

DANMAP 2019



Data used in this figure is based on registered sales to individuals
ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system

During the same period, the number of DDD per prescription increased for females from 9.1 to 10.7 (17%), and for males from 9.6 to 11.2 (17%). Altogether from 2010-2019, the consumption in females decreased from 19.0 DID to 15.8 DID (-17%), and in males from 13.0 DID to 10.7 DID (-18%).

Drugs for treatment of respiratory tract infections. For both females and males a decrease in the consumption of beta-lactamase sensitive penicillins and macrolides was observed over the last decade. From 2010 to 2019, for females, beta-lactamase sensitive penicillins decreased from 5.6 DID to 3.6 DID and macrolides from 2.8 DID to 1.6 DID, respectively. For males, the corresponding changes were from 4.5 DID to 3.0 DID and from 1.9 DID to 1.1 DID, respectively (Figure 5.11b).

Urinary drugs. Figure 5.11b presents the consumption of antimicrobial urinary drugs grouped into pivmecillinam and sulfamethizol (against acute infections) and trimethoprim and nitrofurantoin (more often used in the prevention of UTI in elderly or in recurring infections) for 2010 to 2019. Not presented are the shifting trends in use of the individual drug classes: In 2019, pivmecillinam accounted for 3.6 DID for females and 1.05 DID for males. In 2010, the corresponding numbers were 2.6 DID for females and 0.7 DID for males. For sulfamethizol consumption showed opposite trends: from 0.4 DID in 2010 to 0.2 DID in 2019 in females and from 0.06 DID to 0.05 DID in males, respectively.

Since 2017, consumption of all urinary drug classes has decreased. Most notable are the recent changes in consumption of nitrofurantoin: from a stabile 0.7 DID in women in the years 2009 to 2016, to 0.4 DID in 2017 and 0.2 DID in 2018. In men, the consumption of nitrofurantoin decreased from 0.3 DID in 2010 to 0.13 DID in 2019. In 2017, recommendations regarding the use of nitrofurantoin had been issued, advocating for caution in the use in elderly. Unexplained is the increase in women to 0.4 DID in 2019.

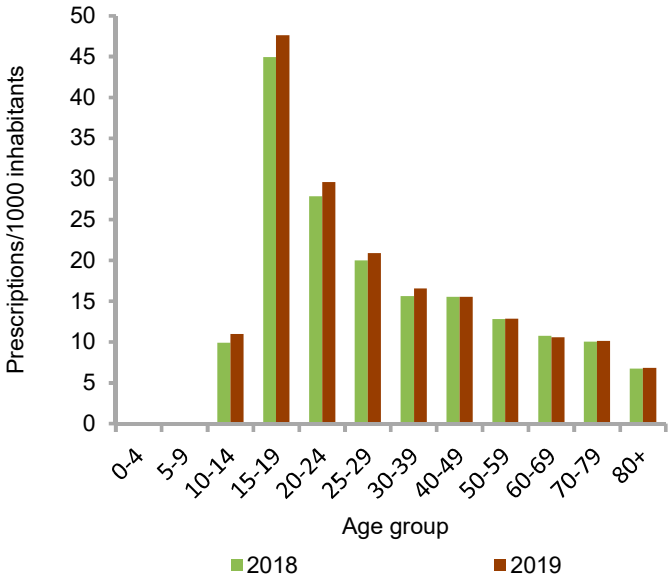
5.3.8 Tetracyclines

In 2019, tetracyclines accounted for 1.48 DID, corresponding to 11% of the total consumption in primary health care. During the last decade, the consumption has decreased by altogether 12.9% from 1.7 DID in 2010. In 2013, the consumption peaked unexpectedly at 1.96 DID. In 2019, the consumption increased again (5.8%), (Figure 5.4). Tetracyclines are used by all age groups above 12 years and by both genders (Figure 5.12).

Tetracyclines account for a considerable part of consumption of antimicrobials among adolescents due to the treatment of acne, (Table 5.6).Treatment lasts long (up to six months) and may even be repeated in a situation of relapse in patients, who may be suffering from the condition for years. Furthermore, within the same family/at the same family doctor, there may be tendency to treat younger siblings as well. There exist clear differences in prescription habits regarding boys and girls. Among girls, the treatment periods are longer and extend into the young adults of up to 24 years, while boys primarily are treated in shorter periods at the age of 15-19 years. In 2010, on average 37 boys aged 15-19 years were treated per 1000 inhabitants (2.1 treatments on average). For girls of the same age the corresponding number was 31 patients per 1000 inhabitants (1.9 treatments on average). In 2019, the number of 15-19 years old boys receiving treatment had declined to 27 treated patients (1.7 treatments) per 1000 inhabitants, while the number of 15-19 year old girls receiving treatment was 31 treated patients (1.6 treatments) per 1000 inhabitants.

In women (all ages) consumption decreased slightly from 1.8 DID in 2010 to 1.7 DID in 2019 and in men from 1.5 DID to 1.2 DID, respectively (not shown). Increases in the occurrence of sexually transmitted infections and changes in the treatment guidelines for these may be a challenge in future years, see chapter 8.3.9 on the occurrence of *N. gonorrhoea* in Denmark.

Figure 5.12 Consumption of tetracyclines in different age groups, prescriptions per 1000 inhabitants, Denmark DANMAP 2019



Data used for this figure is based on registered sales to individuals
ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Table 5.6 Consumption of tetracyclines by clinical indication code DANMAP 2019

Indication written on the prescription	Year		
	2017	2018	2019
Against acne	50.7	54.9	55.6
Prevention of malaria	8.1	6.9	6.0
Against Borrelia infection	2.6	4.2	4.4
Against pelvic inflammatory disease	1.8	1.9	1.9
Against skin and soft tissue infection	1.2	1.5	1.6
Unspecified indications	35.6	30.6	30.4

Data used for this table is based on registered sales to individuals
ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system

5.4 Hospital care

5.4.1 Introduction

Antimicrobial consumption at hospitals is reported to DANMAP once a year through the Register of Medicinal Product Statistics at the Danish Health Data Authority. Reporting is based on deliverances from the hospital pharmacies to the different clinical departments and includes all generic products that are supplied through general trade agreements between the regions and the company Amgros. For more information see chapter 9.8, material and methods.

DANMAP 2019 covers the total sales of systemic antimicrobials (ATC code J01 as well as ATC code P01AB01 and A07AA09) reported from all Danish hospital pharmacies. However, only Figure 5.1a on page 44 includes all consumption, (e.g. also including consumption at private hospitals and psychiatric departments), in 2019 accounting for approximately 2-3% of the total hospital consumption. In all other figures and calculations, only consumption at somatic hospitals with acute care functions is presented.

In DANMAP, data on hospital consumption is kept at a national or regional level. Data on hospital level can be supplied upon request.

Information on consumption at individual patient level is still lacking for the hospital sector. This information is expected to be available through the future national "Hospital Medicine Register", which is currently under development.

The consumption of antimicrobial agents in hospital care is presented as DDD per 100 occupied bed-days (DBD) and as DDD per 100 admissions (DAD) to account for hospital activity. Moreover, data are presented as DID to enable comparison with primary healthcare.

As mentioned, during the past decade the hospitalisation patterns in Denmark changed notably. The shortening of bed-days at hospitals and the increasing ambulatory care function, including increased surgical activity, causes increased pressure on the health system at municipality level, (Figure A5.3 and A5.4 in web annex). Therefore demands arise for more rehabilitation beds for patients dismissed from hospital, but not yet ready for continuing treatment at home.

The increasing number of invasive infections and infections at other sites also induces pressure into the system, increasing the demand for antibiotic treatment (see section 8.1 introduction and Figure 8.3). Since selection pressure for the emergence of antimicrobial resistance follows with increasing hospital activity, the selection pressure has increased considerably from 2010-2019, (Figure A5.3 in web annex).

Table 5.7 presents data on regional and national hospital activity together with information on the size of population for 2010 and 2019. Denmark has a very high bed occupancy rate and overcrowding happens relatively often, especially during winter time and in situations with influenza epidemics.

In 2019, the number of admissions at Danish somatic hospitals is estimated to be 1,364,804, while the number of bed-days is estimated to be 3,898,979. From 2010-2019, the number of bed-days decreased with altogether 16%, while the number of admissions increased with 3.9% and the Danish population with 4.9%. During the decade, activity in ambulatory care increased from 6,454,112 treated patients to 7,984,223 treated patients, (24%). On average, the number of bed-days decreased with an annual 2.0%, while the number of admissions on average increased with 0.8% per year, (Figure A5.3 in web annex).

On a regional level, the number of bed-days decreased in all regions from 2010-2019; Capital Region of Denmark and Region Zealand with -13%, Region of Southern Denmark with -17%, Central Denmark Region with -18% and North Denmark Region with -26%. The number admissions increased from 2010-2019 in Capital Region of Denmark (+5.7%), Region Zealand (+13.7%) and Central Denmark Region (+2.7%). The number of admissions decreased in Region of Southern Denmark and North Denmark Region with -0.8% and -6.7%, respectively.

Table 5.7 Activity at Danish hospitals DANMAP 2019

Region	Number of bed-days at somatic hospitals		Number of admission to somatic hospitals		Population	
	2010	2019*	2010	2019*	2010	2019
Capital Region of Denmark	1,649,961	1,438,898	450,289	475,758	1,680,271	1,835,562
Region Zealand	666,992	583,452	209,301	238,027	820,564	836,738
Region of Southern Denmark	901,209	749,208	258,459	256,324	1,200,277	1,223,348
Central Denmark Region	939,014	771,353	278,027	285,606	1,253,998	1,320,678
North Denmark Region	481,379	356,764	117,697	109,799	579,628	589,755
Denmark	4,638,555	3,898,979	1,313,773	1,364,804	5,534,738	5,806,081

* Data from 2019 are estimated by applying the 10 yr average increase/decrease observed for 2009-2018
Data used in this table is based on the activity at somatic hospitals

5.4.2 Public somatic hospitals - DDD per 100 occupied bed-days (DBD)

In 2019, the consumption of antimicrobial agents at somatic hospitals was 107 DBD, 6.2% higher than the observed 100.7 DBD in 2018 and 49% higher than the consumption measured a decade ago in 2010, (71.6 DBD). The consumption in 2019 was the highest measured this decade, (Table 5.8).

The four penicillin groups accounted for altogether 58.05 DBD, corresponding to 54% of the total somatic hospital consumption of antimicrobials (Figure 5.13, Table 5.8).

In 2019, combination penicillins accounted for 17.3 DBD, making it the largest group consumed in 2019 (16%). In 2017, a shortage of piperacillin with tazobactam had been responsible for a drop in consumption that year. In 2018, delivery had been reestablished, resulting in an increase in the consumption of combination penicillins of altogether 14% since 2016.

Penicillins with extended spectrum are the second largest group consumed at Danish hospitals. In 2019, they accounted for 16.0 DBD (15%) of the consumption in somatic hospitals, a 4.0% increase from 2018 (15.4 DBD). Beta-lactamase sensitive penicillins accounted for 9.75 DBD (9%) and beta-lactamase resistant penicillins for 14.99 DBD (14%), a 42% increase from 2018 (10.52 DBD).

Overall, the consumption of penicillins showed increasing trends for the decade. The combination penicillins increased steeply by 12.5 DBD (259%), the beta-lactamase resistant penicillins and penicillins with extended spectrum less markedly, but still continuously with 7.93 DBD (112%) and 4.54 DBD (40%), respectively, (Figure 5.14a and 5.15). These trends are comparable to the trends observed for the primary sector, apart from changes for 2017-2019, where consumption in the primary sector decreased notably for the combination penicillins.

Table 5.8 Consumption of antimicrobial agents for systemic use in somatic hospitals, DBD, Denmark DANMAP 2019

ATC group	Therapeutic group	Year									
		2010	2011	2012	2013	2014	2015	2016	2017	2018	2019
J01AA	Tetracyclines	1.01	1.09	1.55	1.48	1.65	1.82	2.09	2.05	2.38	3.28
J01CA	Penicillins with extended spectrum	11.45	11.35	12.47	12.88	13.62	14.14	14.50	15.81	15.37	15.99
J01CE	Beta-lactamase sensitive penicillins	8.25	9.03	9.32	9.41	9.32	9.09	9.19	10.20	10.40	9.75
J01CF	Beta-lactamase resistant penicillins	7.06	8.16	8.21	8.78	9.30	9.33	9.95	11.19	10.52	14.99
J01CR	Comb. of penicillins. incl. beta-lactamase inhibitors	4.83	6.48	9.30	10.92	12.78	14.91	15.16	14.02	16.57	17.31
J01DB	1st generation cephalosporins	0.12	0.12	0.12	0.11	0.06	0.04	0.04	0.04	0.04	0.03
J01DC	2nd generation cephalosporins	11.15	14.34	13.36	12.28	11.37	10.14	9.25	11.04	9.00	8.08
J01DD	3rd generation cephalosporins	1.01	1.07	1.03	1.08	1.00	1.04	1.03	1.33	1.20	1.19
J01DF	Monobactams	0.04	0.14	0.15	0.14	0.06	0.03	0.01	0.01	0.01	0.01
J01DH	Carbapenems	1.65	2.38	2.58	2.79	3.30	2.98	2.72	2.91	2.83	2.99
J01EA	Trimethoprim and derivatives	0.32	0.31	0.36	0.38	0.47	0.40	0.37	0.41	0.44	0.40
J01EB	Short-acting sulfonamides	0.28	0.21	0.18	0.16	0.14	0.12	0.10	0.10	0.10	0.09
J01EE	Comb. of sulfonamides and trimethoprim. incl. derivatives	2.56	3.52	3.77	4.40	4.84	5.30	5.45	5.70	6.08	6.72
J01FA	Macrolides	3.17	3.26	3.38	3.27	3.64	4.35	4.70	5.71	6.27	6.70
J01FF	Lincosamides	0.43	0.48	0.61	0.64	0.65	0.57	0.63	0.65	0.76	0.74
J01GB	Aminoglycosides	1.71	2.02	2.10	2.15	2.04	2.22	1.98	2.27	2.20	2.67
J01MA	Fluoroquinolones	8.26	8.42	8.38	8.62	8.63	8.50	7.72	7.43	7.18	6.95
J01XA	Glycopeptides	0.98	1.22	1.25	1.31	1.15	1.16	1.09	1.31	1.27	1.33
J01XB	Polymyxins	0.24	0.22	0.22	0.27	0.22	0.19	0.19	0.19	0.23	0.22
J01XC	Steroid antibacterials (fusidic acid)	0.32	0.25	0.23	0.22	0.23	0.16	0.11	0.07	0.06	0.06
J01XD	Imidazole derivatives	3.51	3.71	3.92	4.09	4.42	4.22	4.52	4.65	4.33	4.09
J01XE	Nitrofuran derivatives (nitrofurantoin)	0.27	0.29	0.33	0.34	0.32	0.27	0.24	0.25	0.27	0.28
J01XX05	Methenamine	0.07	0.09	0.08	0.07	0.06	0.09	0.08	0.07	0.10	0.08
J01XX08	Linezolid	0.20	0.29	0.31	0.36	0.34	0.44	0.36	0.37	0.52	0.53
J01XX09	Daptomycin	0.02	0.01	0.02	0.02	0.05	0.04	0.05	0.08	0.14	0.06
P01AB01	Nitroimidazole derivatives (metronidazole)	2.39	2.34	2.29	2.25	1.98	2.01	2.18	2.03	1.94	1.91
A07AA09	Intestinal antiinfectives (vancomycin)	0.25	0.40	0.47	0.49	0.52	0.47	0.49	0.52	0.50	0.54
J01, P01AB01, A07AA09	Antibacterial agents for systemic use, including metronidazole and vancomycin (total)	71.56	81.20	85.98	88.92	92.16	94.03	94.22	100.39	100.71	106.98

Data used in this table is based consumption at somatic hospitals
ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system
Data from 2019 are estimated by applying the average decrease in bed-days observed in 2009-2018

The consumption of beta-lactamase sensitive penicillins increased with annual fluctuations, presenting fluctuations with decreases in 2014, 2015 and 2019. In 2019, the consumption of beta-lactamase sensitive penicillins was 9.75 DBD.

Notable trends for other antimicrobials for 2010 to 2019 were increases observed for tetracyclines, combinations of sulfonamides and trimethoprim and for macrolides. Although tetracyclines only account for a minor part of the antimicrobials consumed at hospitals, the drug class has been continuously increasing during the past decade; in 2010 they accounted for 1.01 DBD, while in 2019 the consumption had increased to 3.28 DBD. Consumption of combinations of sulfonamides and trimethoprim, increased from 2.56 DBD in 2010 to 6.72 DBD in 2019, a total increase of 163% for the decade. A rise in macrolides was observed from 3.17 DBD in 2010 to 6.70 DBD in 2019 (111%), (Table 5.8, Figure 5.14a and 5.15).

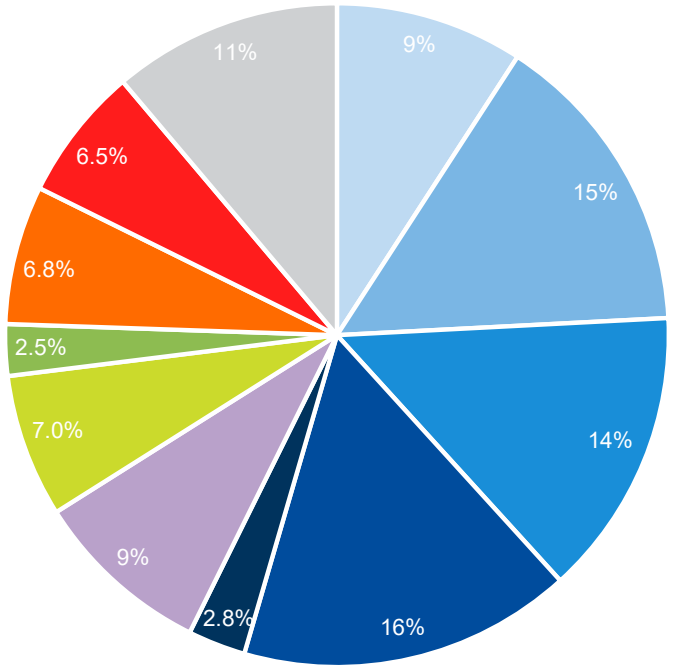
Finally, for linezolid the consumption peaked in 2019 at 0.53 DBD, whereas daptomycin decreased to 0.06 DBD after the observed peak in 2018 (0.14 DBD), (Table 5.8). Although the consumption of both is only minor, these changes are noteworthy: for Linezolid due to high risk of creating resistance, and for daptomycin due to its use in the treatment of invasive vancomycin resistant enterococci (VRE), the number of which has increased dramatically (see chapter 8.2.5 on invasive enterococci and 8.3.3 on VRE). The consumption of linezolid increased with 158% since 2010 (0.2 DBD) but no increase were observed for 2018-2019. The Capital Region of Denmark accounted for 73% of the consumption of linezolid.

Also for daptomycin the main use was in the Capital Region of Denmark (73%), which coincides with the Capital Region having the highest number of clinical cases of VRE in 2019, (section 8.3.3 and 8.3.4).

In 2019, the consumption of leading antimicrobials used in empirical treatment of main infections treated at hospitals continued its increases, a trend following the described increasing trends for the number of invasive isolates, (Figure 5.14a and Figure 8.1). In 2019, these leading antimicrobials constituted 86.7 DBD of the total consumption of 107 DBD (81%). In 2018, it was 81.6 DBD of a total of 100.7 DBD (81%).

Trends in the consumption at hospitals on regional level, measured in DDD per 1000 inhabitants per day and DDD per 100 bed-days, are presented in Figure 5.16, page 58 and 59.

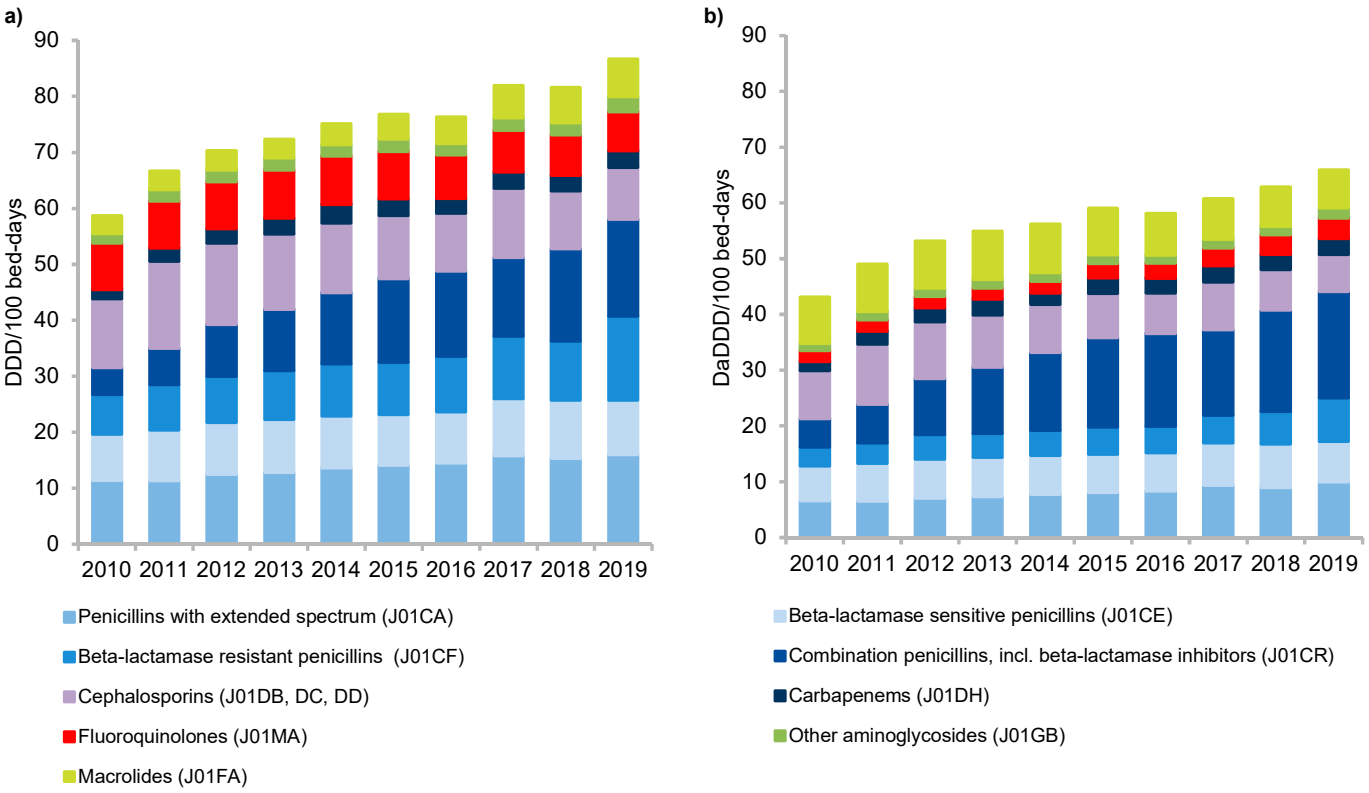
Figure 5.13 Distribution of drug groups within the total consumption of antimicrobial agents in somatic hospitals, in DDD, Denmark DANMAP 2019



Data used in this figure is based on consumption at somatic hospitals
ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 5.14a and b Consumption at somatic hospitals by leading groups of antimicrobial agents (J01), DDD per 100 bed-days and DaDDD per 100 bed-days for comparison, Denmark

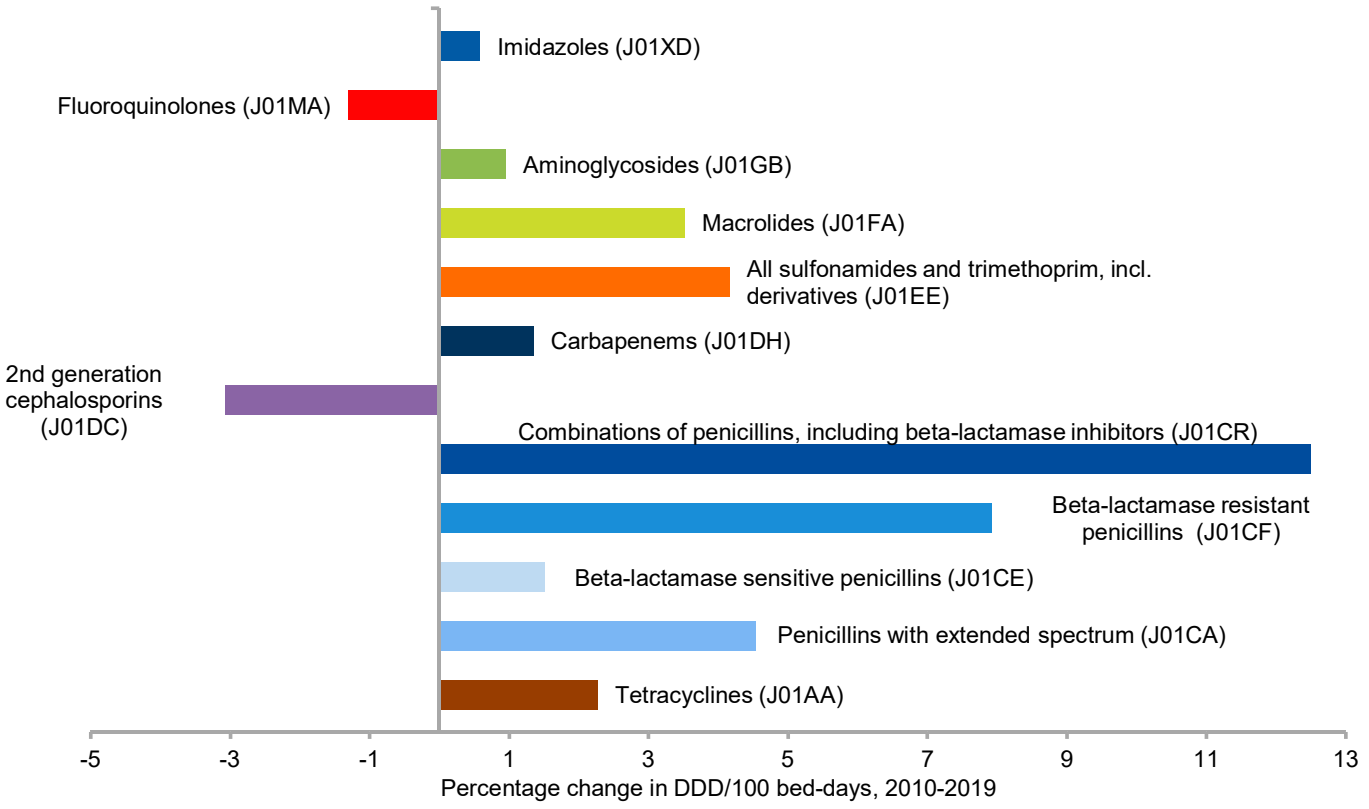
DANMAP 2019



Data used in this figure is based on consumption at somatic hospitals
ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 5.15 Changes in the consumption of leading groups of antimicrobial agents at somatic hospitals, Denmark

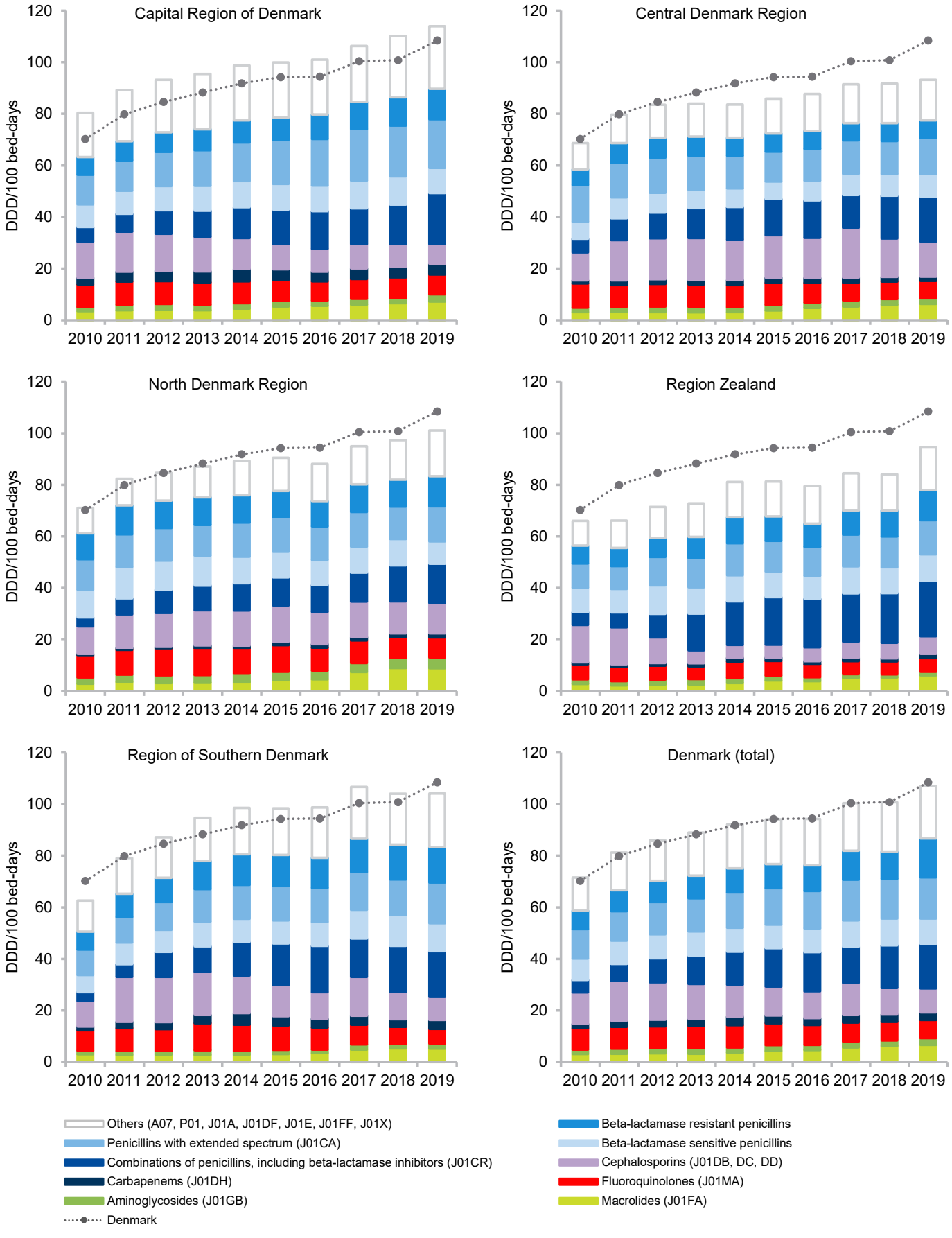
DANMAP 2019



Data used in this figure is based on consumption at somatic hospitals
ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system

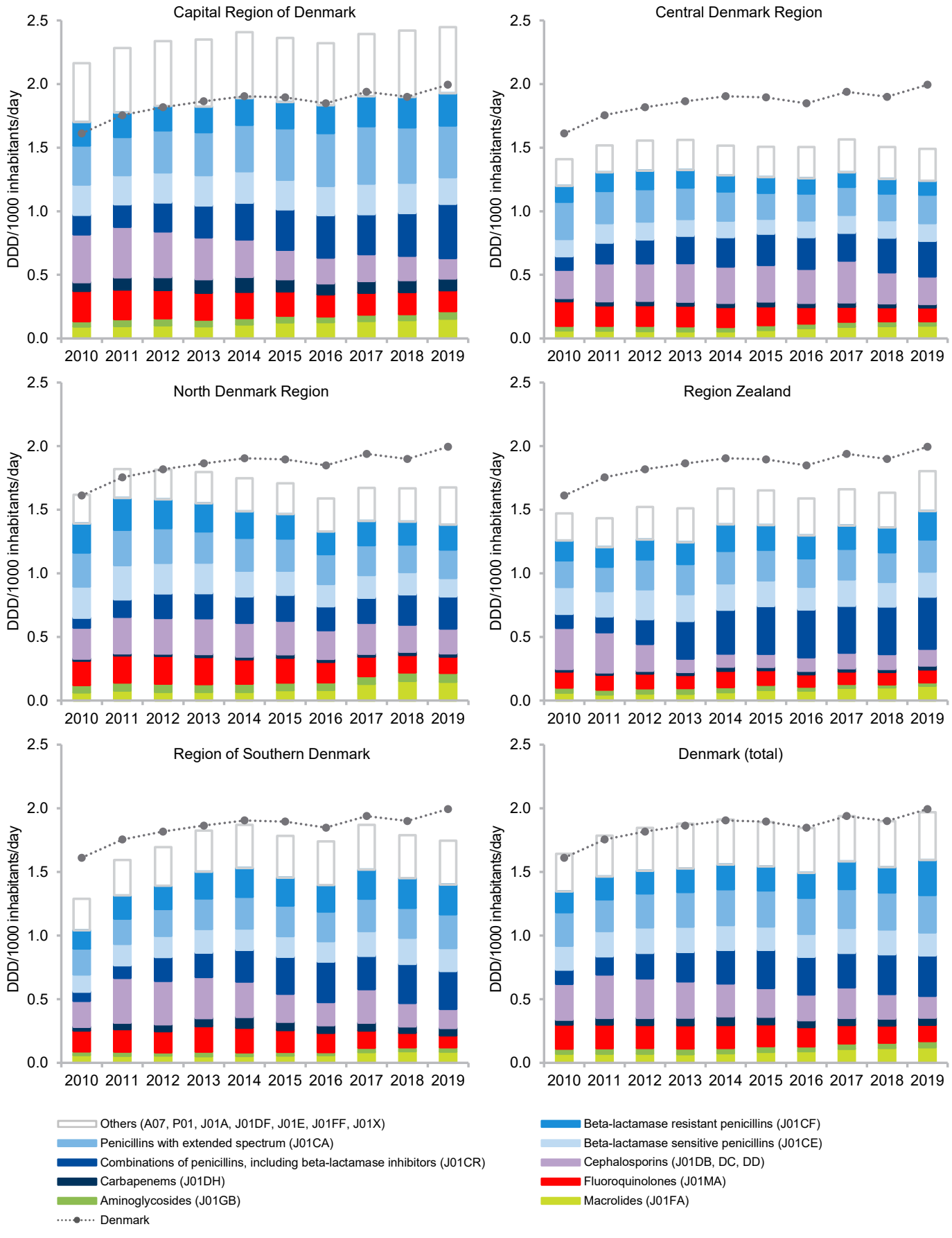
Figure 5.16a Consumption of antimicrobial agents for systemic use in the five health regions, DDD per 100 bed-days, Denmark

DANMAP 2019



Data used in this figure is based consumption at somatic hospitals
ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 5.16b Consumption of antimicrobial agents for systemic use in the five health regions, DDD per 1000 inhabitants per day, Denmark
DANMAP 2019



Data used in this figure is based on consumption at somatic hospitals
ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system

5.4.3 Other measures of consumption at somatic hospitals - DDD per 100 admissions (DAD)

The consumption of antimicrobials at hospitals can also be measured in relation to hospital activity calculated in number of patients "passing through", i.e. DDD per 100 admissions (DAD).

In 2019, the total consumption was 306 DAD, a 3.4% increase from the 296 DAD in 2018 and 21% increase from 253 DAD in 2010. The consumption measured in DDD per 100 admissions has increased since 2016, and in 2019 it reached the highest level ever measured, (Table 5.9). The trends in DDD per 100 admissions reflect for most antimicrobials the trends observed in DBD. However, the observed rates of increases were more marked, when measured in DDD per 100 bed-days than in DDD per 100 admissions for all antimicrobial classes, (Tables 5.8 and 5.9). This could be due to the change in hospital activity, as presented in Figure A5.3 in web annex.

At regional level, the hospital activity measured in admissions mirrors the density of the population, except for the Region of Southern Denmark and the North Denmark Region, where the population over the last decade has increased slightly, but the number of admissions at somatic hospitals has decreased, (Table 5.7). For all five Danish regions, the number of bed-days in somatic hospitals decreased from 2010-2019. Trends in consumption measured in DDD per 1000 inhabitants per day and DDD per 100 bed-days are presented in Figure 5.16a and b.

A comparison of the usage of antimicrobials for the treatment of animals and humans, respectively, measured in kg active substance is presented in Figure 4.1 and Table A5.1 in web annex. For comparison of consumption at hospitals with the consumption in the primary sector measured in DDD per 1000 inhabitants per day see Figure 5.4.

Table 5.9 Consumption of antimicrobial agents for systemic use in somatic hospitals, DDD per 100 admissions, Denmark DANMAP 2019

ATC group	Therapeutic group	Year									
		2010	2011	2012	2013	2014	2015	2016	2017	2018	2019
J01AA	Tetracyclines	3.55	3.69	5.07	4.74	5.11	5.52	6.21	6.01	6.98	9.37
J01CA	Penicillins with extended spectrum	40.42	38.47	40.70	41.41	42.29	43.00	43.14	46.44	45.14	45.68
J01CE	Beta-lactamase sensitive penicillins	29.10	30.59	30.41	30.25	28.93	27.64	27.34	29.96	30.55	27.86
J01CF	Beta-lactamase resistant penicillins	24.92	27.63	26.78	28.23	28.88	28.39	29.62	32.89	30.90	42.82
J01CR	Comb. of penicillins. incl. beta-lactamase inhibitors	17.03	21.97	30.35	35.11	39.68	45.35	45.11	41.18	48.64	49.46
J01DB	1st generation cephalosporins	0.43	0.39	0.39	0.36	0.19	0.13	0.13	0.12	0.11	0.08
J01DC	2nd generation cephalosporins	39.36	48.57	43.58	39.48	35.32	30.85	27.52	32.44	26.43	23.09
J01DD	3rd generation cephalosporins	3.56	3.64	3.37	3.48	3.11	3.16	3.07	3.91	3.52	3.39
J01DF	Monobactams	0.13	0.49	0.47	0.46	0.20	0.08	0.03	0.02	0.02	0.03
J01DH	Carbapenems	5.81	8.06	8.40	8.96	10.26	9.05	8.09	8.54	8.30	8.55
J01EA	Trimethoprim and derivatives	1.14	1.05	1.18	1.22	1.45	1.21	1.11	1.22	1.28	1.13
J01EB	Short-acting sulfonamides	1.00	0.72	0.58	0.52	0.44	0.36	0.30	0.30	0.29	0.25
J01EE	Comb. of sulfonamides and trimethoprim. incl. derivatives	9.04	11.91	12.29	14.14	15.02	16.12	16.23	16.75	17.86	19.21
J01FA	Macrolides	11.19	11.03	11.04	10.52	11.31	13.24	14.00	16.77	18.40	19.13
J01FF	Lincosamides	1.52	1.62	1.97	2.05	2.01	1.74	1.86	1.90	2.24	2.11
J01GB	Aminoglycosides	6.04	6.85	6.87	6.92	6.35	6.75	5.90	6.66	6.47	7.63
J01MA	Fluoroquinolones	29.16	28.51	27.35	27.72	26.81	25.86	22.98	21.83	21.10	19.86
J01XA	Glycopeptides	3.45	4.12	4.09	4.22	3.58	3.52	3.23	3.85	3.72	3.80
J01XB	Polymyxins	0.84	0.76	0.73	0.86	0.69	0.59	0.58	0.57	0.67	0.63
J01XC	Steroid antibacterials (fusidic acid)	1.11	0.85	0.74	0.71	0.71	0.50	0.34	0.20	0.18	0.16
J01XD	Imidazole derivatives	12.40	12.56	12.79	13.15	13.74	12.83	13.44	13.66	12.71	11.68
J01XE	Nitrofuran derivatives (nitrofurantoin)	0.97	0.97	1.06	1.09	0.98	0.82	0.71	0.74	0.79	0.80
J01XX05	Methenamine	0.26	0.31	0.26	0.23	0.17	0.27	0.24	0.21	0.31	0.22
J01XX08	Linezolid	0.72	0.99	1.01	1.14	1.05	1.33	1.08	1.09	1.53	1.51
J01XX09	Daptomycin	0.07	0.05	0.06	0.07	0.17	0.12	0.15	0.24	0.42	0.18
P01AB01	Nitroimidazole derivatives (metronidazole)	8.44	7.93	7.48	7.22	6.14	6.12	6.50	5.97	5.71	5.47
A07AA09	Intestinal antiinfectives (vancomycin)	0.89	1.36	1.52	1.59	1.62	1.43	1.45	1.53	1.45	1.55
J01, P01AB01, A07AA09	Antibacterial agents for systemic use, including metronidazole and vancomycin (total)	252.59	275.09	280.56	285.88	286.22	285.98	280.35	294.97	295.72	305.65

Data used in this table is based consumption at somatic hospitals
ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system
Data from 2019 are estimated by applying the average decrease in bed-days observed in 2009-2018

5.4.4 Changes in the consumption of antimicrobials of critical interest

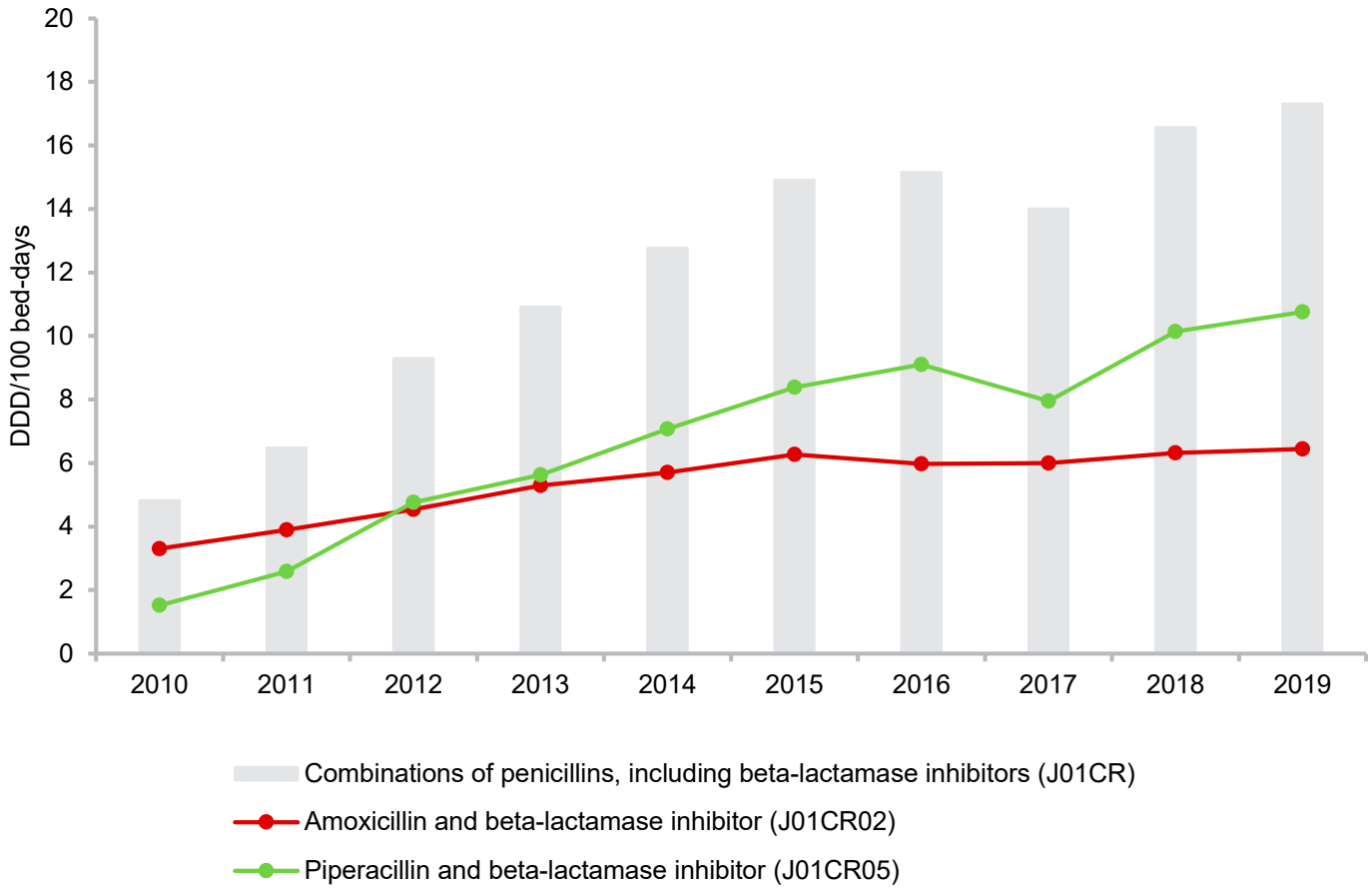
In Denmark, cephalosporins, fluoroquinolones and carbapenems have been collectively termed the antimicrobials of special critical interest due to their important role in the treatment of acutely ill patients suffering from severe infections. Their use is also correlated to a marked risk of resistance, which makes monitoring of the consumption of all three necessary.

For many years, 2nd generation cephalosporins were the main drug in the treatment of patients with sepsis. In an attempt to reduce the consumption of these, the use of piperacillin with tazobactam, a combination penicillin, was recommended as sepsis treatment at all major hospitals during the period of 2005 to 2008. Within recent years, the recommendations on empirical treament in patients with community-acquired sepsis have been further changed to the use of either beta-lactamase sensitive penicillins or penicillins with extended spectrum (in combination with gentamycin). Trends for the consumption of combination penicillins are shown in Figure 5.17. Due to a shortage of piperacillin with tazobactam in 2017, the overall consumption of the drug decreased markedly and was paralleled by a simultaneous increase in the consumption of cephalosporins. This becomes obvious in the regional monitoring of antimicrobials of critical interest for 2017, (Figure 5.17).

In 2019, the antimicrobials of special critical interest constituted altogether 18% of the total consumption at somatic hospitals, measured in DBD. In 2018, it was 20% and ten years ago, in 2010, it was 31%. The trends in the consumption for the five healthcare regions and the average national level during 2010 to 2019 are presented in Figure 5.18.

Cephalosporins accounted for altogether 9.29 DBD, 8.7% of the total consumption, a decrease of 25% from the 12.4 DBD in 2017, (Table 5.8). 2nd generation cephalosporins accounted for the biggest part, 8.08 DBD. Fluoroquinolones accounted for 6.95 DBD, a 3.3% reduction from 7.18 DBD in 2018. The consumption of fluoroquinolones peaked in 2015 and has since shown slight declines. Carbapenems accounted for 2.99 DBD in 2019, a 5.9% increase from 2.83 DBD in 2018.

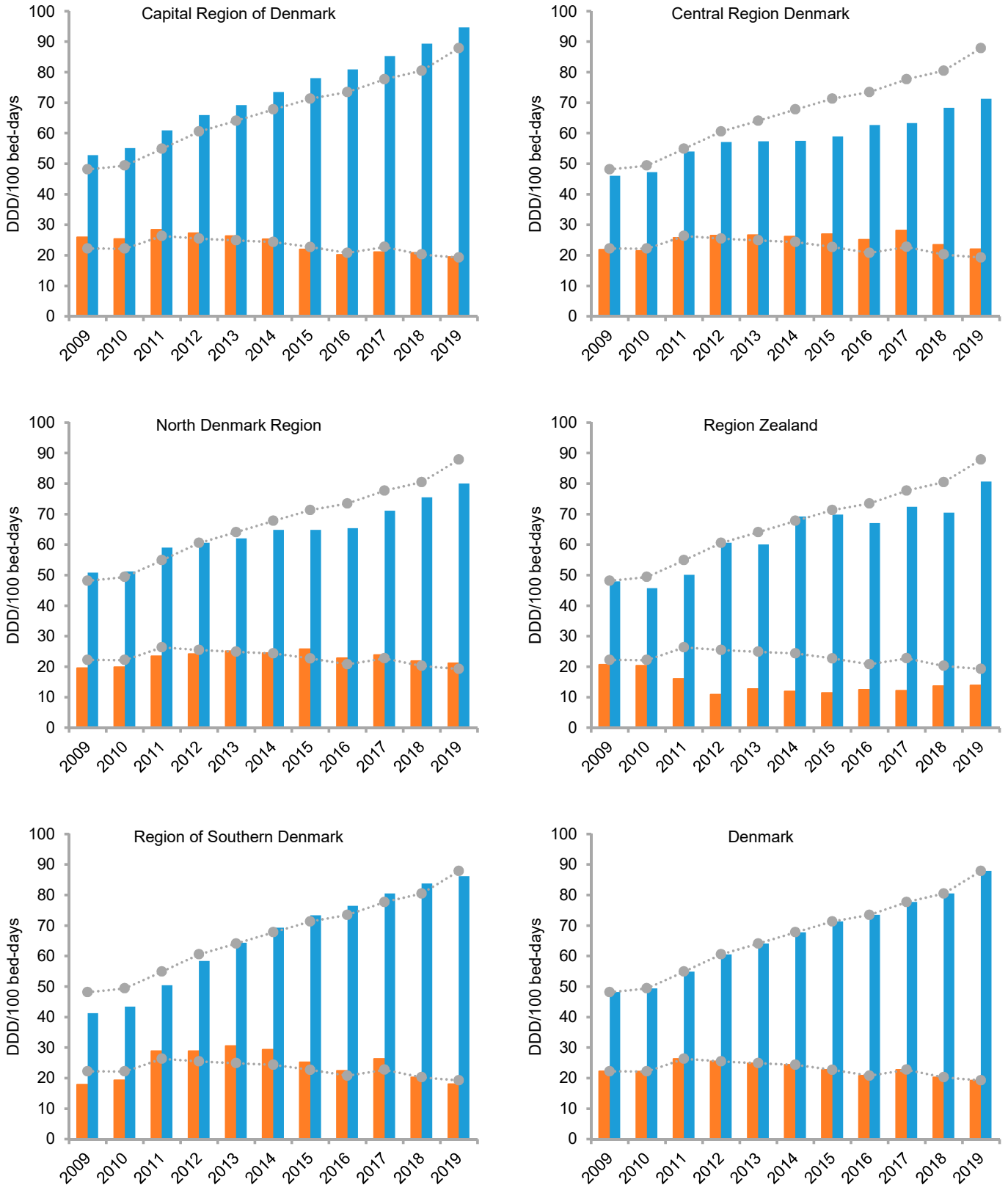
Figure 5.17 Consumption of combination penicillins at somatic hospitals, DDD per 100 bed-days, Denmark DANMAP 2019



Data used in this figure is based consumption at somatic hospitals
ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 5.18 Consumption of antimicrobials of special critical interest in the five health regions, DDD per 100 bed-days, Denmark

DANMAP 2019



Data used in this figure is based on consumption at somatic hospitals
ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system

The consumption of the three antimicrobial groups of critical interest will be monitored closely also in the future. This is due to local, regional and national initiatives. The most important one being the goals developed by working groups under the "National Quality and Learning Teams", an initiative spanning all Danish regions for 2017-2019. Their aim was applying principles of antibiotic stewardship to the main somatic hospitals, primarily focusing on emergency departments and medical departments with a relatively high number of acute patients. For the monitoring of these initiatives the Group developed Danish adjusted DDD for the main antimicrobial classes used at hospitals. When these are applied to the antimicrobial sales reported to DANMAP, the trends in consumption present as shown in Figure 5.14b. For more information on the working group, monitoring and results please see [www.kvalitetsteams.dk] (only available in Danish).

We would like to acknowledge Maja Laursen from the National Health Data Authority in Denmark for data on all antibiotic consumption from primary and hospital care and help in proof reading of this chapter.

We would also like to acknowledge all hospital parmacies in Denmark for data on antibiotic consumption on special delivery at the hospitals.

Majda Attauabi and Ute Wolff Sönksen
For further information: Ute Wolff Sönksen, uws@ssi.dk

Textbox 5.1

National Action Plan on the reduction of antibiotics in humans, 2016-2020

The National Action Plan on the reduction of antibiotics in humans was issued in 2017 by the Danish Ministry of Health and supported by the National Antibiotic Council. Together with the National Action Plan, a One Health Strategy was published. Both are available at the Danish Ministry of Health's homepage at www.SUM.dk. The National Action Plan aims at fulfilling three measurable goals:

- The first goal targets an overall reduction in antimicrobial consumption in primary healthcare, from 462 redeemed prescriptions per 1000 inhabitants in 2016 to 350 redeemed prescriptions per 1000 inhabitants in 2020 (prescriptions issued through general practitioners, medical specialists and dentists)
- The second goal aims at increasing the share of beta-lactamase sensitive penicillins used in primary care to 36% by 2020, thus emphasizing the importance of beta-lactamase sensitive penicillins as the continued drug of choice in many common infections. Also this goal is directed at general practitioners, medical specialists and dentists
- The third goal aims at a 10% reduction in the consumption of the three antimicrobials of special critical interest (cephalosporins, fluoroquinolones and carbapenems) at hospitals from 2016 to 2020, measured in DBD.

The following results have been achieved from 2016-2019:

For goal one the number of prescriptions in primary healthcare (general practitioners, medical specialists and dentists) were reduced to 382 prescriptions per 1000 inhabitants in 2019. Figure 1 shows the number of prescriptions redeemed at pharmacies in Denmark from 2016-2019. Already in 2016, some of the general practioners prescribed less than 350 prescriptions per 1000 inhabitants (DANMAP 2017). By 2019, the percentage of general practioners reaching the goal had increased.

Figure 1 Prescribing trends in the primary sector, prescriptions per 1000 inhabitants, Denmark DANMAP 2019

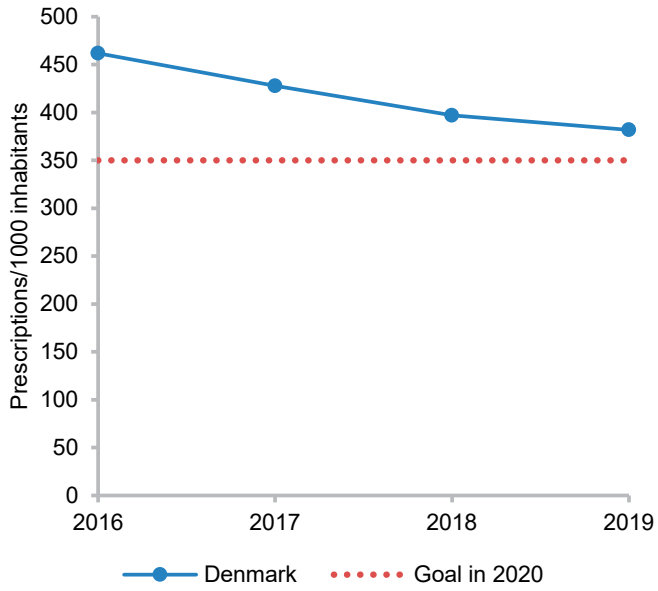
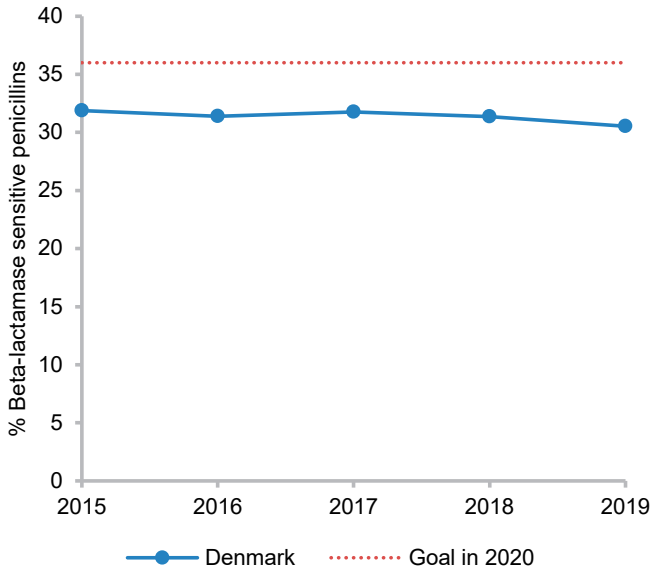


Figure 2 Share of beta-lactamase sensitive penicillins out of total consumption of antimicrobials, primary sector, % DANMAP 2019

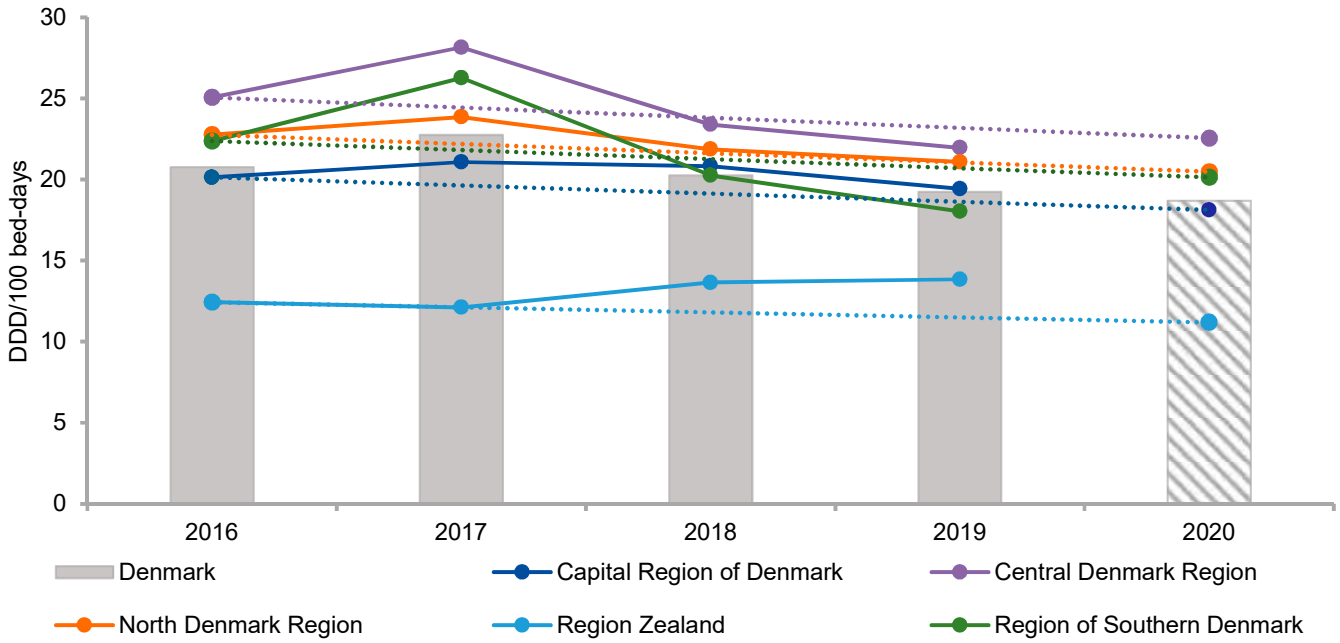


Data used in this figure is based on registered sales to individuals
ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system

For goal two, the proportion of beta-lactamase sensitive penicillin (based on number of prescriptions issued from general practitioners, medical specialists and dentists) remained unchanged (approximately 31%) on national level from 2016 to 2019 (Figure 2).

For goal three, the consumption of antimicrobials of special critical interest at hospitals decreased by 7.4% on national level (from 20.77 DBD in 2016 to 19.24 DBD in 2019), Figure 3. On regional level, the consumption decreased in the Capital Region of Denmark (-3.5%), Central Denmark Region (-12.4%), North Denmark Region (-7.3%) and Region of Southern Denmark (-19.4%). The consumption of antimicrobials of special critical interest increased in the Region Zealand with 11.4%; however, it remained lower than in the other regions.

Figure 3 Consumption of critically important antimicrobials on regional level incl. 10% reduction goal, DDD per 100 bed-days, Denmark
DANMAP 2019



Data used in this figure is based on consumption at somatic hospitals
ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system

In 2017, goal three was challenged through shortages of piperacillin/tazobactam, which brought cephalosporins back as a first line treatment of septic patients. In 2018 and 2019, no difficulties in deliverance of critical important antimicrobials were reported, but for beta-lactams of different formulations it is anticipated to happen again.

The preliminary results from the initiatives based on the National Action Plan highlight the well-known fact that it is easier and faster to achieve overall reductions in consumption than to change habits towards a more prudent use; the latter takes more time and efforts.

Reducing the amount of antimicrobials consumed can only be achieved through parallel actions on the continued improvement of diagnostics and through infection control measures. The National Center for Infection Control (NCIC) at Statens Serum Institut supports many of the national antibiotic initiatives through recommendation guidelines aimed at hospitals and health care settings.

Majda Attauabi and Ute Wolff Sönksen
For further information: Ute Wolff Sönksen, uws@ssi.dk

6

RESISTANCE IN ZOONOTIC BACTERIA AND ANIMAL PATHOGENS

6. Resistance in zoonotic bacteria and animal pathogens

Highlights: In Denmark, antimicrobials are generally not recommended for treatment of self-limiting diarrhoea in humans including salmonellosis and campylobacteriosis. In prolonged or severe cases, treatment may be required and in these cases macrolides (azithromycin) and in hospital settings, ciprofloxacin are recommended.

Erythromycin (macrolide) resistance was present in 4% of *Campylobacter jejuni* isolates from humans with a known travel history. Erythromycin resistance was not observed in human isolates from domestic infections, broiler meat, broilers and cattle.

Resistance to quinolones remained common in *C. jejuni* isolates from humans, Danish broiler meat, broilers and cattle. The levels of ciprofloxacin resistance was 70% in human isolates and 45% and 65% in isolates from broilers and meat hereof, respectively. Resistance towards ciprofloxacin were often accompanied by tetracycline resistance and 51% of the human isolates, and 39% and 30% of the broiler and broiler meat isolates, respectively, were tetracycline resistant.

The level of azithromycin resistance in *Salmonella* Typhimurium isolates was 1% in human isolates and 3% in isolates from Danish pork. Among human cases, resistance to fluoroquinolones was observed in 14% of *S.* Typhimurium isolates from travel-related cases and in 5% of the isolates from domestically acquired cases. Fluoroquinolone resistance has not been recorded in *S.* Typhimurium from Danish pigs and pork since 2010 and 2007, respectively.

Resistance to 3rd generation cephalosporins and carbapenems was not observed in *S.* Typhimurium isolates from animals, food and domestically acquired human cases. Two percent of *S.* Typhimurium from travel-associated cases were resistant to 3rd generation cephalosporins. Carbapenem resistance was not observed.

Surveillance of resistance in animal pathogens was expanded to include pathogens from small animals and mastitis pathogens in addition to the usual porcine pathogens. In all populations and pathogens, the resistance levels remained fairly stable.

6.1 Introduction

Zoonoses are infectious diseases that can be transmitted between animals and humans, either through direct contact with animals or indirectly by contaminated food, water, vectors or the environment. A detailed description of the trends and sources of zoonoses in Denmark and of national surveillance and control programmes can be found in Annual Report on Zoonoses in Denmark 2019 [www.food.dtu.dk].

Campylobacter and *Salmonella* surveillance has been part of the DANMAP programme since 1995. It monitors AMR patterns in broilers, cattle and pigs; isolates from human cases and from fresh meat were included from 1997.

In Denmark, antimicrobials are not recommended for treatment of diarrhoea in patients unless there is prolonged duration or the patient is severely ill. If treatment is required, macrolides (azithromycin) are recommended for treatment of *Campylobacter* infections. There are no general recommendations for treatment of zoonotic *Salmonella* infections in the primary sector, but for infections treated in hospitals, azithromycin or ciprofloxacin is recommended [http://pro.medicin.dk]. The Register of Medicinal Product Statistics at the Danish Health Data Authority does not register the use of antimicrobials specifically for treatment of *Campylobacter* and zoonotic *Salmonella* infections.

Macrolides are used to treat infections in animals in Denmark, whereas flourquinolones and cephalosporins are not used in food-producing animals. In 2019, 12,820 kg of macrolide was prescribed for animals. The majority (93%) of these were used in pigs, whereas cattle and poultry used 190 kg and 228 kg, respectively (Table 4.1).

6.2 *Campylobacter*

For more than a decade, campylobacteriosis has been the most frequently reported bacterial zoonotic disease in Denmark and in the rest of Europe. In 2019, the number of registered cases in Denmark was 5,389 or 92.7 cases per 100,000 inhabitants. Previous studies have shown that the main route of transmission is food, in particular poultry meat, raw milk, contaminated vegetables and water. Other sources are contact with contaminated water during recreational activities and contact with animals [Annual Report on Zoonosis in Denmark 2019].

Several studies on *Campylobacter* sources and their relative impact were carried out in 2015-2017 [Annual Report on Zoonosis in Denmark 2017]. A source attribution study, where isolates from domestically acquired cases were compared to food, animal and environmental isolates, in conjunction with a case control study pointed at chicken meat and cattle/beef as the two major sources. Furthermore, recent analyses of whole-genome sequencing (WGS) data have revealed a large number of small clusters of human cases (comprising 47% of all cases) as well as genetic matching of 30% of the isolates from humans to isolates from food, primarily chicken meat [Joensen et al 2020. Emerg Infect Dis 26: 523].

In humans, campylobacteriosis is a notifiable disease. In order to monitor resistance, a selection of isolates from human *C. jejuni* cases are susceptibility tested. *Campylobacter* isolates were submitted to Statens Serum Institut (SSI) by three clinical microbiological laboratories. The isolates were geographically dispersed and represented both urban and rural areas of Denmark. Roughly, the same number of isolates was susceptibility tested each month and only one isolate per patient was tested. Travel histories of the patients were collected, when possible, and reported to the diagnostic laboratory with submission of patient samples. A human isolate was categorised as domestically acquired if the patient did not travel outside Denmark one week prior to the onset of disease. The DANMAP sampling strategy has been the same in recent years.

The isolates were typed at SSI, and 284 *C. jejuni* isolates were susceptibility tested in accordance with the ECDC recommendations. Of 284 isolates, 278 were assigned a sequence type (ST, 7-locus MLST) [Dingle et al 2001. J Clin Microbiol 39:14]. A total of 86 different ST types was present among the tested isolates. With 44 isolates, ST122 was the most frequent ST type among the tested strains. ST50 (14 isolates), ST19 (13 isolates), ST42 (12 isolates), ST48 (11 isolates), and ST572 (10 isolates) were also frequent ST types. The high occurrence of ST122 isolates was due to a large nationwide outbreak caused by Danish chicken meat that continued throughout 2019, and the isolates selected for DANMAP included 43 of these outbreak isolates.

The animal *Campylobacter* isolates for DANMAP were obtained at slaughterhouse sampling of randomly selected broiler caeca (174 flocks), broiler leg-skin samples (1,248 slaughter batches) and cattle caeca (142 cattle <1 year of age). One isolate per farm or leg-skin sample was susceptibility tested. Sampling, isolation and susceptibility methods followed EFSA's recommendations for animal and food isolates.

MIC distributions for *C. jejuni* from broilers, cattle and humans are available in the web annex (Tables A6.1- A6.2). For further details on methodology, see chapter 9.

6.2.1 Resistance in *Campylobacter jejuni*

Among the domestically acquired human infections, 35% of the isolates were fully sensitive to all antimicrobials tested (Table 6.1). This is the lowest number of fully sensitive *C. jejuni* reported within the last five years, and mainly due to the increase in ciprofloxacin and tetracycline resistance (Figure 6.2). The number of fully sensitive isolates from patients with a known history of travel was 12% and significantly lower than the corresponding number from domestically acquired cases.

Among isolates of broiler origin, the level of fully sensitive isolates continued to decrease. This reflects the continued increase in isolates with resistance to ciprofloxacin and tetracycline from 28% in 2018 to 32% in 2019 in broilers and broiler meat combined (Figure 6.2).

Table 6.1 Resistance (%) in *Campylobacter jejuni* isolates from broilers, cattle and human cases, Denmark DANMAP 2019

	Broilers	Broiler meat	Cattle	Human			
	Danish	Danish	Danish	Domestically acquired	Travel abroad reported	Unknown origin	Total
Antimicrobial agent	%	%	%	%	%	%	%
Ciprofloxacin	45	65	20	63	86	70	70
Erythromycin	0	0	0	0	4	2	1
Gentamicin	0	0	0	0	0	0	0
Nalidixic acid	43	65	20	63	86	70	70
Streptomycin	2	4	3	4	14	4	7
Tetracycline	39	30	11	43	67	52	51
Fully sensitive (%)	55	35	71	35	12	26	27
Number of isolates	56	209	114	155	83	46	284

Note: An isolate is categorised as domestically acquired if the patient did not travel outside Denmark one week prior to the onset of disease. Total number of human cases includes infections of unknown origin

In cattle, the level of fully sensitive isolates remained the same as in previous years (67-72%, Figure 6.2). The level of resistance to ciprofloxacin dropped significantly in 2018 from 30% to 20%, and remained at the lower level in 2019 (Figure 6.1).

Macrolide resistance in *Campylobacter* was monitored using erythromycin, and no erythromycin resistance was observed in *C. jejuni* from cattle, broilers, broiler meat or domestically acquired human cases in 2019. Low levels of resistance were observed among isolates from travel-related cases, and from patients with unknown travel status (Table 6.1).

These low levels of erythromycin resistance were in line with the levels observed over the last decade (Figure 6.1). During the last ten years, macrolide resistance never exceeded 7% in human isolates in any year. Only few erythromycin-resistant *C. jejuni* isolates were identified in poultry and cattle in the last decade, varying between zero and two resistant isolates per year. This suggests that the actual prevalence of macrolide resistance in animal and food isolates remains very close to the limit of detection by the current sampling scheme and is only captured sporadically. Based on the available number of isolates, we are 95% confident that macrolide resistance in 2019 is not exhibited by more than 1.1% and 3% *C. jejuni* from broiler/broiler meat and cattle, respectively (see section 9.7).

A continued increase was observed for ciprofloxacin resistance in *C. jejuni* from broilers and broiler meat, and statistically significant increasing trends have been observed over the last 10 years (Figure 6.1). The increase in ciprofloxacin resistance in isolates from domestically acquired human infections was influenced by the large nationwide outbreak that was caused by a ciprofloxacin-, nalidixic acid- and tetracycline-resistant clone. The implementation of routine-based WGS typing for surveillance of human campylobacteriosis has improved the possibility of identifying epidemiological clusters, and this again has facilitated a more sophisticated analysis of the resistance data. However, clusters of outbreak-related strains have likely been around in the previous years, so it is hard to estimate the influence of outbreaks when establishing trends on resistance. However, high ciprofloxacin resistance was also observed internationally in broilers, broiler meat and humans [EFSA/ECDC 2019. EFSA journal 17(2):5598]. Ciprofloxacin resistance levels were similar in domestically acquired human infections and the main *Campylobacter* sources: broilers and broiler meat.

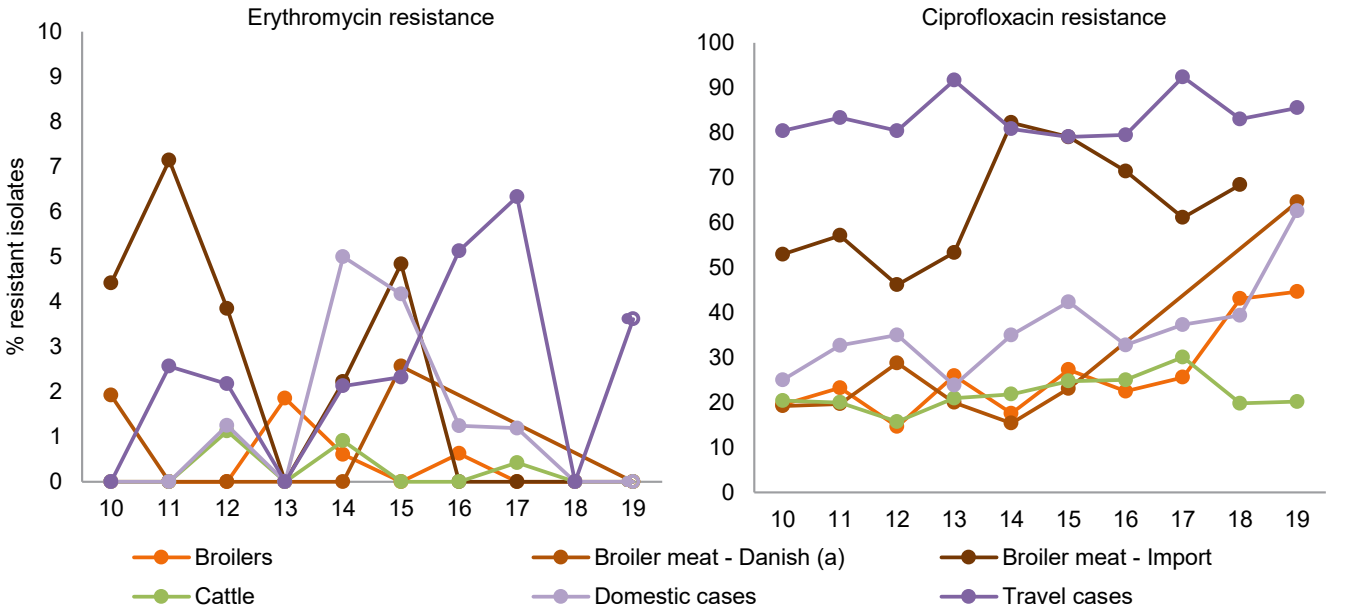
Previously, ciprofloxacin resistance was almost similar in broilers and cattle. However, in 2018 and 2019, the levels in cattle dropped and the levels in broilers increased, resulting in an approximately 20% difference in ciprofloxacin resistance between the two animal reservoirs. Flouroquinolones have not been used for production animals in Denmark for over a decade.

Despite the increase in ciprofloxacin and tetracycline resistance in domestic human *C. jejuni* isolates, the occurrence of resistance to ciprofloxacin and tetracycline was still higher in travel-associated isolates (86% and 67%, respectively) than in isolates from domestically acquired infections (63% and 43%, respectively).

Resistance to ciprofloxacin or ciprofloxacin in combination with tetracycline was the most frequently observed resistance in the human isolates from 2019. Ciprofloxacin resistance was prevalent in 22% and 20% of the domestic and travel related isolates, respectively, and ciprofloxacin in combination with tetracycline was prevalent in 41% and 65% of the domestic and travel-related isolates, respectively (Figure 6.2).

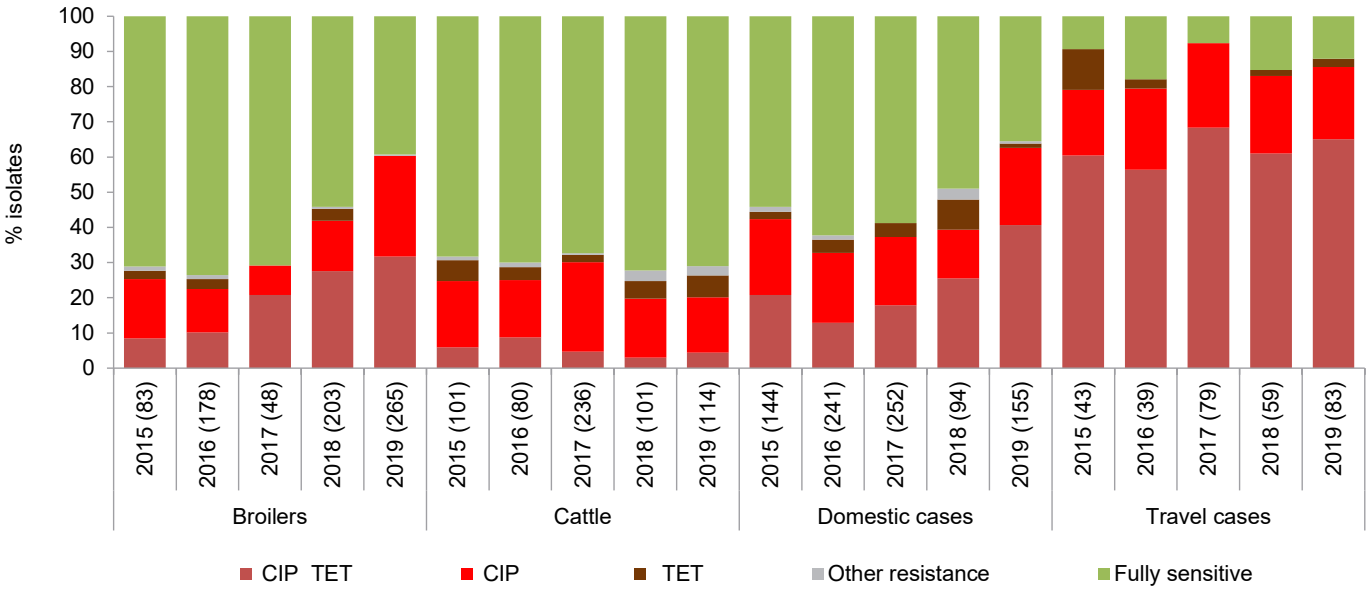
Also among the resistant isolates from animal and meat from 2019, resistance to quinolones only (94/194) or quinolones in combination with tetracycline only (81/194) was most frequent in 2019 (Figure 6.2). The distribution of AMR profiles is presented in the web annex (Table A6.3).

Figure 6.1 Erythromycin and ciprofloxacin resistance (%) among *Campylobacter jejuni* from broilers, broiler meat, cattle and human cases, Denmark DANMAP 2019



Please note the 10-fold difference in the y-axis in the two graphs. Isolates originate from broiler meat ready for retail (2010 to 2015) and leg-skin samples collected after slaughter (2019). No data from 2016-2018

Figure 6.2 Distribution (%) of AMR profiles in *Campylobacter jejuni* from broilers, cattle and human cases, Denmark DANMAP 2019



Note: The number of isolates included each year is shown in parentheses, where broilers include isolates from Danish broiler meat. CIP: all isolates with ciprofloxacin resistance but not tetracycline resistance, TET: all isolates with tetracycline resistance but not ciprofloxacin resistance, CIP TET: all isolates with both ciprofloxacin and tetracycline resistance, Other resistance: all isolates without both ciprofloxacin and tetracycline resistance. CIP TET, CIP and TET isolates may be resistant to erythromycin, nalidixic acid or streptomycin

Fluoroquinolones are not used in food production animals in Denmark, suggesting that the continued increase in ciprofloxacin resistance in broilers is driven by something other than the direct usage of fluoroquinolones. In general, the Danish poultry sector uses only a few antimicrobials, but tetracyclines is the most common antimicrobial used in poultry. The high level of *C. jejuni* isolates with both ciprofloxacin and tetracycline resistance suggests the potential for co-selection of ciprofloxacin resistance by the use of tetracycline in poultry. Whether that is what happens in reality warrants further investigation.

As in 2018, gentamicin resistance was not observed in any of the human isolates, and the level of streptomycin resistance was also in line with the reported levels in the previous years with a total of 7% resistant isolates, and resistance levels of 4% and 14%, respectively, for isolates from domestic and travel-related cases.

Similar to previous years, no resistance to gentamicin was observed in broiler isolates in 2019 (Table 6.1), providing 95% confidence that resistance to the antimicrobial is only present in 1.1% or less of the *C. jejuni* isolates from broilers and broiler meat from Denmark.

Campylobacter jejuni from organic and free-range broilers

During 2019, *Campylobacter* isolates were collected from organic and free-range broiler flocks. At slaughterhouses, leg-skin samples were randomly collected from 123 slaughter batches. One isolate per sample was sequenced using WGS. ResFinder 4.0 was used for detection of antimicrobial resistance genes and the corresponding phenotypic resistance (see section 9.6).

Campylobacter jejuni was recovered from 68% of the samples, and WGS data was available from 83 isolates. No genes conveying resistance to erythromycin or gentamycin was detected, and point mutations in the *gyrA* genes were found to cause quinolone resistance (Table 1). Genotypic resistance to ciprofloxacin and nalidixic acid (51%) was lower than observed in isolates from conventionally produced broiler meat (65%, based om MIC testing). In organic broiler meat, the occurrence of isolates with tetracycline resistance genes was higher than in isolates from conventionally produced broiler meat with phenotypic tetracycline resistance (Tables 1 and 6.1).

This is the first report on resistance in a nationally representative sample of organic broilers in Denmark, and it is uncertain whether the high levels are persistent or just a sporadic finding. Different methods for resistance detection in isolates were used in isolates from conventional and organic broilers, which may contribute to the observed differences.

Table 1 Occurence of genotypic resistance in *Campylobacter jejuni* isolates from organic and free-range broilers, Denmark
DANMAP 2019

Antimicrobial agent	Number (%) of isolates	Resistance genes (ResFinder 4.0)
Ampicillin	1 (1%)	*bla*OXA-61 (n = 1)
Ciprofloxacin	42 (51%)	*gyrA* (p.T86I) (n = 42)
Erythromycin	None	
Gentamicin	None	
Nalidixic acid	42 (51%)	*gyrA* (p.T86I) (n = 42)
Streptomycin	9 (11%)	*ant*(6)-Ia (n = 4), *aad*E-Cc (n = 5)
Tetracycline	48 (58%)	*tet*(O/32/O) (n = 47), *tet*(O) (n = 16)
Number of isolates	83	

Note: ResFinder 4.0 identified resistance genes in 48 of the 83 isolates with available WGS data

6.3 *Salmonella*

Salmonella is the second most frequent zoonotic bacterial pathogen in humans in Denmark as well as in the EU and can have a severe impact on both animal and human health [Annual Report on Zoonoses in Denmark 2019; ECDC/EFSA 2018. EFSA journal 16(12):5500].

In 2019, a total of 1,120 human laboratory-confirmed cases of salmonellosis were reported (19.3 cases per 100,000 inhabitants). The most common serotypes were *S.* Enteritidis and *S.* Typhimurium (including the monophasic variants) with 5.3 and 4.7 cases per 100,000 inhabitants, respectively [Annual report on Zoonoses in Denmark 2019].

In Denmark, human *S.* Typhimurium cases are often associated with contaminated pork, whereas cases caused by *S.* Enteritidis frequently are associated with travel. *S.* Typhimurium often displays a broad spectrum of resistance. Clonal dissemination seems to play an important role for the occurrence of antimicrobial resistance among *S.* Typhimurium [Lucarelli et al. 2010. J Clin Microbiol 48:2103–2109], and the rapid, global dissemination of genomic islands conferring resistance to ampicillin, streptomycin, sulfonamide and tetracycline (the ASSuT profile) among *S.* Typhimurium and its monophasic variants has increased the occurrence of multi-resistant *Salmonella* in Europe [EFSA/ECDC 2019. EFSA journal 17(2):5598].

The Danish *S.* Typhimurium isolates from production animals and humans are often resistant to ampicillin, sulfonamides and tetracycline (ASuT). However, these antimicrobials are not used for treatment of salmonellosis, and thus the public health impact of ASuT resistance is of less direct importance than resistance to critically important antibiotics such as macrolides and fluoroquinioloes that are used for treatment of humans.

Salmonellosis is a notifiable disease in humans, and all clinical isolates were submitted to SSI by the departments of clinical microbiology. The travel history of the patient was collected, when possible, and registered with the patient samples. With exception of S. Entertidtis, all incoming isolates were tested for susceptibility. For *S.* Enteritidis, a selection of strains were tested. Routinely the isolates were analysed by whole-genome sequencing, and the 7-locus MLST (sequence types) were derived (Kidgell et al. 2002, Infect Genet Evol. 2: 39-45.). The isolates were susceptibility tested phenotypically in accordance with the ECDC recommendations. Only one isolate per patient was tested.

Salmonella isolates from pigs were obtained from national surveillance and control programmes at slaughterhouses by sampling randomly selected pig caeca (798 animals) and carcase swabs (10,743 animals). One isolate per farm or pool of swabs was susceptibility tested. The occurrence of *Salmonella* in broilers, layers and cattle is monitored in Denmark, but only few isolates were found in 2019. Susceptibility testing of all *Salmonella* isolates was carried out in compliance with an updated Danish regulation on critically important antimicrobial resistance in *Salmonella* from poultry, cattle and pigs, which came into force 1st of January 2019 (order No. 1424/2018). For animal and food isolates, sampling, isolation, and susceptibility testing followed the methods recommened by EFSA.

In DANMAP, *S.* Typhimurium includes the monophasic variants with antigenic formulas S. 4, [5],12:i:-, unless otherwise stated. MIC distributions and occurrence of resistance among isolates from pigs, pork and humans are presented in the web annex (Tables A6.3 - A6.6).

6.3.1 Resistance in *S.* Typhimurium

S. Typhimurium, including the monophasic variants, was isolated from 45 pig cacae samples and from 59 pork samples, where 80 of the 104 isolates were monophasic variants.

A total of 271 human *S.* Typhimurium isolates, consisting of 86 diphasic and 185 monophasic variants, were susceptibility tested. ST types were derived for all human isolates except one. The monophasic isolates were dominated by ST34 (166 isolates) and the diphasic variants were dominated by ST19

Table 6.2 Resistance (%) in *Salmonella* Typhimurium isolates from pigs, pork and humans, Denmark DANMAP 2019

	Pigs	Pork	Human		
	Danish	Danish	Domestically acquired	Travel abroad reported	Total
Antimicrobial agent	%	%	%	%	%
Ampicillin	76	78	77	63	73
Azithromycin	0	3	2	0	<1
Cefotaxime	0	0	0	2	<1
Ceftazidime	0	0	0	2	<1
Chloram-phenicol	11	12	8	12	7
Ciprofloxacin	0	0	4	14	6
Colistin	0	0	2	5	2
Gentamicin	16	7	3	6	2
Meropenem	0	0	0	0	0
Nalidixic acid	0	0	3	3	2
Sulfonamide	80	83	73	55	66
Tetracycline	80	73	75	57	70
Tigecycline	0	0	4	0	2
Trimethoprim	18	12	12	12	9
Fully sensitive (%)	13	12	14	31	21
Number of isolates	45	59	97	65	271

Note: Includes isolates verified as monophasic variants of *S.* Typhimurium with antigenic formulas S. 4,[5],12:i:-.Total number of human cases includes infections with unknown travel status. An isolate is considered fully sensitive if susceptible to all antimicrobial agents included in the test panel (Table 9.3). None of the colistin resistant strains harboured *mcr* genes

(66), ST34 (9) and ST36 (9). Eighty-nine monophasic isolates were associated with four outbreaks that encompassed 57 (ST34), 14 (ST34), 13 (ST5296), and 5 (ST34) cases. With a few exceptions, the outbreak strains were ASuT-resistant.

Since 2014, macrolide resistance in *Salmonella* has been monitored using azithromycin. In 2019, azithromycin resistance was observed in two *S.* Typhimurium isolates from Danish pork (3%) and in 2% of *S.* Typhimurium isolates from domestically acquired human cases (Table 6.2 and Figure 6.3).

From Danish pigs and pork, between zero and three azithromycin-resistant *S.* Typhimurium isolates per year were detected, and resistance never exceeded 6% annually during 2014-2019 (Figure 6.3). This indicates that the actual prevalence of azithromycin resistance in pigs and pork is close to the detection limit offered by the current sampling scheme.

During the last ten years, ciprofloxacin resistance in *S.* Typhimurium from Danish pigs and pork has rarely been observed, and, in 2019, none of 105 isolates were resistant to ciprofloxacin. The level of ciprofloxacin resistance in isolates from domestically acquired and travel associated human cases was 4% and 14%, respectively. These figures are in line with the observations in the previous years (Figure 6.3).

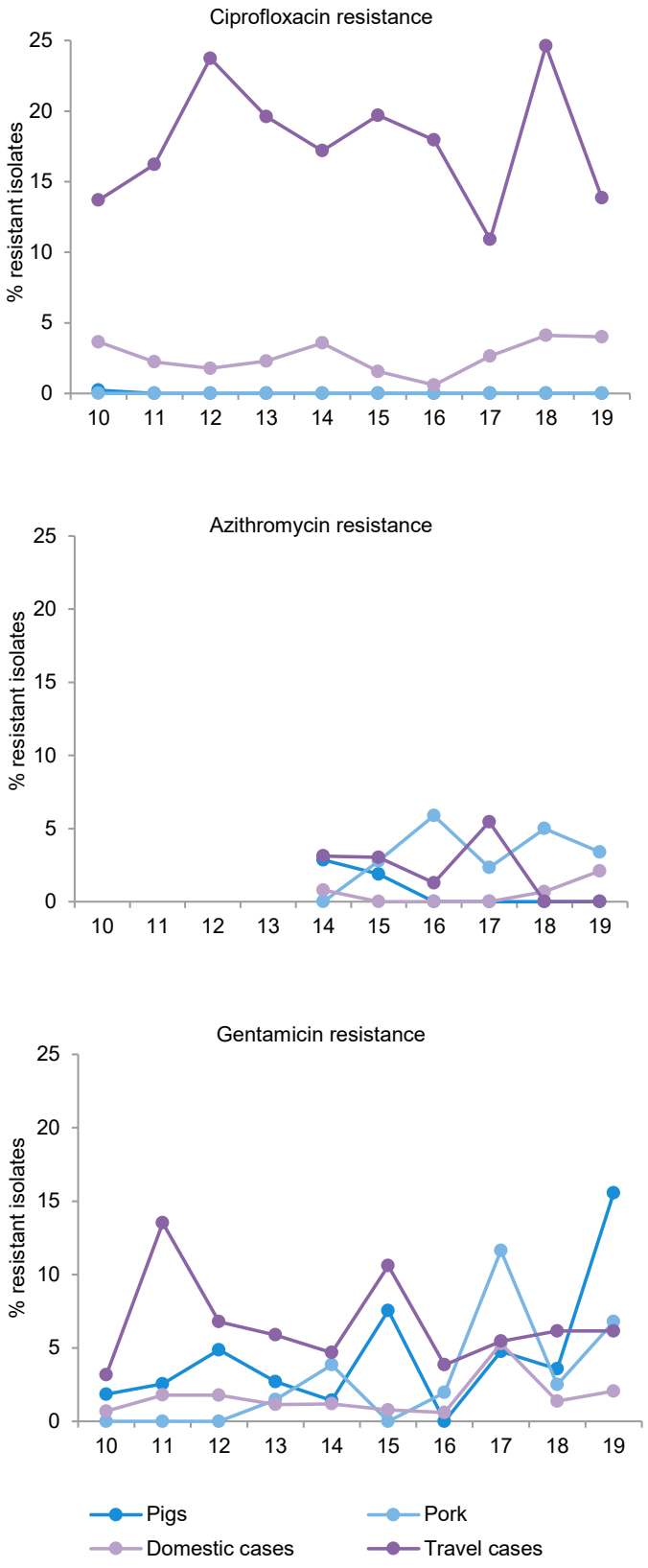
The levels of resistance to gentamycin in isolates from pigs were 16% with 7% resistance in domestically produced pork. This is an increase from previous years and coincides with an increase in use of neomycin in pigs that followed the discontinued use of colistin in 2017. The trend is very uncertain, due to the small number of isolates from pigs and pork, and the coming years will provide more information.

The levels of gentamycin resistance in domestically acquired human isolates have been stable over the last years, and, in 2019, two percent of the isolates were resistant.

Among human cases, the level of cephalosporin resistance was overall <1% for both cefotaxime and ceftazidime, and resistance to 3rd generation cephalosporins was only observed in isolates from travel-related cases. As in the previous years, none of the isolates from pigs or pork were resistant to 3rd generation cephalosporins. Meropenem (carbapenem) resistance was not observed in animal, food or human isolates. The findings for pigs and pork provide 95% confidence that the true prevalence of 3rd generation cephalosporins and carbapenem is less than 2.8% in pigs and pork in Denmark (see section 9.7).

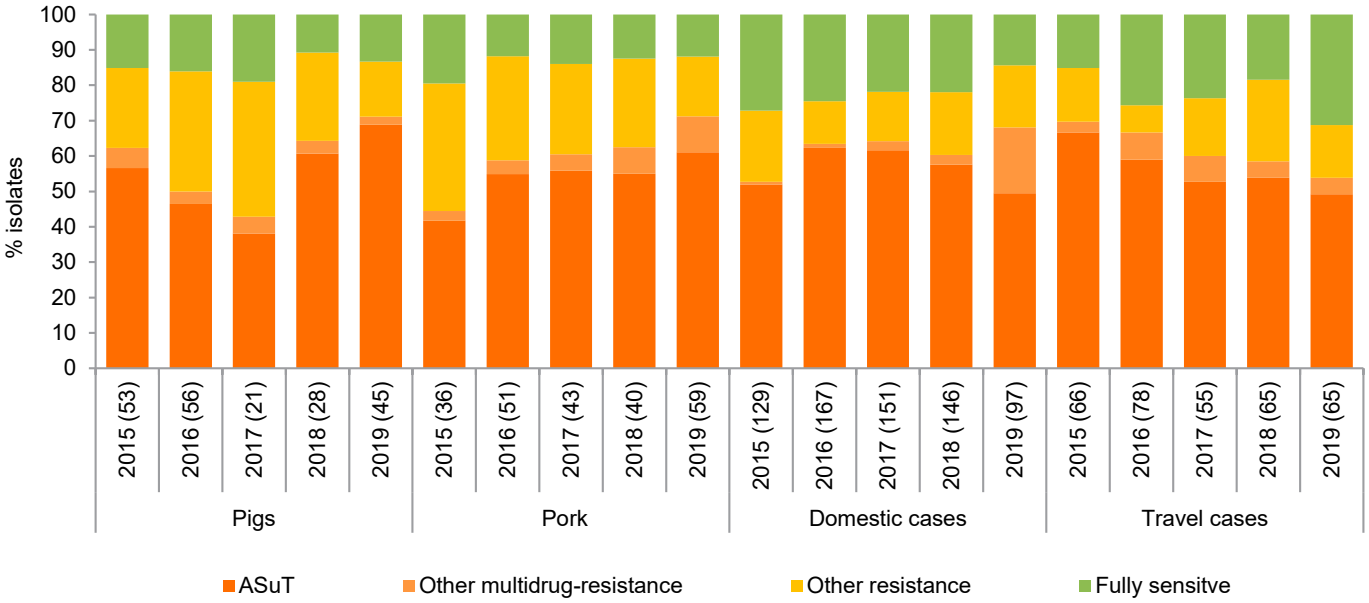
Resistance to tigecycline and colistin in *S.* Typhimurium is rare in Denmark and was not found in pigs or pork in 2019. Two percent of the human cases were resistant to tigecycline (MIC value>= 4 mg/L), and 2% was resistant to colistin. The colistin-resistant human isolates had MIC values that were close to the ECOFF, and none of the isolates harboured *mcr* genes.

Figure 6.3 Ciprofloxacin, azithromycin and gentamycin resistance (%) among *S.* Typhimurium from pigs, domestic pork and human cases, Denmark DANMAP 2019



Note: Includes isolates verified as monophasic variants of *S.* Typhimurium with antigenic formulas *S.* 4,[5],12:i:-. An isolate is categorised as domestically acquired if the patient did not travel outside Denmark one week prior to the onset of disease. Total number of human cases includes infections of unknown origin

Figure 6.4 Distribution (%) of multidrug-resistant, resistant and fully sensitive *S.* Typhimurium from pigs, domestic pork and human cases, Denmark
DANMAP 2019



Note: Number of isolates included each year is presented in the parenthesis. Includes isolates verified as monophasic variants of *S.* Typhimurium with antigenic formulas *S.* 4,[5],12:i:-.An isolate is considered fully sensitive if susceptible to all antimicrobial agents included in the test panel, and multidrug-resistant if resistant to 3 or more of the 12 antimicrobial classes included in the test panel (Table 9.3). ASuT are multidrug-resistant isolates resistant to ampicillin, sulfonamide and tetracycline

Most of the isolates were resistant to one or several antimicrobials. Less than 13% of the isolates from pigs and pork and 14% of the isolates from human domestic cases were fully sensitive to all tested antimicrobials, and the number of fully sensitive isolates from travel-associated cases was 31% (Table 6.2).

Over the last five years, the occurrence of multidrug-resistance among *S.* Typhimurium from Danish pork has increased significantly (Figure 6.4). As in previous years, the ASuT phenotype was the most frequent resistance profile among *S.* Typhimurium from both pigs and pork as well as from human cases (Figure 6.4). The observed levels were similar to most European countries and especially high among the monophasic isolates. See the AMR profile distribution in the web annex (Table A6.7).

The marked reduction in usage of tetracycline over the last ten years in pigs is still not reflected in the 2019 levels of resistance in *S.* Typhimurium from pigs and pork. In pigs, tetracycline resistance continued to increase from 47% in 2010 to 75% in 2018 and 80% in 2019, probably due to spread of *Salmonella* clones. The increased use since 2017 of macrolides for weaner and finisher pigs did not result in a measurable increase in resistance to azithromycin, but this will be monitored closely in the coming years.

6.3.2 Resistance in other *Salmonella* serotypes

***S.* Derby** is common in pigs, but the high prevalence is not reflected in the number of human cases. In pigs, a decrease in fully sensitive isolates was observed from 70% in 2018 to 55% in 2019 (Table 6.3). As expected, resistance to tetracycline, sulfonamides, trimethoprim and ampicillin was most common, either alone or in combination. Gentamycin resistance was found in 1-2% of the S. Derby isolates. Resistance to ciprofloxacin, azithromycin or other antimicrobials of critical importance was not detected (Table 6.3).

Twenty-one human S. Derby isolates were susceptibility tested in 2019. Eleven isolates were part of an outbreak caused by a fully sensitive strain. Two travel-associated isolates exhibited resistance to ampicillin, chloramphenicol, ciprofloxacin, sulfonamide, tetracycline and trimethoprim. One isolate was resistant to tetracycline, and one isolate was resistant to sulfonamides.

***S.* Infantis** isolates from 22 sporadic human cases were susceptibility tested, 17 of which were fully sensitive. Two isolates from domestic cases were resistant to cefotaxime and ceftazidime. Only eight *S.* Infantis were isolated from pigs and pork, seven of which were fully sensitive and one of which had an ASuT profile supplemented with trimethoprim resistance.

***S.* Dublin** is cattle associated. A total of 24 human isolates of *S.* Dublin were susceptibility tested. *S.* Dublin is intrinsically (naturally) resistant to colistin. Most of the isolates, 21, were fully sensitive, and resistance to 3rd generation cephalosporins was not observed. Two strains exhibited multi-resistance that included ciprofloxacin resistance (one isolate) and azithromycin resistance (one isolate). Most of the *S.* Dublin isolates came from cases with no information on travel.

***S.* Enteritidis** is a common cause of salmonellosis in Denmark, where it is often associated with travel. In 2019, 32 *S.* Enteritidis isolates were susceptibility tested. Like *S.* Dublin, *S.* Enteritidis is intrinsically resistant to colistin. Resistance to ciprofloxacin is common in *S.* Enteritidis, with 17 of the 32 isolates being resistant. Four of the susceptibility tested isolates of *S.* Enteritidis were from domestic cases. In 2019, *S.* Enteritidis was found in 11 batches of imported duck meat. Three of the isolates were resistant to ciprofloxacin and the remaining isolates were fully sensitive.

Helle Korsgaard, Johanne Ellis-Iversen and Jeppe Boel
For further information:
Johanne Ellis-Iversen, joell@food.dtu.dk
Jeppe Boel, jebl@ssi.dk

Table 6.3 Resistance (%) in *Salmonella Derby* isolates from pigs and pork, Denmark DANMAP 2019

	Pigs	Pork
Antimicrobial agent	Danish %	Danish %
Ampicillin	13	16
Azithromycin	0	0
Cefotaxime	0	0
Ceftazidime	0	0
Chloramphenicol	4	2
Ciprofloxacin	0	0
Colistin	0	0
Gentamicin	1	2
Meropenem	0	0
Nalidixic acid	0	0
Sulfonamide	21	25
Tetracycline	31	21
Tigecycline	0	0
Trimethoprim	21	23
Fully sensitive (%)	55	64
Number of isolates	67	56

Note: An isolate is considered fully sensitive if susceptible to all antimicrobial agents included in the test panel (Table 9.3)

Textbox 6.1

Antimicrobial resistance in clinical isolates from dogs and cats in Denmark

Background: Most countries, including Denmark, have no systematic surveillance of antimicrobial resistance (AMR) in companion animals and no systematic way of detecting trends and new or emerging resistance problems to guide antimicrobial use practices and policies. In small animal practice today, *Escherichia coli* and *Staphylococcus pseudintermedius* are the most common bacterial pathogens encountered, causing mainly urinary tract and skin infections, respectively. Among these, the increasing occurrence of methicillin-resistant *S. pseudintermedius* (MRSP) and extended-spectrum beta-lactamase (ESBL)-producing *E. coli* is particularly worrying, since they can be resistant to all antimicrobial drugs approved for veterinary use [1]. Furthermore, they can be transmitted to humans, posing a potential risk to household contacts and veterinary staff.

Materials and methods: Antimicrobial susceptibility data were obtained for 1,177 *E. coli* and 1,763 *S. pseudintermedius* isolates from canine and feline clinical specimens submitted to Sund Vet Diagnostik, the veterinary diagnostic microbiology laboratory at the University of Copenhagen. This laboratory processes veterinary samples from all over Denmark, including from referral practices. Isolates were obtained in 2011-2012 and 2016-2019 from predominantly urinary tract infections and skin/ear infections (Table 1). The two time periods were selected to enable comparison of resistance levels before and after the national guidelines for antimicrobial treatment of companion animals were launched in late 2012 [2]. Isolates were tested by broth microdilution using commercial plates (SensiTitre, Thermo Fisher Scientific) according to the Clinical Laboratory Standards Institute standards for susceptibility testing of veterinary pathogens [3].

Table 1 Origin of *E. coli* and *S. pseudintermedius* isolates obtained from clinical specimens in Sund Vet Diagnostik, 2011-2012 and 2016-2019 DANMAP 2019

	Staphylococcus pseudintermedius		*Escherichia coli*	
Origin	Dogs	Cats	Dogs	Cats
Skin, wounds and ears	1358	19	230	13
Urinary tract	111	7	587	149
Other	264	4	176	22
Total	1733	30	993	184

Results: Overall, resistance levels were stable with no major fluctuations over the 8-year time period (Table 2). In *E. coli*, imipenem resistance was not detected, indicating the absence of carbapenemase-producers. Between 4% and 7% of *E. coli* isolates were resistant to cefpodoxime, which is an indicator of ESBL- and AmpC-production. Based on sequence-based typing of a subset of isolates, CTX-M-1, CTX-M-15, and CMY-2 are the most common ESBL/AmpC enzymes in clinical *E. coli* from dogs in Denmark. Approximately 25% and 10% of *E. coli* isolates were resistant to ampicillin and potentiated sulfonamides, respectively (Table 2).

In the national treatment guidelines, these agents are recommended as first choice for lower urinary tract infection (UTI) in dogs and cats. Consumption of potentiated sulfonamides for pets has decreased by 25% between 2011 and 2018, likely due to withdrawal from the Danish market in 2014 of the only sulfonamide/trimethoprim product licensed for these animals. This may explain the significant reduction in levels of resistance to sulfamethoxazole/trimethoprim (15 vs. 9%, $p<0.001$). Today, amoxicillin is the only registered first choice antibiotic for treatment of UTI. The high level of resistance to ampicillin, which is a surrogate antibiotic for testing amoxicillin susceptibility, emphasizes the need for culture and susceptibility testing to guide therapy for this common condition.

Between 6% and 8% of the *S. pseudintermedius* isolates were likely to be MRSP, as they exhibited resistance to oxacillin. Since 2005, the almost pan-resistant MRSP clonal complex CC71 has spread globally among dogs and to a lesser extent among cats [4]. Although this clone also occurs in Denmark, the most prevalent clone in Danish pets is CC258, which is typically susceptible to more drugs than CC71 [5]. Clindamycin is the systemic treatment of choice for superficial canine skin infections, and

continued ... Textbox 6.1

25-28% of *S. pseudintermedius* isolates were resistant to this drug. Even higher percentages of resistance were observed for antibiotics that are not used for treatment of *S. pseudintermedius* infections, such as doxycycline (29-33%) and erythromycin (26-28%), and the already high level of resistance to ampicillin increased even further (58% vs 70%, p<0.001). Notably, most skin and ear infections can be managed by antiseptic topical treatment (e.g. shampoo, gels) without the need for systemic antimicrobial therapy. However, more severe infections including deep pyoderma require systemic therapy, and susceptibility testing is highly recommended in these cases, also in consideration of the treatment period, which is often longer than three weeks.

In order to assess the representativeness of our data, we recently studied resistance in clinical *S. pseudintermedius* from dogs with first-time pyoderma that were not previously treated with antibiotics. Only 14% of isolates in that study were resistant to clindamycin [6], suggesting that the data reported here are likely to overestimate resistance levels encountered in primary practices. We also suspect that the overall lower resistance levels observed in *E. coli* in 2016-2017, as compared to the periods before and after (Table 2), could be due to a higher proportion of isolates from primary practices in that period.

Table 2 Percentage of antimicrobial resistant clinical *E. coli* and *S. pseudintermedius* isolates from dogs and cats in Denmark
DANMAP 2019

	Escherichia coli			*Staphylococcus pseudintermedius*		
	2011-2012	2016-2017	2018-2019	2011-2012	2016-2017	2018-2019
	(N=342)	(N=394)	(N=441)	(N=675)	(N=486)	(N=602)
Antimicrobial agent	%	%	%	%	%	%
Amikacin	1	2	2	1	1	1
Ampicillin[a]	28	14	25	58	59	70
Amoxicillin/clavulanic acid[b]	9	4	5	-	8	7
Cefazolin	-	-	-	6	8	7
Cefpodoxime	7	4	5	-	-	-
Chloramphenicol	4	4	4	14	16	21
Clindamycin	-	-	-	28	25	27
Doxycycline[b]	9	7	8	-	33	29
Enrofloxacin	8	3	4	3	3	2
Erythromycin	-	-	-	28	26	28
Gentamicin	4	4	4	3	3	2
Imipenem	0	0	0	-	-	-
Marbofloxacine	8	3	3	3	3	3
Oxacillin	-	-	-	6	8	6
Sulfamethoxazole/trimethoprim	15	7	9	6	5	6

a) Susceptibility data for ampicillin and amoxicillin/clavulanic acid in *E. coli* have been determined only for isolates from urinary tract infections, as isolates from other infections are unequivocally classified as resistant to these drugs according to CLSI breakpoints
b) Amoxicillin/clavulanic acid and doxycycline susceptibility data from 2011-12 for *S. pseudintermedius* were not included, as concentrations in the MIC panels used cannot be used for interpretation according to the breakpoints available today

Discussion and conclusion: It is difficult to relate AMR in companion animals to national trends of antimicrobial consumption, because VetStat data on antimicrobial usage in companion animals are less complete than for production animals. A detailed investigation of consumption patterns in DANMAP 2016 indicated a small overall decrease in total antimicrobial consumption for companion animals, including a substantial decrease in the use of 3rd generation cephalosporins following the launch of the national treatment guidelines in 2012. This positive trend has continued since 2016, but companion animals still account for 12% and 89% of total kg active ingredient of all 3rd and 4th generation cephalosporins and fluoroquinolones used for animals, respectively. Several studies have hypothesized that exposure to these two antimicrobial classes is a risk factor for colonization or infection with MRSP- and ESBL-producing *E. coli*. Accordingly, a further reduction in their use should be pursued to control the occurrence of these multidrug-resistant bacteria in the future.

In conclusion, AMR levels in clinical *E. coli* and *S. pseudintermedius* isolates from dogs and cats have remained stable for most antimicrobials since 2011. MRSP- and ESBL-producing *E. coli* still occur at fairly low frequency, and it is important to maintain this trend via continued focus on responsible antimicrobial use in small animal practice. A largely untapped resource for implementing responsible antimicrobial use is the implementation of antimicrobial stewardship programmes at the clinic level [7]. Guidelines for developing such programmes tailored to type and size of clinic have now become available in small animal veterinary medicine [8].

Peter Damborg and Luca Guardabassi, University of Copenhagen, Denmark
For further information: Peter Damborg, pedam@sund.ku.dk

References

[1] Damborg P, Broens EM, Chomel BB, Guenther S, Pasmans F, Wagenaar JA, Weese JS, Wieler LH, Windahl U, Vanrompay D, Guardabassi L. 2016a. Bacterial Zoonoses Transmitted by Household Pets: State-of-the-Art and Future Perspectives for Targeted Research and Policy Actions. J Comp Pathol. 2016. 155(1 Suppl 1):S27-40. doi: 10.1016/j.jcpa.2015.03.004.

[2] Jessen LR, Damborg P, Spohr A, Goericke-Pesch S, Langhorn R, Houser G, Willesen J, Schjærff M, Eriksen T, Sørensen TM, Jensen VF, Obling F, Guardabassi L. Antibiotic use guideline for companion animals. 2018. Faggruppe Familiedyr (DDD). Available online: https://www.ddd.dk/faggrupper/faggruppe-familiedyr/vejledninger-og-guidelines/antibiotikavejledning-til-familiedyr/

[3] Clinical and Laboratory Standards Institute (CLSI). 2018. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals. 4th ed. CLSI supplement VET08. CLSI, Wayne, Pa., USA.

[4] Perreten V, Kadlec K, Schwarz S, Grönlund Andersson U, Finn M, Greko C, Moodley A, Kania SA, Frank LA, Bemis DA, Franco A, Iurescia M, Battisti A, Duim B, Wagenaar JA, van Duijkeren E, Weese JS, Fitzgerald JR, Rossano A, Guardabassi L. 2010. Clonal spread of methicillin-resistant *Staphylococcus pseudintermedius* in Europe and North America: an international multicentre study. J Antimicrob Chemother. 65(6):1145-54. doi: 10.1093/jac/dkq078.

[5] Damborg P, Moodley A, Aalbæk B, Ventrella G, Dos Santos TP, Guardabassi L. 2016b. High genotypic diversity among methicillin-resistant *Staphylococcus pseudintermedius* isolated from canine infections in Denmark. BMC Vet Res. 2016 12(1):131. doi: 10.1186/s12917-016-0756-y.

[6] Larsen RF, Boysen L, Jessen LR, Guardabassi L, Damborg P. 2018. Diversity of *Staphylococcus pseudintermedius* in carriage sites and skin lesions of dogs with superficial bacterial folliculitis: potential implications for diagnostic testing and therapy. Vet Dermatol. Online ahead of print. doi: 10.1111/vde.12549.

[7] Guardabassi L, Prescott JF. 2015. Antimicrobial Stewardship in Small Animal Veterinary Practice: From Theory to Practice. Vet Clin North Am Small Anim Pract. 45(2):361-76, vii. doi: 10.1016/j.cvsm.2014.11.005.

[8] UMN. Handbook of Antimicrobial Stewardship in Companion Animal Veterinary Settings, 1st ed. Saint Paul, MN: UMN, 2020. Available at https://arsi.umn.edu/handbook.

Textbox 6.2

MRSA surveys in animals

In 2019, the Danish Veterinary and Food Administration (DVFA) conducted MRSA surveys in conventional breeding pig herds, dairy cattle herds, veal calf herds, broiler flocks, horses and mink feed. For pigs, dairy cattle, veal calves and horses, 25 individual animals were tested per herd/farm and the samples analysed as pools of five samples, whereas sampling of poultry flocks was performed by collecting five sock samples per flock. Sampling of mink feed was done by swabs from ready-mixed feed. From mink feed, five pooled samples of five swabs were obtained from each batch of eight tonnes of feed. The results of the MRSA surveys are summarised in Table 1 and are presented at herd/flock/feed batch level (between-unit prevalence) rather than at the animal level (within-unit prevalence).

Table 1 Prevalence of MRSA in various animal populations and mink feed in Denmark, 2019 DANMAP 2019

Herd type	Number of sampled units*	Number of positive units	% positive units
Conventional breeding pigs	73	69	95
Dairy cattle	131	2	2
Veal calves	115	11	10
Horses	120	13	11
Broiler chicken	83	0	0
Mink feed	10	1	10

*Unit = flock/ herd/ feed batch

The prevalence of MRSA in conventional breeding pig herds increased from 83% in 2018 to 95% in 2019. All isolates belonged to CC398. The prevalence among dairy herds remained low (6% in 2018). One isolate was *spa* type t127/CC1 while the other was *mecC* positive and *spa* type t843/CC130. The prevalence among horse herds was also stable (8% in 2018). Two positive horse samples were *mecC* (*spa* types t843/CC130 and t3256/CC130), while the remaining were CC398. Sampling from mink feed demonstrated one positive feed batch (*spa* type t011/CC398) and suggests that feed may be a route of introduction of MRSA in the mink flocks. In 2018, 25% of mink farms were positive for MRSA, and all but one farm/isolate were positive with *spa* types associated with CC398.

Anders Rhod Larsen & Andreas Petersen, Statens Serum Institut
For further information: Andreas Petersen, AAP@ssi.dk

Textbox 6.3

Antimicrobial resistance in mastitis isolates from dairy cows

Background and data source: Mastitis is the leading cause of antimicrobial usage in the dairy production worldwide. It is estimated that in Denmark, each year, every third dairy cow suffers from mastitis. Some of the major pathogens associated with clinical mastitis are *Staphylococcus aureus*, *Streptococcus uberis*, *Streptococcus dysgalactiae*, and *Escherichia coli*. However, in Denmark, veterinarians are only allowed to prescribe penicillin for treatment of mastitis as long as no antibiotic resistance testing is carried out. The aim of this study was to provide new data of antimicrobial resistance occurrence in udder pathogens from Danish dairy cows with clinical mastitis. The isolates included were submitted from veterinary clinics throughout 2018 and 2019 to Center for Diagnostics, Technical University of Denmark, in relation to various research projects. The antimicrobial susceptibility testing was carried out using the broth microdilution method with SensiTitre. The panels and breakpoints applied for interpreting the minimum inhibitory concentrations (MICs) were those routinely applied at Center for Diagnostics, Technical University of Denmark [1].

Results and discussion: The Gram-positive isolates generally exhibited low resistance levels to the tested antimicrobials (Table 1). The highest resistance level for the *S. aureus* isolates was to sulfamethoxazole (25%, 2018), otherwise low resistance levels were found (<20%). A single isolate was cefoxitin-resistant (8%, 2019), and this will be studied further for potential MRSA identification. All Streptococci isolates were susceptible to penicillin. However, several *S. uberis* isolates showed decreased susceptibility against penicillin and were classified as intermediate (data not shown). This trend is novel and will be investigated further in future projects. Additionally, the *S. uberis* isolates showed high resistance levels to streptomycin (84%, 2018 and 100%, 2019), cefoxitin (63%, 2018 and 50%, 2019) and tetracycline (26%, 2018 and 20%, 2019). For the *S. dysgalactiae* isolates, the highest resistance level was found in tetracycline (24%, 2018 and 19%, 2019) and otherwise, low resistance levels were detected.

Table 1 Resistance occurrence (%) among *S. aureus*, *S. uberis*, *S. dysgalactiae*, and *E. coli* isolates from dairy cows with clinical mastitis DANMAP 2019

	AMP	AUG	APR	CHL	CIP	COL	ERY	FFN	FOT	FOX	GEN	NAL	NEO	PEN	SMX	SPE	STR	SXT	TET	TIA	TMP	XNL
	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
S.aureus																						
2018 (n = 12)	-	-	-	0	0	-	0	-	-	0	0	-	-	8	25	17	0	0	0	0	0	-
2019 (n = 12)	-	-	-	0	0	-	0	-	-	8	0	-	-	0	8	8	0	0	8	0	0	-
S. uberis																						
2018 (n = 19)	-	-	-	0	0	-	5	-	-	63	NA	-	-	0	-	5	84	0	26	5	0	-
2019 (n = 20)	-	-	-	0	0	-	15	-	-	50	NA	-	-	0	-	10	100	0	20	0	0	-
S. dysgalactiae																						
2018 (n = 17)	-	-	-	0	0	-	0	-	-	0	NA	-	-	0	-	0	6	0	24	0	0	-
2019 (n = 16)	-	-	-	0	0	-	6	-	-	0	NA	-	-	0	-	0	6	0	19	0	0	-
E. coli																						
2018 (n = 23)	4	0	0	4	0	4	-	0	0	-	0	4	0	-	17	4	4	-	4	-	4	0
2019 (n = 17)	6	6	0	0	0	0	-	0	0	-	0	0	0	-	6	6	12	-	0	-	0	0

AMP (ampicillin), AUG (amoxicillin + clavulanic acid), APR (apramycin), CHL (chloramphenicol), CIP (ciprofloxacin), COL (colistin), ERY (erythromycin), FFN (florfenicol), FOT (cefotaxime), FOX (cefoxitin), GEN (gentamicin), NAL (nalidixic acid), NEO (neomycin), PEN (penicillin), SMX (sulphamethoxazole), SPE (spectomycin), STR (streptomycin), SXT (sulphamethoxazole-trimethoprim), TET (tetracycline), TIA (tiamulin), TMP (trimethoprim), and XNL (ceftiourfur)

continued ... Textbox 6.3

The *E. coli* isolates exhibited low resistance levels to all tested agents, and no indication of ESBL producing *E. coli* was found in this study. A single isolate was colistin resistant, and this is currently under further investigation. This study showed no major changes in resistance levels from 2018 to 2019, however, we still emphasize the need for incessant surveillance of resistance occurrence and development among mastitis pathogens in the future.

Desiree Corvera Kløve and Lærke Boye Astrup
For further information: Lærke Boye Astrup, lboast@vet.dtu.dk

Reference

[1] Chehabi, C. N., Nonnemann, B., Astrup, L. B., Farre, M., and Pedersen, K., In vitro Antimicrobial Resistance of Causative Agents to Clinical Mastitis in Danish Dairy Cows, 2019, Foodborne Pathogens and Disease, Vol. 16, No. 8, https://doi.org/10.1089/fpd.2018.2560

Textbox 6.4

Resistance in bacteria from diagnostic submissions from pigs

Background and data source: Data on antimicrobial susceptibility of three important veterinary pathogens haemolytic *Escherichia coli, Streptococcus suis,* and *Actinobacillus pleuropneumoniae* were obtained from the routine diagnostic laboratory investigation of isolates from dead and diseased pigs submitted to SEGES Pig Research Centre's Laboratory for Pig Diseases in Kjellerup during 2019. The number of isolates belonging to other bacterial species was insufficient to deduct annual trends.

The antimicrobial susceptibility testing was carried out using the broth microdilution method with SensiTitre. Internationally approved clinical breakpoints were not available for most of the drug-bacterium combinations, so the occurrences of resistant isolates are presented according to the clinical breakpoints that are currently in use at both DTU National Veterinary Institute and Laboratory for Pig Diseases, SEGES. These breakpoints are mainly CLSI breakpoints, preferably porcine, but for *E. coli* the breakpoints are mostly human. When the applied breakpoints are adjusted according to new established breakpoints, these are used retrospectively in this text box.

MIC distributions and occurrence of resistance are presented in the web annex (Tables A6.8-A6.10).

Table 1 Resistance (%) among bacteria from diagnostic submissions from pigs, Denmark DANMAP 2019

Antimicrobial agent	*Actinobacillus pleuropneumoniae*	*Haemolytic Escherichia coli*	*Streptococcus suis*
	%	%	%
Amoxicillin/clavulanic acid	-	4	-
Ampicillin	1	62	-
Apramycin	-	12	-
Cefotaxime	-	0	-
Cefoxitin	-	-	14
Ceftiofur	0	0	-
Chloramphenicol	-	18	1
Ciprofloxacin	0	1	0
Colistin	-	0	-
Erythromycin	100	-	61
Florfenicol	0	10	1
Gentamicin	-	9	0
Nalidixic acid	-	6	-
Neomycin	-	20	-
Penicillin	1	-	0
Spectinomycin	0	51	27
Streptomycin	-	77	37
Sulfamethoxazole	-	-	86
Sulfonamide	-	75	-
Sulfonamid/trimethoprim	0	-	3
Tetracycline	0	69	69
Tiamulin	0	-	16
Tilmicosin	0	-	-
Trimethoprim	-	56	8
Tulathromycin	0	-	-
Number of isolates	116	262	147

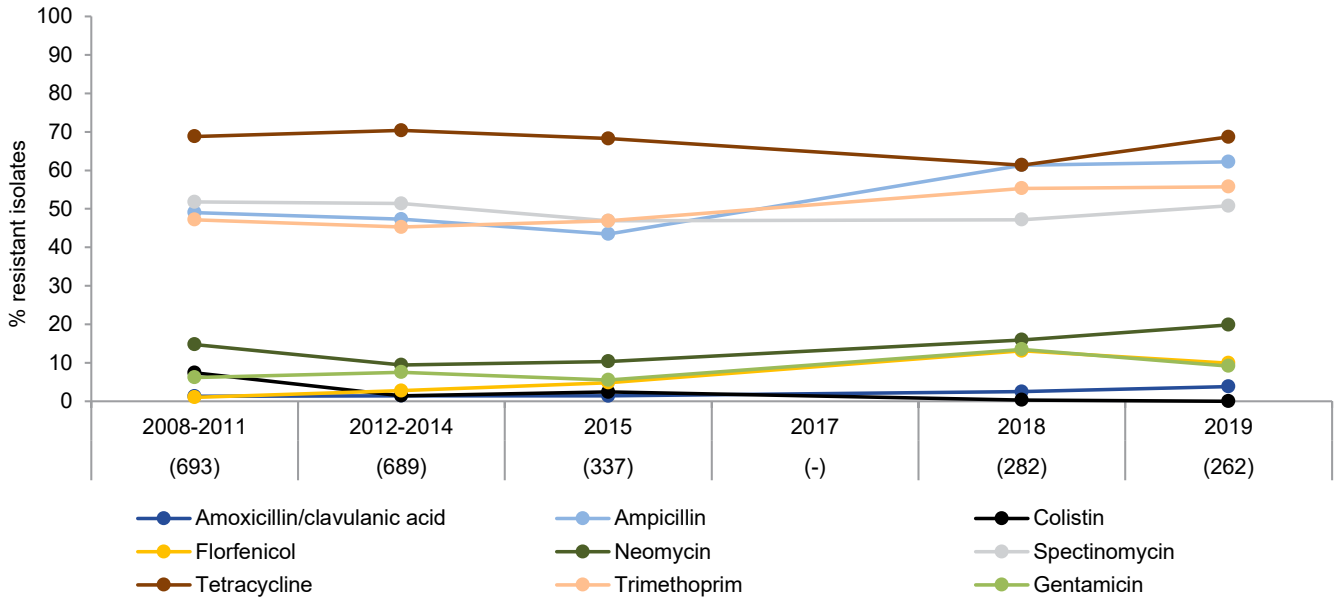
Isolates from the routine diagnostic laboratory investigation of isolates from dead and diseased pigs submitted to SEGES Pig Research Centre's Laboratory for Pig Diseases in Kjellerup. Occurrences of resistant isolates are presented according to the clinical breakpoints that are currently in use at both DTU National Veterinary Institute and Laboratory for Pig Diseases. Clinical breakpoint and MIC distributions are presented in the web annex (Table A6.8 - A6.10)

continued ... Textbox 6.4

E. coli - haemolytic pathogenic strains

Enterotoxigenic *E. coli* (ETEC) in combination with *Brachyspira pilosicoli* and *Lawsonia intracellularis* are the most prevalent causes of bacterial diarrhoeal in Danish pigs. Since 2014, PCR identification has been the most frequent method for identification of the diarrhoeal pathogens in Denmark, including identification of *E. coli* F4 and *E. coli* F18. Before 2018, the *E. coli* isolates were identified by serotyping at the SEGES laboratory, with the most virulent ETEC strains belonging to serovars O138, O139, O141, and O149, which are haemolytic and positive for enterotoxin. These strains are also mostly positive for F4 or F18 fimbrial adhesins, which are used for attachment to the intestinal mucosa. The haemolytic *E. coli* included in these analyses originated almost exclusively from porcine enteritis or oedema disease. The data from 2018 and 2019 presented F4 or F18 positive *E. coli*, while data for 2008-2017 presented the *E. coli* serovars O138, O139, O141, and O149. In general, the F18 positive strains belong to the serovars O138, O139 and O141, while serovar O149 carry the F4 fimbriae. However, this is not a clear cut correlation.

Figure 1 Resistance (%) among haemolytic *Escherichia coli* from pigs, Denmark DANMAP 2019



Note: Isolates from the routine diagnostic laboratory investigation of isolates from dead and diseased pigs submitted to SEGES Pig Research Centre's Laboratory for Pig Diseases in Kjellerup. Occurrences of resistant isolates are presented according to the clinical breakpoints that are currently in use at both DTU National Veterinary Institute and Laboratory for Pig Diseases. Clinical breakpoint and MIC distributions are presented in the web annex (Table A6.8)

As in previous years, high resistance levels were recorded in 2019 for ampicillin, streptomycin, sulfonamide, tetracycline, trimethoprim, and spectinomycin (Figure 1). The level of resistance to these four antimicrobials remained at the same level throughout the past decade (Figure 1). The use of tetracycline has decreased significantly since 2016, but this is not reflected in the occurrence of tetracycline resistance in *E. coli*. There may be several explanations for this, including co-selection (ASSuT complex). Also, the use of tetracycline for diarrhoea might not have decreased as much as for other infections. Many of the other porcine pathogens are susceptible to most antimicrobials, while resistance is much more widespread in the haemolytic *E. coli* F4/F18. Thus, the use of tetracycline has decreased more in finishers than in weaners, but also within weaners, the use of tetracycline might have decreased more for respiratory diseases than for diarrhoea. Most cases of porcine diarrhoea that require treatment occur during the weaning period and tetracycline, neomycin, or aminopenicillin are the compounds of choice for *E. coli* infection. In pigs, the aminoglycosides are only used for gastrointestinal infections, and particularly the use of neomycin has been increasing in recent years, after a reintroduction in 2017. In 2018, a significant increase was observed in the level of resistance to neomycin, gentamicin and apramycin (Figure 1). For neomycin, the increase continued reaching 20% in 2019. For apramycin and gentamicin, the occurrence of resistance in *E. coli* was slightly, but not significantly lower in 2019 compared to 2018, but remained at a high level compared to previous years. The parallel increase of the gentamicin and

apramycin is most likely a result of cross-resistance, as the vast majority of apramycin resistant isolates were also resistant to gentamicin (Table 1). A similar slight (non-significant) decrease was also noted for florfenicol (10% in 2019), after a continuous increase during the last decade (Figure 1).

Amoxacillin-clavulanic acid (amoxiclav) for oral treatment of pigs is a relatively new drug, and the use has been at a low level. However, an increasing trend in resistance to amoxiclav has been observed during the past decade, reaching 4% in 2020.

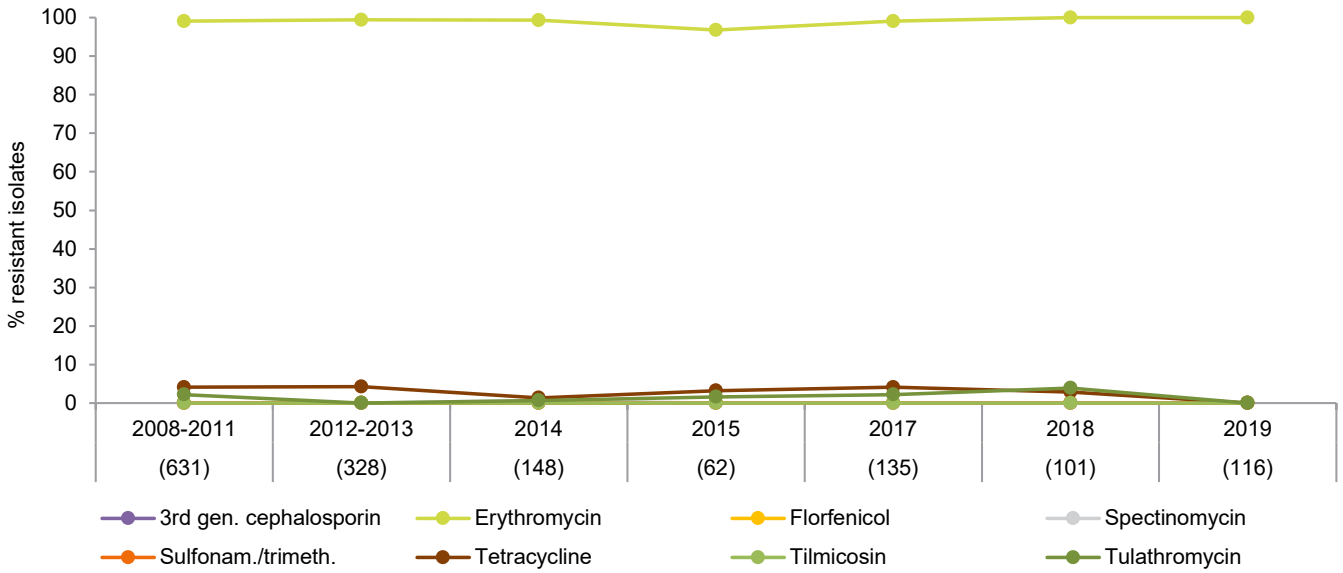
In 2017, the use of colistin was regulated by the authorities (given a high weight in the Yellow Card legislation), and the use of colistin has been close to zero since 2017. Resistance to colistin has since been decreasing (from a low level), and in 2019 no colistin resistant isolates were found.

Actinobacillus pleuropneumoniae

Actinobacillus pleuropneumoniae causes severe pleuropneumonia in pigs, although severity varies between serotypes. Outbreaks usually require rapid onset of treatment to minimise losses. Fortunately, *A. pleuropneumoniae* has very low occurrence of resistance to most of the available antimicrobials. Almost all isolates are resistant to erythromycin but this compound is not available for veterinary use. Macrolides are frequently used for treatment of pneumonia, and the resistance to other macrolides is low (Figure 2). Tulathromycin is frequently used, but in recent years the occurrence of resistance increased nonsignificantly reaching 4% in 2018 (Table 1). However, in 2019, no tulathromycin resistant isolates were identified. Still, as resistance to tulathromycin does occur in Danish *A. pleuropneumoniae*, susceptibility testing is important to monitor the occurrence of resistance and reduce the risk of treatment failure.

No resistance to florfenicol, sulfonamide-trimethoprim and tilmicosin has been observed for the last decade, and the occurrence of resistance remained absent or very low to penicillin, spectinomycin and tiamulin (Table 1). It is also worth noting that no resistance to ciprofloxacin has been observed in Danish isolates for more than 10 years.

Figure 2 Resistance (%) among *Actinobacillus pleuropneumoniae* from pigs, Denmark DANMAP 2019



Note: Isolates from the routine diagnostic laboratory investigation of isolates from dead and diseased pigs submitted to SEGES Pig Research Centre's Laboratory for Pig Diseases in Kjellerup. Occurrences of resistant isolates are presented according to the clinical breakpoints that are currently in use at both DTU National Veterinary Institute and Laboratory for Pig Diseases. Clinical breakpoint and MIC distributions are presented in the web annex (Table A6.9)

continued ... Textbox 6.4

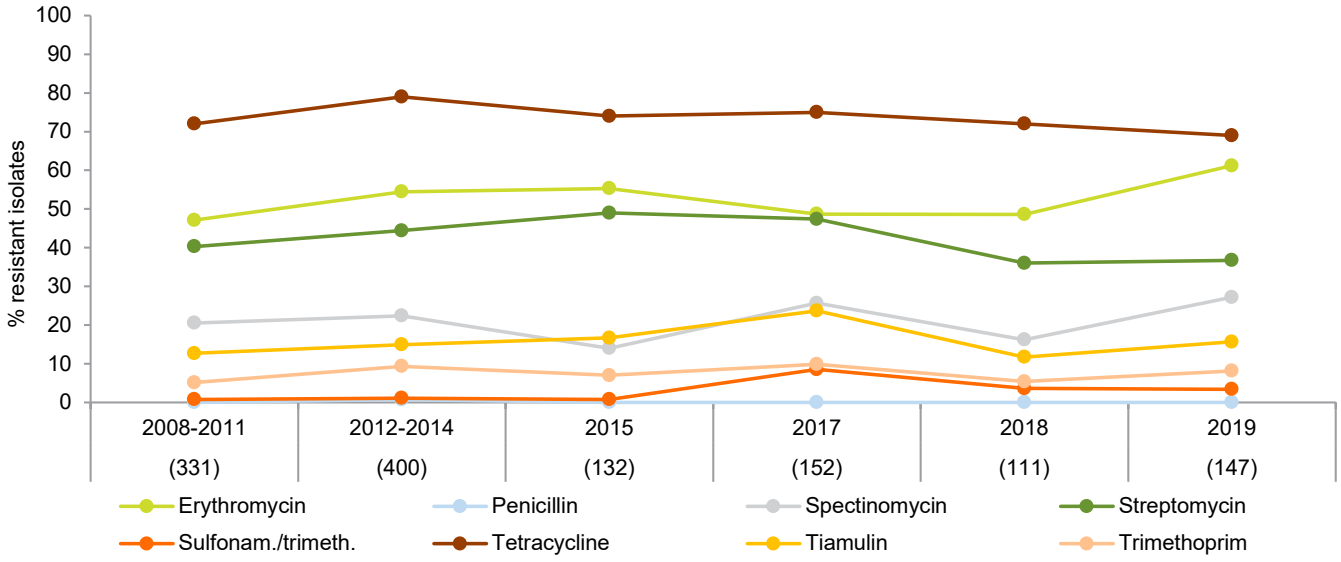
Streptococcus suis

Streptococcus suis may cause several different infectious conditions in pigs, such as meningitis, otitis media, arthritis, pneumonia, and septicaemia, and causes losses to the farmers due to increased mortality and veterinary costs.

This year the breakpoints for tetracycline and penicillin resistance were updated applying the CLSI breakpoints for porcine isolates. As in previous years, the highest levels of resistance was seen for tetracycline, streptomycin and erythromycin, but for erythromycin a significant increase was observed, reaching 61% in 2019 (Figure 3). The occurrence of resistance to tetracycline has remained stable around 70%, despite the decreases in use of tetracycline in pigs.

Resistance to sulfonamide is also very high (86% in 2019), but for pigs, sulfonamides are only available in combination with trimethoprim. Resistance to sulfonamide-trimethoprim remained at a low level, with 3.4% resistant isolates in 2019. For S. suis, there are several treatment options using compounds with very low levels of resistance (Table 1). Almost all isolates were susceptible to sulfonamide-trimethoprim, penicillin and florfenicol in 2019 and the past decade. Of these, penicillin and florfenicol are recommended 1st choice antimicrobials in the official guidelines. The occurrence of resistance to pleuromutilins (tiamulin) and spectinomycin remained at a moderate levels (Figure 3). For these compounds, considerable fluctuations were seen over the past decade, but mostly these fluctuations were non-significant. However, for spectinomycin the increase to 27% resistant isolates was significantly higher than the 2018 level, but similar to the level in 2017.

Figure 3 Resistance (%) among *Streptococcus suis* from pigs, Denmark DANMAP 2019



Note: Isolates from the routine diagnostic laboratory investigation of isolates from dead and diseased pigs submitted to SEGES Pig Research Centre's Laboratory for Pig Diseases in Kjellerup. Occurrences of resistant isolates are presented according to the clinical breakpoints that are currently in use at both DTU National Veterinary Institute and Laboratory for Pig Diseases. Clinical breakpoint and MIC distributions are presented in the web annex (Table A6.10)

Vibeke Frøkjær Jensen, Solveig Harksen and Charlotte Mark Salomonsen
For further information: vfje@dtu.dk

7

RESISTANCE IN INDICATOR BACTERIA

7. Resistance in indicator bacteria

Highlights: In 2019, trends and levels of antimicrobial resistance in indicator *E. coli* from poultry, pigs and cattle were overall very similar to previous years.

The relative distribution of fully sensitive indicator *E. coli* from broilers (64%) and cattle (87%) was comparable to 2018, whereas a decrease from 53% to 42% was observed in fully sensitive isolates from pigs. Compared to 2018, a statistically significant increase (from 30% to 44%) in sulfonamide resistance and a moderate increase (from 23% to 32%) in occurrence of multidrug-resistance was observed in pig isolates. Among broiler and cattle isolates, the occurrence of multidrug-resistance was comparable to 2018 and only minor fluctuations (1%-2%) in resistance were observed between the years in cattle isolates. However, over the last 5-year period, the occurrence of multidrug-resistant *E. coli* isolates from broilers has decreased significantly (from 23% to 11%).

From broilers, only one isolate (<1%) resistant to cefotaxime and ceftazidime was detected by the non-selective method. More phenotypic resistant isolates were detected by the more sensitive selective isolation methods in samples from pigs and cattle, indicating that resistance was also present in a relatively small proportion of commensal *E. coli* in pigs and cattle as well. Furthermore, no colistin, meropenem and tigecycline resistance was detected. The slow but steady increase in resistance to ciprofloxacin/nalidixic acid in *E. coli* from broilers, observed over the last ten years, continued.

In 2019, selective isolation methods showed that trends of *E. coli* producing ESBL/AmpC in animals and meat aligned with the observations from 2015-2018: lower occurrence in cattle compared to pigs (8% vs. 27%), lower levels in domestically produced broiler meat and pork than in imported (5% and 3% vs. 34% and 42%, respectively), and the decreasing occurrence in Danish broiler meat continued. ESBL transferring enzymes often associated with human infections, such as CTX-M-1, CTX-M-14 and/or CTX-M-15 were detected in low or very low numbers in all sources, and chromosomal AmpC mutations in isolates from pigs and cattle were still dominant.

Importantly, again all samples examined for carbapenemase-producing *E. coli* (including OXA-48) were found negative.

In 2019, enterococci from pigs showed no resistance to vancomycin, linezolid, teicoplanin or tigecycline. The *E. faecalis* isolates were resistant to tetracycline (91%), erythromycin (63%) and chloramphenicol (33%) and compared to 2017, an increase in tetracycline (13%) chloramphenicol (9%) and erythromycin (8%) resistance has been observed. Resistance levels in *E. faecium* were comparable to the latest observations from 2010-2012 and the most commonly observed resistance was tetracycline (54%), erythromycin (20%) and ampicillin (12%).

Currently, the zoonotic risk linked to transfer of resistance to critically important antimicrobials from animals to humans appears to be very limited in Denmark.

7.1 Introduction

Escherichia coli and *Enterococcus* are included in the DANMAP programme to monitor occurrence of antimicrobial resistance in different reservoirs through the food chain for the following reasons: i) they are present as commensals in the gut microbiota of healthy animals and humans, ii) they can acquire antimicrobial resistance both via mutations in chromosomal genes and horisontal transfer of antimicrobial resistance genes, and iii) they have the potential to cause infections in both animals and humans and to transfer antimicrobial resistance to pathogenic bacteria of the same or other species.

E. coli exhibiting resistance to 3rd generation cephalosporins via production of extended-spectrum beta-lactamases (ESBLs) and AmpC beta-lactamases (AmpCs) is one of the fastest spreading antimicrobial resistance problems in both humans and production animals worldwide. Several studies report similar ESBL/AmpC genes, plasmids and/or clones of *E. coli* isolates in animals, meat and human infections, which suggests a zoonotic link [Roer et al 2019. J Antimicrob Chemother 74(3):557; Valcek et al 2019. J Antimicrob Chemother 74(8):2171].

Carbapenemase-producing Enterobactericeae (CPE) are a great threat to human health, because carbapenems are last-line antimicrobial drugs for treatment of infections caused by multidrug-resistant Gram-negative bacteria. Currently, CPE have been detected sporadically in production animals in EU but never in Denmark [EFSA/ECDC 2019. EFSA journal 17(2):5598].

Since 2014, isolation and antimicrobial susceptibility testing of indicator *E. coli*, extended-spectrum cephalosporinase- and carbapenemase-producing *E. coli* (ESC and CPE) has been performed according to the EU harmonised monitoring of antimicrobial resistance [Decision 2013/652/EU].

7.2 Indicator *Escherichia coli*

All isolates originated from caecal samples randomly collected from healthy pigs, broilers and cattle at slaughter. Only one isolate per farm was included. Susceptibility to the antimicrobials recommended by EFSA was measured by broth microdilution to determine minimal inhibitory concentrations (MIC). MIC distributions and occurrence of resistance among indicator *E. coli* are presented in the web annex (Table A7.1). These results were obtained using the non-selective isolation procedure. Results obtained by using selective procedures for detection of cefotaxime-resistant *E. coli* are reported in section 7.3.

7.2.1 Indicator *E. coli* from broilers, cattle and pigs

From 168 representative pools of broiler caeca collected at Danish slaughterhouses, 159 *E. coli* isolates were obtained and all 159 isolates were tested for antimicrobial resistance (Table 7.1). More than half (64%) of the broiler isolates were susceptible to all antimicrobials in the test panel. From 186 representative cattle caeca collected at Danish slaughterhouses, 174 *E. coli* isolates were obtained, and 172 of these were tested for antimicrobial resistance (Table 7.1). The vast majority of cattle isolates (87%) was susceptible to all tested antimicrobials. From 195 representative pig caeca collected at Danish slaughterhouses, 192 *E. coli* isolates were obtained and 190 of these were tested for antimicrobial resistance (Table 7.1). Less than the half (42%) of the isolates from pigs were susceptible to all antimicrobials tested.

Among isolates from all animals, no colistin, meropenem or tigecycline resistance was detected. Compared to 2018, a minor increase (13% to 16%) in ciprofloxacin and nalidixic acid (13% to 15%) resistance in broiler isolates was observed, whereas a minor decrease (4% to 1%) in azithromycin resistance was detected in pig isolates. Only one isolate from broilers was resistant to 3rd generation cephalosporins (cefotaxime and ceftazidime), whereas no resistance to these critically important antimicrobials was found in cattle or pigs using non-selective methods (Table 7.1).

Table 7.1 Resistance (%) in *Escherichia coli* isolates from broilers, cattle and pigs, Denmark DANMAP 2019

	Broilers	Cattle	Pigs
Antimicrobial agent	Danish %	Danish %	Danish %
Ampicillin	15	6	35
Azithromycin	0	0	1
Cefotaxime	<1	0	0
Ceftazidime	<1	0	0
Chloramphenicol	<1	6	6
Ciprofloxacin	16	0	3
Colistin	0	0	0
Gentamicin	3	<1	2
Meropenem	0	0	0
Nalidixic acid	15	0	1
Sulfonamide	16	9	44
Tetracycline	11	12	35
Tigecycline	0	0	0
Trimethoprim	9	1	29
Fully sensitive (%)	64	87	42
Number of isolates	159	172	190

Note: An isolate is considered fully sensitive if susceptible to all antimicrobial agents included in the test panel, and multidrug-resistant if resistant to 3 or more of the 12 antimicrobial classes included (Table 9.3)

Among pig isolates, a statistically significant increase (30% to 44%) in sulfonamide resistance was observed, whereas resistance levels to the other antimicrobials were comparable to the observations in 2018. (Figure 7.1). Compared to 2018, only minor fluctuations (1% to 2%) in occurrence of resistance were observed in isolates from cattle and broilers. The occurrence of ampicillin, sulfonamide, tetracycline and trimethoprim resistance was significantly higher in *E. coli* from pigs (35%, 44%, 35% and 29%, respectively) compared to isolates from cattle

(6%, 9%, 12% and 1%, respectively). Resistance to ampicillin and tetracycline was also significantly higher in isolates from pigs than from broilers (15% and 11%, respectively). In contrast, ciprofloxacin resistance was higher in isolates from broilers (16%) compared to isolates from cattle (0%) and pigs (3%).

The relative distribution of fully sensitive isolates from broilers and cattle in 2019 was almost identical to 2018 with a difference of 4% and 2%, respectively. Compared to 2018, a moderate decrease (53% to 42%) in prevalence of fully sensitive *E. coli* from pigs was observed (Figure 7.2). A moderate increase (23% to 32%) in multidrug-resistant isolates from pigs was detected, whereas the prevalence of multidrug-resistant isolates from broilers and cattle was almost identical, compared to 2018. Among the multidrug-resistant isolates, co-resistance to ampicillin, sulfonamide and tetracycline (ASuT) was commonly observed (Figure 7.2).

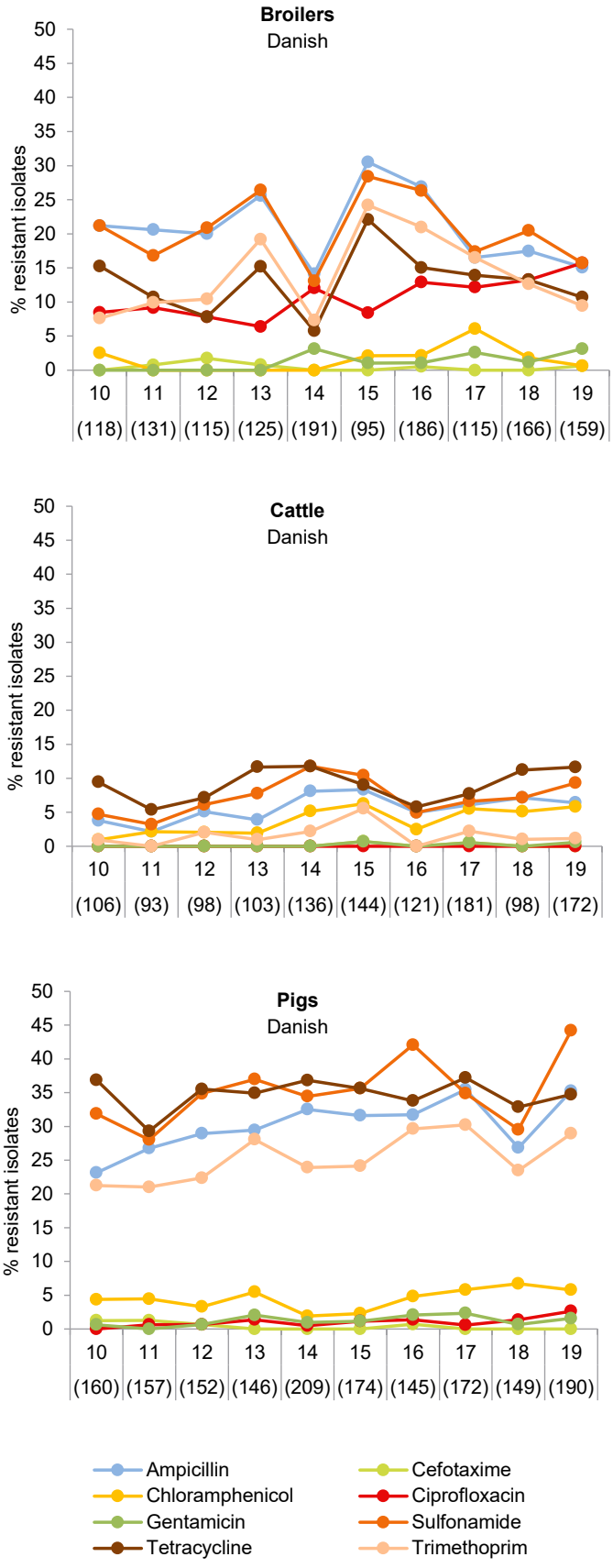
Over the last 5-year period, no statistically significant changes in the relative distribution of fully sensitive isolates from broilers, cattle or pigs have been observed, whereas a significant decrease in the relative occurrence of multidrug-resistant broiler isolates has occurred.

Among all resistant isolates from broilers (n = 58), 19 resistance profiles were observed. In total, 11% of all tested broiler isolates (n = 18) were classified as multidrug-resistant (resistance to ≥3 of the 12 antimicrobial classes in the *E. coli* test panel) and had 8 different resistance profiles (see web annex Table A7.2). The most common profiles included resistance to: i) ciprofloxacin and nalidixic acid, ii) ampicillin, sulfonamide and trimethoprim and iii) tetracycline; and were detected among 26%, 16% and 10% of resistant isolates from broilers, respectively. Noteworthy, a single ESBL-producing isolate with resistance to ampicillin, ceftazidime, cefotaxime, cefepime, sulfonamide and tetracycline was observed in the 2019 monitoring.

A total of, 10 resistance profiles were observed among resistant isolates from cattle (n = 22), and of these isolates, 11 were categorised as multidrug-resistant (see web annex Table A7.2). As in 2018, a high prevalence of tetracycline resistance was detected. The most common profiles included resistance to: i) ampicillin, chloramphenicol, sulfonamide and tetracycline, ii) sulfonamide and tetracycline and iii) tetracycline; and were observed among 36%, 18% and 14% of the resistant isolates from cattle, respectively.

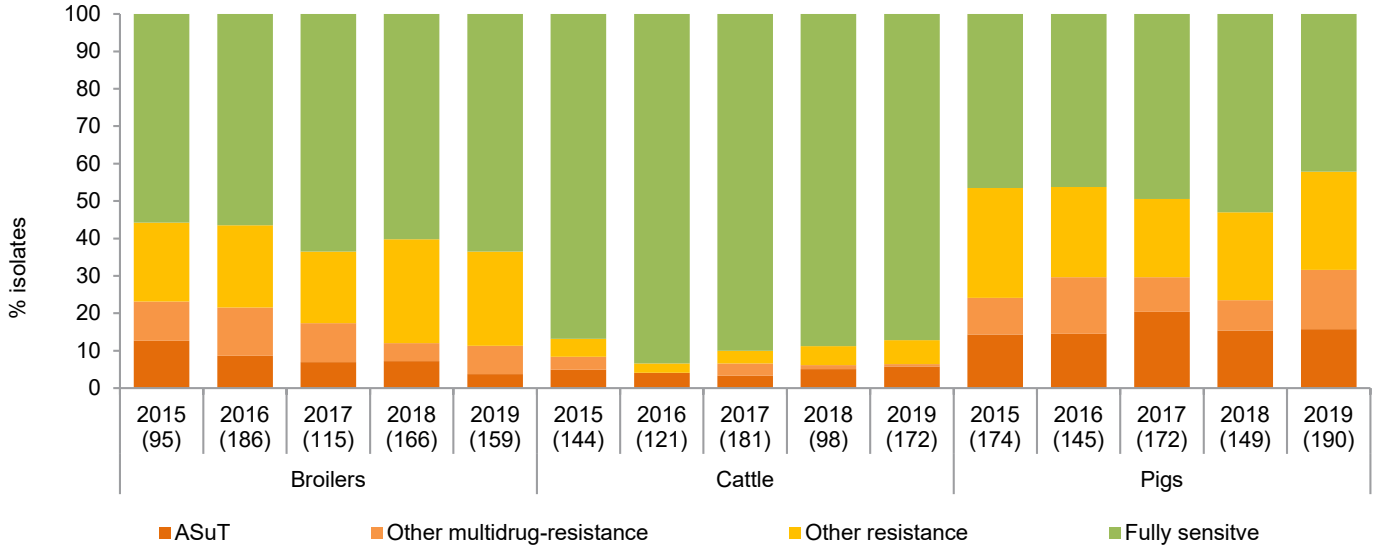
From pigs, a total of 28 resistance profiles were observed among all resistant isolates (n = 110), and 55% of these (n = 60) were categorised as multidrug-resistant (see web annex Table A7.2). Nineteen (17%) of all resistant isolates exhibited resistance to one type of antimicrobial only. Thus, 11%, 5%, 1% and 1% of the resistant isolates showed resistance to tetracycline, sulfonamide, trimethoprim and ampicillin, respectively.

Figure 7.1 Resistance (%) among *Escherichia coli* isolates from broilers, cattle and pigs, Denmark DANMAP 2019



Note: Number of isolates included each year is presented in the parenthesis

Figure 7.2 Distribution (%) of multidrug-resistant, resistant and fully sensitive *Escherichia coli* isolates from broilers, cattle and pigs, Denmark
DANMAP 2019



Note: The number of isolates included each year is shown in parentheses. An isolate is considered fully sensitive if susceptible to all antimicrobial agents included in the test panel, and multidrug-resistant if resistant to 3 or more of the 12 antimicrobial classes included (Table 9.3). ASuT are the multidrug-resistant isolates resistant to ampicillin, sulfonamide and tetracycline, but may also be resistant to other antimicrobials

Over the last 10-year period, there has been a slow but statistically significant increase in resistance to chloramphenicol, nalidixic acid, ciprofloxacin and gentamicin among broiler isolates. For pig isolates, a slow but statistically significant increase in resistance to ampicillin and trimethoprim was observed whereas a statistically significant increase in resistance to ampicillin and chloramphenicol was detected for cattle isolates, over the last 10-year period. Over the last 5-year period, no statistically significant increase in occurrence of resistance was observed among any of the animal isolates.

7.2.2 Perspectives

Antimicrobial resistance monitoring in commensal *E. coli* is considered a useful indicator of the selective pressure exerted by antimicrobial use on the intestinal microbiota of food-producing animals.

From a European perspective, based on the last published data from 2017 and 2018, indicator *E. coli* from Danish broilers and calves <1 year show noticeably low occurrence of resistance to any antimicrobial compared to the indicator *E. coli* from other countries apart from the Nordic countries [EFSA/ECDC 2019. EFSA journal 12(2):5598; EFSA/ECDC 2019. EFSA Journal 2020:18(3):6007]. Denmark is among the countries reporting the lowest occurrence of chloramphenicol and, more importantly, ciprofloxacin resistance in indicator *E. coli* from pigs, whereas the reported occurrence of ampicillin, azithromycin, sulfonamide, trimethoprim and tetracycline resistance was comparable to the average reported in the EU Member States.

The antimicrobial resistance phenotypes detected in animal-origin indicator *E. coli* mostly relevant to human health were ciprofloxacin resistance in *E. coli* from broilers and azithromycin resistance in *E. coli* from pigs, as in 2018. A slow but increasing trend in resistance to ciprofloxacin has occurred in the *E. coli* isolates from broilers over the last ten years (16% in 2019). A single ESBL-producing broiler isolate was identified. Detection of ESBL or AmpC-producing *E. coli* in Danish production animals, using the non-selective isolation procedures only, is rare. Since 2014, the non-selective monitoring has only recovered one isolate from cattle (2015) and one from broilers (2016).

Although the molecular bases of ciprofloxacin resistance have not been investigated, the phenotype indicated chromosomal mutations (in 112 of 116 ciprofloxacin resistant broiler isolates from 2014 to 2019), consequently linking the main risk to human health to the disease-causing potential of these strains. Resistance to azithromycin in isolates from pigs decreased from 4% in 2018 to 1% in 2019. The potential human risk derived by infections with these strains and/or transfer of azithromycin resistance to pathogenic strains remains low - but will be monitored closely the following years. The reduction in use of tetracycline and colistin and the increased use of macrolides and aminoglycosides since 2016, have not caused any measurable changes in the resistance patterns in *E. coli* from pigs.

Resistance to other antimicrobials relevant for human medicine such as colistin, cefotaxime, ceftazidime, meropenem and tigecycline was in general found to be very low (<1%) or was not detected. Based on the number of samples collected, absence of resistant isolates indicates a prevalence of these resistance phenotypes of less than 2% in *E. coli* from broilers, pigs and cattle (see section 9.7). However, cefotaxime- and ceftazidime-resistant *E. coli* were detected, when using selective enrichment, which is more sensitive.

7.3 ESBL/AmpC- and carbapenemase-producing *E. coli*

DANMAP 2019 monitor extended-spectrum cephalosporinase- and carbapenemase-producing *E. coli* (ESC and CPE isolates) from caeca of pigs and cattle at slaughter and from Danish and imported pork and beef at retail, in concordance with the EU regulation on harmonised monitoring of antimicrobial resistance in zoonotic and indicator bacteria from food-producing animals and food (2013/652/EU). In addition, Danish and imported broiler meat at retail was included in DANMAP 2019 as part of the national surveillance strategy. Samples were collected randomly and cultured directly in a selective enrichment for detection of cefotaxime-resistant *E. coli* and carbapenemase-producing *E. coli* (including oxacillinase-producing OXA-48-like enzymes).

Subsequently, obtained *E. coli* isolates were phenotypically antimicrobial susceptibility tested by MIC determination against the panel of defined antimicrobials and ranges of concentrations in concordance with the EU regulation. In parallel, for most isolates, whole genome sequencing (WGS) and *in silico* bioinformatics were applied to detect the ESBL, AmpC and CPE encoding genes. MIC distributions and occurrence of resistance among ESBL/AmpC-producing *E. coli* isolates are presented in the web annex (Tables A7.3 and A7.4).

7.3.1 ESBL/AmpC- and carbapenemase-producing *E. coli* from pigs and domestically produced pork

A total of 330 samples from pigs and 317 samples from domestically produced pork resulted in 89 (27%) and 9 (3%) ESC-positive samples, respectively (Table 7.2). The number of investigated samples and level of ESC isolates were slightly (non-significantly) higher than in 2017 (Figure 7.3). No CPE isolates were recovered, suggesting, with 95% confidence, that CPE isolates may be present in less than 1% of the samples from pigs and domestically produced pork.

Table 7.2 Resistance (%) and beta-lactam resistance phenotype distributions (%) in ESBL/AmpC-producing *Escherichia coli* from broilers and broiler meat recovered by selective enrichment, Denmark DANMAP 2019

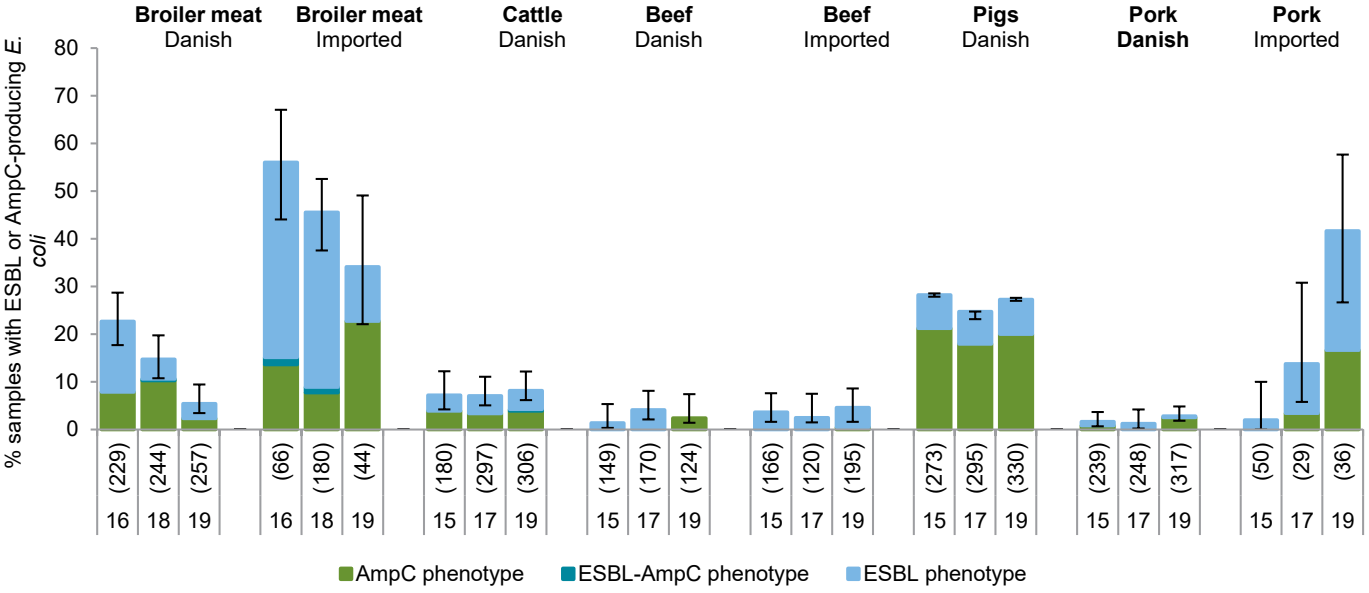
	Broiler meat		Cattle	Beef		Pigs	Pork	
Antimicrobial agent	Danish %	Import %	Danish %	Danish %	Import %	Danish %	Danish %	Import %
Ampicillin	100	100	100	100	100	100	100	100
Azithromycin	0	0	8	0	0	4	0	0
Cefepime[a]	64	53	60	0	89	36	33	87
Cefotaxime[a]	100	100	100	100	100	100	100	100
Cefotaxime/clavulansyre	43	67	48	100	11	73	89	40
Cefoxitin[a]	43	67	52	100	11	73	89	40
Ceftazidime[a]	100	93	84	100	100	100	100	100
Ceftazidime/clavulansyre	43	67	48	100	11	72	89	40
Chloramphenicol	0	0	20	0	33	9	0	0
Ciprofloxacin	57	87	16	0	56	2	11	0
Colistin	0	0	0	0	0	0	0	0
Ertapenem	0	0	0	0	0	0	0	0
Gentamicin	0	7	4	0	11	10	11	7
Imipenem	0	0	0	0	0	0	0	0
Meropenem	0	0	0	0	0	0	0	0
Nalidixic acid	57	93	4	0	44	0	0	0
Sulfonamide	29	93	56	67	33	54	44	60
Temocillin	0	0	0	0	0	0	0	0
Tetracycline	21	87	36	33	89	51	33	33
Tigecycline	0	0	0	0	0	0	0	0
Trimethoprim	7	33	36	33	56	43	44	47
CPE phenotypes	0	0	0	0	0	0	0	0
AmpC phenotypes	43	67	48	100	11	73	89	40
ESBL phenotypes	57	33	48	0	89	27	11	60
ESBL and AmpC phenotypes	0	0	4	0	0	0	0	0
Number of isolates	14	15	25	3	9	89	9	15
Number of samples	257	44	306	124	195	330	317	36

Note: Classification of CPE, ESBL and AmpC phenotypes is based on the MIC results according to the scheme provided by EFSA (see Materials and methods, section 9.7). WGS revealed ESBL genes only in the single isolates that were classified as an ESBL and AmpC phenotype according to MIC results

a) Classification of cephalosporins: 2nd generation (cefoxitin), 3rd generation (cefotaxime and ceftazidime) and 4th generation (cefepime)

Figure 7.3 Occurrence (%) of samples with phenotypic ESBL- or AmpC-producing *E. coli* from animals and meat recovered by selective enrichment, Denmark

DANMAP 2019



Note: Number of samples tested per year is presented in parentheses. Confidence intervals for total proportion of samples positive for phenotypic ESBL- and/or AmpC-producing *E. coli* - calculated as 95% binomial proportions presenting Wilson intervals. Classification of CPE, ESBL and AmpC phenotypes according to the scheme provided by EFSA (see section 9.7)

Among the 89 ESC isolates from pigs, 73% belonged to an AmpC phenotype and 27% to an ESBL phenotype, conferring 100%, 100%, 73%, and 36% resistance to cefotaxime, ceftazidime, cefoxitin and cefepime, respectively (Table 7.2).

Among the nine ESC isolates from domestically produced pork, 8 belonged to an AmpC phenotype and 1 to an ESBL phenotype, conferring 100%, 100%, 89%, and 33% resistance to cefotaxime, ceftazidime, cefoxitin, and cefepime, respectively (Table 7.2).

All, except three, of the 98 ESC isolates from Danish pigs and pork were whole genome sequenced (WGS). Among the AmpC phenotypes, most conferred resistance due to the presence of upregulated AmpC promotor C-42T mutations. One isolate had a T-32A mutation. Two additional isolates of the AmpC phenotype harboured CMY-2 encoding genes. Mainly MLSTs believed to be commensals were observed associated with the AmpC phenotype. Thus, 17, 8, 6, 7 and 5 AmpC-producing *E. coli* out of the 67 exhibiting the C-42T mutation were attributed to ST88, ST23, ST75, ST453, and ST101, respectively. ST23 is known to be observed globally and with a potential zoonotic link [Maluta et al. 2014. PLoS One 12:9(8)]. Similarly, ST48 and ST88 have previously been observed attributed to clinical cases of human and animal origin and as in isolates from domestically produced pork in Denmark, often associated to ESBL-producing *E. coli* [Sallem et al. 2014. Microb Drug Resist 20:495]. The MLST and ESBL and AmpC enzymes combinations are listed in web annex Table A7.6. The isolates which harboured the CMY-2 genotype were attributed to ST88 and ST58.

The 25 ESBL-producing *E. coli* from Danish pigs and pork were attributed to five different enzymes dominated by CTX-M-1 accounting for 20 isolates of which one in addition to CTX-M-1 also harboured CTX-M-175 gene. Similarly to the isolates of an AmpC genotype, also the enzymes of the ESBL-producing *E. coli* were attributed to a long range of MLSTs represented by only one isolate. The MLST mostly represented, but only by three isolates harbouring the CTX-M-1 gene, were ST88 (Table 7.3).

Genotypes (enzymes) of isolates from pigs were not determined in 2017, thus, a comparison to previous years was not possible. The three isolates recovered from domestically produced pork from 2017 all harboured the CTX-M-1 gene.

The ESC isolates exhibited in general a moderate level of antimicrobial resistance with no resistance to colistin, nalidixic acid, tigecycline and temocillin (Table 7.2). Resistance to fluoroquinolones was low, with only three isolates being resistant to ciprofloxacin, all harbouring *qnrS1* genes. Different combinations of additional multidrug resistance including penta-resistance in addition to ESBL- and AmpC-producing *E. coli* was observed in most isolates primarily to ampicillin, chloramphenicol, tetracycline, sulfonamides, and trimethoprim (see web annex Table A7.5).

Interestingly, resistance to gentamicin and azithromycin was observed in a few ESC isolates from pigs (4% azithromycin and 10% gentamicin resistance) and from domestically produced pork (11% gentamicin resistance).

Table 7.3 Number of ESBL and AmpC enzymes detected in *E. coli* isolates from animals and meat recovered by selective enrichment, Denmark DANMAP 2019

	Broiler meat		Cattle	Beef		Pigs	Pork	
Enzymes	Danish	Import	Danish	Danish	Import	Danish	Danish	Import
CMY-2	1					1	1	
CTX-M-1	4		3		3	19	1	7
CTX-M-14	1		6			3		1
CTX-M-15		2	3		2			
CTX-M-175						1		
CTX-M-2			1					
CTX-M-32		1				1		
CTX-M-55						1		
CTX-M-65					2			
SHV-12		1						1
Chromosomal AmpC	5	5	12	3	1	63	5	5
Not available	3	6			1	2	2	2
Number (%) of positive samples	14 (5%)	15 (34%)	25 (8%)	3 (2%)	9 (5%)	90 (27%)	9 (3%)	15 (42%)
Number of tested samples	257	44	306	124	195	330	317	36

Note: Isolates recovered by the selective enrichment methods described in the EURL-AR laboratory protocol (February 2018) and ESBL/AmpC enzymes are determined by WGS. Two isolates with CTX-M-1 contained an additional ESBL gene: CTX-M-175 from pigs and SHV-12 from imported pork. For 2019 data, all MLST and ESBL/AmpC enzymes combinations are listed in web annex Table A7.6

Co-resistance to sulfonamides and trimethoprim is likely attributable to the presence of class 1 integrons, which occasionally also harbour resistance genes encoding resistance to ampicillin, tetracycline and sometimes chloramphenicol. Moderate resistance to sulfonamides and trimethoprim was observed in the ESC isolates from pigs and domestically produced pork to both sulfonamides and trimethoprim as well as to ampicillin and tetracycline.

7.3.2 ESBL/AmpC- and carbapenemase-producing *E. coli* from cattle and domestically produced beef

A total of 306 samples from cattle and 124 samples from domestically produced beef resulted in 25 (8%) and 3 (2%) positive samples, respectively (Table 7.2). Among the isolates, no change was observed in the percentages of the different ESBL- and AmpC -phenotypes from 2017 to 2019 (Figure 7.3). No CPE isolates were recovered, suggesting, with 95% confidence, that CPE isolates may be present in less than 1% of the samples from cattle and in less than 2% of the samples from domestically produced beef.

Among the 25 ESC isolates from cattle, half belonged to an ESBL phenotype and the other half to an AmpC phenotype. The MIC results indicated one isolate of a mixed ESBL-AmpC phenotype, however, WGS analysis only revealed an ESBL gene. In contrast, the three beef isolates represented were all of the AmpC -phenotype. No statistically significant changes were observed between 2017 and 2019 (Table 7.2 and Figure 7.3). The beef isolates conferred 100% resistance to cefotaxime, ceftazidime, and cefoxitin, respectively, with no resistance to cefepime. This differs from the ESBL- and AmpC -producing *E. coli* isolates from cattle, which exhibited 100%, 84%, 52%, and 60% resistance to cefotaxime, ceftazidime, cefoxitin and cefepime, respectively (Table 7.2).

All of the three isolates from Danish beef contained the upregulated chromosomal AmpC mutations C-42T (n = 2) and T-32A (n = 1). The C-42T mutation was also observed in 12 among the 25 isolates from cattle. The remaining isolates from cattle contained the enzyme-encoding genes CTX-M-14 (n = 6), CTX-M-1 (n = 3), CTX-M-15 (n = 3) and CTX-M-2 (n = 1). Allmost all of the 28 isolates were solely attributable to single and different MLSTs. The exceptions were four and two cattle isolates containing either an upregulated chromosomal AmpC mutation, C-42T belonging to ST56 or harbouring the CTX-M-14 attributed to ST43, respectively (see web annex Table A7.6).

The cattle and beef ESC isolates exhibited in general a moderate level of antimicrobial resistance with no resistance to colistin, tigecycline, and temocillin. Moreover, no resistance among the three isolates of domestically produced beef was observed to also azithromycin, chloramphenicol, ciprofloxacin, gentamicin, and nalidixic acid. The 25 ESBL- and AmpC-producing *E. coli* isolates from cattle exhibited similar low to moderate levels of resistance to azithromycin (8%), chloramphenicol (20%), ciprofloxacin (16%), gentamicin (4%), and nalidixic acid (4%). Three of the isolates from cattle harboured *qnrS1* genes and one isolate mutations in *gyrA*.

Five ESC isolates from cattle exhibited a penta-resistance profile besides being ESBL/AmpC -producers. The resistance profile included resistance to ampicillin, chloramphenicol, ciprofloxacin, gentamicin, nalidixic acid, sulfonamides, tetracycline, and trimethoprim.

7.3.3 ESBL/AmpC- and carbapenemase-producing *E. coli* from imported pork and imported beef

A total of 36 and 195 samples from imported pork and imported beef resulted in 15 (42%) and nine (5%) ESC positive samples, respectively (Table 7.2). As in previous years, the packages of meat were selected without stratification on the country of origin. Samples were also specifically examined for the presence of CPE of which none were recovered.

A statistically significant change was observed in the occurrence of ESC isolates from 2015 (2%) to 2019 (42%) among samples from imported pork (Figure 7.3). The level in imported beef was the same as in previous years. All of the 15 pork isolates and 9 beef isolates conferred resistance to cefotaxime and ceftazidime, whereas 11% and 89% of imported beef isolates and 40% and 87% of imported pork isolates were resistant to cefoxitin and cefepime, respectively (Table 7.2).

Among the 15 ESC isolates from imported pork, nine exhibited an ESBL phenotype and six an AmpC phenotype. Two isolates were not viable for WGS and the enzymes were only detected in 13 isolates from imported pork. These harboured CTX-M-1 (n = 7), CTX-M-14 (n = 1), SHV12 (n = 1) and five upregulation of chromosomal *ampC* (mutation C- 42T) (Table 7.3). A number of MLSTs were observed dominated by ST58 and ST88 associated to the CTX-M-1, CTX-M-14, SHV- 12, and upregulation of chromosomal AmpC.

One of the nine ESC isolates from imported beef, was not available for WGS. Among the remaining eight, seven displayed an ESBL phenotype and one an AmpC phenotype. Only CTX-M enzymes were observed: CTX-M-1 (n = 3, in ST117, ST446, and ST1080), CTX-M-15 (n = 2, in ST4981), and CTX-M-65 (n = 2, in ST683), reported and one isolate of an upregulated AmpC promotor, C-42T (ST88). Of the MLSTs observed in 2019, only ST446 was also observed in 2017 among isolates from imported beef.

The ESC isolates from imported beef exhibited a moderate level of antimicrobial resistance besides being ESBL producing compared to ESBL/AmpC-producing *E. coli* isolates from imported pork, which exhibited more susceptibility to many of the antimicrobials tested. No resistance to azithromycin, colistin, temocillin and tigecycline was observed for both types of imported meat with the additional absence of resistance reported for imported pork to chloramphenicol, ciprofloxacin, and nalidixic acid (Table 7.2). Two isolates from imported beef harboured *qnrS1* genes.

7.3.4 ESBL-, AmpC- and carbapenemase-producing *E. coli* from domestically produced broiler meat

A total of 257 samples from retail of domestically produced broiler meat resulted in 14 (5%) ESC positive samples, (Figure 7.3). The prevalence of ESC positive samples in 2019 was significantly lower than in 2016 (15%) and in 2018 (23%). The reason for the general reduction in the prevalence of ESC phenotypes was due to a decline of the ESBL phenotype between 2016 and 2018 from 15% to 4% combined with a decline of the AmpC phenotype between 2018 and 2019 from 10% to 2% (Figure 7.3).

No CPE isolates were recovered, suggesting that we can be 95% certain that CPE isolates are only present in 1% or less of packages of domestically produced broiler meat (see section 9.7).

Overall, the 14 ESC isolates exhibited 100%, 100%, 43%, and 64% resistance to cefotaxime, ceftazidime, cefoxitin and cefepime, respectively (Table 7.2).

A total of 11 of the ESC isolates were whole genome sequenced. The MLST and ESBL and AmpC enzymes combinations are listed in web annex Table A7.6. Among the AmpC phenotypes, five conferred resistance due to the presence of an upregulated AmpC promotor, C-42T, of which four were attributable to ST4663 (Table 7.3) whereas only one isolate harboured the CMY-2 encoding gene. The previously reported introduction of CMY-2 encoded ST2040 *E. coli* to domestically produced broiler meat seemed to disappear in 2019 compared to 2018, indicating a highly changeable population of *E. coli* present in broiler meat of Danish origin. The most common ESBL gene among the five genomes from the broiler meat was as in previous years CTX-M-1 and attributed to three MLSTs of which ST1640 previously has been reported in 2018. (see web annex Table A7.6).

The ESC isolates from Danish broiler meat exhibited varying levels of resistance to other antimicrobials. No resistance to azithromycin, chloramphenicol, colistin, gentamicin, tigecycline, and temocillin, was detected. (Table 7.2). Resistance to quinolones was still moderate but increasing, compared to previous years from 22% in 2018 to 57% in 2019. Concordance between resistance to ciprofloxacin and nalidixic acid was 100%, and chromosomal mutations in the topoisomerases genes *gyrA* were detected in one isolate.

Different combinations of multidrug-resistance were observed among the ESBL and AmpC-producing *E. coli* isolates (web annex Table A7.5). The multidrug-resistance was primarily including ampicillin (100%), ciprofloxacin, nalidixic acid, tetracycline (21%), sulfonamides (29%), and trimethoprim (7%) in addition to the cephalosporins (Table 7.2).

7.3.5 ESBL-, AmpC- and carbapenemase-producing *E. coli* from imported broiler meat

A total of 44 samples from imported broiler meat resulted in 15 (34%) ESC positive samples (Table 7.2), which was a non-significantly decrease from 2018 (46%, Figure 7.3). In contrast to the isolates from Danish broiler meat, the prevalence of ESBL and AmpC phenotypes were higher, with the AmpC phenotype being more common in imported broiler meat than in Danish meat. No CPE isolates were recovered in any of the imported broiler meat samples.

The ESC isolates exhibited 100%, 93%, 67%, and 53% resistance to cefotaxime, ceftazidime, cefoxitin, and cefepime, respectively (Table 7.2).

Only 9 of the 15 ESC isolates were available for WGS. Of those, five isolates exhibited an AmpC phenotype due to the presence of an upregulated AmpC promotor, C-42T (Table 7.3). All the AmpC-producing *E. coli* isolates originated from French products and belonged to a single MLST, ST4710, which was different from those observed in Danish broiler meat.

Interestingly, the CTX-M-1-encoding gene observed as the most commonly found ESBL phenotype in 2018 accounting for 80% of all seemed to have disappeared from the imported broiler meat. However, this could have been due to a small sample size and different producers/countries sampled in the two years. Thus, the four ESBL-producing *E. coli* isolates harboured the SHV-12, CTX-M-15, and CTX-M-32-encoding genes, respectively with three CTX-M isolates belonging to ST10 (see web annex Table A7.6). ST10 has previously been reported being emerging and pathogenic to poultry [Manges and Johnson 2012. CID 55:712; Pires-dos-Santos et al. 2013. Vet Microbiol 162(2-4):873].

The 15 ESC isolates exhibited the same trend as in previous years with higher levels of resistance and co-resistance than those from Danish broiler meat. See AMR profiles listed in web annex Table A7.5. No resistance to azithromycin, chloramphenicol, colistin, tigecycline and temocillin was observed and only low levels of resistance to gentamicin (7%) was detected (Table 7.2). Resistance to quinolones was very high with 87% (n = 13) and 93% (n = 14) resistance to ciprofloxacin and nalidixic acid, respectively. Chromosomal mutations in the topoisomerases genes *gyrA* were observed in 12 isolates. The multidrug-resistance primarily included ampicillin, chloramphenicol, tetracycline, sulfonamides, and trimethoprim in addition to the cephalosporins and often quinolones. Thus, co-resistance, likely attributable to the presence of class 1 integrons and AMR islands, was observed (Table 7.2).

7.3.6 Perspectives

The observed trends seem to be quite consistent with less variation across the years in the occurrence and level of phenotypic resistance with the exception of a significant reduction in the occurrence of ESBL- and AmpC- producing *E. coli* from Danish broiler meat and an increase in the imported pork. The enzymes of the ESBL-, AmpC- and carbapenemase-producing *E. coli* seem to be fairly consistent with the same enzymes being observed each year, but attributed to different MLSTs indicating extensive horizontal gene transfer. The extensive circulation among the commensal *E. coli* of AMR genes of critical importance might be the most interesting observation from 2019. The extensive horizontal gene transfer observed could potentially become a problem, if transferred to a pathogen or contaminating a food product normally not heat-treated.

When comparing the ESC isolates to the isolates from human bloodstream infections collected during 2019, no possible clonal relationships between isolates sharing the same combination of ST and ESBL-/pAmpC-genes, were identified by whole-genome-based single-nucleotide polymorphism (SNP) analysis. Horizontal gene transfer by plasmids encoding ESBL/pAmpC enzymes was not investigated.

Still no carbapenemase-producing *E. coli* were detected in a total of 1,609 samples tested. This is opposed to the increase in reports from other countries in Europe of incidences where carbapenemase-producing *E. coli* is emerging or persistent in the primary production [Bonardi et al. 2019. Ital J Food Saf 8:7956]. Recently, the first OXA-48-producing *E. coli* was isolated from a fattening pig in Germany and since carbapenems are not licensed for clinical use in veterinary medicine in Europe, the increase in carbapenemase-producing *E. coli* in production animals and retail meat could indicate spill-over from humans [Irrgang et al. 2020. Microorganisms 8:855].

7.4 *Enterococcus*

The *Enterococcus faecalis* and *Enterococcus faecium* isolates collected in 2019 originated from randomly collected caecal samples from healthy fattening pigs at slaughter. *E. faecalis* isolates from pigs were previously collected in 2017, whereas *E. faecium* isolates have not been collected since 2012. Since 2014, sampling and testing of *Enterococcus*, have been performed according to the EU harmonised monitoring of antimicrobial resistance [Decision 2013/652/EU] and the antimicrobial test panel recommended by EFSA was applied for MIC testing. MIC distributions and occurrence of resistance among *E. faecalis* and *E. faecium* isolates from pigs are presented in the web annex (Table A7.7). *E. faecalis* is considered intrinsically (i.e. naturally) resistant to streptogramin A and B (quinupristin/dalfopristin), and interpretation of the MIC testing for this antimicrobial agent was not done in DANMAP.

7.4.1 Enterococci from pigs

From 799 representative pig caeca collected at Danish slaughterhouses, 195 *Enterococcus* isolates were obtained and 100 *E. faecium* and 91 *E. faecalis* isolates were tested for antimicrobial resistance (Table 7.4). In total, 40 of the 100 *E. faecium* isolates (40%) showed no resistance to any of the antimicrobials in the test panel (Table 7.4).

In 2019, no linezolid, teicoplanin, tigecycline or vancomycin resistance was detected in any *Enterococcus* isolates from pigs. Among the *E. faecalis* isolates a high proportion of tetracycline (91%) and erythromycin (63%) resistance was observed whereas a more moderate proportion of chloramphenicol (33%) resistance was detected. The prevalence of ciprofloxacin (2%) and daptomycin (3%) resistance was very low in the *E. faecalis* isolates (Table 7.4).

Table 7.4 Resistance (%) in *Enterococcus* isolates from pigs, Denmark DANMAP 2019

Antimicrobial agent	*Enterococcus faecalis* %	*Enterococcus faecium* %
Ampicillin	0	12
Chloramphenicol	33	2
Ciprofloxacin	2	5
Daptomycin	3	0
Erythromycin	63	20
Gentamicin	11	1
Linezolid	0	0
Quinopristin/Dalfopristin	-	6
Teicoplanin	0	0
Tetracycline	91	54
Tigecycline	0	0
Vancomycin	0	0
Number of isolates	91	100

Note: *E. faecalis* are assumed intrinsically resistant to streptogramins and for *E. faecium* a cutoff of >4 was applied for quinopristin/dalfopristin (tradename Synercid) according to investigations presented in DANMAP 2006

The three daptomycin resistant isolates all had a MIC value of 8 mg/L, a level very close to the cutoff of >4 mg/L. The genetic background for the phenotypic daptomycin resistance was not determined.

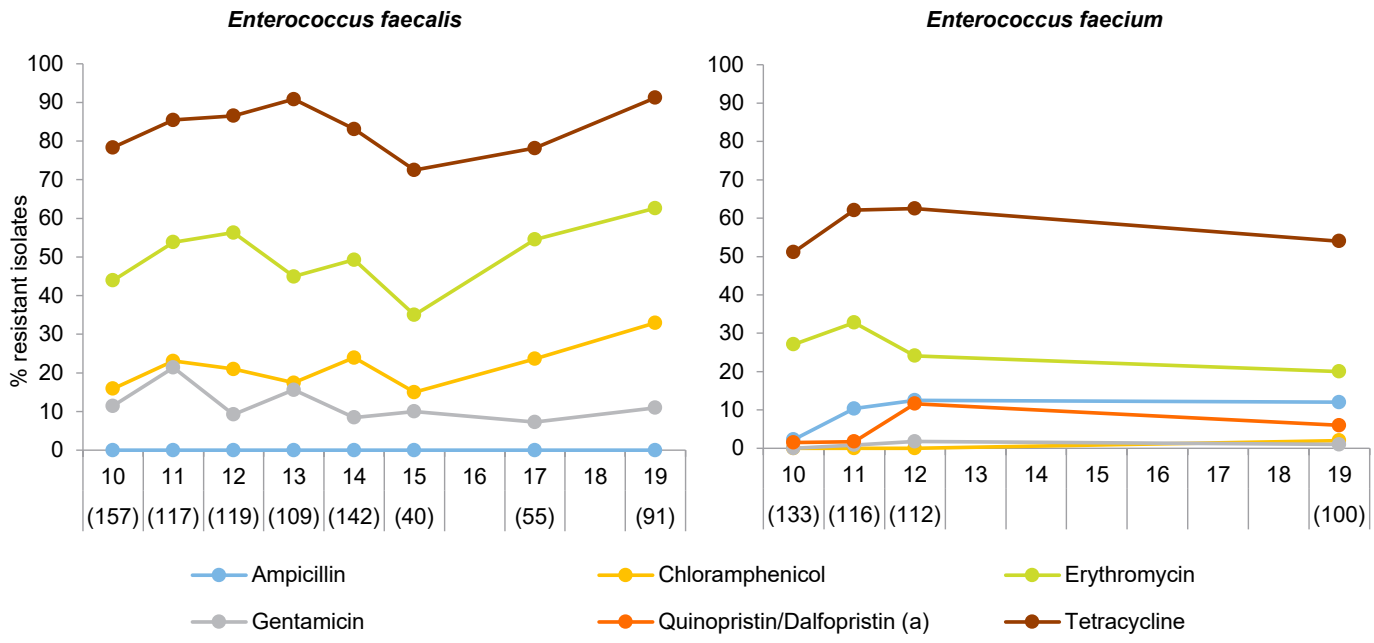
Compared to 2017, a moderate increase in chloramphenicol (9%), erythromycin (8%) and tetracycline (13%) resistance was observed whereas a very low increase in ciprofloxacin (2%), daptomycin (3%) and gentamicin (4%) resistance was detected among the *E. faecalis* isolates. In the *E. faecium* isolates, a moderate proportion of tetracycline (54%) and erythromycin (20%) resistance was found whereas low to very low proportions of ampicillin (12%), quinupristin/dalfopristin (6%), ciprofloxacin (5%), chloramphenicol (2%) and gentamicin (1%) was observed.

During the last 10-year period a statistically significant increase in chloramphenicol and erythromycin resistance have been observed in the *E. faecalis* isolates, and no ampicillin resistance has been found in this group of isolates during the last 10 years (Figure 7.4). Resistance levels in *E. faecium* were comparable to the observations from 2010-2012 (Figure 7.4).

Among the *E. faecalis* isolates, 10 resistance profiles were observed (see web annex Table A7.8). The most common profiles included resistance to i) tetracycline, ii) tetracycline, chloramphenicol and erythromycin and iii) tetracycline and erythromycin, and were observed among 29%, 25% and 21% of all *E. faecalis* isolates (n = 91), respectively.

Among the *E. faecium* isolates that showed resistance to one or more antimicrobials (n = 60), 11 resistance profiles were observed including isolates resistant to quinupristin/dalfopristin only and 15% of these isolates were resistant to three antimicrobials or more (web annex Table A7.8). The most common profiles included resistance to i) tetracycline, ii) tetracycline and erythromycin and iii) tetracycline and ampicillin, and were observed among 47%, 18% and 8% of the resistant *E. faecium* isolates, respectively.

Figure 7.4 Resistance (%) among *Enterococcus* isolates from pigs, Denmark DANMAP 2019



Note: Number of isolates included each year is presented in the parenthesis
a) *E. faecalis* are assumed intrinsically resistant to streptogramins and for *E. faecium* a cutoff of >4 was applied for quinopristin/dalfopristin (tradename Synercid) according to investigations presented in DANMAP 2006

7.4.2 Perspectives

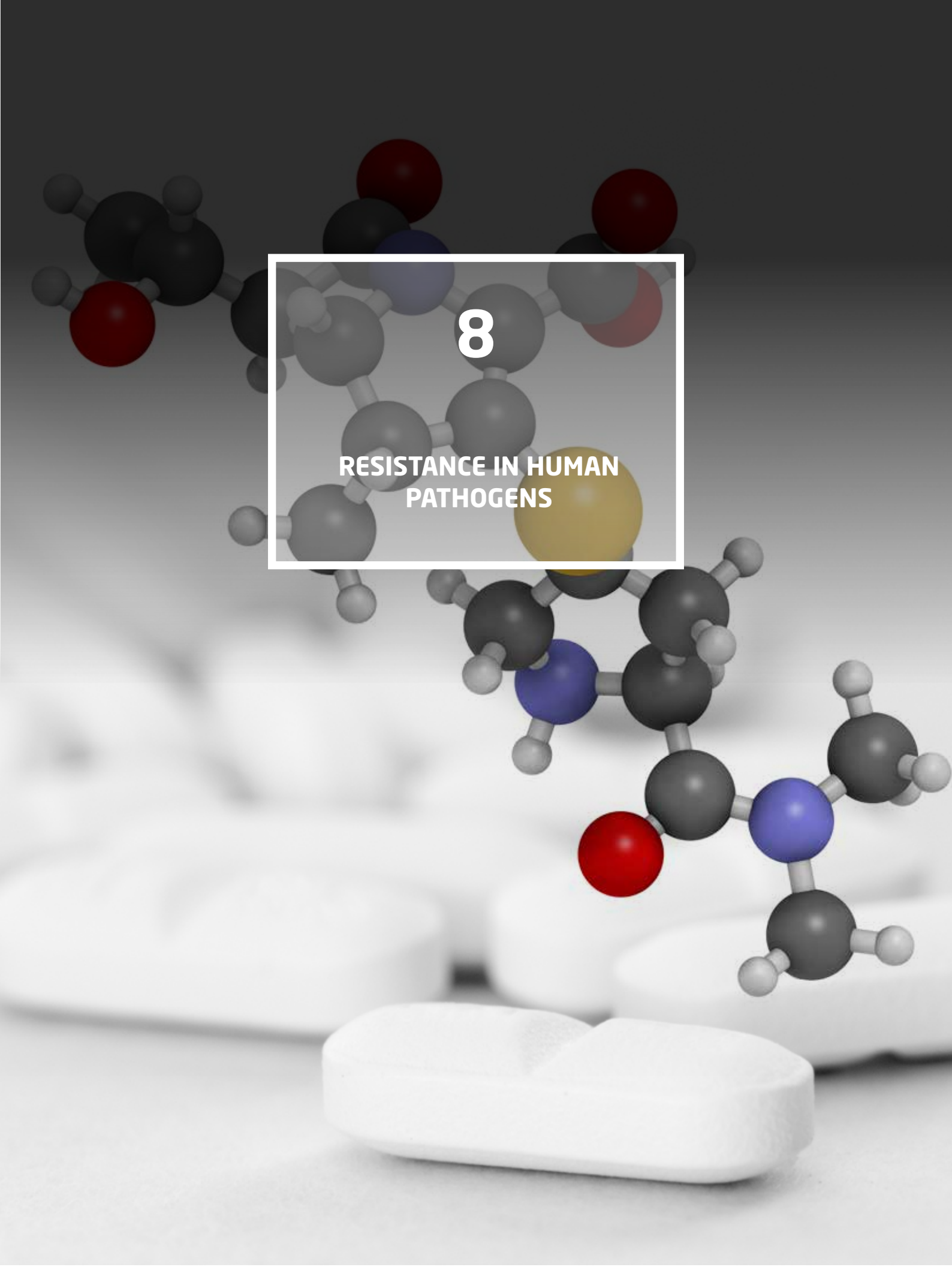
Enterococci are commensal bacteria in the intestine in both animals and humans, however, both *E. faecalis* and *E. faecium* can cause human disease. DANMAP 2019 showed that *E. faecium* or *E. faecalis* isolates from pigs exhibited no resistance to linezolid, teicoplanin, tigecycline and vancomycin (Table 7.4). These antimicrobials are critically important to human medicine and are considered last resort compounds to treat severe infections caused by Gram positive bacteria. During the last decade, an increase in the occurrence of vancomycin-resistant *E. faecium* causing human infections, has been reported in Denmark and very few vancomycin-resistant human *E. faecalis* isolates have also been detected [DANMAP 2019. Figure 8.11].

When comparing the resistance profiles for the human *E. faecium* isolates to the resistance profiles for the *E. faecium* pig isolates from 2019, some differences can be observed. In contrast to the human isolates, the pig isolates showed no linezolid, teicoplanin or vancomycin resistance and had a much lower proportion of ampicillin resistance (12%).

Comparing resistance profiles for the *E. faecalis* isolates a few more similarities can be found. Human isolates exhibited a low proportion of linezolid resistance in contrast to no linezolid resistance among pig isolates. None of the isolates showed teicoplanin, tigecycline or vancomycin resistance. During 2019, SSI reported that vancomycin resistant *E. faecium* isolates from human clinical samples primarily belonged to three types; ST1421-CT1134 *vanA E. faecium*, ST203-CT859 *vanA E. faecium* and ST117-CT36/CT991 *vanB E. faecium* (see section 8.3).

A zoonotic link has not been reported for the VRE from human clinical samples, but studies of VRE from food and fecal samples from animals using selective VRE media are needed in order to reduce the uncertainty of such link.

Rene S. Hendriksen, Troels Ronco and Helle Korsgaard
For further information: Helle Korsgaard, hkor@food.dtu.dk



8

RESISTANCE IN HUMAN PATHOGENS

8. Resistance in human pathogens

Highlights: In the observed decade the number of invasive cases has increased considerably for most species. Concomitantly the number of blood cultures taken each year has increased as well (section 8.1).

Blood infections with ***Staphylococcus aureus*** (subsection 8.3.8) have increased gradually by almost 60% from 1,400 cases in 2010 to 2,233 cases in 2019. In 2019, 2.1% of these were methicillin resistant (MRSA). The number of community acquired (CA-) MRSA continued to increase to 1,536 cases, whereas livestock-associated (LA-) MRSA CC398 cases decreased to 1,122, of which 253 cases presented with infection.

Resistance rates to cephalosporins in ***Escherichia coli*** (subsection 8.2.1) have increased gradually in the past decade primarily caused by the production of extended-spectrum beta-lactamases **(ESBLs)** (subsection 8.3.1). In 2019, the prevalence of the ESBL enzyme CTX-M-15 significantly decreased, whereas the prevalence of CTX-M-14 and CTX-M-55 increased in invasive *E. coli*, compared to 2018. In 2019, a new *E. coli* ST23 CTX-M-14 -producing clone was reported by seven of the ten DCMs, primarily from emergency departments.

In 2019 EUCAST introduced the Area of Technical Uncertainty (ATU) for areas/values in which there is uncertainty of susceptibility categorisation for specific combinations of species and agents. Piperacillin-tazobactam in Enterobacterales is an example and trends should be interpretated with caution. Nevertheless increases in piperacillin-tazobactam resistant *E. coli* and ***Klebsiella pneumoniae*** (subsection 8.2.2) in 2019 were observed.

The number of all types of clinical vancomycin resistant and vancomycin variable ***Enterococcus faecium*** **(VRE/VVE)** (subsection 8.3.3) has increased by 11% in Denmark between 2018 and 2019. For **invasive *E. faecium*** cases (subsection 8.2.5), the increase in the proportion of vancomycin-resistance in 2018 (12.1% compared to 7.1% in 2017) was followed by a minor decrease in 2019 to a proportion of 11%.

During 2019, 221 **carbapenemase-producing organisms (CPO)** (subsection 8.3.2) were detected compared with 177 CPO in 2018. Based on whole genome typing data, 16 carbapenemase-producing Enterobacterales outbreaks were registered with new patients in 2019. Seven of these outbreaks were trans-regional, covering patients from at least two different regions. Epidemiological investigations based on patient hospitalisation data and place of residence identified epidemiological links in nine of the 16 outbreaks. All links were identified in healthcare settings: Patients sharing the same ward or hospital.

The level of non-susceptibility to invasive ***Streptococcus pneumoniae*** (subsection 8.3.5) varies in line with the serotypes that are most prevalent in a given year. In total for penicillin in 2019, 95.1% were susceptible, 4.2% were classified as susceptible increased exposure and 0.7% were resistant. For erythromycin, 96.6% were susceptible and 3.4% were resistant.

A nationwide surveillance of azole resistance in ***Aspergillus fumigatus*** (Textbox 8.3) was initiated in October 2018. Data from the first 18 months showed an azole resistance rate of 6.1% with an overweight of resistance mechanisms associated with azole fungicide use in the environment.

8.1 Introduction

In Denmark all hospitals and general practitioners are serviced by 10 departments of clinical microbiology (DCMs) located at hospitals in the five regions of Denmark. The national surveillance of resistance in human clinical bacteria is based on either data from routine diagnostics performed at the 10 DCMs in Denmark or on resistance and typing results from isolates received at the reference laboratories at SSI for further characterisation. Isolates are received either based on a mutual agreement of voluntary submission of specific species and/or types of resistances or as part of a mandatory surveillance program of diseases made notifiable by the Danish Health Authority (Table 8.1).

Table 8.1 Summary of species/types, sampling and sources of isolates for national resistance surveillance in humans, 2019

DANMAP 2019

Routine diagnostics from all 10 DCMs in Denmark. All data are directly identified and extracted in MiBa	
Species	**Sampling**
Escherichia coli *Klebsiella pneumoniae*	First isolate per patient per year from blood or cerebrospinal fluid, from urine in hospitalised patients and from urine from primary healthcare
Pseudomonas aeruginosa *Acinetobacter species* *Enterococcus faecalis* *Enterococcus faecium*	First isolate per patient per year from blood or cerebrospinal fluid
Voluntary submissions of isolates to the refererance laboratories at SSI	
Species or type	**Sampling**
Staphylococcus areus	One isolate per patient per episode from blood or cerebrospinal fluid
Beta-haemolytic streptococci	One isolate per patient per episode from normally sterile sites
Neisseria gonorrhoeae	One isolate per patient per episode from all sample sites
3rd generation cephalosporin resistant *Escherichia coli*	First isolate per patient within 12 months from blood
Vancomycin-resistant enterococci	First isolate per patient within 12 months irrespective of sample site (excluding screening samples)
Enterococci with exceptional phenotype (e.g. linezolid, daptomycin and tigecycline R)	First isolate per patient within 12 months irrespective of sample site (clinical and screening samples)
All bacterial species with other exceptional phenotypes (e.g. acquired colistin resistance)	One isolate per patient per episode irrespective of sample site
Mandatory submissions of isolates to the reference laboratories at SSI	
Species or type	**Sampling**
Carbapenemase-producing organisms	First isolate per patient within 12 months irrespective of sample site (clinical and screening samples)
Methicillin-resistant *Staphylococcus aureus*	First isolate from all new cases of MRSA postive patients irrespective of sample site (clinical and screening samples)
Streptococcus pneumoniae	One isolate per patient per episode from blood or cerebrospinal fluid
Haemophilus influenzae serotype b, Hib	One isolate per patient per episode from normally sterile sites

Regarding submissions of isolates to the reference laboratories normally more isolates are received, but for the statistics one patient only counts once
For further specification of episodes/cases please see the specific subsections in chapter 9, materials and methods

8.1.1 Surveillance based on MiBa data

The surveillance of resistance in invasive isolates of *Escherichia coli, Klebsiella pneumoniae, Enterococcus faecalis, Enterococcus faecium, Pseudomonas aeruginosa* and *Acinetobacter* species and urine isolates of *E. coli* and *K. pneumoniae* is based on data from routine diagnostics from the DCMs in Denmark. Surveillance has been performed since 1995 - in the very beginning based on reporting from two DCMs, but quickly joined and supported by most DCMs in Denmark. From 2010 to 2014, DANMAP received data from all but one DCM resulting in a coverage of approx. 95% of the population. Since 2015, all DCMs participate in the program resulting in a 100% population coverage. Since 2018, all these data were extracted directly from the Danish Microbiology Database (MiBa) [https://miba.ssi.dk/Service/English.aspx]. A description of MiBa and the usage and validation of MiBa-data is given in (DANMAP 2018, Textbox 8.1). Due to the high quality of data in MiBa, this register-based surveillance provides the most accurate prevalence estimates in Denmark. Materials and methods are described in chapter 9.

8.1.2 Surveillance based on data from the reference laboratories

Another surveillance component is based on submission of specified isolates to the reference laboratories at SSI. Isolate-based surveillance gives the opportunity to further characterise isolates and resistance mechanisms and type the isolates; since 2015-2016, this has been mainly performed by the use of whole genome sequencing (WGS). Voluntary submission of specified isolates has existed since 1957; beginning with the

submission of all isolates of *Staphylococcus aureus* from bloodstream infections. The submission of invasive beta-haemolytic streptococci is also voluntary, while invasive *Streptococcus pneumoniae* and *Haemophilus influenzae* serotype b (Hib) are mandatory to submit. The detection of methicillin-resistant *S. aureus* (MRSA) and *Neisseria gonorrhoeae* from all clinical sites is notifiable but the submission of MRSA isolates is mandatory, while the submission of *N. gonorrhoeae* isolates is voluntary. In addition, the DCMs voluntarily submit isolates of ESBL-producing *E. coli* from blood and vancomycin-resistant enterococci (VRE) from all clinical sites, based on a mutual agreement to survey the development and spread of these often multi-resistant bacteria at Danish hospitals. The Danish Health Authority made carbapenemase-producing organisms (CPO) notifiable, and submission of all clinical and screening isolates irrespective of sample site has been mandatory as of 5th September 2018. Before that, CPO was submitted on a voluntary basis.

8.1.3 Surveillance of invasive cases

A key function in the monitoring of antimicrobial resistance for DANMAP is to surveil the number of resistant bacteria in invasive cases (blood and cerebrospinal fluid). This is harmonised with the monitoring performed by the European Antimicrobial Resistance Surveillance Network (EARS-Net). Figure 8.1 presents the total numbers of invasive cases in Denmark from 2010 to 2019 for the bacterial species included in the surveillance programs for both DANMAP and EARS-Net. Excluded from the figure is *Acinetobacter* species - these have been registered since 2012 in DANMAP and the number of cases are low (55 to 72 cases annually). For all registered species, the following case definitions applies: The first sample, by date of sample collection, of each given bacterial species per unique patient per year of observation are included. Duplicates, within the year of observation, from the same patient are excluded.

Since 2010, the total number of registered invasive cases increased by 46% (from 8,021 to 11,712 cases). The largest increase observed was for *K. pneumoniae* (70%). The only species with an overall decreasing number of cases was *S. pneumoniae* (-33%).

Figure 8.2a shows the number of invasive cases per 100,000 inhabitants in Denmark per year for 2010 to 2019. During this period, the Danish population increased by 4.9% (from 5,534,738 inhabitants in 2010 to 5,806,081 inhabitants in 2019). Figure 8.2b shows the total number of blood cultures taken per 100,000 inhabitants per year for the same period. In addition, the number of unique patients being subjected to blood culture per 100,000 inhabitants per year is shown. In the ten-year period the number of unique patients with at least one blood culture taken per year has increased from 2,059 patients per 100,000 inhabitants in 2010 to 2,914 patients per 100,000 inhabitants in 2019 (an increase of 42%). The total number of blood cultures taken (as registered with a unique sample ID in MiBa) per 100,000 inhabitants has increased even more (63%). Thus, on average more patients have more blood cultures taken each year. 2019 was the first year in the observed decade where less unique patients were blood cultured compared to the previous year but the total number of blood cultures taken increased as previously.

Figure 8.1 Number of submitted invasive isolates for each species under surveillance DANMAP 2019

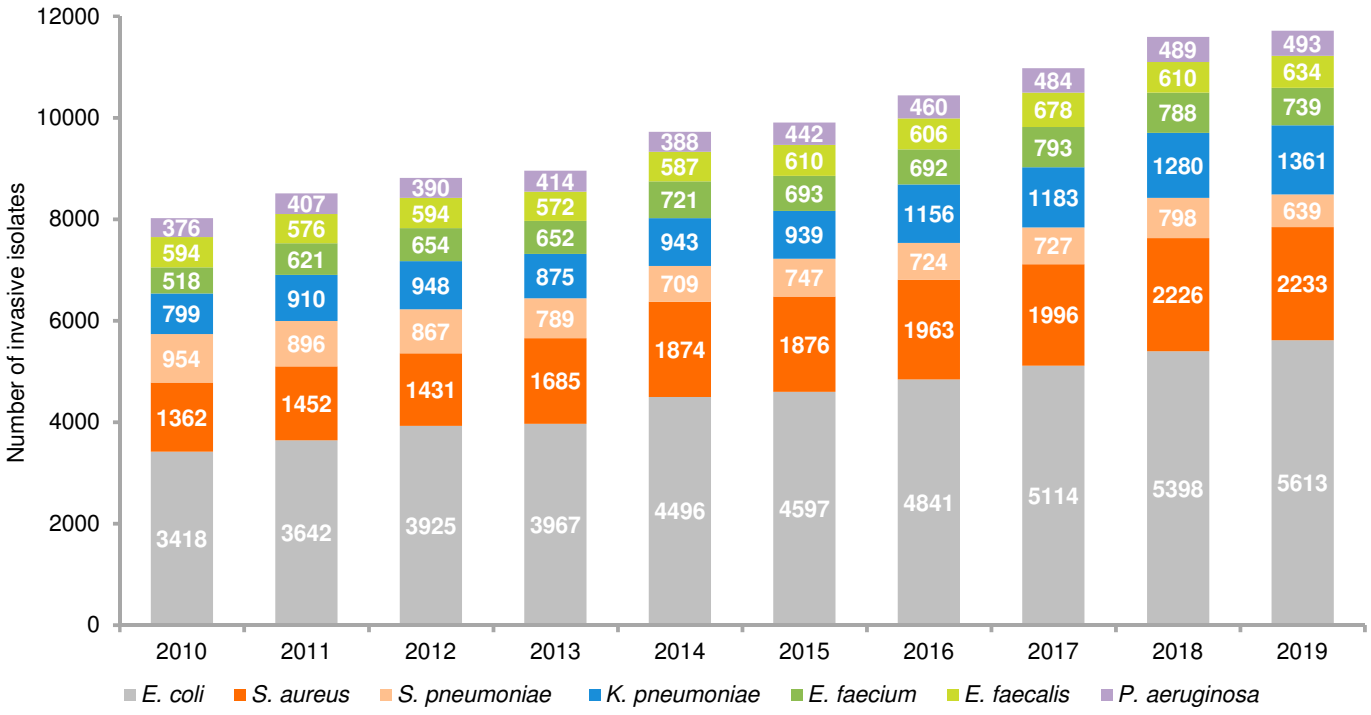
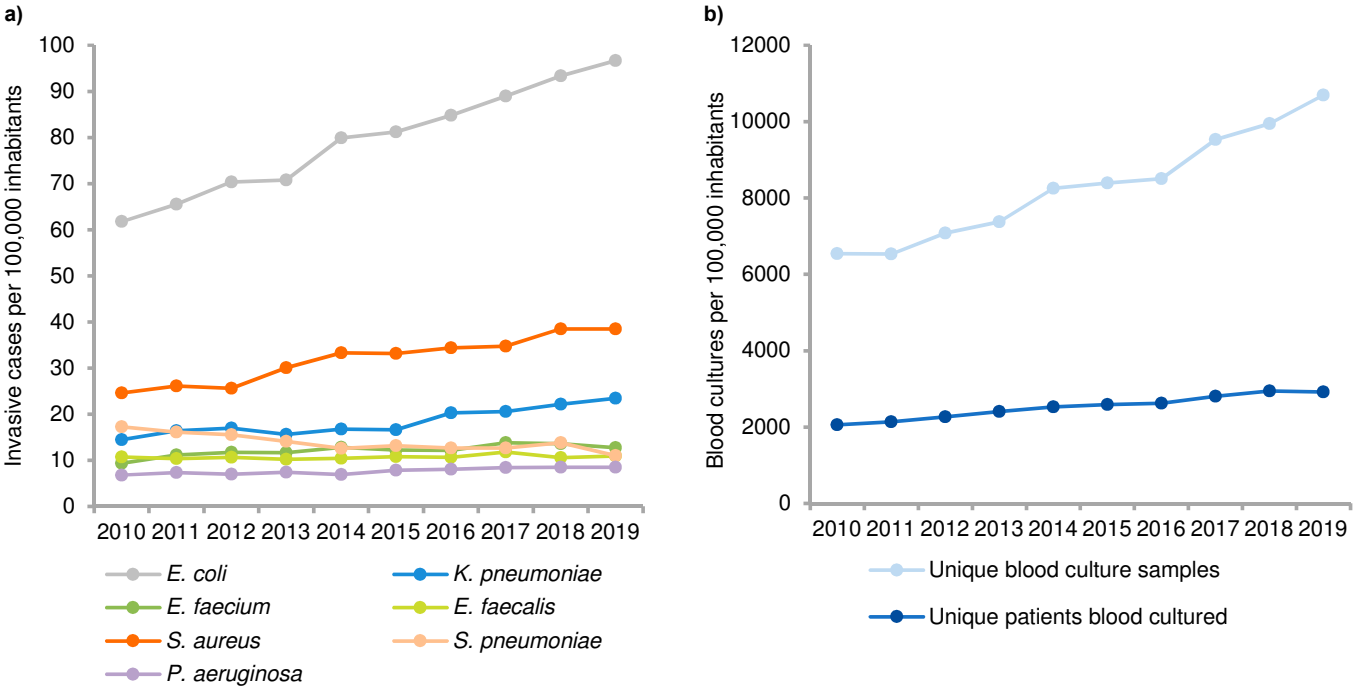


Figure 8.2 Invasive cases and blood cultures taken per 100,000 inhabitants DANMAP 2019



Changes in hospital workflow, improved culturing methods and demographic changes with a growing population of elderly and of chronically ill or immunocompromised patients may explain some of the observed changes. The increasing number of invasive infections is of concern. It demands fast and effective antimicrobial treatment, while simultaneously increasing the risk for the development and selection of resistant bacteria due to a higher consumption of antimicrobials. These resistant bacteria can then be spread in hospital environments with fragile patient populations underlining the need for a health care system with firmly established infection prevention and control. The importance of proper diagnostics combined with a rational use of antimicrobials, reserving the most broad-spectrum antimicrobial classes to the patients with multi-resistant infections is underlined as well.

The following sections in this chapter presents the individual results for the species and/or resistance types under surveillance.

8.2 Surveillance based on MiBa data

8.2.1 *Escherichia coli*

Escherichia coli is by far the most frequent cause of community- and hospital acquired urinary tract infections and of bacteraemia in Denmark accounting for approximately 55%, 45% and 21%, respectively, of all registered positive cultures in MiBa (2018 data). It is part of the normal intestinal flora in both animals and humans, where it comes into close proximity to many other bacterial species. Transferable resistance mechanisms are frequently seen in between these gut bacteria as well as the development of resistance through mutations. Since *E. coli* is the most frequent cause of urinary tract infections and bacteraemia, it is also one of the biggest drivers of antimicrobial use.

Invasive cases from hospital patients

For 2019, a total of 5,613 unique patients with invasive *E. coli* isolates from all departments of clinical microbiology (DCMs) in Denmark, were identified in MiBa. All 10 DCMs routinely (>75% of isolates) registered antimicrobial susceptibility interpretations for ampicillin, ciprofloxacin, piperacillin-tazobactam, gentamicin, cefuroxime, mecillinam and carbapenem in MiBa. In addition, nine DCMs routinely registered antimicrobial susceptibility for 3rd generation cephalosporins and seven routinely registered antimicrobial susceptibility for amoxicillin-clavulanic acid. Tested 3rd generation cephalosporins were ceftazidime, except for two DCMs where it was ceftriaxone and cefpodoxime. The tested carbapenem was meropenem for all DCMs in 2019. Antimicrobial susceptibility testing was mainly performed by disc diffusion. The presented data consist of the registered interpretation results, performed by the DCMs, based on the S-I-R system. Zone diameters have also been registered since 2015 and will be commented upon in specific cases.

Resistance rates for all tested antimicrobials, presented as a national mean for each antimicrobial class, are summarized in Table 8.2. In Figure 8.3 total numbers of invasive isolates and rates of resistance are shown for the past decade. Time trends and significance levels, based on the resistance rates five and ten years back respectively, are presented in Figure 8.3c. Test results for mecillinam resistance in invasive *E. coli* are excluded from Figure 8.3, since the S-I-R interpretation rules for the individual DCM differ and/or have varied over time, making comparison of the results difficult and time trends unreliable. Also amoxicillin-clavulanic acid test results are excluded from Figure 8.3 for which reason resistance rates are commented on later in this section.

Table 8.2 *Escherichia coli*. Resistance (%) in isolates from humans, 2019 DANMAP 2019

	Invasive isolates, hospitals %	Urine isolates, hospitals %	Urine isolates, primary health care %
Ampicillin	46	44	38
Mecillinam	14	8.1	5.3
Piperacillin-tazobactam	5.5	4.4	6.5*
Amoxicillin-clavulanic acid	26	14	6.5*
Sulfonamide		31*	28
Trimethoprim		25	23
Nitrofuratoin		1.2*	1.1
Gentamicin	5.3	4.8	3.9*
Ciprofloxacin	11	11	8.0
Cefuroxime	10	7.8	5.7*
3rd generation cephalosporins	6.9	6.9	5.2
Carbapenem	0.1	0.0	0.0*

The presented resistance rates are means of the resistance rates determined by the individual DCMs. Included are results from all DCMs where >75% of the isolates in each antibiotic/sample group were susceptibility tested. The * marks where less than 6 (out of totally 10 DCM) tested a sufficient percentage of their samples. For carbapenems, piperacillin/tazobactam and gentamicin in urines from primary health care, only one DCM tested >75% of their isolates

A continuous increase in the number of invasive *E. coli* cases was observed throughout the years, from 3,426 cases in 2010 to 5,613 cases in 2019. This corresponds to 61.8 cases and 96.7 cases per 100,000 inhabitants respectively (a 57% increase). Simultaneously, the total number of blood cultures performed also increased steeply with 63% per 100.000 inhabitants (commented on in subsection 8.1.3).

For cefuroxime in invasive *E. coli* a significant increase in resistance was observed for the past decade as well as for the past five years. A minor EUCAST breakpoint change in 2017 influenced 2017 data compared to 2016, but there has not been any breakpoint change since then. The same increase was not observed for 3rd generation cephalosporins. The increased ciprofloxacin resistance rate in 2017 compared to 2016 mainly reflected a relatively large change in EUCAST breakpoints in 2017. Ciprofloxacin breakpoints changed (lowered) once again combined with the introduction of the Area of Technical Uncertainty (ATU) in the EUCAST clinical breakpoint Table v. 9.0 in 2019. Therefore curves for ciprofloxacin resistance should be interpreted with caution and time trend calculations are not conducted. For piperacillin-tazobactam the interpretation should be taken with caution as well, even though there have been no EUCAST breakpoint changes since 2012. Several local rules regarding downgrading piperacillin-tazobactam from I to R or S to R has existed during the years and also technical problems with the testing has been a recurring issue. Furthermore, in 2019 an ATU, for piperacillin-tazobactam was introduced. With a calculation based only on zone diameters 2.5% of the isolates were resistant (<17 mm) in 2018 compared to 3.1% in 2019, indicating a "true" increase in resistance towards piperacillin-tazobactam (Figure A8.1 in web annex). Gentamicin resistance rates have declined in the decade. For more details see Figure 8.3.

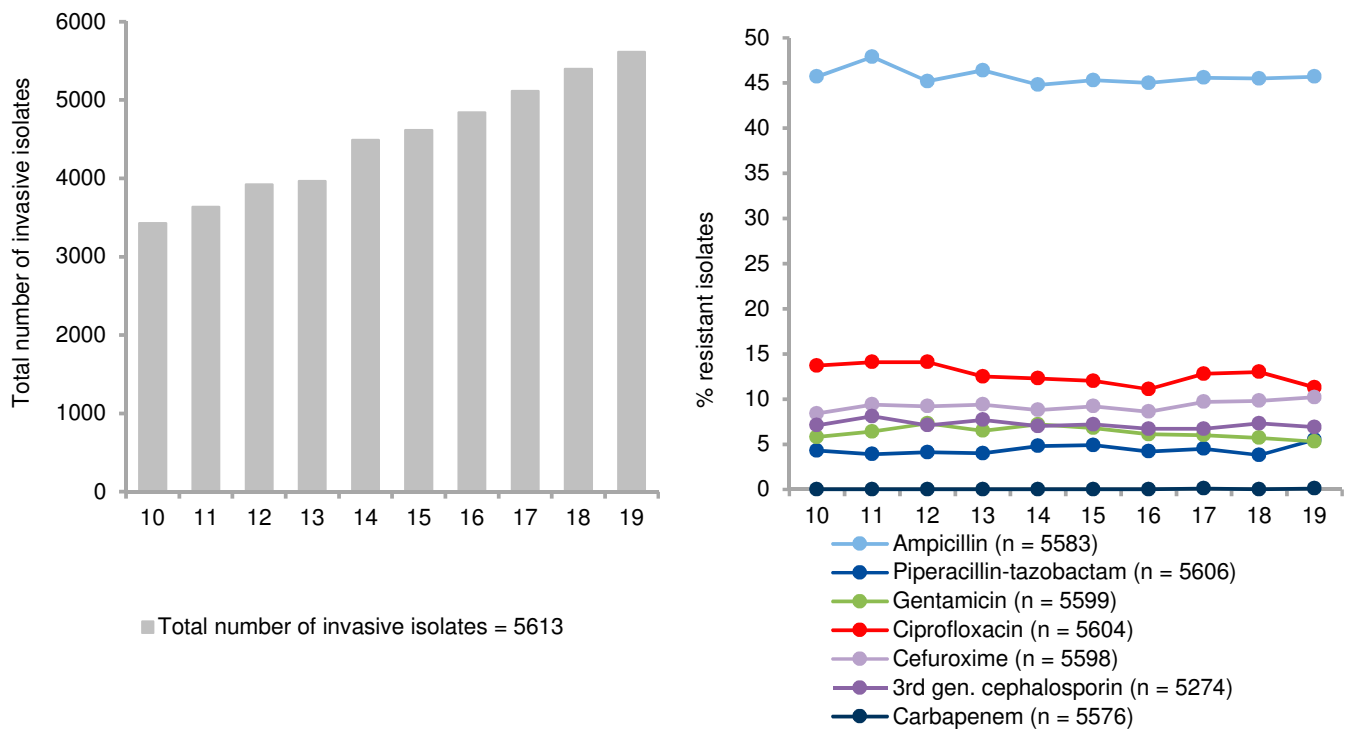
Reporting of amoxicillin-clavulanic acid resistance rates needs special concerns. There are two different breakpoints defined; one more restrictive breakpoint and one less restrictive only to be used in uncomplicated UTI, and there is an ATU that was introduced by EUCAST in 2019. According to the reported S-I-R interpretations, an increase from 19% to 26% resistant cases from 2018 to 2019 was noticed. Looking into the reported zone diameters though, no such change was observed (Figure A8.2 in web annex).

The defined clinical breakpoint by EUCAST for amoxicillin-clavulanic acid is S≥19 mm, R<19 mm, with an ATU of 19-20 mm, and the breakpoint to be used only in uncomplicated UTI is S≥16 mm, R<16 mm (EUCAST v.9.0). Evaluation of the zone distributions reveals changes from 2018 to 2019 only above 18 mm with less isolates in the ATU (19-20 mm) in 2019 compared to 2018, and instead more isolates with reported zones of 21 mm. The isolates for which the results fall in the ATU range, EUCAST suggests the following alternatives depending on the situation: Repeat the test, use an alternative test, downgrade the susceptibility category or include the uncertainty as part of the report. Some DCMs simply do not report any result on amoxicillin-clavulanic acid to the clinicians and only handle it if needed. If all reported zones are interpreted with the same breakpoint of 19 mm, the resistance rates in 2018 and 2019 are 31% and 30% respectively. Here we report interpretations as reported to MiBa by the DCMs in Table 8.2.

The number of carbapenem resistant *E. coli* isolates remained continuously very low with three carbapenem resistant isolates and two additional isolates which were susceptible increased exposure in the invasive cases in 2019. The level of multiresistant (combined resistance to 3rd generation cephalosporins, ciprofloxacin and gentamicin) invasive *E. coli* remained

at around 2%, Table 8.3. For colistin, four of the invasive *E. coli* were registered resistant, however colistin resistance is not tested routinely and in 2019 colistin susceptibility testing was reported for 521 of the isolates, constituting 9.3% of the invasive cases.

Figure 8.3 *Escherichia coli*. Resistance (%) in invasive isolates from humans DANMAP 2019



c)

Substance	Percent resistant invasive *E. coli* isolates										Time trends (Cochran-Armitage test)	
	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	10 years (2010-2019)	5 years (2015-2019)
Ampicillin	45.7	47.9	45.2	46.4	44.8	45.3	45	45.6	45.5	45.7	↓ p = 0.114	↑ p = 0.277
Piperacillin-tazobactam	4.3	3.9	4.1	4	4.8	4.9	4.2	4.5	3.8	5.5	NA	NA
Gentamicin	5.8	6.4	7.3	6.5	7.2	6.8	6.1	6	5.7	5.3	↓ p = 0.001	↓ p = 0.001
Ciprofloxacin	13.7	14.1	14.1	12.5	12.3	12	11.1	12.8	13	11.3	NA	NA
Cefuroxime	8.4	9.4	9.2	9.4	8.8	9.2	8.6	9.7	9.8	10.2	↑ p = 0.005	↑ p = 0.007
3rd gen.cephalosporins	7.1	8.1	7.1	7.7	7	7.2	6.7	6.7	7.3	6.9	↓ p = 0.01	↑ p = 0.459
Carbapenem	0	0	0	0	0	0	0	0.1	0	0.1	↑ p = 0.046	↑ p = 0.303
Total number of isolates	3426	3636	3922	3967	4492	4618	4841	5114	5398	5613		

a) Total numbers of invasive isolates per year. b) Resistance rates among tested isolates c) Resistance rates and time trends and significance levels for the past five and 10 years, respectively (Cochran-Armitage test)
The number (n) in parentheses represents the numbers of isolates tested in 2019. NA = Not applicable

Table 8.3 *Escherichia coli*. Combined resistance to 3rd generation cephalosporins, ciprofloxacin, and gentamicin (multiresistance) in invasive isolates from humans, Denmark DANMAP 2019

	2014 % (N)	2015 % (N)	2016 % (N)	2017 % (N)	2018 % (N)	2019 % (N)
Resistance	1.8 (72)	2.3 (93)	1.8 (87)	1.8 (88)	2.0 (100)	1.8 (93)
Percentage (no.) of isolates tested for combined resistance (multiresistance)	90 (4039)	88 (4071)	98 (4763)	95 (4883)	93 (4997)	94 (5259)
Total number of invasive isolates	4495	4614	4841	5114	5398	5613

Urinary cases from hospitals

For 2019, a total of 47,235 unique patients with *E. coli* isolates, cultured in urine samples from hospital patients from all DCMs in Denmark, were identified in MiBa. A total of 2,090 unique patients with *E. coli* isolates, cultured in urine samples were excluded because it was not possible to categorise them as hospital or primary care urine samples.

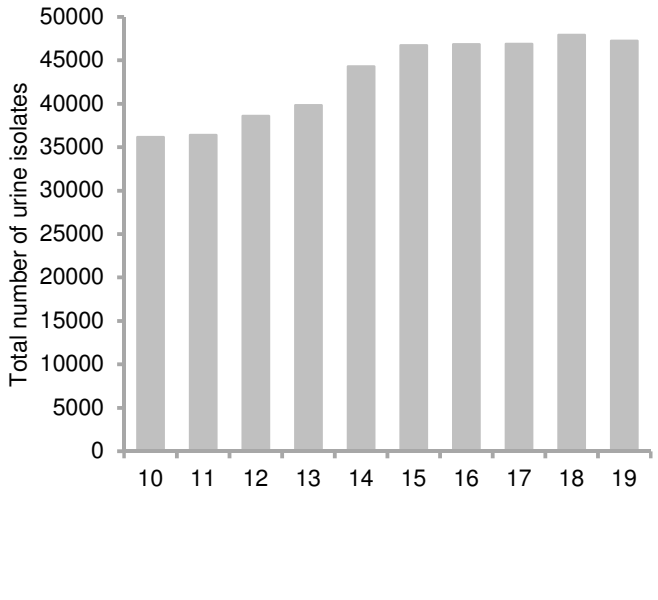
All 10 DCMs routinely (>75% of isolates) registered antimicrobial susceptibility interpretations for ampicillin, mecillinam, piperacillin/tazobactam and ciprofloxacin in MiBa. In addition nine DCMs routinely registered antimicrobial susceptibilities for gentamicin and eight DCMs for cefuroxime and trimethoprim.

Six DCMs routinely registered antimicrobial susceptibilities for amoxicillin-clavulanic acid, 3rd generation cephalosporins and carbapenem and five DCMs for nitrofurantoin. Three DCMs routinely registered antimicrobial susceptibilities for sulphonamide.

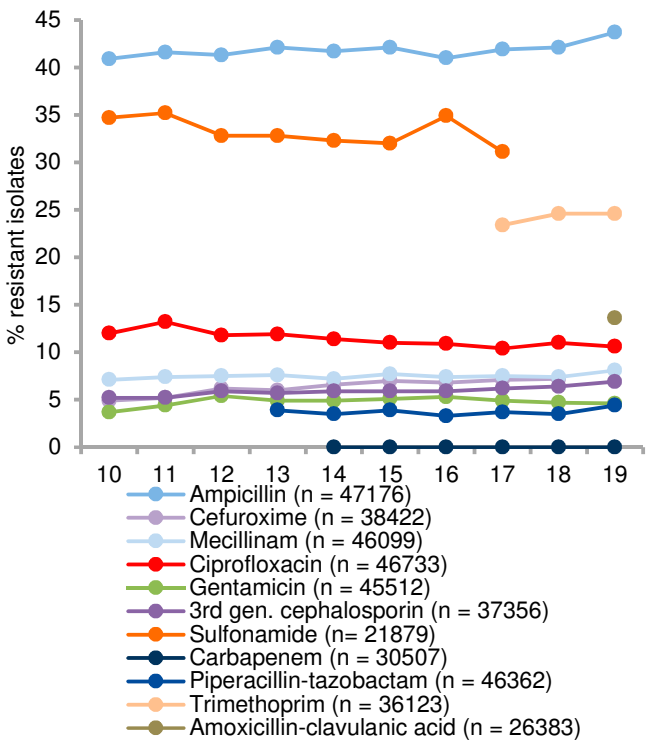
Susceptibility results for all tested antimicrobials are summarized together with the results from the invasive isolates as a national mean for each antimicrobial class in Table 8.2. In Figure 8.4, rates of resistance are plotted for the past decade. Time trends and significance levels, based on the resistance rates for the past five and 10 years respectively, are presented in Figure 8.4c.

Figure 8.4 *Escherichia coli*. Resistance (%) in urine isolates from humans in hospitals DANMAP 2019

a)



b)



c)

Substance	Percent resistant *E. coli* isolates from hospital urine										Time trends (Cochran-Armitage test)	
	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	10 years (2010-2019)	5 years (2015-2019)
Ampicillin	40.9	41.6	41.3	42.1	41.7	42.1	41	41.9	42.1	43.7	↑ p <0.001	↑ p <0.001
Mecillinam	7.1	7.4	7.5	7.6	7.2	7.7	7.4	7.5	7.4	8.1	↑ p <0.001	↑ p = 0.016
Piperacillin-tazobactam				3.9	3.5	3.9	3.3	3.7	3.5	4.4	NA	NA
Sulfonamide	34.7	35.2	32.8	32.8*	32.3*	32.0	34.9	31.1	31.0*	31.4*	NA	NA
Gentamicin	3.7	4.4	5.4	4.9	4.9	5.1	5.3	4.9	4.7	4.6	↓ p <0.001	↓ p = 0.004
Ciprofloxacin	12	13.2	11.8	11.9	11.4	11	10.9	10.4	11	10.6	NA	NA
Cefuroxime	4.9	5.2	6.2	6	6.6	7	6.8	7.1	7.2	7.8	↑ p <0.001	↑ p <0.001
3rd gen. cephalosporins	5.2	5.2	5.9	5.7	5.9	5.9	5.9	6.2	6.4	6.9	↑ p <0.001	↑ p <0.001
Carbapenem					0	0	0	0	0	0	NA	↑ p = 0.004
Total number of isolates	36149	36389	38610	39820	44288	46723	46865	46884	47914	47235		

a) Total numbers of urine isolates per year, b) Resistance rates among tested isolates, c) Resistance rates and time trends and significance levels for the past five and 10 years, respectively (Cochran-Armitage test)
The number (n) in parentheses represents the numbers of isolates tested in 2019. NA = Not applicable
*Indicates that less than 6 DCMs reported rutine testing

For ampicillin, mecillinam, cefuroxime and 3rd generation cephalosporins there are slow but significant increases in resistance for the past decade as well as for the past five years. For more details see Figure 8.4.

In 2019 there was not noted any increase in carbapenem resistance with 19 carbapenem resistant and 16 susceptible increased exposure *E. coli* urine isolates from hospital patients registered, compared to 24 and 20 in 2018.

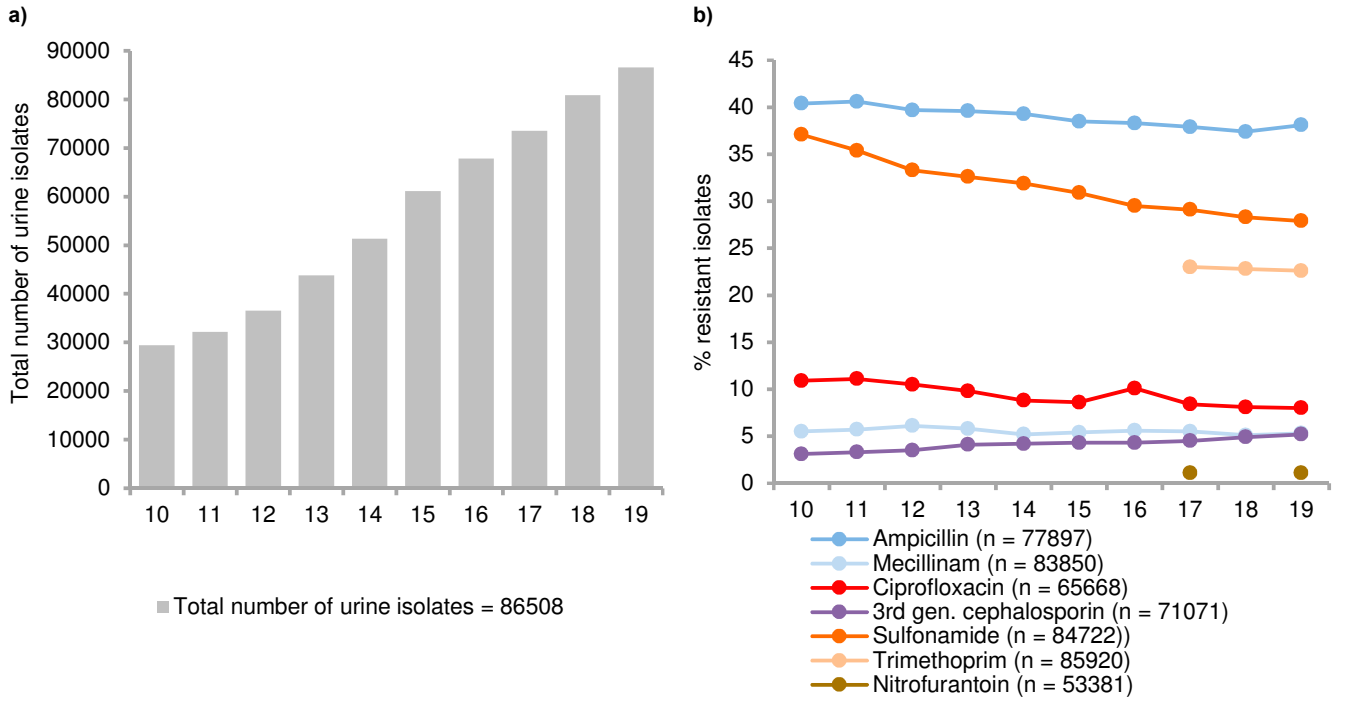
Urinary cases from primary health care

For 2019, a total of 86,508 unique patients with *E. coli* isolates, cultured in urine samples from primary health care (PHC), from nine DCMs in Denmark, were identified in MiBa. The general practitioners (GPs) in Denmark are all serviced by those nine DCMs except for a few GPs in the Capital Region of Denmark whom are serviced by one private laboratory.

All nine DCMs routinely (>75% of isolates) registered antimicrobial susceptibility interpretations for mecillinam, sulphonamide and trimethoprim in MiBa. In addition eight DCMs routinely registered antimicrobial susceptibilities for ampicillin, six DCMs for 3rd generation cephalosporins, ciprofloxacin and nitrofurantoin and four DCMs for amoxicillin-clavulanic acid. Three DCMs routinely registered antimicrobial susceptibilities for cefuroxime and just one DCM routinely registered antimicrobial susceptibilities for piperacillin-tazobactam, gentamicin and carbapenem.

As for the results from invasive isolates and isolates from hospital urines, susceptibility results for all tested antimicrobials are shown as national means in Table 8.2. In Figure 8.5, rates of resistance are plotted for the past decade. Time trends and significance levels, based on the resistance rates for the past five and 10 years respectively, are presented in Figure 8.5c.

Figure 8.5 *Escherichia coli*. Resistance (%) in urine isolates from humans in primary health care DANMAP 2019



c)

Substance	Percent resistant *E. coli* isolates from PHC urine										Time trends (Cochran-Armitage test)	
	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	10 years (2010-2019)	5 years (2015-2019)
Ampicillin	40.4	40.6	39.7	39.6	39.3	38.5	38.3	37.9	37.4	38.1	↓ p <0.001	↓ p = 0.007
Mecillinam	5.5	5.7	6.1	5.8	5.2	5.4	5.6	5.5	5.1	5.3	↓ p = 0.001	↓ p = 0.01
Sulfonamide	37.1	35.4	33.3	32.6	31.9	30.9	29.5	29.1	28.3	27.9	↓ p <0.001	↓ p <0.001
Ciprofloxacin	10.9	11.1	10.5	9.8	8.8	8.6	10.1	8.4	8.1	8	NA	NA
3rd gen. cephalosporins	3.1	3.3	3.5	4.1	4.2	4.3	4.3	4.5	4.9	5.2	↑ p <0.001	↑ p <0.001
Total number of isolates	29421	32117	36475	43770	51272	61083	67798	73497	80851	86508		

a) Total numbers of urine isolates per year, b) Resistance rates among tested isolates, c) Resistance rates and time trends and significance levels for the past five and 10 years, respectively (Cochran-Armitage test)
The number (n) in parentheses represents the numbers of isolates tested in 2019. NA = Not applicable

A continuing slow increase in resistance to 3rd generation cephalosporins, a slow decline in mecillinam resistance and a more pronounced decline in sulphonamide resistance were observed. All changes are significant calculating both 10 and 5 years back. For more details see Figure 8.5.

In 2019, six carbapenem resistant and nine susceptible increased exposure *E. coli* isolates from PHC urinary cases were registered. As noted, registration of carbapenem susceptibility results in urine samples from PHC is only routinely done in one of nine DCMs. However, since carbapenem resistant isolates are often multiresistant, most DCMs recognise them and perform additional testing.

Conclusion

A substantial increase in the total number of invasive and of primary health care urinary *E. coli* cases were observed since 2010. Throughout the same time period, a corresponding increase in the total numbers of blood cultures taken and urinary samples registered from the primary sector occurred. The number of urinary *E. coli* cases from hospitals showed less increase as did the total number of urine samples registered from hospitals. It could be that at least part of the increase in the number of *E. coli* cases was due to an increased number of cultures taken.

A worrisome trend for all three categories of *E. coli* isolates were an increase in cephalosporin (cefuroxime and/or 3rd generation cephalosporins) resistance rates both for the past decade, and for the past five years. One exception was the resistance to 3rd generation cephalosporins in invasive *E. coli*, which showed no increase in resistance rates. In Europe an increase in resistance to 3rd generation cephalosporins in invasive *E. coli* was observed (EU/EEA population-weighted mean 15.1%) [EARS-Net annual report, 2018]. Resistance to 3rd generation cephalosporins often occur through production of extended-spectrum beta-lactamases (ESBLs) (for more information see section 8.3). When the population of ESBL-positive *E. coli* isolates increases, this might lead to increased use of broader spectrum antimicrobials and thereby a vicious circle selecting for even more resistance can run. Whenever possible, a downscaling to narrow spectrum antimicrobials is recommended.

Resistance to carbapenem in invasive and urinary *E. coli* isolates is still contained at an acceptable level. Besides antimicrobial stewardship many initiatives, including active surveillance and infection control programs are effectuated each day to keep it this way. In 2019 a new national surveillance database with registration of all verified and possible outbreaks with carbapenemase-producing Enterobacterales (CPE) in health care settings was established (for further information, please see section 8.3.2).

Sissel Skovgaard, Jonas Kähler and Anna E. Henius
For further information: Sissel Skovgaard, sisk@ssi.dk

8.2.2 *Klebsiella pneumoniae*

Klebsiella pneumoniae is capable of colonising the gastrointestinal and respiratory tract in humans, especially in hospitalised patients. It may cause infections such as urinary tract infections, severe pneumonia and blood stream infections – the latter especially in patients with indwelling devices - and may give rise to nosocomial outbreaks. *K. pneumoniae* frequently displays plasmid-mediated resistance mechanisms which are transferrable and may be transferred to other organisms.

Invasive cases from hospitals

For 2019, a total of 1,361 unique patients with invasive *K. pneumoniae* isolates from all departments of clinical microbiology (DCMs) in Denmark, were identified in MiBa. All 10 DCMs routinely (>75% of isolates) registered antimicrobial susceptibility interpretations for ciprofloxacin, piperacillin-tazobactam, gentamicin, cefuroxime and meropenem in MiBa. In addition, nine DCMs routinely registered antimicrobial susceptibilities for mecillinam and 3rd generation cephalosporins and seven routinely registered antimicrobial susceptibilities for amoxicillin-clavulanic acid. Tested 3rd generation cephalosporins were ceftazidime, except for two DCMs where it was ceftriaxone and cefpodoxime. The tested carbapenem was meropenem for all DCM in 2019. Antimicrobial susceptibility testing was mainly performed by disc diffusion. The presented data consist of the registered interpretation results, performed by the DCMs, based on the S-I-R system. Zone diameters have also been registered since 2015 and will be commented upon in specific cases.

Resistance rates for 2019 for all tested antimicrobials, presented as a national mean for each antimicrobial class, are summarised in Table 8.4. In Figure 8.6 rates of resistance are shown for the past decade. Time trends and significance levels, based on the resistance rates for the past five and ten years respectively, are presented in Figure 8.6c. Test results for mecillinam resistance in invasive *K. pneumoniae* are excluded from Figure 8.6, since the S-I-R interpretation rules for the individual DCM differ and/or have varied over time, making comparison of the results difficult and time trends unreliable.

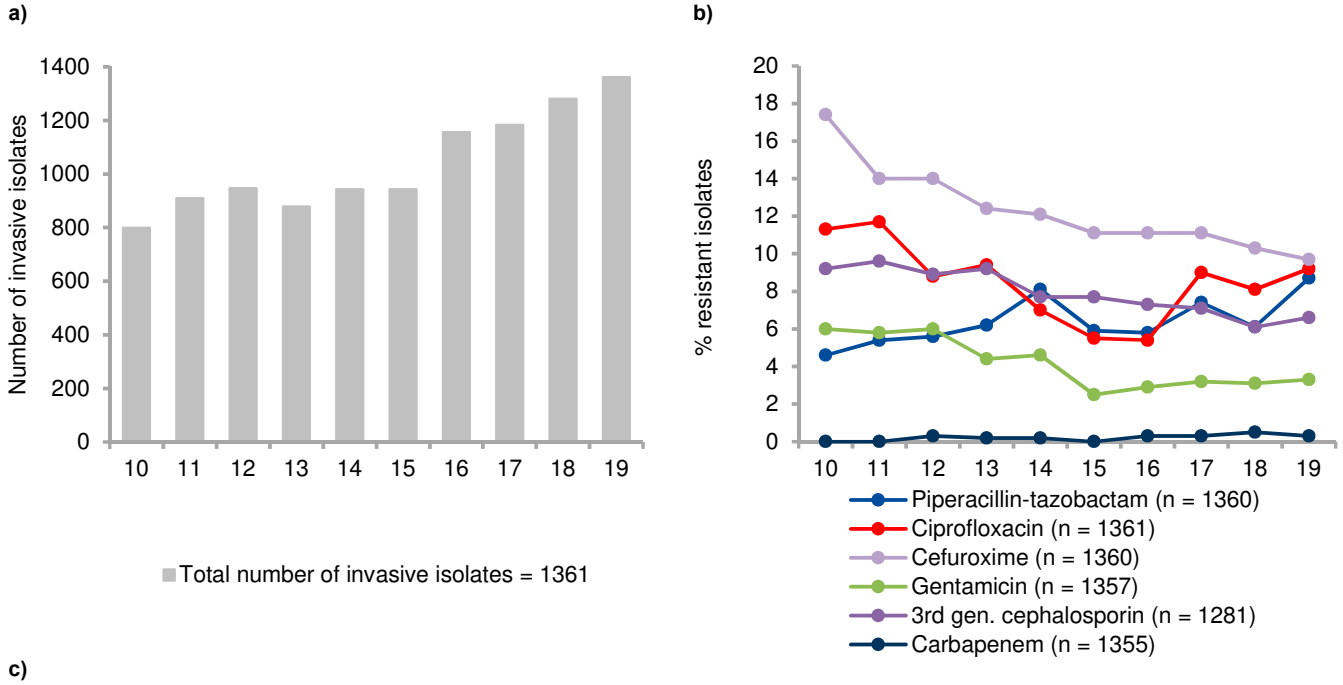
As for invasive *E. coli* cases, a continuous increase in the number of invasive *K. pneumoniae* cases was observed throughout the years, from 799 cases in 2010 to 1,361 cases in 2019. This corresponds to 14.4 cases and 23.4 cases per 100,000 inhabitants respectively (a 62% increase). Simultaneously, the total number of blood cultures taken also increased steeply with 63% per 100.000 inhabitants (commented on in subsection 8.1.3).

Table 8.4 *Klebsiella pneumoniae*. Resistance (%) in isolates from humans, 2019 DANMAP 2019

Substance	Invasive isolates, hospitals %	Urine isolates, hospitals %	Urine isolates, primary healthcare %
Mecillinam	14	13	11
Piperacillin/tazobactam	8.7	8.5	9.4*
Amoxicillin/clavulanic acid	17	12	7.7*
Sulfonamide		20*	19
Trimethoprim		20	20
Nitrofuratoin		33*	33*
Gentamicin	3.3	3.0	2.0*
Ciprofloxacin	9.2	7.4	5.5
Cefuroxime	9.7	8.6	4.5*
3rd generation cephalosporins	6.6	6.0	4.5
Carbapenem	0.3	0.2	0.1*

The presented resistance rates are means of the resistance rates determined by the individual DCM. Included are results from all DCM where >75% of the isolates in each antibiotic/sample group were susceptibility tested. The * markes where less than 6 (out of totally 10 DCM) tested a sufficient percentage of their samples. For carbapenems, piperacillin/tazobactam and gentamicin in urines from primary health care, only one DCM tested >75% of their isolates

Figure 8.6 *Klebsiella pneumoniae*. Resistance (%) in invasive isolates from humans DANMAP 2019



c)

Substance	Percent resistant invasive *K. pneumoniae* isolates										Time trends (Cochran-Armitage test)	
	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	10 years (2010-2019)	5 years (2015-2019)
Piperacillin/tazobactam	4.6	5.4	5.6	6.2	8.1	5.9	5.8	7.4	6.1	8.7	NA	NA
Gentamicin	6.0	5.8	6.0	4.4	4.6	2.5	2.9	3.2	3.1	3.3	↓ p <0.001	↑ p = 0.125
Ciprofloxacin	11.3	11.7	8.8	9.4	7	5.5	5.4	9.0	8.1	9.2	NA	NA
Cefuroxime	17.4	14.0	14.0	12.4	12.1	11.1	11.1	11.1	10.3	9.7	↓ p <0.001	↓ p = 0.097
3rd gen. cephalosporins	9.2	9.6	8.9	9.2	7.7	7.7	7.3	7.1	6.1	6.6	↓ p <0.001	↓ p = 0.1
Carbapenem	0.0	0.0	0.3	0.2	0.2	0	0.3	0.3	0.5	0.3	↑ p = 0.033	↑ p = 0.083
Total number of isolates	799	910	947	879	943	943	1156	1183	1280	1361		

a) Total numbers of invasive isolates per year. b) Resistance rates among tested isolates
c) Resistance rates and time trends and significance levels for the past five and 10 years, respectively (Cochran-Armitage test)
The number (n) in parentheses represents the numbers of isolates tested in 2019. NA = Not applicable

In invasive *K. pneumoniae* resistance rates have decreased over the past 10 years for gentamicin, ciprofloxacin, cefuroxime and 3rd generation cephalosporins, but with insignificant or no decrease in the past five years. For more details see Figure 8.6. The increased ciprofloxacin resistance rate in 2017 compared to 2016 mainly reflected a relatively large change in EUCAST breakpoints in 2017. Ciprofloxacin breakpoints changed (lowered) once again combined with the introduction of the Area of Technical Uncertainty (ATU) in the EUCAST clinical breakpoint Table v. 9.0 in 2019. Therefore curves for ciprofloxacin resistance should be interpreted with caution and time trend calculations are not conducted. For piperacillin-tazobactam, the interpretation should be taken with caution as well, even though there have been no EUCAST breakpoint changes since 2012. Several local rules regarding downgrading piperacillin-tazobactam from I to R or S to R have existed during the years and also technical problems with the testing has been a recurring issue. Furthermore, in 2019 an ATU, for piperacillin-tazobactam was introduced. With a calculation based only on zone diameters 4.3% of the isolates were resistant (<17 mm) in 2018 compared to 5.7% in 2019, indicating a "true" increase in resistance towards piperacillin-tazobactam (Figure A8.3 in web annex).

For carbapenem resistance in invasive *K. pneumoniae* a very small but significant increase was observed over the past 10 years. However total numbers of meropenem resistant invasive isolates are low, and no increase was observed in 2019 with four resistant and two susceptible increased exposure isolates compared to seven resistant and one susceptible increased exposure invasive *K. pneumoniae* isolates in 2018. 2018 was the year with most meropenem resistant invasive *K. pneumoniae* isolates until now. The level of multiresistant (combined resistance to 3rd generation cephalosporins, ciprofloxacin and gentamicin) invasive *K. pneumoniae* remained at around 2%, Table 8.5. For colistin none of the invasive *K. pneumoniae* were registered resistant, though susceptibility to colistin is not routinely tested.

Urinary cases from hospitals

For 2019, a total of 7,926 unique patients with *K. pneumoniae* isolates, cultured in urine samples from hospitalised patients from all DCMs in Denmark, were identified in MiBa. 225 unique patients with *K. pneumoniae* isolates, cultured in urine samples were excluded because it was not possible to categorise them as hospital or primary care urine samples.

All 10 DCMs routinely (>75% of isolates) registered antimicrobial susceptibility interpretations for mecillinam, piperacillin-tazobactam and ciprofloxacin in MiBa. In addition nine DCMs routinely registered antimicrobial susceptibilities for gentamicin and cefuroxime, eight DCMs for trimethoprim, six DCMs for 3rd generation cephalosporins, carbapenem and amoxicillin-clavulanic acid, five DCMs for nitrofurantoin and four DCMs for sulphonamide.

Resistance rates for all tested antimicrobials are summarized together with the results from the invasive isolates as a national mean for each antimicrobial class in Table 8.4. In Figure 8.7 rates of resistance are shown for the past decade. Time trends and significance levels, based on the resistance rates for the past five and 10 years, respectively, are presented in Figure 8.7c.

As for invasive *K. pneumoniae* resistance rates in urine isolates from hospitals have decreased over the past 10 years for gentamicin, ciprofloxacin, cefuroxime and 3rd generation cephalosporins, but with less or no decrease in the past five years. For 2019 a decrease in resistance to mecillinam was observed. Thereby the very steep increase observed in 2017 and confirmed in 2018 seems to be on reverse. For more details see Figure 8.7.

In 2019, in total 15 carbapenem resistant and 11 susceptible increased exposure *K. pneumoniae* isolates from hospital urinary cases were registered compared to 19 and 6 in 2018.

Urinary cases from primary health care

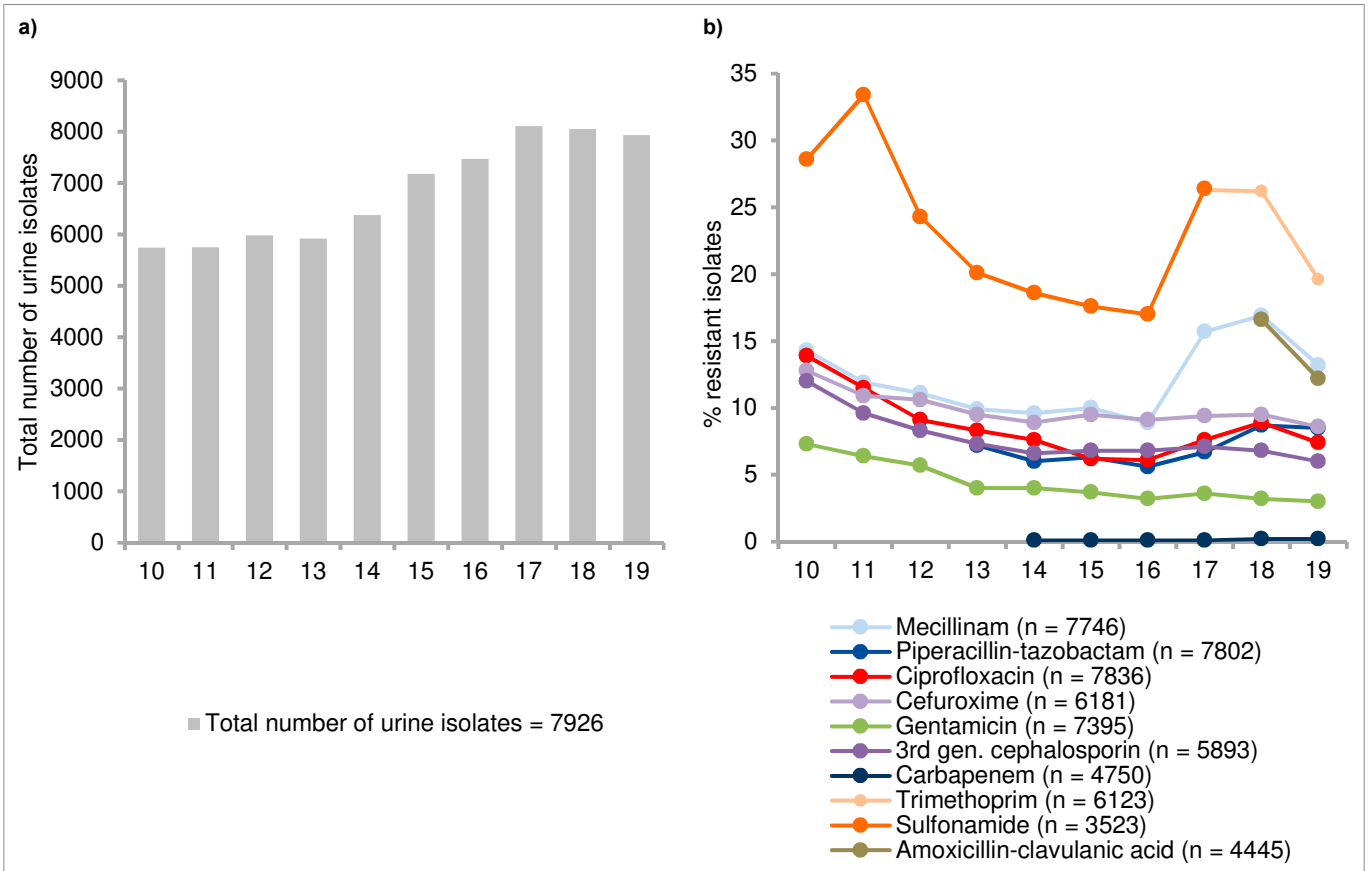
For 2019, a total of 9,696 unique patients with *K. pneumoniae* isolates, cultured in urine samples from primary health care (PHC), from nine DCMs in Denmark, were identified in MiBa. The general practitioners (GPs) in Denmark are all serviced by those nine DCMs except for a few GPs in the Capital Region of Denmark whom a serviced by one private laboratory.

Table 8.5 *K. pneumoniae*. Combined resistance to 3rd generation cephalosporins, ciprofloxacin, and gentamicin (multiresistance) in invasive isolates from humans

DANMAP 2019

	2014 % (N)	2015 % (N)	2016 % (N)	2017 % (N)	2018 % (N)	2019 % (N)
Resistance	3.0 (26)	1.1 (9)	1.6 (18)	2.4 (27)	1.7 (20)	2.4 (30)
Percentage (no.) of isolates tested for combined resistance (multiresistance)	91 (859)	89 (840)	98 (1131)	95 (1122)	93 (1188)	94 (1275)
Total number of invasive isolates	943	943	1156	1183	1280	1361

Figure 8.7 *Klebsiella pneumoniae*. Resistance (%) in urine isolates from humans in hospitals DANMAP 2019



c)

Substance	Percent resistant *K. pneumoniae* isolates from hospital urine										Time trends (Cochran-Armitage test)	
	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	10 years (2010-2019)	5 years (2015-2019)
Mecillinam	14.3	11.9	11.1	9.9	9.6	10	8.9	15.7	16.9	13.2	↑ p <0.001	↑ p <0.001
Piperacillin/tazobactam				7.2	6	6.3	5.6	6.7	8.7	8.5	NA	NA
Gentamicin	7.3	6.4	5.7	4	4	3.7	3.2	3.6	3.2	3	↓ p <0.001	↓ p = 0.024
Ciprofloxacin	13.9	11.5	9.1	8.3	7.6	6.2	6.1	7.6	8.9	7.4	NA	NA
Cefuroxime	12.8	10.9	10.6	9.5	8.9	9.5	9.1	9.4	9.5	8.6	↓ p <0.001	↓ p = 0.126
3rd gen. cephalosporins	12	9.6	8.3	7.3	6.6	6.8	6.8	7.1	6.8	6	↓ p <0.001	↓ p = 0.048
Total number of isolates	5740	5746	5982	5919	6372	7175	7467	8106	8047	7926		

a) Total numbers of invasive isolates per year, b) Resistance rates among tested isolates, c) Resistance rates and time trends and significance levels for the past five and 10 years, respectively (Cochran-Armitage test)
The number (n) in parentheses represents the numbers of isolates tested in 2019. NA = Not applicable

All nine DCMs routinely (>75% of isolates) registered antimicrobial susceptibility interpretations for mecillinam and trimethoprim in MiBa. In addition eight DCMs routinely registered antimicrobial susceptibilities for sulphonamide, six DCMs for 3rd generation cephalosporins and ciprofloxacin, five DCMs for nitrofurantoin, four DCMs for amoxicillin-clavulanic acid, three DCMs for cefuroxime and one DCM for piperacillin-tazobactam, carbapenem and gentamicin.

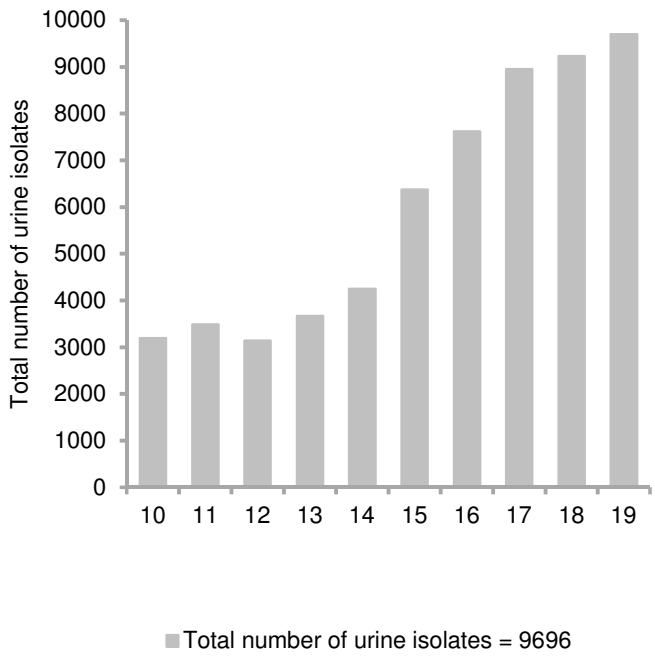
As for the results from invasive isolates and isolates from hospital urines resistance results for all tested antimicrobials are shown as national means in Table 8.4. In Figure 8.8, rates of resistance are shown for the past decade. Time trends and significance levels, based on the resistance rates for the past five and ten years respectively, are presented in Figure 8.8c.

Also in urine isolates from PHC a decrease in resistance to mecillinam was observed in 2019 and thereby the very steep increase observed in 2017 and confirmed in 2018 seems to be on reverse. For more details see Figure 8.8.

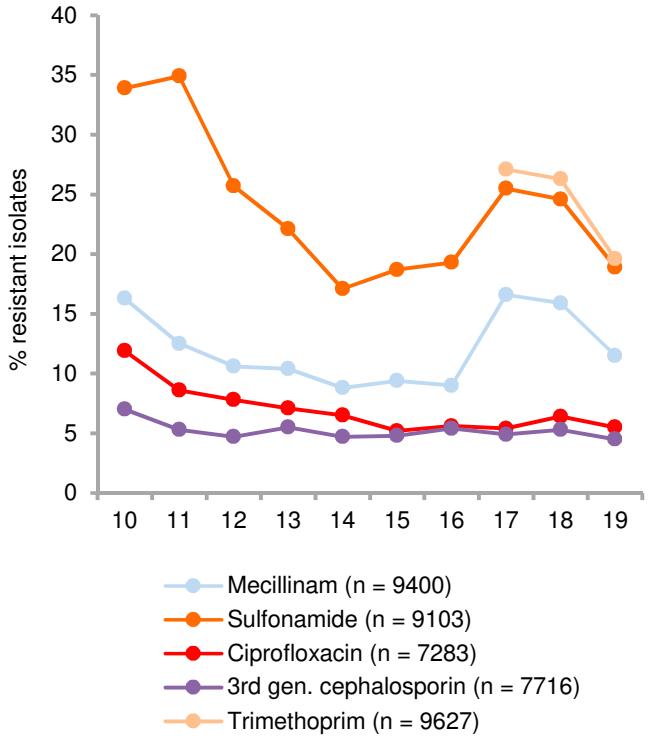
In total numbers three carbapenem resistant and two susceptible increased exposure isolates were registered in 2019 compared to four and two in 2018. Susceptibility results for carbapenem though, is only routinely reported in MiBa from one DCM.

Figure 8.8 *Klebsiella pneumoniae*. Resistance (%) in urine isolates from humans in primary health care DANMAP 2019

a)



b)



c)

Substance	Percent resistant *K. pneumoniae* isolates from PHC urine										Time trends (Cochran-Armitage test)	
	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	10 years (2010-2019)	5 years (2015-2019)
Mecillinam	16.3	12.5	10.6	10.4	8.8	9.4	9	16.6	15.9	11.5	↑ p <0.001	↑ p <0.001
Sulfonamide	33.9	34.9	25.7	22.1	17.1	18.7	19.3	25.5	24.6	18.9	↓ p <0.001	↑ p = 0.074
Ciprofloxacin	11.9	8.6	7.8	7.1	6.5	5.2	5.6	5.4	6.4	5.5	NA	NA
3rd gen. cephalosporins	7	5.3	4.7	5.5	4.7	4.8	5.4	4.9	5.3	4.5	↓ p = 0.001	↓ p <0.001
Total number of isolates	3197	3489	3142	3669	4246	6372	7615	8948	9227	9696		

a) Total numbers of invasive isolates per year, b) Resistance rates among tested isolates, c) Resistance rates and time trends and significance levels for the past five and 10 years, respectively (Cochran-Armitage test)
The number (n) in parentheses represents the numbers of isolates tested in 2019. NA = Not applicable

Conclusion

The general trend for *K. pneumoniae* in all three specimen categories have been declines in resistance rates to important antimicrobials as cephalosporins, gentamicin and ciprofloxacin over a ten years period. These declines have slowed down or stagnated during the past five years. For mecillinam a worrisome increase in resistance in 2017 and 2018 has reversed in 2019. Amoxicillin-clavulanic acid has only been observed since 2017, and in the urinary isolates resistance rates has declined in the period. Even though caution should be taken when interpreting reported susceptibility results towards piperacillin-tazobactam a worrisome change in the zone distributions for invasive isolates of *K. pneumoniae* with more isolates being less than 17mm (resistant) was observed.

The small, but increasing levels of carbapenem resistance in *K. pneumoniae* is worrisome. Often those isolates are also resistant to other important antimicrobials. In 2019 though, no increase in numbers of carbapenem resistant cases was observed. In the southern and south-eastern part of Europe high levels of carbapenem resistance, with Greece peaking at 63.9%, as well as combined resistance to 3rd generation cephalosporins, ciprofloxacin and gentamicin is truly problematic [EARS-Net annual report, 2018].

Sissel Skovgaard, Jonas Kähler and Anna E. Henius
For further information: Sissel Skovgaard, sisk@ssi.dk

8.2.3 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is an opportunistic pathogen causing relatively rare but significant disease in humans. *P. aeruginosa* typically infects the pulmonary tract, urinary tract, burns, wounds, and can cause bloodstream infections as well. It is a relatively frequent colonizer of medical devices (e.g. indwelling catheters). *P. aeruginosa* infection is a serious problem in immunocompromised patients with e.g. cancer and in patients with cystic fibrosis. The case fatality rate in these patients is high. *P. aeruginosa* is intrinsically resistant to the majority of antimicrobial agents. The antimicrobial classes, which can be used for treatment include: some fluoroquinolones, some aminoglycosides, some beta-lactams (piperacillin-tazobactam, ceftazidime and carbapenems) and colistin.

Invasive cases from hospital patients

In 2019, a total of 493 unique patients with invasive *P. aeruginosa* isolates from all 10 departments of clinical microbiology (DCMc) in Denmark, were identified in MiBa. All 10 DCMs routinely (>75% of isolates) registered antimicrobial susceptibility interpretations for ciprofloxacin, piperacillin-tazobactam, gentamicin and meropenem in MiBa and nine DCMs routinely registered antimicrobial susceptibilities for ceftazidime. Antimicrobial susceptibility testing was mainly performed by disc diffusion or E-test. The presented data consist of the registered interpretation results, performed by the DCMs, and are based on the S-I-R system.

Data are presented in Figure 8.9.

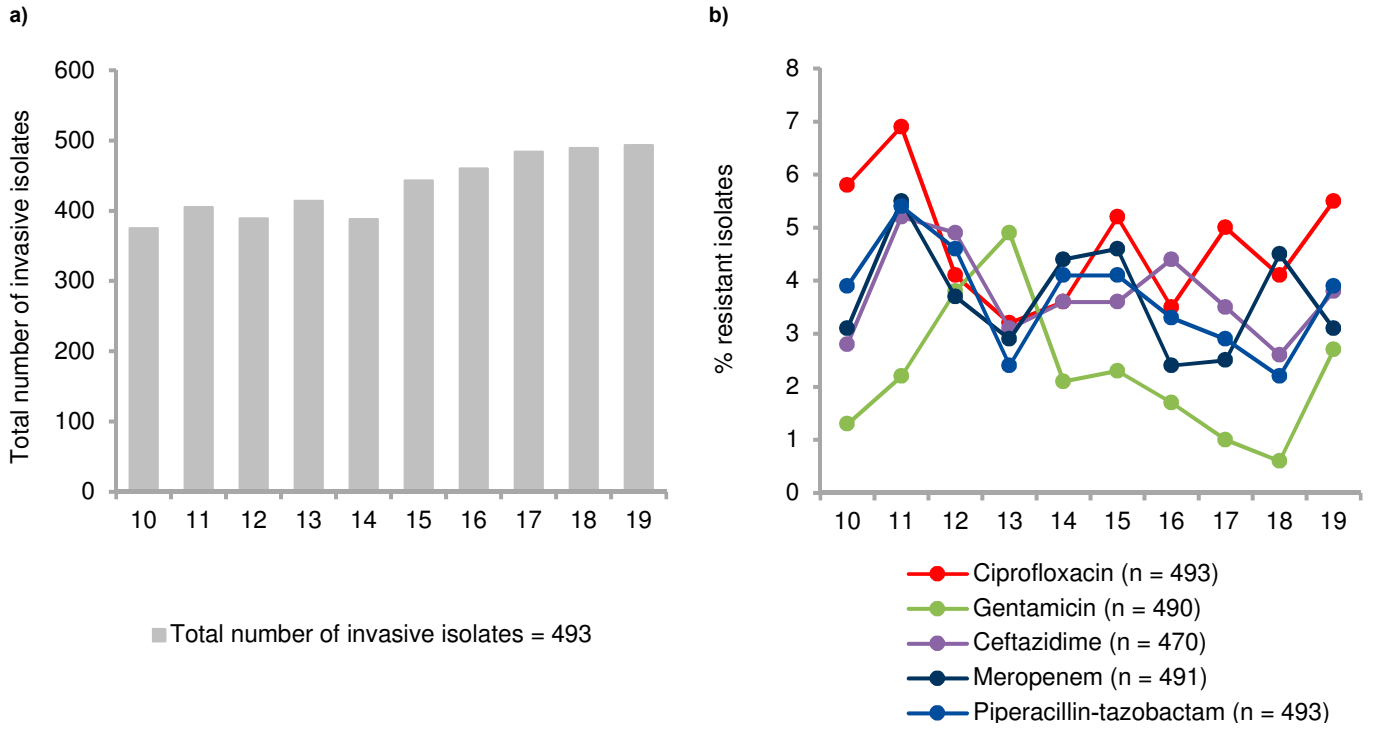
The highest level of resistance was reported for ciprofloxacin with 5.5%. Meropenem resistance was reported in 3.1% of the cases, and only in 1.7% of the cases resistance to three or more of the five antimicrobials under surveillance were reported. None of the invasive *P. aeruginosa* isolates identified in MiBa for 2019 were registered as colistin resistant.

Conclusion

Regarding invasive *P. aeruginosa* the situation in Denmark is quite stable both in number of cases and resistance profiles among those, with relatively low overall prevalence of resistance. EARS-Net 2018 reported a small decreasing trend in resistance in the EU/EEA population-weighted mean for all antimicrobial groups under surveillance during the period 2015-2018. In 2018 EU/EEA population-weighted means for prevalence of resistance were: ceftazidime (14.1%), fluoroquinolones (19.7%), aminoglycosides (11.8%), piperacillin-tazobactam (18.3%), carbapenems (17.2%) and for combined resistance (resistance to three or more of the five monitored antimicrobials) (12.8%). Large inter-country variations are reported between South East Europe and North Europe [EARS-Net annual report, 2018].

Sissel Skovgaard, Jonas Kähler and Anna E. Henius
For further information: Sissel Skovgaard, sisk@ssi.dk

Figure 8.9 *Pseudomonas aeruginosa*. Resistance (%) in invasive isolates from humans DANMAP 2019



a) Total numbers of invasive isolates per year
b) Resistance rates among tested isolates. The number (n) in parentheses represents the numbers of isolates tested in 2019

8.2.4 *Acinetobacter* species

The genus *Acinetobacter* includes several species and is found widespread in nature, in soil, water and/or animals and humans. In humans *Acinetobacter* can colonize the skin and wounds but may also be the cause of hospital-acquired infections like central line-associated bloodstream infections, nosocomial pneumonia, urinary tract infections and wound infections. Many of the different subspecies are phenotypically alike, and thus identification at species level can be difficult. Species belonging to the *A. baumannii* group are considered as the most clinically important. *Acinetobacter* is intrinsically resistant to a broad range of antimicrobials mediated by a low membrane permeability and constitutive expression of various efflux systems. The antimicrobial classes, which can be used for treatment include some fluoroquinolones, aminoglycosides, carbapenems and colistin. Particularly for *A. baumannii*, multiresistant clones are widespread in the hospital environment in many South- and East European countries, where they cause nosocomial outbreaks in fragile patient subpopulations at e.g. intensive care units. Of worldwide concern are severely war-wounded soldiers colonised or infected with multiresistant *A. baumannii*.

Invasive cases from hospitals

In 2019, a total of 72 unique patients with invasive *Acinetobacter* species were identified in MiBa from nine departments of clinical microbiology (DCMs) in Denmark. All nine DCMs routinely (>75% of isolates) registered antimicrobial susceptibility interpretations for ciprofloxacin, meropenem and gentamicin. Antimicrobial susceptibility testing was mainly performed by disc diffusion. The presented data consist of the registered interpretation results, performed by the DCMs, and are based on the S-I-R system.

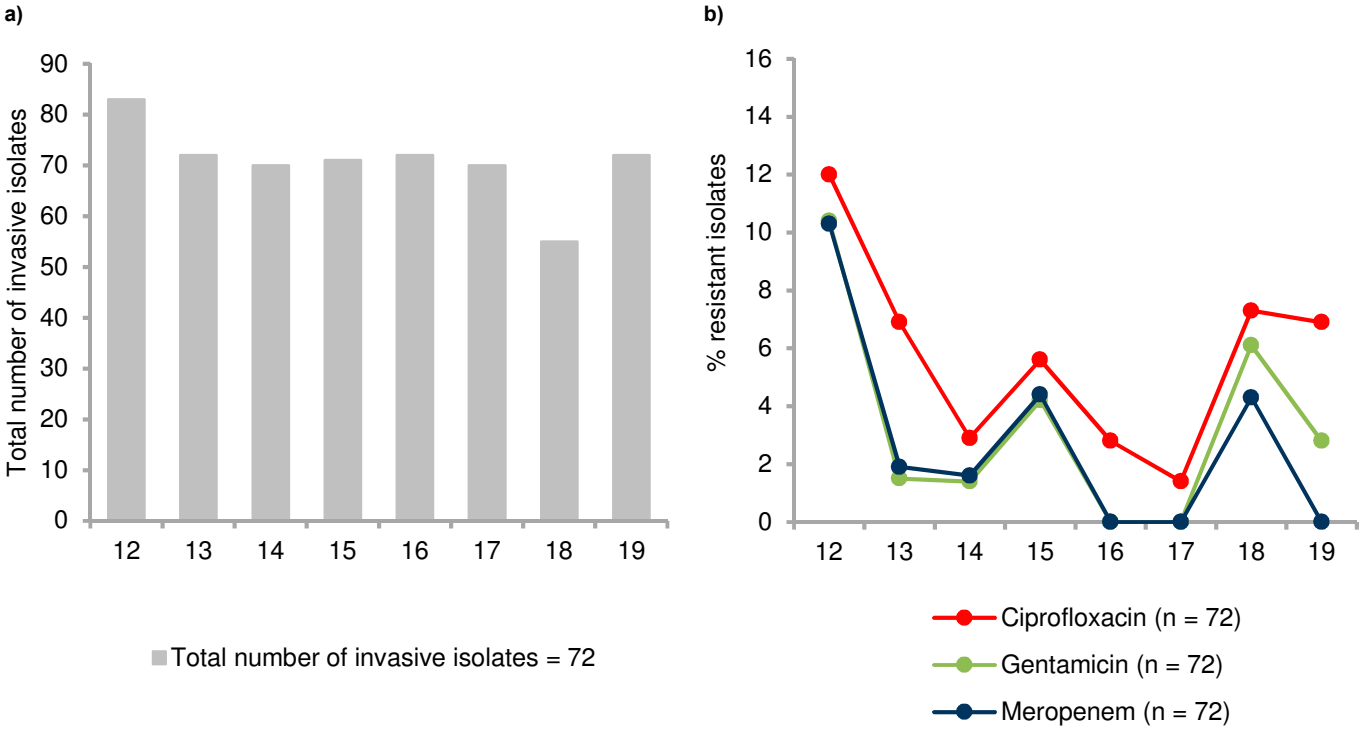
Data are presented in Table 8.6 and in Figure 8.10.

Tabel 8.6 *Acinetobacter* spp. Tested and resistant invasive isolates DANMAP 2019

	2012		2013		2014		2015		2016		2017		2018		2019	
	res.	n	res.	n	res.	n	res.	n	res.	n	res.	n	res.	n	res.	n
Ciprofloxacin	10	83	5	72	2	69	4	71	2	72	1	70	4	55	5	72
Gentamicin	8	77	1	65	1	70	3	71	0	70	0	70	3	49	2	72
Meropenem	6	58	1	52	1	62	3	68	0	69	0	67	2	47	0	72
Total number of invasive isolates	84		72		72		71		72		70		55		72	

res. = number of resistant isolates. n = number of tested isolates

Figure 8.10 *Acinetobacter* species. Resistance (%) in invasive isolates from humans DANMAP 2019



a) Total numbers of invasive isolates per year
b) Resistance rates among tested isolates. The number (n) in parentheses represents the numbers of isolates tested in 2019

The number of invasive *Acinetobacter* cases were similar to the years 2013 – 2017. None of the 72 isolates were resistant to meropenem, 5 isolates were resistant to ciprofloxacin and 2 were resistant to gentamicin. None had combined resistance to ciprofloxacin and gentamicin. None of the invasive *Acinetobacter* species were reported resistant to colistin, however, susceptibility to colistin, is not routinely tested.

Conclusion

In general, low total numbers of invasive *Acinetobacter* species are registered in Denmark, as well as low total numbers of resistant invasive *Acinetobacter* species. In EARS-Net, markedly differences in resistance profiles across Europe have been reported. Particularly the Baltic and Southern and South-Eastern countries of Europe reported on problems with high resistance levels and the most common have been combined resistance to fluoroquinolones, aminoglycosides and carbapenems with up to 91% of isolates with combined resistance in Croatia in 2018 as the highest and a EU/EAA population-weighted mean of 29%. The northern countries reported in between 0% and 4.3% combined resistance in 2018 [EARS-Net annual report, 2018].

Sissel Skovgaard, Jonas Kähler and Anna E. Henius
For further information: Sissel Skovgaard, sisk@ssi.dk

8.2.5 Enterococci

Enterococci constitute a part of the normal intestinal microbiota in humans and animals. More than 54 species belonging to the genus *Enterococcus* have been described, but the majority of the human infections are caused by *E. faecalis* and *E. faecium*. Most common clinical infections include urinary tract infections, bacteraemia and bacterial endocarditis. Enterococci are intrinsically resistant to many groups of antimicrobials and thereby get a selective advantage in e.g. hospitalised patients under antibiotic treatment where they can lead to colonization or infection. The source of hospital infection is often associated with the use of medical supplies, such as catheters, as well as other instruments and medical devices. Use of antimicrobials in these patients increases the risk for an enterococcal infection.

Treatment of enterococcal infections may be challenging. For *E. faecium*, were the vast majority are ampicillin resistant, severe infections are treated with vancomycin. Antimicrobials, such as linezolid and daptomycin are options for treatment of the multiresistant, vancomycin-resistant *Enterococcus* (VRE). Combinational therapy based on a synergistic effect of beta-lactam antibiotics (penicillin/ampicillin) with an aminoglycoside (most often gentamicin) or glycopeptide (vancomycin) is required in cases of endocarditis.

Invasive cases from hospitals

For 2019, a total of 634 unique patients with invasive *E. faecalis* isolates and 739 unique patients with invasive *E. faecium* isolates from all 10 departments of clinical microbiology (DCMs) in Denmark, were identified in MiBa. For *E. faecalis*, all 10 DCMs routinely (>75% of isolates) reported antimicrobial susceptibility interpretations for ampicillin in MiBa. In addition, nine DCMs routinely reported antimicrobial susceptibilities for vancomycin, six DCMs for linezolid, two DCMs for teicoplanin and one DCM for tigecycline. For *E. faecium*, all 10 DCMs routinely (>75% of isolates) reported antimicrobial susceptibility interpretations for ampicillin and vancomycin in MiBa. In addition, six DCMs routinely reported antimicrobial susceptibilities for linezolid, two DCMs for teicoplanin and one DCM for tigecycline. Antimicrobial susceptibility testing was mainly performed by disc diffusion. The presented data consist of the registered interpretation results, performed by the DCMs, based on the S-I-R system. For vancomycin all isolates reported as VRE in MiBa (based on PCR results for *vanA/B* genes) were calculated as vancomycin resistant independently from the actual zone/MIC result and there was not distinguished between VRE and VVE (vancomycin-variable enterococci). High-level resistance to gentamicin was calculated with EUCAST break points (MIC >128 mg/L and/or zone diameters <8 mm) on MIC and/or zone diameters as registered in MiBa. Gentamicin MIC and/or zone diameters were routinely reported from three DCMs.

Resistance to all tested antimicrobials are presented as a national mean of the combined DCMs reporting in Table 8.7. In Figure 8.11, total numbers of invasive isolates of *E. faecalis* and *E. faecium* and the ratio of resistance to vancomycin in both, for the past decade, are shown.

The total number of invasive cases of *E. faecalis* and *E. faecium* have been stable in 2018 and 2019.

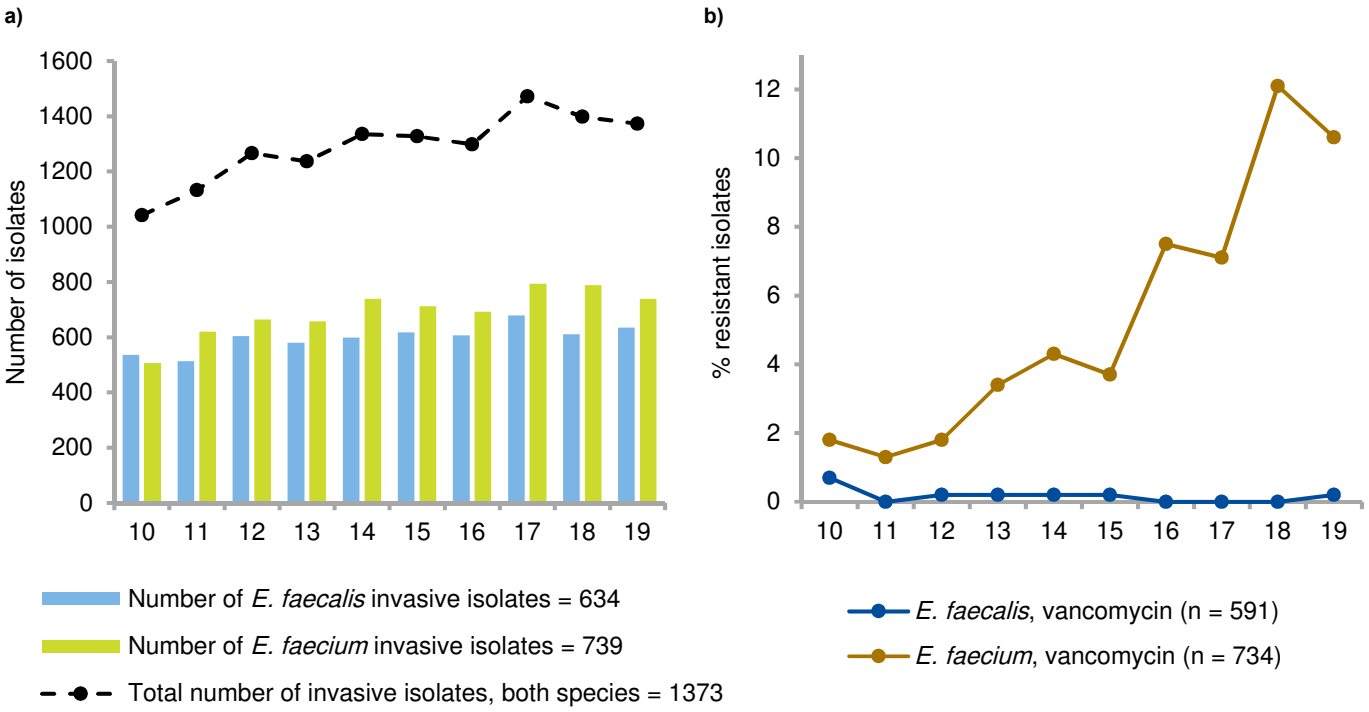
Table 8.7 Enterococci. Resistance (%) in invasive isolates from humans, 2019 DANMAP 2019

	E. faecalis	*E. faecium*	Number of included isolates (number of DCM)	
	%	%	*E. faecalis*	*E. faecium*
Ampicillin	0.2	93	632 (10)	735 (10)
Vancomycin	0.2	11	591 (9)	734 (10)
Linezolid	1.7	0.2	470 (6)	535 (6)
High-level gentamicin	9.4	33	278 (3)	296 (3)
Teicoplanin	1.0	6	198 (2)	218 (2)
Tigecycline	0.0	0.0	95 (1)	91 (1)

All proportions of resistance are presented for each antibiotic as a mean of the resistance rates reported by the DCMs. Included are all DCMs that repports routine testing (>75% of the isolates). The number in parentheses tells the number of included DCMs

Figure 8.11 Enterococci. Number of isolates and rates of resistance to vancomycin (%) in invasive isolates from humans

DANMAP 2019



a) Total numbers of invasive isolates per year, b) Resistance rates among tested isolates. The number (n) in parentheses represents the numbers of isolates tested in 2019

A continuing high prevalence of ampicillin-resistance in invasive *E. faecium* has been observed with rates in-between 92% and 95% since 2010. In 2002 the resistance rate was 65%.

The steep increase in the proportion of vancomycin resistant invasive *E. faecium* (12% compared to 7.1% in 2017) in 2018 was followed by a minor decrease to 11% in 2019. One vancomycin resistant invasive *E. faecalis* were reported in 2019. In total numbers 79 unique patients had a vancomycin resistant *E. faecium*/*E. faecalis* as the first invasive *Enterococcus* isolate in 2019. This number was 97 patients in 2018 and 56 in 2017.

The proportion of high-level gentamicin resistance is based on reporting from one DCM in 2010-2017, four DMCs in 2018 and from three DCMs in 2019. These rather sparse data show a decreasing trend in high-level gentamicin resistance in invasive *E. faecalis* over the decade, from 36% in 2010 to 20% in 2016, 7.1% in 2017 and a levelling with 9.4% in 2019. In *E. faecium* the level has been oscillating between 55% and 75% in the same time period, but a decreasing trend has been observed since 2017 with 43%, and further in 2019 with 33%.

During 2019, eight invasive isolates of *E. faecalis* and one invasive isolate of *E. faecium* from nine unique patients were reported linezolid resistant by the six DCMs routinely reporting interpretations to linezolid in MiBa (Table 8.7). In 2018 the numbers were five *E. faecalis* (five reporting DCMs) and three *E. faecium* (six reporting DCMs). All linezolid resistant invasive isolates identified in MiBa in 2019, were found susceptible to vancomycin.

Conclusion

An increase of invasive enterococci, mainly caused by an increase in invasive *E. faecium*, has been observed since 2002 (Figure 8.1.1. DANMAP 2015). The increase was combined with, an increase in the proportion of ampicillin resistant *E. faecium* (65% in 2002 and more than 90% since 2010) in the beginning, and since 2013 combined with an increase in vancomycin resistant *E. faecium*. In 2018, yet a steep increase in the percentage of vancomycin resistant invasive *E. faecium* was observed while the total number of invasive enterococci did not increase further compared to 2017. In 2019, the percentage of vancomycin resistant invasive *E. faecium* as well as the total number of invasive enterococci did not increase any further. The proportion of invasive vancomycin resistant *E. faecium* is relatively high in Denmark, 11% in 2019, especially when compared to the other Nordic countries with a range of 0-2.3%. But also southern European countries like France and Spain have lower percentages of vancomycin resistant invasive *E. faecium* than Denmark. However, EARS-Net also reported a worrisome increase in vancomycin resistant *E. faecium* in several countries from 2015 to 2018 [EARS-Net annual report, 2018].

Sissel Skovgaard, Jonas Kähler and Anna E. Henius
For further information: Sissel Skovgaard, sisk@ssi.dk

8.3 Surveillance based on data from the reference laboratories

8.3.1 Characterisation of ESBL- and pAmpC-producing *Escherichia coli* from bloodstream infections, 2019 Denmark

Background

Resistance to 3rd generation cephalosporins in *Escherichia coli* often occur through production of extended-spectrum beta-lactamases (ESBLs), carbapenemases, plasmid-mediated AmpC (pAmpC) or through mutations within the promoter/attenuator region of chromosomal AmpC (cAmpC) leading to overexpression/hyperproduction.

Since 2014, the Danish Departments of Clinical Microbiology (DCMs) have, on a voluntary basis, submitted 3rd generation cephalosporin-resistant *E. coli* (3GC-R Ec) from bloodstream infections for further characterization at the National Reference Laboratory for Antimicrobial Resistance at Statens Serum Institut (SSI) by adhering to the EUCAST criteria for reduced susceptibility towards cefpodoxime, ceftriaxone, cefotaxime or ceftazidime.

At SSI, the 3GC-R Ec's collected in Denmark through 2019, were phenotypically tested for ESBL-production. ESBL- and/or pAmpC-positive isolates from January, March, May, July, September, and November were subjected to whole-genome sequencing and characterized according to Multi locus Sequence Types (MLSTs), and the encoding ESBL-, pAmpC- and carbapenemase genes. For isolates with no ESBL-, pAmpC-, or carbapenemase-encoding genes detected, the sequences were investigated for promotor mutations presumed to up-regulate cAmpC production.

Results

In 2019, a total of 375 *E. coli* isolates from unique patients, were identified with phenotypic test, as ESBL, AmpC or carbapenemase-producing isolates. Demographic data was available for all 375 *E. coli* isolates in 2019; 206 (55%) of the patients were men compared to 185 (53%) in 2018, and 169 (45%) were women compared to 167 (47%) in 2018. The average age at diagnosis was 72 years, ranging from below one year to 99 years.

The regional distribution of the 375 isolates with ESBL, AmpC or carbapenemase pheonotype was compared to data from previous years (Table 8.8 and Figure 8.12).

From 2014 to 2019, the reported cases of *E. coli* ESBL/AmpC or carbapenemase-producing isolates from bloodstream infections increased with 53% from 245 to 375, compared to the overall number of *E. coli* bloodstream infections which increased with 25% in the same period.

From 2018 to 2019, the number increased from 352 to 375 isolates, a 7% increase, compared to the overall number that increased with 4%.

In The Capital Region, the number of reported cases decreased from 154 cases in 2018 to 124 cases in 2019 (p = 0.003), whereas the reported number of cases in The Zealand Region increased significantly from 23 cases in 2018 to 53 cases in 2019 (p = 0.0008). For the remaining three regions, the reported number of cases were stable in 2019 compared with 2018.

Whole genome sequencing data were obtained from 197 *E. coli* isolates (as only isolates from every second months were sequenced). Genes encoding ESBL and/or pAmpC were detected in 192 (97%) of the isolates while 5 isolates were cAmpC hyper producers only; these 5 isolates were not investigated further.

In 2019, 15 different ESBL-, and pAmpC-enzymes were detected among the 192 sequenced isolates (Table 8.9). As in previous years, CTX-M-15 was the most prevalent enzyme, decreasing from 57% in 2018 to 43% in 2019 (p=0.0006). The presence of CTX-M-14 increased from 9% in 2018 to 17% in 2019 (p=0.005), and the presence of CTX-M-55 increased from 1% in 2018 to 4% in 2019 (p=0.02). No new ESBL- or pAmpC-enzymes were detected among the isolates in 2019.

No carbapenemase producers were observed among the 197 whole genome sequenced ESBL- and/or pAmpC *E. coli* isolates from blood infection (from every second months were sequenced), but two carbapenemase producing *E. coli* isolates from bloodstream infections were detected in June and October.

Table 8.8 Distribution of ESBL/pAmpC -producing *E. coli* from bloodstream infections, Denmark DANMAP 2019

	DANMAP 2014	DANMAP 2015	DANMAP 2016	DANMAP 2017	DANMAP 2018	DANMAP 2019
Region	Number	Number	Number	Number	Number	Number
The Capital Region of Denmark	110	116	111	112	154	124
The Zealand Region	27	14	36	38	23	53
Region of Southern Denmark	43	45	67	76	75	97
Central Denmark Region	43	59	66	80	74	67
North Denmark Region	22	41	32	31	26	34
Total Numbers	245	275	312	337	352	375

Figure 8.12 Regionwide distribution of ESBL/pAmpC-producing *E. coli* from bloodstream infections, Denmark DANMAP 2019

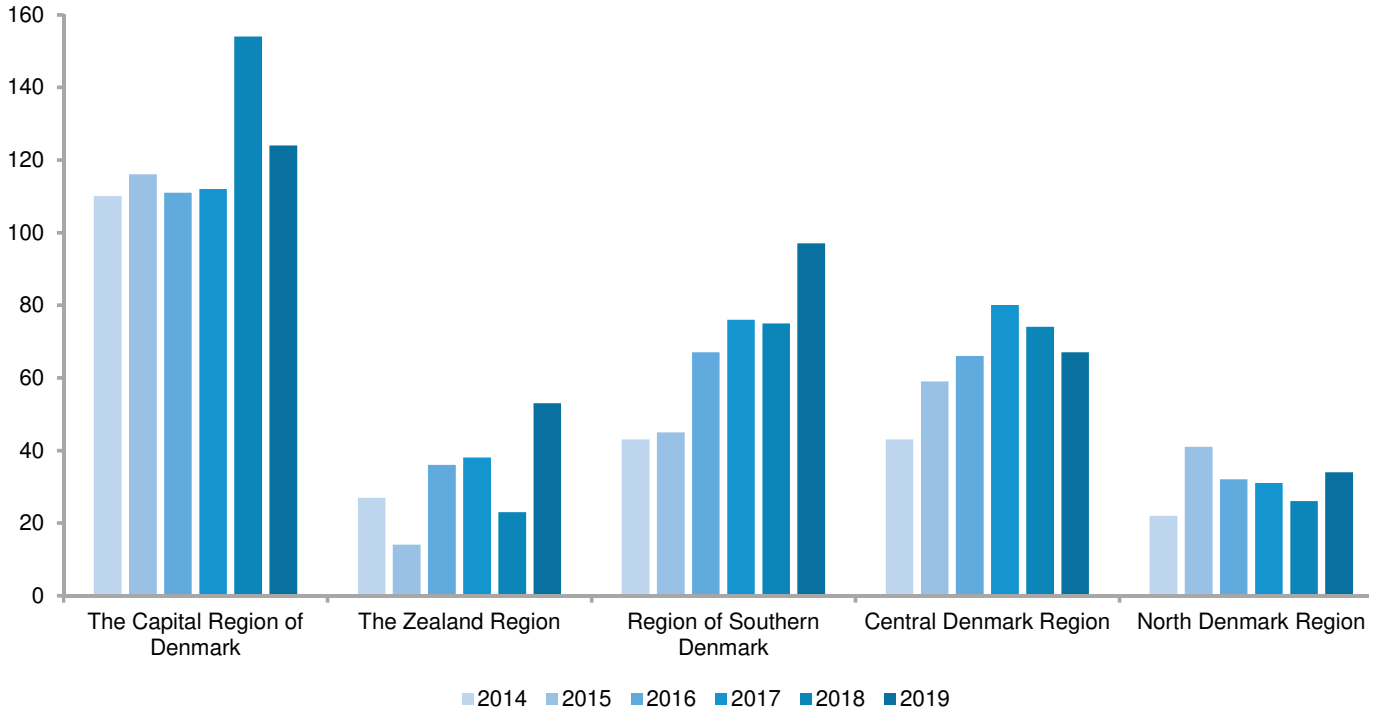


Table 8.9 Most common ESBL enzymes, pAmpC enzymes and carbapenemases detected in *E. coli* from bloodstream infections, Denmark DANMAP 2019

	DANMAP 2014		DANMAP 2015		DANMAP 2016		DANMAP 2017		DANMAP 2018		DANMAP 2019	
Enzyme	Number	%	Number	%	Number	%	Number	%	Number	%	Number*	%
blaCTX-M-1	10	4%	7	3%	8	3%	17	5%	25	7%	8	4%
blaCTX-M-101	12	5%	15	5%	14	4%	9	3%	4	1%	2	1%
blaCTX-M-14	38	16%	33	12%	40	13%	48	14%	31	9%	33	17%
blaCTX-M-14b	5	2%	5	2%	9	3%	3	1%	10	3%	3	2%
blaCTX-M-15	121	49%	139	51%	157	50%	164	49%	200	57%	82	43%
blaCTX-M-27	25	10%	33	12%	44	14%	52	15%	53	15%	37	19%
blaCTX-M-3	4	2%	4	1%	7	2%	8	2%	5	1%	4	2%
blaCTX-M-55	8	3%	14	5%	6	2%	13	4%	4	1%	8	4%
blaCMY-2	10	4%	6	2%	10	3%	7	2%	6	2%	5	3%
blaDHA-1	0	0%	3	1%	5	2%	6	2%	10	3%	4	2%
blaSHV-12	2	1%	5	2%	5	2%	3	1%	4	1%	2	1%
Other CMY variants	4	2%	10	4%	3	1%	3	1%	3	1%	5	3%
Other ESBL enzymes	12	5%	8	3%	17	5%	10	3%	10	3%	3	2%
Carbapenemase enzymes	3	1%	3	1%	1	<1%	1	<1%	5	1%	0	0%

In some isolates more than one enzyme was detected
*Numbers based on sequenced data from odd months

In 2019, the 197 whole genome sequenced *E. coli* isolates belonged to 39 different MLSTs, with the most common sequence type (ST) being ST131 (47%), followed by ST69 (7%) and ST38 (7%) (Table 8.10).

The proportion of ST23 isolates increased from below 1% in 2018 to 6% in 2019 (p=0.00005). Between 2014 and 2018, ST23 was only observed seven times. The 11 isolates from 2019 all produced CTX-M-14, and cgMLST analysis of the clones showed highly identical strains with maximum two allele differences between the isolates. The isolates were reported by seven of the ten DCMs, and primarily from emergency departments.

Among the 93 *E. coli* isolates belonging to ST131, CTX-M-15 (52%) was the most common enzyme, followed by CTX-M-27 (32%), and CTX-M-14 (8%). The presence of CTX-M-15 decreased from 67% in 2018 to 52% in 2019 (p=0.01), whereas the presence of CTX-M-27 increased from 20% in 2018 to 32% in 2019 (p=0.03).

Table 8.10 Distribution of MLSTs in ESBL/pAmpC-producing *E. coli* from bloodstream infections, Denmark DANMAP 2019

	DANMAP 2014		DANMAP 2015		DANMAP 2016		DANMAP 2017		DANMAP 2018		DANMAP 2019	
MLST	Numbers	%	Numbers	%	Numbers	%	Numbers	%	Numbers	%	Numbers*	%
ST131	124	51%	135	49%	177	57%	175	52%	189	54%	93	47%
ST38	18	7%	23	8%	21	7%	23	7%	22	6%	13	7%
ST69	10	4%	10	4%	16	5%	20	6%	27	8%	14	7%
ST648	7	3%	10	4%	5	2%	8	2%	6	2%	4	2%
ST12	5	2%	9	3%	14	4%	6	2%	5	1%	5	3%
ST1193	2	1%	5	2%	10	3%	7	2%	8	2%	6	3%
ST10	0	0%	6	2%	2	1%	4	1%	7	2%	5	3%
ST23	1	<1%	0	0%	2	1%	3	1%	1	<1%	11	6%
ST73	3	1%	2	1%	4	1%	2	1%	6	2%	4	2%
Other STs[1]	75	31%	75	27%	61	20%	89	26%	81	23%	42	21%

[1] Each ST found in less than 2% of the isolates in 2019
*Numbers based on sequenced data from odd months

The whole genome sequencing data from the 192 human bloodstream infections, were compared with the collection of ESBL/AmpC- and carbapenemase-producing isolates of animal origin collected in 2018 and 2019. No clonal relationship were identified by single-nucleotide polymorphism (SNP) analysis for isolates sharing the same combination of ST and ESBL-pAmpC-genes. Horizontal gene transfer of ESBL/pAmpC or carbapenemase genes were not investigated.

Conclusion

In 2019, the number of ESBL- and/or AmpC positive isolates increased from 352 to 375 isolates (7% increase). Changes of ESBL enzymes produced by the isolates were observed in 2019, where CTX-M-15 decreased, and CTX-M-14 and CTX-M-55 increased. In isolates belonging to ST131, the relative abundance of CTX-M-15 decreased while it increased for CTX-M-27.

The relative distribution of sequence types for the 197 whole genome sequences isolates were similar to the distributions in the previous years; the worldwide disseminated ST131 clone was still strongly represented in 2019 (47%), however, a new ST23 CTX-M-14 clone was observed in 2019.

Louise Roer, Frank Hansen, Henrik Hasman and Anette M. Hammerum
For further information: Anette M. Hammerum, ama@ssi.dk

8.3.2 Carbapenemase producing bacteria in Denmark, 2019

Background

Carbapenems comprise one of the only classes of antimicrobial agents that can be used for treatment of infections with multi-resistant Gram negative bacteria like *Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa* and *Acinetobacter baumannii*. Treatment options for infections with carbapenem-resistant bacteria are often none or suboptimal. Resistance can be caused by the presence of various carbapenemases of which the most frequently occurring are *K. pneumoniae* carbapenemase (KPC), Oxacillinase (OXA), Verona integrin encoded metallo-β-lactamase (VIM), New Delhi metallo-β-lactamase (NDM) and Imipenemase (IMP).

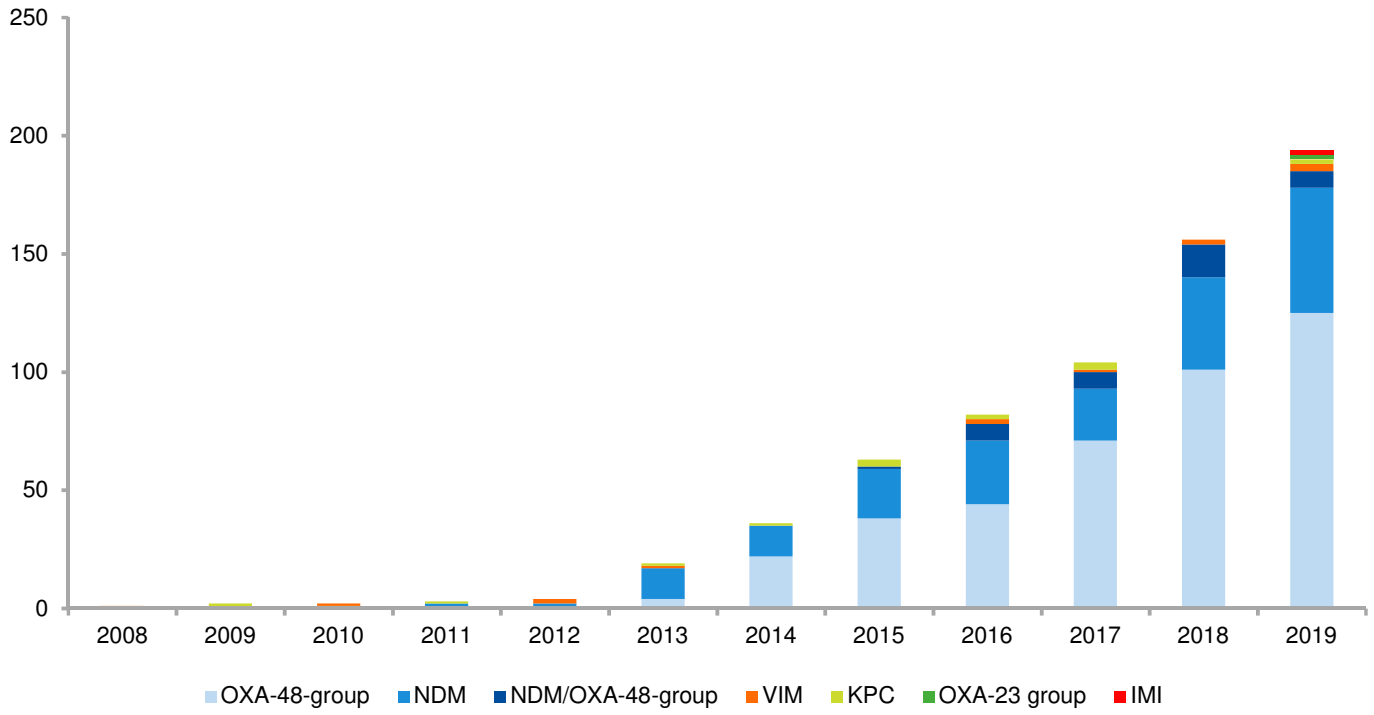
In recent years, Danish departments of clinical microbiology (DCMs) have on a voluntary basis submitted carbapenem-resistant isolates for verification and genotyping at the National Reference Laboratory for Antimicrobial Resistance at Statens Serum Institut (SSI). The Danish Health Authority made CPO notifiable as of 5th September 2018 [https://www.sst.dk/da/udgivelser/2018/~/media/52D5C295BCEA48E6BC596C0083367FF3.ashx]. The present text describes carbapenemase-producing Enterobacterales (CPE), *Pseudomonas* spp. and *Acinetobacter* spp.

During 2019, 221 carbapenemase-producing organisms (CPO) were detected from 187 patients compared with 177 CPO from 160 patients in 2018. More than one isolate from the same patient were included, if the isolates belonged to different bacterial species and/or if the isolates harboured different carbapenemases. Ten of the CPO (nine Enterobacterales and one *Acinetobacter* spp.) were from bloodstream infections compared with 18 of the CPO in 2018.

Enterobacterales

In 2019, 194 CPE isolates were detected from 168 patients compared to 156 CPE from 141 patients in 2018 leading to a 23% increase of submitted CPE isolates compared to 2018. In 2019, seven of the 194 CPE isolates produced both NDM and OXA-48 group enzymes, 125 produced OXA-48-like enzymes and 53 were NDM-producing. Furthermore, three VIM-producing isolates and two KPC-producing isolates were detected. For the first time, two OXA-23 group CPE isolates were detected (Figure 8.13).

Figure 8.13 Numbers of carbapenemase-producing Enterobacterales (CPE), 2008-2019, Denmark DANMAP 2019



OXA-244 belong to the OXA-48-enzyme group. In recent years, an increase in OXA-244-producing *E. coli* isolates has been observed in the national surveillance of CPO in Denmark. Molecular characterisation of 24 OXA-244-producing *E. coli* isolates from January 2016 to August 2019 was initiated and epidemiological investigation of cases, including telephone interviews, was conducted. In conclusion, import of OXA-244 *E. coli* isolates from travelling abroad seemed likely for the majority of cases. Community sources were also possible, as many of the patients had no history of hospitalisation and many of the *E. coli* isolates belonged to STs that are present in the community. It was not possible to point at a single country or a community source as risk factor for acquiring OXA-244-producing *E. coli* [Hammerum et al. 2020, Euro Surveill.;25(18)].

Outbreaks with CPE during 2019

In Denmark, outbreaks with CPE in healthcare settings are registered at SSI in a newly established national database (KURS) for CPE-outbreaks. At SSI, CPE isolates are routinely characterised by whole genome sequencing (WGS). Cluster analysis is conducted to detect possible clustering between the CPE isolates. Isolates from two or more persons sharing the same unique genotype are defined as an outbreak. When epidemiological investigations can establish a link between at least two of the patients in an outbreak cluster, the outbreak is classified as a verified outbreak. When no epidemiological link can be established between the patients, the outbreak is classified as a possible outbreak (Materials and methods, section 9.12)

In total, 16 ongoing CPE-outbreaks were registered with new patients in 2019. In nine of these outbreaks, it was possible to establish an epidemiological link between the patients. All epidemiological links were found in healthcare settings: Patients sharing the same ward or hospital. Of the 16 outbreaks, five of them were new outbreak clusters, each including two patients, identified in the CPE surveillance in 2019. Three of the newly identified outbreak clusters had index patients before 2019 (Table 8.11).

In 2019, an investigation of possible clonal transmission of carbapenemase-producing *K. pneumoniae* complex member isolates in Denmark was carried out using core genome MLST and National Patient Registry Data. Thirteen clusters, including 103 isolates of carbapenemase-producing *K. pneumoniae* from 2014 – 2018, were investigated. In five of these clusters, the patients had stayed at the same ward at the same time, showing a direct epidemiological link. The study found that cgMLST combined with patient hospital admission data and travel information was a reliable and detailed approach for detecting possible transmission of carbapenemase-producing *K. pneumoniae* complex members [Hammerum et al. 2020, Intl J Antimicrob. Agents, 55:105931].

Larger outbreaks with CPE

The ST18 NDM-1-producing *Citrobacter freundii* outbreak, which started in 2012 in the North Denmark Region, continued in 2019 [Hammerum et al. 2016, J. Antimicrob. Agents, 71:3117-3124]. Until the end of 2019, 39 patients have been involved in the outbreak. None of the patients have a prior history of travel. In 2016, an already known patient from the North Denmark Region was detected positive in the Capital Region. During 2018 and 2019, the ST18 NDM-1-producing *C. freundii* also spread in the Central Denmark Region affecting six patients. The origin of the NDM-1-producing *C. freundii* is

Table 8.11 Outbreaks of carbapenemase-producing Enterobacterales (CPE) during 2019 DANMAP 2019

Outbreak ID	Year	Patients total	Patients 2019	Carbapenemase	Type of Outbreak	Species (clonal spread)	Regions	Status
41	2012-2019	39	12	NDM-1	Clonal/ plasmid	ST18 *C. freundii*	Capital Region/Central Denmark Region/North Denmark Region	Verified
48	2013-2019	19	5	OXA-436	Clonal/ plasmid	ST90 *E. cloacae*/ ST22 *C. freundii*	Capital Region/South Denmark Region/Zealand Region	Verified
24	2014-2019	9	2	OXA-181	Clonal	ST410 *E. coli*	Capital Region	Verified
25	2014-2019	6	1	OXA-48	Clonal	ST38 *E. coli*	Capital Region/Zealand Region	Verified
26	2014-2019	3	1	OXA-48	Clonal	ST448 *E. coli*	Capital Region	Possible
49	2014-2019	5	1	NDM-1	Clonal	ST91 *C. freundii*	Central Denmark Region	Possible
21	2015-2019	41	12	NDM-5/OXA-181	Clonal	ST410 *E. coli*	Capital Region/Zealand Region	Verified
22	2015-2019	5	1	OXA-181	Clonal	ST440 *E. coli*	Capital Region/Central Denmark Region	Possible
42	2015-2019	7	2	OXA-48	Clonal	ST65 *C. freundii*	Capital Region/North Denmark Region/Zealand Region	Verified
1*	2016-2019	2	1	OXA-181	Clonal	ST410 *E. coli*	Capital Region	Verified
15	2016-2019	3	1	OXA-48	Clonal	ST38 *E. coli*	Capital Region/South Denmark Region	Possible
33	2016-2019	7	1	OXA-232	Clonal	ST231 *K. pneumoniae*	Central Denmark Region	Verified
40*	2018-2019	2	1	OXA-48	Clonal	ST15 *K. pneumoniae*	Capital Region	Verified
51*	2018-2019	2	1	OXA-48	Clonal	ST73 *E. coli*	Central Denmark Region	Possible
7*	2019	2	2	NDM-5	Clonal	ST167 *E. coli*	Capital Region	Possible
43*	2019	2	2	OXA-48	Clonal	ST323 *C. freundii*	Zealand Region	Possible

*Outbreak clusters identified in 2019

unknown. The NDM-1 encoding plasmid has primarily been detected in ST18 *C. freundii* (56 isolates), but has also been detected in ST161 *E. coli* (3 isolates), ST8 *C. freundii* (1 isolate), ST17 and ST1890 *K. pneumoniae* (2 isolates respectively) and others, indicating plasmid transfer. During 2019, twelve new patients were part of this outbreak.

Since 2015, another large outbreak has been ongoing in the Zealand Region with spread of ST410 NDM-5/OXA-181 *E. coli*. [Roer et al. 2018, mSphere;3(4).]. Until the end of 2019, 41 patients had been involved in this outbreak. From 2018 spread of ST410 NDM-5/OXA-181 *E. coli* were also detected in the Capital Region. During 2019, twelve new patients were part of this outbreak. Apart from the first outbreak patient in 2015, who had been traveling to Egypt [Overballe-Petersen et al. 2018, Genome Announc.6(5): e01542-17] none of the patients had a prior history of travel.

In 2013, OXA-436, a novel carbapenemase belonging to the OXA-48 enzyme group, was detected in Denmark [Samuelsen et al. 2017, Antimicrob. Agents and Chemother 62(1): e01260-17]. The outbreak was still ongoing with five new patients in 2019. In total, 19 patients have been detected with OXA-436-producing CPE isolates (Tabel 8.11), where both plasmid and clonal spread have been seen. Clonal spread of ST90 OXA-436-producing *E. cloacae* isolates was detected in the Region of Southern Denmark (Textbox 8.1).

Besides the twenty-nine new patients in the three larger outbreak registered in 2019, only one or two new patients have been detected in the majority of the other outbreaks (Table 8.11). It seems very likely that the increase in OXA-48-producing CPE was due to plasmid transfer, but this was not investigated further.

For all outbreaks, several infection control measures have been implemented, e.g. extensive screening of patients, enforced hand hygiene, implementation of extensive use of Personal Protective Equipment (PPE), isolation precautions, and implementation of extensive cleaning. Patients are known to be carriers of CPE for years and infection hygiene control measures are important tools to prevent further spread of CPE.

Acinetobacter spp.

In 2019, 20 carbapenemase-producing *Acinetobacter* spp. isolates were detected compared to 18 isolates in 2018. All but three patients had been travelling abroad prior to detection of the carbapenemase-producing *Acinetobacter* spp. In 2019, 19 carbapenemase-producing *Acinetobacter baumannii* with the following enzymes were detected: OXA-23 (13), NDM-1 (1), NDM-1/OXA-23 (2), OXA-58 (2) and OXA-72 (1). Furthermore, one NDM-1/OXA-58/IMP-48-like-producing *Acinetobacter pittii* was detected.

***Pseudomonas* spp.**

In 2019, seven carbapenemase-producing *Pseudomonas* spp. isolates were detected compared to three isolates in 2018. All but one patient had been travelling abroad prior to detection of the carbapenemase-producing *Pseudomonas* spp. isolates. In 2019, six carbapenemase-producing *Pseudomonas aeruginosa* with the following enzymes were detected: VIM-2 (2), NDM-1 (1), KPC-2 (1), IMP-34-like (1) and IMP-45-like (1). Furthermore, one VIM-2-producing *Pseudomonas putida* were detected.

Conclusion

The occurrence of carbapenemase-producing bacteria in Denmark continues to increase, a trend worrisome to patients and clinicians. Especially the spread of CPE among patients in Denmark is of concern, since Enterobacterales can be carried in the intestine for a long time (years) without any symptoms of infections, which makes outbreak control difficult. It is unknown whether part of the observed increase in 2019 could be explained by the new Guidance for prevention of spread of CPO in 2018 resulting in alterations in screening and/or referral procedures.

Henrik Hasman, Frank Hansen, Lone Jannok Porsbo, Anne Kjerulf, Louise Roer, Hülya Kaya, Mette Bar Ilan, Brian Kristensen and Anette M. Hammerum
For further information: Anette M. Hammerum, ama@ssi.dk

Textbox 8.1

Investigation of an *Enterobacter cloacae* OXA-436 carbapenemase-outbreak – Shower drains as reservoirs

In 2013, OXA-436, a novel carbapenemase belonging to the OXA-48 enzyme group, was detected in Denmark [Samuelsen et al. 2017, Antimicrob. Agents and Chemother 62(1): e01260-17]. During 2017-2019, ST90 OXA-436-producing *E. cloacae* isolates were detected from seven patients in the Region of Southern Denmark [ECCMID 2020; abstract 508]. They had all been hospitalized at the same department at some point during 2017-2019. However, there was no direct epidemiological link between several of the patients. Rectal screening of all patients at the involved department was performed several times during the outbreak, but the results were negative. The infection control team audited staff and procedures, but no source or route of transmission was revealed. Finally, an investigation focusing on the department facilities, including sinks and drains, was performed.

Seven drains, 25 sinks and three bedpan boilers/instrument washers were sampled. ST90 OXA-436-producing *E. cloacae* were detected from two shower drains in the patient bathrooms. When comparing the ST90 OXA-436 *E. cloacae* isolates from the two shower drains with the isolate from the index patient, it seems highly plausible that the drains were reservoirs for the ST90 OXA-436 *E. cloacae* isolates. During sampling, staff reported that the shower drains had been partly clogged from time to time, which meant that the patients were standing in water returning from the drains. After the drains were unclogged, no further ST90 OXA-436 *E. cloacae* isolates have been detected in the Danish national surveillance of CPO.

Annette Toft, Mette Marie Nordestgaard, Anette Holm, Anette M. Hammerum, Henrik Hasman and Ulrik Stenz Justesen
For further information: Ulrik Stenz Justesen, Ulrik.Stenz.Justesen@rsyd.dk

8.3.3 Increase in both *vanA* and *vanB E. faecium* in Denmark

Background

Enterococci are intrinsically resistant to a number of first-line antimicrobial agents, including cephalosporins, which makes antibiotic treatment of enterococcal infections challenging. In addition, most hospital-acquired *E. faecium* are resistant to ampicillin, further limiting the treatment options. Vancomycin is an important drug for the treatment of severe *E. faecium* infections, however, an increase in the occurrence of vancomycin resistant enterococci (VRE) has been observed in Denmark and internationally. Newer antibiotics such as linezolid and daptomycin can be used for treatment of VRE, but both antimicrobial agents may lead to potential adverse effects and development of resistance has been reported.

In recent years, *E. faecium* harboring the *vanA* gene complex, but being phenotypically susceptible to vancomycin, have been described in different countries. These enterococci are referred to as vancomycin-variable enterococci (VVE). VVE have caused nosocomial outbreaks and development of reverting mutants becoming vancomycin resistant *in vitro* and *in vivo* has been described. Thus, VVE are clinically relevant and their detection is thus critical in order to avoid treatment failure with vancomycin. However, VVE cannot be selectively cultured on

vancomycin-containing media and can only be detected by molecular methods. In 2015 and 2016, sporadic VVE isolates with different genetic background in relation to concurrent VRE-outbreaks were detected in the Capital Region of Denmark, [Hammerum et al. Euro Surveill. 2020;25(18)]. In 2016, a new VVE clone belonging to ST1421- CT1134, which displays variable vancomycin susceptibility due to a deletion in the *vanX* gene, was detected. [TA Hansen et al., J. Antimicrob. Agents, 2018, 73: 2936–2940].

Surveillance of VRE/VVE

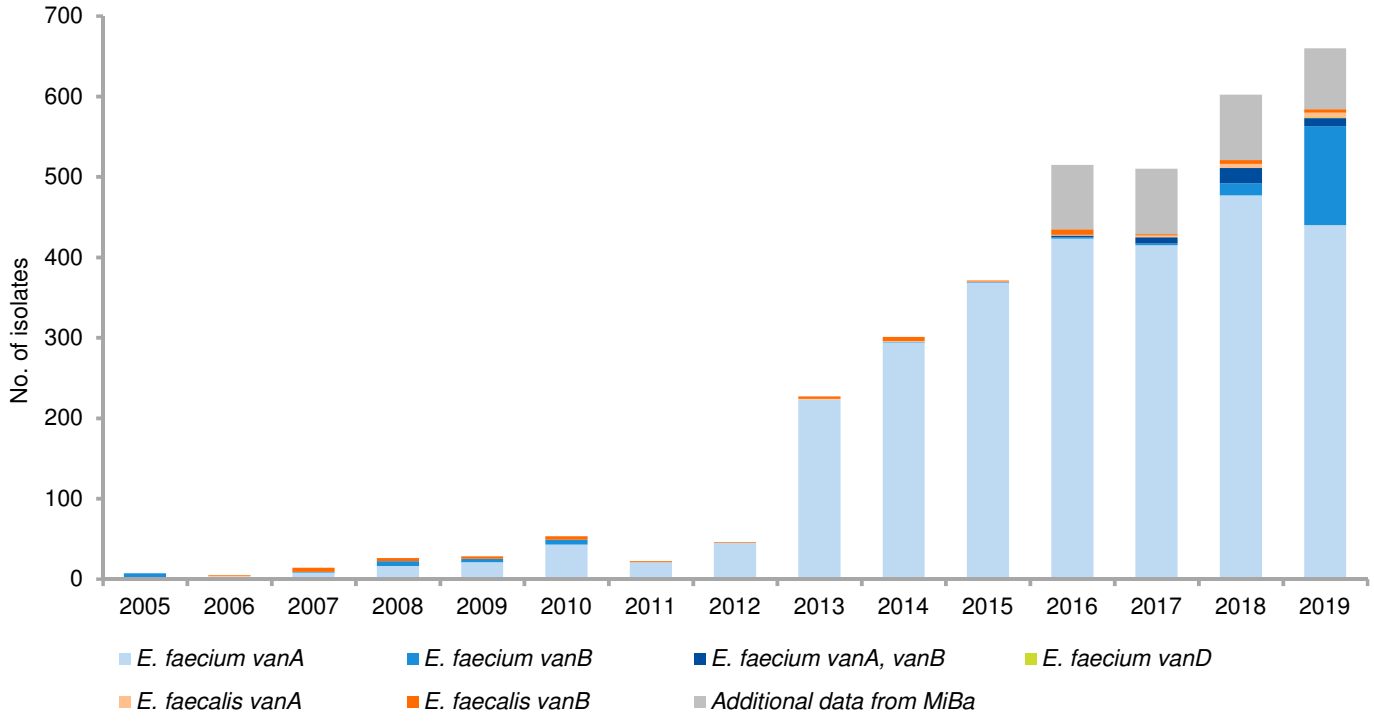
Since 2005, Danish departments of clinical microbiology (DCMs) have voluntarily submitted clinical VRE (one isolate per patient per 12 months) for species identification, genotyping and surveillance to the Antimicrobial Resistance Reference Laboratory at Statens Serum Institut (SSI).

In 2017, testing of phenotypically vancomycin-susceptible *E. faecium* isolates from blood cultures for the presence of *vanA*/*vanB* genes by PCR was introduced in the DCMs in the Capital Region for detection of possible VVE. During 2018, PCR testing was expanded to all clinical *E. faecium* isolates. Similarly in 2018, molecular testing by PCR of *E. faecium* from all clinical samples were implemented in one of the four DCMs in the Region of Southern Denmark. Furthermore, *E. faecium* isolates from blood cultures were tested by PCR for *vanA*/*vanB* genes in a second DCM in the Region of Southern Denmark and in the DCM in the Central Denmark Region in 2018. In 2019, diagnostic algorithms to detect VVE expanded, as most of the DCMs across Denmark as a minimum tested all blood culture *E. faecium* isolates for the presence of *vanA* genes using PCR [Hammerum et al. Euro Surveill. 2020;25(18)].

To determine any underreporting in the submissions, the number of VRE/VVE submitted to SSI in 2016, 2017, 2018 and 2019 were compared to data from clinical VRE reported by the DCMs to MiBa (the Danish Microbiology Database). This comparison showed that the number of submitted VRE/VVE isolates were not complete, since VRE/VVE isolates were missing from 80, 81, 78 and 76 patients in surveillance in 2016, 2017, 2018 and 2019, respectively (Figure 8.14). In 2019, 584 VRE/VVE isolates were submitted to SSI. By adding the 76 VRE/VVE isolates extracted from MiBa, this summed up to 660 VRE/VVE isolates from 660 patients in 2019 compared to 603 VRE/VVE isolates from 599 patients in 2018 (Figure 8.14).

From 2013, a sharp increase in clinical VRE isolates has been observed. Until 2018, the increase was mostly seen for *vanA E. faecium*, but during 2019 an increase was detected for *vanB E. faecium* too (Figure 8.14).

Figure 8.14 Numbers of *Enterococcus faecium* and *Enterococcus faecalis* isolates carrying *vanA* and *vanB* genes from clinical samples submitted to SSI 2005-2019 supplemented with data obtained from MiBa from 2016-2019, Denmark DANMAP 2019



From 2015 through 2019, the clinical VRE/VVE isolates received at SSI have been analysed by whole-genome sequencing (WGS). In 2019, 584 VRE/VVE were analysed by WGS. From the WGS data, species identification, multilocus sequence type (MLST), core genome MLST (cgMLST) and van-genes were identified *in silico*.

Of the 584 clinical VRE/VVE isolates, 440 were *vanA E. faecium*, 123 *vanB E. faecium*, 10 *vanA*/*vanB E. faecium*, one *vanD E. faecium*, six *vanA E. faecalis* and four *vanB E. faecalis* (Figure 8.14). WGS-based cgMLST analysis was performed on the ten *E. faecalis* isolates and the 574 *E. faecium* isolates using SeqSphere+ (Ridom). The ten *E. faecalis* isolates were subdivided into nine different complex types (CTs), whereas the 574 *E. faecium* isolates were subdivided into 72 CTs. Three clones were predominant: ST1421-CT1134 *vanA E. faecium*, ST117-CT36/CT991 *vanB E. faecium* and ST203-CT859/CT1051/CT1507 *vanA E. faecium* (Table 8.12).

ST203-CT859 *vanA E. faecium* isolates were first detected during the end of 2014 [Hammerum et al. J Antimicrob Chemother. 2017 Aug 1;72(8):2184-2190]. This type increased rapidly in numbers and became the most prevalent *vanA E. faecium* type (together with its subtypes CT1051 and CT1507) during 2015 to 2017, but decreased in 2018 and 2019 (Table 8.12). In 2019, only 9% of the VRE/VVE *E. faecium* isolates belonged to ST203-CT859.

In 2017, 3% of the *E. faecium* isolates belonged to the VVE clone, ST1421-CT1134 *vanA E. faecium*. This type was only detected from clinical samples from the Capital Region. In 2018, 34% of the *E. faecium* isolates belonged to ST1421-CT1134, they were detected in the Capital Region, the Region Zealand and from one DCM in the Region of Southern Denmark 2019 [Hammerum et al. Euro Surveill. 2020;25(18)]. During 2019, ST1421-CT1134 *vanA E. faecium* was the most prevalent type observed (50%) (Table 8.12). Furthermore, ST1421-CT1134 *vanA E. faecium* has spread to the Faroe Islands during 2018 and 2019 through patient transferring from Denmark [Hammerum et al. Euro Surveill. 2020;25(18)].

During 2019, the ST117-CT36 *vanB E. faecium* and its subtype ST117-CT991 *vanB E. faecium* increased in Denmark, which was both related to several introductions into Denmark from hospitals abroad and spread between hospitals due to patient transfer. Only two isolates belonged to this type in 2018, whereas 90 isolates were detected during 2019 (Table 8.12).

Conclusion

The increasing number of VRE/VVE cases in 2019 in Denmark is worrying. VRE can be carried in the intestine for a long period without showing any symptoms. Moreover, VRE can persist in the hospital environment, which makes infection control a difficult task. Infection control should include proper cleaning, focus on hand hygiene, VRE/VVE screening and subsequent isolation of patients. The spread of the "VVE clone", ST1421-CT1134 *vanA E. faecium*, in Denmark is of concern, especially because VVE diagnostic is challenging and therefore, the clone is likely to be underdiagnosed. The increase during 2019 of *vanB E. faecium* is also of concern.

Anette M. Hammerum, Louise Roer, Hülya Kaya, Sissel Skovgaard and Henrik Hasman
For further information: Anette M. Hammerum, ama@ssi.dk

Table 8.12 Description of the most common types of *vanA* and/or *vanB Enterococcus faecium* according to MLST and cgMLST, 2015-2019, Denmark

DANMAP 2019

Types[a]	2015		2016		2017		2018		2019	
	(n = 369)		(n = 427)		(n = 425)		(n = 515)		(n = 574)	
ST80-CT14 *vanA*	81	22%	38	9%	15	4%	1	<1%	1	<1%
ST80-CT24 *vanA*	23	6%	19	5%	11	3%	2	<1%	4	<1%
ST80-CT866 *vanA*	14	4%	10	2%	7	2%	N.D.	N.D.	N.D.	N.D.
ST80-CT1064 *vanA/vanB*	N.D.	N.D.	2	<1%	8	2%	23	4%	11b	2%
ST80-CT1729 *vanA*	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	22	4%	2	<1%
ST117-CT1180 *vanA*	N.D.	N.D.	N.D.	N.D.	9	2%	30	6%	14	2%
ST117-CT36/CT991 *vanB*	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	2	<1%	90	16%
ST203-CT859/CT1051/CT1507 *vanA*	188	51%	271	64%	265	63%	161	31%	54	9%
ST1421-CT1134 *vanA*	N.D.	N.D.	2	<1%	13	3%	176	34%	285	50%
Other types	62	17%	85	20%	97	23%	98	19%	113	20%

a) ST, sequence type (MLST); CT, cluster type (cgMLST); N.D., not detected
b) Two isolates were only *vanB* positive

8.3.4 Detection of linezolid resistant enterococci and linezolid-vancomycin resistant enterococci

Background

Linezolid can be used for treatment of infections caused by vancomycin-resistant enterococci. Resistance to linezolid in enterococci is often due to mutations in the V domain of the 23S rRNA genes. The G2576T mutation (*Escherichia coli* numbering) is the most common cause of the linezolid-resistant enterococci (LRE) phenotype. Another mutation in the 23S rRNA, G2505A, (*E. coli* numbering) has also been reported for LRE, but seems to be less frequent than the G2576T mutation. Furthermore, transferable resistance genes (*cfr*, *cfr*(B), *optrA* and *poxtA*) encoding linezolid resistance, have been described in enterococci, whereas amino acid substitutions in the ribosomal proteins L3, L4 and/or L22, suspected to cause decreased susceptibility to linezolid in staphylococci, are rare in enterococci.

In 2018, a web tool for detection of the 23S rRNA mutations (G2576T and G2505A), and the *optrA*, *cfr*, *cfr*(B) and *poxtA* genes from whole genome sequences from enterococci was developed [Hasman et al., J. Antimicrob. Chemother., 2019, 74:1473-1476].

Surveillance of linezolid-resistant enterococci (LRE) Danish departments of clinical microbiology (DCMs) have, on voluntarily basis, submitted LRE for surveillance to the National Reference Laboratory for Antimicrobial Resistance at Statens Serum Institut (SSI).

In DANMAP 2018, LRE isolates from 2015-2018 were investigated. During this period, eight linezolid-resistant *E. faecium* isolates and eight linezolid-resistant *E. faecalis* isolates were send to SSI (only one isolate per patient were included). In 2019, two linezolid-resistant *E. faecalis* isolates were send to SSI.

WGS data from the LRE isolates were investigated for 23S rRNA mutations (G2576T and G2505A), and the *optrA*, *cfr*, *cfr*(B) and *poxtA* genes using the LRE-Finder [https://cge.cbs.dtu.dk/services/ LRE-Finder/].

Among the LRE isolates from 2015-2019, LRE-Finder detected seven *E. faecium* with the G2576T mutation, one *E. faecium* with the G2505A mutation and 10 *E. faecalis* isolates with *optrA* (Table 8.13).

Surveillance of linezolid-vancomycin-resistant Enterococci (LVRE)

In order to detect linezolid-resistant VRE isolates (LVRE), the LRE-Finder was also implemented in the automated surveillance at SSI for detection of LRE mutations and LRE genes in genomes from VRE isolates submitted for the national VRE Surveillance and LRE/LVRE submitted from DCMs directly. During the period 2015-2019, no linezolid vancomycin resistant *E. faecalis* were detected, whereas, 11 linezolid-vancomycin resistant *E. faecium* were identified. Seven linezolid resistant *E. faecium* isolates had the G2576T mutation and were positive for the *vanA* gene encoding vancomycin resistance, three *E. faecium* isolates were positive for *optrA* and *vanA* and one *E. faecium* isolate were positive for *cfr*(B) and *vanB* (Table 8.13).

Conclusion

The findings of LRE and LVRE are of concern. Linezolid is used for treatment of VRE. Only a limited number of antimicrobial agents are available for treatment of infections with LVRE.

Anette M. Hammerum, Louise Roer, Hülya Kaya and Henrik Hasman
For further information: Anette M. Hammerum ama@ssi.dk

Table 8.13 Characterization of the 18 linezolid resistant enterococci (LRE) and the 11 linezolid vancomycin resistant enterococci (LVRE), 2015-2019, Denmark DANMAP 2019

	No. of isolates	Species	Linezolid resistance mechanism	Vancomycin resistant gene
LRE	1	*E. faecium*	G2505A	none
	7	*E. faecium*	G2576T	none
	10	*E. faecalis*	*optrA*	none
LVRE	7	*E. faecium*	G2576T	*vanA*
	3	*E. faecium*	*optrA*	*vanA*
	1	*E. faecium*	*cfr*(B)	*vanB*

8.3.5 *Streptococcus pneumoniae*

The surveillance of pneumococci (*Streptococcus pneumoniae*) causing invasive disease in Denmark is conducted through mandatory submission of clinical isolates to Statens Serum Institut (SSI). At SSI, the isolates are serotyped and tested for antimicrobial susceptibility. Any remaining cases without a submitted isolate are identified through MiBa.

In Denmark, 639 cases of invasive pneumococcal disease (IPD) were registered in 2019. The cases were mainly from pneumococci found in either blood (582) or cerebrospinal fluid (41). For 16 cases, pneumococci had been found in other, normally sterile sites (ascites, joint, pleura, brain), but data from these are by tradition not included in this report. In total 606 isolates were received at the reference laboratory out of the 623 cases of bacteraemia and meningitis identified in MiBa. Four of the 606 received isolates were not viable for susceptibility testing, but serotyping was nevertheless possible. Data for the seventeen remaining cases where isolates were not provided were retrieved from MiBa, and the failure of submitting an isolate for serotyping was mainly found to be due to non-viable isolates or diagnosis through PCR. Antimicrobial susceptibility data for those cases were retrieved, when available, through MiBa. In total, serotypes were available for 606 cases and antimicrobial susceptibility data for both penicillin and erythromycin was available for 612 cases (two additional cases had data for only one of the antimicrobials each registered in MiBa).

The 606 isolates from blood or cerebrospinal fluid belonged to 41 different serotypes. For the 612 cases with fully available susceptibility data, 569 were susceptible to both penicillin and erythromycin (93.0%). For penicillin, 583 out of 613 were susceptible (95.1%), 26 (4.2%) were classified as susceptible increased exposure and four isolates of different serotypes (0.7%) were classified as resistant. For erythromycin, 592 of 613 isolates were susceptible (96.6%) and 21 isolates (3.4%) were resistant.

Table 8.14 Number of invasive isolates and distribution of resistance in the most common sero-types of pneumococci, Denmark

DANMAP 2019

Serotype	N 2019	PEN-S_ ERY-S	PEN-S_ ERY-R	PEN-I_ ERY-S	PEN-I_ ERY-R	PEN-R_ ERY-S	PEN-R_ ERY-R	Unk	% S-S	N (% S-S) 2018	N (% S-S) 2017
8	162	162							100.0%	194 (100%)	192 (99%)
3	69	68						1	100.0%	70 (97%)	57 (100%)
22F	49	47	1	1					95.9%	69 (100%)	58 (100%)
12F	44	42	1	1					95.5%	55 (100%)	69 (99%)
9N	28	27		1					96.4%	62 (98%)	56 (98%)
23B	18	9		8	1				50.0%	14 (7%)	11 (27%)
16F	17	16	1						94.1%	19 (84%)	21 (95%)
11A	17	15	1	1					88.2%	19 (95%)	19 (89%)
24F	15	10	5						66.7%	17 (76%)	19 (79%)
15A	15	13			1			1	92.9%	25 (76%)	16 (63%)
23A	14	14							100.0%	13 (100%)	18 (100%)
35B	13	11		1	1				84.6%	15 (100%)	20 (90%)
33F	13	10	3						76.9%	17 (88%)	13 (92%)
20	13	13							100.0%	24 (100%)	26 (100%)
15C	11	9		1	1				81.8%	4 (100%)	3 (100%)
35F	9	8			1				88.9%	14 (100%)	13 (100%)
10A	9	9							100.0%	15 (100%)	9 (100%)
10B	9	9							100.0%	9 (100%)	9 (100%)
19A	8	4	1	1	1		1		50.0%	11 (82%)	5 (100%)
17F	8	5		3					62.5%	12 (50%)	10 (80%)
15B	8	8							100.0%	9 (100%)	14 (93%)
7C	8	8							100.0%	6 (83%)	3 (100%)
31	6	6							100.0%	15 (100%)	8 (88%)
19F	6	6							100.0%	7 (71%)	13 (69%)
6C	5	4		1					80.0%	5 (40%)	13 (92%)
Other	49	36		1		2	1	9	90.0%	42 (88%)	39 (87%)
Sum	623	569	13	20	6	2	2	11	93.0%	762 (93%)	734 (94%)

N = number of isolates, PEN = penicillin, ERY = erythromycin, % S-S = percentage of isolates that were susceptible to both penicillin and erythromycin

Figure 8.15 Non-wild-type (%) in *Streptococcus pneumoniae* blood and spinal fluid isolates from humans, Denmark DANMAP 2019

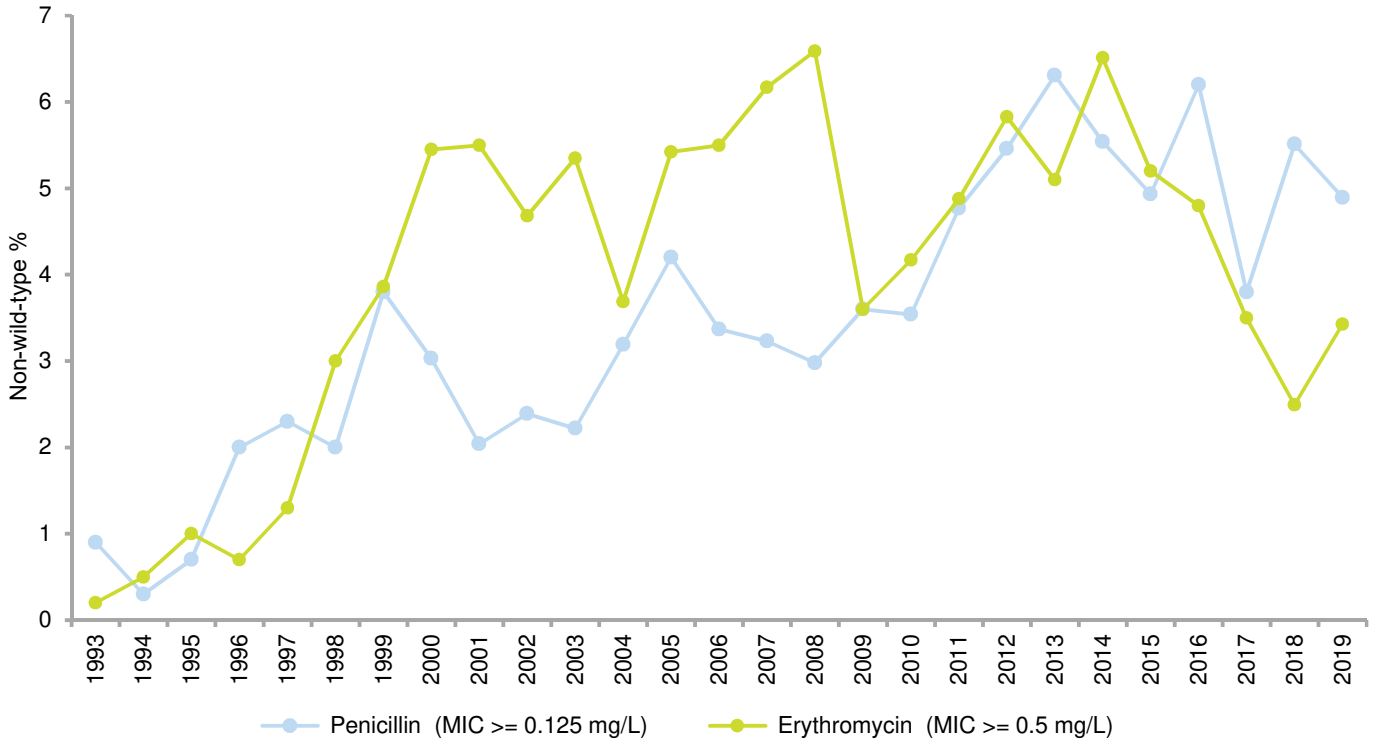
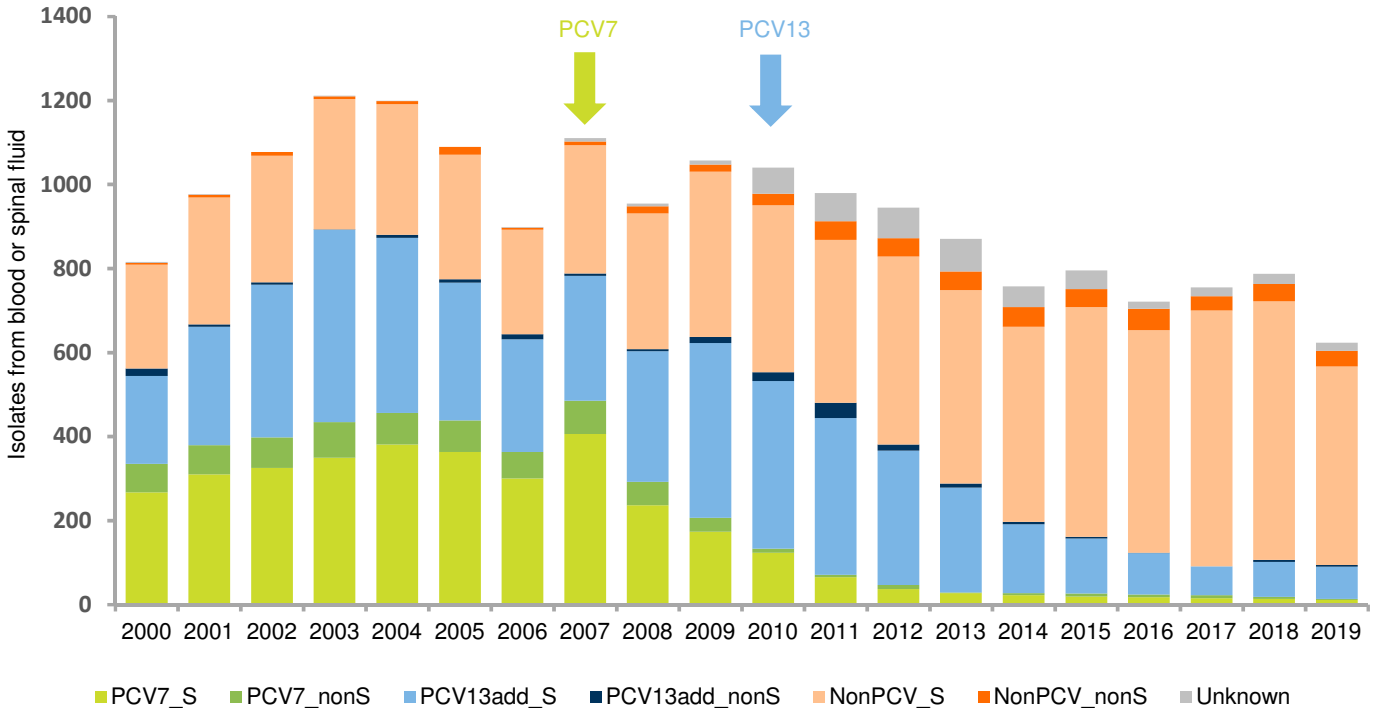


Figure 8.16 *Streptococcus pneumoniae* blood and spinal fluid isolates from humans. Vaccine serotypes and non-vaccine serotypes in combination with susceptibility to penicillin and erythromycin, Denmark DANMAP 2019



PCV7_S : PCV7 serotypes, susceptible to both penicillin and erythromycin
PCV7_nonS : PCV7 serotypes, non-susceptible to either penicillin or erythromycin
PCV13add_S : PCV13 serotypes not in PCV7, susceptible to both penicillin and erythromycin
PCV13add_nonS : PCV13 serotypes not in PCV7, non-susceptible to either penicillin or erythromycin
NonPCV_S : serotypes not included in PCV7 or PCV13, susceptible to both penicillin and erythromycin
NonPCV_nonS : serotypes not included in PCV7 or PCV13, non-susceptible to either penicillin or erythromycin
Unknown : cases where either serotype or susceptibility to penicillin or erythrymycin is unknown
The two arrows indicate when PCV7 and PCV13 were introduced in the Danish childhood immunization programme

Antimicrobial susceptibility in pneumococci is highly correlated to serotypes (Table 8.14), and variations in susceptibility patterns through the years often reflects changes in circulating serotypes. The pneumococcal vaccine introduced in the childhood vaccination programme in 2007 has had a profound effect on the serotypes that are causing invasive pneumococcal disease in Denmark (Figure 8.16), as it is clear that IPD caused by the seven serotypes included in the vaccine (PCV7 vaccine from 2007) and later 13 serotypes (PCV13 vaccine from 2010) has decreased markedly. The current predominant serotype is serotype 8 (26% in 2019), of which all invasive isolates isolated in 2019 and 2018 were fully sensitive to both penicillin and erythromycin.

Comparing the obtained results of antimicrobial susceptibility of IPD in Denmark in 2019 to the data reported in 2018 from our neighbouring countries, the levels of penicillin non-wild-type reported by EARS-Net were: Sweden (5.2%), Norway (5.0%) and Germany (5.3%). The levels of erythromycin resistance were: Sweden (4.5%), Norway (7.6%) and Germany (7.2%). Thus, the results of non-wild-type (I+R) for invasive pneumococci from Denmark in 2019 were similar to the reported values from 2018 from neighbouring countries with respect to penicillin, but markedly lower with respect to erythromycin resistance.

Conclusion

For penicillin, the level of non-wild-type in 2019 was lower than in 2018 (4.9% compared to 5.5%). For erythromycin, the level of resistance in 2019 was slightly higher than the level in 2018 (3.4% compared to 2.5%), but was nevertheless lower than for any of the years from 1999 to 2017, Figure 8.15.

There has been a trend of decreasing resistance to erythromycin since 2013, while the non-wild-type levels to penicillin are more variable. More information on the surveillance of invasive pneumococcal disease in Denmark can be found on the SSI homepage (EPI-NEWS, No 10-2020, https://en.ssi.dk/news/epi-news/2020/no-10---2020).

Tine Dalby and Hans-Christian Slotved
For further information: Tine Dalby, tid@ssi.dk

8.3.6 Beta-haemolytic streptococci

Beta-haemolytic streptococci (BHS) can be divided into serological groups according to antigenic properties of the polysaccharide capsule. In human infections, groups A, B, C and G are the most frequent species. Approximately 10% of humans are asymptomatic throat carriers of BHS of any group.

Streptococcus pyogenes (group A streptococci; GAS) cause pharyngitis, tonsillitis, otitis media, wound infections, and superficial skin infections, but also more severe infections, e.g., bacteraemia, necrotizing myofasciitis, and rarely meningitis. In Denmark, the rate of asymptomatic throat carriage of GAS is approximately 2%.

Streptococcus agalactiae (group B streptococci; GBS) may be present in the vaginal flora of 20-25% of women in the childbearing age. If these bacteria are transferred to the child during labour, meningitis and septicaemia may develop in the newborn. Such infections also occur in elderly or immunocompromised patients.

Streptococcus dysgalactiae subsp. *equisimilis* (group C streptococci; GCS, and group G streptococci; GGS) predominantly cause soft-tissue infections and sometimes bacteraemia.

This report presents data on antimicrobial resistance in non-duplicate invasive isolates (i.e. from normally sterile sites) of BHS submitted in 2019 to the Neisseria and Streptococcus Reference Laboratory. Isolates are received from all DCMs in Denmark. It is voluntary to submit isolates of BHS, and not all DCMs submit BHS of all groups.

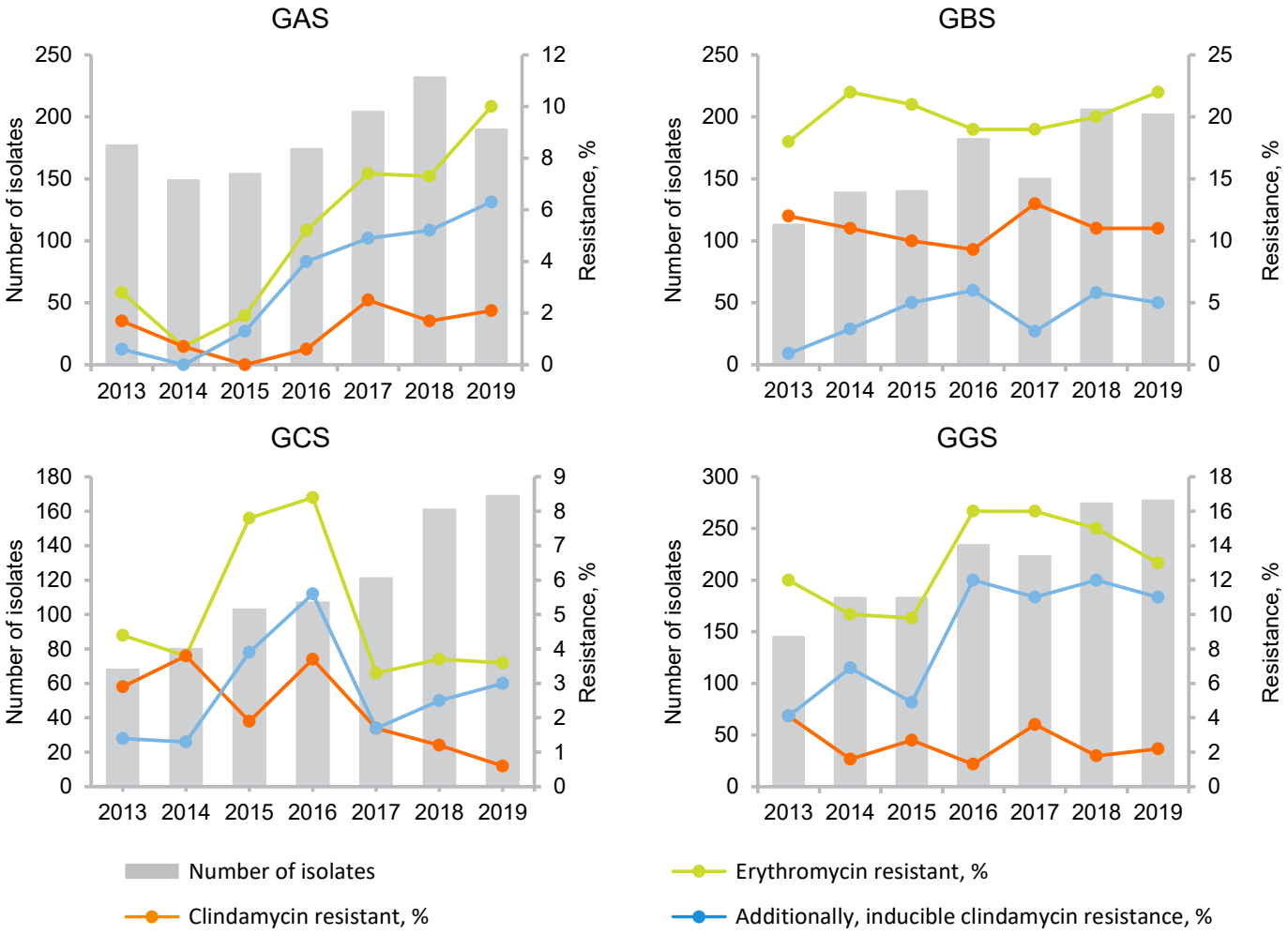
Infections with BHS are usually treated with penicillins or macrolides. All submitted isolates of BHS group A, B, C and G were therefore tested for susceptibility to penicillin, erythromycin, and clindamycin as well as inducible clindamycin resistance. Susceptibility testing was performed with disk diffusion methods. If resistance was observed, MIC was determined with Etest. All results were interpreted according to MIC breakpoints issued by EUCAST. For all isolates of GAS the *emm* type was determined by whole genome sequencing of the portion of the *emm* gene that dictates the M protein serotype.

Figure 8.17 shows the resistance findings for the years 2013 through 2019. In 2019, the number of submitted isolates from unique cases was 838, a slight decrease of 4% compared to 2018 (873). Corresponding changes for individual serogroups were: GAS, -18%; GBS, -2%; GCS, +5%; and GGS, +0.3%.

All isolates were susceptible to penicillin. The erythromycin resistance rate as well as the clindamycin resistance rate remained virtually unchanged compared to 2018 for all four serogroups. The percentage of isolates with inducible clindamycin resistance was: GAS, 6.3%; GBS, 5.0%, GCS 3.0%, and GGS, 11%. These percentages represented a slight increase for GAS and GCS, and a slight decrease for GBS and GGS. The percentage of isolates susceptible to all three antimicrobials was unchanged for all four serogroups.

The GAS isolates belonged to 32 different *emm* types. The majority of the received isolates (124; 65%) belonged to six *emm* types, each of which were represented by at least nine isolates (Table 8.15). The remaining 66 isolates (35%) belonged to 26 different *emm* types.

Figure 8.17 Beta-haemolytic streptococci: Antimicrobial resistance testing results. Numbers of isolates and resistance in percent
DANMAP 2019



GAS = group A streptococci, GBS = group B streptococci, GCS = group C streptococci, GGS = group G streptococci

Table 8.15 Group A streptococci 2019: *emm* types, clindamycin resistance and erythromycin resistance. Number of isolates
DANMAP 2019

	CLI-R		CLI-S		
emm type	ERY-R	ERY-S	ERY-R	ERY-S	Total
1.0	0	0	0	33	33
28.0	1	0	0	28	29
89.0	2	0	0	25	27
12.0	0	0	0	16	16
4.0	0	0	0	10	10
75.0	1	0	0	8	9
Subtotal	4	0	0	120	124
Other	12	0	3	51	66
Total	16	0	3	171	190

Abbreviations: CLI = clindamycin, ERY = erythromycin, R = resistant, S = sensitive, *emm* = the M protein gene
Numbers of isolates are shown for individual *emm* types if ≥9. Otherwise, if <9, the numbers are summarized in the "Other" category

Conclusions

The number of submitted isolates of group A was considerably lower in 2019 than in 2018, but virtually unchanged for the three other serogroups. All isolates were susceptible to penicillin. The erythromycin resistance rate as well as the clindamycin resistance rate remained virtually unchanged compared to 2018 for all four serogroups.

Steen Hoffmann and Hans-Christian Slotved
For further information: Steen Hoffmann, hof@ssi.dk

8.3.7 *Haemophilus influenzae*

Haemophilus influenzae is part of the normal upper respiratory tract flora, where colonisation varies with age. *H. influenzae* can also cause infections, with otitis media and bacterial sinusitis being the most common clinical manifestations. Invasive infections with *H. influenzae* happen relatively rarely and occur predominantly in the very young or elderly patients but may also afflict individuals with underlying conditions, such as chronic obstructive pulmonary disease or cancer. *H. influenzae* is classified into six capsular serotypes (a, b, c, d, e and f, also known as Hia, Hib, Hic, Hid, Hie and Hif), as well as noncapsular (non-typeable, NTHi). Introduction of the polysaccharide type b vaccine in 1993 significantly reduced the number of cases with systemic infection caused by *H. influenzae* type b (Hib) isolates. Before the vaccine was introduced, there were around 80 cases of Hib meningitis annually among infants in Denmark. This has been reduced to 0-2 cases per year. NTHi for which no vaccine yet exists is now the predominant type found among invasive *H. influenzae* infections.

Invasive *Haemophilus influenzae*

Surveillance of invasive Hib is mandatory by submission of the isolate to Statens Serum Institut (SSI). By tradition, most departments of clinical microbiology are voluntarily submitting all isolates of invasive *H. influenzae*. The received isolates are then serotyped and biotyped by the reference laboratory at SSI. Thus, isolates are submitted for the majority of cases, and the remaining cases can be identified through the Danish Microbiological Database (MiBa). Thereby, all invasive infections with *H. influenzae* are registered in the surveillance database, and for the majority of cases serotypes are available. In 2019, all isolates were tested at SSI for antimicrobial susceptibility with disc diffusion assays and the betalactamase test. Whole genome sequencing was also performed on the received isolates, and the data were analysed for the presence of the plasmid-borne beta-lactamase genes TEM-1 and ROB-1. For cases where isolates were not received, antimicrobial susceptibilities were retrieved through MiBa, when available.

The present report includes all episodes of invasive *H. influenzae* as identified through MiBa, where the date of sampling was in 2019. A total of 114 cases were identified, of which isolates from 100 (88%) were received at the reference laboratory. *H. influenzae* were isolated from cerebrospinal fluid in nine of the 114 cases, from blood in 102, from pleural fluid in two and from joint fluid in one of the cases. The serotypes of the received isolates were: six Hib (6%), one Hie (1%), fifteen Hif (15%) and 78 NTHi (78%). The age-distribution of the cases is presented in Figure 8.18.

Figure 8.18 Different serotypes in invasive *H. influenzae* cases according to age, 2019, Denmark DANMAP 2019

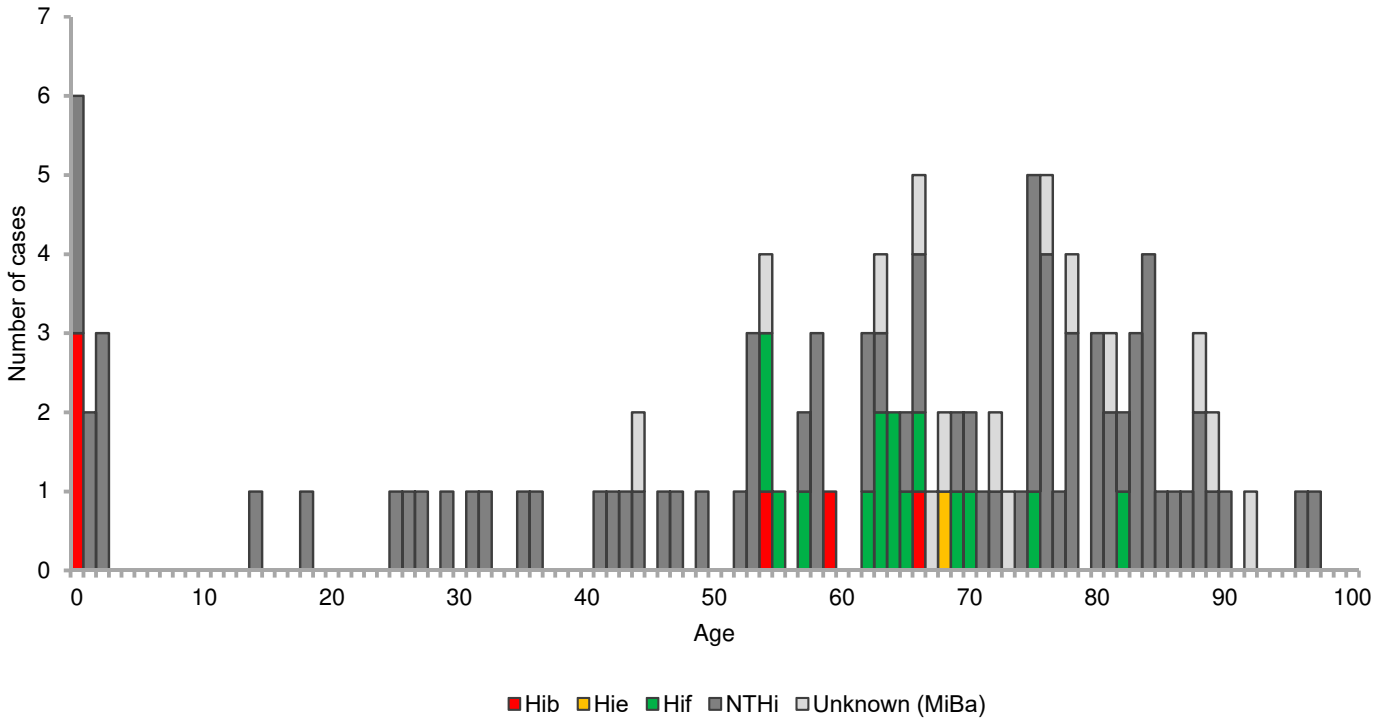


Table 8.16 Distribution of antimicrobial susceptibilities in invasive *H. influenzae* according to serotypes, 2019, Denmark

DANMAP 2019

	Hia	Hib	Hie	Hif	NTHi	Unknown*	All (2019)	All(2018)
Penicillin: no result registered	-	-	-	-	-	1	1	1
Penicillin: I and S	-	5 (83%)	1 (100%)	15 (100%)	57 (73%)	6 (46%)	84 (74%)	89 (74%)
Penicillin: R	-	1 (17%)	-	-	21 (27%)	7 (54%)	29 (26%)	31 (26%)
Ampicillin: no result registered	-	-	-	1	1	3	5	4
Ampicillin: I and S	-	5 (83%)	1 (100%)	14 (100%)	57 (74%)	8 (73%)	85 (78%)	94 (80%)
Ampicillin: R	-	1 (17%)	-		20 (26%)	3 (27%)	24 (22%)	23 (20%)
Cefuroxime: no result registered	-	-	-	-	-	1	1	18
Cefuroxime: I and S	-	6 (100%)	1 (100%)	15 (100%)	69 (88%)	8 (62%)	99 (88%)	87 (84%)
Cefuroxime: R	-	-	-	-	9 (12%)	5 (38%)	14 (12%)	16 (16%)
Amoxi-clav: no result registered	-	-	-	-	-	3	3	29
Amoxi-clav: I and S	-	6 (100%)	1 (100%)	15 (100%)	70 (90%)	8 (73%)	100 (90%)	83 (90%)
Amoxi-clav: R	-	-	-	-	8 (10%)	3 (27%)	11 (10%)	9 (10%)

*The group "unknown" represent the 14 cases that were registered in MiBa, but where an isolate was not received at the reference laboratory at SSI, and therefore not serotyped
Amoxi-clav = amoxicillin-clavulanic acid

Nineteen of the received isolates harboured the TEM-1 gene (one Hib and 18 NTHi) which represented 20% of 93 analysed isolates. All TEM-1 gene positive isolates had corresponding phenotypical betalactamase activity. None of the isolates harboured the ROB-1 gene. Susceptibility results, divided in to serotypes, for penicillin, ampicillin, amoxicillin-clavulanic acid and cefuroxime, are presented in Table 8.16. Due to differences across the departments of clinical microbiology in the reporting of non-resistant isolates, the "S" and "I" interpretations are combined in the table.

The results from antimicrobial susceptibility testings showed that in total there was 26% resistance to penicillin, 22% to ampicillin, 12% to cefuroxime and 10% to amoxicillin-clavulanic acid. Some variation across serotypes was observed. These figures are very similar to what was observed in 2018 (where data were exclusively extracted from MiBa).

In summary, the majority of isolates from invasive infections with *H. influenzae* are of the non-capsular type. This is similar to previous years and also similar to what is observed generally in Europe [R. Whittaker, Emerg Infect Dis, 2017, Mar;23(3):396-404]. The majority of the invasive *H. influenzae* isolates in Denmark in 2019 were found to be non-resistant to the antimicrobials tested, with the non-capsular isolates showing the highest degree of resistance.

Tine Dalby
For further information: Tine Dalby, tid@ssi.dk

8.3.8 *Staphylococcus aureus*

Staphylococcus aureus is part of the normal flora of the skin and mucosa. Approximately 50% of humans will be colonized with *S. aureus* in their nose or throat at any given time, some of these carrying the strain only intermittently, others for longer periods of time. *S. aureus* causes infections ranging from superficial skin infections e.g. impetigo and boils to deeper and more complicated infections. These may be health care associated such as post-operative wound infections and infections related to intravenous catheters and prosthetic devices but they can also be caused by endogenous spread of bacteria causing septic arthritis, osteomyelitis, endocarditis and bacteraemia. Most of these may have a fulminant progress and are associated with high mortality.

In Denmark, a voluntary surveillance program of all *S. aureus* bacteraemia cases was established in 1957. By comparison with the numbers of bacteraemia cases registered in the Danish Microbiology Database (MiBa) since 2010, the number of reported cases and bacterial isolates submitted to SSI has been almost complete (94-97%).

Laboratory and clinical notification of all cases of methicillin-resistant *S. aureus* (MRSA) has existed since November 2006. At SSI, all referred isolates are initially tested using a multiplex PCR detecting: the *spa*, *mecA*, *hsd*, *scn* and *lukF-PV* (PVL) genes. *spa* is used as *S. aureus* specific marker and for subsequent typing by Sanger sequencing, *mecA* to determine MRSA status, and *scn* and *hsd* as markers for human adaptation and relation to the clonal complex (CC) 398, respectively. PVL has been closely linked to skin abscesses and the very rare condition of severe necrotising pneumonia. PVL is rarely found in methicillin-susceptible *S. aureus* (MSSA) causing bacteraemia but has been associated with certain community acquired (CA) MRSA strains. Isolates positive for *mecA* and the CC398 specific *hsd* fragment but negative for *scn* (human adaptive factor) and *pvl* genes are considered typical livestock associated MRSA (LA-MRSA) and are not *spa* typed. All others, including human adapted CC398 isolates, are *spa* typed. In addition, all bacteraemia cases and *mecA* negative presumptive MRSA are tested for presence of the *mecC* gene.

A representative selection of bacteraemia isolates is tested for antimicrobial susceptibility against 17 antimicrobials (see chapter 9 for more information). For MRSA cases, demographic and epidemiological information is registered. Based on the epidemiological information each case is classified with respect to possible place of acquisition: hospital (HA), community (CA), healthcare-associated with a community onset (HACO), import (IMP) and livestock-associated (LA) MRSA. For CA, HACO and LA, classification was separated into known and not known exposure.

Surveillance of bacteraemia

In 2019, altogether 2,233 *S. aureus* bacteraemia cases corresponding to 38.5 cases per 100,000 inhabitants were reported from the departments of clinical microbiology (DCMs) in Denmark. This is almost the same number as in 2018 (2,276). Forty-six (2.1%) of the bacteraemia cases were caused by MRSA. During the last decade the proportion has been between 1.3% (2010 and 2012) and 2.9% (2014) and remains below most other European countries participating in EARS-Net [EARS-Net 2018]. LA-MRSA CC398 caused eight of the 46 MRSA bacteraemia cases. The number of LA-MRSA CC398 bacteraemia cases in 2018 was eight as well. Within 30 days from the bacteraemia onset, 518 (23%) patients died (all cause mortality). The mortality for the MRSA bacteraemia cases was 17% (n = 8, of which 1 was due to CC398 MRSA).

A total of 549 representative isolates were tested for antimicrobial resistance. Results from antimicrobial susceptibility testing in *S. aureus* bacteraemia isolates from 2010–2019 are presented in Table 8.17. Resistance to penicillin in 2019 was 72%. At the beginning of the 1990s resistance to penicillin was around 86% and in 2010 75%, thereby a continuing decreasing trend was observed. The highest frequency of resistance to other antimicrobials than penicillin was observed for fusidic acid (14%), erythromycin (9%), clindamycin (8%) and norfloxacin (5%). For most antimicrobial agents, the susceptibility remained at the same level as the previous years.

Table 8.17 Resistance (%) in isolates from *Staphylococcus aureus bacteraemia* cases 2010-2019, Denmark DANMAP 2019

Antimicrobial agent	2010 %	2011 %	2012 %	2013 %	2014 %	2015 %	2016 %	2017 %	2018 %	2019 %
Methicillin	1.4	1.4	1.2	1.7	2.9	1.5	2.1	2.2	1.6	2.1
Penicillin	75	77	74	76	77	71	71	72	72	72
Erythromycin	5	7	6	7	8	7	7	6	5	9
Clindamycin	4	6	6	6	8	7	6	5	4	8
Tetracycline	3	2	2	3	5	4	3	3	3	2
Fusidic acid	13	13	14	15	15	16	12	14	17	14
Rifampicin	<1	<1	<1	0	<1	<1	<1	<1	<1	<1
Norfloxacin	3	4	4	5	6	6	4	4	4	5
Kanamycin	1	<1	1	2	2	3	1	1	2	<1
Linezolid	0	0	0	0	0	0	0	0	0	0
Mupirocin	<1	<1	<1	<1	<1	<1	0	<1	0	<1
Trimethoprim-sulfamethoxazole	nt	<1	1	1	1	<1	<1	<1	0	<1
Numbers tested	1416	1515	1523	962	381	502	560	551	504	549

nt = not tested. In web annex Table A8.1 the distribution of MICs and resistance for all tested antimicrobial agents are shown

Table 8.18 The ten most prevalent *spa* types demonstrated in SAB and in non-LA-CC398 MRSA cases, Denmark 2019 DANMAP 2019

SAB			MRSA			
spa type	CC group	No. of cases	*spa* type	CC group	No. of cases	No. causing infections (%)
t127	CC1	116	t304	CC8	230	99 (43)
t091	CC7	95	t223	CC22	181	80 (44)
t084	CC15	91	t008	CC8	153	95 (62)
t230	CC45	73	t127	CC1	151	74 (49)
t002	CC5	72	t002	CC5	134	58 (43)
t012	CC30	65	t4549	CC8	109	89 (82)
t021	CC30	65	t044	CC80	69	31 (45)
t008	CC8	59	t005	CC22	54	32 (59)
t701	CC8	56	t019	CC30	48	34 (71)
t015	CC45	36	t021	CC30	43	23 (53)

CC = Clonal complex, SAB = *S. aureus* bacteraemia, spa = *S. aureus*-specific staphylococcal protein A, MRSA = Methicillin-resistant *Staphylococcus aureus*

Typing revealed 632 different *spa* types distributed in 27 different clonal complexes (CCs). The ten most prevalent *spa* types, representing 33% of the total number, are presented in Table 8.17 together with *spa* types from the most prominent types of MRSA. The distribution of the most prevalent *spa* types has been very stable over the last decade with only minor changes in the relative order. The PVL toxin was present in 41 (1.8%) cases of which six were MRSA. The 41 PVL presenting isolates were distributed among 22 different *spa* types and 11 different CCs.

Surveillance of methicillin-resistant *S. aureus*

In 2019, 3,657 MRSA cases were detected (63.0 per 100,000 inhabitants). This was almost the same number as in 2018 (3,669; Figure 8.19). For the last four years, the numbers of new MRSA cases seem to be levelling off. A case was defined when a person for the first time tested positive for a specific MRSA strain regardless of clinical situation (infection or colonisation).

CC398 cases constituted 32% (n = 1,163) of new MRSA cases, of which 1,122 belonged to the LA-MRSA CC398 and the remaining 41 to a human adapted variant harbouring the PVL encoding genes. The number of LA-MRSA CC398 is lower than the previous five years. The decrease in number of cases may be influenced by the fact that only new cases are registered in the surveillance program. Many people in contact with livestock have already been examined and tested positive at an earlier stage and also cases where the clinical situation changes from colonisation to infection will thus not be registered as new cases.

MRSA isolates carrying *mecC* were detected in 35 cases (1.0%). Twenty-seven of the cases (77%) had infections at the time of diagnosis. Two patients reported contact to horses, which previously have been shown to be reservoirs for *mecC* MRSA. The remaining 33 patients reported no known contact to any livestock.

In the course of 2019, 23 MRSA outbreaks were registered at hospitals, nursing homes and other institutions. These outbreaks comprised a total of 157 cases. Seven of the outbreaks occurred in neonatal departments, comprising a total of 85 cases. Additionally, eight outbreaks were observed in nursing homes (counting a total of 38 residents) and in two residential schools (11 pupils).

The total number of cases and the number of cases presenting with infection according to epidemiological classification are shown in Table 8.19. Most of the cases (82%) were acquired in Denmark. At the time of diagnosis, 44% (n = 1,599) of cases had infection, which was similar to 2018 (40%).

Figure 8.19 Number of new MRSA cases with a three years moving average, Denmark DANMAP 2019

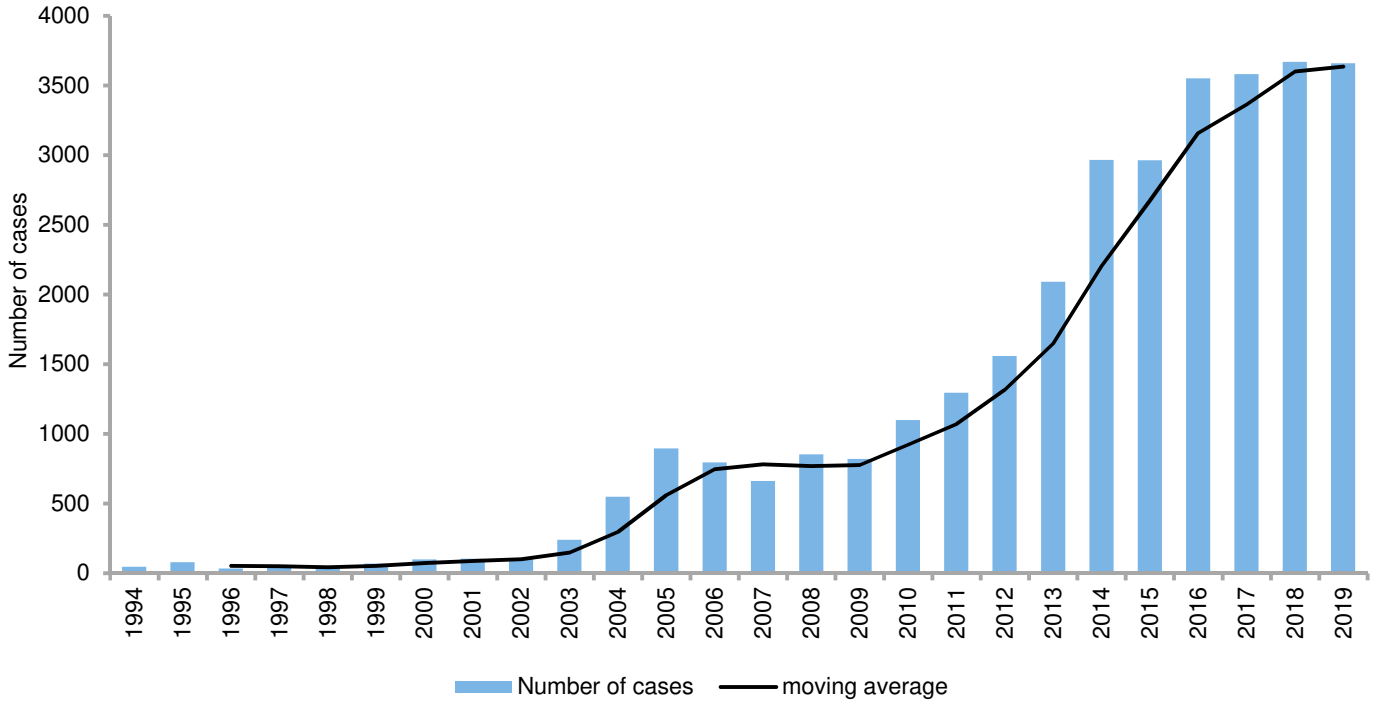
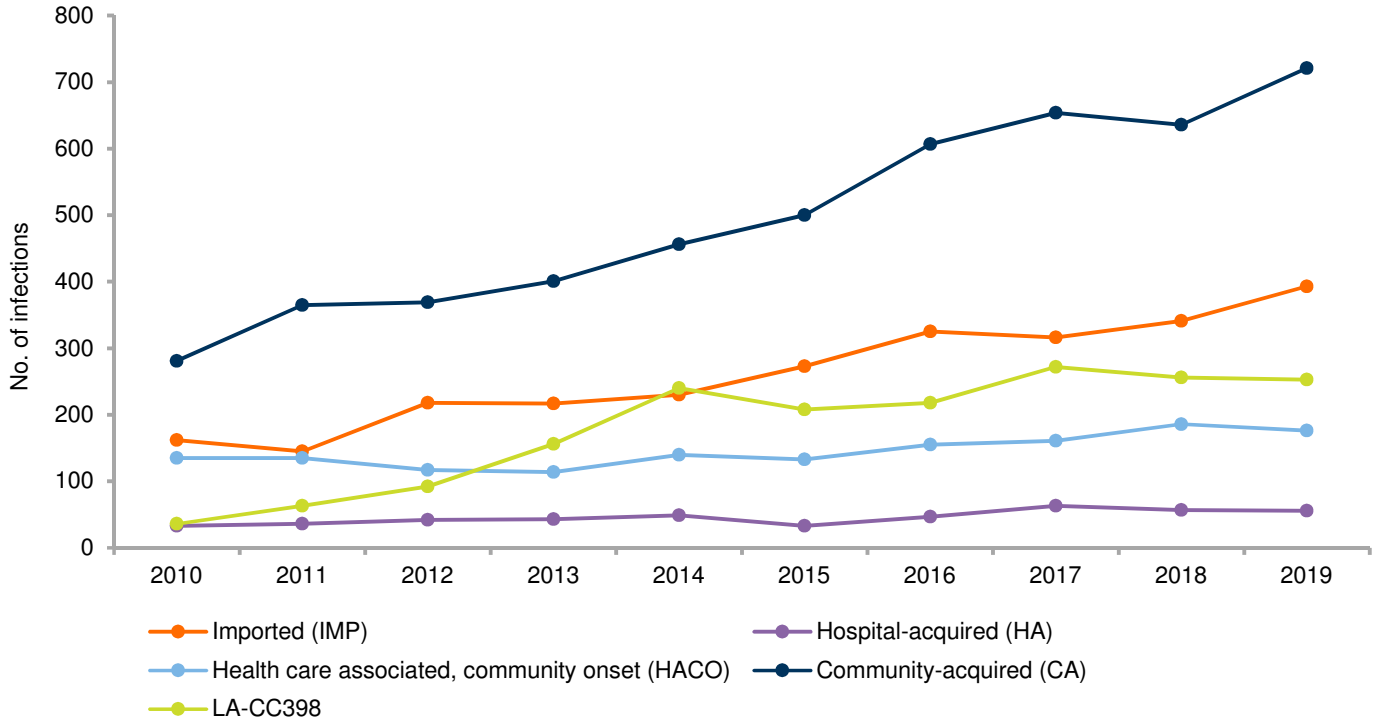


Table 8.19 Epidemiological classification of new MRSA cases, Denmark 2019 DANMAP 2019

Epidemiologic classification	Exposure	No. of cases (% of total)	No. (%) of cases with infections
Imported (IMP)		**651 (18)**	393 (60)
Hospital-acquired (HA)		**75 (2)**	36 (48)
Healthcare associated, community onset (HACO)		**229 (6)**	
	with known exposure	26	12 (46)
	without known exposure	203	162 (80)
Healthcare worker		**44 (1)**	22 (50)
Community-acquired (CA)		**1536 (42)**	
	with known exposure	844	143 (17)
	without known exposure	692	578 (84)
LA-MRSA CC398		**1122 (31)**	
	with known exposure	993	167 (17)
	without known exposure	129	86 (67)

Numbers shown in bold are totals

Figure 8.20 Number of MRSA infections according to epidemiological classification, Denmark DANMAP 2019



The trend of MRSA infections for 2010-2019 based on their epidemiological classification is shown in Figure 8.20. Community-acquired infections increased to 721 cases (corresponding to that 47% of the total number of CA MRSA cases presented with infections in 2019, Table 8.19). Imported cases presented with infections in 60% of the cases and the number of infections in this category has been increasing from less than 100 cases in 2007 to 393 cases in 2019. The number of HA, HACO and LA cases with infection were at a similar level as in 2018.

It should be noted that the average time patients are hospitalised has decreased over the years to approximately three days, which means that some HA-MRSA cases may not be recognized before patients are discharged.

Molecular typing of the MRSA strains

In total, *spa* typing revealed 365 different strain types, not including isolates belonging to LA-CC398. Among the infections, 285 *spa* types were demonstrated. The 10 dominating non-LA-CC398 *spa* types isolated in 2019 are listed in Table 8.18. They constituted 47% of the total number of non-LA-CC398 MRSA isolates. *spa* types t304 and t223 have been among the five most prevalent *spa* types since 2016 and can be linked to the refugee crisis following the civil war in Syria. Table 8.17 does not list *spa* types in CC398, which for many years has been the dominating CC.

The PVL encoding gene was detected in 29% of the infections and in 13% of the asymptomatic carriers and most often in

Table 8.20 Resistance (%) in non LA-CC398 MRSA isolates, 2010-2019, Denmark DANMAP 2019

	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019
Erythromycin	44	37	38	32	33	37	34	34	33	33
Clindamycin	34	27	29	24	23	29	25	27	28	23
Tetracycline	20	17	18	20	21	24	26	24	26	22
Fusidic acid	16	15	17	17	17	19	18	16	18	23
Rifampicin	3	2	1	1	<1	<1	1	1	1	<1
Norfloxacin	32	27	25	23	27	21	19	20	21	21
Kanamycin	33	31	26	29	30	32	28	26	28	31
Linezolid	0	0	0	<1	<1	0	<1	0	<1	0
Mupirocin	4	<1	<1	<1	<1	<1	<1	<1	<1	<1
Trimethoprim-sulfamethoxazole	ND	1	2	3	3	4	2	3	3	4
Number of tested isolates	984	1125	1324	1451	1616	1242	1184	1193	1233	1025

relation to isolates with *spa* types t008 (n = 109), t044 (n = 65), t019 (n = 45), t005 (n = 38) and t021 (n = 37).

Resistance among MRSA isolates

Resistance data for non-LA-CC398 isolates are presented in Table 8.20. Forty percent of non-LA-CC398 isolates received in 2019 was tested (n = 1,025). Resistance prevalences were similar to previous years.

Andreas Petersen and Anders Rhod Larsen
For further information: Andreas Petersen, aap@ssi.dk

8.3.9 *Neisseria gonorrhoeae*

Neisseria gonorrhoeae is the causative agent of the sexually transmitted infection gonorrhoea, which is usually located in the urethra in males and cervix in females. *N. gonorrhoeae* (gonococci) may also sometimes be demonstrated in specimens from the pharynx or rectum in either gender. In females, the finding of gonococci in rectal specimens is usually due to contamination with vaginal secretion, while in men who have sex with men (MSM) it may be due to unprotected anal sex leading to infection. In both genders, the condition is generally asymptomatic. Pharyngeal gonorrhoea is virtually always asymptomatic.

Complications of gonorrhoea include e.g. salpingitis, epididymitis, orchitis, and prostatitis. Furthermore, conjunctivitis may occur in newborns after transmission from an infected mother during labour and rarely in adults following direct or indirect inoculation.

Surveillance

Methods: Since 1962, the departments of clinical microbiology in Denmark have voluntarily submitted isolates of gonococci to the Neisseria and Streptococcus Reference (NSR) laboratory at Statens Serum Institut for national surveillance of antimicrobial resistance.

At the NSR laboratory ceftriaxone, ciprofloxacin and azithromycin MICs were determined using the Etest® on chocolate agar incubated at 35°C in 5% CO_2. The breakpoints used were those defined by EUCAST. The breakpoints for azithromycin were changed as of January 1, 2019 (EUCAST version 9.0) where only an epidemiological cut-off value (ECOFF), MIC >1 mg/L was defined. This is because azithromycin should only be used in conjunction with another efficient agent. Until January 1, 2019, S was defined by MIC ≤ 0.25 mg/L and R by MIC >0.5 mg/L. These breakpoints were used in this report in order to enable comparison of azithromycin data for 2019 with those from previous years.

Both resistant and intermediate susceptible isolates have through the years been categorised as resistant in the DANMAP reports. However, isolates with intermediate susceptibility/susceptible increased exposure are non-existing according to EUCAST breakpoints for ceftriaxone and azithromycin and they are exceedingly rare regarding ciprofloxacin. Penicillinase production was tested for using the Nitrocephin assay.

As part of NSR's participation in ECDC's surveillance of sexually transmitted infections since 2009, 110 - 120 gonococcus isolates are collected consecutively each year and investigated for susceptibility to an expanded panel of antimicrobial agents. This panel includes cefixime and in selected years also spectinomycin and sometimes gentamicin.

Results and discussion: Most of the received isolates were from urethra or cervix, while clinicians only rarely obtain specimens from rectum and pharynx. Occasionally, the NSR laboratory received strains isolated from other anatomical sites, such as conjunctivae, joint fluid, blood, Bartholin's abscess, etc.

The NSR Laboratory received isolates from 1,119 unique cases of gonorrhoea diagnosed in 2019. The annual number increased considerably from 2011 through 2016 (Figure 8.21). This was partly because of the widespread use of combined nucleic acid amplifications tests (NAATs) for *Chlamydia trachomatis* and *N. gonorrhoeae* which have identified unexpected cases of gonorrhoea (sometimes followed by culture), and partly due to an increasing incidence of gonorrhoea, especially among young heterosexual persons, among whom an increas-

ing proportion are women, at least until 2016. A slight decrease in the annual number of isolates from unique cases was observed in 2017 and a more pronounced decrease in 2018 continuing in 2019. It should be recalled, however, that many cases diagnosed by NAATs are not followed-up by culture.

Figure 8.21 Number of submitted gonocccus isolates from males and females, and the percentage of isolates with ciprofloxacin resistance, azithromycin resistance, and penicillinase production DANMAP 2019

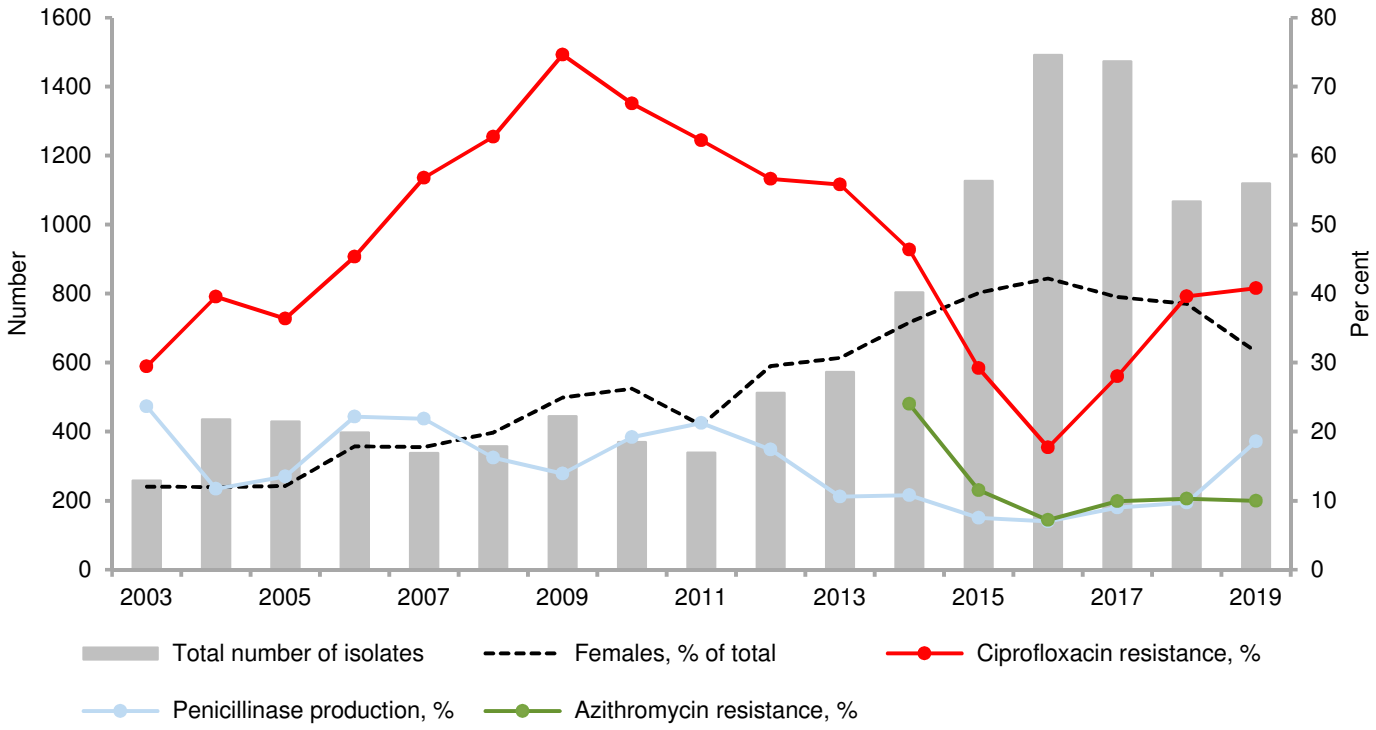
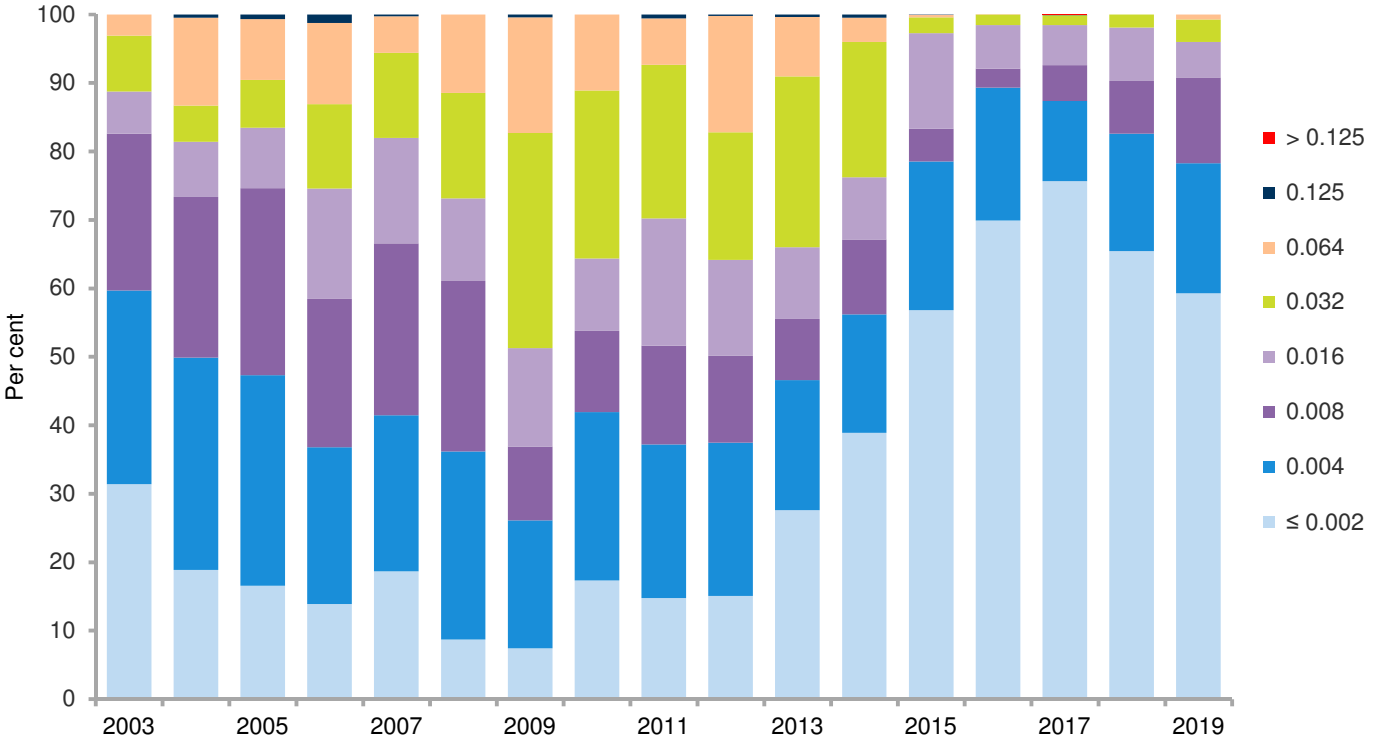


Figure 8.22 Distribution of ceftriaxone MIC (mg/L) values in gonococci DANMAP 2019



The ciprofloxacin resistance rate was 41% in 2019 (40% in 2018, 28% in 2017 and 18% in 2016), thus still considerably lower than the peak of 75% in 2009 (Figure 8.21). The percentage of strains producing penicillinase was 19%, i.e. an increase since 2018 (10%). It has fluctuated between 22% in 2005 and 7% in 2016. Azithromycin non-wild-type (MIC above the present ECOFF >1 mg/L) was found in 4.6% of the tested isolates. Using the old resistance breakpoint on 2019 data (MIC >0.5 mg/L) 10% of the isolates was found resistant, the same as in 2018 (Figure 8.21).

Ceftriaxone resistant gonococci (MIC >0.125 mg/L) as well as ceftriaxone treatment failure in patients with gonorrhoea have occurred in several countries during recent years. During 2003 through 2009, the proportion of isolates with ceftriaxone MIC ≥ 0.008 mg/L gradually increased from 40% to nearly 75% (Figure 8.22). During recent years this trend has nearly reversed, the proportion being 44% in 2014, 11% in 2016 and 17% in 2018, although slightly increasing to 22% in 2019.

The Danish National Board of Health issued guidelines in 2015 for the diagnosis and treatment of sexually transmitted infections. A combination of high dose ceftriaxone (500 mg i.m.) and azithromycin (2 g) is recommended for the treatment of gonorrhoea. Ciprofloxacin (500 mg p.o.) may be used for treatment if the strain is fully susceptible. In 2017, there was a single case among the submitted isolates with ceftriaxone MIC of 0.25 mg/L and azithromycin MIC of 0.25 mg/L. The use of this combination therapeutic regimen has been gradually abandoned during 2019.

In a subset of 119 isolates, resistance against cefixime (MIC >0.125 mg/L) was 0% in 2019, like in 2018. Cefixime is an oral cephalosporin that has never been used in Denmark. Resistance against spectinomycin was 0% in 2019 as well as in the years 2015 through 2018. MIC values for gentamicin were 1 to 4 mg/L, but no breakpoints are defined for this agent against gonococci.

Conclusions

The ciprofloxacin and azithromycin resistance rates and the ceftriaxone MIC distribution were virtually unchanged in 2019 compared to 2018. Although resistance problems among gonococci are still not present in Denmark, the frequent emergence globally of gonococcal resistance to antibiotics underlines the importance of maintaining the centralised national surveillance of antimicrobial resistance in gonococci.

Steen Hoffmann
For further information: Steen Hoffmann, hof@ssi.dk

Textbox 8.2

Incidence of multiresistant bacteria in Greenland

Background: Greenland has a population of 55,992 inhabitants (January 2019) and Nuuk is the capital with around 18,000 inhabitants. Greenland is an autonomous administrative country of Denmark; it has its own Ministry of Health and the country is divided into five health regions. Although sparsely populated, due to its big geographic dispersion, there are five smaller hospitals, one national hospital and 11 health care centres in the five health regions. The national and largest hospital, Dronning Ingrids Hospital (182 beds), is situated in Nuuk. Around 15-16,000 persons are admitted to hospital at least once a year. Patients with specific or serious diseases that cannot be treated at Dronning Ingrids Hospital are transferred to Den-mark or Iceland for further treatment e.g. haemodialysis, cancer treatment, brain surgery etc.

Resistant bacteria: From 2000 to 2019, 54 patients were diagnosed with methicillin-resistant *Staphylococcus aureus* (MRSA), 116 patients with extended spectrum beta-lactamase (ESBL)-producing Enterobacterales, four patients with vancomycin-resistant enterococci (VRE), and 177 patients with *Clostridioides difficile* (formerly known as *Clostridium difficile*) infection among whom 55 had the 027 type.

MRSA: Since 2015, a nearly 4-fold increase in incidence of MRSA has been observed. The largest increase was seen during 2017, and the main reason for this was an outbreak involving 12 persons in Tasiilaq at the East coast of Greenland (described in details in DANMAP 2017). In 2018, only four new persons were reported with MRSA including one premature child whom carried MRSA in nose and throat. The child was colonised with MRSA while hospitalised in Denmark due to an MRSA-outbreak (MRSA t223, CC22) at the neonatal ward. The mother of the child was MRSA-negative at time of the detection in 2018, but in February 2019 she was also tested positive from the nose with the same MRSA-type as her child. In 2019, there were also four new persons with MRSA: three adults (including the above mentioned mother) and one child. The adults were all MRSA-carriers – two of them in the nose and one in the throat. Two adults were husband and wife and the wife was colonised with MRSA while hospitalised in Denmark; one month later the husband was positive with the same MRSA-type (MRSA t902, CC22). The child (3½ years old) had a wound in the nose without any history of hospitalisation or traveling. In all four cases there was no further transmission in Greenland among family/household or at the hospital.

VRE: In spite of ongoing VRE outbreaks in Denmark, only four patients have been diagnosed with VRE in Greenland. Two patients were colonised with VRE in the rectum, one patient had pleurisy and one patient had VRE in the urine. In all four cases VRE occurred after hospitalisation in Denmark. No transmission was observed in the wards.

CPO: In recent years, an increase in incidence of carbapenemase-producing organisms (CPO) in Denmark has been observed but until now, no CPO has been reported in Greenland.

Other resistant bacteria: Most of the other resistant bacteria observed were imported from Denmark or abroad, but in some cases, especially in patients with ESBL-producing Enterobacterales, treatment with broad-spectrum antimicrobial agents in Greenland probably selected for these bacteria. From 2012 to 2013, there were outbreaks with *C. difficile* type 027 in several hospitals, and transmission within the country occurred. But due to a great effort in infection prevention and control from the hospital staff, these outbreaks were quickly stopped. Of the 12 new patients with *C. difficile* infection diagnosed in 2019, one patient was infected with the 027 type.

Conclusion: Continued focus on surveillance of multiresistant bacteria, compliance to screening procedures, the use of broad-spectrum antimicrobial agents, and on compliance to guidelines for infection prevention and control remain necessary in order to combat multiresistant bacteria in Greenland also in the future.

Anne Kjerulf, Anne-Marie Andersen, Anne Birgitte Jensen and Peter Poulsen
For further information: Anne Kjerulf (alf@ssi.dk)

Textbox 8.3

Azole resistance in *Aspergillus fumigatus* – Nationwide surveillance data from the first 18 months

Azole resistance in *Aspergillus fumigatus* complicates patient management with a negative impact on survival (1,2). Resistance is driven by either target gene mutations in *cyp51A*, other gene mutations or efflux (3). The dominating environmentally driven resistance mechanisms are TR_{34}/L98H and TR_{46}/Y121F/T289A (4). Both resistance mechanisms have been detected in *A. fumigatus* from patients and in the environment (5-8).

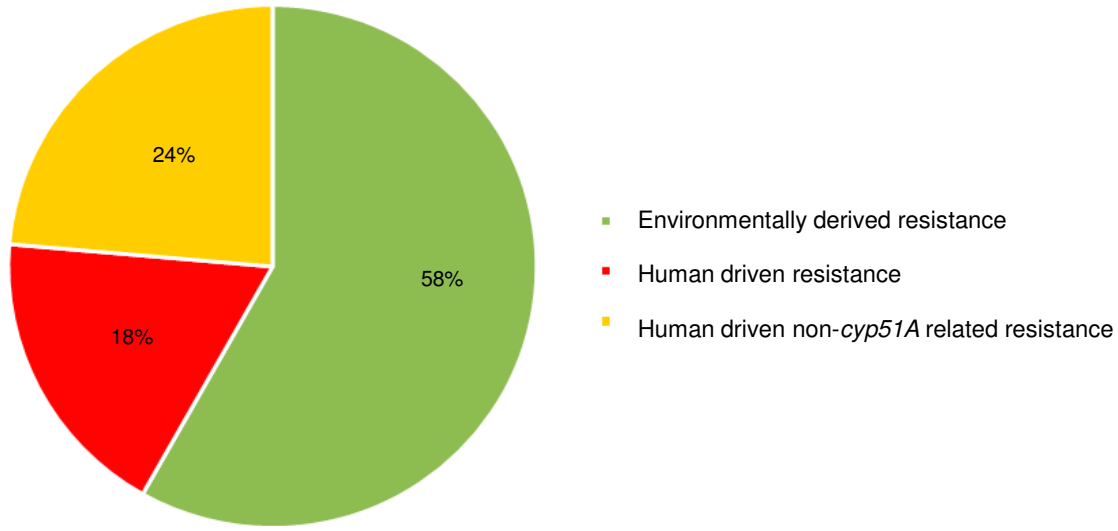
The nationwide surveillance was initiated in October 2018 with participation from all 10 Danish departments of clinical microbiology. The azole resistance rate is based on the following isolates: Clinically relevant *A. fumigatus* isolates and *A. fumigatus* isolates found on a Monday to represent background prevalence, the so-called Monday samples. The EUCAST E.Def 10.1 method using VIPcheck azole agar plates (Mediaproducts BV, Groningen NL) is used for screening and EUCAST E.Def 9.3.2 for susceptibility testing. Susceptibility classification was determined according to the revised EUCAST antifungal clinical breakpoints v 10.0, released in February 2020 (9). All non-susceptible isolates underwent *cyp51A* target gene sequencing. If more than one isolate was received from the same patient within 30 days, only the initial isolate was included in the data analysis unless differential susceptibility and/or resistance mechanism were documented. The azole resistance rate was determined at patient level.

From the first 18 months 1422 *A. fumigatus* isolates from 901 patients were included. Among those, 1419 isolates from 898 patients were susceptibility tested of which 78 isolates (5.5%) were specifically marked as Monday samples. At the patient level, 55 out of 898 patients had one or more resistant *A. fumigatus* isolates resulting in an azole resistance rate of 6.1% nationally. Thirty-two patients (3.6% of all patients and 58% of patients with resistance) had one or more isolates with an environmentally driven mechanism (Figure 1). These patients derived from various regions of Denmark and including both Zealand, Funen and Jutland and with no clear difference over time (Figure 2-3).

We report a nationwide azole resistance rate of 6.1% in *A. fumigatus*, with an overweight of resistance mechanisms associated with azole fungicide use in the environment. This may have an impact on human health.

Figure 1 Classification of the resistant *A. fumigatus* isolates at the patient level according to underlying type and origin of the resistance mechanism

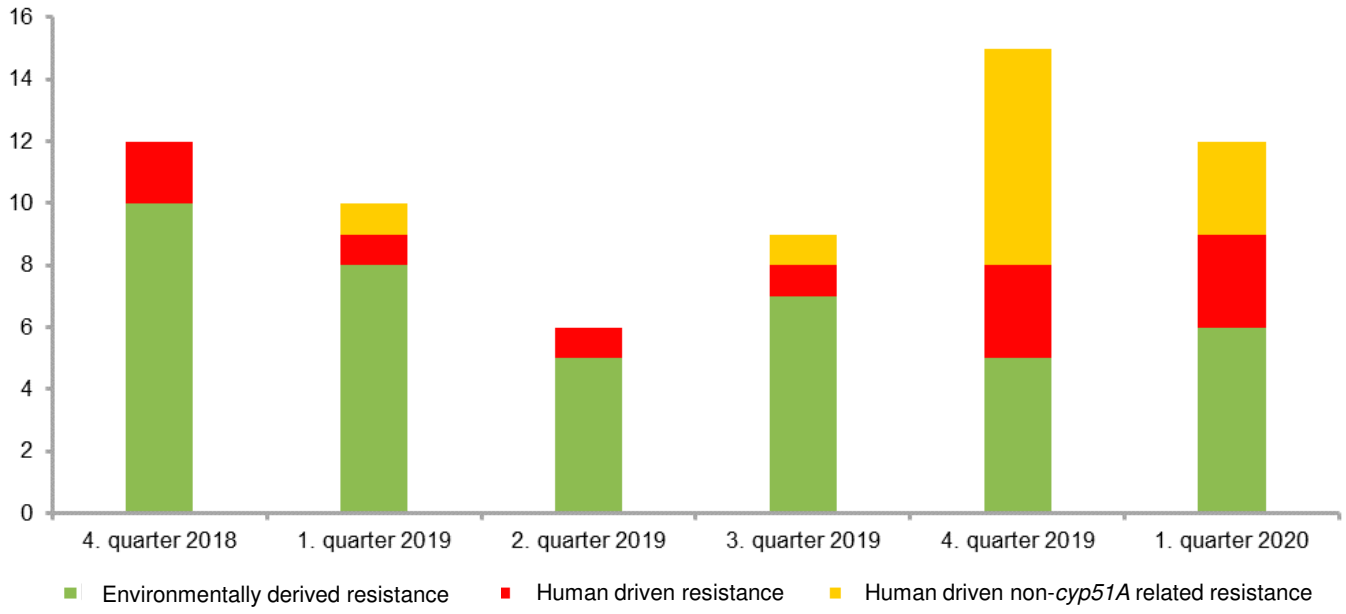
DANMAP 2019



The identified environmental resistance mechanisms consisted of TR_{34}/L98H, TR_{34}/L98H and TR_{34}/L98H/S297T/F495I. The human driven resistance mechanisms were G54R, G54W, M220K, M220R, M220I, P216S and G432S. The non-*cyp51A* related resistance consisted of isolates with *cyp51A* wildtype but elevated voriconazole MICs (≥2 mg/L). Three patients harboured resistant isolates with target gene mutations (TR_{34}/L98H, M220R and P216S, respectively) and also a resistant isolate with *cyp51A* wildtype

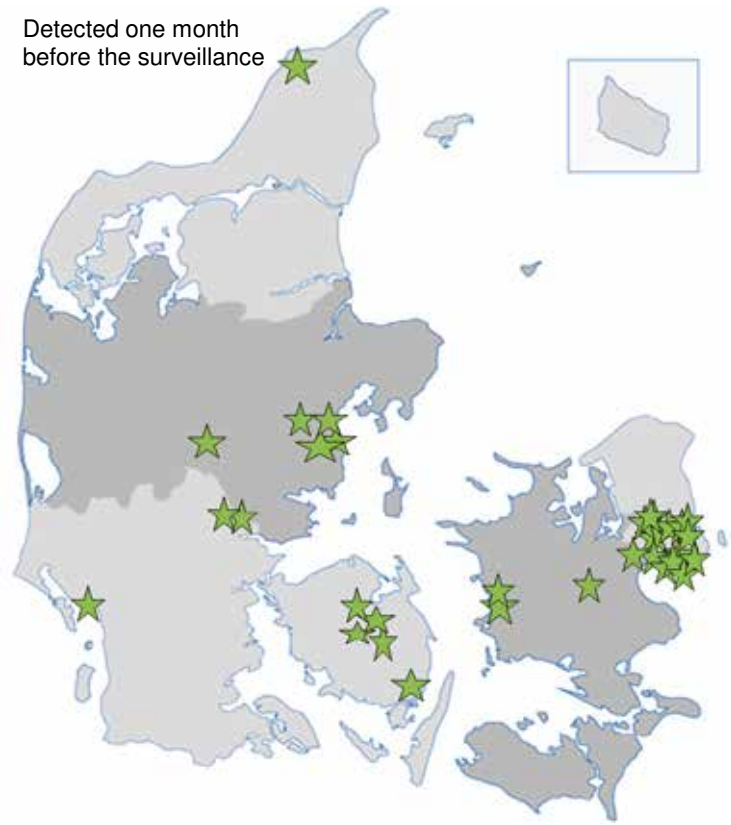
continued ... Textbox 8.3

Figure 2 Number of patients with resistant isolates shown in quarters DANMAP 2019



One patient might be repeated in another quarter. One patient with a human driven target gene mutation in 4. quarter also had a resistant isolate with *cyp51A* wildtype, which is also shown

Figure 3 Geographical areas where resistant *A. fumigatus* isolates from patients with an environmental resistance mechanism have been found DANMAP 2019



The stars mark the locations of hospitals or general practitioners from where the patient samples have been referred

Malene Risum, Rasmus Krøger Hare and Maiken Cavling Arendrup
Unit of Mycology, Statens Serum Institut
For further information: Maiken Cavling Arendrup, maca@ssi.dk

References

[1] Lestrade PP, Bentvelsen RG, Schauwvlieghe AFAD, Schalekamp S, Van Der Velden WJFM, Kuiper EJ, et al. Voriconazole resistance and mortality in invasive aspergillosis: A multicenter retrospective cohort study. Clin Infect Dis. 2019;68(9):1463–71.

[2] Resendiz-Sharpe A, Mercier T, Lestrade PPA, Van Der Beek MT, Von Dem Borne PA, Cornelissen JJ, et al. Prevalence of voriconazole-resistant invasive aspergillosis and its impact on mortality in haematology patients. J Antimicrob Chemother. 2019;74(9):2759–66.

[3] Lestrade PPA, Meis JF, Melchers WJG, Verweij PE. Triazole resistance in *Aspergillus fumigatus*: recent insights and challenges for patient management. Vol. 25, Clinical Microbiology and Infection. 2019.

[4] Stensvold CR, Jørgensen LN, Arendrup MC. Azole-Resistant Invasive Aspergillosis: Relationship to Agriculture. Curr Fungal Infect Rep. 2012 Sep 27;6(3):178–91.

[5] Mortensen KL, Jensen RH, Johansen HK, Skov M, Pressler T, Howard SJ, et al. Aspergillus species and other molds in respiratory samples from patients with cystic fibrosis: a laboratory-based study with focus on *Aspergillus fumigatus* azole resistance. J Clin Microbiol. 2011;49(6):2243–51.

[6] Astvad KMT, Jensen RH, Hassan TM, Mathiasen EG, Thomsen GM, Pedersen UG, et al. First Detection of TR 46 /Y121F/T289A and TR 34 /L98H Alterations in *Aspergillus fumigatus* Isolates from Azole-Naive Patients in Denmark despite Negative Findings in the Environment. Antimicrob Agents Chemother. 2014 Sep;58(9):5096–101.

[7] Mortensen KL, Mellado E, Lass-Florl C, Rodriguez-Tudela JL, Johansen HK, Arendrup MC. Environmental Study of Azole-Resistant *Aspergillus fumigatus* and Other Aspergilli in Austria, Denmark, and Spain. Antimicrob Agents Chemother. 2010 Nov 1;54(11):4545–9.

[8] DANMAP. Azole resistance in Aspergillus spp. Preliminary six months data from the newly established surveillance in Denmark. DANMAP 2018. 2019;Textbox 5.(part 2):77–8.

[9] Arendrup MC, Friberg N, Mares M, Kahlmeter G, Meletiadis J, Guinea J, et al. How to: interpret MICs of antifungal compounds according to the revised clinical breakpoints v. 10.0 European committee on antimicrobial susceptibility testing (EUCAST). Clin Microbiol Infect. 2020;(Online ahead of print).

9

MATERIALS AND METHODS

9. Materials and methods

9.1 General information

For the DANMAP 2019 report, population sizes and geographical data were obtained from Statistics Denmark [www.dst.dk] and data on general practitioners from the Danish Medical Association [www.laeger.dk].

The epidemiological unit for pigs and cattle was defined at the individual farm level, meaning that only one isolate per bacterial species per farm was included in the report. The individual flock of broilers was defined as the epidemiological unit, and for food, the epidemiological unit was defined as the individual meat sample.

For humans, the epidemiological unit was defined as the individual patient and the first isolate per species per patient per year was included. An overview of all antimicrobial agents registered for humans and animals in Denmark is presented in Table 3.2.

9.2 Data on antimicrobial consumption in animals

9.2.1 Data

In Denmark, all antimicrobial agents used for treatment are available on prescription only. Until 2007, antimicrobial agents were exclusively sold by pharmacies or as medicated feed from the feed mills. However, since April 2007, the monopoly was suspended and private companies were given license to sell prescribed veterinary medicinal products for animals, when following strict guidelines, identical to those applied to pharmacies. A pharmacy or company either sells the medicine to veterinarians for use in their practice or for re-sale to farmers, or sells the medicine directly to the animal holder on presentation of a prescription.

In 2019, 96% of all antimicrobial agents were purchased through pharmacies and the drug trading companies, while 4% were purchased from the feed mills. These numbers did not include prescribed zinc oxide from the feeding mills for the pigs. For cattle, 85% of antimicrobial agents used in 2019 were purchased from pharmacies, compared to only 6% in 2004. In aquaculture, approximately two thirds is purchased through the feed mills.

Data on all sales of veterinary prescription medicine from the pharmacies, private companies, feed mills and veterinarians are sent electronically to a central database called VetStat, which is hosted by the Danish Veterinary and Food Administration. Prior to 2001, all data on antimicrobial sales were derived from pharmaceutical companies.

Veterinarians are required by law to report all use of antibiotics and prescriptions for production animals to VetStat monthly. For most veterinarians, the registration of data is linked to the writing of invoices. The electronic registration of the sales at the pharmacies is linked to the billing process and stock accounts at the pharmacy, which ensures a very high data quality regarding amounts and type of drugs. Data are transferred daily from pharmacies to The Register of Medicinal Product Statistics at The Danish Health Authority and to VetStat. However, VetStat does not have any validation on data entry and slight typing errors from veterinarians may occur.

In addition, data on coccidiostatics as feed additives (non-prescription) and antimicrobial growth promoters (not in use since 2000) have also been collected by VetStat, providing an almost complete register of all antimicrobial agents used for animals in Denmark for the past twenty years. In very rare instances, medicines are prescribed on special license and will not be included in VetStat (i.e. medicines not approved for marketing in Denmark).

The VetStat database contains detailed information about source and consumption for each prescription item: date of sale, identity of prescribing veterinarian, source ID (identity of the pharmacy, feed mill, or veterinarian practice reporting), package identity code and amount, animal species, age group, disease category and code for farm-identity (CHR Danish Central Husbandry Register). The package code is a unique identifier, relating to all information on the medicinal product, such as active ingredient, content as number of unit doses (e.g. number of tablets), package size, and code of the antimicrobial agent in the Veterinary Anatomical Therapeutic Chemical (ATCvet) classification system.

Knowledge of the target animal species enables the presentation of consumption data in "defined animal daily doses" (DADD) a national veterinary equivalent to the international defined daily doses (DDDs) system applied in the human field [www.whocc.no]. The data presented in DANMAP 2019 were extracted from VetStat on 3 March 2020.

9.2.2 Methods

In DANMAP, we report use of antimicrobials dispersed in different animal populations. As a first step, the amount of antimicrobial agents used in animals is measured in kg active compound, to enable an overall crude comparison of consumption in different animal species and in the veterinary and human sectors.

A more detailed comparison of antimicrobial use is performed, taking into account their potency, formulation, route of administration and the age of the animals (where relevant), by generating defined animal daily doses (DADDs). For these calculations, we select data with relevant animal and age group codes and relevant codes for dispensation. For example, when calculating the antimicrobial use for systemic treatment in pigs, we select consumption data where the age groups

are defined as finishers, weaners, sows or boars and exclude antimicrobials dispensed as tablets, products for topical use, intramammaries and gynaecologicals. This is described in the footnotes for figures and tables in chapter 4.

Numerator - DADD

Defined animal daily dose (DADD) is the average maintenance dose per day for a drug used for its main indication in the appropriate animal species. The DADD is not defined at product level but as mg active compound per kg live animal for each antimicrobial agent, administration route and animal species. DADD has been specifically defined for use in DANMAP and does not always completely match the "prescribed daily dose" or the recommended dosage in the Summaries of Product Characteristics (SPC).

The following principles are applied when setting the DADDs:

1. Minor inconsistencies are corrected (e.g. due to rounding of numbers);
2. Approved dosage for the most widely used antimicrobial products is given priority above dosage for products that are rarely used;
3. Approved dosage for older products within the group is maintained as the common DADD even if a new product is approved with a higher dosage;
4. If the dosage for a group shows large variation in approved dosages of the products, the dosages provided by "The Veterinary Formulary" [British Veterinary Association 2005, 6th edition] are applied;
5. Dosages may vary within active compound and administration route, if different dosages have been approved for different age groups, indications or formulations.

When principle 3 and 4 are conflicting, principle 5 is applied.

Denominator - live biomass

The number of animals in a population (in epidemiological terms: the population at risk) is represented by their live biomass. The biomass of a species is calculated, taking into account average live bodyweight and the average lifespan in each age group. The estimation of live biomass and thus the number of standard animals at risk per day depends on the available data sources for each species. For DANMAP 2019, only the live biomass for pigs, cattle and mink were updated.

Pig production: The estimation was based on the number of pigs produced, including exports at different ages, productivity data for each year [Statistics Denmark; Danish Agriculture and Food Council] and census data for breeding animals [Statistics Denmark]. The average weight and life span for the growing animals (piglets, weaners and finishers) was estimated from the annual productivity numbers [Danish Agriculture and Food Council]. For DANMAP 2019, productivity data from 2018 were used to estimate the biomasses for pigs, since the 2019 productivity data were not available when estimates were calculated. The estimation methods were developed in cooperation with Danish Agriculture and Food Council. There are no statistics on average weight of breeding animals available, so an estimated average weight had to be assumed. However, the size of the breeding animals has probably increased over the last decade, but this was not accounted for.

Cattle production: The live biomass of the cattle population is estimated from census data [Statistics Denmark] and the average live weight of the different age groups. The Danish cattle population is mainly dairy, particularly Holstein Friesian, but also other breeds such as Jersey and a small population of beef cattle. Most of the cattle slaughtered are dairy cows and bull calves of dairy origin. The average live weight was estimated for 10 different age and gender categories.

Fur animals: The live biomass of mink is estimated from production data [Kopenhagen Fur] and carried out as described by Jensen et al., 2016 [Prev Vet Med. 26:170].

Treatment proportion - DAPD

The treatment proportion is a statistical measure for antimicrobial use in animal populations, calculated as the annual number of DADDs administered in the population divided by the estimated total population live biomass (in tonnes). For a single animal, the mg active compound (e.g. the number of DADDs) given in a daily treatment depends on the body weight, therefore, does treatment proportions also represent the proportion of animals treated daily with an average maintenance dose of a particular antimicrobial agent, and are reported as Defined animal daily dose per 1,000 animals per day (DAPD). For example, 10 DAPDs indicate that an estimated 1% of the population, on average, receives a certain treatment on a given day. In principle, the metric DAPD is parallel to the metric used in pharmaco-epidemiology for the human sector, defined daily dose per 1,000 inhabitants per day (DID), see section 9.8.2.

In 2019, DAPD calculations were carried out for pigs, cattle and fur animals.

Due to a relative high number of pigs exported around 30 kg; an adjusted measure of the average antimicrobial use in all age groups was calculated (DAPDadj). The adjustment is based on the assumption that pigs exported at 30 kg, on average, would have received the same amount of antimicrobial agents as other pigs from farrowing to slaughter.

Antimicrobial use per pig produced (adjusted) is calculated as:

$$DAPD_{adj} = \frac{DADD_{sows} + DADD_{weaners} + (1+Q)*DADD_{finishers}}{\Sigma biomassdays_{all} + N_{export}*biomassdays_{adj}}$$

Σbiomassdays_all is the sum of estimated biomass-days for each age group of pigs, N_export is the number of weaning pigs exported, and biomassdays_adj is the assumed number of lost biomass-days per exported pig.

9.3 Collection of bacterial isolates - animals and meat

9.3.1 Animals

Since 2014, most isolates available for DANMAP have been collected in accordance with the EU harmonised monitoring of antimicrobial resistance in zoonotic and commensal bacteria [Decision 2013/652/EU]. The legislation requires, in addition to sampling for the national *Salmonella* control programmes in poultry farms, sampling of broilers and fattening turkeys at slaughter in even years (2014-2020) and sampling of fattening pigs and cattle <1 year at slaughter in odd years (2015-2019).

In 2019, most of the sampling for DANMAP was allocated to the mandatory sampling of caeca from cattle and pigs, but additional sampling of broilers was also carried out.

Meat inspection staff or abattoir personnel at the slaughterhouses collected caecal samples from healthy broilers, cattle (<1 year) and pigs. For broilers, the samples were collected in August and September at the two major Danish slaughterhouses slaughtering conventionally produced chicken. For pigs and cattle, the samples were collected throughout 2019 at the ten major slaughterhouses in Denmark. The slaughterhouses included in the monitoring handled at least 75% of the total number of broilers, cattle and pigs slaughtered in Denmark during 2019.

Sampling was stratified per slaughterhouse by allocating the number of samples from domestically produced animals collected per slaughterhouse proportionally to the annual throughput of the slaughterhouse. Four intact caeca from each broiler flock were pooled into one sample. For pigs and cattle, samples contained 30-100 g caecal material from a single animal.

All samples were processed at the Danish Veterinary and Food Administration's (DVFA) laboratory in Ringsted. Samples from all three animal species were examined for indicator *E. coli*, broiler and cattle samples were also examined for *Campylobacter jejuni*. ESBL/AmpC/carbapenemase-producing *E. coli* was isolated from the cattle and pig samples, whereas *Salmonella* and enterococci was isolated from pigs samples only (Table 9.1).

All Danish flocks of layers, broilers and turkeys are tested for *Salmonella* on-farm as part of the national *Salmonella* control programme. Due to the low prevalence of *Salmonella* in the Danish poultry production [Annual Report on Zoonoses in Denmark, 2019] these data are not included in DANMAP 2019.

9.3.2 Meat

The EU harmonised monitoring requires, in addition to sampling for the national *Salmonella* control programmes at slaughter, sampling at retail of broiler meat in even years (2014-2020) and sampling of pork and beef in odd years (2015-2019) [Decision 2013/652/EU].

In 2019, ESBL/AmpC/carbapenemase-producing *E. coli* were isolated from packages of fresh, chilled meat from broiler, cattle and pigs collected in Danish wholesale and retail outlets throughout the year by the regional DVFA officers (Table 9.1). Products with added saltwater or other types of marinade as well as minced meat were excluded. Packages of beef and pork were selected at retail without pre-selecting based on the country of origin as requested for the harmonised EU monitoring. In 2019. Additional sampling of broiler meat was carried out the main supermarket chains central storage facilities. The number of establishments and samples selected by each regional DVFA control unit was proportional to the number of establishments in the region in relation to the total number of establishments in the country. One unit of meat (minimum of 200 g) was collected and all samples were processed at the DVFA laboratory. Some of the samples were also examined for *Campylobacter jejuni* (imported broiler meat) and *Salmonella* (imported pork and beef, Table 9.1).

The *Salmonella* isolates from domestically produced broiler meat, beef and pork originate from the national control programme at the slaughterhouses (Table 9.1). For beef and pork, carcases are swabbed in four designated areas (the jaw, breast, back and ham) after min. 12 hours of chilling (covering 10x10cm). For broiler meat, 300 neck-skin samples (1 g) are collected and pooled after slaughter into subsamples of 60 grams. All samples were processed at Industry laboratories. *Salmonella* isolates from cattle and pig carcases were send to the DVFA laboratory, and *Salmonella* isolates from the neck-skin sampling were send to the DTU laboratory.

Campylobacter from domestically produced broiler meat for DANMAP originate from sampling of leg-skins at slaughterhouses receiving either conventionally or organic/free-range broiler flocks. The numbers of samples collected depend on the slaughterhouse capacity and all samples were processed at the DVFA laboratory.

Salmonella from broiler meat and beef are not included in DANMAP 2019 due to low numbers of isolates available from the national surveillance programmes [Annual Report on Zoonoses in Denmark, 2019].

Table 9.1 Legislative and voluntary sampling plans under national control programmes and EU harmonised monitoring that contribute isolates to DANMAP 2019
DANMAP 2019

Bacteria	Origin of isolates	Legislative reporting frequency (2013/652/EU)	Number of tested and positive samples in 2019
Campylobacter spp.	Caecal samples from broilers[a]	Even years	174 flocks (78 positive)
	Caecal samples from cattle <1 yr		142 animals (122 positive)
	Leg skins from conventional broilers[b]		1,248 units (407 positive)
	Leg skins from organic/free-range broilers[b]		123 units (84 positive)
	Fresh broiler meat - ready for retail (imports)[c]		54 units (30 positive)
Salmonella spp.	On-farm samples from laying hens (production flocks)	Even years	411 flocks (8 positive)
	On-farm samples from broilers (production flocks)	Even years	4,012 flocks (12 positive)
	Caecal samples from fattening pigs[d]		798 animals (124 positive)
	Neck skin samples from broilers	Even years	254 units (0 positive)
	Carcase swabs from fattening pigs[e]	Odd years	10,743 animals (1.2% positive)
	Carcase swabs from cattle <1 year[e]	Odd years	4,125 animals (0.1% positive)
	Fresh pork - ready for retail (imports)		141 units (24 positive)
	Fresh beef - ready for retail (imports)		280 units (0 positive)
Enterococcus spp.	Caecal samples from fattening pigs[d]		799 animals (195 positive)
Indicator *E. coli*	Caecal samples from broilers	Even years	168 flocks (159 positive)
	Caecal samples from fattening pigs	Odd years	195 animals (192 positive)
	Caecal samples from cattle <1 yr	Odd years	186 animals (174 positive)
Specific monitoring of ESBL/AmpC and carbapenemase-producing *E. coli*	Caecal samples from cattle <1 yr	Odd years	306 animals (25 positive)
	Caecal samples from fattening pigs	Odd years	330 animals (90 positive)
	Fresh broiler meat - ready for retail (domestic origin)[c]	Even years	257 units (14 positive)
	Fresh broiler meat - ready for retail (imports)[c]	Even years	44 units (15 positive)
	Fresh beef at retail (domestic origin)	Odd years	123 units (3 positive)
	Fresh beef at retail (imports)	Odd years	196 units (9 positive)
	Fresh pork at retail (domestic origin)	Odd years	317 units (9 positive)
	Fresh pork at retail (imports)	Odd years	36 units (15 positive)
	WGS data for collected ESBL/AmpC isolates		164 isolates

Note: Testing for carbapenemase-producing *E. coli* is voluntary according to regulation 2013/652/EU. Carbapenemase-producing *E. coli* was not detected in any of the analysed samples
a) Ceacum samples from broilers were collected during August and September, where the *Campylobacter* prevalence in Danish broilers is highest
b) Collected from carcases at the end of the slaughterline and classified as broiler meat
c) For 2019, broiler meat - ready for retail include both conventional and organic/free-range products
d) Among the 798 fattening pigs tested, the monitoring included animals from 31 of 765 farms more than once
e) Carcase swabs collected at slaughterhouses slaughtering more than 30,000 pigs or 7,500 cattle, swab samples are analysed in pools of 5 samples. When estimating the prevalence of *Salmonella*, both the loss of sensitivity and the probability of more than one positive sample in each pool are taken into consideration

9.4 Microbiological methods - isolates from animals and meat

9.4.1 *Salmonella*

Salmonella was isolated in accordance with the methods issued by the NMKL [NMKL No. 187, 2007] or ISO 6579-1 [ISO6579-1:2017]. Serotyping of isolates was performed by whole genome sequencing using the Illumina MiSeq platform, paired-end sequencing 2x250 cycles. For bioinformatics, a CGE service (Centre for Genomic Epidemiology, DTU) for *Salmonella* serotyping was applied based on the genetic background for antigenic formulas given by the White-Kauffmann-Le Minor scheme. Only one isolate per serotype was selected from each herd, flock or slaughter batch.

9.4.2 *Campylobacter*

Campylobacter from broilers and cattle was isolated and identified according to the methods issued by the NMKL [NMKL No. 119, 2007] followed by species-determination by BAX® rtPCR assay. Pre-enrichment in Bolton broth was used for cattle and broiler meat samples, whereas direct spread of caecal sample on to selective agar was used for broiler samples. Only one *Campylobacter jejuni* isolate per broiler flock, cattle herd or per batch of fresh meat was selected.

9.4.3 Indicator *Escherichia coli*

Indicator *E. coli* from broilers, pigs and cattle was isolated by direct spread of caecal sample material onto violet red bile agar incubated for 24h at 44°C. Presumptive *E. coli* was identified on TBX agar incubated at 44°C o/n. Only one indicator *E. coli* isolate per flock or herd was selected. For specific isolation of ESBL/AmpC and carbapenemase-producing *E. coli* from meat and caecal samples, the present EURL-AR laboratory protocols describing the selective enrichment procedures was applied in accordance with the EU harmonised monitoring. Only one

ESBL/AmpC-producing *E. coli* isolate per cattle herd, pig herd, and meat sample was selected (no isolates of carbapenemase-producing *E. coli* was detected).

9.4.4 Indicator enterococci

Indicator enterococci from pigs was isolated from an adequate amount of caecal material suspended in 2 ml buffered peptone water, and inoculated onto Slanetz Barrtley agar incubated at 41,5°C for 2 days. Three colonies resembling *E. faecium*, or *E. faecalis* if no suspect *E. faecium* were present, were identified by a real-time PCR assay. Only one enterococci per herd, was selected for antimicrobial resistance testing (selecting an *E. faecium* if possible).

9.5 Susceptibility testing – isolates from animals and meat

Antimicrobial susceptibility testing of *Salmonella*, *Campylobacter* and *E. coli* was performed as Minimum Inhibitory Concentration (MIC) determination using broth microdilution by Sensititre (Trek Diagnostic Systems Ltd.). Inoculation and incubation procedures were in accordance with the CLSI guidelines [Clinical and Laboratory Standards Institute, USA] and the European standard [ISO 20776-1:2006]. The isolates were tested for antimicrobial susceptibility in accordance with the Decision 2013/652/EU about the EU harmonised monitoring of antimicrobial resistance.

The relevant quality control strains were used at the laboratories: *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Campylobacter jejuni* ATCC 33560 and *Pseudomonas aeruginosa* ATCC 27853. Isolates from animals and meat were tested for antimicrobial susceptibility at the DVFA laboratory in Ringsted that is accredited by DANAK (the national body for accreditation).

9.6 Whole genome sequencing – isolates from animals and meat

In addition to *Salmonella* serotyping performed by sequencing (section 9.4.1), whole genome sequencing (WGS) and *in silico* bioinformatic tools were also used to detect the genetic background of the ESBL/AmpC and carbapenemase-producing *E. coli*. At the DVFA laboratory in Ringsted, strains were sequenced using the Illumina MiSeq platform and the bioinformatics analysis was conducted at DTU National Food Institute from Centre for Genomic Epidemiology [www.genomicepidemiology.org; https://cge.cbs.dtu.dk//services/all.php]. ResFinder 4.0 was used for detection of antimicrobial resistance genes including chromosomal mutations leading to resistance to beta-lactams, quinolones and colistin as well as acquired resistance genes [Zankari et al. 2012. J Antimicrob Chemother. 67(11):2640; Zankari et al. 2017. J Antimicrob Chemother. 72(10):2764]. ST types were defined using MLST Finder 2.0 [Larsen et al. 2012. J Clin Mico- biol. 50(4):1355].

9.7 Data handling – isolates from animals and meat

For the samples processed at the DVFA laboratory, sampling details and laboratory results were stored in the DVFA Laboratory system. Following validation by DVFA, data were send to DTU National Food Institute (Excel sheets). At the DTU National Food Institute, data were harmonised and one isolate per epidemiological unit was selected for reporting. All data are stored in an Oracle database at isolate level (9i Enterprise Edition®). The database contains all antimicrobial data reported in DANMAP or to EFSA since 2007 (partial dataset from 2001-2006). Variables includes: Bacterial species (subtype where applicable), date of sampling, animal species or food type, herd identifier and country of origin whenever possible.

9.7.1 Interpretation of MIC values

MIC values were retained as continuous variables in the database, from which binary variables were created using the relevant cut-off from 2019 for all years. Since 2007, data have been interpreted using EUCAST epidemiological cut-off values with a few exceptions described in Table 9.2. All MIC-distributions are presented in the web annex at www.danmap.org. Each of the tables provides information on the number of isolates, the applied interpretation of MIC-values and the estimated level of resistance and confidence intervals calculated as 95% binomial proportions presenting Wilson intervals.

An isolate is considered multidrug-resistant if resistant to three or more of the antimicrobial classes defined in Table 9.3 and fully sensitive if susceptible to all antimicrobial agents included in the test panel.

Table 9.3 Definitions of antimicrobial classes for calculation of multidrug-resistance in *Salmonella* and indicator *E. coli*

DANMAP 2019

Antimicrobial classes	*Salmonella* and *E. coli*
Beta-lactam penicillins	Ampicillin
Macrolides	Azithromycin
Cephalosporins	Cefotaxime and/or ceftazidime
Phenicols	Chloramphenicol
Quinolones	Ciprofloxacin and/or nalidixic acid
Polymycins	Colistin
Aminoglycosides	Gentamicin
Carbapenems	Meropenem
Sulfonamides	Sulfonamides
Tetracyclins	Tetracycline
Glycylcyclines	Tigecycline
Trimethoprim	Trimethoprim

Note: An isolate is considered multidrug-resistant if resistant to three or more of the defined antimicrobial classes and fully sensitive if susceptible to all antimicrobial agents included in the test panel

Table 9.2 Interpretation criteriae for MIC-testing by EUCAST epidemiological cut-off values and the corresponding EUCAST clinical breakpoints
DANMAP 2019

Antimicrobial agent	*Salmonella*		*E. coli*		*Enterococcus*		*C. jejuni*		*S. aureus*
	ECOFF µg/ml	Clinical breakpoint µg/ml	ECOFF µg/ml	Clinical breakpoint µg/ml	ECOFF µg/ml	Clinical breakpoint µg/ml	ECOFF µg/ml	Clinical breakpoint µg/ml	Clinical breakpoint µg/ml
Ampicillin	>8	>8	>8	>8	>4	>8			
Azithromycin	>16[a]		>16[a]						
Cefepime	>0.125[a]	>4	>0.125	>4					
Cefotaxime	>0.5	>2	>0.25	>2					
Cefotaxime/ clavulansyre	>0.5[a]		>0.25[a]						
Cefoxitin	>8		>8						>4
Ceftaroline									>1
Ceftazidime	>2	>4	>0.5	>4					
Ceftazidime/ clavulansyre	>2[a]		>0.5[a]						
Ceftobiprole									>2
Chloramphenicol	>16	>8	>16	>8	>32				
Ciprofloxacin	>0.064	>0.064	>0.064	>0.5	>4		>0.5	>0.5	
Clindamycin									>0.5[c]
Colistin	>2[b]	>2	>2	>2					
Daptomycin					>4/8[d]				>1
Ertapenem	>0.064	>1	>0.064	>1					
Erythromycin					>4		>4	>4	>2
Fusidic acid									>1
Gentamicin	>2	>4	>2	>4	>32		>2		>1
Imipenem	>1	>8	>0.5	>8					
Kanamycin									>16
Linezolid					>4	>4			>4
Meropenem	>0.125	>8	>0.125	>8					
Mupirocin									>2
Nalidixic acid	>16		>16				>16		
Norfloxacin									>4
Penicillin									>0.125
Quinupristin/ dalfopristin					>4[d]				
Rifampicin									>0.5
Streptomycin							>4		
Sulfamethoxazole/ trimethoprim									>4
Sulfonamide	>256[a]		>64[a]						
Teicoplanin					>2	>2			
Temocillin	>32		>16						
Tetracycline	>8		>8		>4	>0.5	>1	>2	>2
Tigecycline	>1[e]	>2	>1[f]	>2	>0.25	>0.25			
Trimethoprim	>2	>4	>2	>4					
Vancomycin					>4	>4			

Note: EUCAST epidemiological cut-off values (ECOFFs) and EUCAST clinical breakpoints listed unless noted
a) No current EUCAST ECOFF is available, apply complementary interpretative thresholds as suggested by EFSA [EFSA Supporting publication 2019:EN-1559]
b) No current EUCAST ECOFF is available. For colistin, an ECOFF >2 was applied for *S.* Typhimurium and other serotypes, except for *S.* Enteritidis and *S.* Dublin where an ECOFF >8 was applied according to investigations presented in DANMAP 2011
c) Inducible clindamycin resistance is included
d) For daptomycin, an ECOFF >8 was applied for *E. faecium* [EUCAST, 30 December 2018]. *E. faecalis* are assumed inherent resistant to streptogramins, and ECOFF only applies to *E. faecium*. For *E. faecium*, the EUCAST ECOFF (>1) was not applied for quinopristin/dalfopristin (tradename synercid) according to investigations presented in DANMAP 2006
e) For human isolates, the tigecycline, ECOFF >2 was applied to all serovars
f) The most recent EUCAST ECOFF >0.5 was not applied for *E. coli*

9.7.2 ESBL/AmpC phenotypes

Classification of CPE, ESBL and AmpC phenotypes was done according to the scheme provided by EFSA [EFSA 2018. EFSA Journal 16(2):5182]:

1. CPE phenotype if meropenem MIC >0.12 µg/ml;
2. ESBL phenotype if cefotaxime/ceftazidime MIC >1 µg/ml and meropenem MIC <=0.12 µg/ml and cefoxitin MIC <=8 µg/ml and synergy (clavulanic acid and cefotaxime/ceftazidime);
3. ESBL-AmpC phenotype if cefotaxime/ceftazidime MIC >1 µg/ml and meropenem MIC <=0.12 µg/ml and cefoxitin MIC >8 µg/ml and synergy (clavulanic acid and cefotaxime/ceftazidime);
4. AmpC phenotype if cefotaxime/ceftazidime MIC >1 µg/ml and meropenem MIC <=0.12 µg/ml and cefoxitin MIC >8 µg/ml and no synergy (clavulanic acid and cefotaxime/ceftazidime);
5. Other phenotype if not in 1-4.

Synergy is defined as ≥3 twofold concentration decrease in MIC for clavulanic acid combined with cefotaxime/ceftazidime vs the MIC of cefotaxime/ceftazidime alone.

9.7.3 Statistical tests

Significance tests of differences between proportions of resistant isolates were calculated using Chi-square, or Fisher's Exact Tests as appropriate.

Significance tests for trends in rates of resistance were performed by applying the Cochran-Armitage test. One-sided tests were chosen because of preliminary expected trend directions and a p-value of <0.05 is generally considered significant.

Some types of resistances were looked for, but not found by the DANMAP monitoring system, yielding a prevalence of zero. It is not possible for surveys to prove freedom from diseases or resistances in populations, but with a defined confidence, surveys can identify the maximum possible prevalence given that the survey failed to find any positives [Textbox 6.2, DANMAP 2016]. This maximum prevalence was calculated for the report using 95% confidence and assuming a perfect test by a probability formula to substantiate freedom from disease [Cameron and Baldock 1998, Prev. Vet. Med].

Link to calculation example at epitools.ausvet.com.au.

Analysis were done using SAS®Software, SAS Enterprise Guide 8.2 or Sergeant, ESG, 2018. Epitools Epidemiological Calculators. Ausvet. Available at: http://epitools.ausvet.com.au.

9.8 Data on antimicrobial consumption in humans

9.8.1 Data registration

All antimicrobial consumption in Denmark has since 1997 been reported to DANMAP once a year through the Register of Medicinal Product Statistics at the Danish Health Data Authority. Until 2012, data from hospitals on certain infusion substances was obtained directly from the hospital pharmacies. Since 2013, all data from hospitals are reported to and delivered by The Register of Medicinal Product Statistics at the Danish Health Data Authority.

Reportings on human antimicrobial consumption in Denmark exist from before 1997. These were performed through the Association of Medicine Importers (Medicinimportørforeningen, MEDIF) and the Association of Danish Medicinal Factories (Foreningen af danske Medicinfabrikker, MEFA) based on whole sales data to the pharmacies. This reporting became less reliable over time, since there was an increasing amount of parallel imported drugs from the late 1980's, which were not covered by this registration.

In the primary sector, all antibacterial agents for human use are prescription-only medicines. Sales are reported through the pharmacies by a code relating to the defined package. The information from the code includes information on the active drug, the brand name of the product, formulation, size and number of packages. The sale also reports the age, gender and regional residence of the patient. Since 2004, the sales registration has included the indication code as well. Still, for the treatment of infectious diseases the clinical indications given were often quite unspecific, such as "against infection". Since 2016, the use of more specific indication codes has become more feasible through the implementation of the "common medicine card" (fælles medicinkortet, FMK), mandatory to be used by all medical doctors. In 2019, indication codes were available for 93% of prescriptions, but specific indication codes still accounted for only 72%.

For hospitals, reporting is based on deliverances from the hospital pharmacies to the different clinical departments and includes all generic products that are supplied through general trade agreements between different medical suppliers and Amgros, a private company under agreement with the regions. Amgros is responsible for harmonisation of prices and for ensuring deliverances to all hospitals and work closely together with the Regions' Joint Procurement. Detailed information is given on the different drugs delivered on ATC5 level. For surveillance purposes, it has to be assumed that the amount of delivered antimicrobials is similar to the consumption at the different departments. In reality, antimicrobials may be exchanged between different specialties and departments belonging to the same trust, which makes precise calculations of the consumption on specialty level difficult. In DANMAP, reporting of data on hospital consumption is therefore kept at a national or regional level. Data on hospital level can be supplied upon request. In case of production failures and shortages in deliverance of specific products, the hospitals have to apply for special deliverances through the Danish Medicines Agency (Figure A5.2 in web annex). These special deliverances are reported separately to DANMAP through the hospital pharmacies. An example is the shortages in deliver-

ance of piperacillin with tazobactam and pivmecillinam as well as mecillinam in 2017. The shortage in piperacillin with tazobactam had significant impact on the amount used, while the shortage in (piv)mecillinam could not be clearly tracked in changes in consumption. In 2019, 188,609 DDD (0.6% of total antimicrobial consumption) was consumed at the hospitals through special deliverances. The need of special deliverances might have been caused by shortages in deliverances.

Data on treatment at patient level is available at very few of the hospitals and has so far been used in local quality assurance only but has not been available to the national surveillance system. Thus a national account of the prudence of use of antimicrobials at hospitals has so far not been possible.

9.8.2 Method

Primarily somatic hospitals were included in the DANMAP reporting. Data from private hospitals and clinics, psychiatric hospitals, specialised non-acute care clinics, rehabilitation centres and hospices were excluded from DANMAP in most calculations since their activity and functions are not comparable to public, somatic hospitals and therefor may skew the data. Their consumption accounts for approximately 4% of the antimicrobial consumption at hospitals.

The present report includes data on the consumption of "antibacterials for systemic use", or group J01, of the 2019 update of the Anatomical Therapeutic Chemical (ATC) classification, in primary healthcare and in hospitals as well as consumption of per oral and rectal preparations of metronidazole (P01AB01) and for hospitals oral preparations of vancomycin (A07AA09). As recommended by the World Health Organization (WHO), consumption of antibacterial agents in primary healthcare is expressed as DIDs, i.e. the number of DDDs per 1,000 inhabitants per day.

The consumption in hospital healthcare is expressed as the number of DDDs per 100 occupied beds per day (DDD/100 occupied bed-days or DBD). Since reporting in DBD does not necessarily reflect changes in hospital activity and production, consumption at hospitals is also presented as DAD (the number of DDDs per 100 admissions) and crude DDD. Finally, the consumption of antibacterial agents at hospitals has also been calculated in DIDs, primarily for comparison with primary healthcare.

9.8.3 DDD

Defined daily dose is the assumed average maintenance dose per day for a drug used for its main indication in adults. DDDs provide a fixed unit of measurement independent of price and formulation, enabling the assessment of trends in drug consumption and to perform comparisons between population groups. The DDDs are defined and revised yearly by the WHO Collaborating Centre for Drug Statistics and Methodology [www.whocc.no/atcddd/ index database].

Per January 2019 the WHO updated the DDDs for seven main antimicrobial agents, based on recommendations from an expert working group in collaboration with the European Center for Disease Control (ECDC). New and former DDDs are presented in Table 9.4. From DANMAP 2018, the new DDD values were applied and all tables and figures were updated ten years back.

9.8.4 DBD

DDD/100 occupied bed-days. The number of occupied bed-days are calculated as the date of discharge minus the date of admission and rounded up to nearest 24 hours, e.g. one day. Every new admission to a new hospital department counts as a new bed-day. Number of bed-days was extracted from the National Patient Registry at the Danish Health Data Authority [www.sundhedsdatastyrelsen.dk].

The National Patient Registry was upgraded in 2019, and data in the new registry are not comparable to data in the previous registry. Unification of data is ongoing but not completed. Thus the number of bed-days in 2019 is estimated by applying the average decrease observed in 2009-2018.

Table 9.4 New DDDs assigned by WHO Collaborating Centre per January 2019 DANMAP 2019

ATC5 code	ATC level name	Previous DDD			New DDD		
		Weight	Unit	Route of administration	Weight	Unit	Route of administration
J01CA01	Ampicillin	2.0	g	Parenteral	6.0	g	Parenteral
J01CA04	Amoxicillin	1.0	g	Oral	1.5	g	Oral
J01CA04	Amoxicillin	1.0	g	Parenteral	3.0	g	Parenteral
J01CA17	Temocillin	2.0	g	Parenteral	4.0	g	Parenteral
J01CR02	Amoxicillin and beta-lactamase inhibitor	1.0	g	Oral	1.5	g	Oral
J01DE01	Cefepime	2.0	g	Parenteral	4.0	g	Parenteral
J01DH02	Meropenem	2.0	g	Parenteral	3.0	g	Parenteral
J01MA02	Ciprofloxacin	0.5	g	Parenteral	0.8	g	Parenteral
J01XB01	Colistin	3.0	MU	Parenteral	9.0	MU	Parenteral

9.8.5 DAD

DDD/100 admissions. One admission is registered whenever a patient is admitted to a specific ward (i.e. one patient can be registered as admitted multiple times if transferred between wards during the same hospital stay). The admissions were extracted from the National Patient Registry at the Danish Health Data Authority [www.sundhedsdatastyrelsen.dk].

The National Patient Registry was upgraded in 2019, and data in the new registry are not comparable to data in the previous registry. Unification of data is ongoing but not completed. Thus the number of admissions in 2019 is estimated by applying the average increase observed in 2009-2018.

9.8.6 DaDDD

Danish adjusted daily dose. This unit was developed for DANMAP 2018 as an attempt to better picture the actual dosages used for antibiotic treatment in Denmark. DaDDD units were made by combining recommended dosages in Danish treatment guidelines with data from the prescription register, thus defining a Danish maintenance dose for each given drug. The work with DaDDD was initiated by an expert group under the Danish Regional Learning and Quality teams (LKT) developing measurable units for consumption at Danish hospitals. The DANMAP group further developed these units to also apply to drugs given orally. DaDDD, their counterparts DDD and the conversion factors are presented in Table 9.5 and Table 9.6 for the primary and hospital sector, respectively.

For further information regarding the LKT initiative in 2017-2019, please go to www.kvalitetsteams.dk > Lærings- og kvalitetsteams > Rationelt antibiotikaforbrug på hospitaler (only available in Danish). The LKT report with results from the project can be found at www.kvalitetsteams.dk.

Table 9.5 Danish adjusted DDD for penicillins in the primary sector, 2019 DANMAP 2019

ATC5 code	Antimicrobial agent	WHO DDDs in grams	Danish adjused DDDs in grams	Conversion factor	Primary indication
J01CA02	Pivampicillin	1.05	2.10	0.50	Urinary tract infection
J01CA04	Amoxicillin	1.50	1.50	1.00	Otitis media
J01CA08	Pivmecillinam	0.60	1.20	0.50	Urinary tract infection
J01CE02	Phenoxymethylpenicillin	2.00	1.90	1.05	Upper respiratory tract infection
J01CF01	Dicloxacillin	2.00	3.00	0.67	Skin- and soft tissue infection
J01CF05	Flucloxacillin	2.00	3.00	0.67	Skin- and soft tissue infection
J01CR02	Amoxicillin and beta-lactamase inhibitors	1.50	1.50	1.00	Upper and lower respiratory tract infection

Note: Solely per oral administration routes

Table 9.6 Danish adjusted DDD for main antimicrobials in the hospital sector, 2019 DANMAP 2019

ATC5 code	Antimicrobial agent	WHO DDDs in gram	Danish adjusted DDDs	Conversion factor	Route of administration
J01CA01	Ampicillin	6.00	8.00	0.75	Parenteral
J01CA02	Pivampicillin	1.05	2.10	0.50	Oral
J01CA04	Amoxicillin	1.50	1.50	1.00	Oral
J01CA08	Pivmecillinam	0.60	1.20	0.50	Oral
J01CE01	Benzylpenicillin	3.60	4.80	0.75	Parenteral
J01CE02	Phenoxymethylpenicillin	2.00	2.67	0.75	Oral
J01CF01	Dicloxacillin	2.00	4.00	0.50	Oral
J01CF05	Flucloxacillin	2.0	4.0	0.5	Oral
J01CR02	Amoxicillin and beta-lactamase inhibitor	1.50	1.50	1.00	Oral
J01CR05	Piperacillin and beta-lactamase inhibitor	14.00	11.97	1.17	Parenteral
J01DC02	Cefuroxim	3.00	4.48	0.67	Parenteral
J01DH02	Meropenem	3.00	3.00	1.00	Parenteral
J01FA09	Clarithromycin	0.50	1.00	0.50	Oral
J01FA10	Azithromycin	0.30	1.00	0.30	Oral
J01GB03	Gentamicin	0.24	0.35	0.69	Parenteral
J01MA02	Ciprofloxacin	0.80	0.80	1.00	Parenteral

Figures based on DaDDD can be found in chapter 5, antimicrobials in humans, Figure 5.5b and Figure 5.14b, presenting data from primary sector and hospital sector, respectively.

9.9 *Salmonella* and *Campylobacter* in humans

9.9.1 Data source

Antimicrobial susceptibility was performed on human clinical isolates submitted to Statens Serum Institut (SSI). *Salmonella* isolates were submitted from all clinical microbiology laboratories in Denmark and *Campylobacter* isolates were submitted from clinical microbiology laboratories representing the island of Zealand excluding the Capital Region, Funen, and Northern Jutland. As in previous years, SSI collected information on travel history from the patients. Cases were categorised as "domestically acquired" if the patients had not travelled abroad within the week prior to the onset of disease.

9.9.2 Microbiological methods

Salmonella isolates were analysed by whole genome sequencing and the serotypes were derived from the DNA sequences. In a few cases, the DNA information was supplemented with slide agglutination according to the Kauffman-White Scheme. *Campylobacter* species identification was performed by the use of MALDI-TOFF.

9.9.3 Susceptibility testing

Antimicrobial susceptibility testing of *Salmonella* and *Campylobacter* was performed as Minimum Inhibitory Concentration (MIC) determination using the Sensititre broth microdilution system (Trek Diagnostic Systems Ltd.). Inoculation and incubation procedures were in accordance with the CLSI guidelines [Clinical and Laboratory Standards Institute, USA] and the European standard ISO 20776-1:2006.

9.9.4 Data handling

Data on *Salmonella* and *Campylobacter* infections are stored in the Danish Registry of Enteric Pathogens (SQL database) that is maintained by SSI. This register includes only one isolate per patient within a window of six months and includes data on susceptibility testing of gastrointestinal pathogens.

9.10 *E. coli, K. pneumoniae, P. aeruginosa, Acinetobacter* spp., *E. faecium* and *E. faecalis* in human patients

9.10.1 Data source

The surveillance of invasive isolates of *Escherichia coli, Klebsiella pneumoniae, Enterococcus faecalis, Enterococcus faecium, Pseudomonas aeruginosa* and *Acinetobacter* species and urine isolates of *E. coli* and *K. pneumoniae* was based on data from routine diagnostics at the 10 departments of clinical microbiology (DCMs) in Denmark. For 2019, all these data were extracted directly from the Danish Microbiology Database (MiBa) [https://miba.ssi.dk]. Before 2018, data were reported from the individual DCMs to SSI. A description of MiBa and the usage and validation of MiBa-data is given in Textbox 8.1 in DANMAP 2018 [https://www.danmap.org].

9.10.2 Microbiological methods

All microbiological analyses including species identification, susceptibility testing and interpretation of test results, were performed by the DCMs. Since November 2015, all Danish DCMs used the EUCAST terminology with the EUCAST breakpoints and the EUCAST methods for roughly all species. Few exceptions exist at some DCMs were local rules were applied on the susceptibility interpretations in specific cases - e.g. susceptibility to mecillinam in invasive cases. In 2019 EUCAST introduced the Area of Technical Uncertainty (ATU) for some combinations of species and agents reflecting problematic areas regarding variations and uncertainty of susceptibility categorisation. Piperacillin-tazobactam, ciprofloxacin and amoxicillin-clavulanic acid (in systemic breakpoints) and Enterobacterales are examples where ATUs were applied. ATUs can be handled differently by the DCMs and may influence interpretation results and was commented on when necessary in the affected sections.

9.10.3 Data handling

Cases were identified in MiBa and susceptibility results extracted. Before 2018, cases were identified based on the reported data from the individual DCMs.

The case definition has been harmonised with the definition by the European Antimicrobial Resistance Surveillance Network (EARS-Net): The first sample, by date of sample collection, of each given bacterial species per unique patient per year of observation. Duplicates from the same patient, within the year of observation, were removed. Thereby only resistance data on the first isolate per patient per specimen per year were included. Resistance data from the individual DCMs were excluded if not tested or registered in MiBa routinely (minimum 75% of the specific species/antimicrobial agent combination). Samples were either invasive (including blood cultures or cerebrospinal fluid) or urinary samples from hospital patients or from primary healthcare patients.

9.10.4 Statistical test

Significance tests for trends in rates of resistance in human bacteria were performed by applying Cochran-Armitage test. One-sided tests were chosen because of preliminary expected trend directions. Cochran-Armitage test calculates probability in binomial proportions across one single, levelled variable. In this report, the test has been performed on susceptibility data from the past 10 and five years. The significance levels were calculated using the DescTools v0.99.19 package in R version 3.5.0. A p-value of <0.05 was considered significant. The resulting p-values are reported supplemented by an arrow indicating trend direction. Note that the significance levels serve to support the graphs and thus should be interpreted with caution.

9.11 ESBL-producing bacteria in human patients

9.11.1 Data source

Since 2014, the Danish DCMs have on a voluntary basis submitted 3rd generation cephalosporin resistant *Escherichia*

coli isolates from bloodstream infections for verification and genotyping at the Antimicrobial Resistance Reference Laboratory, Statens Serum Institut.

9.11.2 Microbiological methods of isolates from patients

Since 2014, whole genome sequencing (WGS) and *in silico* bioinformatics analysis have been applied for isolates predicted to carry ESBL and/or AmpC genes based on initial phenotypic tests, to characterize the genetic background of the ESBL and/or AmpC phenotypes. Only one isolate from each patient was included if less than 12 months were between isolation of the two isolates.

9.11.3 Data handling

The quality of the raw sequencing and assembly data was ensured using the analytical tool BIFROST [https://github.com/ssi-dk/bifrost/]. An in-house bacterial analysis pipeline, building on a weekly update of the ResFinder database [https://bitbucket. org/genomicepidemiology/resfinder] was used for the *in silico* detection of acquired ESBL genes, pAmpCs, carbapenemase genes and MLST from assembled WGS data. For isolates with no ESBL-, pAmpC-, or carbapenemase-encoding genes detected, the sequences were investigated for promotor mutations presumed to up-regulate chromosomal AmpC by the use of myDb-Finder version 1.2 [https://cge.cbs.dtu.dk]. Possible clonal clusters were detected using the SeqSphere+ (Ridom) software to call cgMLST types (*E. coli* scheme).

9.12 CPO in human patients

9.12.1 Data source

The Danish DCMs have on a voluntary basis submitted carbapenem resistant isolates for verification and genotyping at the Antimicrobial Resistance Reference Laboratory, Statens Serum Institut. Since September 5th 2018, CPO has had mandatory notification in Denmark. For outbreak investigation data from The National Patient Register (LPR), information gathered at the hospitals, information of residence from the Danish Civil Registration System (CPR) and telephone interviews was conducted.

9.12.2 Microbiological methods

All submitted isolates (originating both from screening and clinical samples) predicted to carry a carbapenemase based on initial phenotypic tests were subjected to WGS. More than one isolate from the same patient were included, only if the isolates belonged to different bacterial species and/or if isolates within the same species harboured different carbapenemases.

9.12.3 Data handling

The quality of the raw sequencing and assembly data was ensured using the analytical tool BIFROST [https://github.com/ssi-dk/bifrost/]. An in-house bacterial analysis pipeline, building on a weekly update of the ResFinder database [https://bitbucket.org/genomicepidemiology/resfinder], was used for the *in silico* detection of acquired CPO genes and MLST from assembled WGS data. Possible clonal clusters were detected using the SeqSphere+ (Ridom) software to call cgMLST types where such schemes are available (*E. coli, Klebsiella pneumoniae* and *Acinetobacter baumannii*). For outbreak investigation identified clonal clusters were linked with patient data like time and place of hospitalisation, place of residence and telephone interviews. Isolates from two or more persons (cases) sharing the same unique genotype was defined as an outbreak. In verified outbreaks, an epidemiological link could be established between two or more cases in the cluster, e.g. the patients had been at the same hospital ward at the same time or they live in the same geographical location such as a nursing home. When no epidemiological link could be establish between cases with the same unique genotype, the outbreak was classified as a possible outbreak. A possible outbreak can be reclassified as a verified outbreak, if new cases or information is providing an epidemiological link between two or more of the cases. Outbreak investigations of a cluster of cases is closed when no new cases has appeared within 6 months, but can be reopened, if new cases is detected in the surveillance of CPO.

9.13 VRE in human patients

9.13.1 Data source

The Danish DCMs have on a voluntary basis, submitted VRE for species identification, genotyping and surveillance to the Antimicrobial Resistance Reference Laboratory, Statens Serum Institut.

9.13.2 Microbiological methods

All clinical VRE isolates have been whole-genome sequenced. Only one isolate from each patient was included if less than 12 months were between isolation of the two isolates.

9.13.3 Data handling

The quality of the raw sequencing and assembly data was ensured using the analytical tool BIFROST [https://github.com/ssi-dk/bifrost/]. An in-house bacterial analysis pipeline, building on a weekly update of the ResFinder database [https://bitbucket.org/genomicepidemiology/resfinder], was used for the *in silico* detection of genes related to vancomycin resistance in enterococci and MLST from assembled WGS data. Possible clonal clusters were detected using the SeqSphere+ (Ridom) software to call cgMLST types.

9.14 Invasive *Streptococcus pneumoniae* in humans

9.14.1 Data source

Invasive pneumococcal disease is a notifiable disease in Denmark, and it is mandatory to submit all invasive isolates of *S. pneumoniae* to SSI for serotyping and susceptibility testing. For cases, where isolates could not be submitted, identification and registration is conducted by use of the Danish Microbiology Database (MiBa).

9.14.2 Microbiological methods

Identification or confirmation of the species *S. pneumoniae* was based on: visual evaluation of colonies, positive optochin test and test with either latex omni test (ImmuLex™ *S.*

pneumoniae Omni, SSI Diagnostica, Denmark) or Neufeld based Omni serum (SSI Diagnostica, Denmark). If challenging results occurred, MALDI-TOF, bile solubility test, or whole genome sequencing were performed to further confirm the correct species identification. For non-viable isolates, species identification was based on the detection of the *lytA* gene by the use of PCR.

Serotype identification of invasive *S. pneumoniae* was performed by the use of latex agglutination (ImmuLex™ Pneumotest Kit, SSI Diagnostica, Denmark) and serotype specific antisera by the Neufeld test (SSI Diagnostica, Denmark). For non-viable isolates, serotyping was often possible by the use of PCR.

9.14.3 Susceptibility testing

Screening for penicillin- and erythromycin-resistant *S. pneumoniae* was performed with 1 µg oxacillin discs and 15 µg erythromycin discs (Oxoid, Roskilde, Denmark), respectively, on Mueller-Hinton agar (Mueller-Hinton plate, 5% blood, 20 mg beta- NAD, SSI Diagnostica, Denmark). Isolates, that were found non-susceptible by screening were further analysed for penicillin and erythromycin MICs by broth microdilution using the STP6F plate, Sensititre (Trek Diagnostic Systems, Thermo Scientific) as recommended by the manufacturer. All breakpoints used were as defined by EUCAST (Eucast Clinical Breakpoint Tables v.9.0). Isolates that were either resistant or susceptible increased exposure were categorised together as non-wild-type. For cases, where an isolate was not received at the reference laboratory, susceptibility data could often be found in MiBa.

9.14.4 Data handling

Only cases with isolates from blood or spinal fluid were included in the DANMAP report. Repeated samples within a 30 days window were classified as duplicates and were omitted from the analysis.

9.15 Invasive beta-haemolytic streptococci (group A, B, C and G streptococci) in humans

9.15.1 Data source

Isolates of beta-haemolytic streptococci (BHS) from normally sterile sites (e.g., blood, cerebrospinal fluid, synovial fluid, pleural fluid, ascites, and tissue obtained during surgery) were submitted to SSI from the departments of clinical microbiology on a voluntary basis.

9.15.2 Microbiological methods

Identification of streptococcal group was performed by latex agglutination (Streptococcal Grouping Reagent, Oxoid, Denmark). DNA from all isolates was extracted using DNeasy Blood & Tissue kit as described by the manufacturer (Qiagen, Valencia, CA). Fragment libraries were constructed using the Nextera XT DNA Library Preparation Kit (Illumina, San Diego, CA), followed by 150-bp paired-end sequencing on a NextSeq (Illumina) according to manufacturer's instructions. The sequencing reads were assembled using SKESA [https://github.com/ncbi/SKESA]. The isolates were species typed using Kraken [https://ccb.jhu.edu], and MLST typed using https://github.com/tseemann/mlst. The isolates were *emm* typed by performing a BLAST search to all published *emm* types by CDC [http://www.cdc.gov/streplab/protocol-emm-type.html].

9.15.3 Susceptibility testing

Screening for penicillin, erythromycin and clindamycin resistance was performed with 1 unit penicillin G discs, 15 µg erythromycin discs and 2 µg clindamycin discs (Oxoid, Denmark) on Mueller-Hinton agar (Mueller-Hinton plate, 5% blood, 20 mg beta-NAD, SSI Diagnostica, Denmark). Isolates were also tested for inducible clindamycin resistance. For non-susceptible streptococci the MIC was determined with ETEST (Biomérieux), with either benzylpenicillin, erythromycin or clindamycin on Mueller-Hinton agar. The breakpoints used were as defined by the EUCAST (EUCAST Clinical Breakpoint Tables v. 9.0). Isolates that were either resistant or susceptible increased exposure were categorised together as resistant.

9.15.4 Data handling

A case of invasive BHS disease was defined as the isolation of BHS from a normally sterile site. A new case was defined as an invasive isolate with a different Lancefield group within 30 days from the first one or an invasive isolate of any Lancefield group more than 30 days after the first episode, or the isolation of a new type (T-type, *emm*-type or GBS type) if the group was identical on both occasions.

Only one isolate from each unique case of BHS infection was included in the DANMAP report.

9.16 Invasive *Haemophilus influenzae* in humans

9.16.1 Data source

Invasive infections with *Haemophilus influenzae* type b (Hib) is a notifiable disease in Denmark and all invasive isolates nationwide are sent to the reference laboratory at SSI. By tradition, invasive isolates of other serotypes have also been submitted on a voluntary basis, and thus a broader picture of invasive *H. influenzae* in Denmark can be obtained. All cases were identified through MiBa and registered in the surveillance database at SSI. Cases, where isolates were not submitted to the reference laboratory were registered as "unknown serotype".

9.16.2 Microbiological methods

At SSI the isolates were serotyped and biotyped. Identification or confirmation of the species *H. influenzae* was based on: visual evaluation of colonies, the satellitism test and biochemical reactions. Serotypes were determined by latex agglutination (ImmuLexTM *H. influenzae*, SSI Diagnostika, Denmark). Biotypes were determined by a series of biochemical reactions.

9.16.3 Susceptibility testing

Susceptibility-testing of the 2019 isolates was performed by betalactamase test and disc diffusion assays. The presence

of beta-lactamase encoding plasmids TEM-1 and ROB-1 were moreover found through whole-genome sequencing. Where isolates were not submitted, data from antimicrobial susceptibility testing at the departments of clinical microbiology were extracted from MiBa, when available.

9.16.4 Data handling

A case was defined as isolation of *H. influenzae* from normally sterile sites (e.g. blood, spinal fluid, pleura, joint). Repeated samples within a 30 days window were classified as duplicates and were omitted from the analysis.

9.17 *Staphylococcus aureus* including MRSA in humans

9.17.1 Data source

Blood isolates were referred on a voluntary basis by all DCMs to the National reference laboratory for antimicrobial resistance at SSI. Detection of methicillin-resistant *Staphylococcus aureus* (MRSA) is a notifiable condition in Denmark and therefore all MRSA isolates from all sample types were sent to the reference laboratory.

9.17.2 Microbiological methods

At SSI, all isolates were initially tested using a multiplex PCR detecting the *spa*, *mecA*, *hsd*, *scn* and *pvl* (LukF-PV) genes [Larsen et al. 2008. Clin Microbiol Infect. 14: 611-614; Stegger et al. 2012. Clin Microbiol Infect. 18: 395-400]. *spa* was used as *S. aureus* specific marker and for subsequent typing by Sanger sequencing [Harmsen et al. 2003. J Clin Microbiol. 41: 5442-5448], *mecA* to determine MRSA status, and *scn* and *hsd* as markers for human adaptation and relation to CC398, respectively. All bacteremia cases and *mecA* negative presumed MRSA were tested for presence of the *mecC* gene. *spa*-negative isolates were confirmed as *S. aureus* by MALDI-TOF. Based on the *spa* type and known association with MLST typing, each isolate was assigned to a clonal complex (CC).

9.17.3 Susceptibility testing

Antimicrobial susceptibility testing of *Staphylococcus aureus* was performed by Minimum Inhibitory Concentration (MIC) determination using a custom-made panel (DKSSP2, Trek Diagnostic Systems Ltd.). Inoculation and incubation procedures were in accordance with the CLSI guidelines [Clinical and Laboratory Standards Institute, USA] and the European standard ISO 20776-1:2006. The isolates were tested for antimicrobial susceptibility in accordance with the Decision 2013/652/EU about the EU harmonised monitoring of antimicrobial resistance.

Staphylococcus aureus ATCC 29213 was included as quality control for each batch of resistance determination.

9.17.4 Data handling

For blood isolates a case was defined as a patient with a positive blood culture. Subsequent isolates from the same patient was only included if the positive blood cultures were obtained at least one month apart (new episode).

For MRSA, data on the characteristics of the isolates and the clinical/epidemiological information were obtained from the Danish MRSA register at SSI (mandatory reportable). Patients were registered, regardless of colonisation or clinical infection, the first time they were diagnosed with MRSA or when a new subtype was demonstrated. Based on the reported information, MRSA cases were classified as colonisation/active screening (i.e. surveillance samples to detect nasal, throat, gut or skin colonisation), imported infection (i.e. acquired outside Denmark), infection acquired in a Danish hospital, defined as diagnosed >48 hours after hospitalisation with no sign of infection at admittance (HA-MRSA) or infection diagnosed outside hospitals (community onset). MRSA cases with community onset were further classified according to risk factors during the previous 6 months as either health-care associated with community onset (HACO) or community-acquired (CA). Health-care associated risk factors included prior hospitalisations, stay in long-term care facilities and being a health-care worker. Community risk factors included known MRSA-positive household members or other close contacts. Due to the increasing numbers of cases belonging to CC398, this type was treated separately as both epidemiology and relevant exposure are different from other CA cases.

9.18 Gonococci in humans

9.18.1 Data source

Isolates of gonococci (*Neisseria gonorrhoeae*) were submitted from the departments of clinical microbiology to SSI on a voluntary basis. The isolates were obtained by culture of specimens from a variety of anatomical locations, most often urethra, cervix, rectum, throat, and rarely from other sites, e.g. eyes, joint fluid, and blood.

9.18.2 Microbiological methods

The bacteriological identification of the received isolates was performed by MALDI-TOF.

9.18.3 Susceptibility testing

For all isolates the MICs of azithromycin, ceftriaxon and ciprofloxacin were determined with ETEST (Biomérieux) on chocolate agar. The MICs of cefixime, gentamicin, and spectinomycin were determined for 118 consecutive isolates as part of an ECDC project on gonococcal antimicrobial resistance. The breakpoints used were those defined by EUCAST (EUCAST Clinical Breakpoint Tables v. 9.0). A cefinase disc technique was used to examine the isolates for beta-lactamase production.

9.18.4 Data handling

Only one isolate from each unique case of gonorrhoea were included in the DANMAP report. Laboratory demonstration of gonococci in repetitive specimens was considered to represent a new case of gonorrhoea if the specimens were obtained with an interval of more than 21 days.

10

TERMINOLOGY

List of abbreviations

AGP	Antimicrobial growth promoter
AMU	Antimicrobial use
AMR	Antimicrobial resistance
ATC	Anatomical Therapeutic Chemical Classification System
ATCvet	Anatomical Therapeutic Chemical Classification System for veterinary medicines
ATU	Area of Technical Uncertainty
CA	Community-acquired
CC	Clonal complex
CDI	Clostridium difficile infections
CHR	Central Husbandry Register
CPE	Carbapenemase producing Enterobacterales
CPO	Carbapenemase producing organisms
CPR	Danish Civil Registry, register for social security numbers
DAD	Defined Daily Doses per 100 admissions
DADD	Defined Animal Daily Dose
DaDDD	Danish adjusted Defined Daily Doses
DAPD	Defined Animal Daily Dose per 1,000 animals per day
DBD	Defined Daily Doses per 100 occupied bed-days
DCM	Department of clinical microbiology
DDD	Defined Daily Dose
DID	Defined Daily Doses per 1,000 inhabitants per day (DDD/1000 inhabitants/day)
DTU	Technical University of Denmark
DVFA	Danish Veterinary and Food Administration
EARS-Net	The European Antimicrobial Resistance Surveillance Network
ECDC	European Centre for Disease Prevention and Control
EFSA	European Food Safety Authority
ESC	Extended Spectrum cephalosporinase
EUCAST	European Committee on Antimicrobial Susceptibility Testing
GP	General Practitioner
HAI	Hospital-acquired infections
HCAI	Health care associated infections
HACO	Health care associated community onset
HAIBA	Hospital Acquired Infections Database
MiBa	The Danish Microbiology Database
MIC	Minimum inhibitory concentration
MDR	Multidrug-resistant
MRSA	Methicillin-resistant *Staphylococcus aureus*
NAAT	Nucleic acid amplification test
OIE	World Organisation for Animal Health
PCR	Polymerase chain reaction
PHC	Primary health care
RFCA	Regional Veterinary and Food Control Authorities
SEGES	Knowledge Centre for Agriculture
SSI	Statens Serum Institut
ST	Serotype/Sequence type
VASC	Veterinary advisory service contracts
VMP	Veterinary medicinal products
VetStat	Danish Register of Veterinary Medicines
VRE	Vancomycin-resistant enterococci
VVE	Vancomycin-variable enterococci
WGS	Whole-genome sequencing
WHO	World Health Organization

Glossary

Anatomical Therapeutic Chemical (ATC) classification. An international classification system for drug consumption studies. The ATC code identifies the therapeutic ingredient(s) of each drug for human use according to the organ or system on which it acts and its chemical, pharmacological and therapeutic properties. Antibacterials for systemic use are known as ATC group J01. The ATC classification is maintained by the WHO Collaborating Centre for Drug Statistics and Methodology (Oslo, Norway) [www.whocc.no/atcddd/indexdatabase/]. The ATC classification for veterinary medicinal products, ATCvet, is based on the same main principles as the ATC classification system for medicines for human use and is also maintained by the WHO Collaborating Centre for Drug Statistics and Methodology [www.whocc.no/ atcvet/database/].

Antibacterial agents. Synthetic (chemotherapeutics) or natural (antibiotics) substances that destroy bacteria or suppress bacterial growth or reproduction [Source: Dorland's Illustrated Medical Dictionary]. In the section on human consumption, 'antibacterial agents' are referred to as 'antimicrobial agents' (see below).

Antimicrobial agents. The term 'antimicrobial agents' covers antibacterial, antiviral, coccidiostatic and antimycotic agents. In the section on veterinary consumption, the broad term 'antimicrobial agents' is generally used because coccidiostats are included. Antiviral substances are not used in veterinary medicine, and antimycotics are only registered for topical veterinary use and used mainly in companion animals. Antimycobacterial agents are not included. The term 'antibacterial agents' is only used in the veterinary section for precision, to distinguish from use of coccidiostats as feed additives (poultry only). In the chapter on human consumption, the term 'antimicrobial agents' refers to all antibacterial agents for systemic use (J01 in the ATC system) including metronidazole and vancomycin, which are used for systemic treatment but registered under the ATC code P01AB01 and A07AA09, respectively.

Broiler. A type of chicken raised specifically for meat production. In Denmark, the average weight after slaughter is 1.51 kg.

CentralHusbandryRegister (CHR). This is a register of all Danish farms defined as geographical sites housing production animals. It contains information concerning ownership, farm size, animal species, age groups, number of animals and production type. Each farm has a unique farm identity number (CHR-number).

Defined Daily Dose (DDD). This is the assumed average maintenance dose per day for a drug used for its main indication in adults. It should be emphasised that the Defined Daily Dose is a unit of measurement and does not necessarily reflect the recommended or prescribed daily dose. DDDs provide a fixed unit of measurement independent of price and formulation, enabling the assessment of trends in drug consumption and to perform comparisons between population groups. The DDDs are defined and revised yearly by the WHO Collaborating Centre for Drug Statistics and Methodology [www.whocc.no/atcddd/ index database].

Defined Daily Dose per 100 admissions (DAD). DAD measures the amount of daily doses consumed per 100 admitted patients at hospitals during a given timeframe (one year). It is used for benchmarking consumption related to the hospital activity and will typically be compared to the consumption measured in DBD (see below). DAD and DBD will generally be applied to comparing individual hospitals and consumption of individual drug classes over time. In DANMAP DAD covers all patients attended at somatic hospitals only. Admission-days are extracted from the National Patient Registry (Landspatientregistret, LPR). Time of reporting differs between hospitals and closing time for reporting is later than extraction time for the DANMAP report.

Defined animal daily dose (DADD). DADD is the average maintenance dose per day for a drug used for its main indication in the appropriate animal species. DADD has been specifically defined for use in DANMAP and does not always completely match the "prescribed daily dose" or the recommended dosage in the Summaries of Product Characteristics (SPC). The DADD is defined as mg active compound per kg live biomass for each antimicrobial agent, administration route and animal species. In DANMAP 2012, the DADD replaced the ADD (as defined in VetStat and assigned at product level). For more details, see section 9.2, Materials and Methods and the applied DADD's are listed in the web annex.

DADD per 1,000 animals per day (DAPD). Trends in veterinary consumption, both within and across species, are presented in DAPD, which allows for comparison between sectors and adjustment for changes in live biomass. The estimated live biomass is expressed as the number of standard animals with an estimated average weight and lifetime. This may also be referred to as the 'standard-animals-at-risk' and takes into account species differences in body-mass and lifespan. The DAPD is a statistical measure, providing an estimate of the proportion of animals (in thousands) treated daily with a particular antimicrobial agent. For example, 10 DAPDs indicate that an estimated 1% of the population, on average, receives a certain treatment on a given day (see section 9.2, Materials and Methods).

Defined Daily Doses per 100 occupied bed-days (DBD). DBD is the consumption calculated in defined daily doses at hospitals, divided through the number of bed-days. This allows comparison of hospitals related to the length of patient stays.

The number of bed-days is extracted from The National Patient Registry (Landspatientregistret, LPR). Time of reporting differs between hospitals and closing time for reporting is later than extraction time for the DANMAP report. Every patient admitted to hospital accounts for one bed-day, independent of the actual length of stay within every 24 hours. This corresponds to the actual hours at hospital divided by 24 hours and rounded up to the next whole number. For patients transferred between wards each transfer will count as a new bed-day. Non-ended hospital stays are not included.

DDD per 1,000 inhabitants per day (DID). Consumption in both primary health care, hospital care and the overall total consumption is presented in DID, allowing for comparison between sectors and for illustration of the consumption in hospital care without taking hospital activity (discharges and length of stays) into account. Data presented in DID provide a rough estimate of the proportion of the population within a defined area treated daily with certain drugs. For example, 10 DIDs indicate that 1% of the population on average gets a certain treatment daily. In figures presented as DDD/1000 inhabitants/day.

ESBL. In the DANMAP report, 'ESBL' describes the clinically important acquired beta-lactamases with activity against extended-spectrum cephalosporins; including the classical class A ESBLs (CTX-M, SHV, TEM), the plasmid-mediated AmpC and OXA-ESBLs [Giske et al. 2009. J. Antimicrob. Chemother. 63: 1-4].

Finishers. Pigs from 30-100 kg live weight from after the weaner stage to time of slaughter.

Fully sensitive. An isolate will be referred to as fully sensitive if susceptible to all antimicrobial agents included in the test panel for the specific bacteria.

Human clinical samples/isolates. In the DANMAP report, human clinical samples and/or isolates refers to the sample being taken in a clinical situation, meaning in the course of diagnosing and treating a possible infection in a patient.

Human screening samples/isolates. In the DANMAP report, human screening samples and/or isolates refers to sampling being performed for monitoring purposes in a defined group of asymptomatic individuals. Examples are rectal swaps to determine carriage of multi-resistant bacteria in the intestine or swaps from the throat, nostrils or perineum to determine carriage of methicillin-resistant *Staphylococcus areus* (MRSA).

Intramammaries. Antimicrobial agents for local application in the mammary gland (udder) to treat mastitis.

Layer. A hen raised to produce eggs for consumption.

Minimum inhibitory concentration (MIC). This is the lowest concentration of antimicrobial agent in a given culture medium, e.g. broth or agar, below which growth of the bacteria is not inhibited.

Multi-resistant. A *Salmonella*, *Campylobacter*, *Enterococcus* or *E. coli* isolate is assumed multi-resistant if it is resistant to three or more of the main antimicrobial classes. The number of antimicrobial classes and antimicrobial agents included in this definition depend on the test panel for each bacterium.

Pets or pet animals. Dogs, cats, birds, mice, Guinea pigs and more exotic species kept at home for pleasure, rather than kept for work or food. Horses are not included as pet animals. The live biomasses of Danish pets used for estimating veterinary consumption only include dogs and cat.

Piglet. The new-born pig is called a piglet from birth till they are permanently separated from the sow at 3-4 weeks of age. The weight of the piglet at weaning is approximately 7 kg.

Poultry. The major production species are fowl *Gallus gallus* (broilers, layers, including breeding and rearing) and turkey. Regarding antimicrobial consumption, 'poultry' also includes domesticated ducks, geese, game birds and pigeons.

Sow. Any breeding female pig on the farm.

Weaner. Any pig of 7–30 kg live weight after it has been weaned (dry diet and water only).

DANMAP 2019

Printed matter
5041 0751