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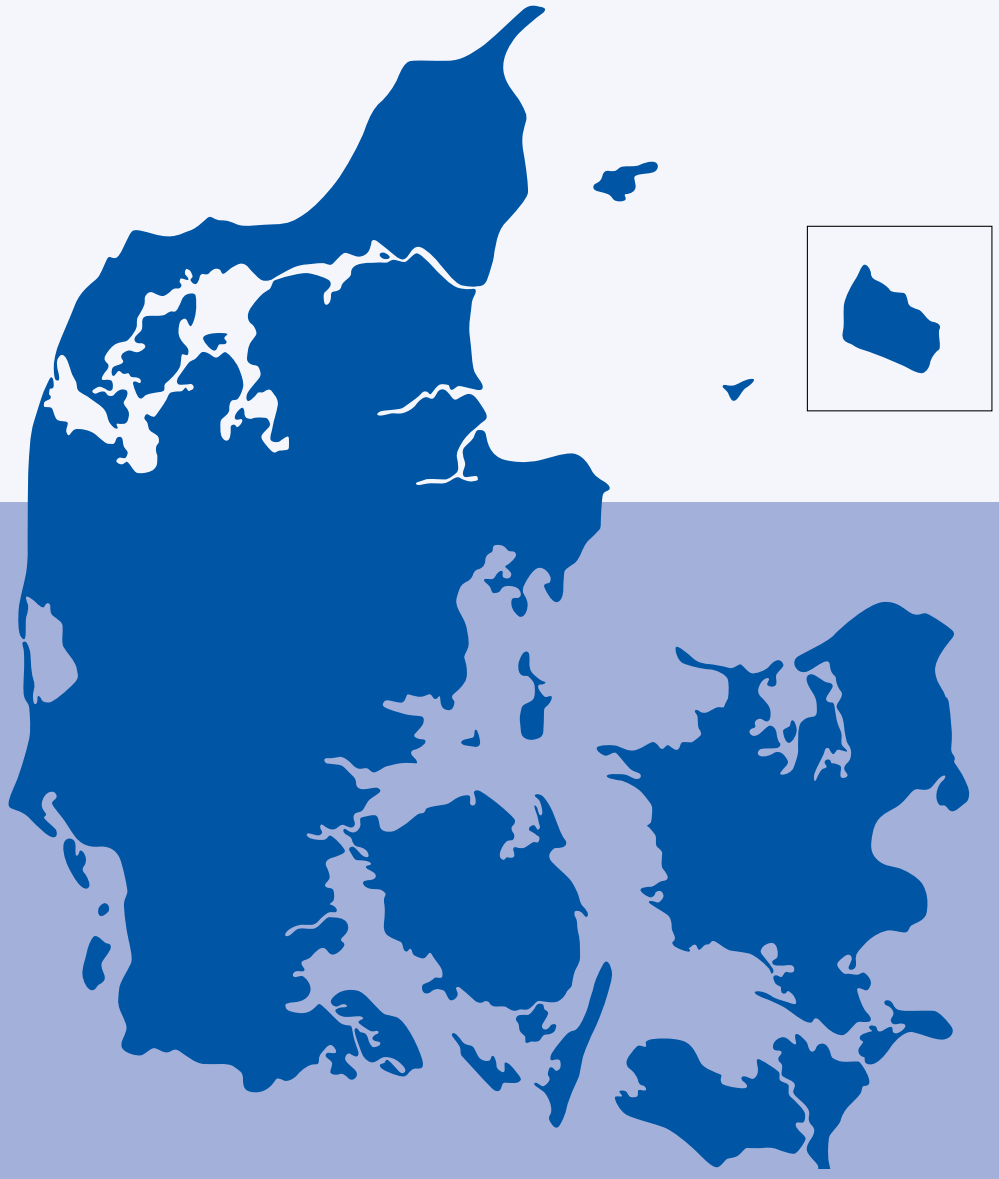
STATENS SERUM INSTITUT

DANMAP 2023

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



Diagnostic Infectious Disease Preparedness, Statens Serum Institut
National Food Institute, Technical University of Denmark

DANMAP 2023

Editors:
Ana Sofia Ribeiro Duarte
Joana Pessoa
Majda Attauabi
Mikkel Lindegaard
Ute Wolff Sönksen

DANMAP Steering Committee:
National Food Institute: Frank Møller Aarestrup, Marianne Sandberg
Statens Serum Institut: Anders Rhod Larsen, Ute Wolff Sönksen

Contributing authors:
Anette M. Hammerum
Anne Kjerulf
Asja Kunøe
Brian Kristensen
Charlotte M. Salomonsen
Frank Hansen
Hans-Christian Slotved
Henrik Hasman
Jeppe Boel
Jesper Larsen
Kasper Thystrup Karstensen
Kurt Fuursted
Lina M. Cavaco
Lone Jannok Porsbo
Louise Roer
Marianne Sandberg
Patrick Munk
Peter Damborg
Pia T. Hansen
Steen Hoffmann
Svend Haugegaard
Tinna Urth
Vibe Dalhoff Andersen

Contact:
Ana Sofia Ribeiro Duarte (asrd@food.dtu.dk)
National Food Institute,
Technical University of Denmark
Henrik Dams Allé, Building 204, DK-2800 Kgs. Lyngby

Ute Wolff Sönksen (uws@ssi.dk)
Diagnostic Infectious Disease Preparedness
Statens Serum Institut
Artillerivej 5, DK-2300 Copenhagen

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

DANMAP 2023 - November 2024 - ISSN 1600-2032

The report is available from www.danmap.org.

DANMAP 2023

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

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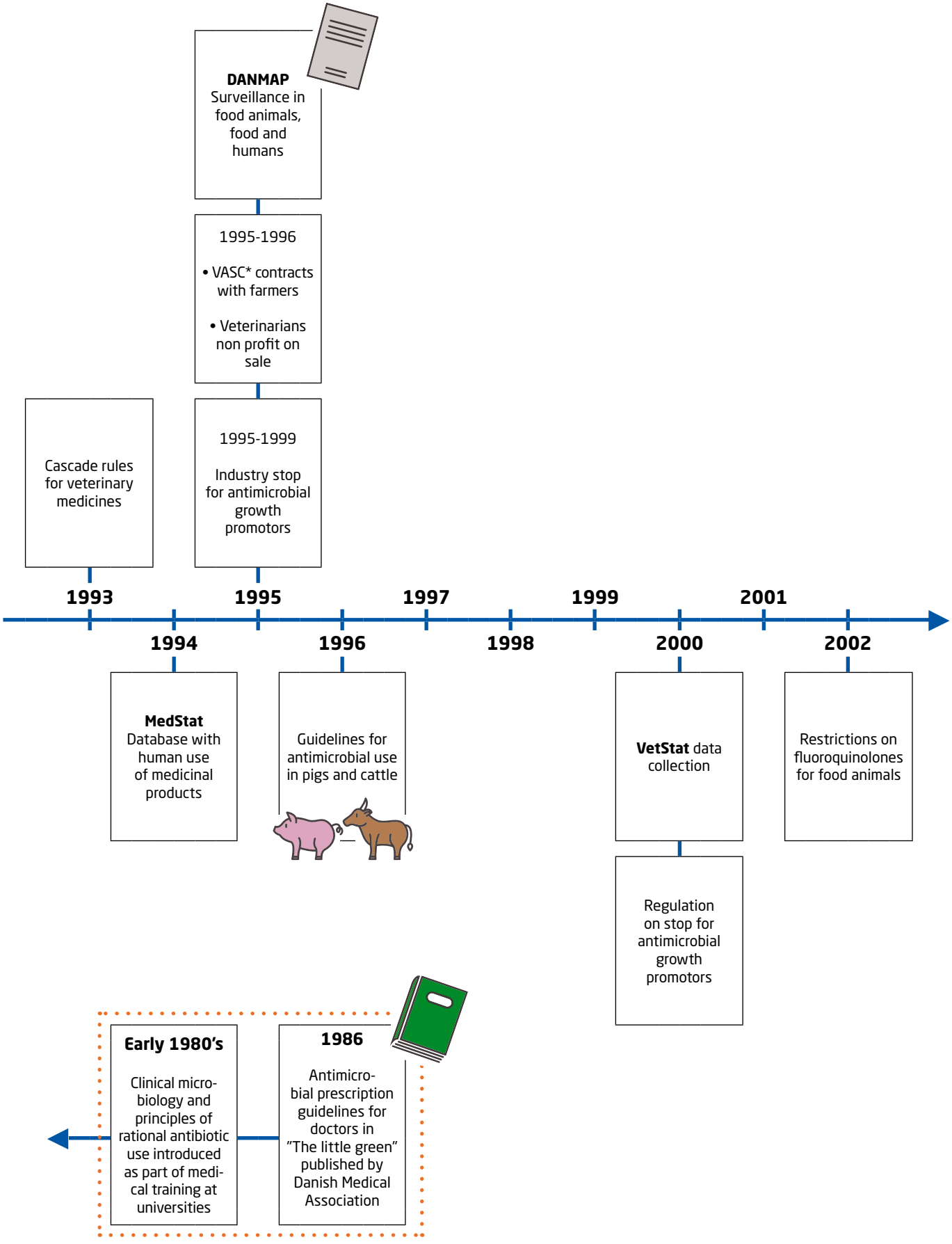
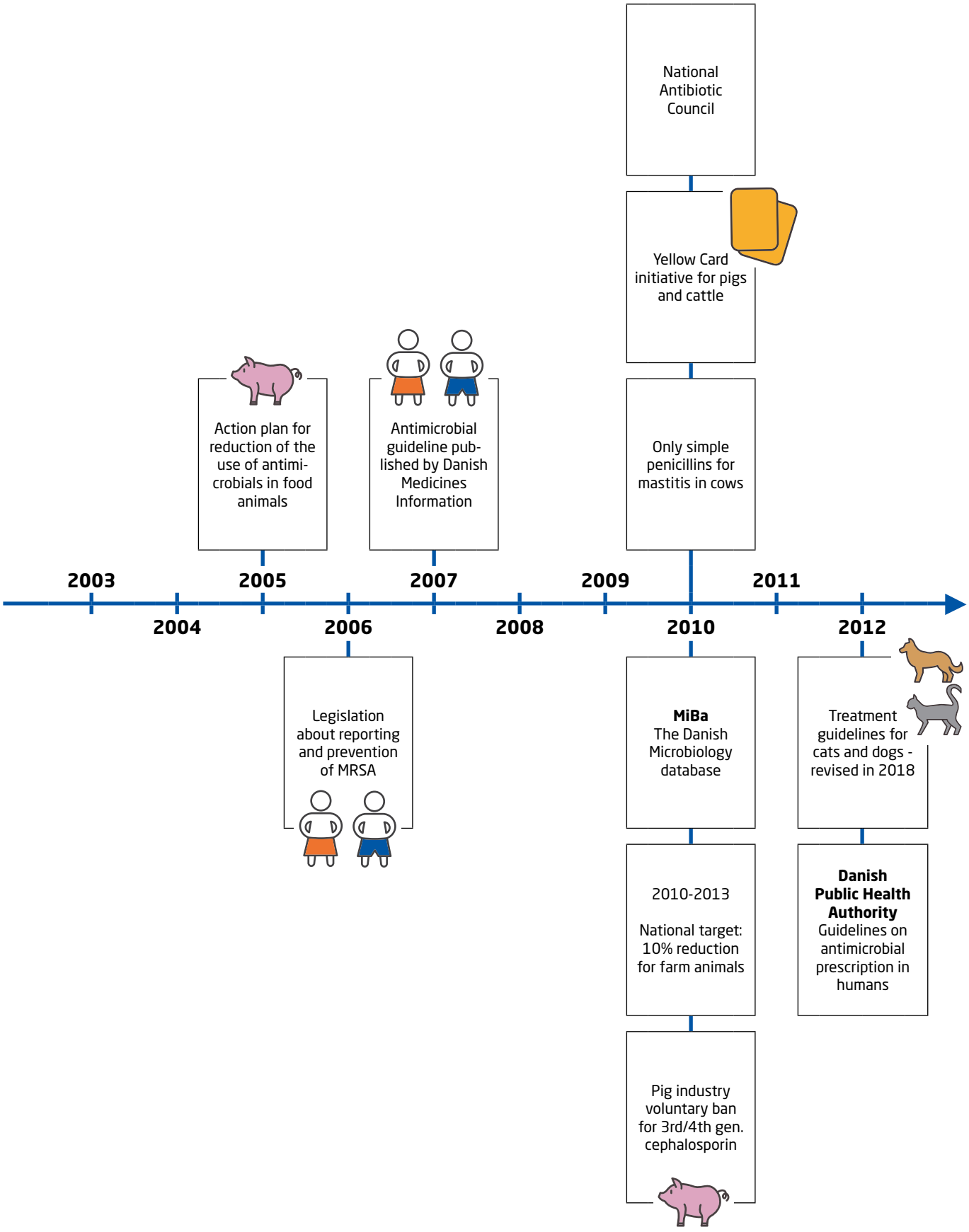


* Veterinary Advisory Service contracts


2003
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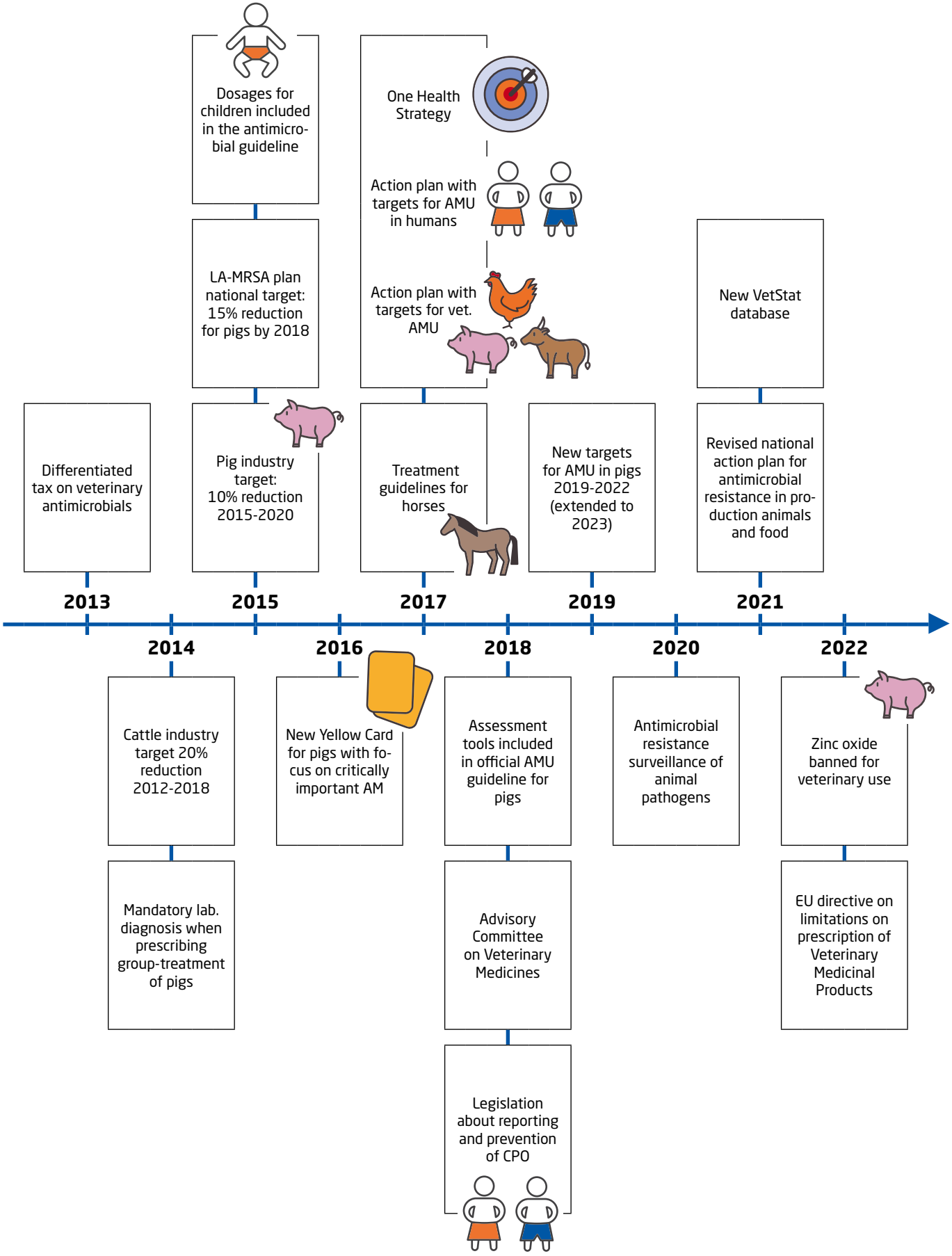
Action plan for reduction of the use of antimicrobials in food animals
Antimicrobial guideline published by Danish Medicines Information
Legislation about reporting and prevention of MRSA
National Antibiotic Council
Yellow Card initiative for pigs and cattle
Only simple penicillins for mastitis in cows
MiBa The Danish Microbiology database
2010-2013 National target: 10% reduction for farm animals
Pig industry voluntary ban for 3rd/4th gen. cephalosporin
Treatment guidelines for cats and dogs - revised in 2018
Danish Public Health Authority Guidelines on antimicrobial prescription in humans

continued ... Timeline of DANMAP



1. Editorial

2024, as the year is nearing its end, the world stands between hope and challenge in regards to implementing the changes and actions needed to preventing, managing and controlling antimicrobial resistance (AMR) to which the United Nations members committed themselves at the UN General Assembly in New York in September.

Denmark, with its long-standing tradition of high-quality diagnostics, robust health data systems and effective public health responses, is well-positioned to endorse some of the agreed actions. Denmark also has strong experience in working across the human and animal sectors in an integrating One Health approach, which offers a unique opportunity to support both national and global efforts against AMR. Finally, Denmark is characterized by flat hierarchical structures and a strong collaborative spirit in the planning, coordination and response to possible AMR threats.

2023, the year of the data collected and presented in this DANMAP report, seems to be an equally important year. While 2022 was a year pulling out of the COVID-19 pandemic, having to enforce, strengthen and reorganize much of health care systems and work, 2023 saw proof of the former years investigations into antibiotic stewardship and infection prevention and control programs. Although nothing seems to be fully back to normal yet, the trends in the report start picking up at where we left in 2019. An example are continued efforts at long-term care facilities in working with better prevention of infections in the elderly, which are demonstrated by a reduced antibiotic usage and fewer infections than in former years. Another example is the continued reductions in use of the antibiotics of "special critical interest", cephalosporins and fluoroquinolones, at hospitals.

But not everything is solely positive, as demonstrated by a higher use of antibiotics in practically all other age groups than the elderly, continuously increasing use of piperacillin-tazobactam at hospitals, or the backlash in resistance in *E. coli* and *K. pneumoniae* in clinical samples after years of overall decreasing trends. 2023 also found an increase in the number of outbreaks with Carbapenemase-producing Enterobacterales at Danish hospitals, which demands increased screening, cleaning and other preventive efforts.

The veterinary side also saw interesting changes in 2023, which should not be left unnoticed. Following the ban of zinc oxide in the pig production from June 2022, increases were observed in usage of antimicrobials in weaners, leading to marked increases for aminoglycosides, particularly neomycin and apramycin, and a seemingly direct association to observed increasing resistance levels to the different types of aminoglycosides, particularly neomycin and gentamicin, in haemolytic *E. coli* and *Salmonella enterica* recovered from pigs.

It remains positive that no clear association could be found in analyses investigating the dispersion of resistance genes (Extended spectrum beta-lactamases, ESBL) and sequence types among bacterial strains of *E. coli* from invasive cases in humans and healthy animals, which points towards only little if any direct transmission between the examined populations. However, further genomic analysis of the isolate collection has identified some ESBL genes with probable zoonotic links, which calls for further research.

In conclusion, in 2023 Denmark continued overall low trends of resistance in bacterial strains, and a comparably low usage of antimicrobials in both the veterinary and human sector, but this should not be misinterpreted as "nothing needs to be done". Continuously low levels of antibiotic resistance demand continuous efforts in prevention and rational antibiotic use. High output of pigs produced demands high and sustained input of initiatives ensuring animal health and biosecurity. Movements of goods and people across countries with varying levels of AMR demand attention and screening efforts.

All countries are now – again – encouraged to work with national action plans in a One Health approach. The Danish Veterinary and Food Administration recently published their newest version, the human side is in a consultation process regarding theirs. What is left to do is to renew the Danish One Health AMR Strategy, the overarching umbrella needed upon all efforts.

The DANMAP team

Acknowledgements

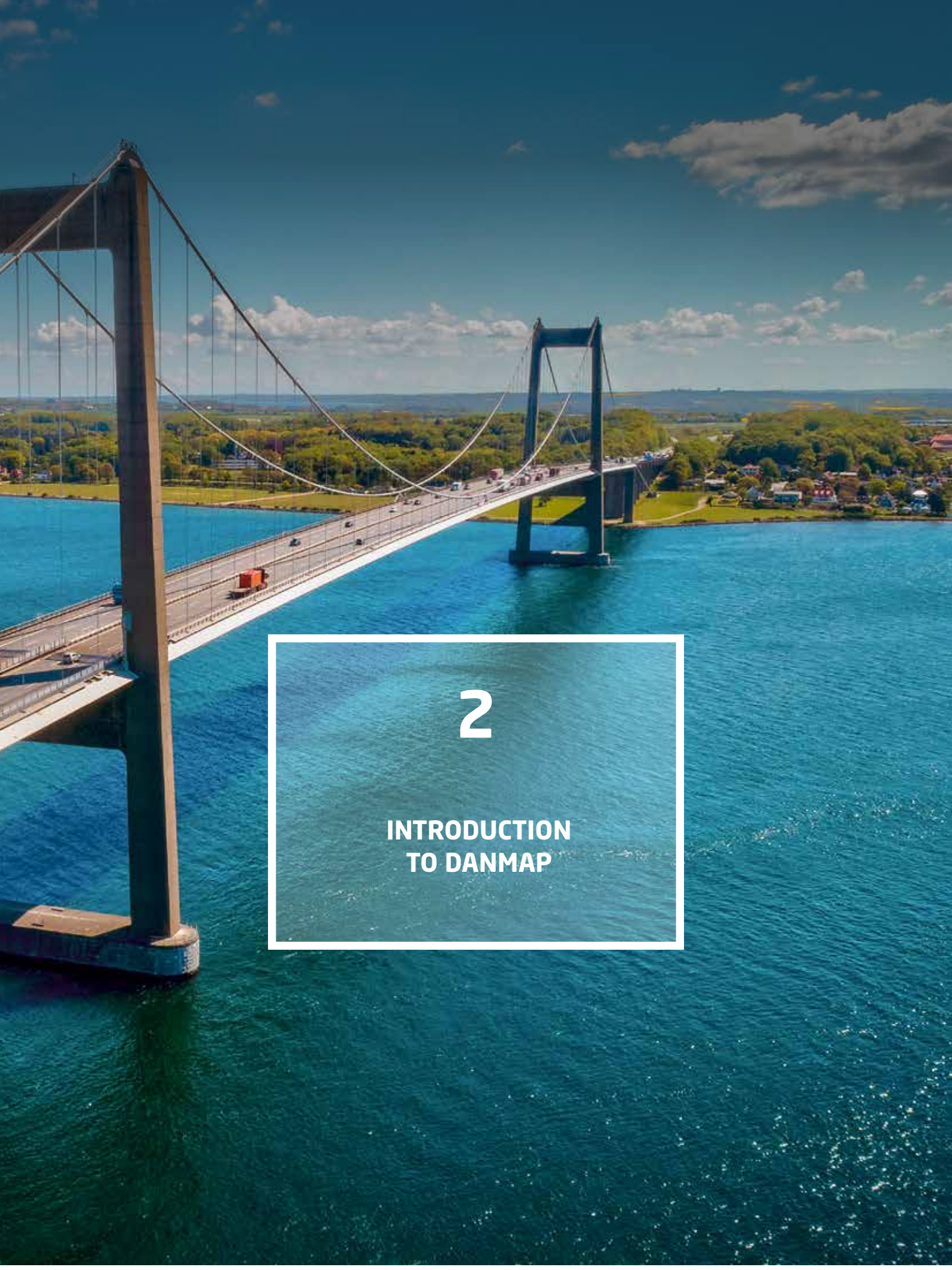
DANMAP is based on a strong collaboration between several institutions and on the contributions from highly skilled staff from many specialties and professions. Without their knowledge and engagement, there would be no DANMAP surveillance.

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

- The meat inspection staff and company personnel at the participating slaughterhouses for collecting samples from animals at slaughter
- The staff of local Veterinary and Food inspections units for collecting food and animal samples
- The staff of the Danish Veterinary and Food Administration's Laboratory, Ringsted for analysing animal and food samples
- The Danish Veterinary and Food Administration's Food and Feed Safety Division, for collecting and transmitting data on antimicrobial resistance in food and animal samples and for discussing data interpretation
- The Danish Veterinary and Food Administration's Animal Medicine and Veterinary Trade Division, for collecting and transmitting data on veterinary consumption of antimicrobial agents from VetStat and discussing the interpretation of the data
- The Danish Agriculture and Food Council for collaboration regarding the estimation of live biomass of production animals
- Statistics Denmark for providing data necessary for the estimation of live biomass of poultry
- The Danish Aquaculture Producer Organisation for providing data necessary for the estimation of live biomass of fish
- Colleagues at the National Food Institute, DTU, for valuable discussions on many topics related to the report
- All data providers for textboxes and textbox authors

Statens Serum Institut would like to thank the following:

- The Departments of Clinical Microbiology and the DANRES group - Danish Study Group for Antimicrobial Resistance Surveillance - for providing data on resistance in bacteria from human clinical samples and discussing many of the topics included in the report
- The staff of the Neisseria and Streptococcus Typing Unit at SSI for providing data on samples and resistance in beta-haemolytic 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 the Unit of Mycology at SSI for providing resistance data for human *Candida* and *Aspergillus*
- Colleagues at the Infectious Disease Epidemiology & Prevention Unit at SSI
- Colleagues at the Data Integration and Analysis Secretariat at SSI
- The Danish Health Data Authority and the Register of Medicinal Products Statistics for providing data on antimicrobial consumption on healthcare activity
- All Danish hospital pharmacies for providing data on antimicrobials consumed at hospitals through special deliverances



2

INTRODUCTION TO DANMAP

2. Introduction to DANMAP

2.1 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-producing animals and humans
- To carry out surveillance of the occurrence of antimicrobial resistance in bacteria isolated from food-producing animals, food of animal origin (meat) and humans
- To identify areas for further research, e.g. antimicrobial resistance transmission or possible associations between antimicrobial consumption and antimicrobial resistance
- To deliver data to veterinarians, medical doctors and other health professionals for the development of antibiotic treatment guidelines
- 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

Since 2021, DANMAP also provides an integrated analysis of resistance in bacteria from humans and food animals.

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 programme.

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.

These categories of bacteria are 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
- Clinical isolates from sick food-producing animals to monitor resistance

The National Food Institute at the Technical University of Denmark (DTU) and the National AMR reference laboratory at Statens Serum Institut (SSI) are responsible for data interpretation and output communication mainly via the annual DANMAP report and seminar. 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 Food, Agriculture and Fisheries. Support from the ministries has also helped build the databases and maintaining the registers, which the current surveillance system relies upon.

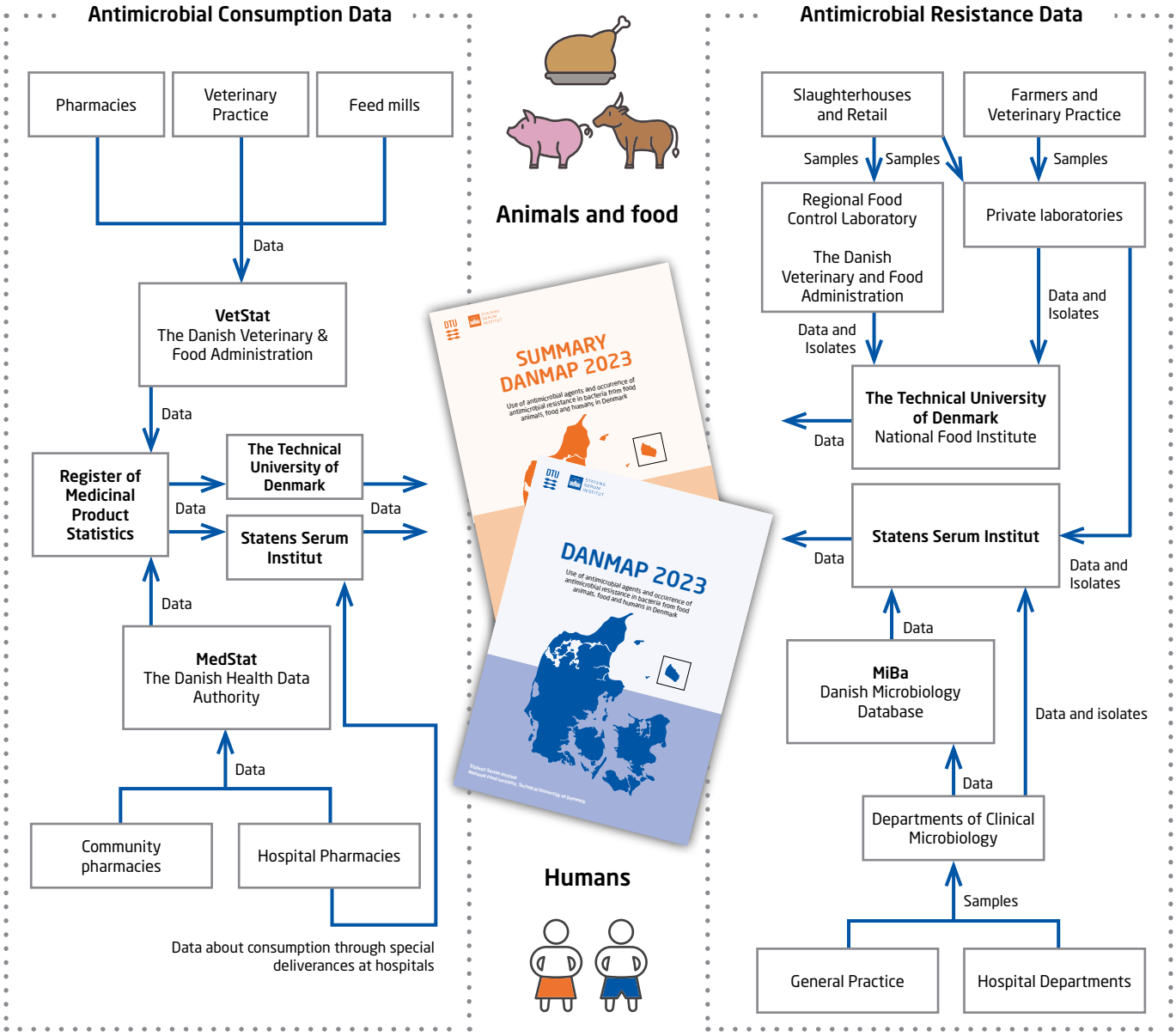
For further information on the development and history of DANMAP, please read Chapter 2, "DANMAP – A 20 year perspective" in DANMAP 2015 and Chapter 1, "DANMAP - the beginning" in DANMAP 2020.

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 2.1.

The 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 many clinical laboratories and most reference laboratories. However, phenotypical testing is still considered relevant, more feasible, cheaper and sometimes faster, especially in a clinical setting. Phenotypical testing also continues to be 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.

Figure 2.1 Organisation DANMAP regarding data and data flow DANMAP 2023



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 Institut, respectively, for further phenotypic and genotypic characterisation (Figure 2.1). 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.

Figure 2.2 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 national reference laboratories (NRL) situated at Statens Serum Institut DANMAP 2023

North Denmark Region

No. of inhabitants	594,634
No. of inhabitants/km^2	75
No. of inhabitants/GP	1,780

Central Denmark Region

No. of inhabitants	1,358,879
No. of inhabitants/km^2	104
No. of inhabitants/GP	1,655

Capital Region of Denmark

No. of inhabitants	1,891,871
No. of inhabitants/km^2	738
No. of inhabitants/GP	1,721

Region Zealand

No. of inhabitants	849,857
No. of inhabitants/km^2	118
No. of inhabitants/GP	1,918

Region of Southern Denmark

No. of inhabitants	1,237,413
No. of inhabitants/km^2	101
No. of inhabitants/GP	1,543

GP = General Practioner
DCM = Department of Clinical Microbiology
NRL = National Reference Laboratories

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

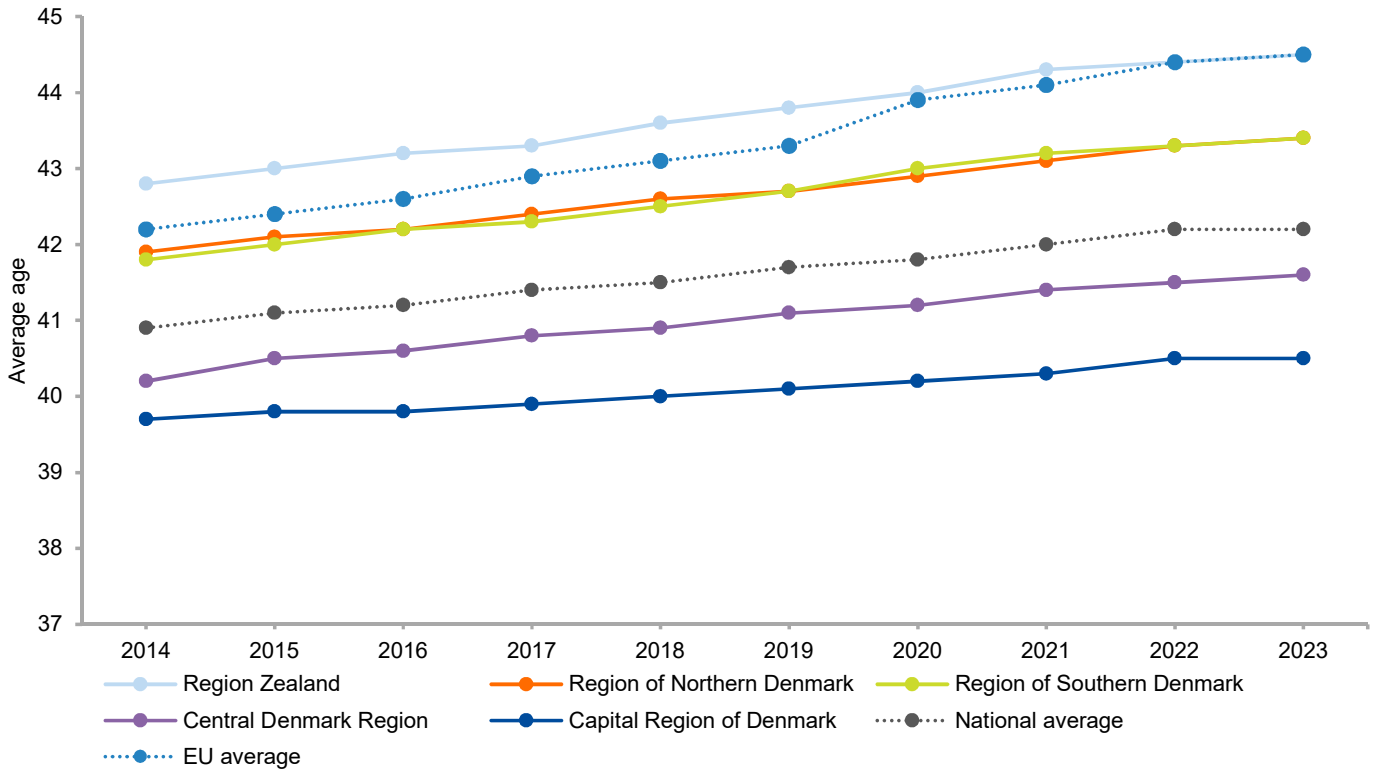
2.2 Information on demographics and health care system

During the past 27 years, the human population in Denmark has increased from approximately 5.2 million inhabitants in 1995 to 5.9 million in 2023 [www.dst.dk]. Simultaneously, the average age has increased gradually. In 2023, the national average age was 42,2 years. The population and the respective regional distribution, in 2023, is presented in Figure 2.2, while regional differences and changes in age are presented in Figure 2.3.

In Denmark, microbiological analyses are carried out by ten hospital departments of clinical microbiology (DCMs) situated at the main regional hospitals, Figure 2.2. 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.

Data on regional and national health care activity at hospitals in 2014 and 2023 are presented in Table 2.1. Denmark has a very high bed occupancy rate at hospitals and can reach maximum capacity during winter time for example due to high influenza activity. In 2023, the number of admissions at Danish somatic hospitals was registered to be 693.169 and the number of bed-days was registered to be 2,911,257. From 2014-2023, the number of bed-days decreased by 26%, the number of admissions decreased by 11% whereas the Danish population grew by 5%.

Figure 2.3 Changes in average age, Denmark and EU, 2014-2023 DANMAP 2023



Data source: Statistics Denmark and Eurostat

Table 2.1 Activity at Danish hospitals, 2014 and 2023 DANMAP 2023

Region	Number of bed-days in somatic hospitals		Number of admission to somatic hospitals		Population	
	2014	2023	2014	2023	2014	2023
Capital Region of Denmark	1,594,168	948,225	273,023	229,441	1,749,405	1,891,871
Region Zealand	504,223	443,558	105,106	102,149	816,726	849,857
Region of Southern Denmark	755,226	587,570	162,134	142,407	1,202,509	1,237,413
Central Denmark Region	726,610	602,411	168,010	147,794	1,277,538	1,358,879
North Denmark Region	370,362	329,494	73,370	71,378	581,057	594,634
Denmark	3,950,589	2,911,257	781,643	693,169	5,627,235	5,932,654

Data: Activity at somatic hospitals
Data source: The National Patient Register

2.3 Information on animal population and food production system

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

The production of food-producing animals as well as the production of meat and milk are presented in Table 2.2 and 2.3.

2.4 Registered antimicrobial agents

Table 2.4 shows the antimicrobial agents registered to treat bacterial infections in humans and animals. Some of these are listed on the highest priority list of medically important antimicrobials for the treatment of bacterial infections in humans, according to definitions made by the World Health Organization [WHO 2024]. In order to be considered critically important or highest priority critically important an antimicrobial class or subclass with authorized use in humans and animals must meet two criteria; 1) be the only - or one of a limited number of compounds available to treat serious human infections 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.

Furthermore, when both criteria are met, two prioritization factors are applied: 1) the antimicrobial class contains at least one antimicrobial that is both on the WHO Essential Medicines List (EML) and is classified as Watch or Reserve on the AWaRe classification list; 2) the antimicrobial class is used to treat human infections, often invasive and life-threatening, for which there is extensive evidence of transmission of resistance from non-human sources. When both prioritization factors are met, the antimicrobial is Highest Priority Critically Important (HPCIA), otherwise it is classified as Critically Important (CIA). Thus, in the newest list revision from 2024, four drug classes were considered highest priority critically important: 3rd and 4th generation cephalosporins, quinolones, polymyxins and phosphonic acid derivatives. Additionally, three antimicrobial classes were considered critically important: aminoglycosides, macrolides and ansamycins. In Denmark, the use of HPCIA classes in food-producing animals has generally been absent or reduced through either voluntary or legislative restrictions, while there is some use of the CIA classes aminoglycosides and 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, see Chapter 5.

Growth promoters are no longer used for animals in Denmark and are shown in parentheses in Table 2.4. Most of these influenced Gram-positive bacteria. Since 1995, the indicator enterococci from animals and meat have been used to monitor resistance towards former growth promoters.

Table 2.2 Production (1,000 heads) of food animals, Denmark DANMAP 2023

	Pigs		Cattle		Poultry	
Year	Total	Exported [a]	Slaughter cattle	Dairy cows	Broilers	Turkeys [b]
2014	30002	11120	556	563	115497	595
2015	30874	12133	511	561	114238	598
2016	31660	13280	540	572	120685	834
2017	31662	14173	509	570	117602	601
2018	32571	14449	533	575	122268	642
2019	31694	14897	518	567	123976	661
2020	32018	14736	500	567	120508	684
2021	32646	14092	506	564	118431	467
2022	31669	13856	493	557	114698	427
2023	29353	14865	486	..[c]	123803	358

Source: Statistics Denmark. Export data for poultry from Statistics Denmark, personal communication until 2022 and from www.dst.dk in 2023
a) Export of live pigs. These are included in total number of heads
b) Since 2006, more than 99% of the turkeys have been exported for slaughter
c) .. indicates that the observation is missing, discretionary or too uncertain to state

Table 2.3 Production (mill kg) of meat, milk and fish, Denmark DANMAP 2023

						Farmed fish [c]	
Year	Pork	Beef	Broiler meat [a]	Turkey meat	Milk [b]	Land based	Marine net ponds
2014	1944	143	174	9	5592	32	14
2015	1954	135	172	9	5744	36	16
2016	1943	142	182	10	5892	36	12
2017	1896	135	178	7	6088	37	14
2018	1967	142	185	10	6305	38	14
2019	1864	137	187	8	6323	41	14
2020	1952	133	195	8	6394	36	11
2021	2079	134	144	6	6390	37	12
2022	1956	128	200	6	6392	32	14
2023	1663	126	207	6	6377	-	-

Source: Statistics Denmark. Export data for poultry from Statistics Denmark, personal communication until 2022 and from www.dst.dk in 2023
a) Average weight after slaughter for poultry from Statistics Denmark, personal communication until 2022. In 2022, a final slaughtered weight of 1.74 kg per broiler produced and 12.93 kg per turkey produced was estimated. The same weight estimates were used in 2023
b) Conventional and organic
c) The numbers for 2023 are not final. Data are based on accounts statistics for aquaculture. The production of farmed fish includes fish transferred from one production facility to another

Table 2.4 Antimicrobial agents registered for systemic and veterinary intramammary therapeutic use in animals and humans, Denmark DANMAP 2023

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, eravacyclin
QJ01BA	Amphenicols	Florfenicol	
J01CA / QJ01CA	Penicillins with extended spectrum	Ampicillin, amoxicillin,	Ampicillin, pivampicillin, amoxicillin, pivmecillinam, mecillinam, benzathin benzylpenicillin
J01CE / QJ01CE	Beta-lactamase sensitive penicillins	Benzylpenicillin, phenoxymethylpenicillin, procaine penicillin, penethamate hydroiodide, benzathin benzylpenicillin	Benzylpenicillin, phenoxymethylpenicillin
J01CF / QJ51CF / QJ51RC	Beta-lactamase resistant penicillins	Cloxacillin, nafcillin	Dicloxacillin, cloxacillin, flucloxacillin
J01CR / QJ01CR	Comb. of penicillins and beta-lactamase inhibitors	Amoxicillin/clavulanate	Amoxicillin/clavulanic acid, piperacillin/tazobactam
J01DB / QJ01DB,QJ51DB	First-generation cephalosporins	Cefalexin, cefadroxil, cefapirin	Cefalexin, cefazolin
J01DC	Second-generation cephalosporins		Cefuroxime
J01DD / QJ01DD,QJ51DD	Third-generation cephalosporins incl. comb. with beta-lactamase inhibitors	Cefoperazone, ceftiofur, cefovecin	Cefotaxime, ceftazidime, ceftriaxone, ceftazidime/avibactam
J01DE / QJ51DE / QJ01DE	Fourth-generation cephalosporins	Cefquinome	Cefepime
J01DF	Monobactams		Aztreonam
J01DH	Carbapenems		Meropenem, ertapenem, imipenem and cilastatin
J01DI	Fifth-generation cephalosporins incl. comb. with beta-lactamase inhibitors		Ceftaroline fasamil, ceftolozan/tazobactam, ceftobiprol
J01EA	Trimethoprim and derivatives		Sulfathiazole, sulfadiazine, sulfamerazine, trimethoprim
J01EB / QJ01EQ	Short-acting sulfonamides	Sulfadimidine, sulfathiazole, sulfadiazine, sulfamerazine	Sulfamethizole
J01EE / QJ01EW / QJ51RE	Comb.of sulfonamides and trimethoprim, incl. derivatives	Sulfadiazine/trimethoprim, sulfadoxine/trimethoprim, sulfatroxazole/trimethoprim, sulfadimidine/trimethoprim, sulfamethoxazole/trimethoprim	Sulfamethoxazole/trimethoprim
J01FA / QJ01FA	Macrolides	Spiramycin, tylosin, tilmicosin, tylvalosintartrat, tulathromycin, gamithromycin	Erythromycine, roxithromycine, clarithromycine, azithromycine, spectinomycin, pirlimycin
J01FF / QJ01FF / QJ51FF	Lincosamides	Clindamycin, lincomycin, spectinomycin, pirlimycin	Clindamycin
QJ01XX [b]	Streptogramins	(Virginiamycin)	Framycetin
J01GB / QJ01RA,QJ01GB, QJ01RV, QJ51RG, QJ51RC	Aminoglycosides	Streptomycin, dihydrostreptomycin, gentamicin, neomycin, apramycin, framycetin	Tobramycin, gentamicin, amikacin
J01MA / QJ01MA	Fluoroquinolones	Enrofloxacin, marbofloxacin, difloxacin, ibafloxacin, pradofloxacin, danofloxacin, orbifloxacin	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
J01XB / QJ51RD [b]	Polypeptides (incl. polymyxins)	(Colistin, bacitracin)	Colistin
J01XC	Steroid antibacterials		Fusidic acid
J01XD,P01AB [c]	Imidazole derivatives		Metronidazole
J01XE	Nitrofurane derivatives		Nitrofurantoin
J01XX	Other antibacterials		Methenamine, linezolid, daptomycin, 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

3

ONE HEALTH AMR

3. One Health AMR

3.1 Introduction

One Health is defined as a unified approach to optimize the health of people, animals and the environment, under which multiple sectors must collaborate at varying levels [www.who.int].

DANMAP was established with the aim of understanding the drivers of AMR in humans and in the livestock industry and their interconnectedness. The monitoring programme has always been considered an integrated approach to research and surveillance, but with integration taking place at the coordination level rather than at the level of data collection and management. Hence, data are stored in separate databases by the animal and human sectors, however interpretation of results is done in cooperation. Moreover, integration happens when discussing resistance findings in indicator and pathogenic bacteria and using it as a basis for recommendations and treatment guidelines among different participants of the programme. Finally, DANMAP supports the development and definition of strategies and action plans to reduce AMR in a collaborative manner, and by fostering the dialogue between different actors and stakeholders across sectors.

However, there has always been the wish to get a more in depth understanding of the possible relationship between the veterinary, food-producing and human sectors concerning antimicrobial usage (AMU) and development of antimicrobial resistance (AMR). To be able to foresee if changes in one sector will have a possible significant impact on the other sector, it requires knowledge of the probable routes of transmission and the size and speed of transfer. This again calls for further harmonised data collection, to define common denominators and units and to be able to perform cross-analyses on data from both the veterinary and human sectors.

At the EU level, an attempt to perform cross-analysis has been made since 2015 in the JIACRA reports [JIACRA IV, 2019-2021, ECDC, EFSA, EMA; 2024]. At the national level, even in a country such as Denmark with a long-established detailed monitoring system based on stable delivery of high quality data, there are a number of challenges in the implementation of integrated data analysis. Data are collected in the animal, food and human sectors often under different premises - following different legislation, with varying sampling strategies and magnitudes.

Here we cross-analyse antimicrobial resistance data from monitoring in livestock animals and humans in Denmark. We map the frequency of multi-locus sequence types (MLST) and resistance genes and mutations of extended spectrum beta-lactamase-producing *E. coli* (here abbreviated to ESBL Ec) recovered from livestock animals and meat and from humans with bloodstream infections. Furthermore, we study the genomic context of selected resistance genes detected in those isolates, which may have been horizontally transferred between reservoirs. Several recent studies [Aziz, et al 2024. *Microbiology Spectrum*. 12. e0341523; Nadimpalli et al 2023, *Frontiers in Ecology and the Environment*. 21. 10.1002/fee.2639; Liu et al 2023. *One Health*, 16: 100518; Roer, et al. 2019. J Antimicrob Chemother 74(3):557- 560; Valcek, et al. 2019. J *Antimicrob Chemother* 74(8):2171- 2175] report possible zoonotic transmission of ESBL Ec, both in high-income and low- and middle-income countries, underlining the importance of monitoring the occurrence of these bacteria in animals and humans, and assessing the possibility of transfer across sectors.

The annual number of bloodstream infections in humans in Denmark caused by ESBL Ec has been decreasing since 2019 (see Chapter 8, section 8.2.1). Similarly, a significant reduction in ESBL Ec has been observed in Danish broilers (DANMAP 2022, Chapter 7, section 7.3.1), cattle and pigs (see Chapter 7, section 7.3.1), as well as among domestic and imported meat (DANMAP 2022, 2023, Chapter 7, section 7.3.1). In this chapter, we demonstrate, as previously done, possible relationships between ESBL Ec from different sources in Denmark, and we furthermore deepen the genomic analyses to further investigate the possible occurrence of zoonotic transmission.

3.2 Genotypic comparison of ESBL/AmpC-producing *E. coli* from humans, animals and food

3.2.1 Abundance distribution of MLSTs and ESBL/AmpC genotypes

Since 2022 (DANMAP 2021, Chapter 3), DANMAP has compared the distributions of multi-locus sequence types (MLSTs or STs) and ESBL/AmpC genes and mutations among ESBL Ec from humans, food-producing animals and meat to identify any major overlaps between sectors suggesting a zoonotic link.

In the present report, we added new data from 2023; 91 isolates of animal origin and 225 isolates from humans, totaling a dataset of 1,964 ESBL isolates from humans and animals from 2018 through 2023. The 1,282 human isolates were clinical isolates from bloodstream infections sent voluntarily from the departments of clinical microbiology to the SSI reference laboratory for antimicrobial susceptibility testing. The animal and meat isolates (broilers: 90, broiler meat: 145, cattle: 56, beef: 41, pigs: 219, pork: 50, and turkey meat: 81) stem from the EU mandatory screening programme from healthy animals and meat products (see Chapter 10 for more information).

Each isolate had been sequenced as part of the surveillance activities, and the MLST and ESBL/AmpC genotype were extracted from the whole genome sequence. For an overview of sequence types and ESBL/AmpC genotypes detected in 2023 in *E. coli* from bloodstream infections, and from cattle, pigs, beef and pork, see Chapter 8 (Tables 8.15 and 8.16), and Chapter 7 (Table 7.3), respectively. For the purpose of the visual demonstration of the abundance of STs and resistance

genes in the different reservoirs, we selected only flows of five or more isolates, thus limiting the analysis to a selection of data (Figure 3.1). The results described below refer to the selected isolates.

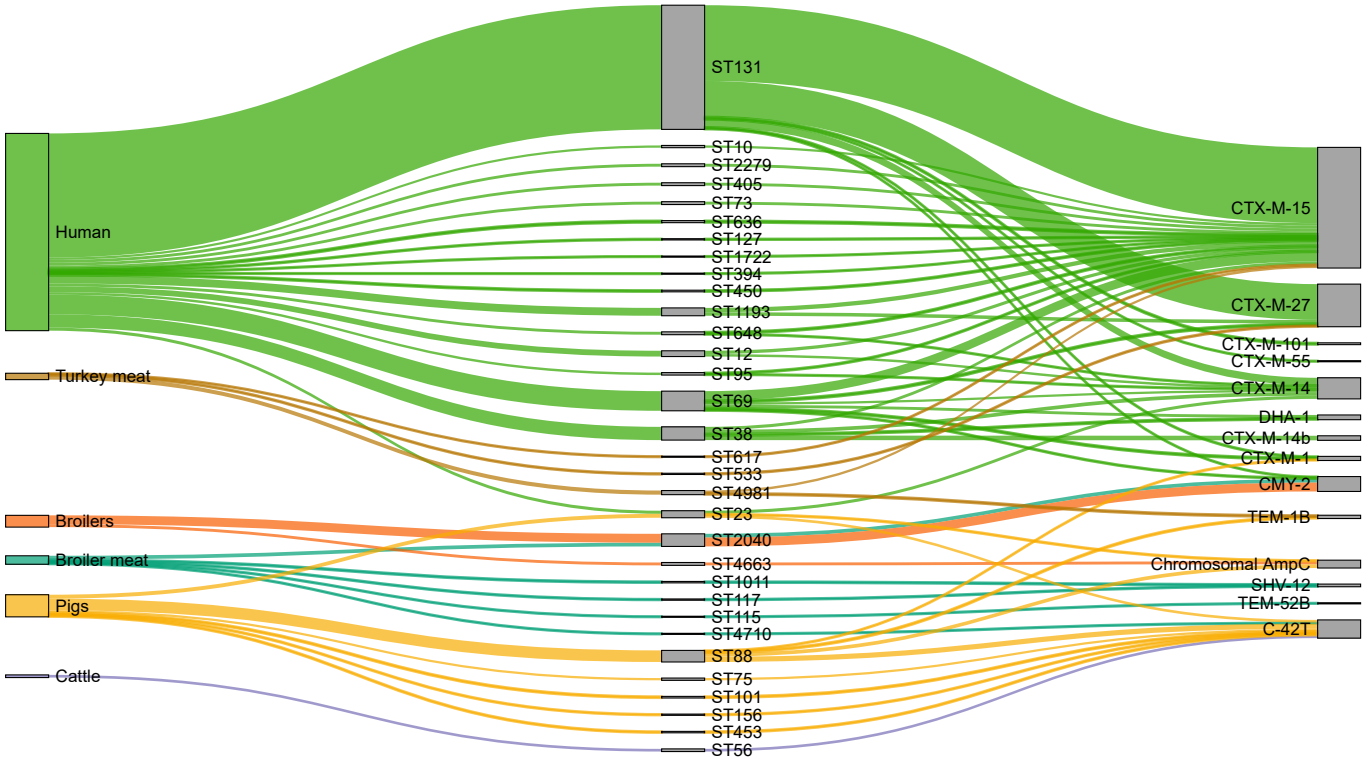
As in the previous years, limited overlap was found in both STs and ESBL/AmpC genes and mutations in isolates from humans vs. animals and food (Figure 3.1).

Regarding the distribution of MLSTs, one or few sequence types predominated among the isolates of each source. Isolates from humans were mostly from ST131, followed by ST69 and ST38 (see also Chapter 8, Table 8.16). The most abundant STs of animal and meat isolates were ST2040 for broilers and broiler meat, ST4981 for turkey meat, ST88 for pigs and ST56 for cattle. In accordance with former findings (see DANMAP 2015, Textbox 7.3), ST23 was found in both humans and pigs, although the ESBL/AmpC genotype differed between the human and pig strains. The pig isolates from ST23 harboured AmpC C-42T mutations, whereas the human isolates harboured the ESBL gene *CTX-M-14*.

Only the AmpC plasmid-mediated gene *CMY-2*, and the ESBL genes *CTX-M-1*, *CTX-M-15* and *CTX-M-27* were found in both humans and food-producing animals or meat. Interestingly, turkey meat isolates were those with the largest overlap with human isolates regarding the detected ESBL genotypes, including carriage of *CTX-M-15* and *CTX-M-27* (the two most common ESBL genes among human isolates; see also Chapter 8, Table 8.15), although by different MLSTs. The *CMY-2* AmpC gene was almost exclusively found among isolates from broilers and broiler meat from ST2040, but also in human isolates of ST69. The *CTX-M-1* gene was mostly found among human isolates, but also in pig isolates of ST88. All isolates from cattle harboured a C-42T mutation, which was not detected among the selected human isolates. Notably, isolates from broilers were of two types; ST2040 carrying a *CMY-2* gene or ST4663 harbouring a chromossomal AmpC mutation.

In general, sequence types seem to strongly associate with species, whereas there is more variance in combinations of STs and ESBL/AmpC genes and mutations. In the 2018 DANMAP report Textbox 7.2, Roer, et al., used whole genome sequencing on a similar, but smaller, dataset to investigate for possible zoonotic links. A possible link was found in ST69/*CTX-M-1*, but the SNP-distance was not indicative of an outbreak or a direct transmission, but rather of a clonal relationship. In conclusion, it remains challenging to find clear evidence of zoonotic transmission of ESBL Ec between animals and humans in Denmark, within the investigated time frame of five years, and when considering the occurrence of ESBL/ AmpC genotypes in different sequence types. Further research into slow transmission over longer time spans, as well as more in depth genomic analyses could be of interest.

Figure 3.1 A Sankey diagram comprised of 1202 ESBL Ec isolates from humans, animals and food showing the relationship between the isolates' source, sequence type and ESBL/AmpC gene or mutation DANMAP 2023



The flows between nodes are coded according to source. Only flows of five or more isolates are shown

3.2.2 Genomic context of ESLB/AmpC genes

As described above and in DANMAP 2022 (Chapter 3, Textbox 3.1), it is difficult to establish a clearly distinct transmission link for most ESBL genes between animals, meat and humans. Rarely the same acquired antimicrobial resistance genes (ARGs) are detected in different reservoirs, but for these, the analysis conclusion of the DNA sequence of the ARGs flanking region, i.e. the genetic code that comes before and after a gene, can assist in the determination of a common ARG source.

Here we applied the bioinformatics tool Flankophile [http://www.genomicepidemiology.org/services], for analysis and visualization of flanking region synteny of selected ESBL/AmpC genes, to a selected dataset of 1,557 sequenced ESBL Ec isolates from humans, animals and meat, gathered in the period 2018-2023 (see section 3.1 above).

We focused the analysis on resistance genes also present in figure 3.1, i.e. those identified in at least five isolates from each combination of source and MLST. This included the ESBL genes *CTX-M-1*, *CTX-M-14*, *CTX-M-15*, *CTX-M-27*, *CTX-M-55*, *TEM-52B*, and the AmpC genes *DHA-1* and *CMY-2*. Considering hierarchical clustering of variants according to both resistance gene and flanking region sequences, the results showed the following:

CTX-M-1: two variants were identified exclusively in broiler meat and broilers; three variants where present in both broilers or broiler meat, and pigs or pork; two variants were identified in pigs and humans, with one of those being also present in turkey meat.

CTX-M-14: most variants were exclusively identified in humans; one variant was identified in humans and broilers, broiler meat, pigs and duck meat.

CTX-M-15: several variants were present in both humans and an animal host, including three clusters with pigs, cattle, and turkey meat; two clusters showed the presence of the same variant in turkey meat and in beef and cattle.

CTX-M-27: there were no identical variants identified among human- and animal isolates; all variants were identified in humans, except for a single cluster observed in turkey meat.

CTX-M-55: two variants were identified in pork and humans; one cluster included turkey meat and broiler meat.

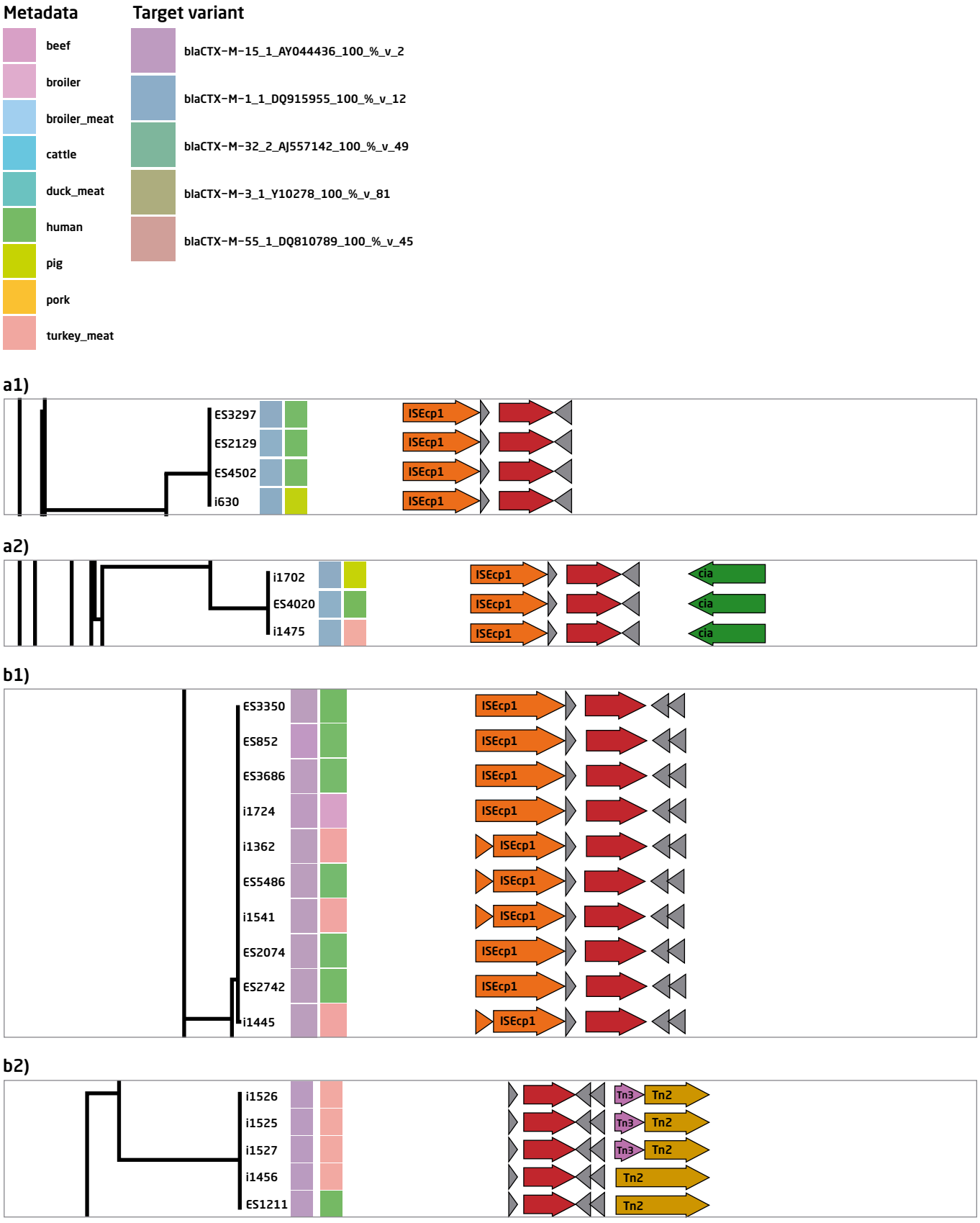
TEM-52B: there were no identical variants identified among human- and animal isolates.

DHA-1: two variants were identified in both humans and animal hosts, one in pigs only, and another in broilers, cattle, pigs and pork.

CMY-2: there were no identical variants identified among human- and animal isolates; one variant was identified in broilers, pork and beef.

In sum, among those ARGs included in the analysis, the comparison of the gene sequence and its flanking region showed some examples where the gene variant and its entire 2,500 base pair flanking region was identical in isolates from humans and from one or more animal hosts. There were also examples where no such overlap was observed. Furthermore, there were cases of overlap between different animal hosts, but not with humans.

Figure 3.2 ESBL genes *CTX-M-1* (a1, a2) and *CTX-M-15* (b1, b2) identical in both gene and flanking region sequences between *E. coli* isolates of animal origin and human origin DANMAP 2023



Details from Flankophile plots, showing a1 – clustering of *CTX-M-1* genes of human- and pig origin; a2 - clustering of *CTX-M-1* genes of human-, pig- and turkey meat origin; b1 - clustering of *CTX-M-15* genes of human-, turkey meat and beef origin; b2 - clustering of *CTX-M-15* genes of human- and turkey meat origin. From left to right: distance tree of the gene's flanking regions (straight vertical lines indicate that the flanking regions are 95% identical); color annotation columns representing the target variant (left) and the host species (right); arrows depicting the gene synteny, with the target sequence in red

3.3 Conclusion and future perspectives

This One Health chapter presents integrated analysis of surveillance data for antimicrobial resistance (AMR) from the human and animal/food sectors.

The comparison of ESBL/AmpC-producing *E. coli* isolates from livestock animals, meat and human bloodstream infections suggests, as previously, limited overlap between the sources with regards to the combination of sequence type and ESBL/AmpC-genes.

The further analysis of the genomic background of the ESBL/AmpC-genes, including the gene sequence and the flanking regions, contributed to further investigate the potential common origin of the genes, regardless of the sequence type of the isolates. The results of both analyses are in accordance regarding *CTX-M-1* being possibly shared between isolates from pigs and humans, as well as *CTX-M-15* being possibly shared between humans and turkey meat. The flanking region analysis clarified that the *CTX-M-27* present in turkey meat isolates, as well as the *CMY-2* present in isolates from broilers and broiler meat do not overlap with the variant present in human isolates.

While the comparison of sequence type and ESBL/AmpC genes combinations shows little to no overlap between ESBL Ec of different hosts, the gene sequence and flanking region analysis indicates a probable transmission of ESBL/AmpC genes across *E. coli* from different hosts via horizontal transfer. The zoonotic transmission of ESBL/AmpC-producing *E. coli* thus warrant continued monitoring and further studies, to provide a deeper comprehension of transmission between sectors.

Ana Sofia Ribeiro Duarte, Mikkel Lindegaard, Patrick Munk and Ute Wolff Sönksen
For further information: Ana Sofia Ribeiro Duarte, asrd@food.dtu.dk

4

ANTIMICROBIAL CONSUMPTION IN ANIMALS

4. Antimicrobial consumption in animals

Highlights: In 2023, the total consumption of antimicrobials in animals amounted to 86.69 tonnes of active compounds approved for animals.

The **pig sector** consumed 84.03% of all prescribed veterinary antimicrobials, equal to 72.85 tonnes of active compound. Calculated in treatment proportions, an estimated 3.21% (32.08 DAPD) of all pigs, on average, received antimicrobial treatment per day in 2023. In sows and piglets and in finishers, the treatment proportions increased by 3.41% and 14.12%, respectively, in 2023 compared to 2022. The highest treatment proportion was observed in the treatment of weaners: 11.92%, corresponding to 119.3 DAPD. The treatment of weaners increased by 14.86% in 2023 compared to 2022, continuing the increasing trend observed in 2022, with a 7.61% increase compared to 2021. This increase is most likely a result of the ban of prescribed zinc oxide usage in pig production implemented June 2022. In addition, the previous ceased use of colistin, and the implementation of Order 2019/6 on veterinary medicinal products applied since January 2022 could also have affected the antimicrobial use. The increase seen in 2022 and 2023 was mostly due to an increased use of neomycin and apramycin to treat post-weaning diarrhea.

Over time, the antimicrobial classes used in the treatment of pigs have changed notably. The critically important antimicrobials 3rd and 4th generation cephalosporins, glycopeptides, polymyxins, and fluoroquinolones have been phased out. However, over the last decade, there has been an increase in the consumption of macrolides from 6.07 DAPD to 8.23 DAPD, aminoglycosides from 1.42 DAPD to 6.65 DAPD, and simple penicillins from 2.82 DAPD to 3.46 DAPD. During the same period, the consumption of tetracyclines has decreased from 9.20 DAPD in 2014 to 4.47 DAPD in 2023.

In 2023, antimicrobial consumption in **cattle** amounted to 7.96 tonnes. Approximately two thirds of the consumption were used to treat older cattle (>1 year). Over the past decade, the total antimicrobial consumption has decreased for older cattle (>1 year), from 2.79 DAPD to 1.91 DAPD. During the same period, an increase in the total consumption from 5.43 DAPD to 7.04 DAPD was observed in young cattle. Also in cattle, the changes in usage of antimicrobial classes are noticeable i.e., there has been an increased consumption of aminoglycoside, amphenicols, macrolides, and simple penicillins (beta-lactamase sensitive penicillins) for treatment of younger cattle and increased consumption of simple penicillins (beta-lactamase sensitive penicillins) for intramammary treatment.

The antimicrobial consumption in **poultry** was 1305.80 kg active compound and has decreased by 40.55 kg from 2022 to 2023. In 2023 the consumption of macrolides decreased by 257.62 kg of active compound compared to 2022, while consumption of penicillins and tetracyclines increased.

In 2023, cephalosporins were prescribed mainly for **pets and horses** (58.49 kg) or as intramammary treatment for **cattle** (50.09 kg). Furthermore, fluoroquinolones (13.80kg) were prescribed almost exclusively for horses and pets.

4.1 Introduction

The DANMAP programme began monitoring the national consumption of antimicrobials in humans and animals in 1995.

Since the early 1990s, there has been increased political and public focus on the consumption of antimicrobials in the Danish animal production. This has resulted in discontinued usage of antimicrobials for growth promotion combined with several other initiatives, including voluntary bans on the use of 3rd and 4th generation cephalosporins in the pig and cattle production, as well as regulatory legislation regarding therapeutic use.

Figure 4.1 presents the total consumption of antimicrobials in animals and humans since 1990 and 1997, respectively. Increases in, and intensification of, pig production has also had a significant impact on the overall consumption during this time.

The observed decrease in antimicrobial consumption after 1994 was foremost due to the discontinued usage of antimicrobials for growth promotion and most likely also 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 to promote preventive veterinary strategies and optimize antimicrobial consumption; and 3) enforcement of the so-called "cascade rule" [Order (DK) 142/1993], limiting the prescription of (cheaper) extemporaneously produced medicines.

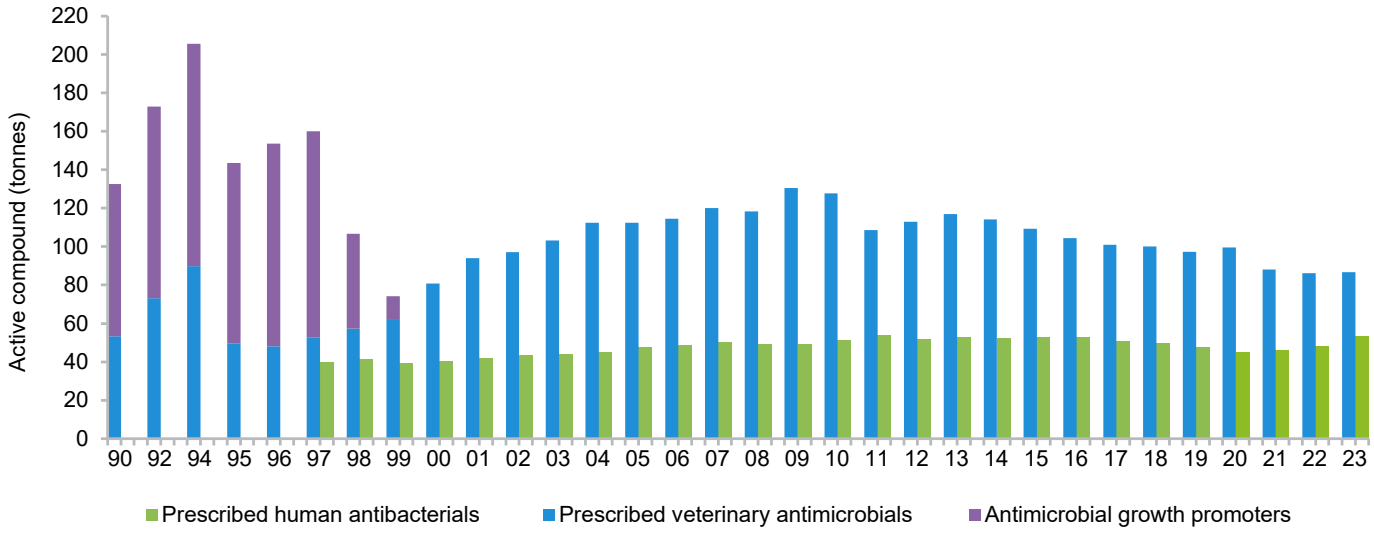
Other important interventions were the restriction on the use of fluoroquinolones in production animals through legislation implemented in 2002 and 2003, and the voluntary ban on use of cephalosporins in pig production in 2010, followed by a similar initiative in dairy cattle production in 2014.

Furthermore, the cattle production implemented a ban on use of 3rd and 4th generation cephalosporins for cattle from 2019.

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

To achieve the action plan goals, the Yellow Card initiative was established in 2010, introducing surveillance at pig herd level. In practice, antimicrobial consumption is monitored in individual herds in relation to legislation-supported thresholds, which enables legal action on individual farmers with high antimicrobial consumption per pig [DANMAP 2010]. As a result, a distinct decrease in antimicrobial consumption has been observed at national level both when the Yellow Card was implemented (from 2010 to 2011), and when it was revised (from 2016 to 2018). In 2016, the Yellow Card initiative was revised, adding multiplication factors to adjust the consumption of certain antimicrobials. Tetracyclines were multiplied by 1.2, and the factor was increased to 1.5 in 2017. Fluoroquinolones, cephalosporins and colistin (added in 2017) were given the highest multiplication factor of 10 [DANMAP 2017].

Figure 4.1 Antimicrobial consumption for humans and all animal species, tonnes of active compound, Denmark DANMAP 2023



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 2023, consumption data were extracted from the VetStat on 1 July 2024 and include all antimicrobials approved for use in animals

Effects from other legislative actions 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 peroral treatment of groups of pigs through water or feed rather than injection treatment of individual pigs.

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) in both animals and humans. At the same time, a new national action plan to reduce AMR in animals was introduced, setting specific targets to further reduce the antimicrobial consumption for animals in the coming years (from 2019 to 2023).

Also, to reduce the need for disposal of excess antimicrobials, veterinarians and pharmacies were permitted to split packages of veterinary medicine as from 2019 [Order (DK) 1655/2018]. This initiative may also enhance surveillance by reducing the difference between amounts of antimicrobials prescribed and amounts consumed.

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, https://foedevarestyrelsen.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.

Order 2019/6 on veterinary medicinal products (VMPs) has been applied since 28 January 2022. There is a particular focus on reducing the risk of antimicrobial resistance [Order (DK) 6/2019], and it includes relevant provisions on the prescription and use of VMPs such as limiting the quantity prescribed to the amount required for the treatment or therapy concerned, limiting the prescription of antimicrobials for metaphylaxis or prophylaxis, ensuring that VMPs are used in accordance with the marketing authorisations (SPC), restricting the routine use of antimicrobials to compensate for e.g. poor hygiene and reserving the use of antimicrobials listed in Order 2022/1255 for treatment of certain infections in humans [DANMAP 2022, Textbox 4.2].

Following the recommendations from the European Medicines Agency (EMA), and the consequent decision issued by the European Commission in 2017, Denmark and all Member States had to ban the use of VMPs containing zinc oxide for food-producing animals by June 2022. The use of prescribed zinc oxide in pig production has been banned in Denmark since then [DANMAP 2022, Textbox 4.1].

4.1.1 Data sources

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

Since 2001, data on all medicines prescribed for consumption in animals, including vaccines, antimicrobial growth promoters, and coccidiostatica have been recorded in the national database VetStat. Since 2010, the VetStat database has been hosted and maintained by DVFA. In June 2021, DVFA launched an updated platform for VetStat. The 2023 data presented in this report were extracted from this new VetStat on 1. July 2024. The data were extracted, analysed, and interpreted for DANMAP by the National Food Institute, Technical University of Denmark.

4.2 Total antimicrobial consumption in animals

Together with the introduction of the new VetStat database in 2021, the criteria for allocating antimicrobial consumption to the different animal species and age groups were revised i.e., consumption is allocated to the species and age group combinations from the categories defined in VetStat [Order (DK) 2542/2021]. This affected the calculated amounts per species while the overall trends of antimicrobial consumption remained the same.

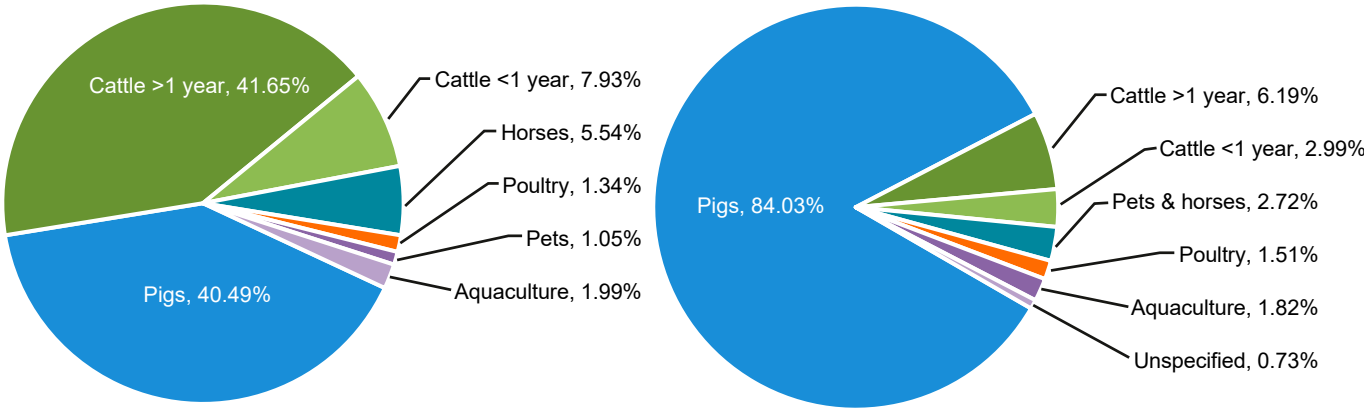
The total consumption of antimicrobials in all animals amounted to 86.69 tonnes of active compound, representing a 0.61% (523.18 kg) increase compared to 2022 (Figure 4.1). The 2023 consumption in pigs, cattle and poultry comprised approximately 84.03%, 9.18%, and 1.51%, respectively, of the total antimicrobial consumption in animals (Figure 4.2).

The pig production is the main driver of consumption of antimicrobials in animals in Denmark. Cattle comprises the largest live biomass followed by the live biomass of pigs. However, the vast proportion of cattle live biomass consists of dairy cows, which have very low consumption of antimicrobials compared with growing animals such as slaughter pigs.

Historically, the overall consumption of kg active compound of antimicrobials was 57.85% lower in 2023 compared to 1994. A major part of this reduction can be explained by the discontinued consumption of growth promoters from 1994 to 1999.

Between 2000 (start of VetStat) and 2009, the amount of kg active compound of antimicrobials used in animals increased by 61.7% (Figure 4.1). During this period, the number of finishers produced also increased, as did the proportion of exported live pigs at approximately 30 kg. Until 2021, the proportion of these pigs continued to increase, while there was an overall gradual decrease in the consumption of antimicrobials in animals. In 2022 and 2023, the number of produced finishers decreased by 4.33% and 17.93%, respectively.

Figure 4.2 Distribution of live biomass and antimicrobial consumption in main animal species, tonnes, Denmark DANMAP 2023



The live biomass is estimated from census data (pigs, cattle, and companion animals) and production data (poultry, and aquaculture). The live biomass estimates for poultry (turkeys and broilers), aquaculture, horses and pets are based on 2012 data and may well be underestimated. The estimation procedures are described in Chapter 10, Section 10.2

Table 4.1 Antimicrobial consumption by animal species and age group, kg active compound, Denmark DANMAP 2023

	Aminoglycosides	Amphenicols	Cephalosporins [a]	Fluoroquinolones	Lincosamides	Macrolides	Other antimicrobials [b]	Other quinolones	Penicillins, b-lactamase sensitive	Penicillins, others	Pleuromutilins	Sulfonamides and trimethoprim	Tetracyclines	2022	2023
Pigs	19236.6	608.9	-	-	1768.0	11114.8	-	-	11120.8	7255.4	6466.3	4712.9	10567.6	71356.2	72851.2
Sows, piglets, gilts and boars	2007.8	272.4	-	-	351.5	428.7	-	-	5903.8	2146.4	627.3	3658.5	1074.6	17082.9	16470.8
Weaners, =<30kg	17137.0	320.7	-	-	949.7	7469.5	-	-	1620.4	4368.9	2315.8	946.8	6936.8	39023.5	42065.6
Finishers and polts	91.8	15.8	-	-	466.8	3216.7	-	-	3596.6	740.1	3523.2	107.7	2556.1	15249.8	14314.8
Cattle	801.3	931.2	50.1	-	4.3	229.6	4.5	-	4263.0	563.4	-	363.3	750.5	8164.9	7961.2
Intramammaries	21.3	-	50.1	-	3.6	-	0.0	-	227.1	155.4	-	0.0	-	451.0	457.6
Cows, bulls, heifers and steers >24 months	184.1	10.3	-	-	0.5	73.1	0.2	-	3459.9	292.9	-	280.4	469.5	4851.5	4770.9
Calves <12 months	579.6	907.8	-	-	0.1	154.2	4.3	-	496.0	107.8	-	81.3	264.3	2589.3	2595.4
Young cattle btw 12 and 24 months	16.3	13.1	-	-	0.0	2.4	0.0	-	80.0	7.3	-	1.6	16.7	273.1	137.2
Poultry	50.2	-	-	-	15.3	176.3	-	-	315.9	203.1	16.9	31.2	496.9	1346.4	1305.8
Broilers	21.3	-	-	-	9.2	67.7	-	-	168.0	43.8	-	26.4	327.0	653.5	663.4
Layer hens	3.5	-	-	-	-	89.8	-	-	51.7	10.7	16.9	0.6	20.8	267.8	193.9
Turkeys	20.2	-	-	-	5.6	16.5	-	-	96.2	134.4	-	-	97.0	295.2	369.9
Other poultry	5.3	-	-	-	0.4	2.4	-	-	-	14.2	-	4.2	52.1	130.0	78.6
Other production animals	2.0	125.1	0.0	-	0.7	0.2	0.0	525.2	1.0	2.5	-	931.8	0.8	2460.2	1589.3
Aquaculture	-	124.9	0.0	-	-	-	-	525.2	-	-	-	931.7	-	2452.0	1581.9
Fur animals	1.3	-	-	-	0.7	-	-	-	-	1.0	-	0.0	-	0.6	3.1
Other	0.6	0.2	0.0	-	-	0.2	0.0	-	1.0	1.5	-	0.1	0.8	7.6	4.4
Companion animals	3.6	1.1	58.5	13.1	73.4	2.2	74.0	-	15.9	477.5	0.2	1595.1	39.3	2159.3	2353.5
Horses	0.3	0.0	0.0	0.0	0.0	-	0.3	-	5.9	0.6	-	166.6	7.7	150.2	181.3
Pets	2.2	0.6	21.6	4.3	17.7	2.2	24.5	-	10.0	86.5	0.2	194.4	18.8	345.8	383.0
Unspecfied	1.0	0.4	36.9	8.7	55.6	-	49.2	-	-	390.4	-	1234.1	12.8	1663.3	1789.3
Unknown [c]	85.6	11.0	0.5	0.7	3.5	7.6	0.4	6.0	342.9	84.2	2.8	12.7	73.4	682.3	631.3
Total	20179.3	1677.2	109.1	13.8	1865.1	11530.9	78.8	531.2	16059.4	8586.1	6486.1	7647.0	11928.4	86169.1	86692.3

Data for 2023 were extracted from VetStat on 1 July 2024
Combination products are split into active compounds
a) In 2023, 3rd cephalosporins were only used in pets (0.93 kg), and registered as "unspecified"
b) Including other antiinfectives, dermatologicals, ontological, opthalmologicals, polymyxin, quinolones, and sulfonamides, plain
c) Including data with no information on animal species/age group, or mismatch between animal species and age group

4.3 Antimicrobial consumption by animal species

4.3.1 Antimicrobial consumption in pigs

Most of the antimicrobials used for animals were consumed within the pig production in 2023. The total consumption in pigs was 72.85 tonnes of active compound, which was 1494.98 kg more than in 2022 (Table 4.1). The estimated live biomass of pigs decreased by 11.31% in 2023 compared to 2022 primarily due to a decrease in finishers slaughtered in Denmark.

The national MRSA action plan aimed to reduce antimicrobial consumption in pigs by 15% in 2018 compared to 2014. This goal was reached in 2019, where the achieved reduction was 16%. A revised action plan with new targets was agreed upon in August 2021, establishing that antimicrobial consumption in the pig production should decrease by 2% each year from 2019-2022 compared to the consumption level in 2018 (74.00 tonnes). That action plan was extended until 2023, however targets were not met by the end of 2023. A new food and veterinary agreement has been established in June 2024 aiming for an 8% reduction in antimicrobial use from 2024 to 2027 compared to the consumption level in 2018.

The **treatment proportion** (DAPD) of the total population reflects the trends in selection pressure within the population. The DAPDs in the pig population overall and by age group are presented in Figures 4.3 a.-c. and 4.4. The distribution of parental and peroral administration for the overall population and by age group are shown in Figures 4.3 b.-c. Finally, The DAPDs by age group at indication level are presented i Figure 4.5.

Historically, DAPD increased from 2004 to 2009, followed by a clear decrease in 2010 and 2011 with the introduction of the Yellow Card initiative. From 2013 to 2021, an overall slightly decreasing trend in treatment proportion has been observed (Figure 4.3 a).

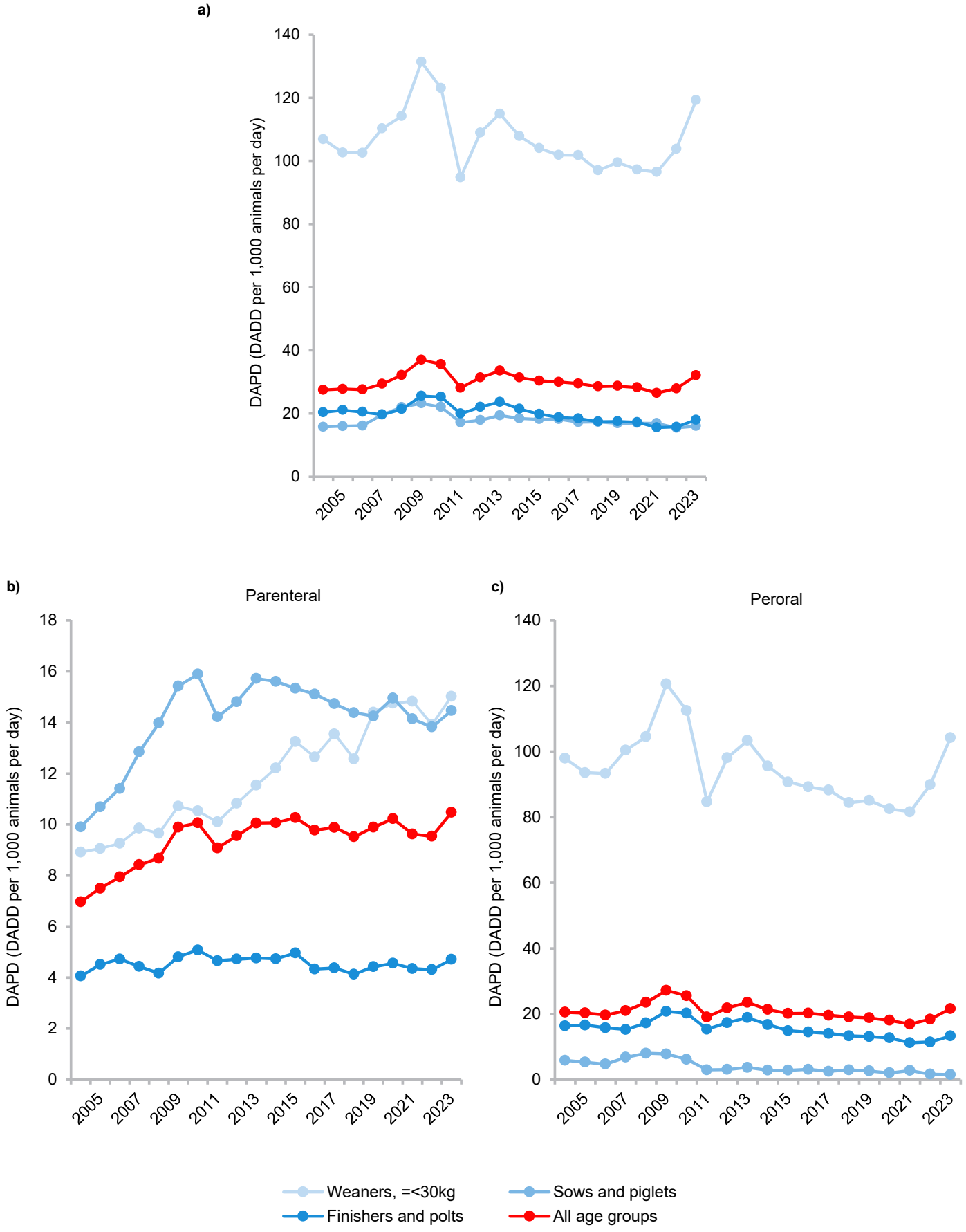
In 2023, the increase in antimicrobial consumption measured as kg active compound in pigs when inspecting crude consumption data was 2,10% (Table 4.1), while changes in the overall treatment proportion are more subtle and vary between age groups and antimicrobial classes. When comparing 2023 to 2022, the overall DAPD increased by 3.41% in sows and piglets, while DAPD for weaners and finishers increased by 14.86% and 14.12%, respectively (Figure 4.3 a.). On a given day in 2023, approximately 1.60% and 1.80% of sows, piglets and finishers respectively, as well as 11.92% of weaners were treated with antimicrobials. The main prescription indication of antimicrobial consumption was for diarrhea in weaners (Figure 4.5).

In contrast to the decreasing trend in DAPD observed from 2013 to 2021, a substantial increase was observed from 2021 to 2023. Thus, DAPD of all age groups increased by 5.31% and 14.86% from 2021 to 2022 and from 2022 to 2023, respectively. The overall treatment proportion increased by 9.85% and 17.46% for parenteral and peroral usage, respectively, from 2022 to 2023 (Figure 4.3 b and c). When comparing 2022 to 2023 the increased use can be observed for all antimicrobial classes; Aminoglycosides increased by 48.92%, amphenicols increased by 18.60%, lincosamides increased by 2.21%, macrolides increased by 11.63%, penicillins, beta-lactamase sensitive, increased by 9.39%, penicillins, other, increased by 3.90%, pleuromutilins increased by 2.45%, sulfonamides and trimethoprim increased by 13.58% and tetracyclines increased by 9.22%. The main cause was increased DAPD use in weaners (Figure 4.4).

Since the ban of prescribed zinc oxide usage in pig production by June 2022, the ceased use of colistin and the Order 2019/6 on veterinary medicinal products applied since 28 January 2022, the use of aminoglycosides, primarily neomycin and apramycin, for weaners has increased by 145% from 16.87 DAPD in 2021 to 41.39 DAPD in 2023 (Figure 4.4).

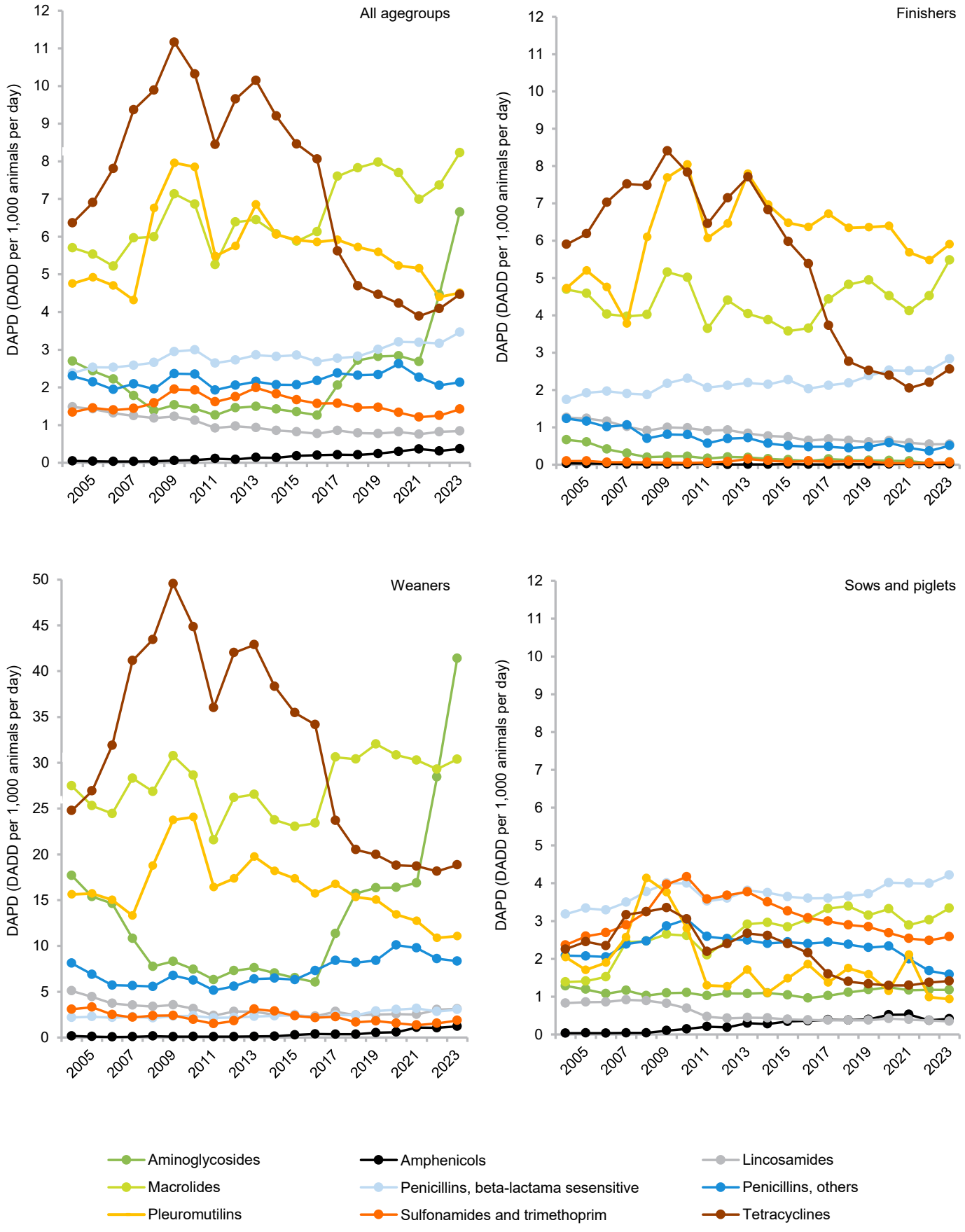
In 2023, no consumption of the critical important antimicrobials 3rd and 4th generation cephalosporins was registered in pigs (Table 4.1).

Figure 4.3 a Total antimicrobial consumption in the pig production, DAPD, Denmark. b. and c. Total antimicrobial consumption in the pig production at administration level, DAPD, Denmark
DANMAP 2023



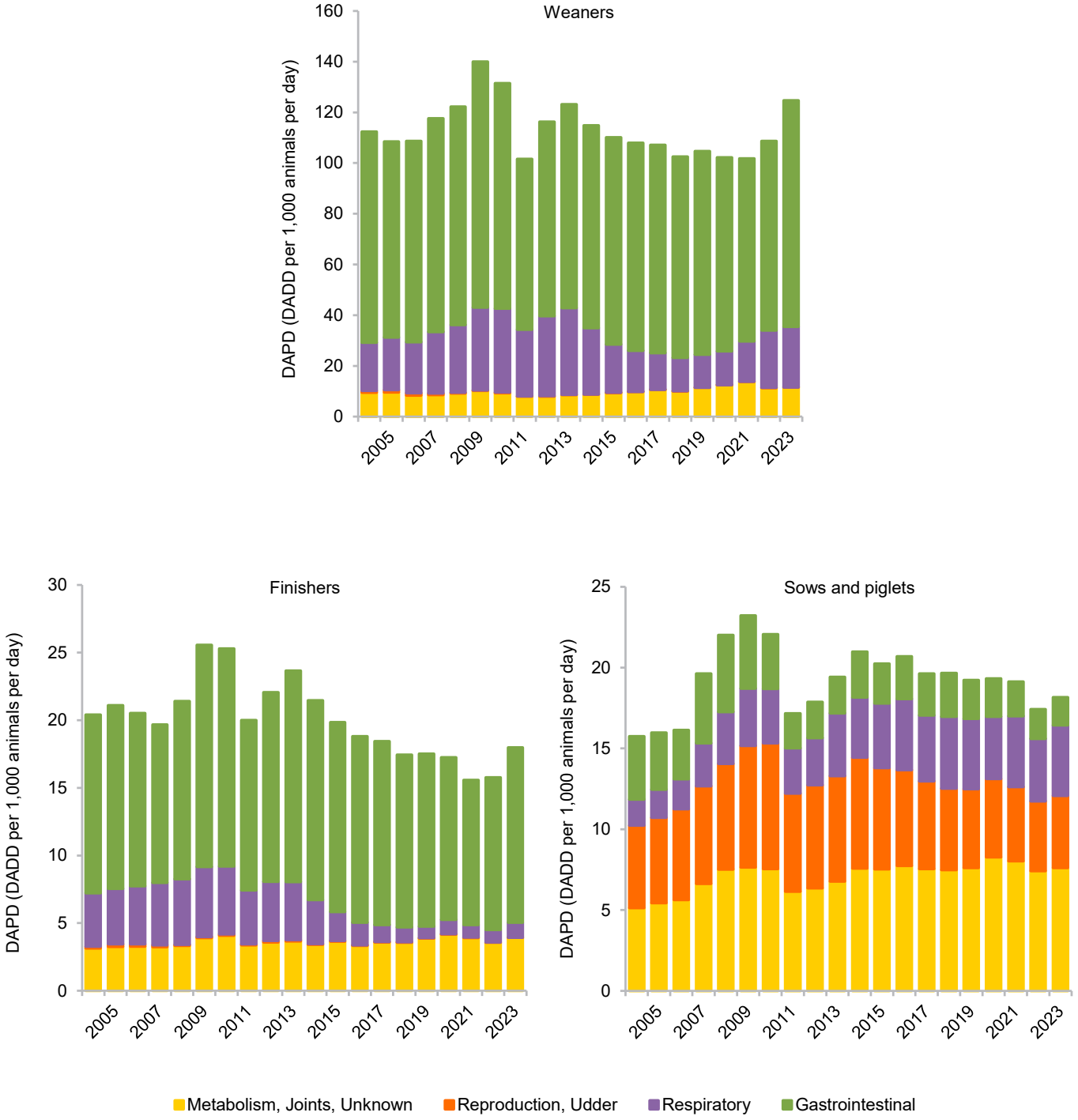
"Sows and piglets" include treatment in boars, where boars constitute 4-5% of the estimated live biomass for the age group. DAPDs are calculated as the number of standard doses for one kg animal divided by the estimated live biomass in the age group of the total population (in tonnes)

Figure 4.4 Antimicrobial consumption in the total pig production and in each age group at antimicrobial class level, DAPD, Denmark
DANMAP 2023



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)
The age group “sows and piglets” includes treatment in boars, where boars constitute 4-5% of the estimated live biomass for the age group

Figure 4.5 Antimicrobial use in each age group at indication level DANMAP 2023



Note: Intramammaries, gynecologicals and topical drugs not included

4.3.2 Antimicrobial consumption in cattle

Legislation-supported thresholds for antimicrobial consumption in cattle have been in place since 2011. In 2023, approximately 7.96 tonnes were recorded for use in cattle, of which approximately 457.63 kg of active compound were used for intramammary therapeutic or dry-cow treatment. Treatments with penicillins, beta-lactamase sensitive accounts for 52.21% of the total usage for cattle (Table 4.1).

About 32.60% of the antimicrobial consumption for systemic treatment was used for young cattle (<12 months), and the rest was used to treat adult cattle (>12 months) (Table 4.1). The production of veal, beef and milk has remained relatively stable over the past 5 years (Chapter 2, Table 2.3).

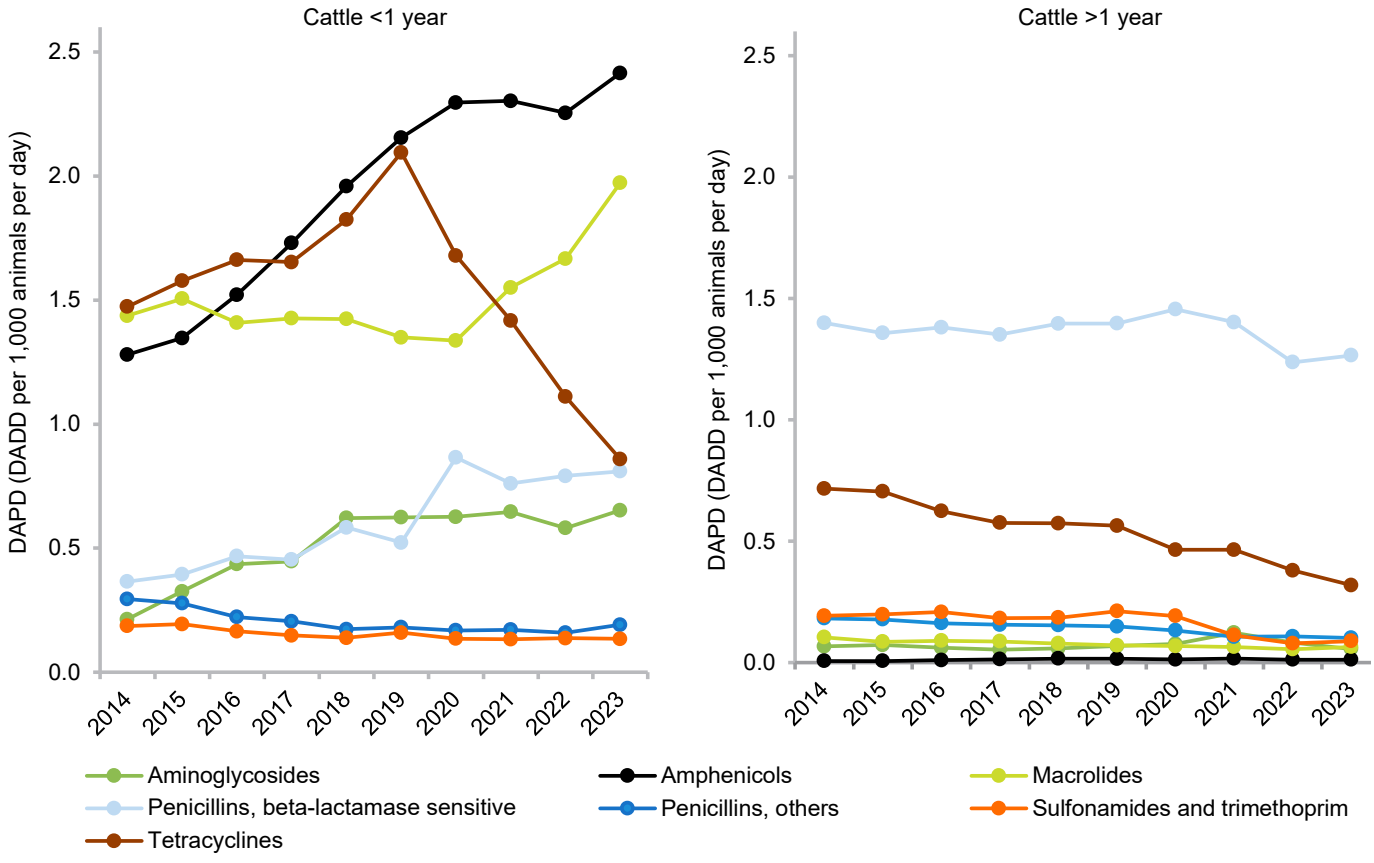
Measured in kg active compound, in adult cattle, the consumption was 2.49% lower in 2023 than in 2022. Moreover, there has been a gradual decrease in the overall use of antimicrobials for systemic treatment in adult cattle over the past decade. Consumption was 20.74% lower in 2023 compared to 2019 and 21.78% lower than in 2014.

Measured as treatment proportions, the use in adult cattle was 2.79 and 1.95 DAPD in 2014 and 2022 respectively. In 2023, the treatment proportion was 1.91 DAPD.

The main indication for systemic treatment in adult cattle was mastitis (Reproduction, Udder). Systemic treatment of adult cattle was carried out using parenteral antimicrobial products only. The main indication for systemic treatment in young cattle was respiratory diseases. Overall, parenteral products accounts for 92.4% of systemic treatment of cattle (Figure 4.6 and 4.7).

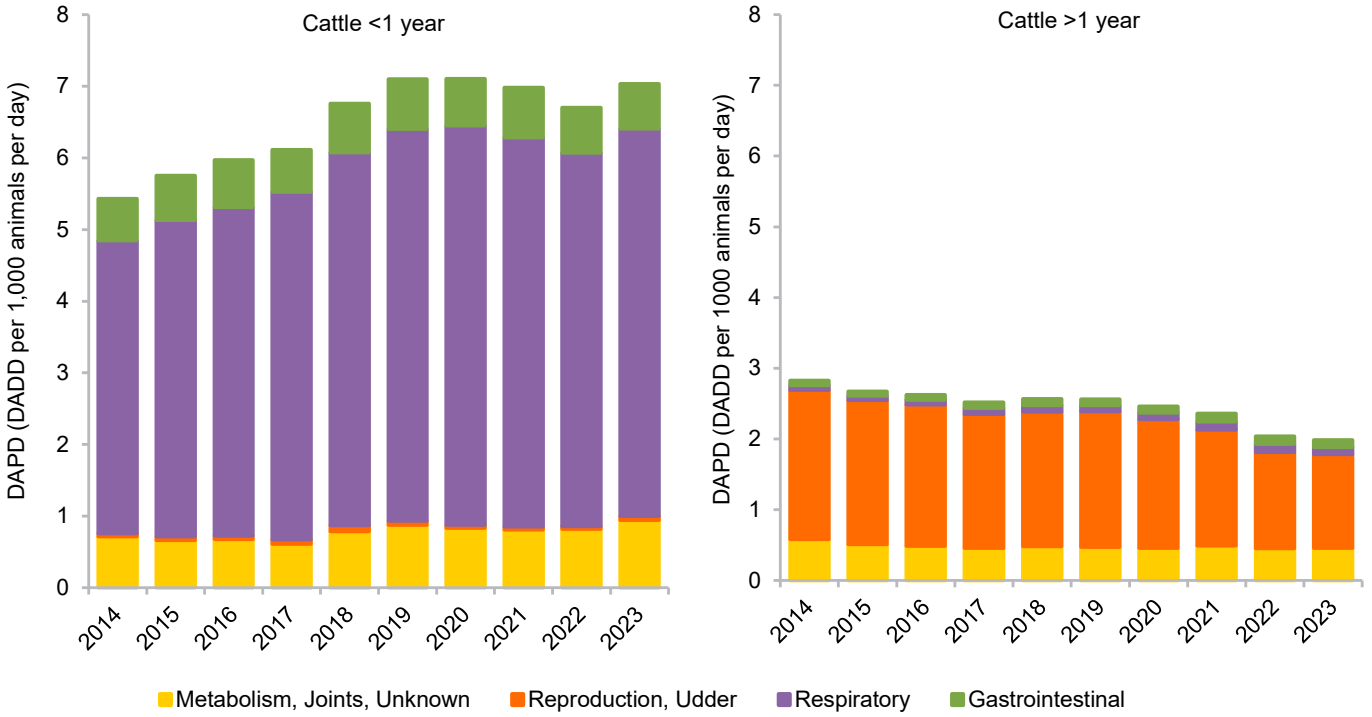
Historically, the antimicrobial systemic consumption in young cattle increased until 2019, followed by a slight decrease in the following years. DAPDs at antimicrobial class level of cattle <1 year and cattle >1 year are presented in Figure 4.6.

Figure 4.6 Antimicrobial consumption in cattle production by age groups at antimicrobial class level, DAPD, Denmark DANMAP 2023



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)
Intramammary applications are not included (doses needed for calculating DAPD not available)
The DAPDs of amphenicols in cattle <1 year differ from previous reports, due to missing data in the old VetStat

Figure 4.7. Antimicrobial use in each age group at indication level, DAPD, Denmark DANMAP 2023



Note: Intramammaries, gynecologicals and topical drugs not included

In young cattle, treatment (DAPD) with amphenicols (florfenicol) has increased steadily since 2014 and are still the most frequently prescribed antimicrobial (34.33%), followed by macrolides and tetracyclines, 28.04% and 12.20%, respectively in 2023.

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. The use of fluoroquinolones in food-producing animals is also notifiable to the DVFA. No fluoroquinolones were registered in VetStat for consumption in cattle in 2023.

In 2014, the cattle production began to phase out the use of 3rd and 4th generation cephalosporins used for systemic treatment, resulting in a significant drop in 2015. In 2019, cattle production implemented a ban on use of 3rd and 4th generation cephalosporins in all cattle, and no use has been registered since 2020.

By the year of 2020, the board of Danish dairy and beef producers' strategy for good udder health aimed at a 20% reduction in the use of antimicrobials for treatment of mastitis and other cattle diseases compared to 2012, as well as lowering the geometric mean bulk tank cell counts to 150,000. The dairy industry also aims to promote the use of simple penicillins (beta-lactamase sensitive penicillins) when dry-cow therapy or mastitis treatment is required.

The board of Danish dairy and beef producers renewed its strategy for disease prevention in calves and cows, including good udder health objectives for the period 2021-2023. The goals are, for the given period, a 10% annual reduction in use of antimicrobials for treatment of cattle <1 year old and a 3% annual reduction in use of antimicrobials for treatment of cattle >1 year old. Moreover, the new strategy aims to reduce the proportion of milk producers with a cell count >200,000 from 60% to 30%.

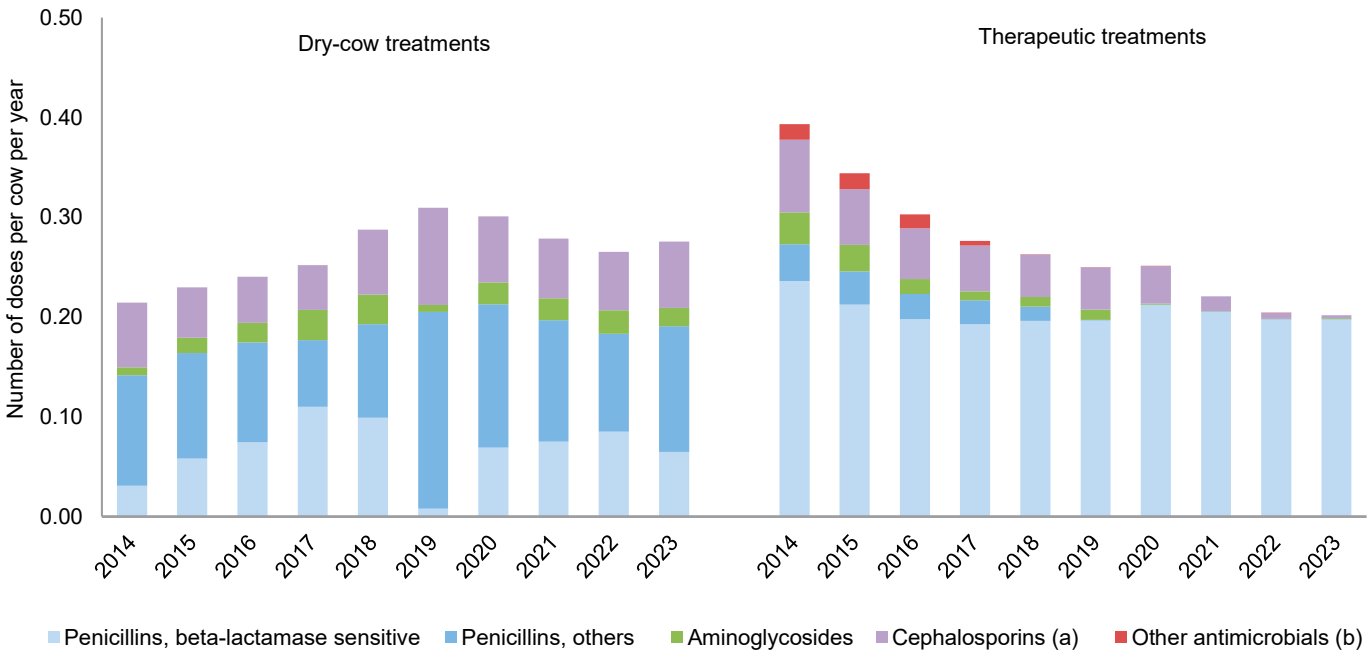
In 2023, the overall antimicrobial consumption in cattle was 24.40% (2569.88 kg) lower than in 2012 and the bulk tank milk counts were at 185.400 in February 2022. However, in this period systemic treatments of young cattle increased by 35.60% (680.28 kg), while the systemic treatment of adult cattle decreased by 36.83% (2857.74 kg). The use of intramammary applications decreased by 16.25% in the given period.

The consumption of intramammary treatment, measured as doses per cow per year, is shown in Figure 4.8. The consumption of simple penicillins (beta-lactamase sensitive penicillins) has increased, whereas the consumption of 1st generation cephalosporins has decreased.

In 2019, there was a remarkable shift in the dry-cow treatments and the use of the beta-lactamase sensitive penicillins for this purpose almost ceased, while the use of the other penicillins, especially cloxacillin, increased. This shift was caused by a product shortage, where the only beta-lactamase sensitive penicillin for dry-cow treatment was unavailable for longer periods during 2019, and other penicillins especially products containing cloxacillin, had to be used instead [Personal communication; Michael Farre, Danish Agriculture and Food Council]. In 2020 and onwards, it again shifted to the use of only beta-lactamase sensitive penicillins (Figure 4.8).

For therapeutic treatments, beta-lactamase sensitive penicillins remained the most used antimicrobial class in 2023.

Figure 4.8 Consumption of antimicrobials for intramammary application in cattle, treatments per cow per year, Denmark DANMAP 2023



For intramammary treatment, the consumption has been estimated as the number of doses, Combination products are split into active compounds

a) 1st generation cephalosporins only

b) Includes lincomycin for dry-cow treatments. For therapeutic treatment, mainly 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 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).

Both a substantial increase and decrease in consumption of antimicrobials is very likely caused by disease in several flocks in a single farm [personal communication, Susanne Kabel, Danish Agriculture and Food Council].

Previously, VetStat did not allow easy differentiation of antimicrobial use in different types of poultry production. However, this has been amended in the new VetStat. From June 2021 antimicrobial use has been reported in more detail, subsequently within some years it will be possible to follow trends in antimicrobial usage in the different types of poultry production.

In 2023, the total antimicrobial usage has decreased by 40.55 kg of active compound compared to 2022. While the consumption of penicillins and tetracyclines has increased by 146.38 and 54.49 kg active compound, respectively, the macrolide consumption has decreased by 257.62 kg active compound (Table 4.2).

For the past decade, cephalosporins have not been used in poultry production, and the use of fluoroquinolones stopped in 2021. Colistin has not been used since 2016.

Table 4.2 Consumption of antimicrobials in poultry, kg active compound, Denmark DANMAP 2023

	Aminoglycosides	Amphenicols	Fluoroquinolones	Lincosamides	Macrolides	Other antimicrobials [a]	Penicillins, beta-lactamase sensitive	Penicillins, others	Pleuromutilins	Sulfonamides and trimethoprim	Tetracyclines	Total
2014	21.42	8.51	0.11	10.49	402.83	2.60	133.27	373.78	0.38	82.26	604.11	1639.75
2015	258.47	4.37	1.00	129.12	133.31	9.96	204.43	565.96	0.63	445.46	818.09	2570.78
2016	60.19	4.83	-	23.77	175.58	8.00	264.55	257.65	0.38	111.00	764.56	1670.51
2017	64.87	5.06	-	31.75	244.87	1.00	355.55	334.77	0.45	84.60	487.45	1610.37
2018	50.56	-	-	25.28	194.95	-	357.83	242.58	0.83	36.60	521.12	1429.73
2019	54.80	0.23	0.01	27.36	274.83	-	368.37	234.30	0.64	64.25	694.27	1719.07
2020	58.19	-	-	29.01	156.91	-	334.10	237.34	0.23	54.60	1590.93	2461.31
2021	58.87	-	-	27.69	168.86	-	115.38	204.10	0.38	34.80	656.61	1266.68
2022	50.42	-	-	14.98	433.96	-	232.70	139.88	19.38	12.60	442.43	1346.35
2023	50.23	-	-	15.28	176.34	-	315.90	203.06	16.88	31.20	496.92	1305.80

Data for 2023 were extracted from VetStat on 1 July 2024
VetStat does not differentiate between consumption in the different sectors of poultry production
Combination drugs are divided into active compounds
a) Other antibacterials also include other quinolones and polymyxins

4.3.4 Antimicrobial consumption in aquaculture, and companion animals

Aquaculture

Antimicrobial consumption in aquaculture is mainly driven by the summer air temperatures and hours of summer sunlight because bacterial diseases are more likely to occur when water temperatures are high [Villumsen and Bojesen, 2022. Microbiol Spectr. 10(5):e0175222]. Although the aquaculture production continues to focus on developing improved vaccination strategies to reduce the risk of bacterial diseases that may require treatment with antimicrobials, the antimicrobial consumption varies significantly from year to year. In 2023 the antimicrobial consumption decreased by 35.62% compared to the average consumption in the previous five years. The decrease was solely due to decreased usage of combination products of sulfonamides and trimethoprim (Table 4.3).

In 2023, mainly three antimicrobial classes were used to treat bacterial infections in aquaculture: 58.90% of sulfonamides and trimethoprim, 33.20% of other quinolones (oxolinic acid), and 7.89% of amphenicols (florfenicol) (Table 4.3).

Table 4.3 Consumption of antimicrobials in aquaculture, kg active compound, Denmark DANMAP 2023

	Amphenicols	Other antibacterials [a]	Other quinolones	Penicillins, others	Sulfonamides and trimethoprim	Tetracyclines	Total
2014	297.1	-	1678.3	9.8	3131.9	-	5117.0
2015	311.1	-	1004.5	5.1	1655.0	0.7	2976.4
2016	315.3	0.0	893.1	13.6	1085.9	0.4	2308.2
2017	350.3	0.0	636.8	35.0	679.3	0.1	1701.6
2018	323.5	-	899.3	51.6	2292.6	0.5	3567.4
2019	292.6	-	446.9	43.9	1720.9	22.0	2526.3
2020	341.2	-	565.3	27.1	1030.2	1.0	1964.7
2021	295.4	1.8	366.3	19.5	1091.3	1.4	1775.8
2022	144.1	0.0	366.5	-	1940.8	0.6	2452.0
2023	124.9	0.0	525.2	-	931.7	-	1581.9

Data for 2023 were extracted from VetStat on 1 July 2024
Combination products are split into active compounds
a) Other antibacterials also includes lincosamides

Companion animals - horses and pets

The information available on antimicrobial consumption in companion animals is not as accurate as for production animals, since VetStat allows registration of antimicrobials for companion animals without defining animal species. Table 4.4 shows the antimicrobial consumption registered for companion animals in three categories: horses, pets, and "unspecified".

The total amount of antimicrobials estimated for consumption in companion animals in 2023 was 2353.54 kg (Table 4.1). As in previous years, a substantial amount of sulfonamide/trimethoprim registered as used for pets or unspecified is oral paste, a product normally used for horses. Thus, a substantial amount of sulfonamide/trimethoprim included in Table 4.4 is likely to have been used for horses (1400.7 kg in 2023).

A large proportion of antimicrobials 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, repeated use of critically important antimicrobials may pose a risk to the owners, and the use of these antimicrobials is therefore monitored carefully. Since the treatment guidelines by DVA were published in 2012 (revised in 2018), the use of cephalosporins has been reduced from 272.70 kg in 2012 to 58.49 kg of active compound in 2023 (Table 4.1 and 4.4).

In 2023, the consumption of fluoroquinolones in companion animals, mainly dogs and cats, was 13.07 kg active compound and represented the majority (94.71%) of fluoroquinolones used in all animals (Table 4.1 and 4.4). Similarly, the companion animals accounted for 53.63% (58.49 kg) of all the cephalosporins consumed in animals (Table 4.1 and 4.4). In 2023, 3rd generation cephalosporins were only used in pets (0.93 kg) and registered as "unspecified" (Table 4.1).

Table 4.4 Estimated consumption of antimicrobials for horses, pets and unspecified animals, kg active compound, Denmark

DANMAP 2023

	Aminoglycosides	Amphenicols	Cephalosporins	Fluoroquinolones	Lincosamides	Macrolides	Other antimicrobials [a]	Other quinolones	Penicillin's, b-lactamase sensitive	Penicillin's, others	Pleuromutilins	Sulfonamides and trimethoprim	Tetracyclines	Total
Horses														
2014	1.4	-	0.4	0.0	-	0.1	0.0	-	9.5	0.3	-	98.2	6.7	116.7
2015	2.8	-	0.4	0.0	0.0	0.1	0.0	-	6.9	0.1	-	114.6	4.8	129.6
2016	0.8	-	0.1	0.0	-	-	0.0	-	5.2	0.0	-	108.0	5.3	119.5
2017	0.9	0.1	0.1	0.0	-	-	0.0	-	5.4	0.1	-	106.4	3.0	116.0
2018	0.7	0.0	0.2		-	0.1	0.0	-	6.0	0.0	-	100.6	3.8	111.3
2019	0.9	-	0.1	0.0	-	0.0	0.0	-	4.9	0.1	-	94.2	3.8	104.1
2020	1.7	-	0.0	0.0	0.0	-	0.0	-	5.3	0.0	-	111.5	3.5	122.1
2021	0.2	-	0.0	0.0	0.0	0.0	0.1	-	5.2	0.1	-	105.8	2.0	113.4
2022	0.3	0.0	0.0	0.0	0.0	1.0	0.2	-	4.5	0.2	-	136.6	7.3	150.2
2023	0.3	0.0	0.0	0.0	0.0	-	0.3	-	5.9	0.6	-	166.6	7.7	181.3
Pets														
2014	5.6	0.2	81.3	5.0	19.0	5.0	6.8	1.0	12.1	122.3	0.4	260.9	13.3	532.9
2015	4.8	0.1	61.8	5.6	21.8	3.3	6.8	-	13.2	123.4	1.8	226.2	20.5	489.2
2016	3.4	0.4	55.3	5.4	21.8	2.3	7.4	0.1	9.8	131.2	0.3	269.1	21.5	527.8
2017	3.8	0.7	41.7	5.2	18.4	1.7	8.3	-	9.2	125.8	0.1	272.4	19.3	506.6
2018	3.9	0.3	35.9	4.9	17.5	1.7	14.3	1.0	10.0	113.7	0.5	253.2	21.1	478.1
2019	3.7	0.3	32.3	4.5	17.2	7.4	15.0	0.0	10.3	108.4	0.6	236.8	14.8	451.4
2020	4.3	0.6	30.7	5.1	19.1	3.8	17.6	-	12.9	103.4	0.5	262.3	17.7	478.0
2021	3.2	0.7	28.0	4.7	19.2	2.2	20.8	-	11.5	100.1	0.1	270.8	23.7	485.0
2022	1.7	0.0	22.3	3.9	16.5	0.2	24.4	-	9.4	79.5	0.1	165.5	22.3	345.8
2023	2.2	0.6	21.6	4.3	17.7	2.2	24.5	-	10.0	86.5	0.2	194.4	18.8	383.0
Unspecfied														
2014	48.3	0.0	132.0	8.2	50.2	0.0	26.7	-	2.6	435.5	-	968.9	20.4	1692.8
2015	41.3	0.3	95.9	8.7	46.8	0.0	25.1	1.0	1.5	429.2	-	946.8	17.0	1613.7
2016	37.4	0.4	81.6	9.7	48.9	0.3	26.3	-	2.2	468.7	-	1015.3	17.0	1707.9
2017	33.2	0.2	69.1	9.3	50.1	0.0	28.3	-	1.9	469.8	-	1071.7	14.7	1748.3
2018	31.8	1.3	61.4	9.8	45.8	-	34.8	-	1.8	453.0	-	1136.6	13.0	1789.3
2019	29.2	0.2	60.6	9.9	48.8	0.1	36.8	-	1.9	442.6	0.1	1140.7	16.0	1787.1
2020	22.6	0.4	56.9	10.7	52.2	0.1	40.2	-	2.9	446.9	-	1221.7	15.6	1870.3
2021	17.3	0.4	49.4	10.1	57.5	-	47.3	-	0.8	457.5	-	1284.6	14.5	1939.4
2022	1.3	0.4	38.8	8.8	54.3	-	51.9	-		395.3	-	1100.6	11.9	1663.3
2023	1.0	0.4	36.9	8.7	55.6	-	49.2	-		390.4	-	1234.1	12.8	1789.3

Data for 2023 were extracted from VetStat 1 July 2024.
Combination products are split into active compounds
The estimates include all veterinary approved antimicrobials, for use in horses, pets, as well as products without a specified animal species (unknown)
a) Other antibacterials also include other otologicals, pleuromutilins, polymyxins and sulfonamides, plain

Vibe Dalhoff Andersen and Marianne Sandberg
For further information:
Marianne Sandberg, marsan@food.dtu.dk

Textbox 4.1

A shift in the use of aminoglycosides following the ceased use of zinc oxide

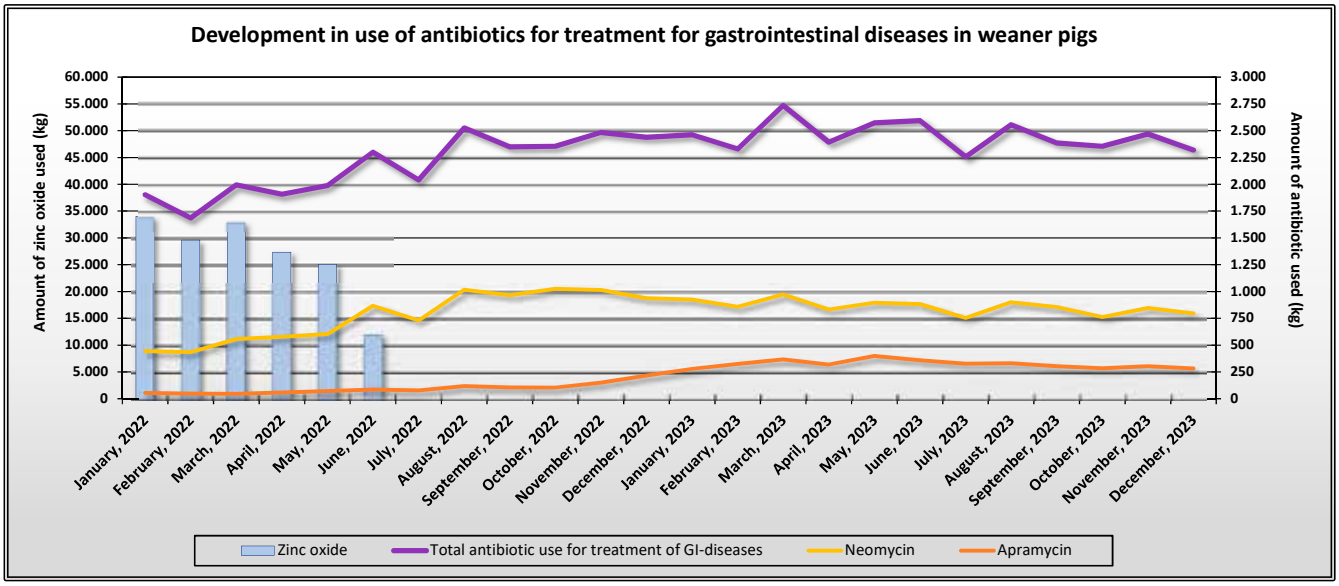
The use of zinc oxide in Veterinary Medicinal Products (VMPs) was banned across Europe from June 26th 2022, due to a consideration of the risks for the environment. Prior to this, VMPs containing zinc oxide had been used to control the occurrence of post-weaning diarrhea in piglets. The political ambition in Denmark was that the ban of zinc oxide should happen without an increase in antimicrobial use. While the Danish pig industry investigated strategies to find suitable alternatives for managing post-weaning diarrhea in the years leading up to the ban, the complete discontinuing use of zinc oxide led to an acute rise in the use of neomycin in 2022; as described in the DANMAP 2022 report.

Neomycin is a first-choice antibiotic for treating post-weaning *E. coli* associated diarrhea in the Danish national antibiotic treatment guidelines for pigs and was used in some capacity for treating post-weaning diarrhea before the ban on zinc oxide. With the increased use came increasing problems with resistance towards neomycin, which led to an increased use of apramycin towards the end of 2022 and the beginning of 2023. In 2023, the levels of use of both neomycin and apramycin have stabilized, but to a higher level than prior to the ban of zinc oxide.

The increase in neomycin resistance and increased use of apramycin can be problematic, due to the impaired effectiveness and unfavorable alternative antibiotic treatment options. A range of new initiatives to decrease antibiotic use in pig production have been launched in 2024, as specified in the political food and veterinary agreement 2024-2027 and in the Danish Veterinary and Food Administration's 'National action plan on Antimicrobial Resistance in animals and food' for 2024-2027.

The Initiatives includes updates of the national antibiotic treatment guidelines, new thresholds for the Yellow Card initiative, focusing on weaned piglets up to 30 kg and an investigation of a benchmarking-model for veterinarians along with a continued focus on investigating alternatives to antibiotic treatment.

Figure 1 Development in use of antibiotics for treatment for gastrointestinal diseases in weaner pigs DANMAP 2023



Frederik Fabricius and Jensine Wilm, The Danish Veterinary and Food Administration
For further information: Frederik Fabricius, NIFAB@fvst.dk, Jensine Wilm, JENWI@fvst.dk

Textbox 4.2

Veterinary medicines and antibiotic resistance

In recent decades, veterinary medicine has focused on a One Health approach that integrates considerations regarding the health of both animals and humans. This has led to new guidelines for the responsible use of antibiotics in animals, aiming to reduce the development of resistance. As part of these efforts, the EU's Veterinary Medicines Regulation (2019/6) was implemented in 2022 with the goal of harmonizing the use of veterinary medicines, including reserving critical drugs for humans and generally ensuring responsible use. Veterinarians support these initiatives, but unfortunately, certain provisions in the regulation, especially Article 106, cause significant challenges for many veterinarians, which may impact a responsible use in a negative way. Veterinarians are required to prescribe medications strictly according to the Summary of Product Characteristics (SPC) which, depending on the available drugs, limits their ability to tailor treatments to individual animals or herds.

Outdated product summaries
One of the biggest challenges is that the SPCs for many veterinary medicines, such as older narrow-spectrum antibiotics, are not updated in line with new knowledge. This forces veterinarians to follow outdated dosage recommendations and treatment durations, which can lead to unnecessary use of medication and, consequently, increase the risk of antibiotic resistance. Marketing authorization holders rarely see a benefit in updating SPCs to reflect new indications, agents, or treatment regimens, as this is resource-demanding, and the risk is that more veterinary medicines will be withdrawn from the market.

Failing access to important medicines
The regulation aims to reduce administrative burdens, strengthen the market, and promote innovation in the veterinary pharmaceutical industry, but there are still challenges in ensuring a stable supply of necessary veterinary medicinal products. And although EU harmonization is intended to reduce discrepancies, in several countries, including small markets like Denmark, shortages of essential products such as certain vaccines and narrow-spectrum antibiotics have been reported. This can drive veterinarians to use broad-spectrum antibiotics instead, increasing the risk of resistance. In addition, Danish veterinarians still face difficulties accessing products like autogenous vaccines, which their colleagues in other EU countries can use. This, combined with the lack of marketed vaccines risks compromising preventive efforts and leading to more disease outbreaks that require antibiotic treatment.

Consequences for animal treatment
Article 106 of the Veterinary Medicines Regulation thus creates practical problems in many treatment situations, including in pig herds. For instance, the strict interpretation of SPCs for medications may result in longer antibiotic treatments with higher doses, even though new empirical knowledge and evidence shows that lower doses for shorter periods are just as effective. This challenges the goals of reducing antibiotic use in this sector.

Similar challenges are seen with companion animals and horses, where veterinarians traditionally base treatment durations on clinical signs, on translating experience from human medicine and scientific guidelines to minimize antibiotic use and unnecessarily long treatments. This is no longer possible under the current requirements, affecting both animal health and welfare.

The need for flexibility
There is a great need to hand back to veterinarians the flexibility to deviate from SPCs when new research supports it. This will ensure better treatment for animals and reduce the risk of antibiotic overuse, which is a major threat in the fight against antimicrobial resistance. Veterinarians want to base their decisions on the latest scientific knowledge rather than being bound by outdated guidelines and rigid legal requirements. This flexibility will help reduce antibiotic use and lower the risk of resistance development, which affects both animals and humans.

Karin Melsen (DVM, chief political advisor) and Pia Rindom (responsible editor), The Danish Veterinary Association
For further information: Karin Melsen, km@ddd.dk, Pia Rindom, pr@ddd.dk

5

ANTIMICROBIAL CONSUMPTION IN HUMANS

5. Antimicrobial consumption in humans

Highlights

Antimicrobial consumption in Denmark was 16.54 DID in 2023, 6.3% lower than consumption in 2014 (17.64 DID) and 6.6% higher than in 2022 (15.51.44 DID) underlining that consumption has resurged since the COVID-19 related decreases in 2020 and 2021.

In primary health care, total antimicrobial consumption was 14.56 DID in 2023, 7.2% higher than the 13.59 DID in 2022 and 6.9% lower than in 2014 (15.64 DID). The increase was driven by a 23% increase in beta-lactamase sensitive penicillins in primary health care. The four groups of penicillins constituted 67% of the consumption and beta-lactamase sensitive penicillins were the most used group of antimicrobials (accounting for 27% of total consumption in primary health care).

Antimicrobials prescribed for respiratory tract infections dropped sharply with the emergence of COVID-19 in 2020 due to the implemented societal restrictions. In 2022 and 2023, the usual winter peak in antimicrobial consumption reached a higher level than observed for 2018-2019. This was due to high rates of viral infections, in particular an early and more severe RSV and influenza season, and an outbreak of Group A streptococci.

Antimicrobials prescribed to children demonstrated marked increases in the recent two years. Among the 0-4 year olds, consumption in 2023 was 298 treated patients per 1,000 inhabitants, a 30% increase compared to 229 treated patients per 1,000 inhabitants in 2022. For the 5-9 year olds, 197 patients per 1,000 inhabitants were treated in 2023 compared to 122 patients per 1,000 inhabitants in 2022 (60% increase).

Elderly inhabitants living at care homes during 2023 received 90% more antimicrobials than elderly inhabitants living in their own homes (1,819 prescriptions per 1,000 inhabitants at long term care facilities compared to 957 prescriptions per 1,000 inhabitants in their own homes). Urinary tract infections were the main cause of the observed difference in the treatment frequency. However, consumption for elderly inhabitants living at care homes has decreased by 28% from 2017 to 2023, while consumption for elderly living in their own homes has decreased by 16%.

Antimicrobial consumption in hospital care measured in DID (i.e. not accounting for hospital activity) was 1.91 DID in 2023, 3% higher than in 2022 (1.86 DID). When measuring in DDD per 100 bed-days (DBD), the consumption in 2023 (135.6 DBD) was 5.6% higher than in 2022 (128.42 DBD) and 36% higher than in 2014 (99.44 DBD).

Product shortages are of increasing concern in antimicrobial supply. In 2023, nitrofurantoin was unavailable in several months due to product shortage. Simultaneously, the supply through special deliveries increased. Also other antimicrobials for urinary tract infection treatment increased at the same time.

AWaRe classification of antimicrobials used in Denmark showed that 84% consisted of access antimicrobials (WHO's goal is 60% access antimicrobials).

5.1 Introduction

In Denmark, antimicrobials are available by prescription from medical doctors, veterinarians and dentists. Sale is restricted to licensed pharmacies who have exclusive rights to sell prescription-only medicines. Thus no over-the-counter sale takes place. All consumption of medicinal products for humans is recorded through the Register of Medicinal Product Statistics at the Danish Health Data Authority (Figure 2.1). This includes sales data from all public and private healthcare providers. Antimicrobial sales data have been submitted from the primary care sector since 1994 and from the hospital sector since 1997.

Registration of medicines consumption in the primary care sector covers sales from pharmacies to individuals and private clinics. Sales data contain an identifier of the prescriber and the patient in addition to information about the prescribed antimicrobial, including ATC code, formulation, package size and number of packages sold. Since 2004, the Register of Medicinal Product Statistics also receives information on the indication for prescribing. This allows a very detailed and near-complete surveillance of all systemic antimicrobials used in Denmark in the primary health care.

For the hospital sector, antimicrobial consumption data from all public somatic hospitals with acute care function (referred to as somatic hospitals) are included in the report. Data from psychiatric hospitals, private hospitals and hospices are excluded, since they only account for a minor share of the consumption and no reliable denominator for measuring antimicrobial consumption in these facilities is available.

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

Changes in consumption of antimicrobials often mirror initiatives promoting prudent use of antibiotics and changes in health care organization. In recent years fluctuations in recorded sales also owe to shortages of the generic products (further described in textbox 5.1 on page 71). Initiatives regarding prudent use of antibiotics were undertaken through the years with particular focus on better diagnostics guiding antibiotic prescribing by general practitioners and working with antibiotic stewardship at hospitals. The former led to the establishment of the Danish Research Center for General Medicine while the latter was supported by the establishment of a network based on experiences from the Learning and Quality Teams at the bigger regional hospitals.

Reorganization of the Danish healthcare system has led to functions being reassigned from hospital ambulatory care to smaller health units, rehabilitation centers and general practitioners. The resulting changes in activity across the healthcare sector may affect the consumption of antimicrobials. Also, the COVID-19 pandemic had significant impact on the spread of a multitude of different infectious diseases, and associated treatments, which still could be observed three years later in 2023. These changes need to be considered when interpreting antimicrobial consumption surveillance data.

As many other European countries Denmark has also worked with annual antibiotic awareness campaigns since 2013, - except for in the pandemic years 2020-2022 - many of which can be found at www.antibiotikaellerej.dk.

5.2 Total antimicrobial consumption in the Danish healthcare system

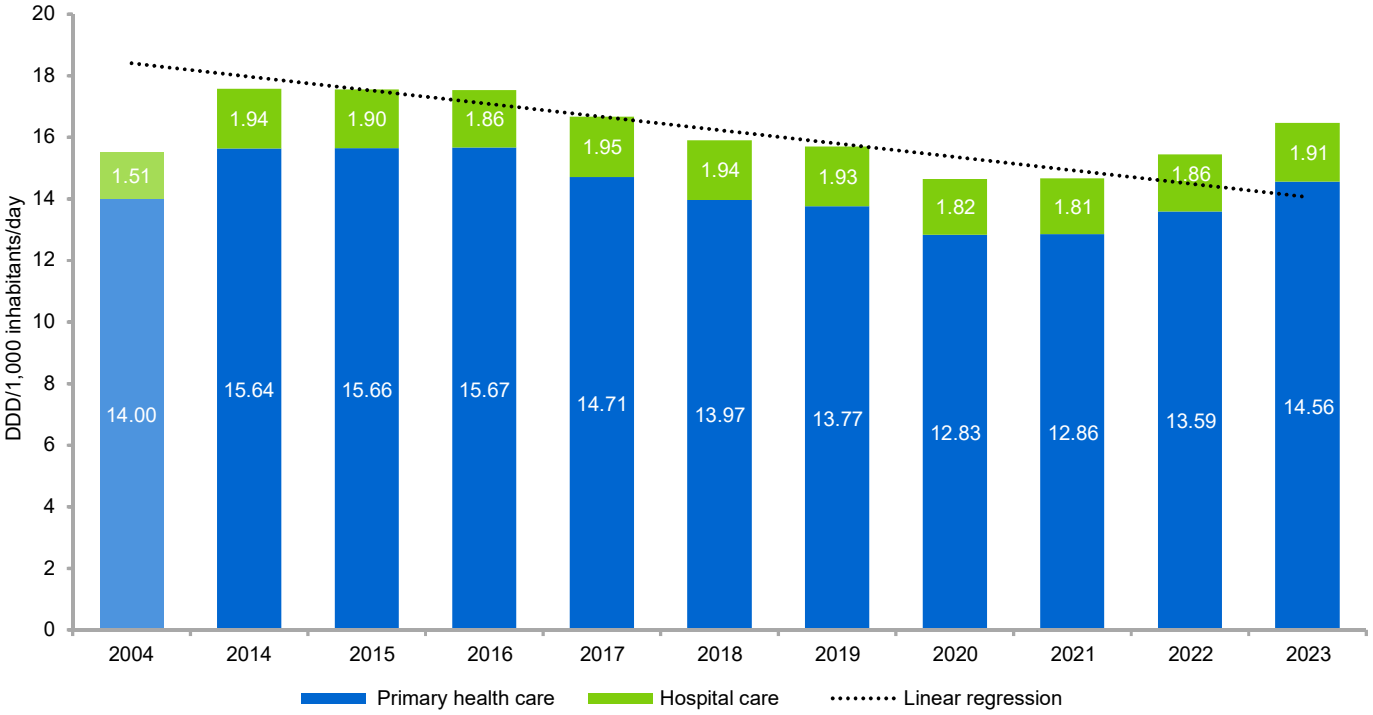
During the first five years of surveillance from 1996 to 2000, the consumption of systemic antimicrobials in Denmark showed no significant changes and consumption was estimated to be at 13 to 14 Defined Daily Doses per 1,000 inhabitants per day (DID). These first five years of reporting are not fully comparable to later years due to changes in reporting and in data systems. Between 2001 and 2011, consumption of antimicrobials increased steadily and peaked at a total of 18.95 DID in 2011 (not shown). From 2011 to 2021, consumption decreased markedly (Figure 5.1). However, from 2022 to 2023, the consumption increased by 6.6% from 15.51 DID to 16.54 DID. The primary care sector accounted for the majority of the consumption in Denmark (88%).

The decrease in total antimicrobial consumption since 2013 in Denmark has mainly been driven by reduced prescribing in primary health care. Measured in DID and not adjusted for hospital activity, antimicrobial consumption at hospitals fluctuated over the years; moving between the lowest levels of 1,86 DID in 2016 to highest levels of 1,95 DID in 2017. The notably lower levels in 2020 and 2021 are considered exceptions due to the COVID-19 pandemic. The hospital share of the total antimicrobial consumption was 12% in 2023.

Consumption of antimicrobials in primary health care and somatic hospitals in the five Danish health regions is presented in Figure 5.2. The trends in consumption are similar in all five regions. Region Zealand showed the highest total consumptions of 17.06 DID in 2023, whereas Central Region of Denmark had the lowest total consumption of 14.66 DID.

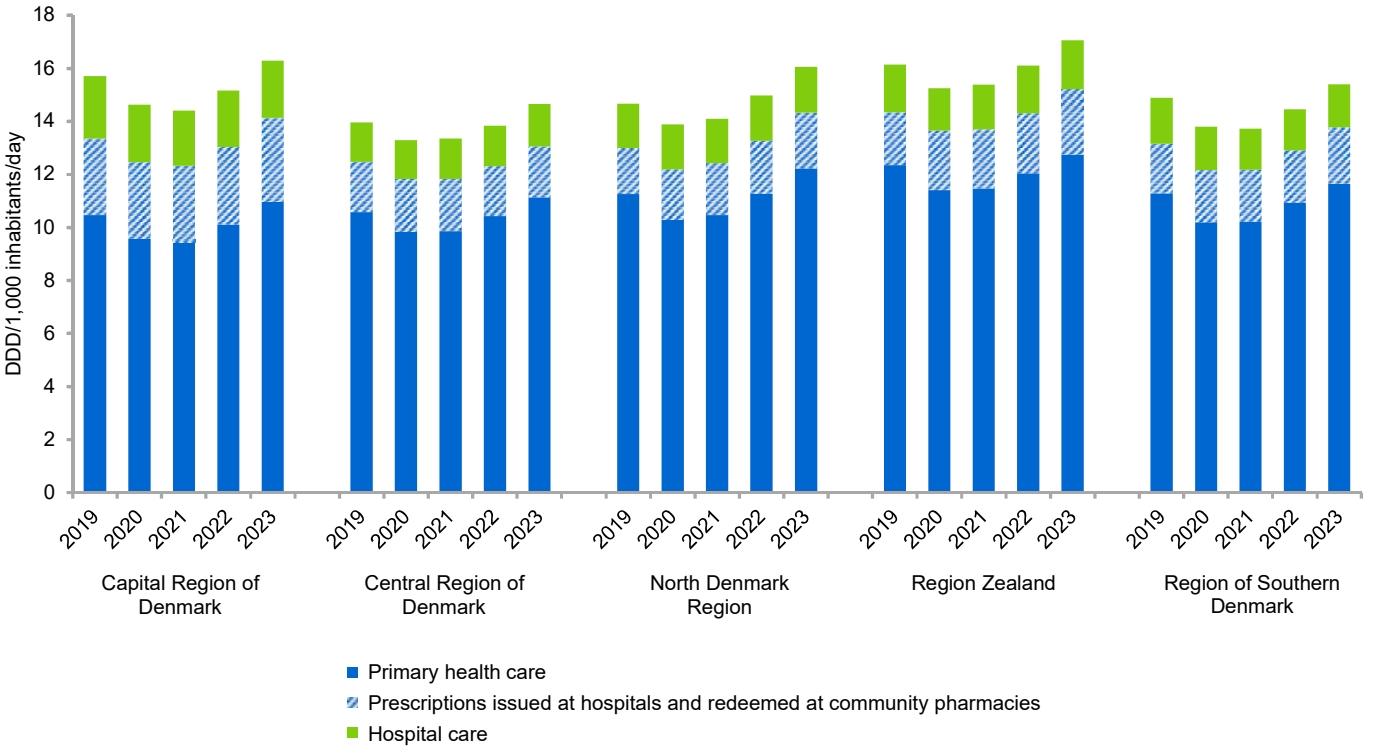
The main antimicrobial drug classes and their consumption in primary health care and at somatic hospitals are presented in Figure 5.3. Most notable are high use of beta-lactams in both health care sectors and low to none use of cephalosporins/aminoglycosides and of carbapenems in primary health care (Figure 5.4).

Figure 5.1 Total consumption of systemic antimicrobial agents in humans, DDD per 1,000 inhabitants per day, Denmark, 2004 and 2014-2023
DANMAP 2023



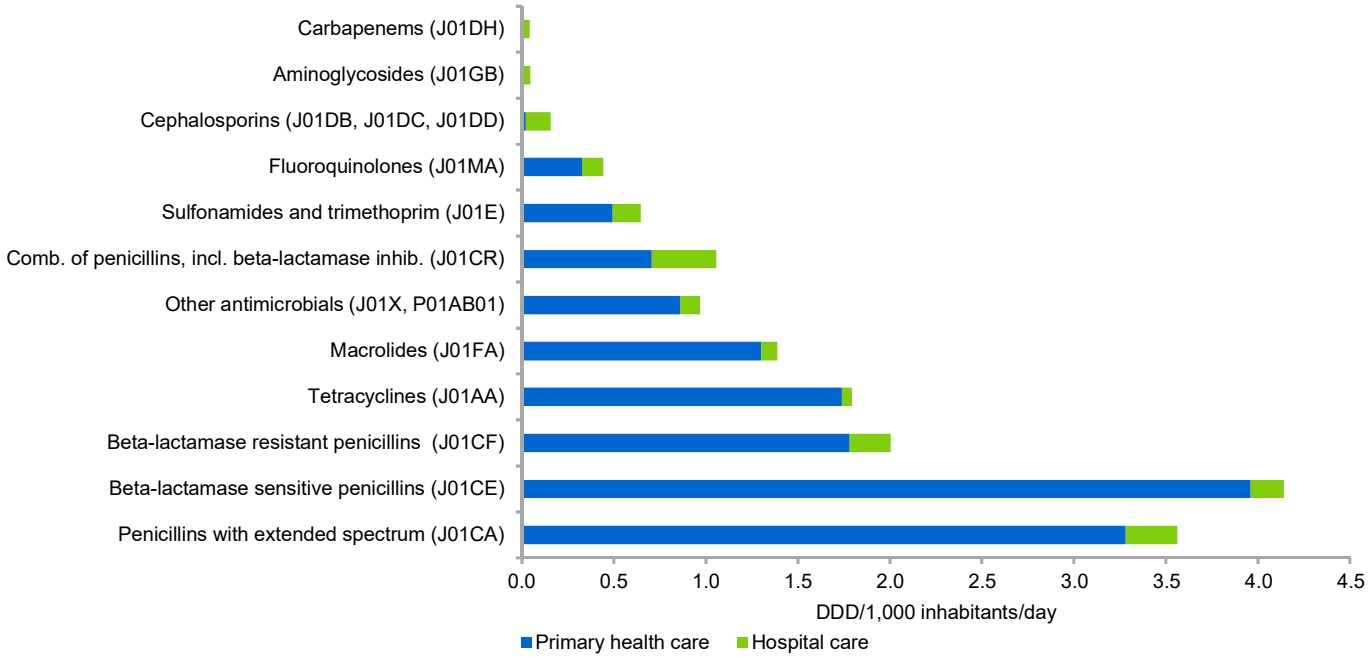
Data: Total sale of antimicrobials in Denmark
Data source: Register of Medicinal Product Statistics and 2024 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 5.2 Consumption of systemic antimicrobial agents in primary health care and at somatic hospitals, DDD per 1,000 inhabitants per day, by Danish region, 2019-2023
DANMAP 2023



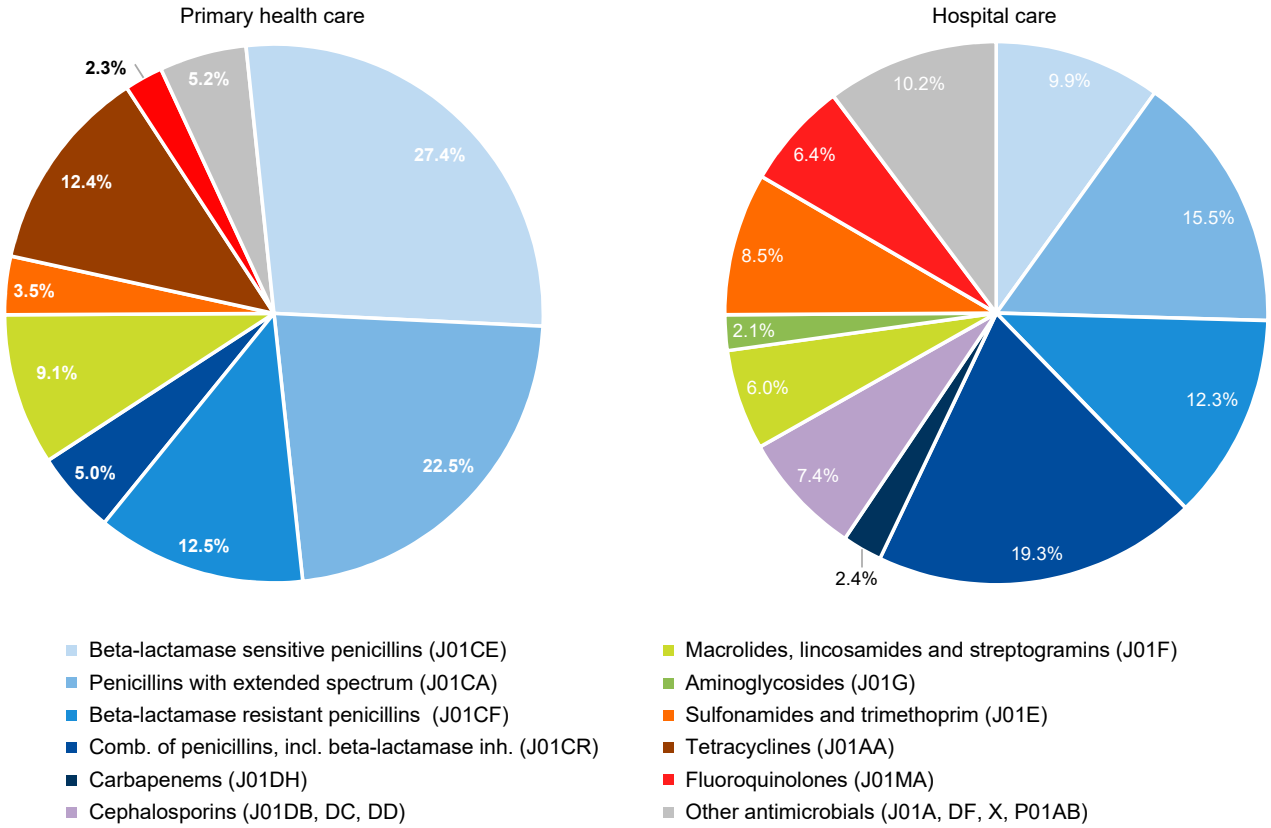
Data: Registered sale of antimicrobials to individuals and antimicrobial consumption at somatic hospitals
Data source: Register of Medicinal Product Statistics and 2024 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 5.3 Distribution of main antimicrobial classes used for humans in primary and hospital care, DDD per 1,000 inhabitants per day, Denmark, 2023 DANMAP 2023



Data: Registered sale of antimicrobials to individuals and antimicrobial consumption at somatic hospitals
Data source: Register of Medicinal Product Statistics and 2024 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 5.4 Percentage distribution of antimicrobial agents in primary health care and hospital care, DDD, Denmark, 2023 DANMAP 2023



Data: Registered sale of antimicrobials to individuals and antimicrobial consumption at somatic hospitals
Data source: Register of Medicinal Product Statistics and 2024 edition of the Anatomical Therapeutic Chemical (ATC) classification system

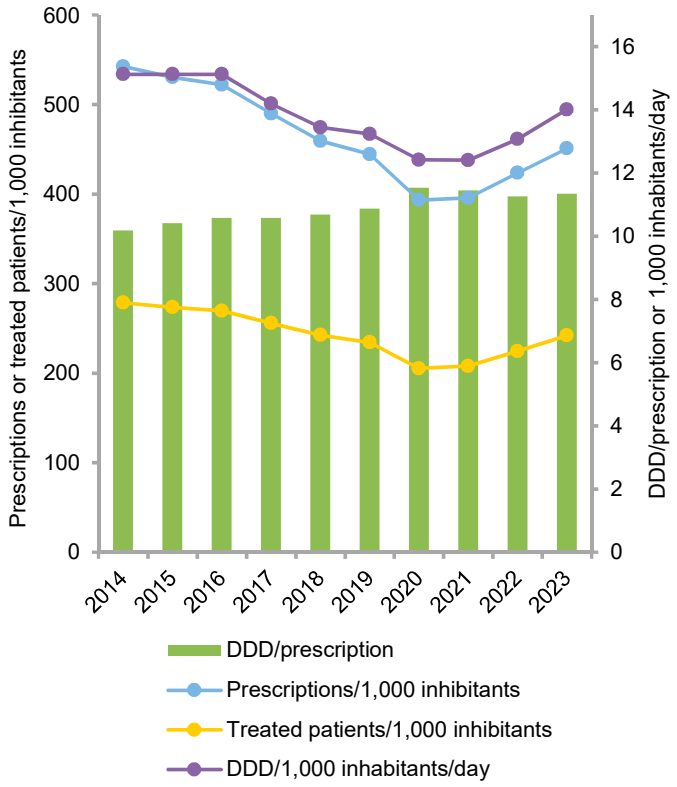
5.3 Antimicrobial consumption in primary health care

In the following sections, the consumption of antimicrobials in primary health care is described by the units DDD per 1,000 inhabitants per day, number of prescriptions per 1,000 inhabitants and number of treated patients per 1,000 inhabitants. The estimates are thus based on sales to individuals and do not include the approximately 4% of antimicrobials, mainly penicillins, sold to clinics and doctors on call.

5.3.1 Overall antimicrobial consumption in primary health care

Comparison of trends over time by different indicators showed decreased consumption from 2014-2020, no change from 2020-2021 and increased consumption from 2021-2023 (Figure 5.5). Thus, the COVID-19 related decrease in consumption was caught up in 2023. In 2023, the average DDD/prescription was 11.3, 11% higher than 2014 (10.2 DDD per prescription). The total number of prescriptions was 451 per 1,000 inhabitants in 2023, a 6% increase since 2022 and a 17% reduction from the 543 prescriptions per 1,000 inhabitants in 2014.

Figure 5.5 Consumption of systemic antimicrobial agents in primary health care, Denmark, 2014-2023 DANMAP 2023



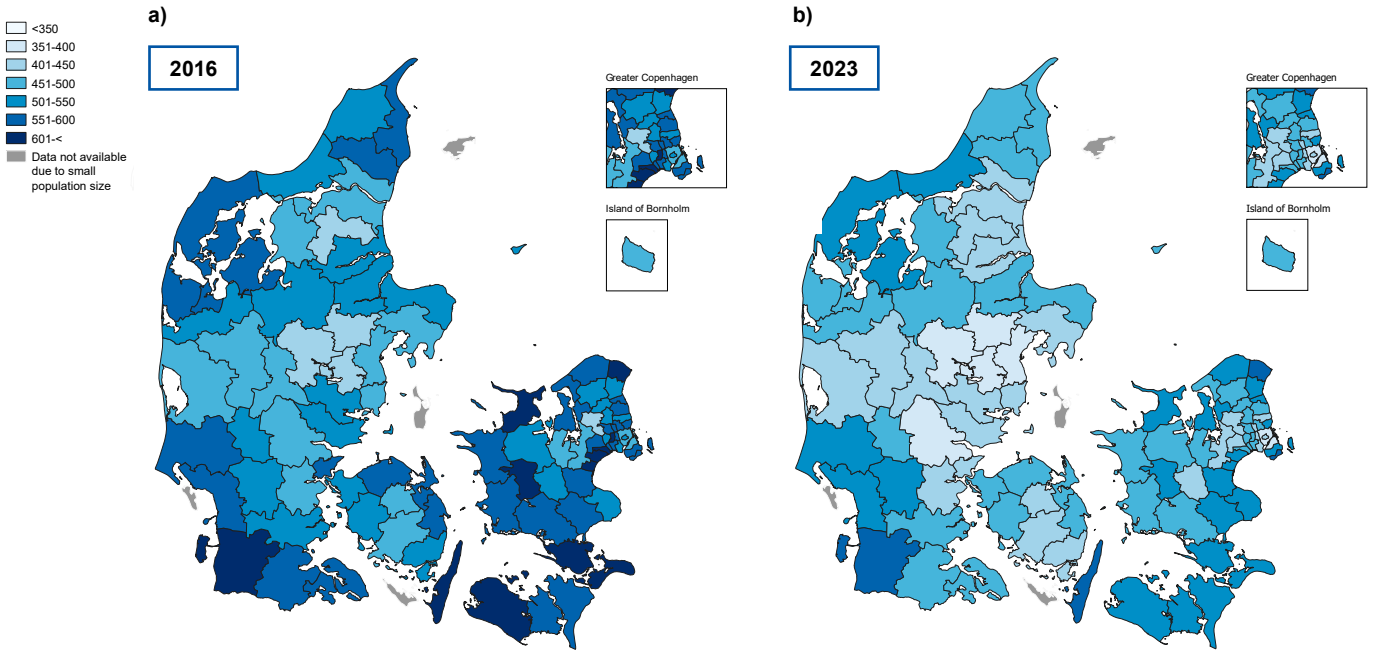
Data: Registered sale of antimicrobials to individuals
Data source: Register of Medicinal Product Statistics and 2023 edition of the Anatomical Therapeutic Chemical (ATC) classification system

The number of treated patients and prescriptions has decreased over the decade, probably due to raising awareness among prescribers and the public. However, doses per prescription have increased, partly due to switch to antibiotics that contribute with more DDDs per treatment, e.g. the switch to pivmecillinam as drug of choice in the treatment of urinary tract infections and the switch to tetracycline as drug of choice in the treatment of chlamydia.

Interregional differences in the levels of prescribing have been described in DANMAP since 2017 (Table 5.1). In general, the Danish population is relatively homogenous and health care is of standardized quality, which, combined with several initiatives to educate GPs in appropriate prescribing, diminishes potential differences in prescribing trends. However, observed variations in prescribing may owe to differences in population density (distance to nearest general practitioner), differences in age and comorbidity of the population (younger populations in bigger cities and in the capital region) as well as behavioral differences between urban and rural populations.

Figure 5.6 shows the number of prescriptions per 1,000 inhabitants at municipality level in 2016 and 2023, respectively. In 2023, the consumption ranged from 384 to 596 prescriptions per 1,000 inhabitants. In 2016, the range was 434-727 prescriptions per 1,000 inhabitants. Of note is that prescribers in all municipalities reduced their prescribing activities in the shown period. Demographic differences might impact the range of prescribing. Distribution of elderly inhabitants above 60 years in the municipalities follows almost the distribution of prescriptions per 1,000 inhabitants with higher prescription rates in municipalities with bigger population of elderly inhabitants above 60 years (data not shown).

Figure 5.6 Number of prescriptions in primary health care per 1,000 inhabitants in Danish municipalities in a) 2016 and b) 2023
DANMAP 2023



Data: Registered sale of antimicrobials to individuals
Data source: Register of Medicinal Product Statistics and 2024 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Table 5.1 Consumption of antimicrobial agents for systemic use in primary health care at regional level, Denmark, 2019-2023 DANMAP 2023

Region	Indicator	Year				
		2019	2020	2021	2022	2023
Capital Region	DDD/1,000 inhabitants/day	13.35	12.47	12.32	13.03	14.14
	Prescriptions/1,000 inhabitants	441	382	378	409	443
Region Zealand	DDD/1,000 inhabitants/day	14.34	13.65	13.71	14.31	15.21
	Prescriptions/1,000 inhabitants	482	436	440	466	489
Region of Southern Denmark	DDD/1,000 inhabitants/day	13.15	12.17	12.17	12.91	13.78
	Prescriptions/1,000 inhabitants	455	401	405	434	460
Central Denmark Region	DDD/1,000 inhabitants/day	12.47	11.82	11.83	12.32	13.07
	Prescriptions/1,000 inhabitants	417	374	380	402	425
North Denmark Region	DDD/1,000 inhabitants/day	12.99	12.20	12.42	13.27	14.33
	Prescriptions/1,000 inhabitants	436	390	400	432	458
Denmark (total)	DDD/1,000 inhabitants/day	13.24	12.42	12.40	13.07	14.01
	Prescriptions/1,000 inhabitants	445	393	396	424	451

Data: Registered sale of antimicrobials to individuals
Data source: Register of Medicinal Product Statistics and 2024 edition of the Anatomical Therapeutic Chemical (ATC) classification system

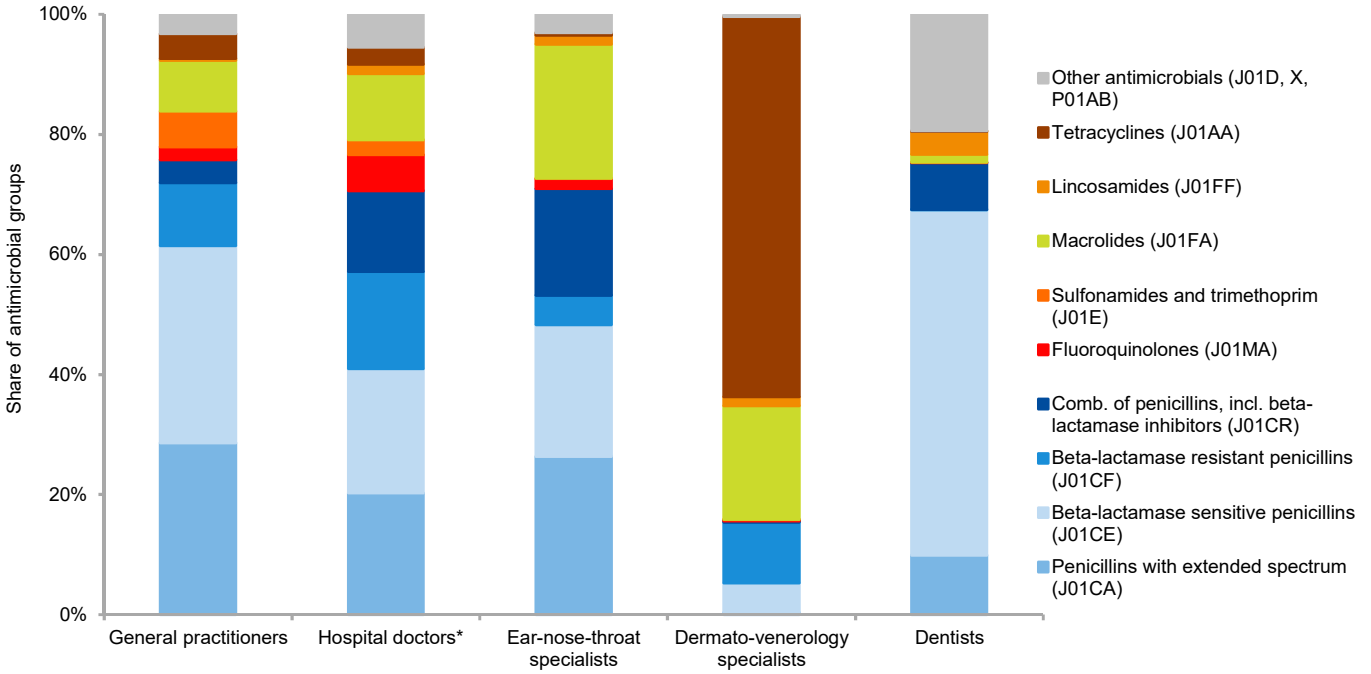
5.3.2 Antimicrobial consumption by prescriber

Prescribing trends in primary health care also clearly differ by prescriber's specialty. Table 5.2 shows an overview of number of prescriptions issued by different specialists, including hospital doctors issuing prescriptions for patients at hospitals, which then are redeemed at a community pharmacy.
Figure 5.7 shows the main antimicrobial groups prescribed by medical specialty in primary health care in 2023. In 2023, 63% of antimicrobial prescriptions from dermato-venerology specialists were tetracyclines, which are indicated for treatment of severe acne and sexually transmitted chlamydia/mycoplasma infections.

Majority of prescriptions by dentists were narrow-spectrum beta-lactamase sensitive penicillins (58%) reflecting adherence to the recommended first-line treatment for common dental infections in primary health care.

General practitioners have access to their own prescribing data through ordiprax+, an online dashboard with personal log-in which visualises prescribing data and enables comparisons with other practices on regional level (DANMAP 2020 Textbox 5.2). Additionally, general practitioners are organized in quality clusters where improving rational antimicrobial prescribing is discussed among other topics.

Figure 5.7 Antimicrobial groups prescribed by main medical specialities in primary health care, Denmark, 2023 DANMAP 2023



* Hospital doctors issuing prescriptions for patients in ambulatory care or upon discharge from hospital
Data: Registered sale of antimicrobials to individuals
Data source: Register of Medicinal Product Statistics and 2024 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Table 5.2 Number of prescriptions per 1,000 inhabitants by main medical specialities, Denmark, 2019-2023 DANMAP 2023

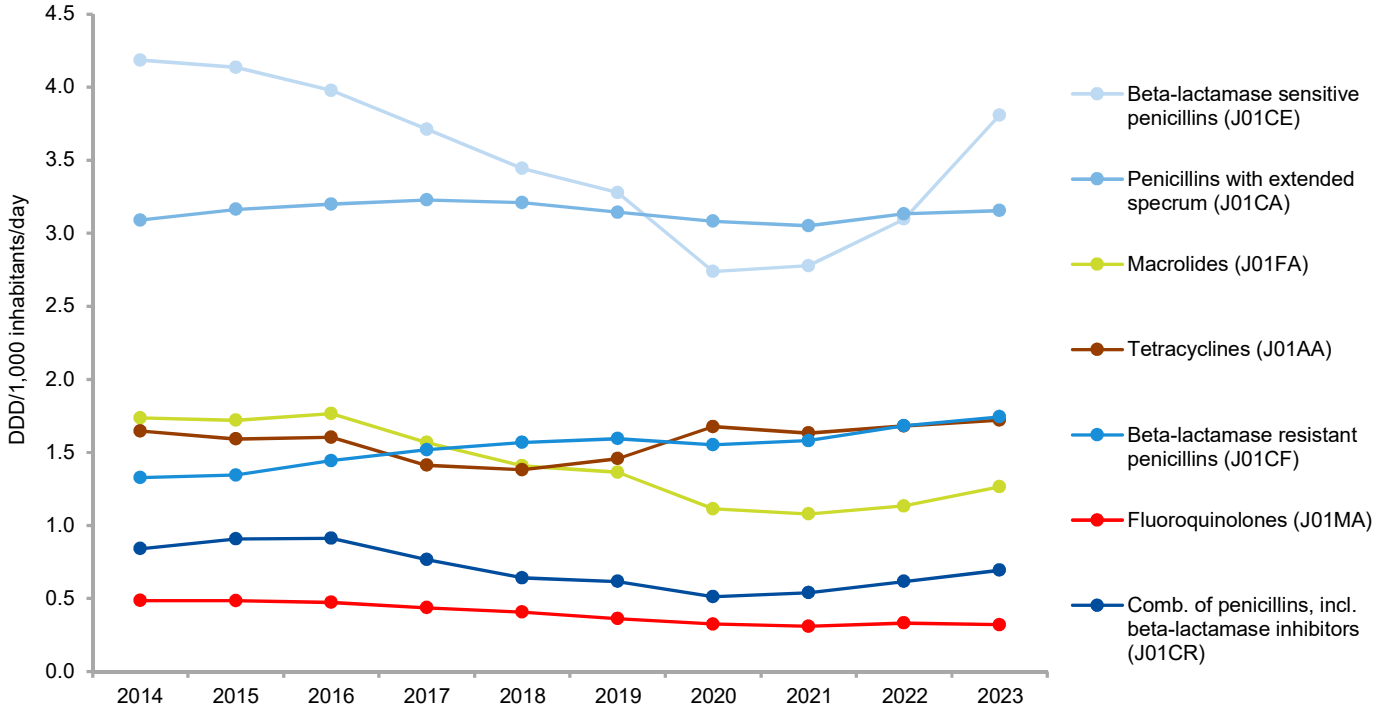
Prescriber		Year				
		2019	2020	2021	2022	2023
General practitioners	Prescriptions per 1,000 inhabitants	327.1	280.4	279.0	300.9	323.1
	DDD per prescription	10.4	11.1	11.0	10.8	10.9
Hospital doctors*	Prescriptions per 1,000 inhabitants	63.2	64.6	63.5	62.0	65.6
	DDD per prescription	12.7	13.0	13.2	13.6	13.7
Ear-nose-throat specialists	Prescriptions per 1,000 inhabitants	7.8	6.1	6.9	8.1	8.5
	DDD per prescription	8.1	8.9	8.3	8.1	8.0
Dermato-venerology specialists	Prescriptions per 1,000 inhabitants	5.4	5.3	5.0	4.6	4.4
	DDD per prescription	33.4	33.8	35.4	35.0	34.6
Dentists	Prescriptions per 1,000 inhabitants	28.8	25.6	28.9	34.4	33.1
	DDD per prescription	7.9	7.9	7.7	7.7	8.2

* Hospital doctors issuing prescriptions for patients in ambulatory care or upon discharge from hospital
Data: Registered sale of antimicrobials to individuals
Data source: Register of Medicinal Product Statistics and 2024 edition of the Anatomical Therapeutic Chemical (ATC) classification system

5.3.3 Consumption of antimicrobial groups

In compliance with treatment guideline, beta-lactamase sensitive penicillins were the most used antimicrobials in primary health care in Denmark for decades (Figure 5.8). In 2023, beta-lactamase sensitive penicillins accounted for 27% of total consumption in primary health care. Altogether the four penicillin groups (penicillins with extended spectrum; beta-lactamase sensitive penicillins; beta-lactamase resistant penicillins; combinations of penicillins, including beta-lactamase inhibitors) accounted for 9.73 DID corresponding to 67% of antimicrobials consumed in primary health care in 2023. Other beta-lactams such as cephalosporins, monobactams and carbapenems were either used at extremely low level or restricted to hospital use only.

Figure 5.8 Consumption of leading antimicrobial groups for systemic use in primary health care, DDD per 1,000 inhabitants per day, Denmark, 2014-2023
DANMAP 2023



Data: Registered sale of antimicrobials to individuals
Data source: Register of Medicinal Product Statistics and 2024 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Table 5.3 Consumption of antimicrobial agents for systemic use in primary health care, DDD per 1,000 inhabitants per day, Denmark, 2004 and 2014-2023
DANMAP 2023

ATC group	Therapeutic group	Year										
		2004	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023
J01AA	Tetracyclines	1.18	1.66	1.60	1.62	1.42	1.40	1.48	1.69	1.64	1.70	1.74
J01CA	Penicillins with extended spectrum	2.28	3.20	3.28	3.33	3.36	3.35	3.28	3.19	3.17	3.26	3.28
J01CE	Beta-lactamase sensitive penicillins	5.25	4.38	4.33	4.16	3.88	3.61	3.44	2.84	2.89	3.23	3.96
J01CF	Beta-lactamase resistant penicillins	0.93	1.36	1.38	1.48	1.56	1.60	1.63	1.58	1.61	1.72	1.78
J01CR	Combinations of penicillins, including beta-lactamase inhibitors	0.04	0.87	0.95	0.95	0.79	0.66	0.63	0.52	0.55	0.63	0.71
J01D	Cephalosporins and other betalactam antibiotics	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.02	0.02
J01EA	Trimethoprim and derivates	0.41	0.55	0.56	0.56	0.56	0.53	0.45	0.43	0.42	0.39	0.41
J01EB	Short-acting sulfonamides	0.36	0.21	0.18	0.16	0.15	0.14	0.13	0.11	0.09	0.09	0.08
J01EE	Combination of sulfonamides and trimethoprim, including derivates	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01
J01FA	Macrolides	2.25	1.79	1.77	1.82	1.62	1.46	1.41	1.15	1.11	1.17	1.30
J01FF	Lincosamides	0.01	0.05	0.05	0.06	0.06	0.06	0.06	0.07	0.07	0.07	0.08
J01GB	Aminoglycosides	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
J01MA	Fluroquinolones	0.29	0.50	0.49	0.48	0.44	0.41	0.37	0.33	0.32	0.34	0.33
J01XC	Steroid antibacterials (combination fusidic acid)	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00
J01XE	Nitrofuran derivates (nitrofurantoin)	0.43	0.48	0.45	0.43	0.26	0.15	0.27	0.27	0.28	0.27	0.12
J01XX	Other antibacterials (metheamine >99%)	0.30	0.24	0.25	0.27	0.28	0.29	0.32	0.34	0.39	0.42	0.47
J01XD and P01AB01	Nitroimidazole derivates (metronidazole)	0.20	0.28	0.28	0.28	0.25	0.24	0.24	0.23	0.24	0.24	0.25
J01 and P01AB01	Antimicrobial agents for systemic use (total)	14.00	15.64	15.66	15.67	14.71	13.97	13.77	12.83	12.86	13.59	14.56

Data: Registered sale of antimicrobials to individuals
Data source: Register of Medicinal Product Statistics and 2024 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Table 5.4 Number of treated patients per 1,000 inhabitants for leading antimicrobial agents in primary health care, Denmark, 2004 and 2014-2023

DANMAP 2023

ATC group	Therapeutic group	Year										
		2004	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023
J01AA	Tetracyclines	11.60	12.20	11.32	11.04	10.35	9.69	10.10	14.43	12.99	13.64	14.06
J01CA	Penicillins with extended spectrum	70.56	75.32	74.87	74.05	74.04	73.56	71.97	67.14	68.60	71.45	72.42
J01CE	Beta-lactamase sensitive penicillins	171.23	134.79	130.06	125.69	119.32	110.90	104.70	84.93	87.69	100.09	116.67
J01CF	Beta-lactamase resistant penicillins	27.11	29.24	28.85	29.70	29.96	31.10	31.06	30.52	30.89	32.92	33.77
J01CR	Combinations of penicillins, including betalctamase inhibitors	1.28	20.52	22.03	22.17	19.89	17.73	17.33	14.43	15.50	17.90	20.28
J01E	Sulphonamides and trimethoprim	36.39	24.65	22.45	21.17	19.87	18.42	16.63	15.04	13.66	12.67	12.47
J01FA	Macrolides	65.89	51.38	51.75	53.21	46.01	40.11	38.45	25.13	24.97	27.16	30.06
J01MA	Fluoroquinolones	10.83	15.30	15.04	14.37	13.36	12.26	10.74	9.01	8.52	9.10	8.87
J01X	Other antibacterials (methenamine >99%)	7.10	7.16	7.35	7.47	5.01	3.62	5.66	5.80	5.95	5.91	2.65
P01AB01	Nitroimidazole derivatives (metronidazole)	12.58	16.31	16.47	16.03	14.84	14.05	13.57	13.36	13.77	13.94	14.11
J01 and P01AB01	Antimicrobial agents for systemic use (total)	306.28	278.62	273.49	269.72	255.72	242.55	234.34	205.27	207.85	224.57	242.07

Data: Registered sale of antimicrobials to individuals
Data source: Register of Medicinal Product Statistics and 2024 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Table 5.5 Number of prescriptions per 1,000 inhabitants for leading antimicrobial agents in primary health care, Denmark, 2004 and 2014-2023

DANMAP 2023

ATC group	Therapeutic group	Year										
		2004	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023
J01AA	Tetracyclines	19.21	20.00	17.90	17.18	15.89	14.63	15.11	20.19	18.25	18.71	19.10
J01CA	Penicillins with extended spectrum	102.84	113.83	113.53	113.16	114.37	114.31	112.19	105.93	107.97	112.19	114.04
J01CE	Beta-lactamase sensitive penicillins	226.56	170.70	163.09	157.13	148.52	136.81	128.77	104.07	107.28	122.87	145.45
J01CF	Beta-lactamase resistant penicillins	38.66	41.04	40.81	41.87	41.87	43.35	43.16	42.87	43.17	45.66	47.05
J01CR	Combinations of penicillins, including betalctamase inhibitors	1.95	29.02	30.73	31.13	27.09	23.71	23.07	19.14	20.36	23.45	26.32
J01E	Sulphonamides and trimethoprim	55.02	41.51	38.39	36.41	34.29	31.74	28.14	25.59	23.07	21.26	21.30
J01FA	Macrolides	90.05	68.01	68.00	68.85	60.00	52.64	50.71	33.66	33.80	36.94	40.32
J01MA	Fluoroquinolones	14.16	19.67	19.50	18.74	17.37	15.97	13.99	12.07	11.41	11.96	11.57
J01X	Other antibacterials (methenamine >99%)	15.08	16.73	16.28	15.82	10.18	6.76	10.29	10.62	10.70	10.72	5.47
P01AB01	Nitroimidazole derivatives (metronidazole)	14.72	19.06	19.15	18.63	17.26	16.31	15.78	15.62	16.00	16.17	16.25
J01 and P01AB01	Antimicrobial agents for systemic use (total)	579.54	542.53	530.56	522.19	490.08	459.39	444.53	393.34	395.76	423.70	451.05

Data: Registered sale of antimicrobials to individuals
Data source: Register of Medicinal Product Statistics and 2024 edition of the Anatomical Therapeutic Chemical (ATC) classification system

5.3.4 Antimicrobial consumption by patient case mix

Antimicrobial consumption is highly affected by patient case mix. The need for antimicrobials is different throughout life and for the two genders. Antimicrobial consumption is also affected by other sociodemographic factors (DANMAP 2022, Textbox 5.2). Figure 5.9a-c presents consumption of antimicrobials by age group based on different denominators: Figure 5.9a presents consumption in DDD per 1,000 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 1,000 inhabitants. Figure 5.9d presents population size by age group. Children and adolescents are presented in five-year age groups, while adults are clustered in 10-year age groups.

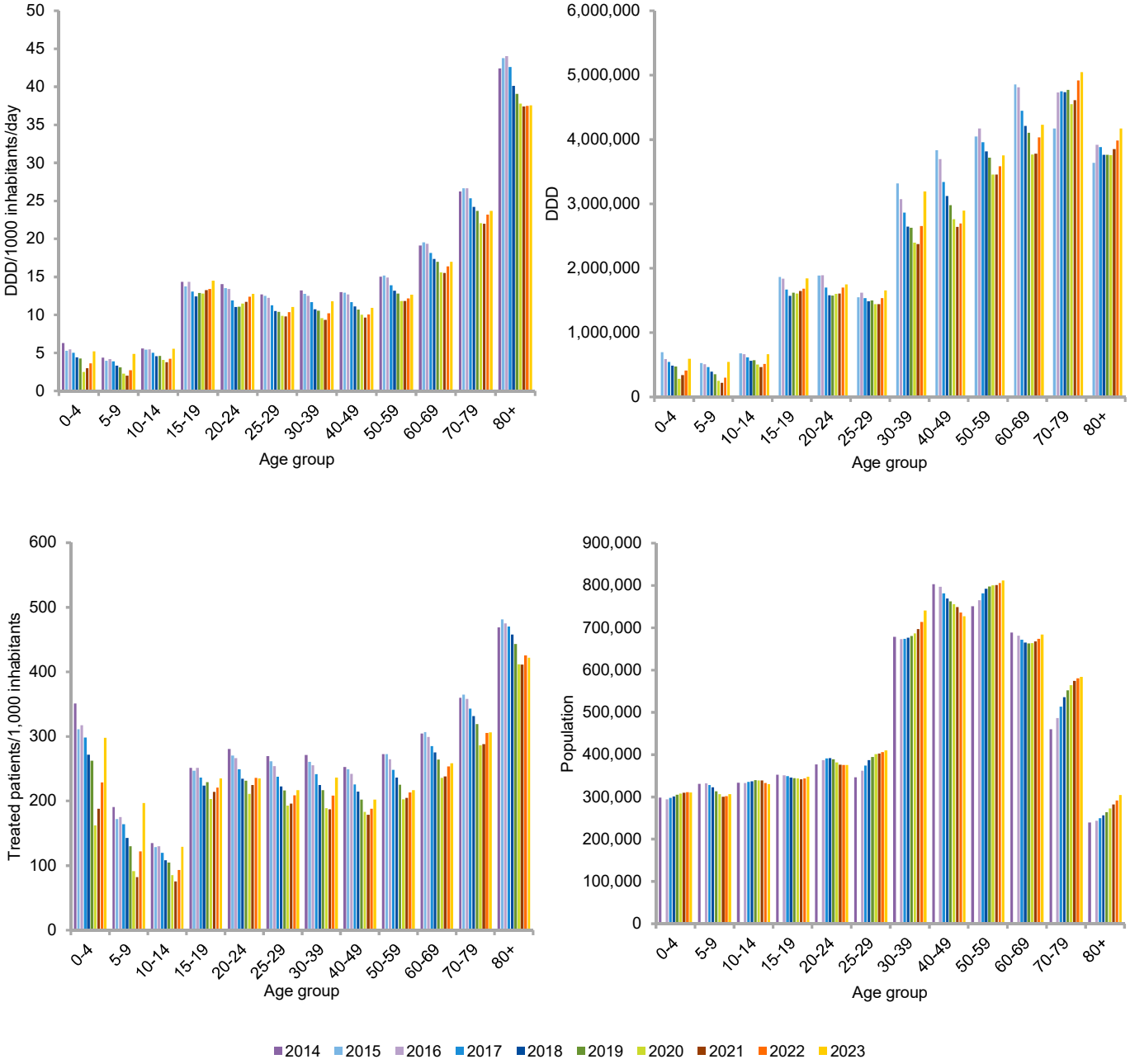
Estimates of antimicrobial consumption for children using DDD need to be interpreted with caution since the DDD is defined as "maintenance dose per day for its main indication in adults". The maintenance dose per day for children may differ from the one for adults due to different pharmacodynamics and -kinetics. Furthermore, infants and young children in the same age group might be treated with different doses based on body weight. Therefore, other units of measurement might be more suitable to monitor consumption in children, e.g. number of treated patients per 1,000 inhabitants and number of prescriptions per 1,000 inhabitants.

In 2023, 242 patients per 1,000 inhabitants were treated with antimicrobials, receiving 451 prescriptions per 1,000 inhabitants. In 2022, the corresponding numbers were approximately 6-8% lower, 225 treated patients and 424 prescriptions per 1,000 inhabitants. Since 2014, the consumption decreased from 279 treated patients per 1,000 inhabitants and 543 prescriptions per 1,000 inhabitants (reduction by 13% and 17% from 2014 to 2023, respectively).

Macrolides play an important role in the treatment of bacterial respiratory tract infections in children and adolescents (Figure 5.10). Macrolides were also used as first-line treatment for chlamydia infections until the change in guidance (already described in DANMAP2020) which may be the reason for the relatively high consumption of macrolides in the 15-24 year olds. However, penicillins are the most used antimicrobial agents for children and adolescents, constituting between 47% and 89% of all antimicrobials prescribed depending on age group (Figure 5.10).

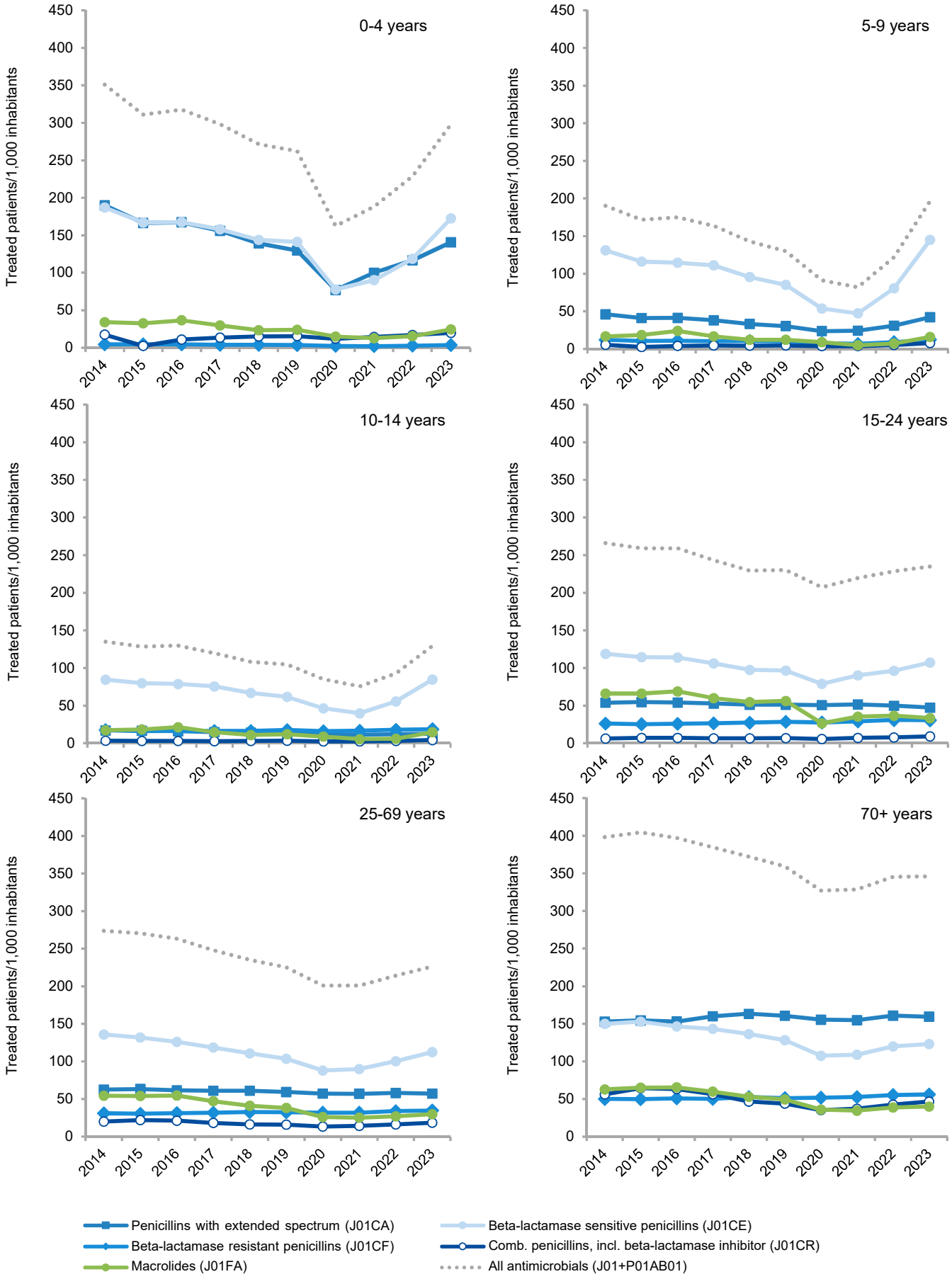
Differences in antimicrobial consumption between genders are well known. In 2023, the number of treated females (all age groups) was 282 per 1,000 inhabitants and the number of treated males was 202 per 1,000 inhabitants. In general, females receive more treatment – a trend driven by higher incidence of urinary tract infections and different healthcare-seeking behavior. Thus, the consumption of pivmecillinam, sulfonamides, trimethoprim and nitrofurantoin, all indicated for treatment of urinary tract infections, is approximately three times higher for females than for males (Figure 5.11). The reduction in consumption of these antimicrobials was primary driven by fewer prescriptions for elderly women (80+ years), who were the most frequently treated (578 prescriptions per 1,000 females above 80 years).

Figure 5.9 Consumption of systemic antimicrobial agents in primary health care by age group, measured in a) DDD per 1,000 inhabitants per day, b) DDD, c) treated patients per 1,000 inhabitants and d) population size, Denmark, 2014-2023 DANMAP 2023



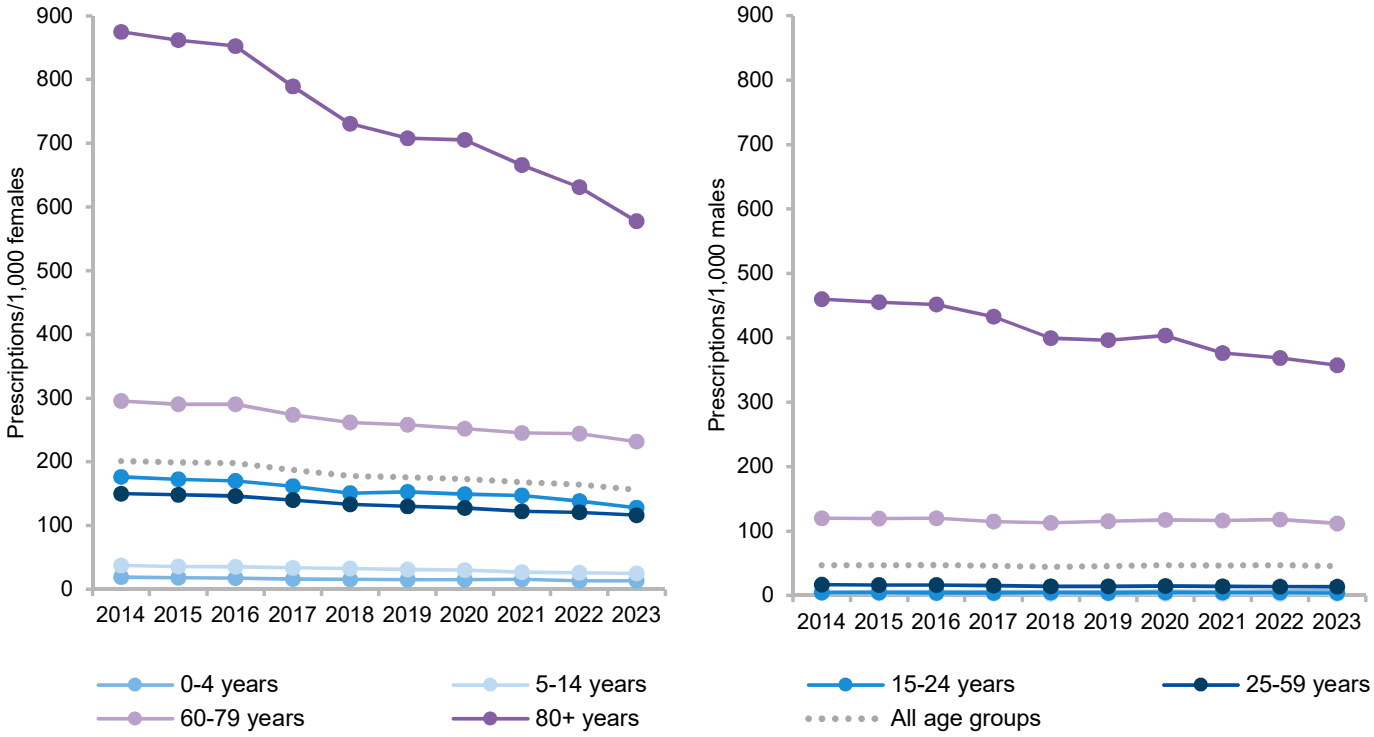
Data: Registered sale of antimicrobials to individuals
Data source: Register of Medicinal Product Statistics, 2024 edition of the Anatomical Therapeutic Chemical (ATC) classification system and Statistics Denmark

Figure 5.10 Consumption of main antimicrobial agents by age group, treated patients/1,000 inhabitants, Denmark, 2014-2023 DANMAP 2023



Data: Registered sale of antimicrobials to individuals
Data source: Register of Medicinal Product Statistics and 2024 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 5.11 Consumption of antimicrobials primarily used for treatment of urinary tract infections* in primary health care for a) females and b) males, prescriptions per 1,000 inhabitants, Denmark, 2014-2023 DANMAP 2023



* Pivmecillinam, sulfonamides, trimethoprim and nitrofurantoin
Data: Registered sale of antimicrobials to individuals
Data source: Register of Medicinal Product Statistics and 2024 edition of the Anatomical Therapeutic Chemical (ATC) classification system

5.3.5 Antimicrobial consumption for treatment of respiratory tract infections

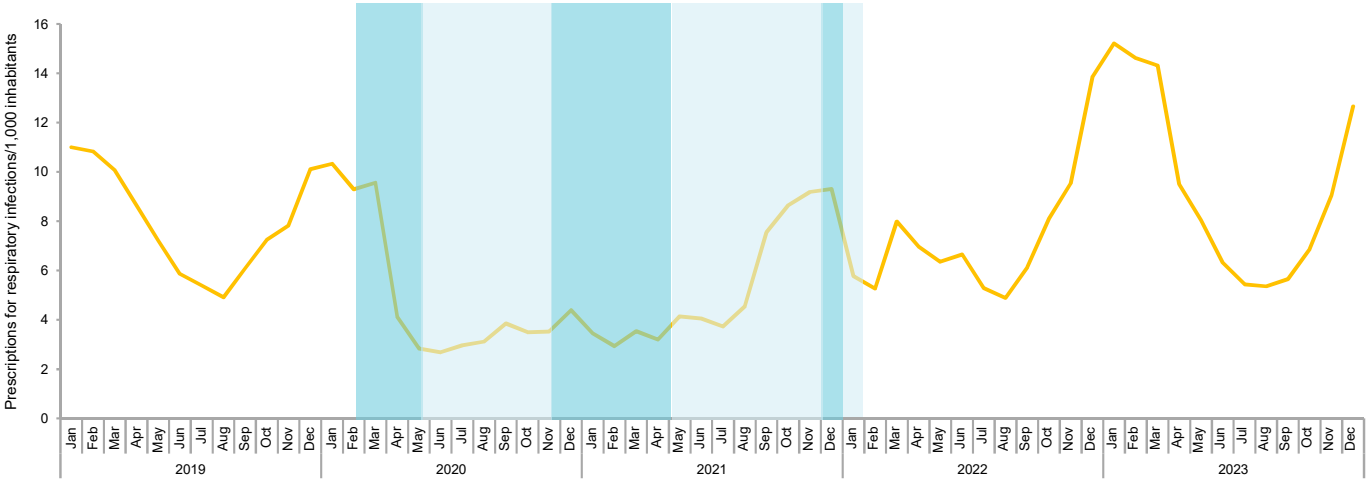
One of the main indications provided by prescribers in primary health care for treatment with antimicrobials is upper and/or lower respiratory tract infections. In 2020, consumption of antimicrobials prescribed for treatment of respiratory tract infections started slightly lower compared to previous years, and was followed by a sharp drop in consumption from April 2020 to July 2021 (Figure 5.12). This coincided with a sharp decrease in number of laboratory confirmed influenza and RSV infections, most likely due to the societal restriction implemented in March 2020 due to the COVID-19 pandemic. However, from August 2021 the consumption went back to levels similar to the corresponding pre-pandemic months in 2019, again coinciding with the Respiratory Syncytial Virus (RSV) summer epidemic in 2021. Antimicrobial consumption during the winter 2022-2023 reached a higher level than observed in 2018-2019. This coincided with an early RSV and influenza season as well as an outbreak of Group A streptococci, as also observed in other European countries.

5.3.6 Antimicrobial consumption for elderly inhabitants

One of the recent surveillance approaches added to the DANMAP program is surveillance of antimicrobial consumption in elderly inhabitants aged 65 years and above. Close surveillance of antimicrobial consumption in this population is necessary as it is one of the most fragile populations in society. Surveillance contributes to high quality treatment of infections and thereby prevents emergence of antimicrobial resistant pathogens. The surveillance is based on the Danish Care Home Register and Danish Civil Registry. By combining these registries, it is possible to divide elderly inhabitants into two populations; elderly inhabitants living in their own homes and elderly inhabitants living at long term care facilities.

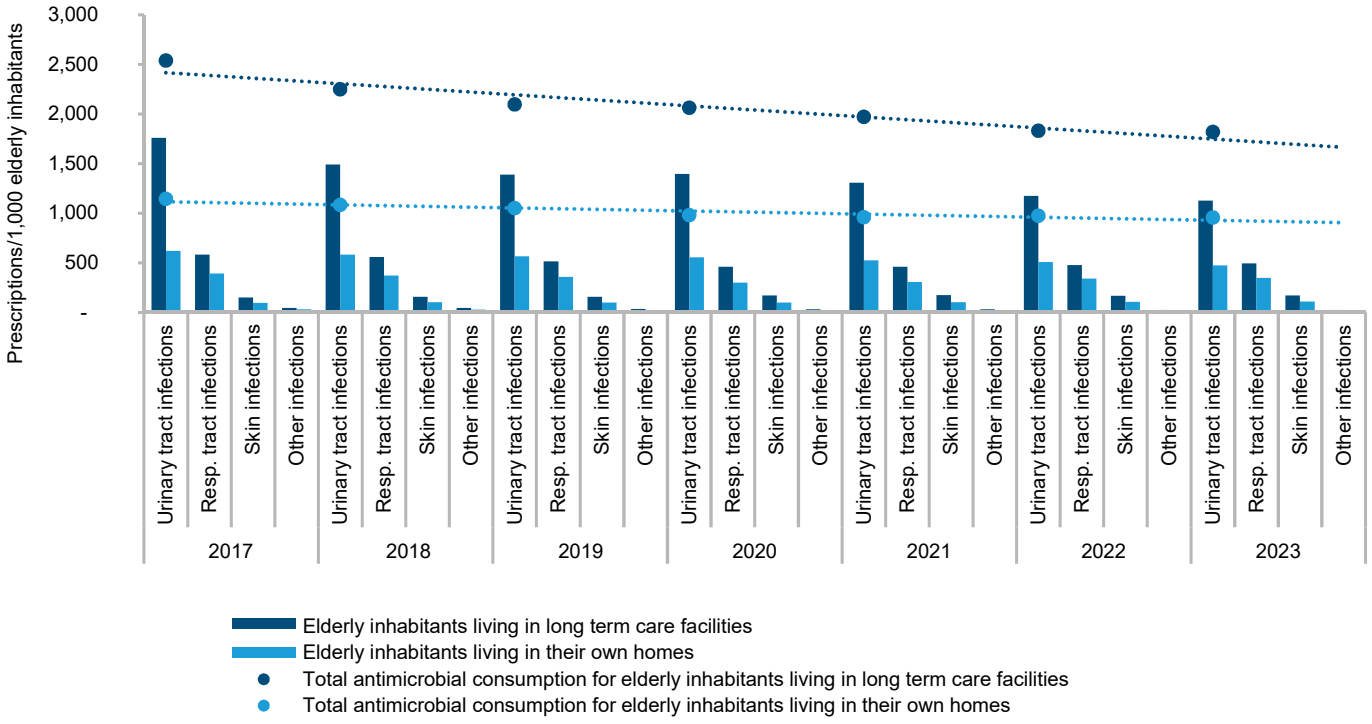
Figure 5.13 shows antimicrobial consumption for elderly inhabitants aged 65 years and above in 2017-2023. Elderly inhabitants living at care homes received 90% more antimicrobials than elderly inhabitants living in their own homes in 2023. The figure also compares treatment of specific infections in the two populations as it is well known that treatment of urinary tract infections is the main cause of the difference observed in the treatment frequency of the two populations of elderly inhabitants. These differences in treatment of elderly inhabitants are observed despite a continuous decrease in the antimicrobial consumption for elderly inhabitants living at long term care facilities.

Figure 5.12 Monthly antimicrobial prescriptions indicated for treatment of respiratory tract infections in primary health care, prescriptions per 1,000 inhabitants, Denmark, 2019-2023 DANMAP 2023



COVID-19 restrictions in place
Fewer restrictions in place
Data: Registered sale of antimicrobials to individuals
Data source: Register of Medicinal Product Statistics and 2024 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 5.13 Consumption of antimicrobials (J01 and P01AB01) in primary health care for elderly inhabitants living in long term care facilities and for elderly inhabitants living in their own homes, Denmark, 2017-2023 DANMAP 2023



Elderly inhabitants living in long term care facilities
Elderly inhabitants living in their own homes
Total antimicrobial consumption for elderly inhabitants living in long term care facilities
Total antimicrobial consumption for elderly inhabitants living in their own homes

Data: Registered sale of antimicrobials to individuals
Data source: Register of Medicinal Product Statistics, 2024 edition of the Anatomical Therapeutic Chemical (ATC) classification system, Care Home Register and Danish Civil Registry

5.4 Antimicrobial consumption in hospital care

Surveillance of antimicrobial consumption in hospital care is based on sale of systemic antimicrobials (ATC code J01, P01AB01 and A07AA09) from Danish hospital pharmacies to hospitals, excluding private hospitals and psychiatric departments (approximately 2-3% of the total hospital consumption). Antimicrobial consumption data are presented as DDD per 100 occupied bed-days (DBD) and per 100 admissions (DAD) to account for hospital activity.

Hospital activity has changed over the years with decreasing number of bed-days and admissions, but with different rates (Figure 5.14). Earlier discharge of patients, increasing ambulatory care functions in the community as well as in care homes and changed workflow due to new electronic systems all affect the activity and need to be considered when interpreting antimicrobial consumption trends in hospitals (see Table 2.1 in Chapter 2 'Introduction').

Information on consumption at patient level is currently not available to DANMAP for the hospital sector. This information is expected to become available to DANMAP through the "Hospital Medicine Register" in coming years.

5.4.1 Antimicrobial consumption at public somatic hospitals accounting for hospital activity

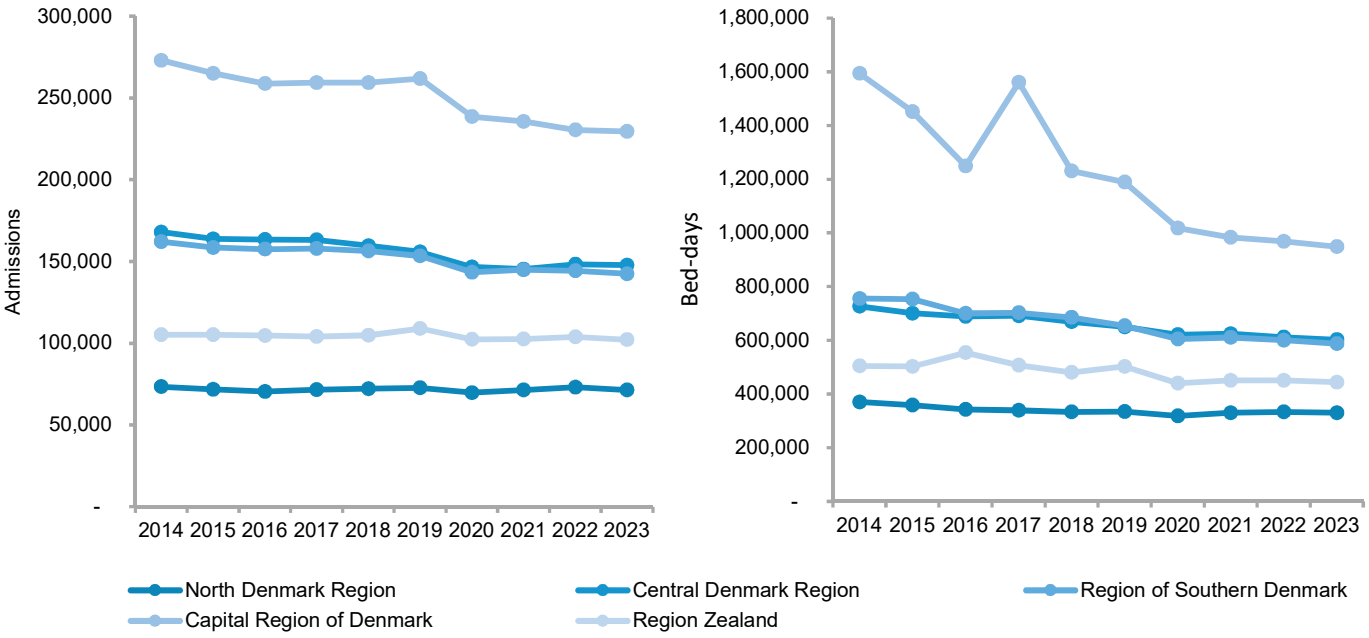
In 2023, the consumption of antimicrobial agents at somatic hospitals was 135.6 DBD. This is 5.6% higher than in 2022 (128.42 DBD) and 36% higher than a decade ago (99.44 DBD in 2014) (Table 5.6).

The four penicillin groups (penicillins with extended spectrum, beta-lactamase sensitive penicillins, beta-lactamase resistant penicillins and combinations of penicillins, including beta-lactamase inhibitors) accounted for 77.12 DBD, corresponding to 57% of the total consumption of antimicrobials at somatic hospitals in Denmark in 2023. The main group of antimicrobials in 2023; combinations of penicillins, including beta-lactamase inhibitors increased by 89.5% since 2014.

Linezolid consumption has increased to 0.65 DBD in 2023 which is the highest level observed the last decade. Over the past decade, the consumption of linezolid increased by 77% (0.37 DBD in 2014). Consumption of daptomycin peaked in 2018 (0.17 DBD), and has since been fluctuating over the years reaching 0.12 DBD in 2023 (Table 5.6). Although the overall consumption of both antimicrobials is low, these changes are of concern since both are reserved for treatment of serious infections caused by vancomycin-resistant enterococci (VRE) or methicillin-resistant *Staphylococcus aureus*.

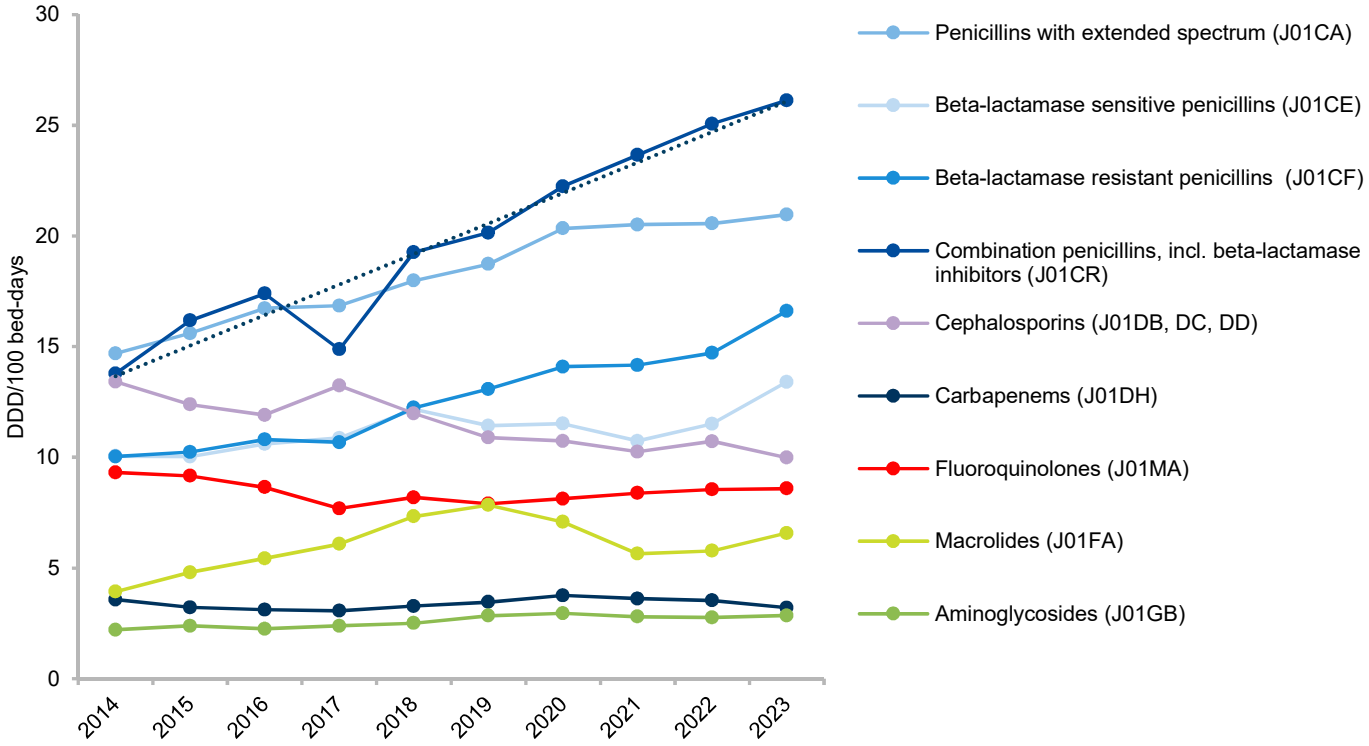
The consumption of antimicrobials at hospitals can also be measured in relation to the number of patients being admitted, i.e. DDD per 100 admissions (DAD) (Table 5.7).

Figure 5.14 Activity at somatic hospitals, bed-days and admissions, Denmark, 2014-2023 DANMAP 2023



Data source: The National Patient Register

Figure 5.15 Consumption of leading groups of antimicrobial agents at somatic hospitals, DDD per 100 bed-days, Denmark, 2014-2023
DANMAP 2023



Data: Antimicrobial consumption at somatic hospitals
Data source: Register of Medicinal Product Statistics, 2024 edition of the Anatomical Therapeutic Chemical (ATC) classification system and The National Patient Register

Table 5.6 Consumption of antimicrobial agents for systemic use in somatic hospitals, DDD per 100 bed-days, Denmark, 2014-2023

DANMAP 2023

ATC group	Therapeutic group	Year									
		2014	2015	2016	2017	2018	2019	2020	2021	2022	2023
J01AA	Tetracyclines	1.78	2.00	2.42	2.18	2.78	3.67	3.13	3.25	3.53	4.00
J01CA	Penicillins with extended spectrum	14.69	15.60	16.73	16.85	17.98	18.73	20.34	20.51	20.56	20.97
J01CE	Beta-lactamase sensitive penicillins	10.05	10.03	10.60	10.87	12.17	11.42	11.52	10.74	11.51	13.41
J01CF	Beta-lactamase resistant penicillins	10.03	10.24	10.80	10.67	12.24	13.08	14.09	14.17	14.72	16.61
J01CR	Comb. of penicillins. incl. beta-lactamase inhibitors	13.79	16.18	17.40	14.87	19.27	20.15	22.24	23.66	25.07	26.13
J01DB	1st generation cephalosporins	0.07	0.05	0.05	0.04	0.04	0.03	0.04	0.03	0.03	0.03
J01DC	2nd generation cephalosporins	12.27	11.19	10.67	11.77	10.53	9.47	9.32	8.84	9.16	8.50
J01DD	3rd generation cephalosporins	1.08	1.15	1.19	1.42	1.40	1.39	1.38	1.38	1.53	1.46
J01DF	Monobactams	0.07	0.03	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01
J01DH	Carbapenems	3.57	3.22	3.12	3.06	3.27	3.46	3.76	3.61	3.53	3.20
J01EA	Trimethoprim and derivatives	0.50	0.44	0.43	0.44	0.51	0.47	0.52	0.49	0.46	0.43
J01EB	Short-acting sulfonamides	0.15	0.13	0.12	0.11	0.12	0.10	0.07	0.07	0.06	0.04
J01EE	Comb. of sulfonamides and trimethoprim. incl. derivatives	5.22	5.76	6.19	5.97	7.01	7.77	8.42	9.29	9.62	10.64
J01FA	Macrolides	3.93	4.80	5.43	6.08	7.33	7.84	7.08	5.64	5.78	6.57
J01FF	Lincosamides	0.70	0.63	0.72	0.69	0.89	0.86	0.83	0.79	0.82	1.51
J01GB	Aminoglycosides	2.21	2.38	2.25	2.38	2.51	2.85	2.95	2.80	2.76	2.85
J01MA	Fluoroquinolones	9.31	9.16	8.65	7.69	8.19	7.90	8.13	8.38	8.54	8.59
J01XA	Glycopeptides	1.24	1.28	1.25	1.40	1.48	1.56	1.73	1.74	1.70	1.58
J01XB	Polymyxins	0.24	0.21	0.22	0.21	0.27	0.26	0.28	0.27	0.28	0.34
J01XC	Steroid antibacterials (fusidic acid)	0.25	0.18	0.13	0.07	0.07	0.07	0.06	0.07	0.05	0.05
J01XD	Imidazole derivatives	4.77	4.66	5.21	4.96	5.06	4.79	4.94	4.57	4.45	4.34
J01XE	Nitrofuran derivatives (nitrofurantoin)	0.34	0.30	0.27	0.27	0.31	0.33	0.40	0.36	0.37	0.44
J01XX05	Methenamine	0.06	0.10	0.09	0.08	0.12	0.09	0.10	0.13	0.13	0.15
J01XX08	Linezolid	0.37	0.48	0.42	0.40	0.61	0.62	0.57	0.58	0.65	0.65
J01XX09	Daptomycin	0.06	0.04	0.06	0.09	0.17	0.08	0.11	0.14	0.13	0.12
P01AB01	Nitroimidazole derivatives (metronidazole)	2.13	2.21	2.52	2.17	2.28	2.23	2.30	2.22	2.18	2.17
A07AA09	Intestinal antiinfectives (vancomycin)	0.56	0.52	0.56	0.55	0.58	0.64	0.77	0.67	0.79	0.83
J01, P01AB01, A07AA09	Antimicrobial agents for systemic use, incl. metronidazole and vancomycin	99.44	102.96	107.53	105.29	117.20	119.85	125.11	124.42	128.42	135.60

Data: Antimicrobial consumption at somatic hospitals
Data source: Register of Medicinal Product Statistics, 2024 edition of the Anatomical Therapeutic Chemical (ATC) classification system and The National Patient Register

Table 5.7 Consumption of antimicrobial agents for systemic use in somatic hospitals, DDD per 100 admissions, Denmark, 2014-2023
DANMAP 2023

ATC group	Therapeutic group	Year									
		2014	2015	2016	2017	2018	2019	2020	2021	2022	2023
J01AA	Tetracyclines	9.00	9.86	11.35	10.96	12.54	16.22	13.43	13.92	14.96	16.78
J01CA	Penicillins with extended spectrum	74.25	76.87	78.34	84.73	81.26	82.84	87.16	87.91	87.08	88.07
J01CE	Beta-lactamase sensitive penicillins	50.80	49.41	49.65	54.65	54.99	50.53	49.36	46.03	48.75	56.31
J01CF	Beta-lactamase resistant penicillins	50.72	50.45	50.58	53.68	55.28	57.87	60.39	60.71	62.33	69.77
J01CR	Comb. of penicillins. incl. beta-lactamase inhibitors	69.68	79.72	81.46	74.80	87.05	89.12	95.31	101.38	106.18	109.73
J01DB	1st generation cephalosporins	0.34	0.24	0.23	0.22	0.20	0.14	0.16	0.15	0.14	0.11
J01DC	2nd generation cephalosporins	62.01	55.14	49.97	59.19	47.58	41.87	39.91	37.88	38.80	35.69
J01DD	3rd generation cephalosporins	5.45	5.65	5.57	7.13	6.34	6.14	5.92	5.91	6.46	6.13
J01DF	Monobactams	0.35	0.15	0.06	0.04	0.03	0.05	0.04	0.03	0.06	0.03
J01DH	Carbapenems	18.02	15.84	14.60	15.41	14.79	15.29	16.12	15.47	14.96	13.45
J01EA	Trimethoprim and derivatives	2.55	2.16	2.02	2.22	2.31	2.06	2.23	2.09	1.94	1.81
J01EB	Short-acting sulfonamides	0.78	0.65	0.55	0.55	0.53	0.45	0.32	0.31	0.25	0.19
J01EE	Comb. of sulfonamides and trimethoprim. incl. derivatives	26.38	28.40	29.00	30.01	31.66	34.37	36.10	39.82	40.76	44.69
J01FA	Macrolides	19.86	23.67	25.42	30.59	33.12	34.69	30.32	24.18	24.47	27.60
J01FF	Lincosamides	3.53	3.10	3.38	3.46	4.03	3.82	3.57	3.39	3.46	6.32
J01GB	Aminoglycosides	11.15	11.74	10.54	11.96	11.34	12.58	12.63	11.99	11.69	11.97
J01MA	Fluoroquinolones	47.07	45.14	40.51	38.65	36.99	34.94	34.83	35.92	36.18	36.08
J01XA	Glycopeptides	6.29	6.29	5.87	7.03	6.70	6.89	7.42	7.47	7.21	6.64
J01XB	Polymyxins	1.22	1.05	1.05	1.03	1.20	1.14	1.18	1.17	1.20	1.42
J01XC	Steroid antibacterials (fusidic acid)	1.25	0.89	0.62	0.36	0.33	0.29	0.26	0.29	0.22	0.23
J01XD	Imidazole derivatives	24.13	22.94	24.42	24.92	22.88	21.19	21.15	19.57	18.83	18.24
J01XE	Nitrofuran derivatives (nitrofurantoin)	1.72	1.46	1.28	1.36	1.42	1.45	1.73	1.53	1.55	1.83
J01XX05	Methenamine	0.30	0.48	0.43	0.38	0.55	0.41	0.45	0.56	0.57	0.61
J01XX08	Linezolid	1.85	2.38	1.97	1.99	2.76	2.74	2.43	2.49	2.74	2.72
J01XX09	Daptomycin	0.30	0.21	0.27	0.44	0.75	0.33	0.48	0.61	0.55	0.52
P01AB01	Nitroimidazole derivatives (metronidazole)	10.77	10.91	11.80	10.92	10.28	9.88	9.87	9.51	9.21	9.09
A07AA09	Intestinal antiinfectives (vancomycin)	2.84	2.55	2.63	2.79	2.62	2.81	3.30	2.88	3.33	3.47
J01, P01AB01, A07AA09	Antimicrobial agents for systemic use, incl. metronidazole and vancomycin	502.61	507.35	503.57	529.46	529.52	530.10	536.06	533.19	543.88	569.52

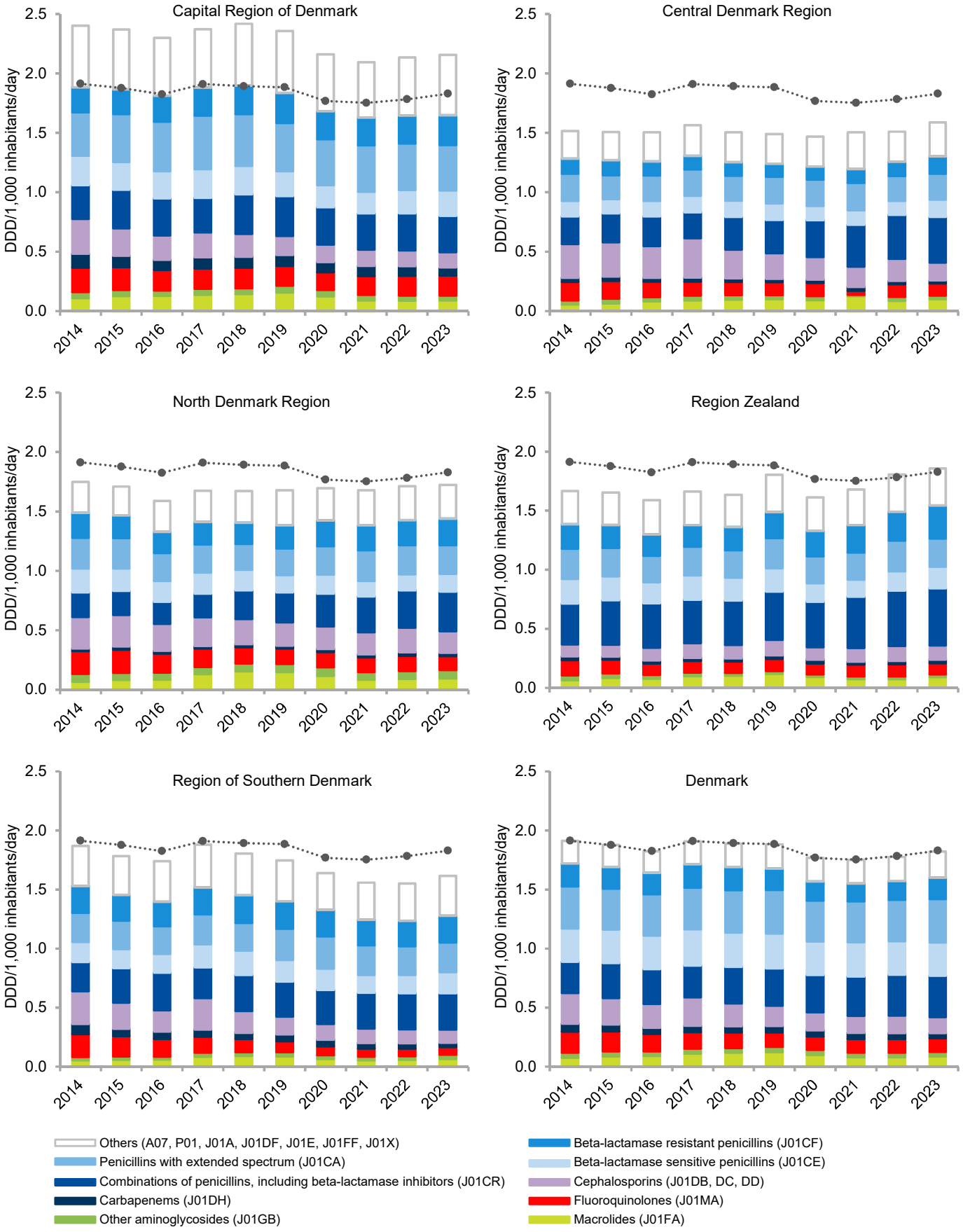
Data: Antimicrobial consumption at somatic hospitals
Data source: Register of Medicinal Product Statistics, 2024 edition of the Anatomical Therapeutic Chemical (ATC) classification system and The National Patient Register

5.4.2 Antimicrobial consumption at regional level at public somatic hospitals

Trends in hospital consumption at regional level measured in DDD per 100 bed-days are presented in Figure 5.17. The Capital Region of Denmark shows the highest level of consumption when compared to the other regions in 2023. It is also notable that consumption increased for each region the last decade when measured in DBD (Figure 5.17) but remains almost unchanged over the same period when measured in DID (Figure 5.16). This reflects that hospital activity changes during the years and more antimicrobials were used in relation to hospital patients' bed-days.

Figure 5.16 Consumption of antimicrobial agents for systemic use at hospitals in the five health regions, DDD per 1,000 inhabitants per day, Denmark, 2014-2023

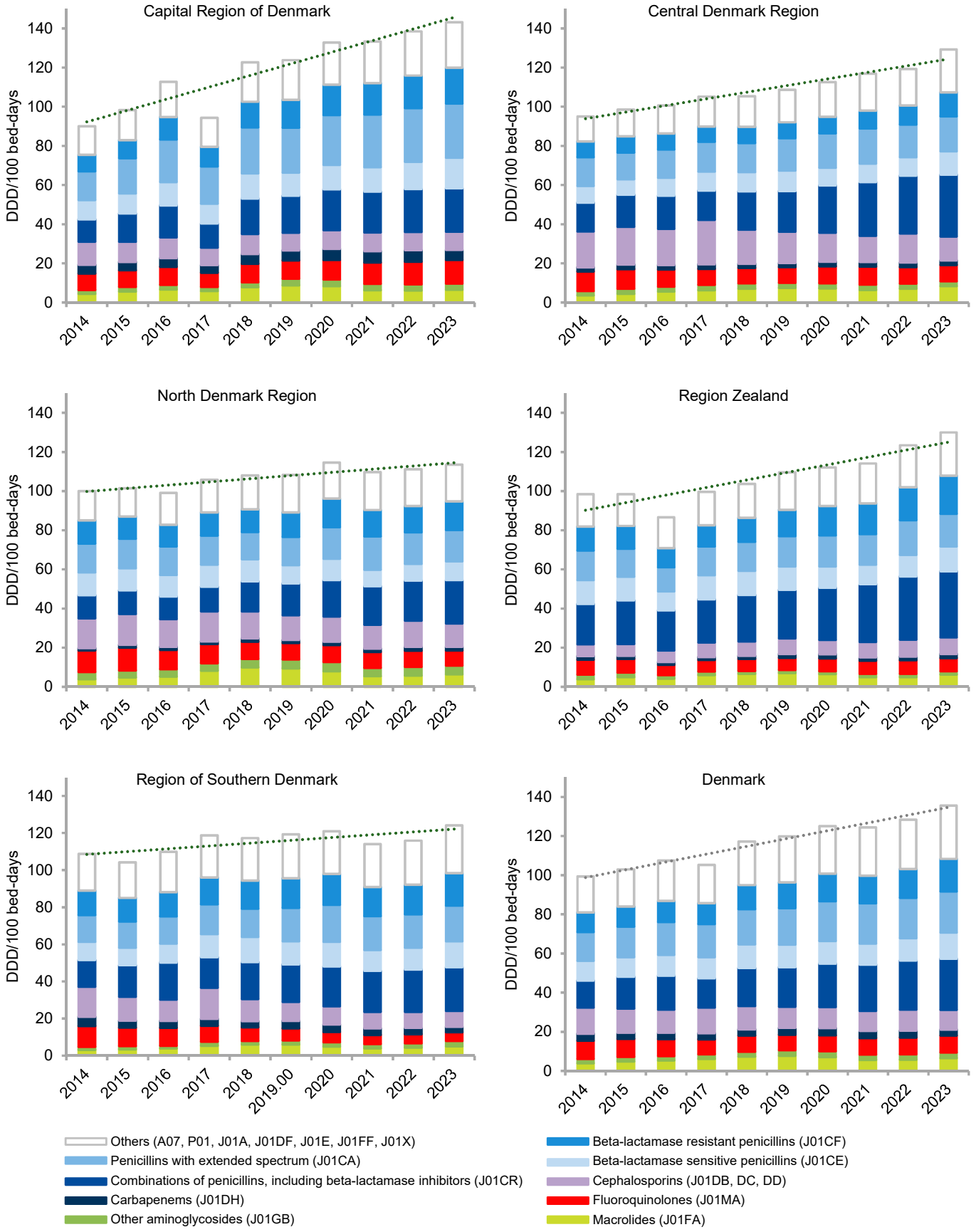
DANMAP 2023



Data: Antimicrobial consumption at somatic hospitals
Data source: Register of Medicinal Product Statistics, 2024 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 5.17 Consumption of antimicrobial agents for systemic use at hospitals in the five health regions, DDD per 100 bed-days, Denmark, 2014-2023

DANMAP 2023



Data: Antimicrobial consumption at somatic hospitals
Data source: Register of Medicinal Product Statistics, 2024 edition of the Anatomical Therapeutic Chemical (ATC) classification system and The National Patient Register

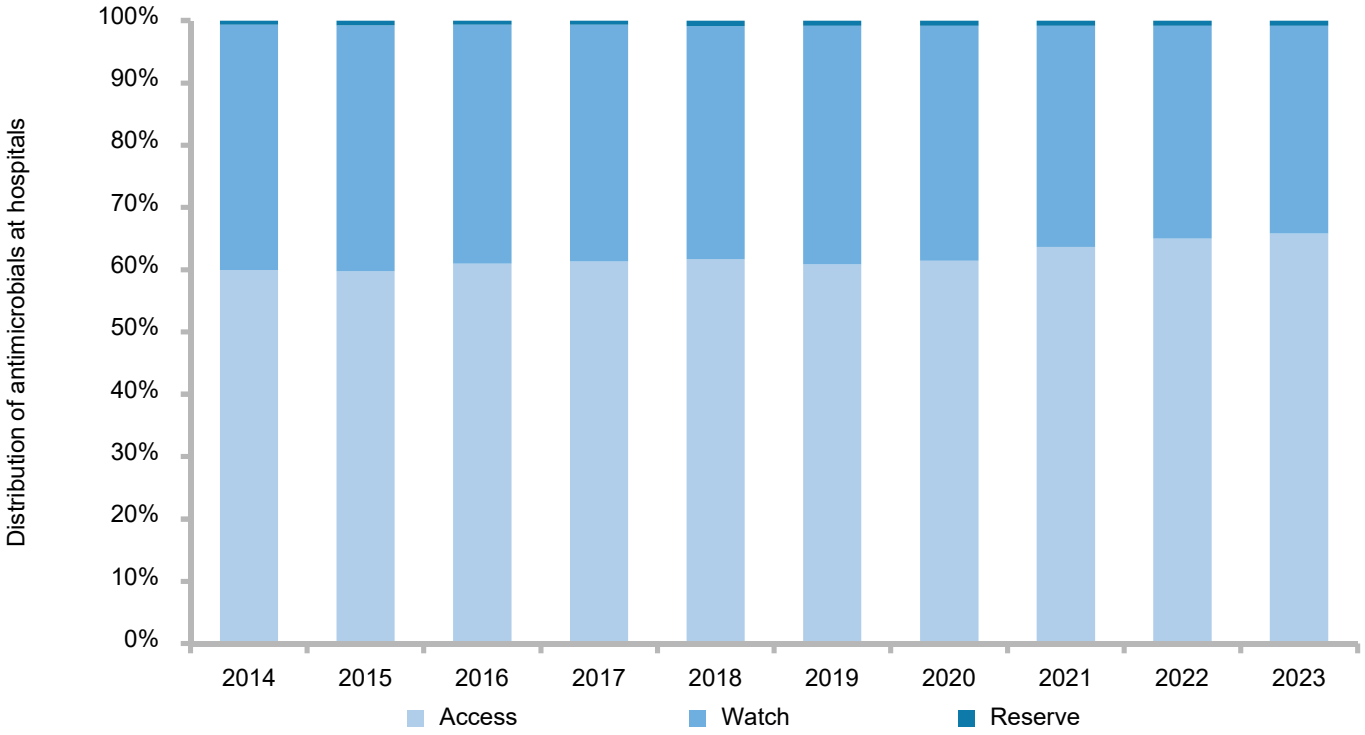
5.4.4 AWaRe classification of antimicrobials at Danish somatic hospitals

The World Health Organization (WHO) has developed the AWaRe classification system as a tool to assist antibiotic stewardship and to reduce antimicrobial resistance. Antibiotics are classified into three groups to emphasise the importance of their appropriate use:

- **Access:** Antibiotics used to treat common susceptible pathogens with lower resistance potential than antibiotics in the other groups. 60% of total antimicrobial consumption should consist of access agents.
- **Watch:** Antibiotics that have higher resistance potential, including most of the highest priority agents. These antibiotics should be prioritised as key targets of stewardship programs and monitoring.
- **Reserve:** Antibiotics reserved for treatment of confirmed or suspected infections due to multi-drug resistant organisms. These antibiotics should be considered as "last resort" options.

Antimicrobial consumption at somatic hospitals has consisted of more than 60% "access antimicrobials" since 2016, whereas "reserve antimicrobials" consituted 1% in all years (Figure 5.18). On national level, i.e. including antimicrobial consumption in primary health care, "access antimicrobials" consitituted at least 80% since 2014 (84% in 2023).

Figure 5.18 Percentage distribution of antimictobials at somatic hospitals according to WHO AWaRe classification, DDD, Denmark, 2014-2023
DANMAP 2023



Data: Antimicrobial consumption at somatic hospitals
Data source: Register of Medicinal Product Statistics, 2024 edition of the Anatomical Therapeutic Chemical (ATC) classification system

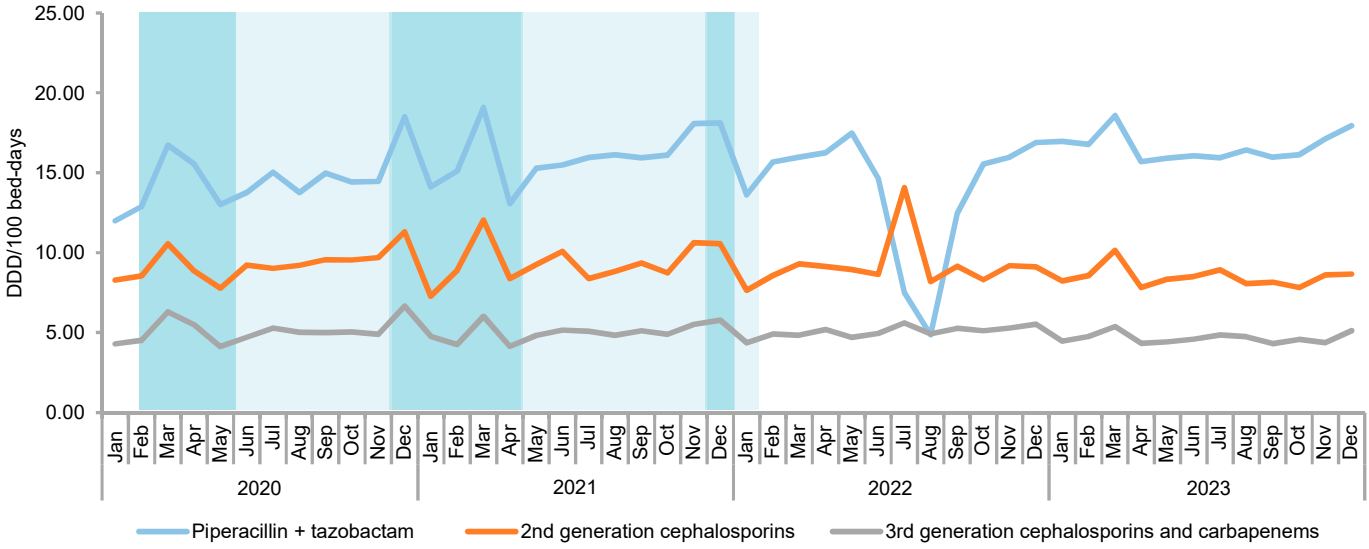
5.4.5 Shortage of antimicrobials

Issues with supply chains of antimicrobials can lead to product shortages. Some shortages do not reach the patient due to the substitution rules in Denmark. Shortages can also be solved by using antimicrobials on special delivery, which requires permission from the Danish Medicines Agency.

Monthly consumption of the main antimicrobial groups for treatment of critically ill patients at hospitals from 2019 to 2022 are shown in Figures 5.19. In 2022, penicillin/beta-lactamase inhibitor combinations decreased sharply in July and August due to product shortages. However, prescribers had access to penicillin/beta-lactamase inhibitor combinations via special deliveries. Approximately 70,000 DDD penicillin/beta-lactamase inhibitor combinations were purchased through special delivery in 2022, whereas in 2019-2021 the number was approximately 4,000 DDD (Table 5.8).

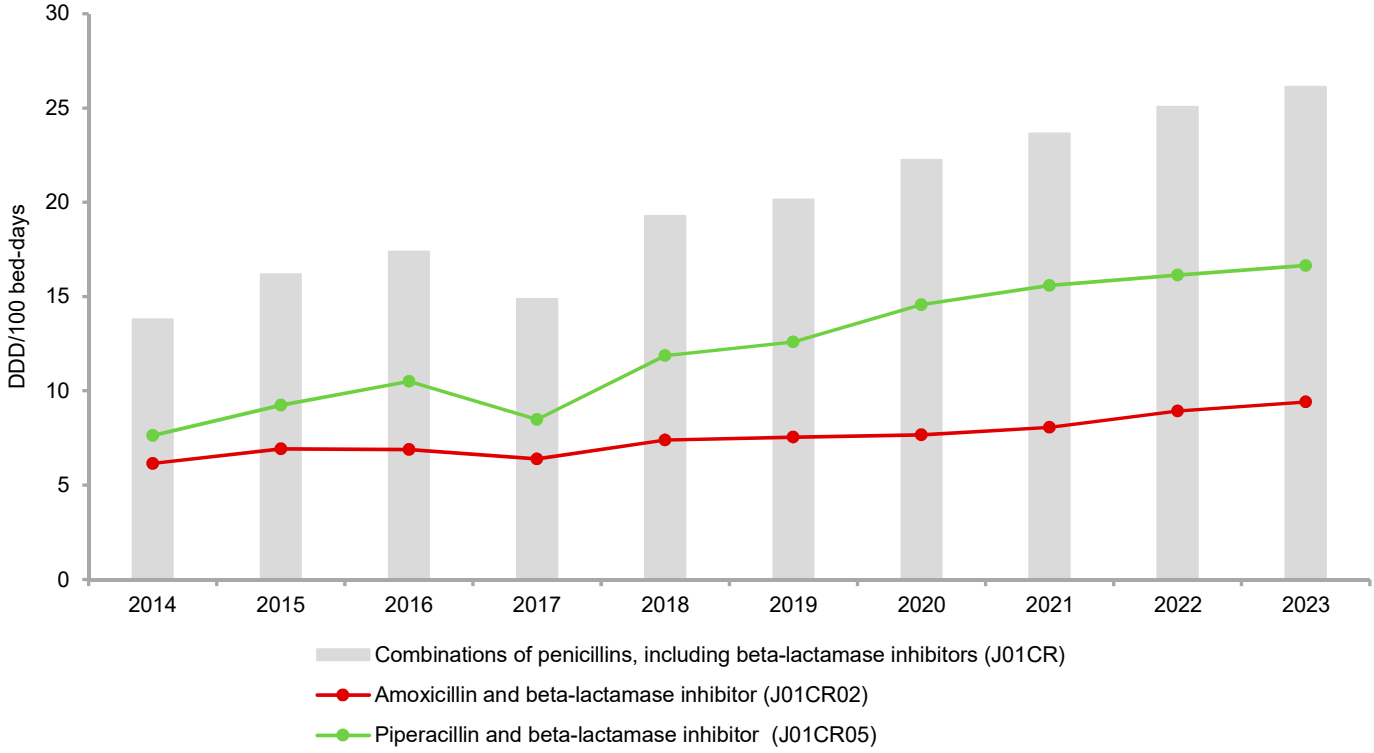
Special deliveries could not solve the shortage of penicillin/beta-lactamase inhibitor combinations in 2017, which lead to a significant decrease in consumption that year (Figure 5.20). Simultaneously, an increase in consumption of cephalosporins was observed (Figure 5.15). In 2023, nitrofurantoin was hit by shortage (Figure 5.21) and even with increased supply via special deliveries, it was not possible to cover the whole need of it, why other antimicrobials were chosen for treatment (Table 5.8).

Figure 5.19 Consumption of key antimicrobials used for treatment of seriously ill patients in hospital, DDD per 100 bed-days, Denmark, 2020-2023 DANMAP 2023



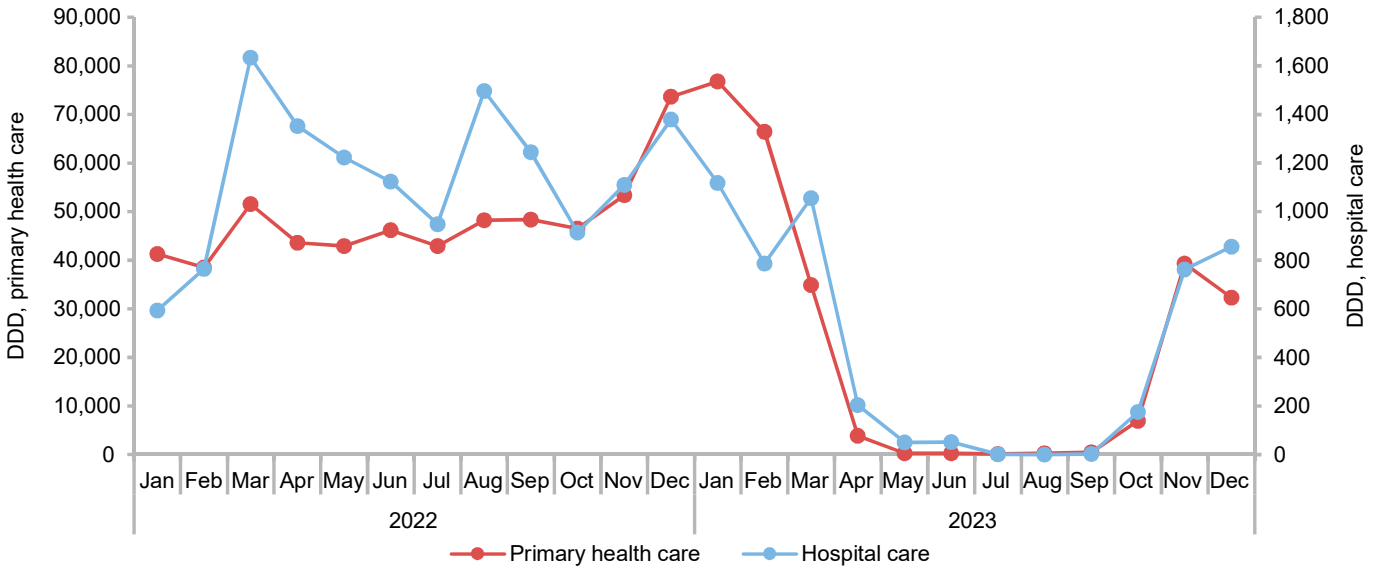
Data: Antimicrobial consumption at somatic hospitals
Data source: Register of Medicinal Product Statistics, 2024 edition of the Anatomical Therapeutic Chemical (ATC) classification system and The National Patient Register

Figure 5.20 Consumption of combination penicillins at somatic hospitals, DDD per 100 bed-days, Denmark, 2014-2023 DANMAP 2023



Data: Antimicrobial consumption at somatic hospitals
Data source: Register of Medicinal Product Statistics, 2024 edition of the Anatomical Therapeutic Chemical (ATC) classification system and The National Patient Register

Figure 5.21 Monthly consumption of nitrofurantoin by health care sector, DDD, Denmark, 2022-2023 DANMAP 2023



Data: Antimicrobial consumption in Denmark
Data source: Register of Medicinal Product Statistics, 2024 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Table 5.8 Consumption of selected antimicrobials on special delivery to hospitals, DDD, 2014-2023 DANMAP 2023

Antimicrobial	Year									
	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023
J01MA12 Levofloxacin	4,470	7,240	8,080	8,180	6,710	7,360	20,370	44,200	41,530	45,360
J01XE01 Nitrofurantoin										7,950
J01EE01 Sulfamethoxazol and trimethoprim	6,820	6,590	6,704	8,188	7,596	7,136	3,094	8,585	2,610	3,760
J01CE02 Phenoxymethylpenicillin				5,085	417			5,183		2,792
J01GB01 Tobramycin						6,895	6,840	4,790	3,850	2,620
J01CF05 Flucloxacillin	2,690	2,313	2,275	2,200	1,783	1,790	1,665	1,873	2,540	2,233
J01CR02 Amoxicillin and beta-lactamase inhibitor	721	10,743	3,276	2,579	3,882	4,348	4,277	3,934	4,177	1,726
J01MA02 Ciprofloxacin	710	1,155	1,195	690	766	726	1,028	908	935	890
J01CR05 Piperacillin og beta-lactamaseinhibitor				16,465	4,457				63,808	
J01CE08 Benzathine benzylpenicillin	316	562	372	1,514	618	538	748	544	524	652

Data: Consumption of antimicrobials on special delivery
Data source: Danish Hospital Pharmacies

We would like to acknowledge Maja Laursen from the National Health Data Authority in Denmark for data on antimicrobial consumption and activity in hospital care. We would also like to acknowledge all hospital pharmacies in Denmark for data on special delivery of antimicrobials to the hospitals.

Finally, we would like to acknowledge the panel of experts from clinical microbiology laboratories and from research centers for general practice for their valuable input to this chapter.

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

Textbox 5.1

International approach to improve supply of antibiotics

What makes a market vulnerable? Which factors need to be considered when trying to strengthen a market? It seems like repeated shortages and length of shortages are not necessarily a sign of nor does sales volume necessarily correlate with an elevated risk of withdrawal[1]. The deregistration problems and shortages issues must then have roots elsewhere. Some of them will be mentioned here, but it's a multifactual problem.

Antimicrobial stewardship programs aim at promoting rational prescribing of antimicrobials in accordance with treatment guidelines. Thus, the programs will result in decreased use of antibiotics, resulting in a smaller and less attractive market. However, antimicrobial stewardship programs are essential[2] in the fight against antimicrobial resistance and initiatives to solve supply chain problems therefor need to take this into account.

Development of new antibiotics is not profitable, as they often are to be preserved for last treatment option. On the other hand, old antibiotics, e.g. phenoxymethylpenicillin have difficulties getting registered in new markets because updated legislation has new requirements on indication and doses.

All countries have individual legislation on registration of medicinal products. Different legislative requirements make the production difficult, costly and inefficient. A report published by PLATINEA showed that within the Nordic market (Finland, Norway, Sweden and Denmark) phenoxymethylpenicillin is marketed in 10 different strengths, 41 different package sizes – of which 33 packages are only marketed in one single country[3]. The Nordic market combined makes up a quarter of the Italian antibiotic market – thus a high fragmentation as seen with phenoxymethylpenicillin in a small market must be accounted as a major risk factor for future shortages.

EU-JAMRAI-2 WP9 ACCESS

JAMRAI-2 is a One Health initiative and the biggest AMR investment from the EC EU4Health: 50 million Euros, 30 countries (27 European countries and Iceland, Norway and Ukraine), more than 100 partners. From 1st of January 2024 till 31st of December 2027 the countries will work together to attack the AMR-problems from different perspectives. Ten work packages are covering the update or development of National Action Plans (NAP), including important activities such as Infection Prevention and Control (IPC) programmes with the implementation of pilot studies, surveillance of resistance in humans, animals and the environment and access to antibiotics and vaccines.

EU-JAMRAI 2 receives funding from the European Union's EU4Health programme under grant agreement n°101127787.

Work package 9 on ANTIBIOTIC ACCESS (WP9) has 14 participating countries with the objective to help the member states in regards to strengthened/improved access to selected AMR-related products (antibiotics and vaccines) both for human and for veterinary use. WP9 will primarily focus on country-specific needs and demands.

The work package consists of several tasks and subtasks. The first task for each participating country is to develop a national list of vulnerable AMR-related products (antibiotics and/or vaccines). The aim is to focus on older, narrow-spectrum antibiotics used as first-in-line treatment or to treat a small group of critically ill patients and vaccines used to prevent bacterial infections; the products chosen must have a vulnerable supply and include both human and veterinary products.

Once the priority products have been chosen, each country must try to look at the demand and supply barriers. What are the main reasons for clinicians not to prescribe drug A? Is the drug not registered? Is it a more expensive choice? Does the country experience lots of shortages? Why is drug B not available in the country? Could harmonization between different participating countries be an option to increase the demand of a drug needed for the participating countries?

[1] Läkemedelsverket/ SWEDICH MEDICAL PRODUCTS AGENCY: "*Indicators that reveal antibiotics at risk of withdrawal*"
[2] amr_2017_action-plan_0.pdf (europa.eu)
[3] 240212_Mappingtheantibioticmarket_PLATINEA.pdf (uu.se)

continued ... Textbox 5.1

When mapping the demand and supply barriers each participating country will work in close collaboration with WP9 but also national stakeholders to look and implement possible interventions to strengthen the supply of the prioritized products. The lists from all participating countries will be compared, and countries with similar demands will be encouraged to work together to over time achieve a strengthened access to and more secured supply of their chosen products.

An important aspect of the work package is thus to make individual countries aware of similar challenges and bring common demands as well as commonly detected causes of impacted supply to the attention of the EU for a better understanding and possible cross-country interventions.

In Denmark, hopes are high for better future supply of many of the small-spectrum penicillins, particularly in pediatric formulations, an interest that has been brought up also by other countries. The synergy that stems from developing demand lists and supply challenges in parallel should not be overlooked. Bringing together technical as well as political levels of different countries on these topics brings the advantage of attracting more attention to the problem as well as solving it. We thus find that EU-JAMRAI II offers an opportunity to make a change, not only within a country but across.

Signe Miang Jensen and Ute Wolff Sönksen
For further information: Signe Miang Jensen,smij@ssi.dk

EU-JAMRAI Joint Action Antimicrobial Resistance and Healthcare-Associated Infections

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Textbox 5.2

HALT 4 – An audit on infections and consumption of antibiotics among residents in Danish nursing homes

HALT 4 (https://hygiejne.ssi.dk/overvaagning/halt-4-projekt) (Healthcare Associated Infections in European Long Time Care Facilities 4) is a European project managed by ECDC (The European Center for Disease Prevention and Control).

In 2023, the National Center for Infection Control (CEI) at Statens Serum Institut coordinated an audit (HALT 4) on the prevalence of infections and consumption of systemic antibiotics (incl. antifungal and antiviral agents) among residents in Danish nursing homes. This was performed in collaboration with local personnel responsible for infection prevention and control (IPC) and staff at participating nursing homes in the periods May to June and August to November.

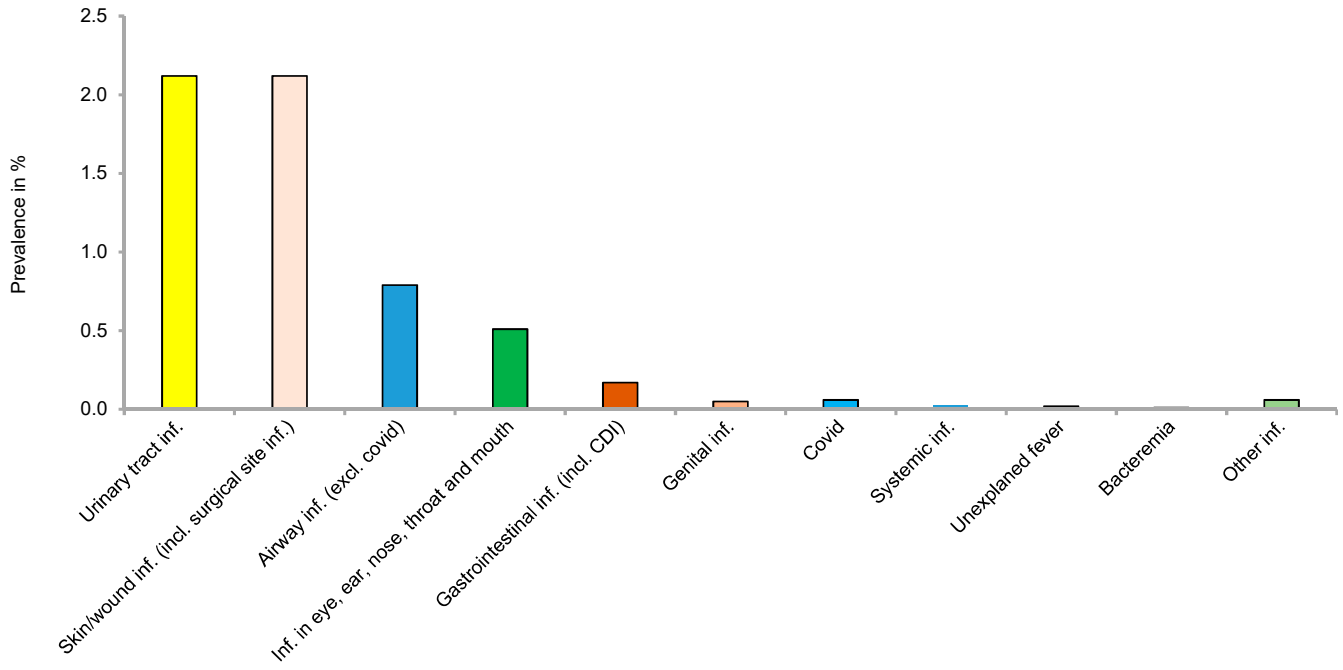
Purpose

- To focus on IPC, infections and consumption of systemic antibiotics and/or antifungal and/or antiviral agents in nursing homes
- To increase knowledge about the above conditions and the resources used on IPC in nursing homes
- To elucidate the conditions in nursing homes, both nationally and internationally, in order to be able to target future preventive interventions.

Method

CEI invited by mail and newsletter announcement all identified Danish nursing homes and all Danish municipalities and regions to participate. Participation was voluntarily and not randomized. Data collection and registration were performed by regional and/or municipal IPC personnel and/or by local staff at the participating nursing homes. Data were collected and recorded on one given day for each participating nursing home. Therefore, results represent a single point in time and do not provide information on a larger temporal scale.

Figure 1 Distribution of the various infections DANMAP 2023



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Results

In all, 294 nursing homes from 47 municipalities from all five regions participated, amounting to approx. 30% of the approx. 950 nursing homes in Denmark. Out of a total of 11,909 residents, 11,751 were included. 36.4% of the residents were men and 63.6% were women. The average age was 83.3 years, and 49.9% of residents were 85 years or older ; 29.8% of the residents had been living in the nursing home for less than a year.

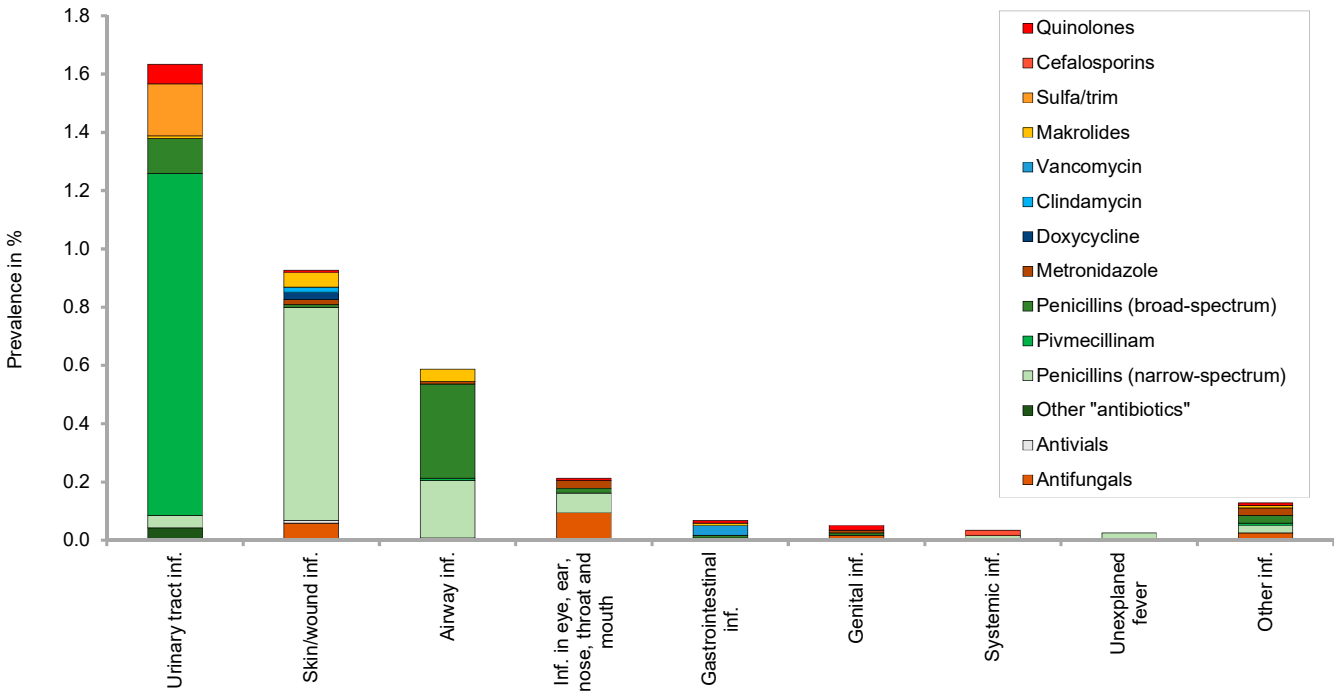
Infections

In total, 672 residents (5.72%) had an infection (defined as signs/symptoms of and/or received systemic antimicrobial therapeutic treatment against infection): 649 residents had one infection, 22 residents had 2 and one resident had three different infections, which amounted to a total of 696 infections. The distribution of infections can be seen in Figure 1.

Antimicrobial treatment

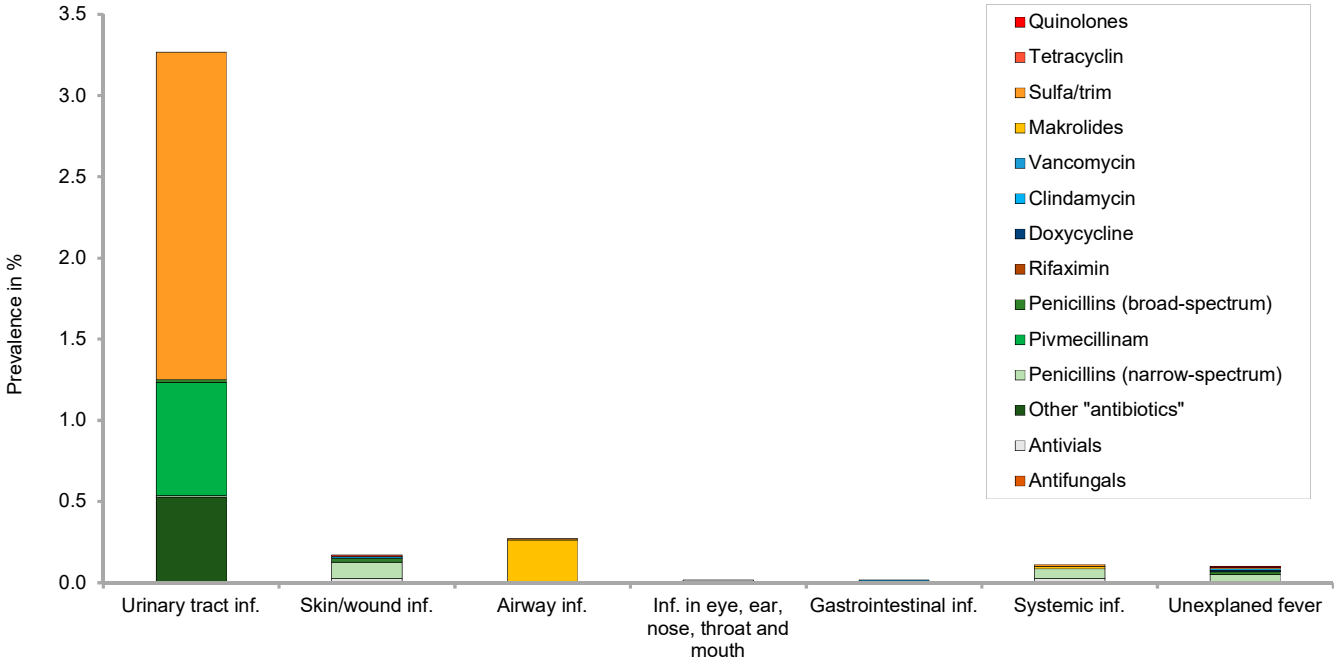
In total, 842 residents (7.17%) received systemic antibiotics and/or antifungal and/or antiviral agents. For 386 residents (3.28%), the indication was treatment of infection, for 445 (3.79%) it was prophylaxis, and for 11 (0.09%) it was both. Details are shown in Figures 2 and 3.

Figure 2 Distribution of 431 antimicrobials for systemic treatment of 403 infections DANMAP 2023



Quinolones include ciprofloxacin. Sulfa/trim includes sulfamethizole, sulfamethoxazole/trimethoprim, and trimethoprim. Macrolides include azithromycin. Broad-spectrum penicillins include amoxicillin and amoxicillin/clavulanic acid. Narrow-spectrum penicillins include dicloxacillin and phenoxymethylpenicillin. Other "antibiotics" include nitrofurantoin and methenamine. Antifungals include fluconazole. Antivirals include aciclovir, dolutegravir/rilpivirine and valaciclovir

Figure 3 Distribution of 465 antimicrobials for systemic prophylaxis in 457 cases DANMAP 2023



Quinolones include ciprofloxacin and moxifloxacin. Cephalosporins include ceftriaxone and cefuroxime. Sulfa/trim includes sulfamethizole and trimethoprim. Macrolides include azithromycin, clarithromycin, and roxithromycin. Broad-spectrum penicillins include amoxicillin, amoxicillin/clavulanic acid, and piperacillin/tazobactam. Narrow-spectrum penicillins include dicloxacillin and phenoxymethylpenicillin. Other "antibiotics" include nitrofurantoin. Antiviral agents include aciclovir. Antifungals include fluconazole, nystatin, and terbinafine

Comparing data from HALT 4 with data from the previously conducted and similar audit HALT 3 from 2017, the prevalences of residents with infection and both residents in therapeutic and prophylactic treatment are lower in HALT 4 than in HALT 3 (https://hygiejne.ssi.dk/overvaagning/halt-3---projekt). In particular, the prevalence of prophylactic treatment of urinary tract infection is significantly lower, see Table 1.

However, as the participating nursing homes in the two audits were not randomised, a comparison and a conclusion should be done with causion.

Table 1 Yearly number of isolates and patients DANMAP 2023

	HALT 4 – 294 perticipating nursing homes with 11,751 incl. residents	HALT 3 – 95 perticipating nursing homes with 3,346 incl. residents
Residents with infection	672 (5.72%)	227 (6.78%)
Residents in therapeutic treatment	392 (3.34%)	129 (3.86%)
Residents in therapeutic and prophylactic treatment	22 (0.19%)	5 (0.15%)
Residents in prophylactic treatment	446 (3.80%)	225 (6.72%)
Residents in prophylactic treatment of urinary tract infection	384 (3.27%)	213 (6.37%)

Christian Stab Jensen
For further information: csj@si.dk

Textbox 5.3

Infection Prevention and Control and prevention of Antimicrobial Resistance goes hand in hand

In Denmark there are numerous activities concerning infection prevention and control (IPC) and antimicrobial resistance (AMR) - both on the national and on the international level.

Across Europe as well as globally it is increasingly stressed that controlling AMR in human health must be based on aligning efforts within surveillance, antimicrobial stewardship (AMS) and IPC.

In June 2023, the European Union recommended to step up EU actions to combat antimicrobial resistance with a One Health approach. In the Council recommendations[1] they encourage member states to (extract):

- Have in place by the 14th of June 2024 and regularly update and implement National Actions Plans against AMR.
- Ensure that IPC measures in human health are put in place and continuously monitored to limit the spread of antimicrobial resistant pathogens.
- Ensure that measures are put in place in human health to support the prudent use of antimicrobial agents in health care settings including primary care and long-term care facilities.

To support the countries in stepping up the AMR actions the European Commision has invested 50 million Euros in EU-JAMRAI 2[2] which is a project under the EU4health programme[3].

EU-JAMRAI 2 (European Joint Action on Antimicrobial Resistance and Healthcare-Associated Infections) 2024 to 2027 seeks to implement concrete actions to monitor, prevent and effectively tackle AMR across human, animal and environmental health domains through a "One-Health" approach and to make Europe a best practice region. The project focuses on multiple areas, such as AMS, surveillance, awareness raising, capacity building, IPC and behavioral science. An essential part of the project is to support the countries in developing or updating their national AMR action plans with a focus on both AMR and IPC[4].

Among the 10 work packages (WP) in EU-JAMRAI 2, Denmark participates in WP5 (National Action Plans), WP6 (Antimicrobial Stewardship), WP7 (Infection Prevention and Control), WP8 (One Health Surveillance) and WP9 (Acces to antibiotics), see more in textbox 5.1. "International approach to improve supply of antibiotics" in DANMAP 2023.

WP7 is about improving IPC actions with a One Health approach. WP7 has tasks within the human, veterinary and environmental sectors and has a general focus on behavior change strategies to support further uptake of IPC recommendations. Several subtasks are described within the human activities: Development of frameworks for implementation of IPC competencies and prioritizing EU standards in IPC programs, support the participating member states and associated countries in the implementation of IPC core components, give access to an IPC toolbox and, finally, use peer-to-peer exchange programs including mentorships and observerships. Topics which constitute a challenge for IPC of today as e.g. lack of educated workforce, specialized care moving out of the hospitals and replaced by care at home, and IPC in the green transition will be included in the work. As part of the EU-JAMRAI 2 project the Danish National Center for Infection Control (CEI) in close collaboration with our national IPC partners aim at improving the acces to IPC knowledge and tools, strengthening IPC networking and knowledge-sharing, supporting pilot projects and other implementation activities. A general focus will be on how to improve and maintain IPC competencies. Read about the Danish IPC activites at CEI SSI subsite[5].

EU-JAMRAI 2 receives funding from the European Union's EU4Health programme under grant agreement n°101127787.

EU-JAMRAI Joint Action Antimicrobial Resistance and Healthcare-Associated Infections

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The Danish IPC guidelines are in place

All healthcare professionals are expected to be familiar with and act in compliance with the national guidelines for IPC (published by the National Centre for Infection Control at SSI); in Danish "NIR Generelle forholdsregler for sundhedssektoren"[6]. The supplemental national guideline includes specific guidance on VRE, ESBL and other multidrug-resistant microorganisms (MDRO) and should be followed when being in contact with a patient, for which either clinical infection or carriage of MDRO is suspected or known; "NIR Supplerende forholdsregler ved infektioner og bærertilstand i sundhedssektoren"[7].

The Guidance on Preventing the Spread of MRSA by the Danish Health Authority was issued in 2006, the year MRSA became notifiable. The applicable 3rd edition of the guideline is from 2016; Guidance on Preventing the Spread of MRSA - The Danish Health Authority (sst.dk)[8]. The guideline represents the national recommendations and strategic framework for preventing the spread of MRSA at hospitals and in other healthcare settings and nursing homes. A 16-year MRSA surveillance report[9] has shown that the national MRSA strategy has been successful in controlling the spread of MRSA at hospitals as the primary goal of the MRSA guideline and to stabilize the spread of livestock-associated MRSA.

The first national guideline on preventing the spread of CPO by the Danish Health Authority was issued in 2018 (only in Danish); "Vejledning om forebyggelse af spredning af CPO"[10]. The guideline provides a national strategic framework for detection and management of CPO in hospitals. The main purpose of the guideline is to maintain a low prevalence of disease caused by CPO associated with certain high-risk situations. Despite this national guideline CPO is increasing in Denmark (see chapter 8.3.2, carbapenemase-producing organisms, CPO) indicating that more needs to be done in order to combat the outbreaks at hospitals and at long-term care facilities. There are a lot of challenges in controlling these outbreaks as they are long-lasting.

The purpose of both guidelines is to minimize the spread of these often highly resistant bacteria to the ill and weak patients at hospitals and in long-term care facilities, simultaneously keeping the occurrence of these bacteria on a continued low level. The guidelines contain recommendations for active screening of patients on admission to hospital, based on assessment of certain risk situations, e.g. admission to a hospital abroad during the last six months. Both guidelines are free of charge and easy to download from the Danish Health Authority website www.sst.dk.

In hospitals, nursing homes and home care, it is necessary to supplement the general precautions with transmission based (isolation) precautions in the case of an outbreak: "Infektionshygiejniske retningslinjer for MRSA"[11] and "Infektionshygiejniske retningslinjer for CPO"[12].

All IPC guidelines emphasize the importance of all healthcare staff, irrespective of profession, to contribute to the management and prevention of infections and to prescribe antibiotics with care.

Asja Kunøe and Anne Kjerulf, Statens Serum Institut
For further information: Asja Kunøe, asku@ssi.dk

[1] https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32023H0622(01)
[2] https://eu-jamrai.eu/
[3] https://health.ec.europa.eu/funding/eu4health-programme-2021-2027-vision-healthier-european-union_en
[4] https://ec.europa.eu/info/funding-tenders/opportunities/portal/screen/opportunities/projects-details/43332642/101127787/EU4H
[5] https://hygiejne.ssi.dk/formidling/eu-jamrai-2
[6] https://hygiejne.ssi.dk/NIRgenerelle
[7] https://hygiejne.ssi.dk/NIRsupplerende
[8] https://www.sst.dk/en/english/publications/2022/Guidance-on-Preventing-the-Spread-of-MRSA
[9] https://hygiejne.ssi.dk/overvaagning/mrsa
[10] https://www.sst.dk/da/udgivelser/2018/vejledning-og-bekendtgoerelse-om-forebyggelse-mod-spredning-af-cpo
[11] https://hygiejne.ssi.dk/retningslinjer/infektionshygiejniske-retningslinjer-for-mrsa
[12] https://hygiejne.ssi.dk/retningslinjer/infektionshygiejniske-retningslinjer-for-cpo

Textbox 5.4

Consumption of antimicrobials in the Faroe Islands

Background

The Faroe Islands (FI) consist of 18 islands inhabited by approximately 54,000 inhabitants, approximately 22,000 of whom live in the capital Tórshavn. Sjúkrahúsverkid consists of the main hospital (Landssjúkrahúsid, LS, with 130 beds), located in Tórshavn, and two smaller hospitals in Klaksvík (22 beds) and Suduroy (26 beds). The Faroese healthcare system is comparable to the Danish healthcare system with general practitioners responsible for primary care and hospitals providing secondary care. LS has a local as well as a centralised function. In the case of specific diseases, demanding highly specialised care, patients are referred to hospitals in Denmark or other hospitals abroad.

Data and data sources: Data on antimicrobial consumption (purchase data) for FI and for the three hospitals were supplied by the Chief Pharmaceutical Office. Data on somatic bed-days were obtained from LS.

Antimicrobial consumption at the three hospitals

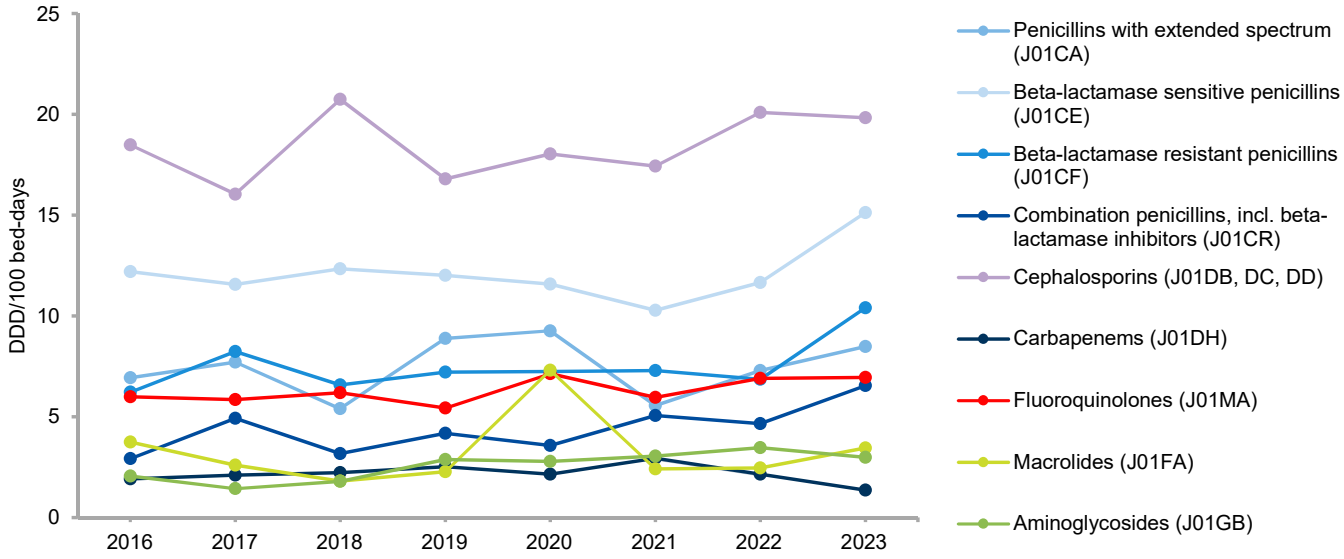
The total antimicrobial consumption was 104.13 DDD/100 bed-days (DBD), a 13% increase compared to 2022, a 26% increase compared to 2021 (82.66 DBD), and a 33% increase compared to 2016 (78.58 DBD). Special attention to three broad-spectrum antimicrobials, cephalosporins, carbapenems and fluoroquinolones is still required: In 2023, the consumption of cephalosporins was 19.83 DBD (19% of the total hospital antimicrobial consumption), slightly lower than in 2022 (20.08 DBD), but increased by 14% and 10% compared to 2021 (17.43 DBD) and 2020 (18.02 DBD), respectively. Fluoroquinolone was at the same level (6.95 DBD) as in 2022 (6.90 DBD), but has shown an increasing trend over the years with a 16% increase compared to 2016 (5.99 DBD). Problems with supply due to product shortage might be part of the explanation.

Use of carbapenems (1.36 DBD) decreased by 37% and 54% compared to 2022 (2.14 DBD) and 2021 (2.94 DBD) and is only at 71% of the level in 2016, which is a positive development.

Likewise, it is encouraging to observe a 30% increase in the consumption of beta-lactamase sensitive penicillins (15.12 DBD in 2023 vs. 11.65 DBD in 2022). A similar development is seen in the consumption of beta-lactamase resistant penicillins (10.40 DBD in 2023 vs. 6.85 DBD in 2022) (Figure 1).

Figure 1 Consumption of leading groups of antimicrobial agents at somatic departments in the three Faroese hospitals, DDD per 100 bed-days, 2016-2023

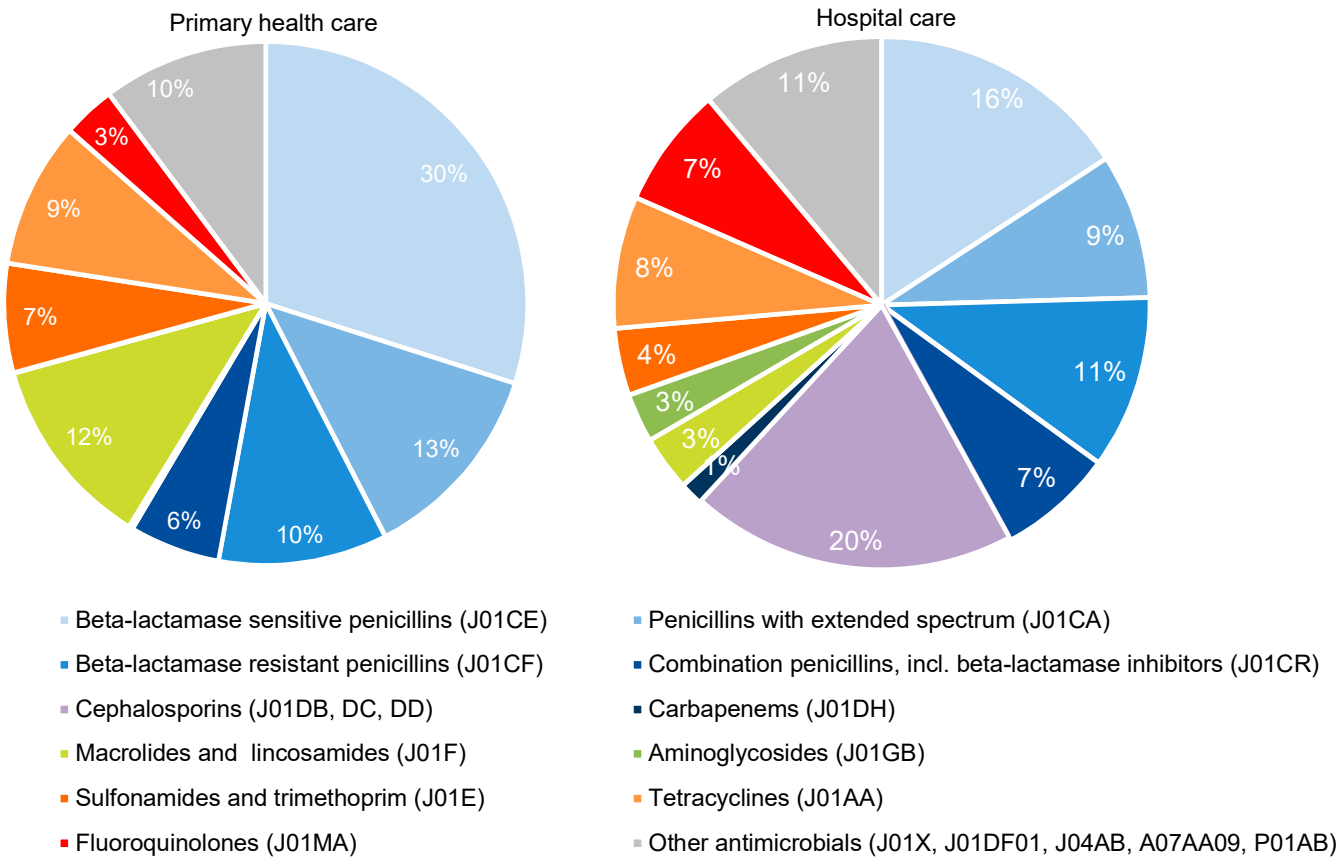
DANMAP 2023



Antimicrobial consumption in primary healthcare

In 2023, the total antimicrobial consumption in primary healthcare was 15.65 DDD/1,000 inhabitants/day (DID) – comparable to 2022 (15.26 DID), but representing a 19% increase since 2016 (13.11 DID). The distribution of antimicrobial agents used is shown in Figure 2 and compared to distribution of similar antimicrobials in hospital consumption.

Figure 2 Percentage distribution of antimicrobial agents in primary healthcare and hospital care, DDD, 2023 DANMAP 2023



Consumption of macrolides and lincosamides increased by 27% compared to 2022 (1.86 DID in 2023 vs. 1.45 DID in 2022). An encouraging observation was a 14% decrease in the use of fluoroquinolones (0.51 DID in 2023 vs. 0.59 DID in 2022).

Total antimicrobial consumption in hospitals was 2.27 DID, accounting for 13% of the total consumption at FI, whereas the consumption in primary healthcare accounted for 87%.

Resistant microorganisms

In 2023, eight patients were identified with MRSA (six were clinical specimens, two were obtained by screening). The corresponding patient numbers for VRE were two and five, respectively, and for CPO one and two respectively.

Conclusion

Focus on broad-spectrum antimicrobials as well as infection prevention and control remains crucial, as there is still a high-level consumption of antimicrobials such as the cephalosporins.

Elsebeth Tvenstrup Jensen, Ann Winther Jensen, Anne Kjerulf, Lena Lambaa, and Marianna Konoy
For further information: Elsebeth Tvenstrup Jensen, etj@ssi.dk and Anne Kjerulf, alf@ssi.dk

6

RESISTANCE IN ZOONOTIC BACTERIA

6. Resistance in zoonotic bacteria

Highlights: As in previous years, resistance levels in *Campylobacter jejuni* isolated from humans were higher than in isolates from broilers and cattle, and among human isolates, resistance was higher in travel-associated cases compared to domestically acquired cases.

Compared to 2022, the percentage of fully sensitive *C. jejuni* increased in isolates from broilers (from 59% to 61%) and decreased in isolates from cattle (from 76% to 70%).

Erythromycin resistance remained rare in *C. jejuni* from humans (1%) and resistance was not observed in isolates from broilers or cattle.

Fluoroquinolone (ciprofloxacin) resistance remained common in *C. jejuni* isolates from human cases (54%) and broilers (34%) and increased by 7% in cattle (29%). Tetracycline resistance was common in *C. jejuni* from humans (30%) and broilers (27%), and less common in cattle (7%).

Resistance to ciprofloxacin and tetracycline in *C. coli* from pigs increased compared to the levels observed in 2021 (from 20% to 25% and 26% to 35%, respectively). Resistance to ertapenem was not observed in *C. coli* from pigs. *C. coli* isolates from humans exhibited generally more resistance than isolates from pigs, indicating that the human isolates likely originate from sources other than Danish pigs. Erythromycin resistance was seen in 11% of the tested human *C. coli* isolates.

A total of 697 human clinical *Salmonella* spp. isolates representing 88 different serotypes were tested. Most *Salmonella* spp. isolates from humans were fully sensitive (64%), while lower levels of full susceptibility were found in pigs (46%) and pork (25%).

Macrolide (azithromycin) resistance in *Salmonella* spp. remained low in isolates from human travel-associated (3%) and domestic cases (1%). No azithromycin resistance was reported in isolates from domestic pork and a single *S.* Typhimurium resistant isolate was recovered from pigs.

Resistance to ciprofloxacin in *Salmonella* spp. was observed in 15% of the isolates from humans. Ciprofloxacin resistance in *S.* Typhimurium and monophasic *S.* Typhimurium increased in 2023 (from 3% to 12% and 8% to 11%, respectively). Fluoroquinolone resistance has not been recorded in *S.* Typhimurium from Danish pork since 2007 however, a single monophasic *S.* Typhimurium isolate from pigs was resistant to ciprofloxacin in 2023.

Resistance to 3rd generation cephalosporins, cefotaxime and ceftazidime, in *Salmonella* spp. is rare in human isolates (2%), and no resistance was found in isolates from pigs and domestic pork. No carbapenem resistance (meropenem) was observed in *Salmonella* spp. from humans, pigs and domestic pork.

After a three-year decreasing trend, multidrug resistance (MDR) in *S.* Typhimurium and its monophasic variant from domestic pork increased in 2023 by 27%, with an increase in the ratio between the prevalence of other MDR profiles and the combined resistance to ampicillin, sulfamethoxazole and tetracycline (ASuT).

6.1 Resistance in zoonotic bacteria

6.1.1 Introduction to resistance in zoonotic bacteria

Zoonoses are infectious diseases transmitted between animals and humans, either through direct contact or indirectly by ingesting contaminated food or water or by contact with a contaminated environment. A description of the trends and sources of zoonoses in Denmark and national surveillance and control programmes can be found in the Annual Report on Zoonoses in Denmark 2023 [www.food.dtu.dk].

Surveillance of antimicrobial resistance (AMR) in the zoonotic bacteria *Campylobacter* and *Salmonella* from food-producing animals, food products, and humans has been part of the DANMAP programme since 1995. Phenotypic antimicrobial resistance is monitored in isolates from human clinical cases, broilers, cattle, pigs, and derived meat.

In Denmark, antimicrobials are generally not recommended for treating human patients with diarrhoea unless the illness is prolonged, or the patient is severely ill. If treatment is necessary, macrolides (azithromycin) are recommended for treating *Campylobacter* infections. No specific recommendations for antibiotic treatment exist for *Salmonella* infections in the primary sector. In hospitals, intravenous treatment is recommended for septic patients and per oral azithromycin for less severe cases. In case of prolonged or recurrent infections, a combination therapy can be used by adding ciprofloxacin or sulfamethoxazole and trimethoprim. The Register of Medicinal Product Statistics at the Danish Health Data Authority does not register the specific pathogen treated with antimicrobials.

Macrolides are often used to treat infections in food-producing animals in Denmark, particularly in pigs. Fluoroquinolones are not used in production animals, and there is a limited use of 2nd generation cephalosporins in cattle, while no use of 3rd and 4th generation cephalosporins occurs. The use of antimicrobials in the Danish poultry sector is low and limited to only a few antimicrobial classes, primarily tetracyclines (see Chapter 4, Table 4.1).

In humans, antimicrobial resistance is monitored in clinical *Salmonella* isolates and for *C. jejuni* and *C. coli*, a geographically stratified selection of clinical isolates is tested. The testing is performed in accordance with the ECDC recommendations (see Chapter 10, section 10.9). Travel histories of the patients are collected, when possible.

Campylobacter isolates were obtained from healthy animals at slaughter (caecal samples from broilers, cattle and pigs), while *Salmonella* isolates were obtained from caecal samples and carcasses of healthy pigs at slaughter. *C. jejuni* is always reported for broilers and cattle, and *C. coli* is always reported for pigs, and occasionally for broilers and cattle (see Chapter 10, Table 10.1 for further details). Since 2021, the antimicrobial susceptibility testing of *Campylobacter* and *Salmonella* from animals and meat has been done in accordance with the Commission Implementing Decision 2020/1729/EU of 17 November 2020 on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria (see Chapter 10 for further details).

6.1.2 *Campylobacter*

A total of 279 human *C. jejuni* isolates were susceptibility tested. The isolates represented 226 domestically acquired cases and 53 travel-associated cases. Additionally, 57 *C. coli* isolates were tested of which 40 and 17 isolates were from domestic and travel-associated human cases, respectively. All *C. jejuni* isolates recovered from broilers (41), cattle (174), and pigs (1) and all *C. coli* isolates from broilers (1), cattle (8) and pigs (127) were susceptibility tested.

Resistance in *Campylobacter jejuni*

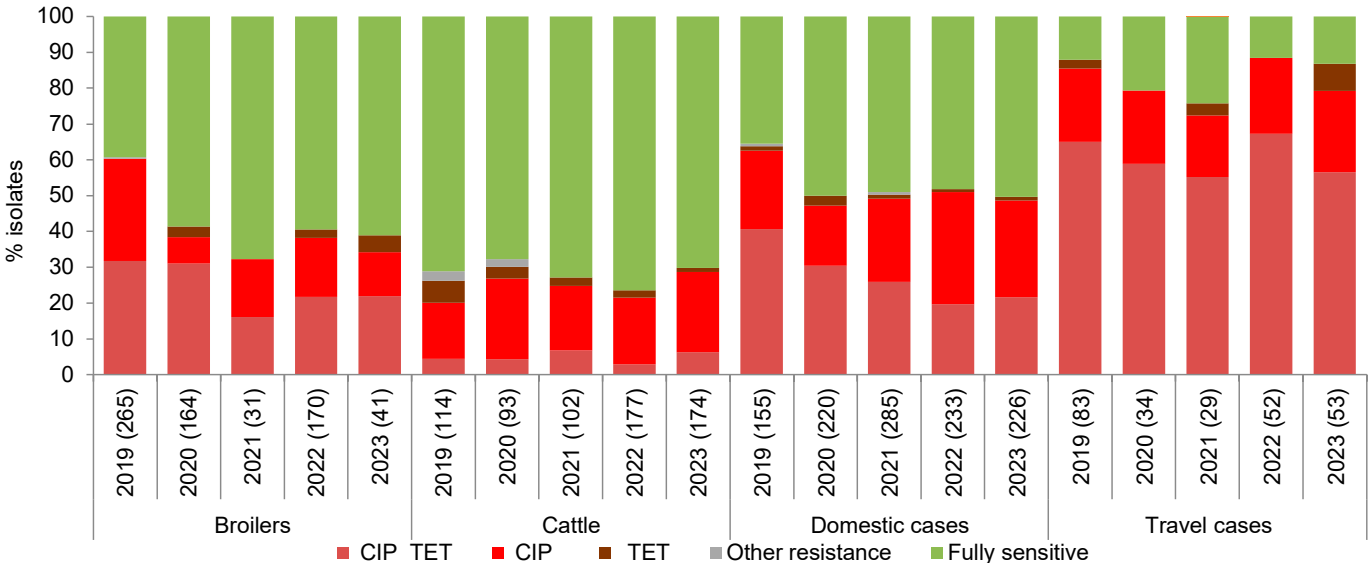
The resistance levels in *C. jejuni* isolates from humans, and Danish broilers and cattle at slaughter are presented in Table 6.1. Resistance to ciprofloxacin and tetracycline was common in isolates from humans, broilers, and cattle (Table 6.1 and Figure 6.1). Like in the previous years the resistance level to erythromycin was low in human isolates (1%) and no erythromycin resistance was observed in isolates from broilers or cattle. Resistance to chloramphenicol and gentamicin was not recorded in 2023.

Ertapenem resistance has been monitored since 2021. However, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) has not proposed an epidemiological cut-off (ECOFF) or clinical breakpoint for ertapenem resistance, which is crucial for identifying whether an isolate is susceptible or resistant. The EU Reference Laboratory for AMR (EURL-AR) and the European Food Safety Authority have established a provisional ECOFF of 0.5 mg/L (Chapter 10, Table 10.2), which DANMAP adopted in 2021. Resistance levels for this antimicrobial in human and broiler isolates were in line with the findings from 2021 and 2022. A total of 6% of all isolates from humans were resistant, with domestic- and travel-related cases presenting 4% and 13% of ertapenem resistance, respectively. Of all *C. jejuni* isolates from broilers, 2% were resistant to ertapenem. Unlike in 2021 and 2022 when no ertapenem resistance was observed in *C. jejuni* isolates from cattle, in 2023, 1% of these isolates were resistant.

In 2023, 61% of *C. jejuni* from broilers, 70% from cattle and 43% from human cases were sensitive to all antimicrobials tested. The percentage of fully sensitive *C. jejuni* isolates from domestic human cases was in line with previous years. Also, resistance levels to ciprofloxacin and tetracycline were higher in infections from travel-related cases compared to domestically acquired cases (Figure 6.2). The occurrence of fully sensitive isolates was similar to that found in 2022 for *C. jejuni* isolates from broilers, while a decrease was observed among isolates from cattle (Figure 6.1).

Figure 6.1 Distribution (%) of AMR profiles in *Campylobacter jejuni* from broilers, cattle and human cases, Denmark, 2019-2023

DANMAP 2023



The number of isolates included each year is shown in parentheses. A human isolate is categorised as domestically acquired if the patient did not travel outside Denmark one week prior to the onset of disease. 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, Fully sensitive: all isolates susceptible to all antimicrobial agents included in the test panel. CIP TET, CIP and TET isolates may be also resistant to other antimicrobials in the test panel (see Table 6.1)

Table 6.1 Resistance (%) in *Campylobacter jejuni* isolates from broilers, cattle and human cases, Denmark, 2023 DANMAP 2023

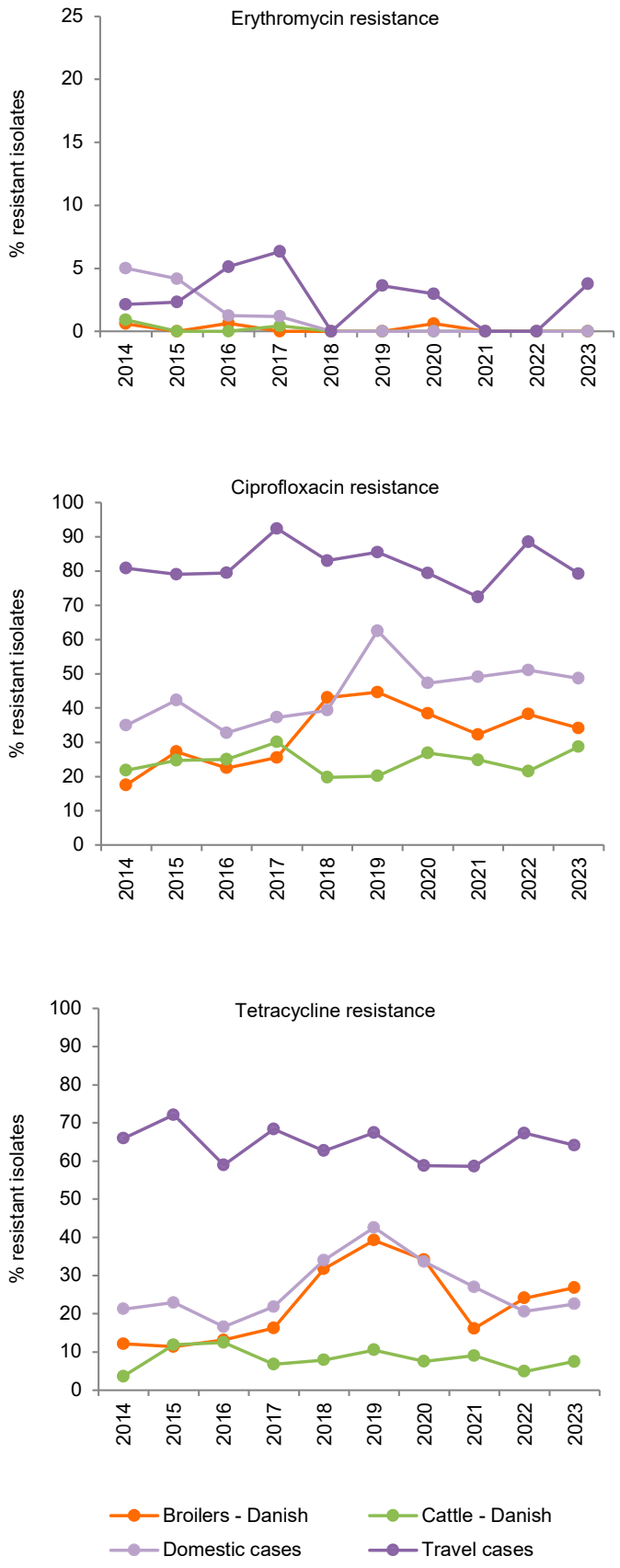
	Broilers	Cattle	Human		
	Danish	Danish	Domestically acquired	Travel abroad reported	Total
Antimicrobial agent	%	%	%	%	%
Chloramphenicol	0	0	0	0	0
Ciprofloxacin	34	29	49	79	54
Ertapenem	2	1	4	13	6
Erythromycin	0	0	0	4	1
Gentamicin	0	0	0	0	0
Tetracycline	27	7	23	64	30
Fully sensitive (%)	61	70	50	13	43
Number of isolates	41	174	226	53	279

An isolate is categorised as domestically acquired if the patient did not travel outside Denmark one week prior to the onset of disease

In 2023, the percentage of *C. jejuni* isolates from broilers with resistance to ciprofloxacin but not tetracycline decreased when compared to the previous year (from 16% to 12%), while the percentage of isolates with combined resistance to ciprofloxacin and tetracycline remained the same (22%). In cattle, *C. jejuni* showed an increase in the combined resistance to ciprofloxacin and tetracycline (from 3% to 6%), compared to 2022. Isolates exhibiting combined resistance towards ciprofloxacin and tetracycline were found in 22% of the human isolates from domestically acquired infections and in 57% of isolates from travel-associated cases Fig 6,1). Resistance to tetracycline but not ciprofloxacin remained rare in isolates from animals in 2023, as did resistance to antimicrobials other than ciprofloxacin and/or tetracycline (Figure 6.1).

Ciprofloxacin resistance has overall increased in *C. jejuni* isolates from Danish broilers over the last decade until 2019. However, the trend shifted to a decrease in 2020 and 2021. In 2022 the occurrence of ciprofloxacin resistance increased again, stabilising in 2023 at a level similar to that observed in 2019 (34%; Figure 6.2). As previously observed, the shift in the trend of resistance to ciprofloxacin coincided with the shifts in resistance to tetracycline (Figure 6.2) and combined resistance to both antimicrobials (Figure 6.1). Fluoroquinolones are not used in food-producing animals in Denmark, and their use is not allowed in broiler production across the EU. This suggests that the development and spread of ciprofloxacin resistance in *C. jejuni* isolates from broilers is driven by mechanisms other than the direct use of fluoroquinolones.

Figure 6.2 Erythromycin, ciprofloxacin and tetracycline resistance (%) among *Campylobacter jejuni* from broilers, cattle and human cases, Denmark, 2014-2023 DANMAP 2023



An isolate is categorised as domestically acquired if the patient did not travel outside Denmark one week prior to the onset of disease

Resistance in *Campylobacter coli*

The resistance levels in *C. coli* isolates from humans, and Danish pigs at slaughter are presented in Table 6.2. *C.* coli constitutes less than 10% of human cases and the DANMAP report has not previously focused on *C.* coli from humans. Therefore, it is not possible to compare the recorded resistance levels with previous years.

The occurrence of resistance to ciprofloxacin in *C. coli* from pigs increased when compared to the levels observed in 2021 (from 20% to 25%). Likewise, tetracycline resistance was comparatively higher (from 26% to 35%). When looking at *C. coli* isolates from humans, the resistance levels to ciprofloxacin and tetracycline are comparatively higher than those seen in pig isolates and *C. jejuni* isolates (Tables 6.1 and 6.2).

Resistance to macrolides, monitored through erythromycin, was six and 11% in isolates from pigs and humans, respectively. All erythromycin-resistant human isolates were from domestically acquired cases.

Resistance to chloramphenicol was not observed in *C. coli* isolates from pigs and humans in 2023. Similarly, resistance to gentamicin was not observed in *C. coli* from pigs and was rare in human isolates (4%). Unlike in 2021, resistance to ertapenem was not observed in *C. coli* from pigs. High resistance levels in human isolates (37%) were observed. *C. coli* isolates from human cases exhibited generally higher resistance levels than isolates from pigs, indicating that human isolates can originate from sources other than Danish pigs.

6.1.3 *Salmonella* spp.

The resistance data for *Salmonella enterica subsp. enterica* for a panel of 15 antimicrobials representing 11 antimicrobial classes are presented in Table 6.3 for pigs, domestic pork and human isolates. In 2023 a total of 697 human clinical *Salmonella* spp. isolates, representing 88 different serotypes were tested for antimicrobial susceptibility. The predominant serotypes were *S.* Typhimurium (93), monophasic S. Typhimurium with the antigenic formula 4,[5], 12:i:- (81), and *S.* Enteritidis (76). Except for *S.* Enteritidis, the tested isolates represented all clinical Danish isolates and included outbreak isolates. The largest outbreak encompassed 31 patients, and three other outbreaks included more than 10 patients. Also in 2023, a total of 82 and 65 *Salmonella* spp. isolates from pigs and domestic pork, respectively were tested for antimicrobial susceptibility. The most common serotypes reported in pigs were *S.* Derby (40), monophasic *S.* Typhimurium variant 4,[5], 12:i:- (31), and *S.* Typhimurium (6). Similarly, in domestic pork, the serovar distribution followed the same order, with 25, 18 and 12 *S.* Derby, monophasic *S.* Typhimurium variant 4,[5], 12:i:- and *S.* Typhimurium, respectively.

Table 6.2 Resistance (%) in *Campylobacter coli* from fattening pigs and human cases, Denmark, 2023 DANMAP 2023

	Pigs	Human		
	Danish	Domestically acquired	Travel abroad reported	Total
Antimicrobial agent	%	%	%	%
Chloramphenicol	0	0	0	0
Ciprofloxacin	25	78	71	75
Ertapenem	0	38	35	37
Erythromycin	6	15	0	11
Gentamicin	0	3	6	4
Tetracycline	35	70	65	68
Fully sensitive (%)	53	18	24	19
Number of isolates	127	40	17	57

An isolate is categorised as domestically acquired if the patient did not travel outside Denmark one week prior to the onset of disease

Most *Salmonella* spp. isolates from humans were fully sensitive (64%), while isolates from pigs showed a lower level of fully sensitive isolates (46%). Domestic pork showed a marked difference, with only 25% of the *Salmonella* spp. isolates sensitive to all antimicrobials tested. This difference can be attributed to the higher proportion of *S.* Derby from pigs when compared to domestic pork isolates, as resistance levels are usually lower in *S.* Derby compared to *S.* Typhimurium and its monophasic variant. Multidrug resistance is defined as resistance to three or more of the 11 tested antimicrobial classes. In 2023, 22% of human isolates were multidrug-resistant (MDR), with comparable levels observed in domestic and travel-associated cases. Higher MDR levels were reported in pigs (48%) and domestic pork (52%).

Since 2014, macrolide resistance in *Salmonella* has been monitored using azithromycin, which is used for the treatment of human *Salmonella* infections in Denmark. Low levels of azithromycin resistance were reported from human travel-associated (3%) and domestic cases (1%), and from pig isolates (1%), while no resistance was reported in isolates from domestic pork. Fluoroquinolones may also be used for the treatment of human *Salmonella* infections and resistance is monitored using nalidixic acid and ciprofloxacin. In 2023, resistance to ciprofloxacin was observed in 15% of the isolates from humans, while in pigs and domestic pork, resistance levels were markedly lower (1% and 0%, respectively). Resistance towards 3rd generation cephalosporins, cefotaxime and ceftazidime, was found in 2% of the human isolates, and no resistance was found in pig and domestic pork isolates. Carbapenem resistance (meropenem) was not observed in *Salmonella* spp. from humans, pigs and domestic pork. Colistin resistance was not reported in *Salmonella* spp. isolates from domestic pork and a low resistance level was found in isolates from pigs (one monophasic *S.* Typhimurium isolate) and human isolates (6%). The majority of these isolates, 37 out of 39 (95%), were *S.* Enteritidis or *S.* Dublin, which are intrinsically resistant to colistin. Gentamicin resistance was found in 2% of the human isolates. Similar levels were observed in pigs (2%), while in domestic pork a higher level was recorded (8%). Similar amikacin resistance levels were found in human isolates (2%) however, most of the amikacin-resistant isolates exhibited MIC values close to the ECOFF. Tigecycline resistance was comparable across *Salmonella* spp. isolates from humans (3%), pigs (2%) and domestic pork (3%) however, many of these isolates were reported with MICs within one dilution range of the new ECOFF established in 2021 (ECOFF changed from MIC >1 mg/L to MIC >0.5 mg/L).

The most frequently observed resistance among isolates from humans, pigs and domestic pork was found for ampicillin (19%, 45% and 46%, respectively), tetracycline (19%, 46%, and 54%, respectively), and sulfamethoxazole (18%, 49% and 68%, respectively).

DANMAP focuses particularly on phenotypic resistance in *S.* Typhimurium and the related monophasic variant 4,[5],12:i:-, as these serotypes are predominant in clinical human isolates and isolates from food-producing animals, especially pigs. Clonal dissemination plays an important role in the occurrence of antimicrobial resistance among *S.* Typhimurium and monophasic *S.* Typhimurium. The global dissemination of genomic islands conferring resistance to ampicillin, sulfamethoxazole and tetracycline (the ASuT multidrug-resistance profile) among monophasic *S.* Typhimurium continues to contribute to a high level of multidrug-resistance among isolates from animals and humans in Denmark. In previous versions of DANMAP, the reported data included both *S.* Typhimurium and the monophasic variant with antigenic formula S. 4, [5],12:i:-, but **in 2023 antimicrobial resistance in these serotypes is presented separately**, unless otherwise indicated.

Table 6.3 Resistance (%) in *Salmonella* spp. isolates from pigs, domestic pork and humans, Denmark, 2023 DANMAP 2023

	Pigs	Pork	Human		
	Danish	Danish	Domestically acquired	Travel abroad reported	Total
Antimicrobial agent	%	%	%	%	%
Amikacin	0	2	2	2	2
Ampicillin	45	46	23	15	2
Azithromycin	1	0	1	3	1
Cefotaxime	0	0	1	3	2
Ceftazidime	0	0	1	3	2
Chloramphenicol	9	9	6	4	5
Ciprofloxacin	1	0	14	18	15
Colistin	1	0	5	5	6
Gentamicin	2	8	2	3	2
Meropenem	0	0	0	0	0
Nalidixic acid	1	0	13	17	14
Sulfamethoxazole	49	68	21	16	18
Tetracycline	46	54	22	18	19
Tigecycline	2	3	3	5	3
Trimethoprim	22	34	4	3	4
Fully sensitive (%)	46	25	65	62	64
Multidrug resistance (%)	48	52	25	24	22
Number of isolates	82	65	355	266	697

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 travel cases and infections of unknown origin. An isolate is considered fully sensitive if susceptible to all antimicrobial agents included in the test panel An isolate is categorised as multidrug resistant if resistant to three or more of the 11 tested antimicrobial classes (Chapter 10, Table 10.3)

Resistance in *S.* Typhimurium and monophasic *S.* Typhimurium

In 2023, a total of 93 *S.* Typhimurium and 81 monophasic *S.* Typhimurium from humans were susceptibility tested. Of these, 28 *S.* Typhimurium and 20 monophasic *S.* Typhimurium were from travel associated cases. Also in 2023, six and 12 *S.* Typhimurium and 31 and 18 monophasic *S.* Typhimurium isolates from pigs and Danish pork, respectively, were tested. Two monophasic *S.* Typhimurium variants with different antigenic formulas were also recovered and tested. However, results are only reported for isolates identified exactly as variant 4,[5],12:i:-.

The resistance data for *S.* Typhimurium and monophasic *S.* Typhimurium from pigs, domestic pork and human isolates are presented in Table 6.4.

Ampicillin-, sulfamethoxazole- and tetracycline resistance are common in isolates from humans, pigs and domestic pork. As in previous years, the occurrence of resistance continued to be overall higher in isolates from domestic pork than in isolates from humans. Notably, no fully sensitive isolates were observed in Danish pork in *S.* Typhimurium and the monophasic variant, contrary to the 10% observed in the previous year. The level of full sensitivity in *S.* Typhimurium isolates from pigs and human cases was 50% and 66%, respectively and the level of full sensitivity among pig and human monophasic *S.* Typhimurium isolates was comparable at lower levels (10% and 6%, respectively).

Among monophasic *S.* Typhimurium isolates from humans, the level of resistance towards 3rd generation cephalosporins, cefotaxime and ceftazidime, was low in domestically acquired cases (2% to 4%) and higher in travel-associated cases (10%). Similarly, and as in previous years, resistance was not observed in *S.* Typhimurium and monophasic *S.* Typhimurium isolates from pigs and domestic pork (Table 6.4).

After a decreasing trend from 77% to 56% between 2020 and 2022, the occurrence of resistance to tetracycline in *S.* Typhimurium and monophasic *S.* Typhimurium from Danish pork increased markedly to 83% and 89% in 2023, respectively, and was similarly high among monophasic *S.* Typhimurium isolates from pigs (83%). However, resistance to tetracycline in *S.* Typhimurium from pigs was markedly lower (33%), but from a very small number of isolates. After a steady reduction in the use of tetracyclines in pig production observed since 2014, an increase in use has been observed in all age categories in 2022 and 2023 (see Chapter 4, Figure 4.4), which may explain the observed increase in tetracycline resistance in monophasic *S.* Typhimurium from pigs and domestic pork.

Table 6.4 Resistance (%) in *Salmonella* Typhimurium and monophasic *S.* Typhimurium isolates from pigs, domestic pork and humans, Denmark, 2023 DANMAP 2023

	S. Typhimurium		Monophasic *S. Typhimurium*		*S. Typhimurium*			Monophasic *S. Typhimurium*		
	Pigs	Pork	Pigs	Pork	Human					
	Danish	Danish	Danish	Danish	Domestically acquired	Travel abroad reported	Total	Domestically acquired	Travel abroad reported	Total
Antimicrobial agent	%	%	%	%	%	%	%	%	%	%
Amikacin	0	0	0	0	0	0	0	2	0	2
Ampicillin	50	42	84	94	24	11	19	91	80	89
Azithromycin	17	0	0	0	0	7	2	2	0	1
Cefotaxime	0	0	0	0	0	0	0	4	10	5
Ceftazidime	0	0	0	0	0	0	0	2	10	4
Chloramphenicol	17	8	13	22	16	4	12	11	15	11
Ciprofloxacin	0	0	3	0	15	7	12	11	15	11
Colistin	0	0	3	0	0	0	0	0	0	0
Gentamicin	0	8	6	17	0	0	0	9	10	9
Meropenem	0	0	0	0	0	0	0	0	0	0
Nalidixic acid	0	0	3	0	13	7	11	9	5	7
Sulfamethoxazole	50	92	87	100	23	36	26	73	65	72
Tetracycline	33	83	87	89	26	43	30	82	75	79
Tigecycline	0	8	3	0	3	21	9	5	5	5
Trimethoprim	33	33	26	33	3	0	2	11	5	9
Fully sensitive (%)	50	0	10	0	71	50	66	4	15	6
Number of isolates	6	12	31	18	62	28	93	56	20	81

Results are shown separately for *S.* Typhimurium and monophasic *S.* Typhimurium with antigenic formula *S.* 4,[5],12:i:-. Isolates from Danish pork were recovered from carcass swabs collected at slaughter. 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 travel cases and infections of unknown origin. An isolate is considered fully sensitive if susceptible to all antimicrobial agents included in the test panel (Chapter 10, Table 10.3)

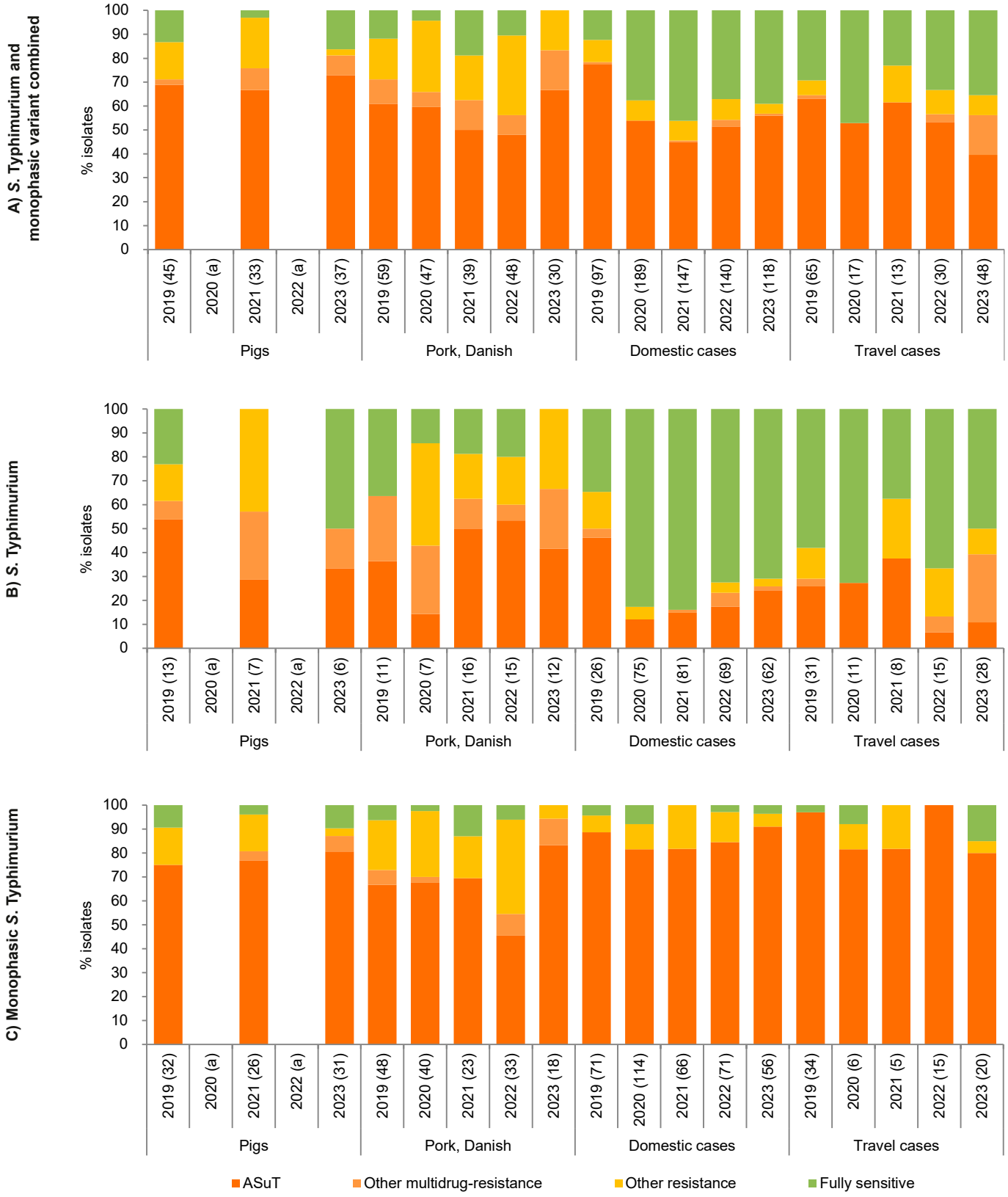
Figure 6.3 presents the relative distribution (%) of AMR profiles for *S.* Typhimurium and monophasic *S.* Typhimurium from pigs, domestic pork and human domestic- and travel-related cases. Data are presented as combined for both *S.* Typhimurium and monophasic *S.* Typhimurium (Fig 6.3A) and separately for *S.* Typhimurium (Fig 6.3B) and monophasic *S.* Typhimurium (Fig 6.3C). Please note that the number of isolates varies and in some cases is low.

Overall, MDR in *S.* Typhimurium and monophasic *S.* Typhimurium from domestic pork showed a 27% increase compared to what was observed in 2022, with an increase in the ratio between the prevalence of other MDR profiles and ASuT. This finding is opposed to the decrease in the occurrence of MDR in *S.* Typhimurium and monophasic *S.* Typhimurium from domestic pork observed between 2019 and 2022 (Figure 6.3.A).

Most *S.* Typhimurium isolates recovered from pigs and domestic pork were resistant to several antimicrobials, with MDR levels reaching 50% and 67%, respectively. In isolates from human domestic-related cases, MDR was found at lower levels (26%), while in travel-related cases a higher MDR level was observed (39%) (Fig 6.3B). The ASuT MDR profile was found in the majority of the MDR S. Typhimurium isolates from pigs, pork and human domestic cases (33%, 42% and 24%, respectively) while for human travel-related cases, other MDR profiles were more commonly found (29%; Figure 6.3.B). When looking at monophasic *S.* Typhimurium, MDR was found at markedly higher levels, with 87% and 94% of isolates from pigs and domestic pork resistant to three or more antimicrobial classes and ASuT the most found MDR profile (81% and 83%, respectively). Similarly, MDR in monophasic *S.* Typhimurium from human domestic- and travel-related cases was reported at high levels (91% and 80%, respectively), with all isolates showing the ASuT resistance profile (Figure 6.3.C).

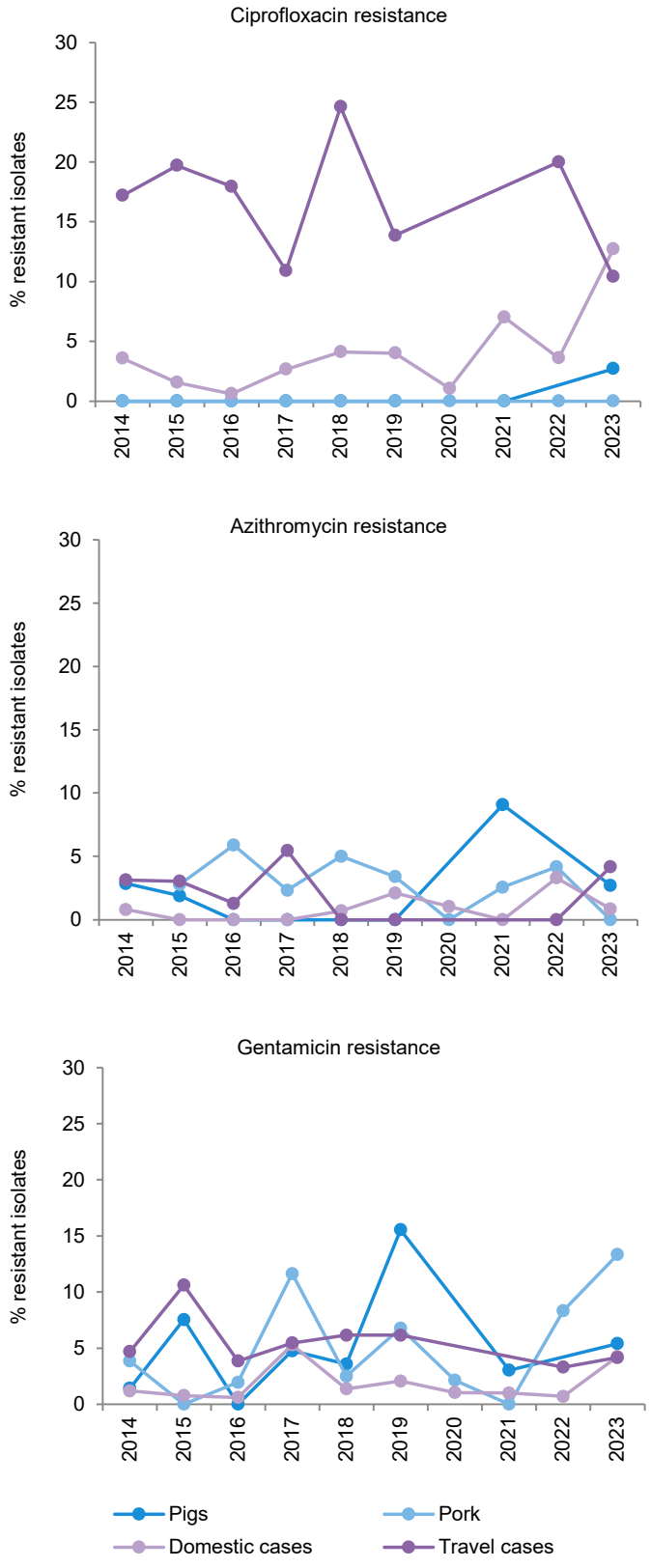
The new approach of analysing *S.* Typhimurium and monophasic *S.* Typhimurium separately reveals marked differences in their levels of MDR and especially ASuT. These are most evident in human isolates. However, caution in data interpretation should be taken in years when a small number of isolates were recovered from domestic pork, pigs and travel-associated cases.

Figure 6.3 Relative distributions (%) of AMR profiles among *Salmonella* Typhimurium and its monophasic variants combined (A), *S.* Typhimurium (B) and monophasic *S.* Typhimurium (C) from pigs, pork and human cases, Denmark, 2019-2023 DANMAP 2023



Number of isolates included each year is presented in parentheses. Includes isolates verified as monophasic variants of *S.* Typhimurium with antigenic formula *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 three or more of all antimicrobial classes included in the test panel (See Chapter 10, Table 10.3). ASuT are multidrug-resistant isolates resistant to ampicillin, sulfamethoxazole and tetracycline. Caution in data interpretation should be taken in years when a small number (n <15) of isolates were recovered from domestic pork, pigs and travel-associated cases.
a) No data;

Figure 6.4 Ciprofloxacin, azithromycin and gentamycin resistance (%) among *S.* Typhimurium and monophasic *S.* Typhimurium combined in isolates from pigs, domestic pork and human cases, Denmark, 2014-2023 DANMAP 2023



Includes isolates of *S.* Typhimurium and monophasic *S.* Typhimurium with antigenic formula 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. Due to the low number of isolates (N<15), travel-associated cases are not shown separately for 2020 and 2021. No data available for pigs in 2020 and 2022

The levels of ciprofloxacin azithromycin and gentamycin resistance (%) among *S.* Typhimurium combined with monophasic *S.* Typhimurium from pigs, domestic pork and human cases from 2014 to 2023 are presented in Figure 6.4.

Historically, ciprofloxacin resistance has predominantly been observed in isolates from travel-associated cases, however, in 2023 the opposite was observed. Ciprofloxacin resistance was observed in 13% of the isolates from domestically-acquired infections compared to 4% in 2022 and 10% of the isolates from travel-associated cases. Ciprofloxacin resistance in *S.* Typhimurium combined with its monophasic variant from pigs and Danish pork continues to be rare. In 2023, ciprofloxacin resistance was observed in a single isolate (3%) from pigs, and it was not found among isolates from domestic pork (Figure 6.4, Table 6.4).

Resistance to azithromycin in *S.* Typhimurium including monophasic *S.* Typhimurium remains at a low level in human isolates, and in 2023 it was only found in three isolates (2%). In 2021, azithromycin resistance was detected in a higher than usual (9%) percentage of isolates from pigs. However, in 2023 the occurrence in pigs returned to the low level that had been previously observed (a single resistant isolate, corresponding to 3%), and azithromycin resistance was not observed among *S.* Typhimurium and monophasic *S.* Typhimurium from domestic pork (Figure 6.4).

The levels of gentamicin resistance have been low over the last years in *S.* Typhimurium including monophasic *S.* Typhimurium from human isolates but in 2023 an increase was observed in human monophasic *S.* Typhimurium with 9% of the isolates showing resistance (Table 6.4). In domestic pork, after a two-year decrease in 2020-2021, the occurrence of resistance to gentamicin increased in 2022 (from 0% in 2021 to 8%) and again in 2023 (to 13%). Although more moderate, resistance levels to gentamicin also increased from 3% in 2021 to 5% in 2023 among *S.* Typhimurium combined with its monophasic variant isolates from pigs (Figure 6.4).

Resistance in other *Salmonella* serotypes

The resistance levels in 76 *S.* Enteritidis isolates from humans are presented in Table 6.5. Except for ciprofloxacin, nalidixic acid and colistin, the resistance levels are generally low in isolates from domestic and travel-related cases. Resistance to ciprofloxacin was observed in 39% of the isolates, with levels in domestic cases showing higher resistance levels than in travel-associated cases (47% and 32%, respectively).

S. Derby was the second most prevalent serotype in domestic pork and pigs with 25 and 40 isolates recovered, respectively.

The occurrence of resistance in *S.* Derby is generally lower than in *S.* Typhimurium and monophasic *S.* Typhimurium. In 2023, 52% of *S.* Derby isolates from domestic pork and 70% from pigs were sensitive to all tested antimicrobials. This

represents a decrease compared to the levels found in 2022 when 69% of pork isolates were sensitive. In 2023, resistance to ampicillin, tetracycline, sulfamethoxazole, and trimethoprim increased compared to the previous year (Figure 6.5). Resistance levels in *S.* Derby from pigs were similar to those found in domestic pork (Table 6.6).

Resistance to critically important antimicrobials remained rare in 2023 in *S.* Derby isolates from pigs and domestic pork. There was no resistance to azithromycin, and only 4% of pork isolates were resistant to tigecycline. Additionally, *S.* Derby isolates from pigs and domestic pork showed no resistance to amikacin, 3rd generation cephalosporins, colistin, gentamicin, meropenem, or fluoroquinolones (Table 6.6).

Table 6.5 Resistance (%) in *Salmonella* Enteritidis isolates from humans, Denmark, 2023 DANMAP 2023

	Human		
Antimicrobial agent	Domestically acquired %	Travel abroad reported %	Total %
Amikacin	0	0	0
Ampicillin	8	11	9
Azithromycin	0	0	0
Cefotaxime	0	0	0
Ceftazidime	0	0	0
Chloramphenicol	0	0	0
Ciprofloxacin	47	32	39
Colistin	42	32	37
Gentamicin	0	0	0
Meropenem	0	0	0
Nalidixic acid	47	32	39
Sulfonamide	5	0	3
Tetracycline	3	5	4
Tigecycline	5	3	4
Trimethoprim	0	0	0
Fully sensitive (%)	39	49	45
Number of isolates	38	37	76

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

Figure 6.5 Resistance (%) among *Salmonella* Derby from domestic pork, Denmark, 2014-2023 DANMAP 2023

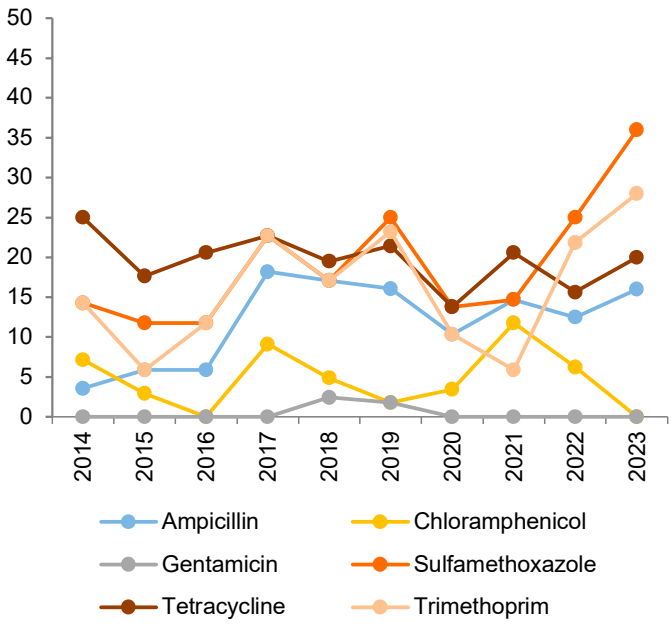


Table 6.6 Resistance (%) among *Salmonella* Derby from pigs and domestic pork, Denmark, 2023 DANMAP 2023

	Pigs	Pork
Antimicrobial agent	Danish %	Danish %
Amikacin	0	4
Ampicillin	18	16
Azithromycin	0	0
Cefotaxime	0	0
Ceftazidime	0	0
Chloramphenicol	5	0
Ciprofloxacin	0	0
Colistin	0	0
Gentamicin	0	0
Meropenem	0	0
Nalidixic acid	0	0
Sulfonamide	23	36
Tetracycline	20	20
Tigecycline	0	4
Trimethoprim	20	28
Fully sensitive (%)	70	52
Number of isolates	40	25

Isolates of Danish pork were recovered from carcass swabs collected at slaughter. An isolate is considered fully sensitive if susceptible to all antimicrobial agents included in the test panel (Chapter 10, Table 10.3)

Joana Pessoa, Ana Sofia Ribeiro Duarte and Jeppe Boel
For further information:
Animal and Food data - Joana Pessoa, joapes@food.dtu.dk
Human data - Jeppe Boel, jebl@ssi.dk

Textbox 6.1

Trends in phenotypic- and genotypic fluoroquinolone resistance in *Campylobacter jejuni* from broilers and broiler meat in Denmark

Background

Despite annual fluctuations, the occurrence of fluoroquinolone (ciprofloxacin) resistance in *Campylobacter jejuni* recovered from Danish broilers at slaughter has increased over a decade [1]. A similar trend has been observed since 2014 in several other EU member states [2]. However, such increase is not driven by direct selection pressure, since fluoroquinolones are not used in broiler production in Denmark.

The phenotypic resistance profile and the genomic background of fluoroquinolone resistance in *Campylobacter jejuni* from broilers and broiler meat in Denmark were investigated retrospectively to identify possible shifts in resistance patterns, and to explore possible explanations for the observed trend.

Materials and methods

Two datasets were gathered for the study - one consisting of minimal inhibitory concentrations (MIC) for ciprofloxacin from 2014 to 2022 for *Campylobacter jejuni* isolates from broilers; the other containing whole-genome sequencing (WGS) results from *C. jejuni* isolates collected from broilers and broiler meat in Denmark, between 2019 and 2024. All data were provided by the Danish Veterinary and Food Administration.

The MIC dataset consisted of 1,333 *C. jejuni* isolates, comprising 1028 isolates from faecal samples from Danish broilers, and 535 isolates from broiler meat from Denmark (n=305) and other four EU countries (n=230). The WGS dataset consisted of 1,051 isolates, 24 originating from Danish broilers and 1,027 from retail broiler meat, including 808 from Danish meat and 219 from imported broiler meat from seven EU countries. Isolates from imported broiler meat were aggregated under the category "EU" for further analyses.

The multilocus sequence type (MLST) and the clonal complex (CC) were determined for 955 of the sequenced isolates. Furthermore, the total 1,051 sequences were mapped against the PointFinder database for identification of point mutations and a prediction of resistance phenotypes using the ResFinder platform version 4.5.0 [3].

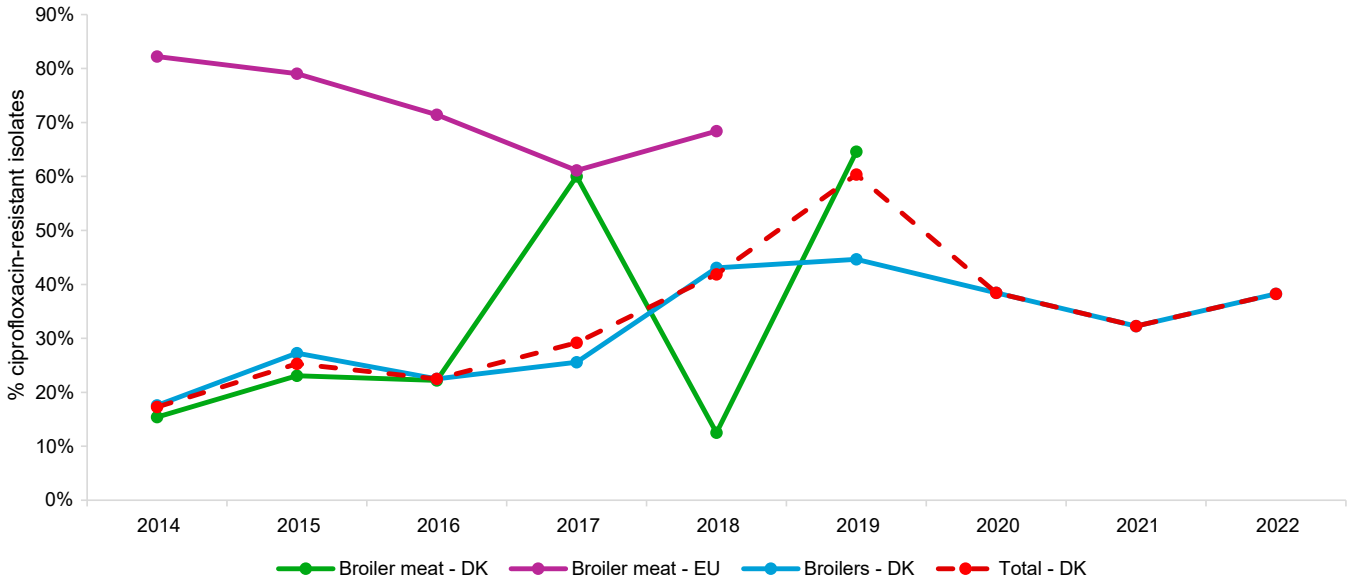
The statistical significance of the increasing trend in phenotypic ciprofloxacin resistance, and of the difference between MLST frequencies among isolates predicted as sensitive and resistant was assessed. Further analyses were performed and are available for consultation [4].

Results and discussion

Phenotypic resistance

The percentage of ciprofloxacin-resistant isolates recovered from the three different sample categories is shown in Figure 1. Between 2014 and 2016, the occurrence of ciprofloxacin resistance among isolates from imported meat was markedly higher than that observed in isolates from domestic meat. Within that period, while the first followed a decreasing trend, the latter showed an increase over time, and both reached the same value by 2017. Phenotypic ciprofloxacin resistance in *C. jejuni* from Danish broilers showed a significant increasing trend between 2014 and 2022. The same was true when considering resistance occurrence among all isolates of Danish origin (Figure 1).

Figure 1 Percent occurrence of ciprofloxacin resistance among *C. jejuni* isolates from broilers and broiler meat sampled in Denmark, 2014-2022
DANMAP 2023



The number of isolates tested from each source varied between years. The total number of isolates of Danish origin (broilers + broiler meat) tested each year were as follows: 2014 (191), 2015 (83), 2016 (178), 2017 (48), 2018 (203), 2019 (265), 2020 (164), 2021 (31), 2022 (170). In 2017 and 2018, there were less than ten isolates tested from domestic broiler meat. MIC results from *C. jejuni* isolates from imported- and Danish broiler meat were only available until 2018 and 2019, respectively

Genotypic resistance

In the period between 2019 and 2023, a total of 364 out of 1,044 (35%) *C. jejuni* isolates were predicted as quinolone-resistant, and no significant increasing or decreasing trend in the occurrence of resistance was detected, with the percentage of isolates predicted as resistant being stable at around 30-40% in the four years considered.

There were 49 different mutations of the *gyr*A gene detected among the sequenced isolates. However, only three mutations were present among isolates predicted as quinolone-resistant – predominantly mutation T86I, followed by T86A and P104S. Isolates with the mutation P104S are usually double mutants, also having T86I.

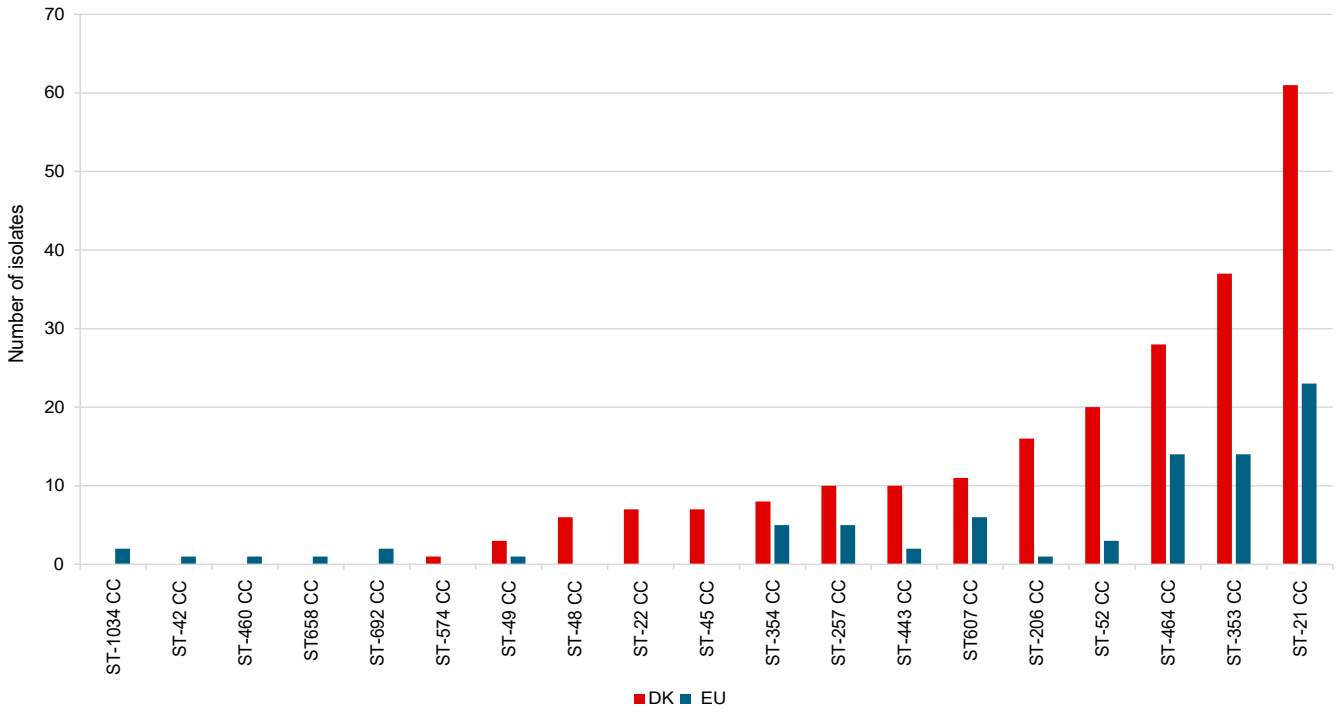
Phylogenetic typing

Among the 955 isolates with an identified sequence type (ST), 329 presented mutations in the *gyr*A gene and were predicted resistant to quinolones, belonging to 51 different STs. The relative distribution of STs was statistically different between the isolates predicted as sensitive to quinolones and those with a mutation conferring quinolone resistance.

The 51 STs identified among resistant-predicted isolates were grouped into 19 clonal complexes (CC). Fourteen CCs were found among isolates from Denmark, three of which exclusively. Likewise, five CCs were only present in isolates from imported EU broiler meat, although in very low numbers (Figure 2).

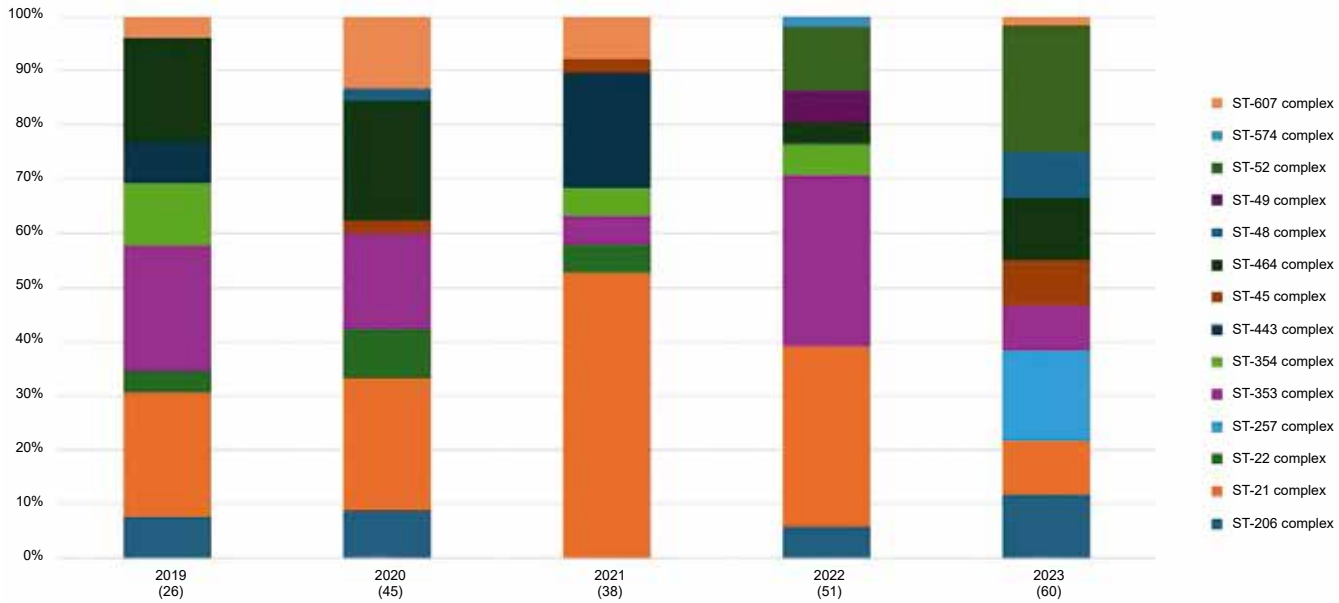
continued ... Textbox 6.1

Figure 2 Distribution of clonal complexes (CCs) among resistant-predicted *C. jejuni* isolates of Danish- and EU origin DANMAP 2023



The distribution of CCs among resistant-predicted isolates of domestic- and EU origin were mostly similar, with the exception of CCs ST-52 and ST-206, which were the fourth and fifth most common among Danish isolates, respectively, but less common among isolates from imported meat. The distribution of CCs among resistant *C. jejuni* mutants from Denmark between 2019 and 2023 is shown in Figure 3.

Figure 3 Percentage of clonal complex abundance among *C. jejuni* resistant mutants of Danish origin, 2019-2023 DANMAP 2023



Total number of resistant-predicted isolates per year is shown in parentheses

While predominant clonal complexes such as ST-21 CC and ST-353 CC appeared every year as expected, other CCs such as ST-45 CC, ST-48 CC, ST-49 CC and ST-574 CC were observed only occasionally. The relative distribution of CCs among quinolone resistant-predicted isolates from Denmark appears more balanced in 2023 compared to previous years, with an increased presence of ST-206, ST-22, ST-48 and ST-45, and a decreased presence of ST-21 CC and ST-353 CC. Interestingly, in 2023, ST-52 CC was the most abundant among Danish resistant mutants ST-52 has been associated with a recent long-lasting outbreak of human campylobacteriosis in Denmark [5], which began in 2021. A phylogenetic analysis comparing ST-52 resistant mutants from broilers and the outbreak ST-52 isolates would be of interest to investigate whether resistant *C. jejuni* has been involved in the outbreak. Also, in 2023, the ST-257 complex appeared for the first time during the period considered. In a recent retrospective study of *C. jejuni* isolates from human clinical cases in the United Kingdom, the clonal complex ST-257 appears among many CCs that showed an increase in the levels of fluoroquinolone resistance over the last two decades [6].

Conclusion

The phenotypic analysis confirmed the existence of an increasing trend of ciprofloxacin-resistant *C. jejuni* isolates in Danish broilers over the last decade, however this trend was not observed for isolates predicted as quinolone-resistant based on the presence of a *gyr*A point mutation. Further studies are needed in order to investigate the cause for this discrepancy.

The phylogenetic typing of the resistant-predicted isolates from Danish broiler meat revealed a shift in variability in 2023, including an increased presence of the clonal complexes ST-52 and ST-257, compared to previous years. Previous studies have shown that resistance patterns within certain clonal complexes can change over time.

Ana Sofia Ribeiro Duarte
For further information: asrd@food.dtu.dk

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7

RESISTANCE IN INDICATOR BACTERIA

7. Resistance in indicator bacteria

Highlights: Over the last five-year monitoring period, there have been no statistically significant trends in the prevalence of fully sensitive **indicator *E. coli*** from broilers or pigs. However, in the same period, there was a significant decrease in fully sensitive *E. coli* from cattle.

As in previous years, no colistin, meropenem or tigecycline resistance were detected in indicator *E. coli*. Amikacin resistance was detected in single isolates from broilers and pigs. Resistance to ciprofloxacin continued to be low in cattle and pigs, and after the increase observed in the previous year, it has decreased by 3% in broilers. Similarly to 2022, in 2023, azithromycin resistance was detected in a small number of isolates from pigs (3%) and additionally in 2% of broiler isolates. Additionally, in cattle, increases between 2% and 7% were observed in the resistance to ampicillin, chloramphenicol, sulfamethoxazole, tetracycline and trimethoprim.

The relative occurrence of multidrug-resistant indicator *E. coli* compared to the previous year increased in broilers and cattle, however a significant increasing trend over the past five years was only detected for cattle. Combined resistance to ampicillin, sulfamethoxazole, and tetracycline (ASuT) continued to be the most common multidrug-resistance profile among *E. coli* from cattle and pigs, however the relative occurrence of other profiles has increased in isolates from cattle in the past five years.

Importantly, as in previous years, samples from pigs, cattle and their meat examined for **carbapenemase-producing (CP) *E. coli*** (including OXA-48) were found negative.

The occurrence of **beta-lactamase-producing *E. coli***, obtained through selective procedures, continued the decreasing trend observed since 2019 in Danish cattle and pigs, and in imported pork, and was also lower in beef (domestic and imported) in 2023, compared to 2021. Antimicrobial susceptibility testing of ESBL-/AmpC-producing *E. coli* from imported beef showed, like in previous years, a very high (100%) occurrence in resistance to fourth generation cephalosporins (cefepime). Isolates from pigs and pork (domestic and imported) showed a decrease in occurrence of resistance to cefepime, compared to 2021. A single isolate from imported pork was found resistant to ertapenem, while one isolate from cattle and three isolates from pigs were found resistant to imipenem.

Whole genome sequencing of beta-lactamase-producing *E. coli* revealed ESBL, AmpC and ESBL+AmpC genotypes. All but one AmpC genotypes encoded upregulated AmpC promotor C-42T mutations, which was also observed in two ESBL+AmpC genotypes, one from pigs and another from cattle. Among the ESBL genotypes, 11 different ESBL genes were detected, with the most frequent being *CTX-M-1* and *CTX-M-15*.

In 2023, 22% of ***E. faecalis*** isolated from pigs were fully sensitive. None of the isolates showed resistance to ampicillin, ciprofloxacin, linezolid, teicoplanin, tigecycline or vancomycin. Combined resistance to chloramphenicol, tetracycline and erythromycin was the most common resistance profile. The overall decrease in resistance observed in 2021 did not continue in 2023.

7.1 Introduction

Escherichia coli and *Enterococcus* are included in the DANMAP programme to monitor the occurrence of antimicrobial resistance (AMR) in different reservoirs throughout 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 horizontal 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 the production of extended-spectrum beta-lactamases (ESBLs) and AmpC beta-lactamases (AmpCs) is among the fastest spreading antimicrobial resistance mechanisms in both humans and food-producing animals worldwide. Some studies have suggested a zoonotic transmission of ESBL/AmpC-producing *E. coli* [Liu et al 2023. One Health, 16: 100518; Roer et al 2019. J Antimicrob Chemother, 74(3):557; Mughini-Gras et al 2019. Lancet Planet Health, 3(8): e357-e369; Liu et al 2018. mBio, 9(4): e00470-18], thus the occurrence of ESBL/AmpC- producing *E. coli* in food-producing animals and their meat is considered of public health relevance. Accordingly, in the harmonized EU monitoring of AMR in zoonotic and indicator bacteria, this is considered a key outcome indicator [EFSA/ECDC 2024, EFSA Journal 2024; 22(2):e8583], for which trends are assessed annually at EU- and Member State level. The zoonotic nature of ESBL/AmpC-producing *E. coli* isolated in Denmark from humans, animals and meat is addressed in Chapter 3.

Carbapenemase-producing Enterobacteriaceae (CPE) pose a great threat to human health, as carbapenems are last-line antimicrobial drugs for the treatment of infections caused by multidrug-resistant Gram-negative bacteria. The monitoring of CP-producing *E. coli* is mandatory in healthy food-producing animals (broilers, fattening turkeys, fattening pigs and calves) and their derived meat. In recent years, CP-producing *E. coli* have been increasingly detected in food-producing animals in the EU, which raises the concern that animals might become a CPE reservoir in the future [EFSA/ ECDC 2024, EFSA Journal 2024; 22(2):e8583].

Isolation and antimicrobial susceptibility testing of indicator *E. coli*, indicator enterococci and ESBL-/AmpC-producing and carbapenemase (CP)-producing *E. coli* are performed in accordance with the EU harmonised monitoring of antimicrobial resistance [Decision 2020/1729/EU]. In 2023, isolates were obtained from randomly selected caecal samples collected from healthy broilers, cattle (calves under one year of age), and fattening pigs at slaughter. Additionally, for the specific monitoring of ESBL-/AmpC- and CP-producing *E. coli*, fresh meat from pigs and cattle was collected at retail and at border control posts (BCPs). Details on sampling, analysis, susceptibility testing and interpretation of results are presented in Chapter 10.

7.2 Indicator *Escherichia coli*

Indicator *E. coli* isolates were obtained from 97% of caecal samples from broiler flocks (125/129), 98% of samples from pigs (173/176) and 98% of samples from cattle (169/172). These isolates were obtained with a non-selective isolation procedure. Results obtained by selective procedures for specific monitoring of ESBL-/AmpC- and CP-producing *E. coli* are presented in Section 7.3.

7.2.1 Indicator *Escherichia coli* from broilers, cattle and pigs

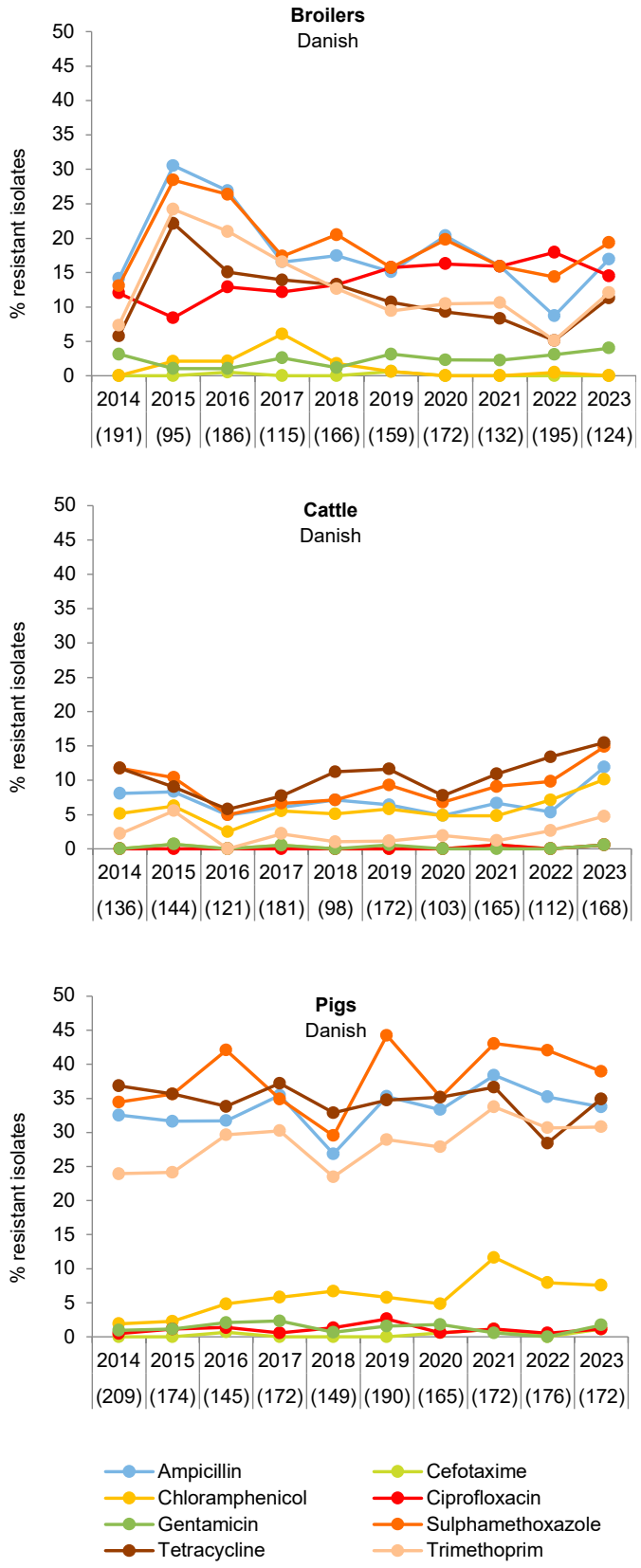
There has been no statistically significant increasing or decreasing trend in the annual prevalence of fully sensitive *E. coli* isolates from broilers or pigs during the past five years of monitoring (Figure 7.2) (p-values of 0.36 for broilers, and 0.10 for pigs). After a consecutive increase to 64% in 2021 and to 67% in 2022, in 2023 the percentage of broiler isolates susceptible to all antimicrobials in the test panel decreased to 60%, while compared to 2022, the percentage of fully sensitive pig isolates (48%) decreased by 1%. A significant decrease in the proportion of fully sensitive *E. coli* between 2019 and 2023 was detected in cattle (p-value=0.04). The percentage of fully sensitive cattle isolates (82%) continued in 2023 the decrease already observed in 2021 (to 87%) and 2022 (to 84%) (Table 7.1).

Table 7.1 Resistance (%) in *Escherichia coli* isolates from broilers, cattle and pigs, Denmark, 2023 DANMAP 2023

	Broilers	Cattle	Pigs
	Danish %	Danish %	Danish %
Amikacin	<1	0	<1
Ampicillin	17	12	34
Azithromycin	2	0	3
Cefotaxime	0	<1	1
Ceftazidime	0	<1	<1
Chloramphenicol	0	10	8
Ciprofloxacin	15	<1	1
Colistin	0	0	0
Gentamicin	4	<1	2
Meropenem	0	0	0
Nalidixic acid	15	0	0
Sulphamethoxazole	19	15	39
Tetracycline	11	15	35
Tigecycline	0	0	0
Trimethoprim	12	5	31
Fully sensitive (%)	60	82	48
Number of isolates	124	168	172

An isolate is considered fully sensitive if susceptible to all antimicrobial agents included in the test pane (Chapter 10, Table 10.3)

Figure 7.1 Resistance (%) among *Escherichia coli* isolates from broilers, cattle and pigs, Denmark, 2023 DANMAP 2023



The number of isolates included each year is shown in parentheses

Compared to 2022, the occurrence of resistance to most antimicrobials in the test panel suffered none or mostly minor fluctuations (1 to 3%). A few exceptions were found in isolates from broilers, where resistance to ampicillin, sulfamethoxazole, tetracycline and trimethoprim increased back to levels like those observed in 2021, after the decrease observed in 2022. The inverse was detected for resistance to fluoroquinolones, which decreased in 2023 back to levels similar to those of 2021. In isolates from pigs, resistance to sulfamethoxazole continued the decrease by 3% also observed in the previous year, while resistance to tetracycline increased by 11%, returning to a level similar to 2021. Although by a limited magnitude (between 2% and 5%), an increase in the percentage of resistance to chloramphenicol, sulfamethoxazole, tetracycline and trimethoprim was observed in *E. coli* from cattle, similarly to the trend seen in 2022. Additionally, the occurrence of resistance to ampicillin among cattle isolates increased by 7% from 2022 to 2023 (Figure 7.1).

As in previous years, no isolates resistant to colistin, meropenem or tigecycline were detected. In 2023, amikacin resistance was detected in a single isolate from cattle and in a single isolate from broilers. Azithromycin resistance was, similarly to previous years, detected in 3% of the isolates from pigs, and notably detected in 2% of the broiler isolates. Azithromycin resistance has been previously detected in broiler isolates in 2015 (1%) and 2020 (<1%). As in previous years, resistance to 3rd generation cephalosporins was not detected or detected at very low levels (up to 1%) in indicator *E. coli* using non-selective methods (Table 7.1).

Resistance to fluoroquinolones continues to be very low (up to 1%) or not observed in *E. coli* from cattle and pigs, and it has notably decreased by 3% in 2023 compared to 2022 among broiler isolates (Figure 7.1). The following monitoring years will disclose whether this represents a shift in the trend of fluoroquinolone resistance or a simple oscillation on the significant increasing trend of the past 10-years of monitoring (p-value=0.01).

The occurrence of resistant and multidrug-resistant (MDR) *E. coli* in broilers did not continue the decrease observed in the two previous years. Instead, MDR increased from 10% in 2022 to 16% in 2023, mostly due to an increase (from 8% to 13%) in occurrence of MDR patterns other than resistance to ampicillin, sulfamethoxazole and tetracycline (ASuT). In pigs, occurrence of MDR and other resistance patterns continues to appear relatively stable, with a 2% increase in the percentage of ASuT isolates in 2023 compared to 2022. In cattle, there is a significant increasing trend in the occurrence of multidrug-resistant *E. coli* since 2019 (p-value=0.005), and in 2023 there was a particularly marked increase in the percentage of ASuT isolates (9%), compared to the previous four monitoring years (Figure 7.2). ASuT resistance continues to be the predominant MDR profile in isolates from pigs (20%), and isolates from cattle (9%).

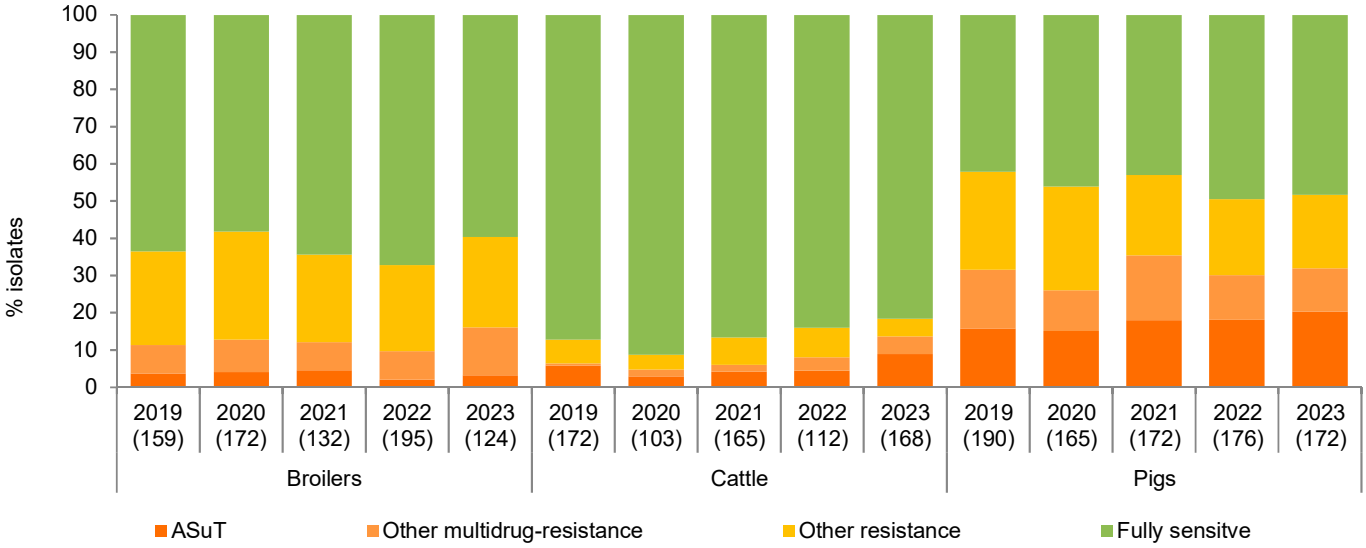
Among indicator *E. coli* isolated with a non-selective procedure, presumptive ESBL/AmpC-producing isolates were found in two samples from pigs and one sample from cattle (Table 7.1) After testing with the second antibiotic panel for confirmation of ESBL/AmpC-producing phenotype, one of the isolates from pigs did not show resistance to third- and fourth-generation cephalosporins.

7.2.2 Perspectives

At the EU level, full sensitivity in indicator *E. coli* isolated from broilers, pigs and cattle varies greatly between countries. The levels observed in 2023 in Denmark are all well above the observed EU median values in 2022 (19% in broilers, 38% in pigs and 54% in cattle). Overall, at EU level, and in many individual European countries, a significant increase in the occurrence of fully-sensitive isolates has been detected in broilers between 2014 and 2022. A similar trend has been observed in fewer individual countries, but not at the EU level, among isolates from pigs and cattle. No significant increasing or decreasing trends in full sensitivity were reported for Denmark between 2014 and 2022, individually, in the latest European Summary Report [EFSA and ECDC 2024, EFSA Journal 2024;22:e8583].

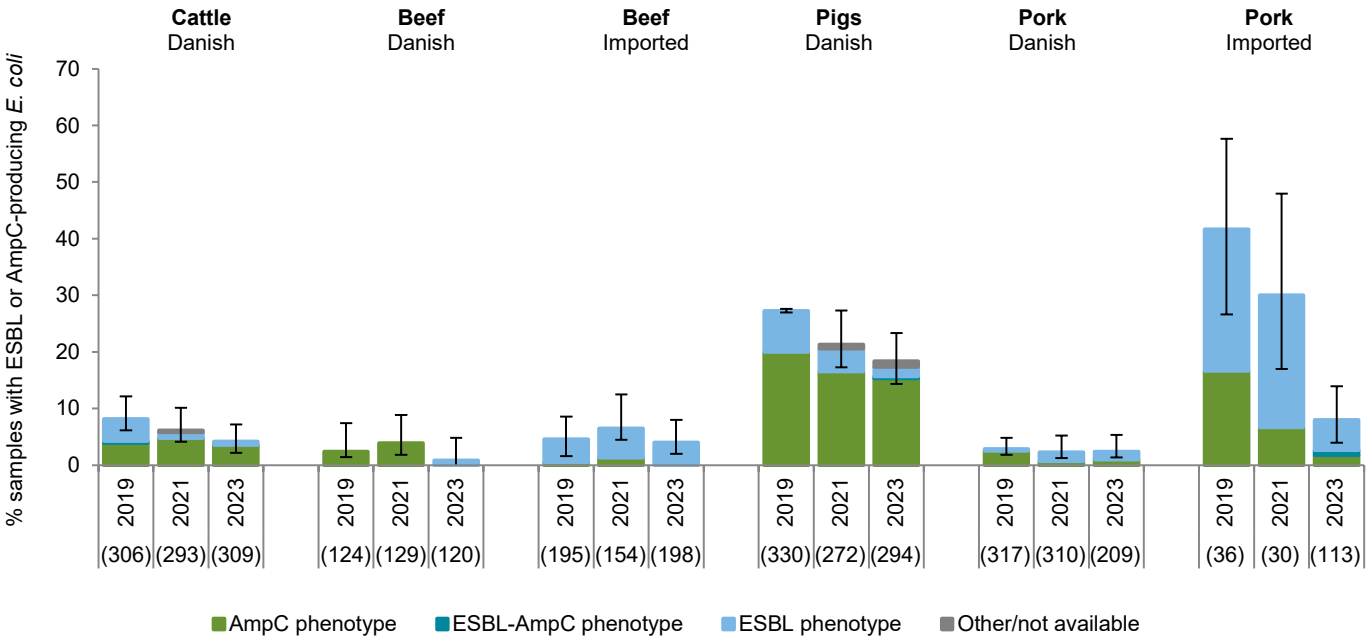
Accordingly, and as in previous years, in DANMAP 2023 no significant increasing or decreasing trends were observed in the occurrence of fully sensitive indicator *E. coli* recovered from broilers or pigs in the last five-year monitoring period (in DANMAP 2023, between 2019 and 2023). An increase of 36% in the systemic antibiotic treatment of young cattle has been observed since 2012. Also, the use of amphenicols and macrolides in calves, has been increasing steadily sinde 2014 and 2020, respectively (Chapter 4, Section 4.3.2). Such increases in consumption may explain the increasing trend in the occurrence of resistant and multidrug-resistant *E. coli* in calves. It is possible that different trends are detected when different monitoring periods are included in the analysis, and/or different methods are applied. In DANMAP 2022, no significant trend was observed among the same isolates in the period 2014 to 2022 [DANMAP 2022, Textbox 7.1].

Figure 7.2 Relative distributions (%) of fully sensitive, resistant and multidrug-resistant *Escherichia coli* isolates from broilers, cattle and pigs, Denmark, 2023
DANMAP 2023



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 three or more of the 12 antimicrobial classes included in the test panel (Chapter 10, Table 10.3). ASuT are the multidrug-resistant isolates resistant to ampicillin, sulfamethoxazole and tetracycline

Figure 7.3 Occurrence (%) of samples with phenotypic ESBL- or AmpC-producing *E. coli* from animals and meat recovered by selective enrichment, Denmark, 2019-2023 DANMAP 2023



Number of samples tested per year is presented in parentheses. Classification of ESBL and AmpC phenotypes is based on the MIC results (Chapter 10, section 10.7.2). The total number of samples of imported beef include 192 samples collected at retail and 6 samples collected at border control posts (BCPs). The total number of samples of imported pork include 110 samples collected at retail and 3 samples collected at BCPs

At the EU level, a significant negative association has been again determined in the most recent JIACRA IV report between the probability of full sensitivity in indicator *E. coli* and the overall consumption of antimicrobials by food-producing animals, observed between 2018 and 2021 [ECDC, EFSA and EMA 2024, EFSA Journal 2024; 22:e8589]. The same report determined a significant decreasing trend in overall antimicrobial consumption by food-producing animals in 20 countries and a corresponding increasing trend in the proportion of fully sensitive indicator *E. coli* in 10 of those countries, including Denmark. JIACRA IV also showed a systematic trend analysis, with results indicated by combination of bacterial species and antimicrobial class. Here, in food-producing animals, statistically significant associations between decrease in antimicrobial use and decrease in antimicrobial resistance in indicator *E. coli* were detected for third- and fourth-generation cephalosporins (3 countries), fluoroquinolones (4 countries), polymyxins (3 countries), aminopenicillins (4 countries) and tetracyclines (14 countries). For Denmark, a significant association between use and resistance in indicator *E. coli* was only observed for polymyxins, specifically for the decrease in colistin use and resistance between 2014 and 2021. This was most likely due to the abrupt decrease to almost zero in the use of colistin in pigs that occurred in 2017 after the increase of the multiplication factor for colistin in the Yellow Card.

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

In 2023, caecal samples collected from pigs and cattle at slaughter, from packages of fresh, chilled pork and beef collected from Danish wholesale and retail outlets, and consignments of pork and beef meat sampled at Danish border control posts were monitored for the presence of extended-spectrum beta-lactamase (ESBL)- , cephalosporinase (AmpC)-, and carbapenemase(CP)-producing *E. coli*. In accordance with the harmonised EU monitoring rules [Decision 2020/1729/EU], packages of meat were collected at retail and border control posts without pre-selecting by country of origin. Of the samples randomly collected at retail, 34% of pig meat and 62% of cattle meat were imported products. Additionally, three samples of imported pork and six samples of imported beef were collected at border control posts.

As in previous years, the selective procedures for detection of CP-producing *E. coli* (including oxacillinase-producing OXA-48-like enzymes), recovered no isolates.

7.3.1 ESBL-/AmpC-producing *E. coli* from pigs, pork, cattle and beef

Following selective enrichment, ESBL/AmpC-producing isolates, i.e. *E. coli* resistant to 3rd generation cephalosporins (cefotaxime and/or ceftazidime), were obtained from 54/294 samples from pigs (18%; CI 95%: 14-23%), 5/209 samples from Danish pork (2%; CI 95%: 1-5%), 9/113 samples from imported pork (8%; CI 95%: 4-14%) , 13/309 samples from cattle (4%; CI 95%: 2-7%), 1/120 samples from Danish beef (1%; CI 95%: 0-5%) and 8/198 samples from imported beef (4%; CI 95%: 2-8%) (Table 7.2, Figure 7.3). ESBL/AmpC-producing *E. coli* was not detected in samples collected at border control posts.

In 2023, in comparison to 2021, the prevalence of ESBL/AmpC-producing *E. coli* has overall decreased in all tested animal/food categories. Specifically, the proportion of cattle and beef samples that tested positive decreased by a magnitude of 2%, while prevalence among samples from Danish pigs decreased by 3% since 2021, and showed a significant decrease over the three last monitoring years (p-value = 0.02). Notably, the occurrence of ESBL/AmpC-producing *E. coli* decreased in imported pork from 42% in 2019 and 30% in 2021 to 8% in 2023. This large decrease needs to be interpreted with care due to the low number of samples tested in 2019 and 2021 (n=36 and n=30, respectively) in comparison to 2023 (n=113). In 2021, the 95% confidence interval for the proportion of positive imported pork samples was 17-48%, thus the occurrence of a decreasing trend may be a possibility, however it needs to be confirmed in the upcoming monitoring years. As observed previously, the occurrence of ESBL/AmpC-producing *E. coli* continues to be higher in imported meat compared to domestic meat (Figure 7.3). This difference is particularly obvious for pork, with a difference of 10% in ESBL/AmpC occurrence.

In 2023, the relative frequency of ESBL-producing and AmpC-producing phenotypes remained mostly unchanged in comparison to 2021, except among isolates from Danish beef, which showed a predominance of the ESBL-producing phenotype in 2023, unlike the two previous monitoring years (Figure 7.3).

All the recovered ESBL/AmpC-producing isolates were resistant to both 3rd generation cephalosporins (cefotaxime and ceftazidime) and to ampicillin. Isolates from imported beef were additionally 100% resistant to the 4th generation cephalosporin cefepime. In the previous monitoring year, imported beef isolates also presented the highest occurrence of cefepime resistance among all animal and meat categories sampled (90%). Compared to 2021, in 2023 the observed proportions of resistance to cefepime remained similar in isolates from cattle (with a 2% decrease) and decreased among isolates from pigs (by 19%), domestic pork (by 26%) and imported pork (by 11%) (Table 7.2).

In 2023, resistance to azithromycin was observed in one out of nine isolates (8%) from cattle, one out of eight isolates (13%) from imported beef, nine out of 54 (17%) isolates from pigs and in two out of five (40%) isolates from domestic pork, which represents an overall increase, compared to the proportions observed in 2021. The prevalence of resistance to ciprofloxacin was also higher in 2023 than in 2021 among isolates from cattle (three out of 13), imported beef (five out of eight), and imported pork (three out of nine).

As in 2021, no resistance to colistin or meropenem was observed in the specific monitoring of ESBL/AmpC-producing *E. coli*. However, ertapenem resistance was observed in one out of nine isolates (11%) from imported pork, while imipenem resistance was observed in one out of 13 isolates (8%) from cattle and in three out of 54 isolates (6%) from pigs.

As in previous years, resistance to tigecycline was not observed among the isolates collected in 2023, and temocillin resistance was detected in a single isolate from pigs. Resistance to sulfamethoxazole, tetracycline and trimethoprim remained common among the recovered ESBL/AmpC-producing isolates (Table 7.2).

The genetic basis for ESBL and AmpC enzymes was detected in all isolates recovered by selective enrichment. The detected enzymes corresponded to the phenotypes derived from the susceptibility testing for the majority of the isolates. In one isolate from cattle, whole genome sequencing revealed a ESBL and AmpC-producing genotype, even though this was not detected with susceptibility testing, while another cattle isolate presented a AmpC phenotype and a ESBL genotype. One isolate from imported beef showed a ESBL phenotype, but a AmpC genotype. Among isolates from pigs, one isolate with AmpC phenotype did not show a AmpC genotypic profile, and three isolates without a ESBL or AmpC phenotype, were determined as ESBL-producers with whole genome sequencing. Finally, one isolate from imported pork showed a ESBL- and AmpC-producing phenotype, which was not confirmed in the genotype (Tables 7.2 and 7.3).

Among the AmpC-producing isolates recovered in 2023, resistance was, as observed in previous years, mainly conferred by upregulated AmpC promotor C-42T mutations (10 isolates from cattle, one from imported beef, forty-four from pigs, and two from Danish and imported pork). In a single isolate from pigs the mutation T-32A was observed instead.

Among all ESBL-producing isolates, 11 different ESBL genes were detected. Overall, the most commonly observed ESBL encoding genes across all categories of animals and meat sampled in 2023 were *blaCTX-M-1* and *blaCTX-M-15*, with the latter being most abundant among isolates from imported beef. The ESBL genes *blaCTX-M-32* and *blaSHV-12* were only detected among isolates from imported beef, while *blaOXA-10*, *blaTEM-15* and *blaTEM-52B* were only detected in isolates from cattle (Table 7.3).

Among the two isolates that harboured both ESBL and AmpC genotypes, upregulated AmpC promotor C-42T mutation was detected, together with the ESBL genes *blaOXA-1* in the pig isolate, and *blaOXA-10* in the cattle isolate (Table 7.3).

In total, 50 MLSTs were observed among all ESBL/AmpC- producing *E. coli* isolates. The most common MLST was ST88, followed by ST23.

7.3.2 Perspectives

There is an ongoing decreasing trend since 2019 in the occurrence of ESBL/AmpC-producing *E. coli* in cattle (although non-significant), pigs and imported pork (significant) (Figure 7.3). Similar decreasing trends have not been observed at EU-level between 2015 and 2021 [EFSA and ECDC 2024, EFSA Journal 2024;22:e8583].

Table 7.2 Resistance (%) and beta-lactam resistance phenotype distribution in ESBL/ApmC-producing *Escherichia coli* recovered from animals and meat by selective enrichment, Denmark, 2023 DANMAP 2023

	Cattle	Beef	Pigs	Pork	
Antimicrobial agent	Danish %	Import %	Danish %	Danish %	Import %
Amikacin	0	0	0	0	0
Ampicillin	100	100	100	100	100
Azithromycin	8	13	17	40	0
Cefepime	31	100	24	60	78
Cefotaxime	100	100	100	100	100
Cefotaxime/clavulanic acid	85	0	89	40	22
Cefoxitin	85	0	85	40	33
Ceftazidime	100	100	100	100	100
Ceftazidime/clavulanic acid	85	0	89	40	22
Chloramphenicol	15	38	19	0	0
Ciprofloxacin	23	63	6	0	33
Colistin	0	0	0	0	0
Ertapenem	0	0	0	0	11
Gentamicin	8	13	11	0	11
Imipenem	8	0	6	0	0
Meropenem	0	0	0	0	0
Nalidixic acid	8	25	4	0	33
Sulfamethoxazole	38	63	70	80	56
Temocillin	0	0	2	0	0
Tetracycline	54	50	57	80	67
Tigecycline	0	0	0	0	0
Trimethoprim	31	38	46	60	22
Number of AmpC phenotypes	11	0	45	2	2
Number of ESBL phenotypes	2	8	5	3	6
Number of ESBL+AmpC phenotypes	0	0	1	0	1
Other phenotypes	0	0	3	0	0
Number of isolates (%)	13 (4%)	8 (4%)	54(18%)	5 (2%)	9 (8%)
Number of samples	309	198	294	209	113

Classification of ESBL-, AmpC- and AmpC+ESBL-producing phenotypes is based on the MIC results (Chapter 10, Section 10.7.2). AmpC, ESBL and AmpC+ESBL phenotypes indicate the number of isolates expressing each specific phenotype. Results for Danish beef are not shown since only a single ESBL-producing isolate was detected

At the EU-level, for pigs, the association between consumption of 3rd and 4th generation cephalosporins and cefotaxime resistance in indicator *E. coli* was significant both for 2019 and 2021. Similarly, despite the observed variations between countries in the consumption of 3rd and 4th generation cephalosporins and the reported occurrence of ESBL- and/or AmpC-producing *E. coli* under specific monitoring, significant positive associations were also observed between consumption and resistance, considering data from broilers, turkeys, pigs and cattle, collected in the period of 2018 to 2021 [ECDC, EFSA and EMA 2024, EFSA Journal 2024; 22:e8589].

In Denmark, 3rd and 4th generation cephalosporins are not used in the treatment of pigs or cattle. Only 1st generation cephalosporins have been used for intramammary treatments in cattle, and their use has been drastically decreasing since 2014, and was nearly absent in 2023 (Chapter 4, Table 4.1). Thus, a decrease in the consumption of 3rd and 4th generation cephalosporins is not likely to be the cause of the decrease in ESBL/AmpC-producing *E. coli* occurrence observed in Danish cattle and pigs, but it could justify the observed decreased in imported pork meat.

There is a large variation in the prevalence of ESBL-/AmpC-producing *E. coli* recovered from animals and meat in different EU countries. In 2021, prevalence ranged from 3% to 77% in pigs, 6% (in Denmark) to 59% in cattle, 0% to 19% in pork, and 0% to 31% in beef [EFSA and ECDC 2024, EFSA Journal 2024;22:e8583]. With the prevalence levels observed in 2023 (Table 7.2), Denmark continues to be among the countries with the lowest occurrence of beta-lactamase-producing *E. coli* in pigs, cattle and meat thereof.

The enzymes of the ESBL and AmpC-producing *E. coli* observed in 2023 are consistent with the enzymes detected in previous years, and also with the distribution of enzymes among isolates from pigs and cattle observed at EU-level [EFSA and ECDC 2024, EFSA Journal 2024;22:e8583].

The zoonotic transmission of beta-lactamase-producing *E. coli* continues to be investigated, with studies presenting different conclusions.

In the EU, a statistically significant association was found between resistance to 3rd generation cephalosporins in invasive *E. coli* from humans and in indicator *E. coli* from calves (2019) and broilers (2020), but not from pigs [ECDC, EFSA and EMA 2024, EFSA Journal 2024; 22:e8589]. In Chapter 3, ESBL/AmpC-producing *E. coli* isolates collected in Denmark between 2018 and 2023 from animals and meat and from human bloodstream infections were compared to address the likelihood of transmission between animals/meat and humans in Denmark.

Still, no carbapenemase-producing *E. coli* were detected in any of the samples tested in 2023. In the EU, in 2021, presumptive CP-producing *E. coli* was detected under specific phenotypic monitoring in two samples from fattening pigs. Additionally, CP-producing genotypes were detected with whole genome sequencing in 23 isolates from pigs and five isolates from cattle [EFSA and ECDC 2024, EFSA Journal 2024;22:e8583]. Since 2021 was the first year of mandatory specific monitoring of CP-producing *E. coli* in pigs and cattle (according to Decision 2020/1729/EU), it is possible that a higher number of isolates will be reported for 2023 at EU-level in the forthcoming EU Summary Report.

Table 7.3 Number of ESBL and AmpC enzymes detected in beta-lactamase-producing *E. coli* isolates from animals and meat recovered by selective enrichment, Denmark, 2023 DANMAP 2023

	Cattle	Beef		Pigs	Pork	
Enzymes	Danish	Danish	Import	Danish	Danish	Import
blaCTX-M-1		1	1	5	3	2
blaCTX-M-15	1		3			1
blaCTX-M-27						1
blaCTX-M-32			2			
blaCTX-M-55				2		3
blaDHA-1	1			2		
blaOXA-1			1	1		
blaOXA-10	1					
blaSHV-12			1			
blaTEM-15	1					
blaTEM-52B	1					
Chromossomal AmpC (T-32A)				1		
Chromossomal AmpC (C-42T)	10		1	44	2	2
Number of AmpC genotypes	9	0	1	44	2	2
Number of ESBL genotypes	3	1	7	9	3	7
Number of AmpC+ESBL genotypes	1	0	0	1	0	0
Number (%) positive samples	13 (4%)	1 (1%)	8 (4%)	54 (18%)	5 (2%)	9 (8%)
Number of tested samples	309	120	198	294	209	113

Number (%) positive samples are isolates recovered by selective enrichment methods for monitoring of beta-lactamase-producing *E. coli*. ESBL/AmpC enzymes were determined by whole genome sequencing of the recovered isolates (Chapter 10, Section 10.6)

7.4 Indicator *Enterococci*

Enterococci were obtained from 169 (34%) out of 492 faecal samples taken from pigs at slaughter, and antimicrobial susceptibility testing was subsequently performed on 87 *E. faecalis* isolates. The identified *E. faecium* isolates (n=85) were not tested for antimicrobial susceptibility.

7.4.1 *E. faecalis* from pigs

Overall, 22% of the *E. faecalis* isolates from pigs were susceptible to all antimicrobials in the test panel, a level similar to that observed in 2021, the previous year of monitoring in pigs (Table 7.4).

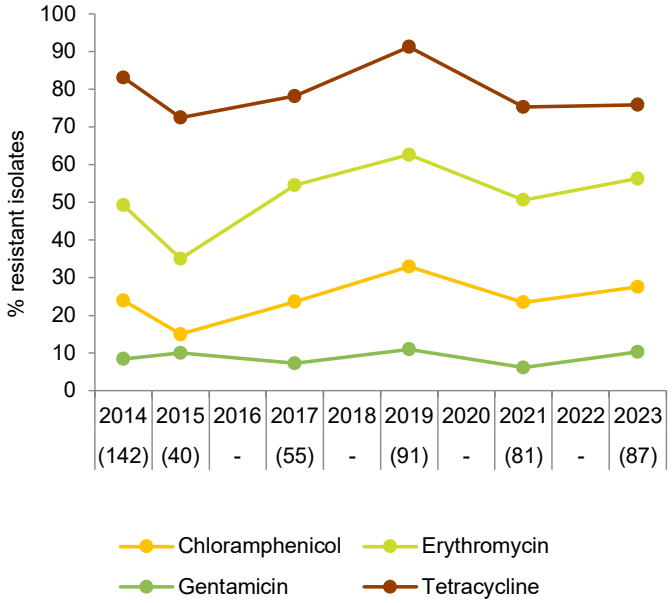
As in 2021, no *E. faecalis* isolates showed resistance to ampicillin, ciprofloxacin, linezolid, teicoplanin, tigecycline or vancomycin. Unlike 2021, in 2023, two isolates (2%) showed resistance to daptomycin. Similar low proportions of daptomycin-resistant isolates have been occasionally observed in previous years (2010, 2014 and 2019). Resistance to tetracycline (76%) and erythromycin (56%), followed by resistance to chloramphenicol (28%) continued to be the most common, and resistance to gentamicin was also observed in 10% of the isolates. Notably, after the decrease observed in 2021, the proportion of resistance to each of these four substances has increased in 2023, by magnitudes of 1% (tetracycline), 4% (gentamicin) and 5% (chloramphenicol and erythromycin) (Figure 7.4).

Table 7.4 Resistance (%) in *Enterococcus faecalis* isolates from pigs, Denmark, 2023 DANMAP 2023

Antimicrobial agent	*Enterococcus faecalis* %
Ampicillin	0
Chloramphenicol	28
Ciprofloxacin	0
Daptomycin	2
Erythromycin	56
Gentamicin	10
Linezolid	0
Teicoplanin	0
Tetracycline	76
Tigecycline	0
Vancomycin	0
Fully sensitive (%)	22
Number of isolates	87

E. faecalis are assumed inherently resistant to streptogramins (Quinopristin/Dalfopristin)

Figure 7.4 Resistance (%) among *Enterococcus faecalis* isolates from pigs, Denmark, 2023 DANMAP 2023



Number of isolates included each year is presented in parentheses

Among the resistant *E. faecalis* (N=65), four different multidrug-resistance profiles were observed in 29 isolates (33%). Combined resistance to chloramphenicol, tetracycline and erythromycin was the most common (22%; 19 isolates), followed by additional resistance to gentamicin (6%; 5 isolates). Four isolates (5%) were resistant to gentamicin, erythromycin and tetracycline, and a single isolate (1%) was resistant to erythromycin, tetracycline and daptomycin.

7.4.2 Perspectives

Enterococci are commensal gut bacteria in both animals and humans and can occasionally cause human disease. In Denmark, most human infections are caused by *E. faecalis* and *E. faecium*. While invasive infections by *E. faecium* in humans have seen a marked decrease in 2022 and 2023 after an increasing trend, those caused by *E. faecalis* have been relatively more constant over the last decade, and suffered a more modest decrease in the past two monitoring years [Chapter 8, Section 8.2.5].

Monitoring of resistance in *E. faecalis* from pigs has occurred in odd years since 2015. In contrast to the decrease observed in 2021, resistance levels in 2023 have overall increased (Figure 7.4).

In 2023, *E. faecalis* isolates recovered from pigs exhibited no resistance to ampicillin or vancomycin, antimicrobials often used to treat complicated infections in humans caused by enterococci. Furthermore, the isolates showed no resistance to linezolid and very low occurrence of resistance to daptomycin (Table 7.4). Both substances are used to treat multidrug-, vancomycin-resistant enterococci infections.

Given the resistance patterns observed in 2023 in *E. faecalis* from pigs and those causing invasive infections in humans, there is no strong suggestion that pigs are a potential zoonotic origin for the selected human isolates. However, the zoonotic nature of enterococci cannot be assessed solely based on antimicrobial resistance profiles. Recently, a phylogenetic study with Danish *E. faecium* isolates showed an almost absent overlap between animal- and food-associated strains and the hospital-associated clade of human isolates, while the overlap was far more extensive with the community-associated *E. faecium* [Roer et al., 2024. Microbiol Spectr 12:e03724-23.]. This again suggests that zoonotic transmission of enterococci may occur, but does not seem to be associated with the strains causing serious infections in humans.

Ana Sofia Ribeiro Duarte
For further information:
Ana Sofia Ribeiro Duarte, asrd@food.dtu.dk

Textbox 7.1

Ecogenomics of Danish cattle *E. coli* between 2001 and 2019

Background

The genomic evolutionary trends of antimicrobial resistance and virulence in indicator *Escherichia coli* within Danish cattle from 2001 to 2019 were recently investigated, focusing on the prevalence, distribution and evolution of different strains. Specifically, the study focused on changes in antimicrobial resistance genes, virulence genes, and phylogeny, and what factors might have contributed to such changes in *E. coli* from Danish cattle.

Materials and methods

The study employed whole genome sequencing of 1,359 indicator *E. coli* isolates from 598 Danish cattle farms, followed by the profiling of their antimicrobial resistance and virulence genes. Furthermore, correlations of resistance and virulence profiles to either year of collection or farm of origin were also explored. Lastly the phylogenetic relatedness among *E. coli* isolates was investigated to draw their ecogenomic signature in cattle over time and space.

Results and discussion

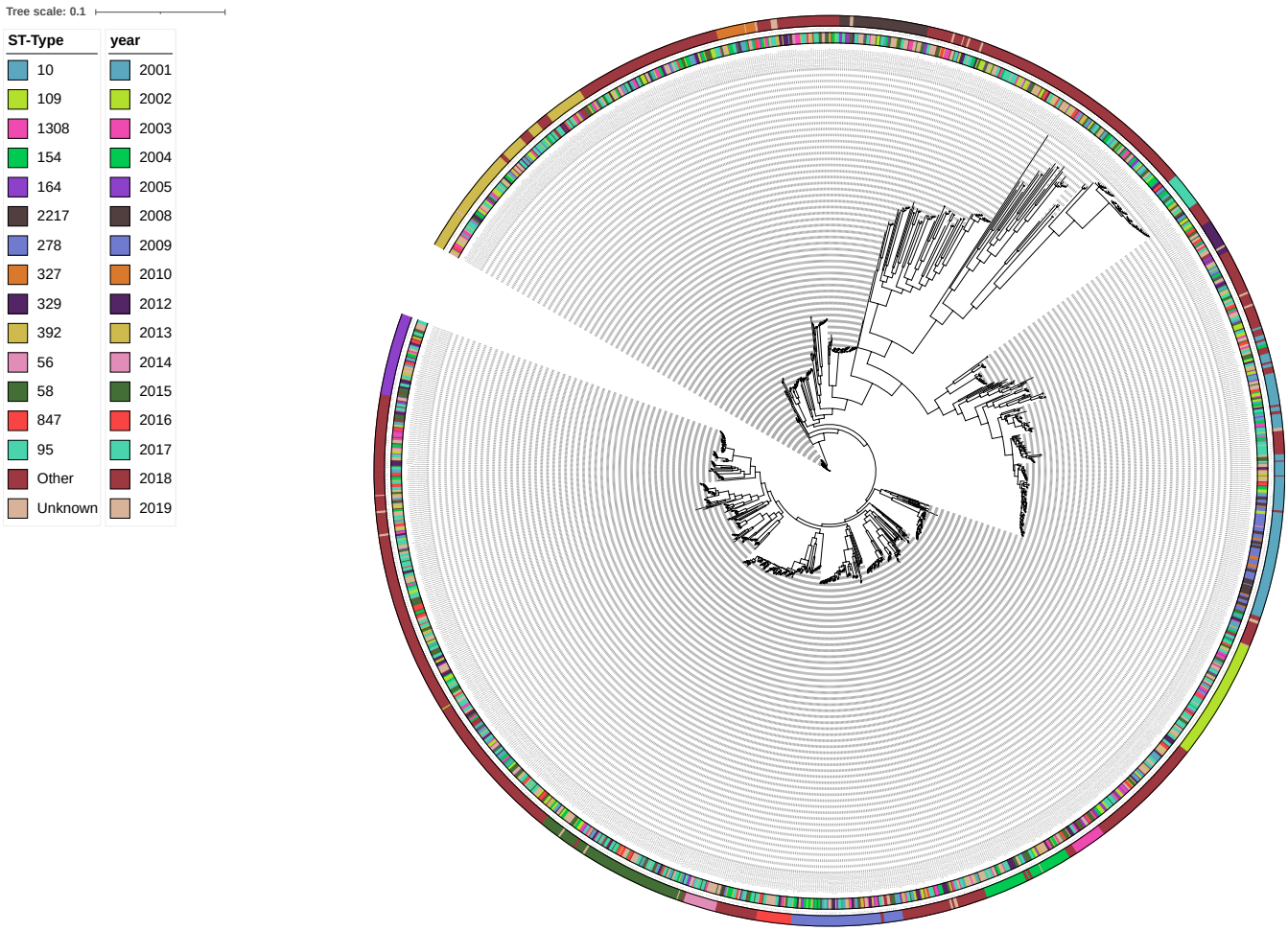
The results showed a rich genomic landscape characterised by the presence of 263 different sequence types among the analysed *E. coli* isolates. The phylogenetic analysis further highlights substantial genetic diversity, with clustering primarily based on sequence types rather than temporal and spatial factors (Figure 1).

Subsequently, we separated the *E. coli* isolates into two groups to create a more homogeneous distribution of number of isolates across farms and reduce biases associated with frequent collections from some locations. Genome-wide comparisons of the isolates indicated that a few single-copy orthologs may exhibit a spatio-temporal pattern, although the significance did not suggest a strong association. Notably, there was a significant reduction in the occurrence of certain genes primarily related to DNA transfer. This pattern was more pronounced in isolates collected after 2015, indicating that the temporal divide between the two groups likely falls within the 2014-2015 period. We are currently investigating the reasons for such separation before and after this period.

The antimicrobial resistance analysis revealed that 22% of isolates harbour resistance genes, with the highest rates observed in the aminoglycoside (15%), beta-lactam (11%), folate pathway antagonist (11%), and tetracycline (12%) classes. Correlation analyses showed diverse resistance gene dynamics across the years, with notable deviations in 2008-2010 and 2014, where there was an increase in resistance observed across multiple antimicrobial classes. Virulence analysis identified key virulence genes associated with the pathotypes enteropathogenic *E. coli* and Shiga toxin-producing *E. coli*.

Further results of this study are available for consultation [1].

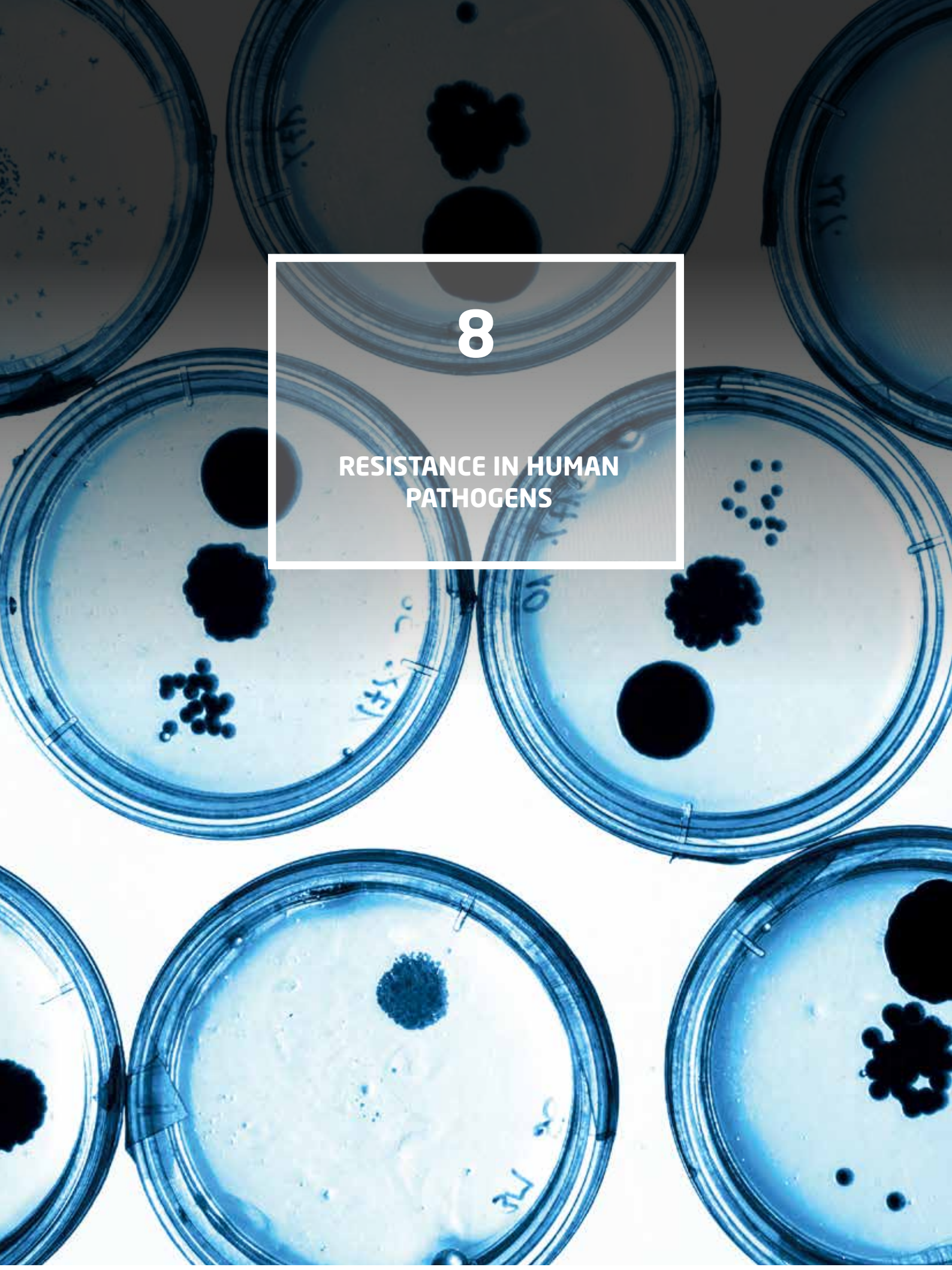
Figure 1 Overview of phylogenetic relationships between isolates, serotype and collection year (color-coded) DANMAP 2023



Saria Otani and Panos Sapountzis
For further information: Saria Otani, saot@food.dtu.dk

References

[1] E. Eberhardt, 2024. Genomic evolutionary dynamics and trends of antimicrobial resistance and virulence in *Escherichia coli* in Danish Cattle over 19 years, Master Thesis, National Food Institute, Technical University of Denmark.



8

RESISTANCE IN HUMAN PATHOGENS

8. Resistance in human pathogens

Highlights

Invasive infections. After years of increasing invasive infections, the numbers stagnated with a total of 12,205 cases. However, invasive infections with *Klebsiella pneumoniae* (1,399 cases) and *Staphylococcus aureus* (2,559 cases) continued to rise. Invasive infections with *Streptococcus pneumoniae* (602 cases) saw a steep decline after the introduction of vaccinations for young children and during the COVID-19 pandemic, but have since risen to near pre-pandemic levels.

Escherichia coli. Resistance levels have generally been stable over the past decade. However, resistance to piperacillin/tazobactam in invasive infections climbed from 4.8% in 2014 to 6.3% in 2023 for Denmark as a whole. These increases were driven by rising trends in the Capital Region, Region Zealand and the Central Denmark Region, whereas the North Denmark Region had a decreasing trend. Combined resistance to 3rd generation cephalosporins, ciprofloxacin and gentamicin remained low at 1.2%. Combined resistance to ampicillin and gentamicin was at 4.1% in 2023, decreasing from 6.9% in 2014. Carbapenem-resistance remained below 1% with no noticeable trend.

Klebsiella pneumoniae. While resistance levels have been decreasing for the past decade, piperacillin/tazobactam-resistance in invasive infection in 2023 surpassed 10% for Denmark as a whole and in four out of five regions. Combined resistance to 3rd generation cephalosporins, ciprofloxacin and gentamicin increased from 1.1% in 2015 to 1.9% in 2023. Additionally, cefuroxime-resistance increased from 7.7% to 9.8% from 2022 to 2023. The rising resistance coupled with an increase in invasive infections with *K. pneumoniae* warrants increased efforts to curb the development.

Carbapenemase-producing organisms/Enterobacterales (CPO/CPE). CPE were increasingly spreading in Danish hospitals, with cases rising 43% from 2022 to 2023, reaching 436 individual cases. CPE, historically linked to travel, are now increasingly found domestically. CPE outbreaks increased in hospitals despite extensive screening and cleaning efforts. 138 new CPO patients were associated with outbreaks in Denmark in 2023. Although bloodstream infections remained rare, the rise in hospital-acquired CPE indicates the emergence of domestic reservoirs. For non-outbreak related cases, 86 patients reported travelling outside Nordic countries, however, the number of cases with no travel information reported was high (130 cases). Additionally, 36 cases were patients from Ukraine.

Staphylococcus aureus. The number of S. aureus bacteraemia cases was 2,571 in 2023 and at the same level as in 2022. Of these, 39 cases (1.5%) were caused by methicillin-resistant *S. aureus* (MRSA) with nine being livestock-associated-MRSA (LA-MRSA). Resistance to penicillin continued to decrease and was 68% in 2023. There were 3,649 cases of MRSA from both screening (45% of cases) and infections (55%), which was a 20% increase compared to 2022. Forty-one MRSA outbreaks were registered at hospitals, nursing homes and other institutions for a total of 199 cases with 80 being infections.

Neisseria gonorrhoeae. Over the decade the number of received isolates and of reported cases increased significantly. In 2023, the reference laboratory at SSI received 2,653 isolates from 2,152 unique cases. Ciprofloxacin resistance was at 45%. Azithromycin-resistance was found in 6% of tested isolates in 2023 compared to 2.9% of tested isolates in 2022.

8.1 Introduction

The Danish national surveillance programme of antimicrobial resistance in human clinical bacteria collects data on routine diagnostics from all of Denmark. It is an active catchment system collecting results from all clinical and screening samples from patients. Data coverage is high; microbiology data from all hospitals and the majority of general practitioners feed into the system, hereby covering a close to complete proportion of microbiological analyses performed in Denmark.

In addition, the programme includes phenotypic resistance and typing results from the reference laboratories at SSI. Isolates are submitted to SSI either as part of the programme on mandatory notifiable diseases or based on voluntary submission of species carrying resistance mechanisms of concern (Table 8.1).

Table 8.1 Summary of species/types, sampling and sources of isolates for national resistance surveillance in humans, 2023

DANMAP 2023

Routine diagnostics from all 10 DCM in Denmark. All data are extracted from EpiMiBa	
Species	**Inclusion criteria**
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 health care
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 reference laboratories at SSI	
Species or type	**Inclusion criteria**
Staphylococcus aureus Beta-haemolytic streptococci	One isolate per patient per episode from blood or cerebrospinal fluid
Neisseria gonorrhoeae	One isolate per patient per episode from any sample site
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 from any sample site (excluding screening samples)
Enterococci with specific concerning phenotype (e.g. linezolid, daptomycin and tigecycline R)	First isolate per patient within 12 months from any sample site (clinical and screening samples)
All bacterial species with other exceptional phenotypes (e.g. acquired colistin resistance)	One isolate per patient per episode from any sample site
Mandatory submissions of isolates to the reference laboratories at SSI	
Species or type	**Inclusion criteria**
Carbapenemase-producing organisms	First isolate per patient within 12 months from any sample site (clinical and screening samples)
Methicillin-resistant *Staphylococcus aureus*	First isolate from all new cases of MRSA postive patients from any sample site (clinical and screening samples)
Streptococcus pneumoniae	One isolate per patient per episode from blood or cerebrospinal fluid
Haemophilus influenzae serotype b, Hib	All invasive isolates

Regarding submissions of isolates to the reference laboratories often more isolates per patient are received, but for the statistics only one isolate per patient is counted

8.1.1 Surveillance based on data from the Danish Microbiology Database (MiBa)

An important part of the national surveillance programme consists of systematically extracted data from a register (EpiMiBa) linking to the Danish Microbiology Database (MiBa). MiBa was established in 2010 with the aim to build a cross-national database that included and made available all microbiology analyses performed by the individual DCM. MiBa thus simultaneously delivers real time patient data to the DCM for clinical use and data to SSI for national surveillance. Until the establishment of MiBa, surveillance was based on voluntary manual reporting from the individual DCM, beginning with data from just two DCM in 1995, but quickly expanding to include more than half of the DCM. Since 2015, all DCM have contributed to the program resulting in a 100% coverage of hospitalised patients and – since the DCM perform microbiology analyses for GPs and private specialists as well – also a very high coverage of results from the primary sector.

A description of MiBa and the usage and validation of data from the associated database EpiMiBa can be found on the following homepage [https://mibaen.ssi.dk/] and in DANMAP 2018, Textbox 8.1.

The MiBa-based surveillance includes all invasive isolates of *Escherichia coli, Klebsiella pneumoniae, Enterococcus faecalis, Enterococcus faecium, Pseudomonas aeruginosa* and *Acinetobacter* species and all urine isolates of *E. coli* and *K. pneumoniae*. For inclusion, the following case definition applies: The first sample of each given bacterial species per individual patient per year, by date of sample collection, an approach identical to the surveillance performed by the European Antimicrobial Resistance Surveillance Network (EARS-Net). All duplicates, patient-related or sample-related within the year of observation, are excluded.

Materials and methods are further described in paragraph 10.10 in Chapter 10.

8.1.2 Surveillance based on data from the reference laboratories

Antimicrobial resistance is also monitored in isolates submitted to the reference laboratories at SSI.

Submission of isolates began in 1957 with the voluntary referral of *S. aureus* from bloodstream infections. Still today, many isolates are sent as part of voluntary programmes; however, the majority of isolates are referred as part of surveillance programmes for notifiable diseases.

The mandatory programme includes methicillin-resistant *S. aureus* (MRSA) and carbapenemase-producing organisms (CPO) from clinical and screening samples, *S. pneumoniae* and *Haemophilus influenzae* serotype b (Hib) from invasive infections and *Nesseria gonorrhoeae* from all clinical samples.

The latter three irrespective of antimicrobial resistance, but resistance mechanisms are investigated both locally and at SSI (Table 8.1).

The voluntary programme includes day-to-day referral of vancomycin-resistant enterococci and monthly or periodically reporting of ESBL-positive invasive *E. coli*, invasive beta-haemolytic streptococci and invasive *S. aureus*.

In addition, the reference laboratory for antibiotic resistance at SSI receives highly resistant strains or strains carrying atypical antimicrobial resistance mechanisms of specific concern irrespective of bacterial species.

Since 2015/2016, characterisation of antimicrobial resistance mechanisms in bacteria has been performed primarily by whole genome sequencing (WGS).

8.1.3 Number of invasive cases

The total numbers of invasive cases of the monitored bacterial species included in the surveillance programmes for both DANMAP and EARS-Net from 2014 to 2023 are presented in Figure 8.1. Invasive cases caused by *Acinetobacter* species are not shown in the figure due to the small numbers of isolates (ranging from 55 to 97 cases/year between 2014 and 2023).

Between 2014 and 2023, the number of registered individual invasive cases increased by 25% from 9,790 to 12,205 cases in Denmark: *E. coli* 4,496 to 5,835 cases (30% increase), *S. aureus* 1,874 to 2,559 cases (37%) and *K. pneumoniae* 943 to 1,399 cases (48%).

Figure 8.1 Number of invasive cases for bacterial species under surveillance, Denmark, 2014-2023 DANMAP 2023

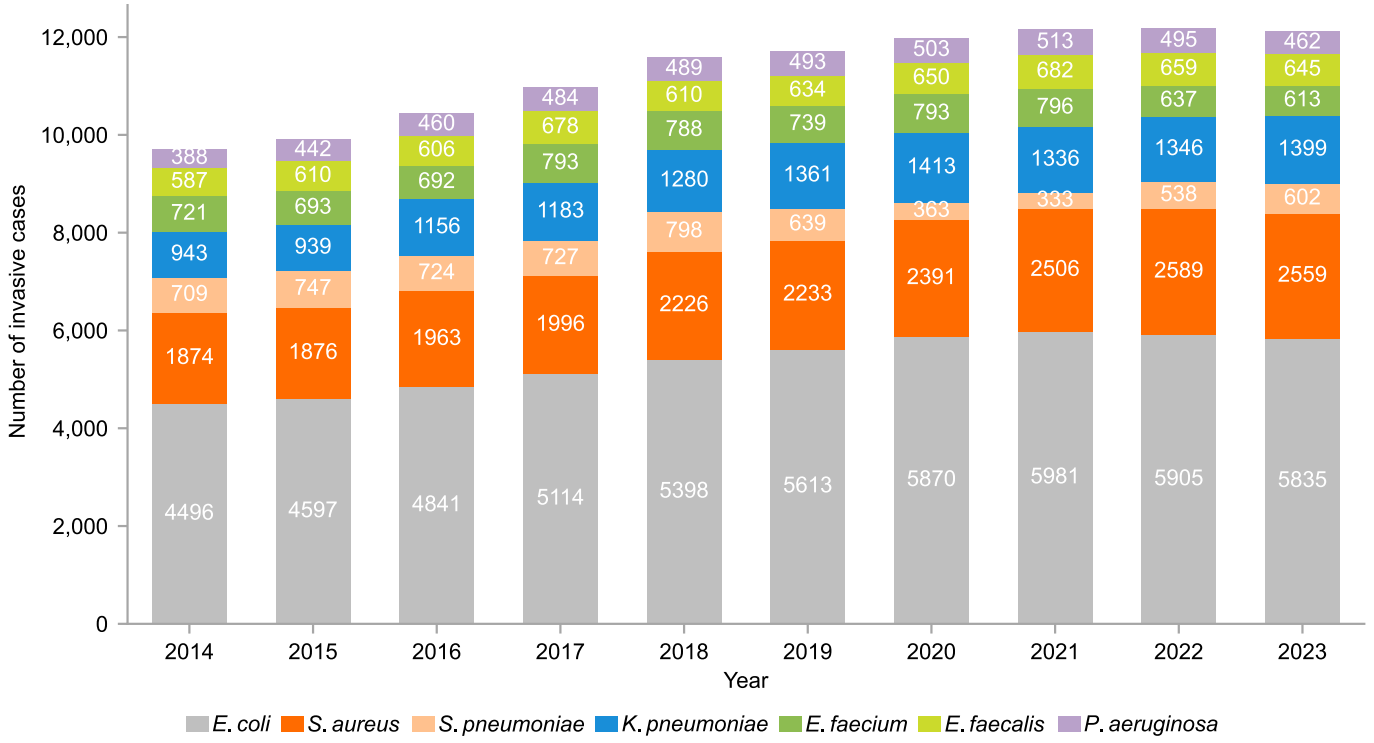
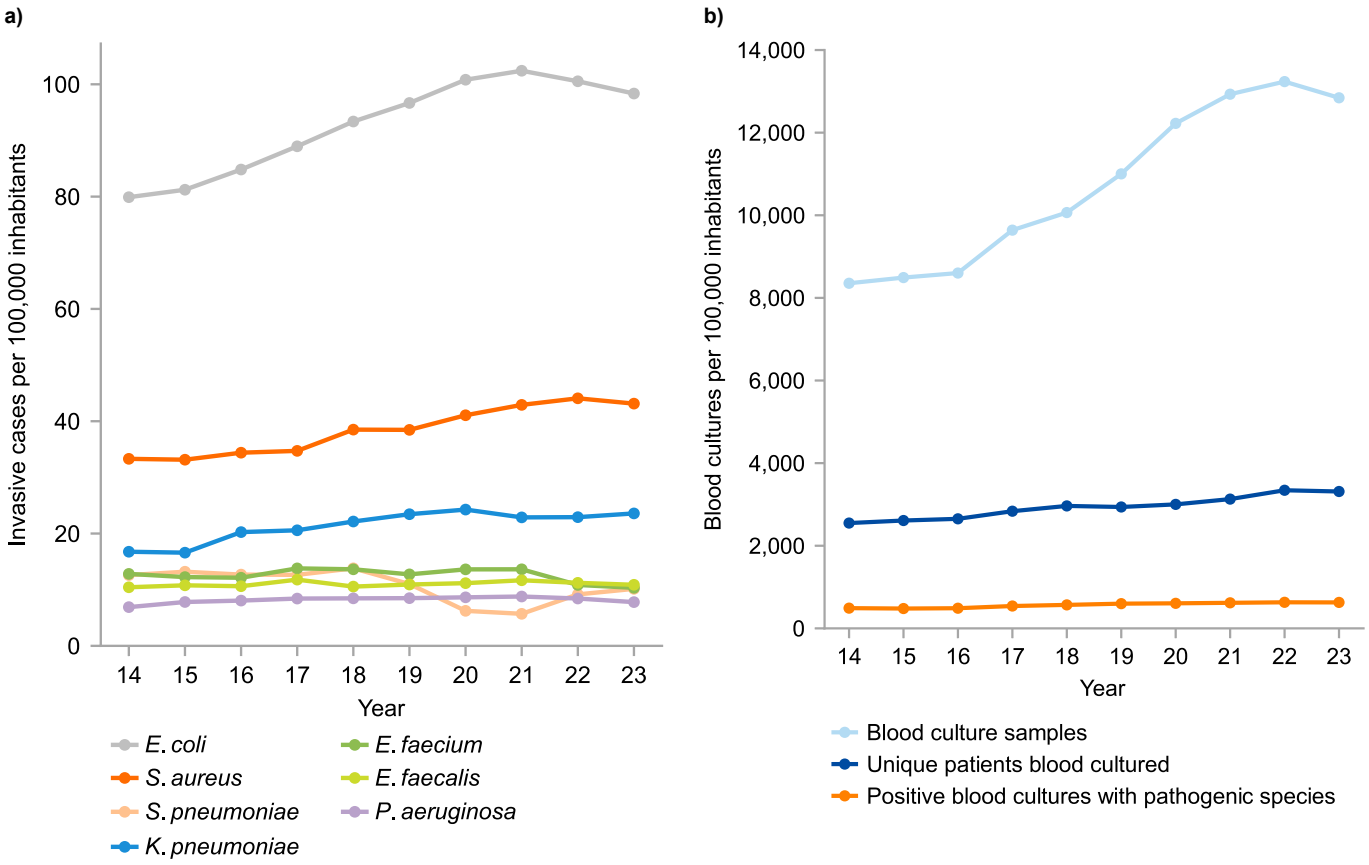


Figure 8.2a shows the incidence of invasive cases of the seven monitored species per 100,000 inhabitants in Denmark per year from 2014 to 2023. During this period, the Danish population increased by 5.4% (from 5,627,235 inhabitants in 2014 to 5,932,654 inhabitants in 2023). For comparison, Figure 8.2b shows the total number of blood cultures taken per 100,000 inhabitants per year in the same period. Additionally, the number of individual patients with minimum one blood culture taken per 100,000 inhabitants per year is shown. In the ten-year period the number of individual patients with at least one blood culture taken per year increased from 2,549 patients per 100,000 inhabitants in 2014 to 3,313 patients per 100,000 inhabitants in 2023 (an increase of 30%). The total number of blood samples (unique samples in MiBa) taken per 100,000 inhabitants increased even more (54%). The incidence of positive blood cultures with pathogenic species increased from 490 per 100,000 inhabitants in 2014 to 628 per 100,000 inhabitants in 2023.

The following sections present results of resistance monitoring in Denmark for the individual bacterial species and/or resistance types under surveillance.

Mikkel Lindegaard and Ute Wolff Sönksen
For further information: Ute Wolff Sönksen, uws@ssi.dk

Figure 8.2 a) Invasive cases and b) blood cultures taken per 100,000 inhabitants, Denmark, 2014-2023 DANMAP 2023



8.2 Results from MiBa data surveillance

8.2.1 *Escherichia coli*

Escherichia coli is by far the most frequent cause of community and hospital acquired bacteraemia and urinary tract infections (UTI) in Denmark. The pathogen 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 these gut bacteria as well as resistance conferred by mutations. Since *E. coli* is the most frequent cause of UTI and bacteraemia, it is also responsible for a large proportion of the antimicrobials used to treat these conditions.

The percentages of resistance in invasive *E. coli* isolates for each key antimicrobial are presented in Table 8.2, as are urine samples from hospitals and primary health care (see details in later paragraphs).

Invasive cases from hospital patients

In 2023, a total of 5,835 individual patients with invasive *E. coli* isolates were identified in EpiMiBa. Antimicrobial susceptibility test results for key antimicrobials were also extracted for these isolates (See Chapter 10 'Material and methods').

Figure 8.3 and Table 8.3 shows the total annual number of invasive isolates and proportion of resistant isolates by region between 2014 and 2023. Most resistance levels decreased or stagnated over the last decade, apart from resistance to piperacillin-tazobactam, which increased in three of the five healthcare regions, leading to an overall increase from 4.5% in 2014 to 6.3% in 2023.

Resistance to carbapenems remained below 1%. The percentages of multidrug resistant invasive isolates are presented in Table 8.4. Combined resistance to ciprofloxacin, cephalosporins and gentamicin decreased from 2.3% in 2015 to 1.2% in 2023 and combined resistance to ampicillin and gentamicin from 6.3% to 4.1%.

Urinary cases from hospitals

In 2023, *E. coli* was isolated from urine samples of 44,389 individual hospital patients. In Table 8.2 summarized antimicrobial susceptibility results for *E. coli* isolates (invasive and urine specimen) are shown. In Figure 8.4 and Table 8.5, resistance for *E. coli* urine isolates from hospitalised patients are shown for 2014-2023.

Urinary cases from primary health care

In 2023, *E. coli* were isolated from urine samples from 106,236 unique patients in primary health care. Susceptibility results for all tested antimicrobials are shown in Table 8.2. In Figure 8.5 and Table 8.6, resistance for *E. coli* urine isolates from primary health care are shown for the past decade.

Conclusion

The number of invasive cases of *E. coli* appears to have reached a plateau. Resistance levels are generally stable for all specimen types, however, the slow rise in piperacillin-tazobactam resistance in *E. coli* from invasive infections and hospital urines are cause for concern.

Table 8.2 *Escherichia coli*. Resistance (%) in isolates from humans, Denmark, 2023 DANMAP 2023

Substance	Invasive isolates, hospitals %	Urine isolates, hospitals %	Urine isolates, primary health care %
Ampicillin	41	39	34
Mecillinam	6.5	6.5	4.0
Piperacillin/tazobactam	6.3	5.4	4.3 (1)
Amoxicillin/clavulanic acid	28.8 (4)	10.1	6.7
Sulfonamide		31 (4)	26
Trimethoprim		21	20
Nitrofuratoin		0.8	0.6
Gentamicin	4.4	4.5	3.7 (1)
Ciprofloxacin	10.6	9.9	7.5
Cefuroxime	9.6	7.7	6.1 (3)
3rd generation cephalosporins	5.8	6.5	5.3
Carbapenem	0.0	0.1	0.0 (2)
Max. number of isolates tested for resistance to the presented antibiotics	5,830	44,263	106,203

Note: Included are results from all DCM where >75% of the isolates in each antibiotic/sample group were susceptibility tested
Numbers in parentheses indicate the number of DCM included if less than six

Figure 8.3 Antimicrobial resistance in invasive *Escherichia coli* isolates from humans by region, Denmark, 2014-2023 DANMAP 2023

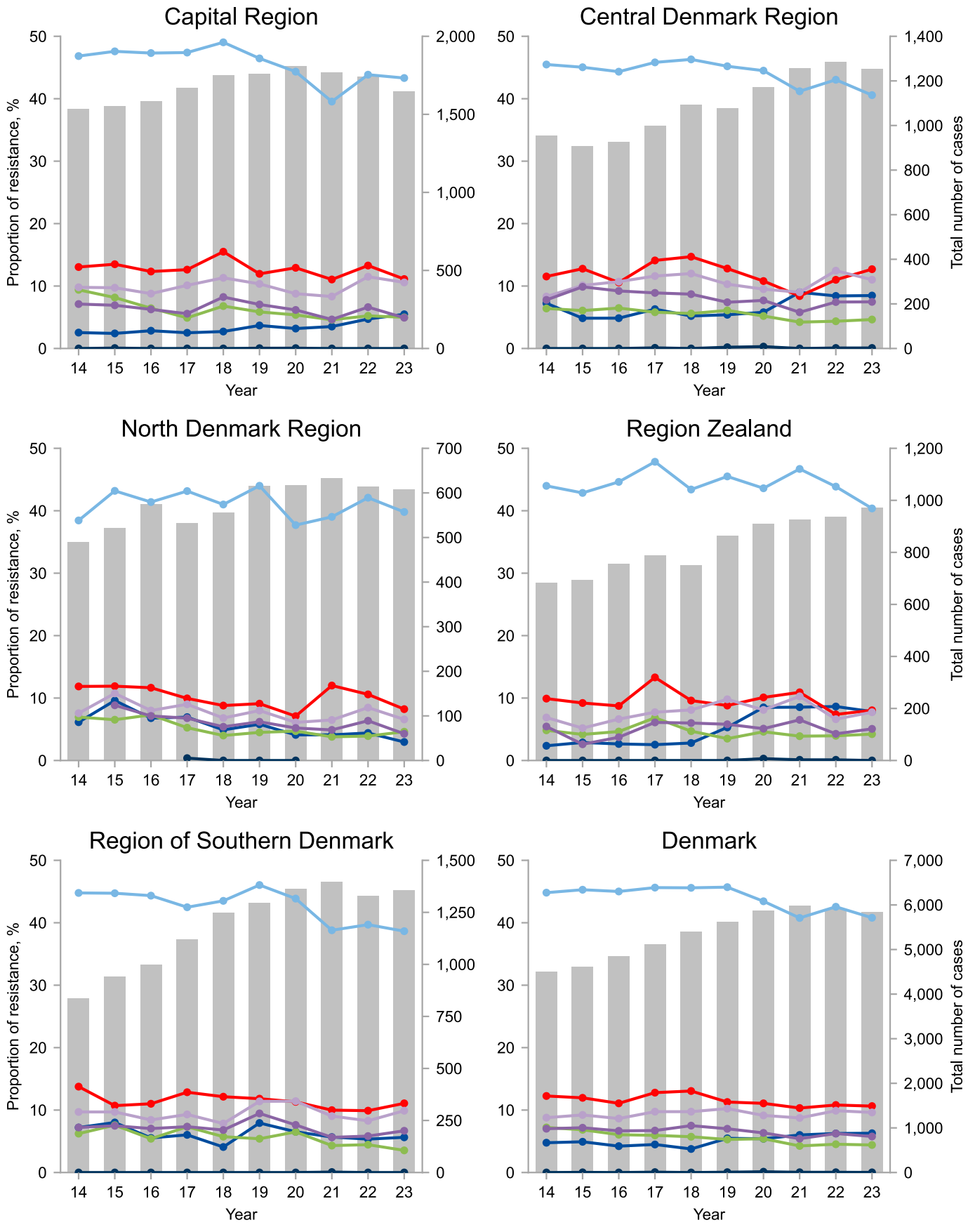


Table 8.3 *Escherichia coli* from invasive infections. Table of resistance percentages, Denmark, 2014-2023 DANMAP 2023

Substance	Percent resistant invasive *E. coli* isolates									
	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023
Ampicillin	44.8	45.3	45	45.6	45.5	45.7	43.4	40.7	42.6	40.8
Piperacillin-tazobactam	4.8	4.9	4.2	4.5	3.8	5.5	5.4	6.0	6.3	6.3
Gentamicin	7.2	6.8	6.1	6.0	5.7	5.3	5.4	4.3	4.5	4.4
Ciprofloxacin	12.3	12.0	11.1	12.8	13.0	11.3	11.1	10.3	10.8	10.6
Cefuroxime	8.8	9.2	8.6	9.7	9.8	10.2	9.2	8.7	9.9	9.6
3rd gen.cephalosporins	7.0	7.2	6.7	6.7	7.3	6.9	6.2	5.4	6.2	5.8
Carbapenem	0.0	0.0	0.0	0.1	0.0	0.1	0.1	0.0	0.0	0.0
Total number of isolates	4,492	4,618	4,841	5,114	5,398	5,613	5,870	5,981	5,905	5,835

Table 8.4 *Escherichia coli*. Combined resistance to 1) ampicillin and gentamicin and 2) 3rd generation cephalosporins, ciprofloxacin, and gentamicin (multiresistance) in invasive isolates from humans, Denmark, 2014-2023 DANMAP 2023

Combination		2014 % (N)	2015 % (N)	2016 % (N)	2017 % (N)	2018 % (N)	2019 % (N)	2020 % (N)	2021 % (N)	2022 % (N)	2023 % (N)
AMP/GEN	Resistance	6.9 (284)	6.3 (254)	5.8 (278)	5.8 (289)	5.5 (284)	5.2 (275)	5.1 (287)	4.0 (229)	4.1 (233)	4.1 (231)
	Percentage (no.) of isolates tested	92 (4,138)	87 (4,009)	99 (4,816)	98 (5,015)	96 (5,170)	95 (5,308)	95 (5,583)	96 (5,745)	96 (5,679)	96 (5,602)
3GC/CIP/ GEN	Resistance	1.8 (72)	2.3 (93)	1.8 (87)	1.8 (88)	2.0 (100)	1.8 (93)	1.5 (82)	1.1 (60)	1.3 (70)	1.2 (63)
	Percentage (no.) of isolates tested	90 (4,039)	88 (4,071)	98 (4,763)	95 (4,883)	93 (4,997)	94 (5,259)	93 (5,470)	93 (5,564)	93 (5,474)	93 (5,417)
	Total number of invasive isolates	4,495	4,614	4,841	5,114	5,398	5,613	5,870	5,981	5,905	5,835

Table 8.5 *Escherichia coli* from hospital urines. Table of resistance percentages, Denmark, 2014-2023 DANMAP 2023

Substance	Percent resistant *E. coli* isolates from hospital urine									
	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023
Ampicillin	41.7	42.1	41.0	41.9	42.1	43.7	40.6	39.2	40.0	39.4
Mecillinam	7.2	7.7	7.4	7.5	7.4	8.1	7.3	6.9	6.8	6.5
Piperacillin-tazobactam	3.5	3.9	3.3	3.7	3.5	4.4	4.3	4.5	5.4	5.4
Sulfonamide	32.3*	32.0	34.9	31.1	31.0*	31.4*	29.4*	28.5*	28.2*	30.9*
Gentamicin	4.9	5.1	5.3	4.9	4.7	4.6	4.6	4.2	4.3	4.5
Ciprofloxacin	11.4	11.0	10.9	10.4	11.0	10.6	9.6	8.7	9.3	9.9
Cefuroxime	6.6	7.0	6.8	7.1	7.2	7.8	7.2	6.7	7.5	7.7
3rd gen. cephalosporins	5.9	5.9	5.9	6.2	6.4	6.9	6.3	5.8	6.2	6.5
Carbapenem	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
Total number of isolates	44,288	46,723	46,865	46,884	47,914	47,235	48,962	49,986	48,559	44,389

* Indicates less than 6 DCM reported rutine susceptibility testing

Table 8.6 *Escherichia coli* from urines from primary health care. Table of resistance percentages, Denmark, 2014-2023 DANMAP 2023

Substance	Resistance in *E. coli* urine isolates from primary health care									
	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023
Ampicillin	39.3	38.5	38.3	37.9	37.4	38.1	36.5	34.3	34.6	34.2
Mecillinam	5.2	5.4	5.6	5.5	5.1	5.3	4.9	4.6	4.3	4.0
Sulfonamide	31.9	30.9	29.5	29.1	28.3	27.9	26.7	25.2	25.0	25.5
Ciprofloxacin	8.8	8.6	10.1	8.4	8.1	8.0	7.5	6.9	6.9	7.5
3rd gen. cephalosporins	4.2	4.3	4.3	4.5	4.9	5.2	5.0	4.4	4.8	5.3
Total number of isolates	51,272	61,083	67,798	73,497	80,851	86,08	88,462	99,077	104,376	106,236

Figure 8.4 Antimicrobial resistance in *Escherichia coli* isolates from urine in humans (hospitals) by region, Denmark, 2014-2023

DANMAP 2023

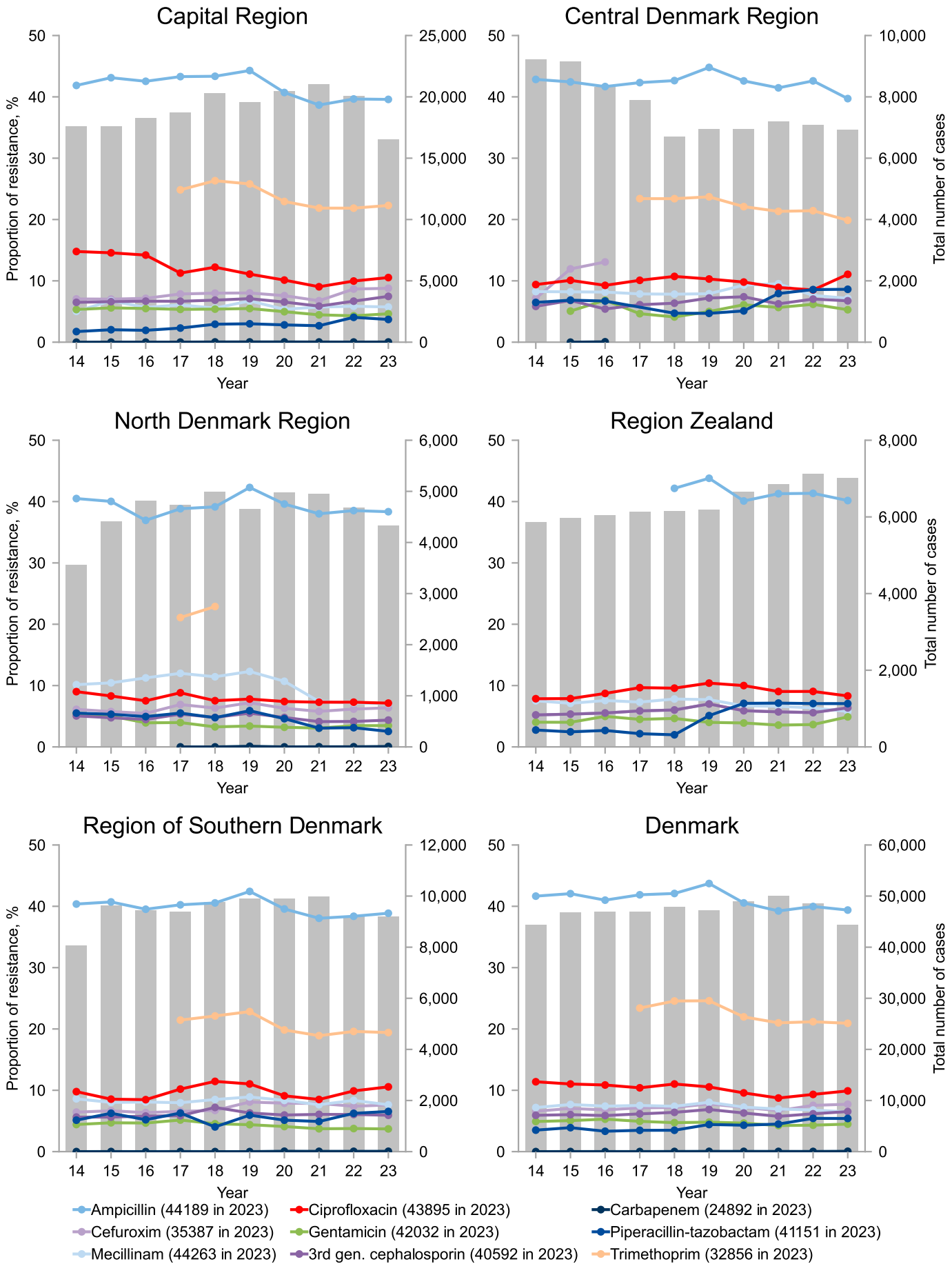
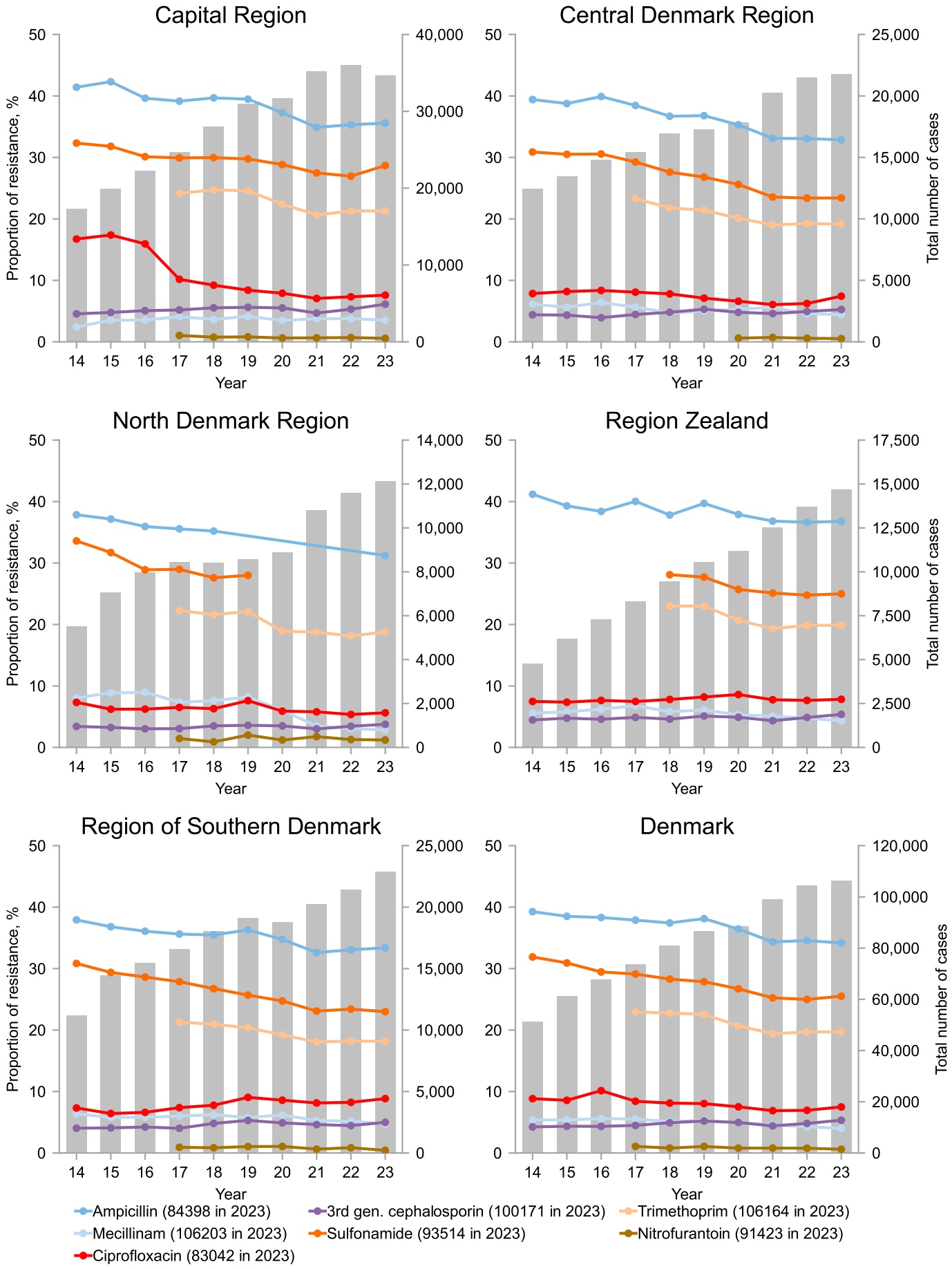


Figure 8.5 Antimicrobial resistance in *Escherichia coli* isolates from urine in humans (primary health care) by region, Denmark, 2014-2023

DANMAP 2023



8.2.2 *Klebsiella pneumonia*

Klebsiella pneumoniae is part of the human intestinal tract. The bacteria cause urinary tract infections, intra-abdominal infections, bacteraemia and ventilator-associated pneumonia (VAP). *K. pneumoniae* may cause nosocomial outbreaks. *K. pneumoniae* frequently displays plasmid-mediated resistance mechanisms, which are transferrable to other bacterial species.

The percentage of resistance in *K. pneumoniae* isolates for key antimicrobials in isolates from invasive infections, urines from hospitals and urines from general praxis, respectively, is presented in Table 8.7.

Invasive cases from hospitals

In 2023, a total of 1,399 unique patients were registered in MiBa with invasive *K. pneumoniae* isolates. Figure 8.6 shows total annual numbers and numbers per region of invasive isolates and percentages of resistance in invasive isolates between 2014 and 2023. From 2014 to 2021, particularly for cephalosporins decreasing trends in resistance were observed. From 2022 to 2023, resistance levels reverted and showed increases for most of the monitored antibiotics. Of particular interest is the almost continous increase in resistance levels to piperacillin-tazobactam, which increased from 8.1% in 2014 to 10.5% in 2023 in total, an increase that was observed for all regions. Resistance levels to carbapenems showed sligth increases over the decade (0.2% to 0.5%) as did combined resistance to ciprofloxacin, cephalosporins and carbapenems (1.1% to 1.9%).

The proportions of isolates resistant to key antimicrobials for the decade are presented in Table 8.8. The percentages of multidrug resistant invasive isolates are presented in Table 8.9.

Table 8.7 *Klebsiella pneumoniae*. Resistance (%) in isolates from humans, Denmark, 2023 DANMAP 2023

Substance	Invasive isolates, hospitals %	Urine isolates, hospitals %	Urine isolates, primary health care %
Mecillinam	8.5	10.0	7.2
Piperacillin/tazobactam	10.5	10.5	9.0 (1)
Amoxicillin/clavulanic acid	16 (5)	7.5	4.7 (5)
Sulfonamide		19.6 (3)	13.4
Trimethoprim		13.9	12.3
Nitrofuratoin		36 (5)	30
Gentamicin	2.8	2.3	1.6 (1)
Ciprofloxacin	7.4	7.5	5.2
Cefuroxime	9.8	9.4	5.9 (3)
3rd generation cephalosporins	5.9	5.7	4.6
Carbapenem	0.5	0.4 (5)	0.2 (2)
Max. number of isolates tested for resistance to the presented antibiotics	1,398	7,459	11,502

Note: Included are results from all DCM where >75% of the isolates in each antibiotic/sample group were susceptibility tested
Numbers in parantheses indicate the number of DCM included if less than six

Figure 8.6 Invasive *Klebsiella pneumoniae* isolates from humans: proportion of resistant isolates and annual number of isolates from unique cases, Denmark, 2014-2023

DANMAP 2023

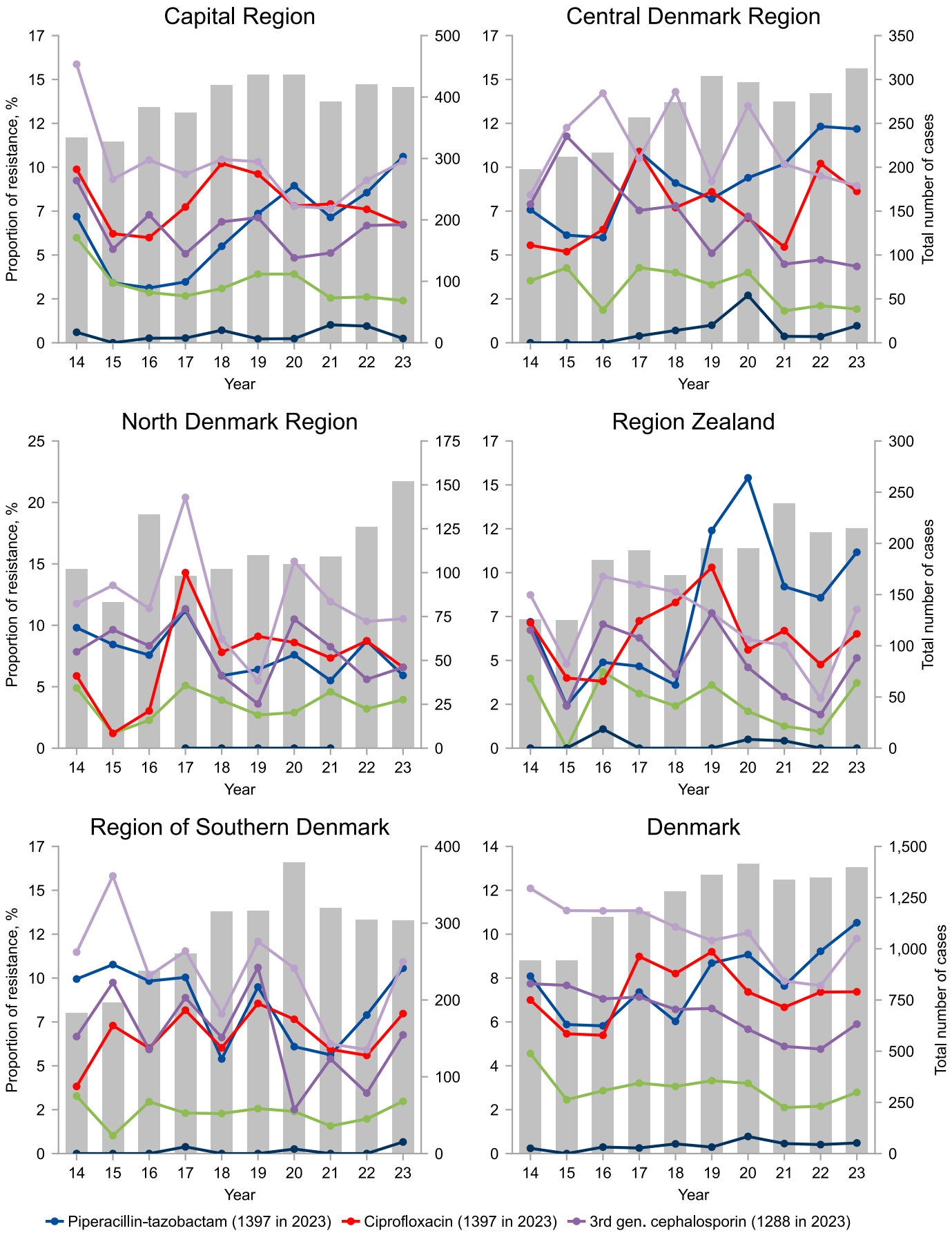


Table 8.8 Invasive *Klebsiella pneumoniae*. Table of resistance percentages, 2014-2023 DANMAP 2023

Substance	Percent resistant invasive *K. pneumoniae* isolates									
	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023
Piperacillin/tazobactam	8.1	5.9	5.8	7.4	6.1	8.7	9.1	7.5	9.2	10.5
Gentamicin	4.6	2.5	2.9	3.2	3.1	3.3	3.2	2.1	2.2	2.8
Ciprofloxacin	7.0	5.5	5.4	9.0	8.1	9.2	7.4	6.7	7.4	7.4
Cefuroxime	12.1	11.1	11.1	11.1	10.3	9.7	10.1	7.9	7.7	9.8
3rd gen. cephalosporins	7.7	7.7	7.3	7.1	6.1	6.6	5.3	4.9	4.8	5.9
Carbapenem	0.2	0.0	0.3	0.3	0.5	0.3	0.8	0.5	0.4	0.5
Total number of isolates	943	939	1,156	1,183	1,280	1,361	1,413	1,336	1,346	1,399

Table 8.9 Invasive *Klebsiella pneumoniae*. Combined resistance to 3rd generation cephalosporins, ciprofloxacin, and gentamicin (multidrug-resistance) in invasive isolates from humans, Denmark, 2015-2023 DANMAP 2023

	2015 % (N)	2016 % (N)	2017 % (N)	2018 % (N)	2019 % (N)	2020 % (N)	2021 % (N)	2022 % (N)	2023 % (N)
Resistance	1.1 (9)	1.6 (18)	2.4 (27)	1.7 (20)	2.4 (30)	1.5 (19)	1.0 (13)	1.0 (13)	1.9 (24)
Percentage (no.) of isolates tested for combined resistance (multiresistance)	89 (840)	98 (1,131)	95 (1,122)	93 (1,188)	94 (1,275)	93 (1,308)	93 (1,248)	94 (1,259)	92 (1,287)
Total number of invasive isolates	943	1,156	1,183	1,280	1,361	1,413	1,336	1,346	1,399

Table 8.10 *Klebsiella pneumoniae* from hospital urines. Table of resistance percentages, Denmark, 2014-2023 DANMAP 2023

Substance	Resistance in *K. pneumoniae* urine isolates from hospitals									
	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023
Mecillinam	9.6	10.0	8.9	15.7	16.9	13.2	11.7	10.6	9.4	10.0
Piperacillin/tazobactam	6.0	6.3	5.6	6.7	8.7	8.5	8.0	8.7	9.9	10.5
Gentamicin	4.0	3.7	3.2	3.6	3.2	3.0	2.9	2.4	2.3	2.3
Ciprofloxacin	7.6	6.2	6.1	7.6	8.9	7.4	7.2	7.2	7.2	7.5
Cefuroxime	8.9	9.5	9.1	9.4	9.5	8.6	8.5	8.4	8.1	9.4
3rd gen. cephalosporins	6.6	6.8	6.8	7.1	6.8	6.0	5.5	5.4	4.7	5.7
Total number of isolates	6,372	7,175	7,467	8,106	8,047	7,926	7,814	7,701	7,911	7,492

Table 8.11 *Klebsiella pneumoniae* from urines from primary health care. Table of resistance percentages, Denmark, 2014-2023 DANMAP 2023

Substance	Resistance in *K. pneumoniae* urine isolates from primary health care									
	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023
Mecillinam	8.8	9.4	9.0	16.6	15.9	11.5	9.4	8.6	7.9	7.2
Sulfonamide	17.1	18.7	19.3	25.5	24.6	18.9	15.5	13.8	11.9	13.4
Ciprofloxacin	6.5	5.2	5.6	5.4	6.4	5.5	5.2	4.9	5.1	5.2
3rd gen. cephalosporins	4.7	4.8	5.4	4.9	5.3	4.5	4.4	3.7	3.4	4.6
Total number of isolates	4,246	6,372	7,615	8,948	9,227	9,696	9,387	10,196	11,039	4,246

Figure 8.7 *Klebsiella pneumoniae* isolates from urines in humans (hospitals): proportion of resistant isolates and annual number of isolates from unique cases, Denmark, 2014-2023

DANMAP 2023

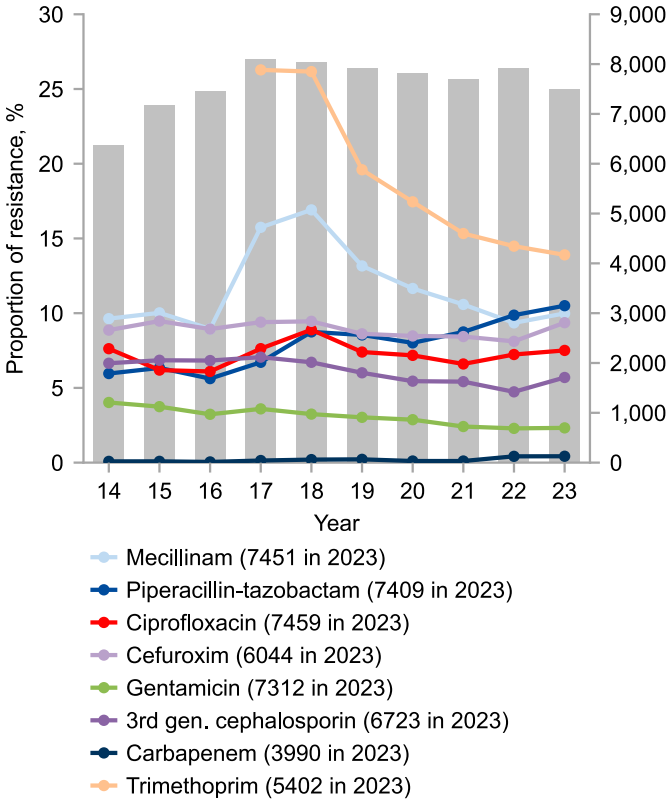
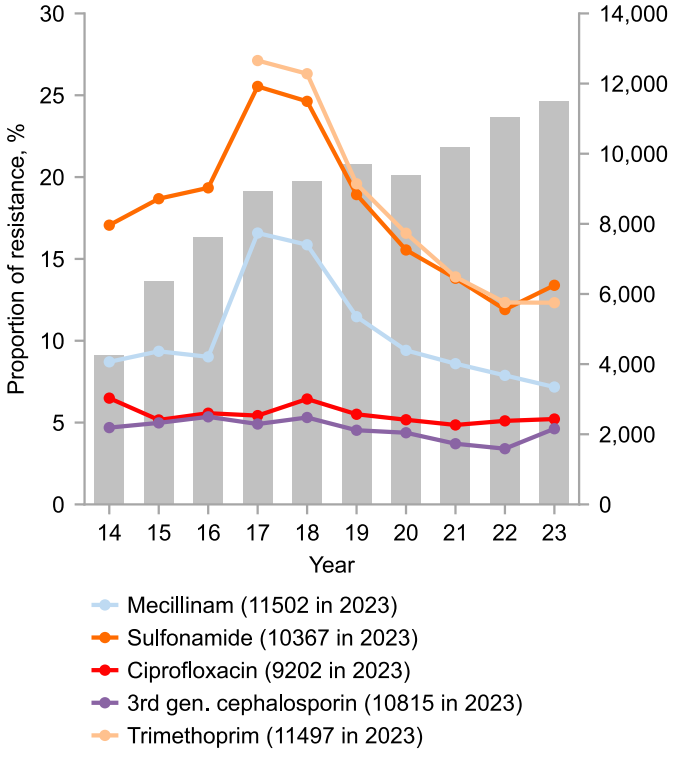


Figure 8.8 *Klebsiella pneumoniae* isolates from urines in humans (primary health care): proportion of resistant isolates and annual number of isolates from unique cases, Denmark, 2014-2023

DANMAP 2023



Urinary cases from hospitals

In 2023, *K. pneumoniae* from urine samples were isolated from 7,492 unique hospital patients in Denmark.

Summarized antibiotic-specific susceptibility results for *K. pneumoniae* isolates (invasive and urine specimen) for 2023 are shown in Table 8.7. In Figure 8.7 and Table 8.10, resistance for *K. pneumoniae* urine isolates from hospitals is shown for 2014-2023.

Urinary cases from primary health care

In 2023, *K. pneumoniae* was isolated from urine samples of 11,039 unique patients in primary health care. As for the results from invasive isolates and isolates from hospital urine samples susceptibility results for all tested antimicrobials are shown in Table 8.7. In Figure 8.8 and Table 8.11, resistance for *K. pneumoniae* urine isolates from primary health care is shown for the past decade.

Conclusion

The number of invasive cases of *K. pneumoniae* have increased by 38% since 2014. Resistance levels to cephalosporins have decreased steadily over the decade but reverted from 2022 to 2023, a change that should be followed closely. As for *E. coli*, a concerning trend is seen with regards to piperacillin-tazobactam for which resistance levels have now surpassed 10% for both invasive infections and hospital urines.

8.2.3 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is an opportunistic pathogen that can colonise the lung, urinary tract, burn wounds, superficial wounds and cause invasive infections. The pathogen is capable of biofilm formation and has the ability to colonise medical devices (e.g. indwelling catheters and implants) which in many cases causes complicated infections requiring prolonged and combination treatment. *P. aeruginosa* infections are more prevalent in certain at-risk groups such as immunocompromised, diabetes and cystic fibrosis patients. *P. aeruginosa* exhibits intrinsic resistance to various antimicrobial agents through chromosomal gene mutations and has the ability to acquire β-lactamases (extended-spectrum β-lactamases (ESBLs) and carbapenemases (especially class B carbapenemases or metallo-β-lactamases [MBLs]) by horizontal transmission.

Invasive cases from hospital patients

In 2023, a total of 495 unique patients with invasive P. *aeruginosa* isolates were registered in Denmark. Figure 8.9 shows the total annual number of invasive isolates and proportion of resistant isolates by region between 2014 and 2023.

Conclusion

The occurrence of invasive *P. aeruginosa* in Denmark is stable regarding the number of cases reported and resistance levels are low.

8.2.4 *Acinetobacter* species

The genus *Acinetobacter* includes several species and is found widespread in nature (soil and water) as well as in animals and humans. In humans, *Acinetobacter* species (spp.) can colonise the skin and wounds but may also cause hospital-acquired infections such as central line-associated bloodstream infections, nosocomial pneumonia, UTIs and wound infections, most often in severely ill patients. 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* spp. is intrinsically resistant to a broad range of antimicrobials mediated by a low membrane permeability and constitutive expression of various efflux systems.

Invasive cases from hospitals

In 2023, a total of 90 unique patients with invasive *Acinetobacter* species were reported. Data are presented in Table 8.12 and in Figure 8.10.

Conclusion

The number of invasive *Acinetobacter* spp. appears to have stabilized at a new level compared to the marked increase from 2020 to 2021; however, the proportion of invasive isolates resistant to key antimicrobials remains low in Denmark.

Figure 8.9 Invasive *Pseudomonas aeruginosa* isolates from humans: proportion of resistant isolates and annual number of isolates from unique cases, Denmark, 2014-2023 DANMAP 2023

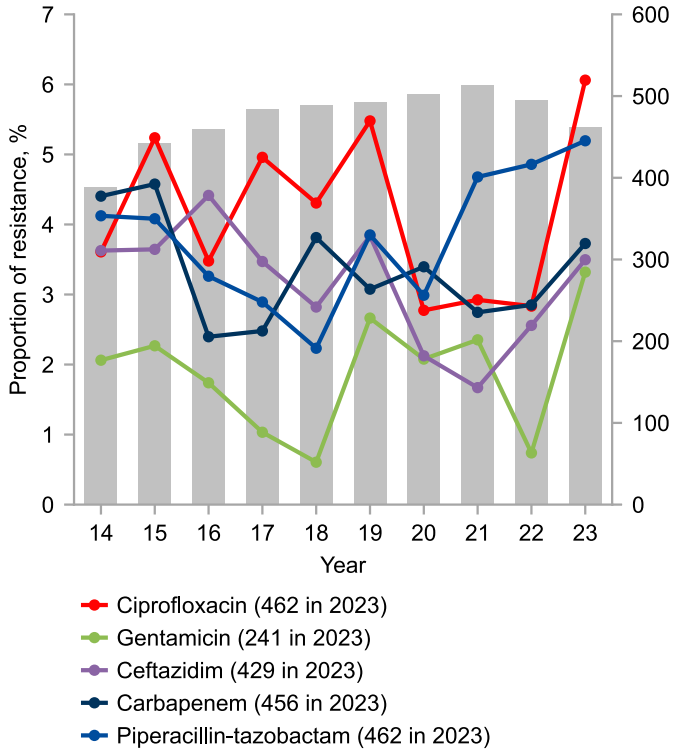


Figure 8.10 Invasive *Acinetobacter* spp. isolates from humans: a) annual number of isolates from unique cases and b) proportion of resistant isolates, Denmark, 2014-2023 DANMAP 2023

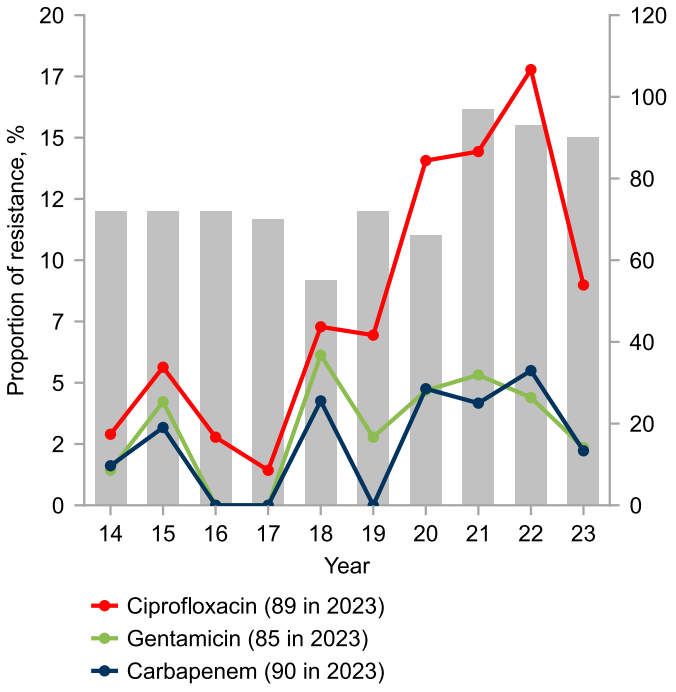


Table 8.12 *Acinetobacter* spp. tested and resistant invasive isolates, Denmark, 2014-2023 DANMAP 2023

	2014		2015		2016		2017		2018		2019		2020		2021		2022		2023	
	res.	n	res.	n	res.	n	res.	n	res.	n	res.	n	res.	n	res.	n	res.	n	res.	n
Ciprofloxacin	2	69	4	71	2	72	1	70	4	55	5	72	9	64	14	97	16	92	8	89
Gentamicin	1	70	3	71	0	70	0	70	3	49	2	72	3	64	5	94	4	92	2	85
Carbapenem	1	62	3	68	0	69	0	67	2	47	0	72	3	63	4	96	5	93	2	90
Total number of invasive isolates	72		71		72		70		55		72		66		97		93		90	

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

8.2.5 Enterococci

Enterococci are part of the normal intestinal microbiota in humans and animals. More than 54 species belonging to the genus *Enterococcus* have been described. The majority of human infections are caused by *E. faecalis* and *E. faecium* and in rare cases by *E. casseliflavus*, *E. gallinarum* and *E. raffinosus*. Most common clinical infections include urinary tract infections, intra-abdominal infections, bacteraemia and infective endocarditis.

Enterococci are intrinsically resistant to many groups of antimicrobials which gives them a selective advantage in e.g. hospitalised patients under antibiotic treatment, leading to colonization or infection. The source of hospital infection is often associated with invasive medical devices.

Invasive cases from hospitals

In 2023, *E. faecalis* isolated from 645 unique patients and *E. faecium* isolated from 613 unique patients were reported in MiBa. Table 8.13 shows resistance percentages towards the most important antibiotics for both species for 2023 and Figure 8.11 shows the annual numbers of invasive isolates and resistance to vancomycin.

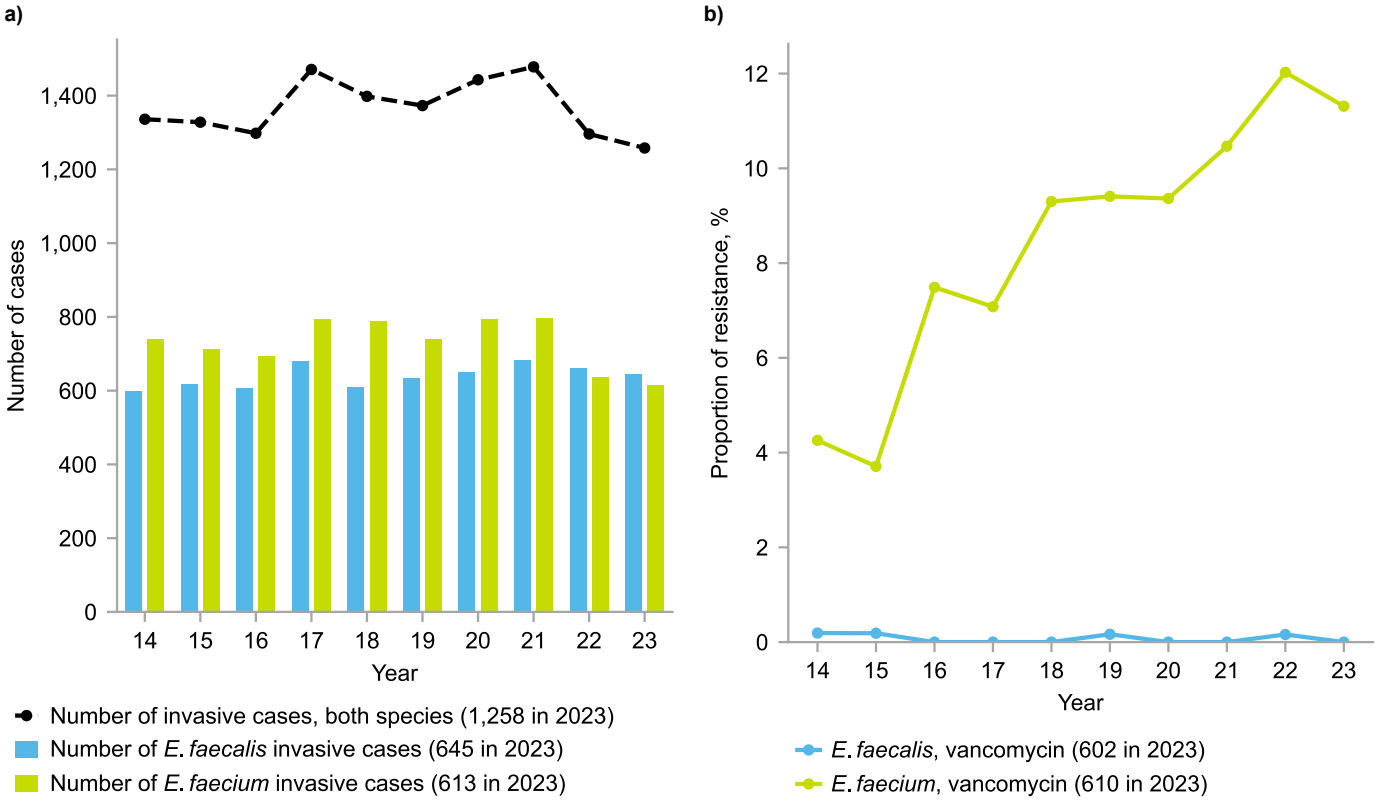
Table 8.13 Enterococci. Resistance (%) in invasive isolates from humans, 2023 DANMAP 2023

	E. faecalis	*E. faecium*	*E. faecalis*	*E. faecium*
	%	%	Number of included isolates (number of DCM)	
Ampicillin	0.2	93.1	645 (10)	592 (9)
Vancomycin	0.0	11.3	602 (9)	610 (10)
Linezolid	0.8	0.0	504 (7)	483 (6)
Teicoplanin	0.4	0.5	227 (2)	196 (2)
Tigecycline	0.0	0.0	110 (1)	104 (1)

All proportions of resistance are presented for each antibiotic as a mean of the resistance rates reported by the DCM. Included are all DCM that report routine testing (>75% of the isolates)

Figure 8.11 Invasive Enterococci faecalis/faecium isolates from humans:
a) annual number of isolates from unique cases and b) proportion of vancomycin resistant isolates, Denmark, 2014-2023

DANMAP 2023



Conclusion

The number of invasive infections with *E. faecium* seems to be decreasing while the number of infections with *E. faecalis* remains stable and resistance to vancomycin is non-existent in *E. faecalis,* however for *E. faecium* is has been increasing for the last decade and has surpassed 10% for the past three years.

Mikkel Lindegaard and Ute Wolff Sönksen
For further information regarding the above chapters 8.1 and 8.2:
Ute Wolff Sönksen, uws@ssi.dk

For further information regarding EpiMiBa data and data analyses:
Mikkel Lindegaard, ldd@ssi.dk

8.3 Results from the reference laboratories

8.3.1 Characterization of ESBL- and pAmpC-producing *Escherichia coli* from bloodstream infections

Background

Resistance to third-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 (DCM) have, on a voluntary basis, submitted third-generation cephalosporin-resistant *E. coli* (3GC 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 EC's collected in Denmark through 2023, 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 Multilocus Sequence Types (MLSTs) and the encoding ESBL-, pAmpC- and carbapenemase genes.

Results

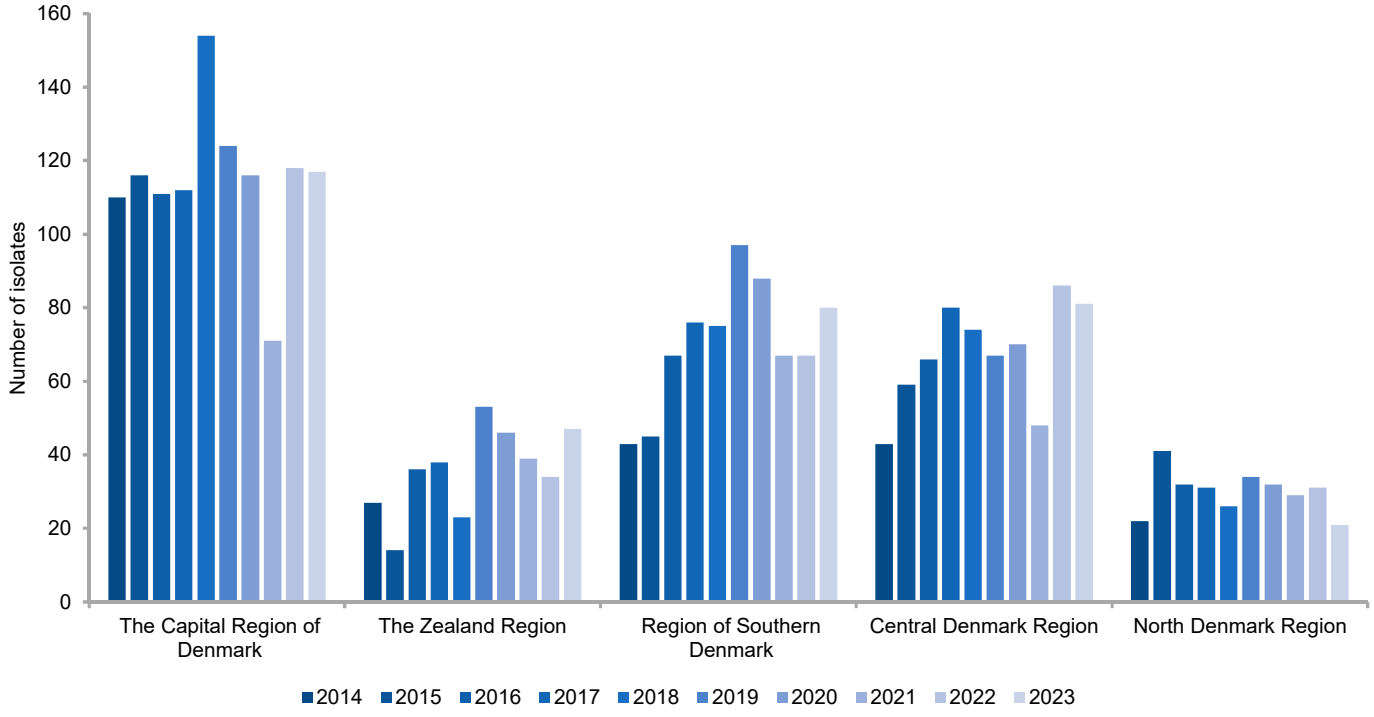
In 2023, a total of 346 *E. coli* blood isolates from unique patients, were identified with phenotypic test, as ESBL and/or AmpC positive isolates. Demographic data was available for all 346 *E. coli* isolates; the median age at diagnosis was 71 years, ranging from below one year to 99 years. In 2023, 164 (47%) of the patients were men compared to 187 (56%) in 2022. The change in men/women distribution observed in 2023 was statistically significant, compared to 2022. Further, it was the first time during surveillance of 3GC EC, that women outnumbered men.

The regional distribution of the 346 isolates with ESBL- and AmpC phenotype was compared to data from previous years (Figure 8.12 and Table 8.14). Following the notable increase in numbers of reported cases of ESBL/pAmpC *E. coli* in bloodstream infections observed from 2021 to 2022 (32% from 254 to 336 isolates), the number reported in 2023 remained stable compared to the previous year, with no significant changes within regions.

Whole genome sequencing data were obtained from 188 *E. coli* isolates (as only isolates from every second month and/or carbapenemase producing *E. coli* were sequenced). Genes encoding ESBL, pAmpC and/or carbapenemase were detected in 179 isolates, and nine isolates were AmpC hyperproducers caused by cAmpC mutations. The AmpC hyperproducers were not included in the further analysis.

In 2023, 16 different genes associated with ESBL-, and pAmpC enzymes were detected among the 179 sequenced isolates encoding ESBL and/or pAmpC genes, (Table 8.15). As in previous years, CTX-M-15 was the most prevalent enzyme, remaining relatively stable in occurrence at 58% in 2023, compared to 52% in 2022. In addition, the carbapenemase enzyme OXA-48 was observed only once during 2023 among the 179 whole genome sequenced bloodstream isolates.

Figure 8.12. ESBL/pAmpC producing *E. coli* from bloodstream infections by region, 2014-2023, Denmark DANMAP 2023



In 2023, the 179 analyzed whole genome sequenced *E. coli* isolates belonged to 45 different known MLSTs. In 2023, the most common sequence type (ST) was still ST131, however a decrease was observed for this type as only 68 (38%) of the isolates belonged to this type in 2023 compared to 89 (50%) in 2022. Additionally, an increase in isolates belonging to ST69 was observed from 9 (5%) in 2022 to 27 (15%) in 2023 (Table 8.16).

Among the 68 *E. coli* isolates belonging to ST131, CTX-M-15 (59%) was the most common enzyme, followed by CTX-M-27 (32%). Furthermore, among the 27 *E. coli* isolates belonging to ST69, CTX-M-15 (48%) was also the most common enzyme, followed by CTX-M-14 (15%), DHA-1 (15%) and CTX-M-27 (11%).

Table 8.14 Distribution of ESBL and carbapenemase producing *E. coli* from bloodstream infections, Denmark, 2015-2023 DANMAP 2023

Region	DANMAP 2015 Numbers	DANMAP 2016 Numbers	DANMAP 2017 Numbers	DANMAP 2018 Numbers	DANMAP 2019 Numbers	DANMAP 2020 Numbers	DANMAP 2021 Numbers	DANMAP 2022 Numbers	DANMAP 2023 Numbers
The Capital Region of Denmark	116	111	112	154	124	116	71	118	117
The Zealand Region	14	36	38	23	53	46	39	34	47
Region of Southern Denmark	45	67	76	75	97	88	67	67	80
Central Denmark Region	59	66	80	74	67	70	48	86	81
North Denmark Region	41	32	31	26	34	32	29	31	21
Total Numbers	275	312	337	352	375	352	254	336	346

Table 8.15 Most common ESBL enzymes and carbapenemases detected in *E. coli* from bloodstream infections, Denmark, 2015-2023 DANMAP 2023

Enzyme	DANMAP 2015 Number	%	DANMAP 2016 Number	%	DANMAP 2017 Number	%	DANMAP 2018 Number	%	DANMAP 2019 Number	%	DANMAP 2020 Number*	%	DANMAP 2021 Number*	%	DANMAP 2022 Number*	%	DANMAP 2023 Number*	%
blaCTX-M-1	7	3%	8	3%	17	5%	25	7%	8	4%	7	4%	6	4%	1	<1%	3	2%
blaCTX-M-14	33	12%	40	13%	48	14%	31	9%	33	17%	15	8%	12	9%	17	9%	17	9%
blaCTX-M-14b	5	2%	9	3%	3	1%	10	3%	3	2%	4	2%	0	0%	3	2%	2	1%
blaCTX-M-15	139	51%	157	50%	164	49%	200	57%	82	43%	100	52%	63	46%	94	52%	103	57%
blaCTX-M-27	33	12%	44	14%	52	15%	53	15%	37	19%	36	19%	29	21%	34	19%	32	18%
blaCTX-M-3	4	1%	7	2%	8	2%	5	1%	4	2%	1	1%	3	2%	1	<1%	0	0%
blaCTX-M-55	14	5%	6	2%	13	4%	4	1%	8	4%	4	2%	5	4%	3	2%	1	<1%
blaCMY-2	6	2%	10	3%	7	2%	6	2%	5	3%	5	3%	2	1%	2	1%	3	2%
blaDHA-1	3	1%	5	2%	6	2%	10	3%	4	2%	7	4%	3	2%	11	6%	9	5%
blaSHV-12	5	2%	5	2%	3	1%	4	1%	2	1%	5	3%	3	2%	3	2%	3	2%
Other CMY variants	10	4%	3	1%	3	1%	3	1%	5	3%	0	0%	1	1%	1	<1%	1	<1%
Other ESBL enzymes	23	8%	17	5%	10	3%	10	3%	3	2%	8	4%	6	4%	7	4%	2	1%
Carbapenemase enzymes	3	1%	1	<1%	1	<1%	5	1%	0	0%	7	4%	4	3%	5	3%	1	<1%

In some isolates more than one enzyme was detected
* Numbers based on sequenced data from odd months

Table 8.16. Distribution of MLSTs in *E. coli* from bloodstream infections, Denmark, 2015-2023 DANMAP 2023

MLST	DANMAP 2015 Number	%	DANMAP 2016 Number	%	DANMAP 2017 Number	%	DANMAP 2018 Number	%	DANMAP 2019 Number*	%	DANMAP 2020 Number*	%	DANMAP 2021 Number*	%	DANMAP 2022 Number*	%	DANMAP 2023	
ST131	135	49%	177	57%	175	52%	189	54%	93	47%	89	46%	64	49%	89	50%	68	38%
ST69	10	4%	16	5%	20	6%	27	8%	14	7%	20	10%	7	5%	9	5%	27	15%
ST12	9	3%	14	4%	6	2%	5	1%	5	3%	2	1%	5	4%	4	2%	8	4%
ST38	23	8%	21	7%	23	7%	22	6%	13	7%	8	4%	1	1%	11	6%	7	4%
ST1193	5	2%	10	3%	7	2%	8	2%	6	3%	9	5%	9	7%	5	3%	7	4%
ST95	ND	ND	5	2%	4	1%	4	1%	3	2%	4	2%	3	2%	2	1%	5	3%
ST2279	ND	ND	0	0%	0	0%	2	1%	0	0%	3	2%	1	1%	2	1%	5	3%
ST73	2	1%	4	1%	2	1%	6	2%	4	2%	8	4%	1	1%	5	3%	5	3%
Other STs[1]	91	33%	65	21%	100	30%	89	25%	59	30%	50	26%	39	30%	51	29%	47	26%

[1] Found in less than 2% in 2022
* Numbers based on sequenced data from odd months

Conclusion
In 2023, the number of ESBL- and/or AmpC positive isolates remained stable compared to 2022, even though a significant shift in the gender distribution was observed. CTX-M-15 remained by far the most prevalent ESBL enzyme in Danish 3GC EC in 2023 (58%). Isolates belonging to ST131 decreased, whereas an increase was observed for isolates belonging to ST69.

In 2023, only one carbapenemase producer was observed among the 179 sequenced ESBL- and/or pAmpC blood infection isolates. Even though, the relative distribution of sequence types for the whole genome sequenced isolates changed compared to previous years; the worldwide disseminated ST131 clone was still strongly represented in 2023 (38%).

Louise Roer, Frank Hansen, Henrik Hasman and Anette M. Hammerum
For further information: Louise Roer, loro@ssi.dk

8.3.2 Carbapenemase-producing organisms (CPO)

Background
Carbapenems comprise one of the only classes of antimicrobial agents that can be used for treatment of infections caused by multidrug-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 expressed by the presence of various carbapenemases of which the five 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).

Carbapenemase-producing organisms (CPO) comprise two main groups: 1) Intestinal bacteria: Carbapenemase-producing Enterobacterales (CPE), e.g. *E. coli* and *K. pneumoniae*, and 2) environmental bacteria: *P. aeruginosa* and *A. baumannii*. The term CPE is used to designate intestinal bacteria. Thus, CPE are a subset of CPO.

CPO have been notifiable in Denmark since 5th September 2018 [https://www.retsinformation.dk/eli/lta/2018/1091]. Before this date, Danish departments of clinical microbiology (DCM) have submitted carbapenem-resistant isolates for verification and genotyping at the National Reference Laboratory for Antimicrobial Resistance at Statens Serum Institut on a voluntary basis. This section describes findings for carbapenemase-producing Enterobacterales (CPE), *Pseudomonas* spp. and *Acinetobacter* spp.

During 2023, 589 CPOs were identified from 458 patients compared with 392 CPO isolates from 335 patients in 2022, an increase in isolates of 50%. More than one isolate from the same patient was included if the isolates belonged to different bacterial species and/or if the isolates harbored different carbapenemases.

Collection of travel information is part of the CPO surveillance in Denmark. Thus, information of travelling abroad in the period of 6 months prior to testing positive for CPO is requested and reported to SSI, together with the clinical sample information.

Carbapenemase-producing Enterobacterales
In 2023, a total of 552 CPE isolates were reported from 436 patients compared with 350 CPE from 304 patients in 2022, resulting in a 58% increase of CPE isolates and a 43% increase in numbers of patients compared to 2022. In 2023, 37 of the 552 CPE isolates produced both NDM and OXA-48 group enzymes, 385 produced OXA-48-like enzymes alone and 109 were only NDM-producing. Furthermore, 12 KPC-, three VIM-, three KPC-/NDM- two VIM/OXA-48- group as well as one VIM/NDM-producing CPE isolate(s) were identified (Figure 8.13).

The large increase in CPE in 2023 was caused by a combination of an increase in the number of both clinical and screening isolates in most of the five regions (Figure 8.14). However, especially in one region (Region Zeeland), an intensified screening regime was implemented in 2023 in order to adhere to the national guidelines issued by the Danish Health Authorities to handle hospital outbreaks caused by CPO. This intensified screening is therefore an important contributing factor in explaining the increase in CPE in this particular region, together with an increase in clinical isolates in the region.

Among the species most frequently detected in CPE outbreaks the dominating bacterial species were *C. freundii* and *E. coli*, (table 8.17), while in travel-related findings the most dominating species were *K. pneumoniae* and *E. coli* (not shown).

No information is available as to the mortality in these cases.

Figure 8.13 Numbers of carbapenemase-producing Enterobacterales (CPE), Denmark, 2008-2023 DANMAP 2023

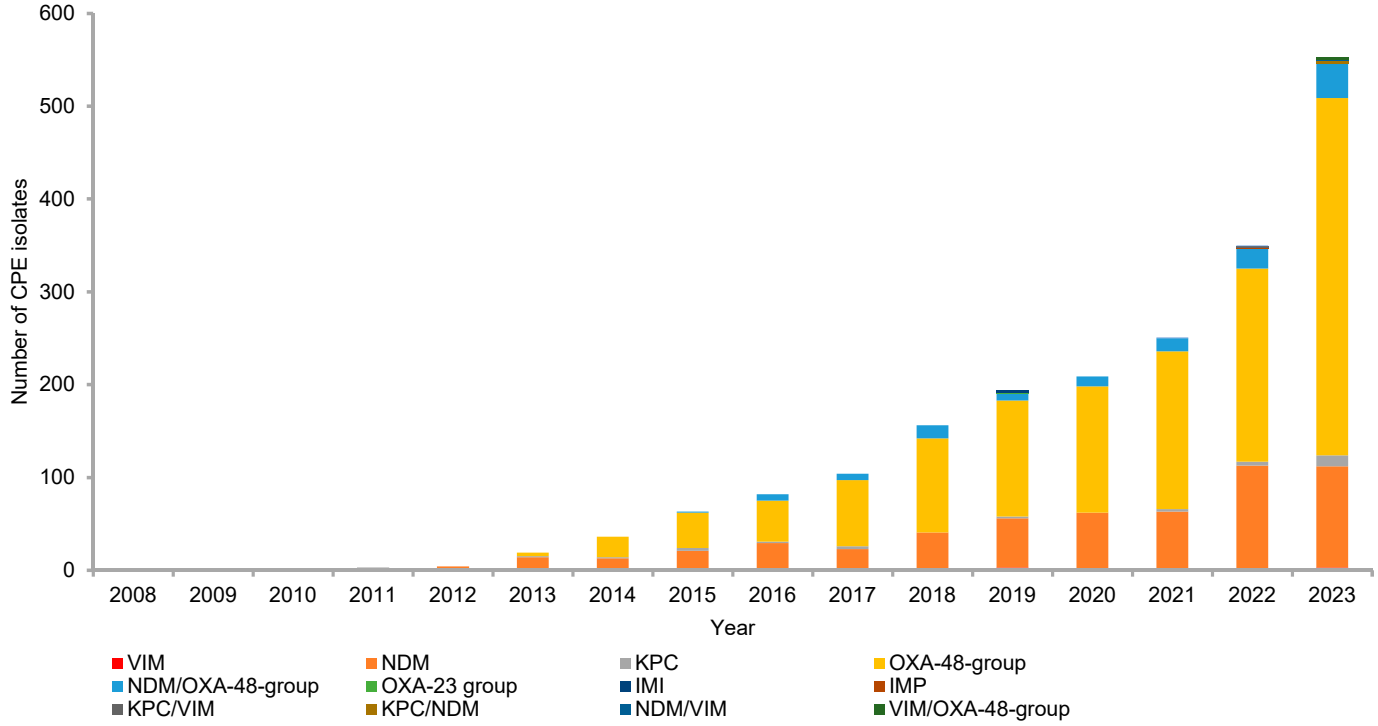
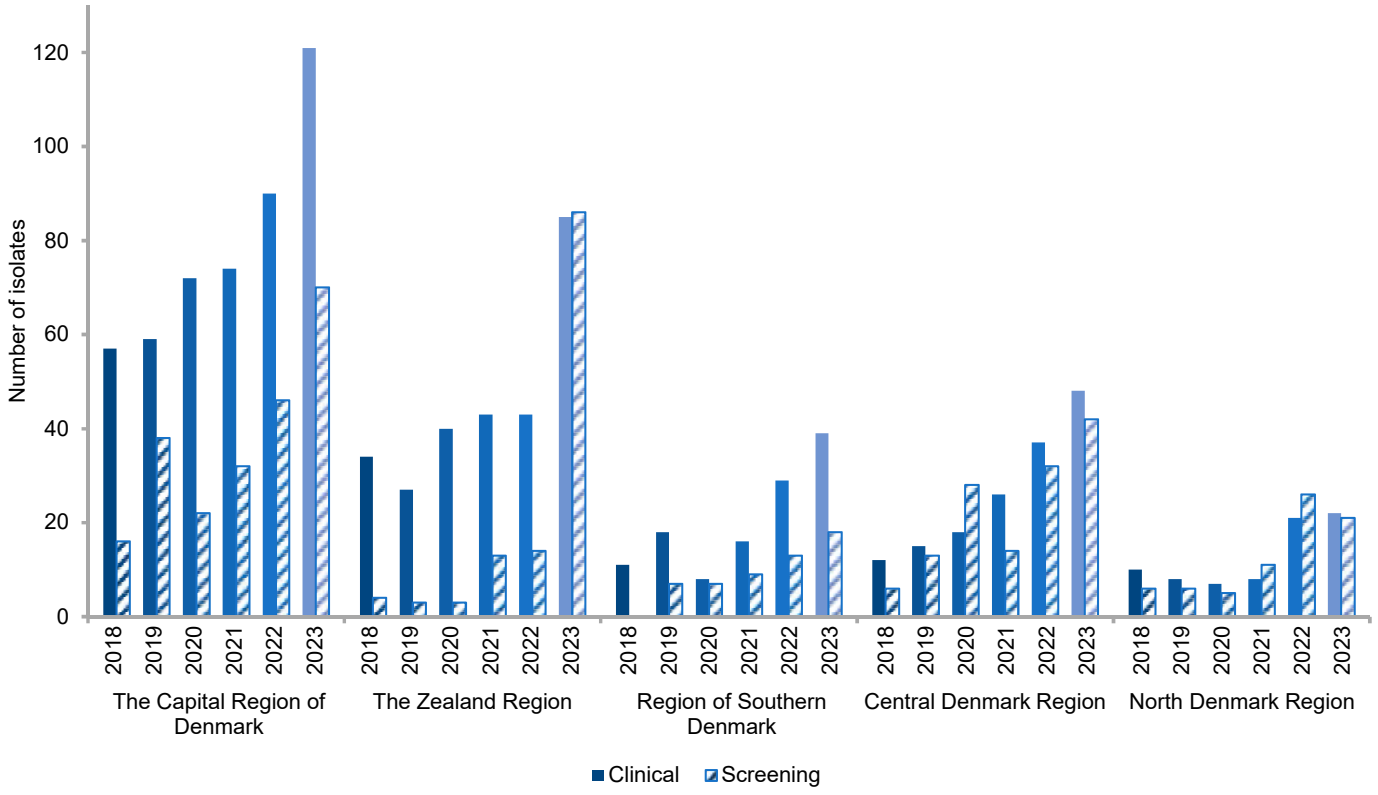


Figure 8.14 Number of clinical and screening CPE isolates per region, Denmark, 2018-2023 DANMAP 2023



Carbapenemase-producing *Acinetobacter* spp.

In 2023, 21 carbapenemase-producing *Acinetobacter* spp. isolates were reported from 21 patients, compared with 23 isolates from 23 patients in 2022. Of these 21 patients, eight patients had been travelling abroad and seven patients had relation to Ukraine prior to identification of the carbapenemase-producing *Acinetobacter* spp. No travel information was reported for six of the patients. In 2023, 18 carbapenemase-producing *Acinetobacter baumannii* with the following enzymes were identified: OXA-23 (12), OXA-72 (2), OXA-23/OXA-72 (3) and NDM-5/OXA-23 (1). Furthermore, one OXA-253- producing *Acinetobacter bereziniae*, one VIM-producing *Acinetobacter monteilii*, one NDM/OXA-58 *A. bereziniae* and one NDM-1-producing *A. lwoffii* were identified. (Figure 8.15).

Carbapenemase-producing *Pseudomonas* spp.

In 2023, 16 carbapenemase-producing *Pseudomonas* spp. isolates from 16 patients were reported compared to 19 isolates in 2022. Of these 16 patients, one patient had not been travelling, four patients had been travelling abroad, eight patients had relation to Ukraine and no information was given for three patients prior to identification of the carbapenemase-producing *Pseudomonas* spp. In 2023, 15 carbapenemase-producing *Pseudomonas aeruginosa* with the following enzymes were identified: NDM-1 (3), VIM (6) and IMP (6). In general, the number of carbapenemase-producing *Pseudomonas* spp. seems to be relatively stable over the years until the onset of Covid-19, which led to a large decrease in the number of isolates and then the onset of the war in Ukraine, which again has led to a large increase (Figure 8.16).

Figure 8.15 Carbapenemase-producing *Acinetobacter* spp. and enzymes identified, Denmark, 2008-2023 DANMAP 2023

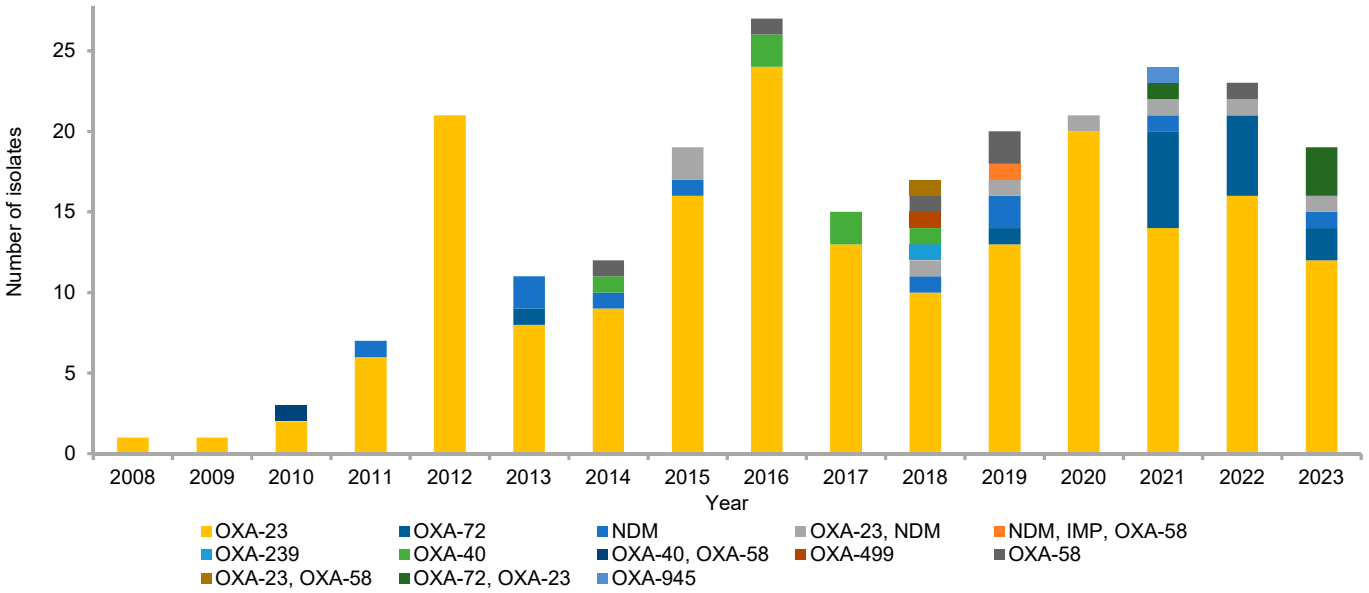
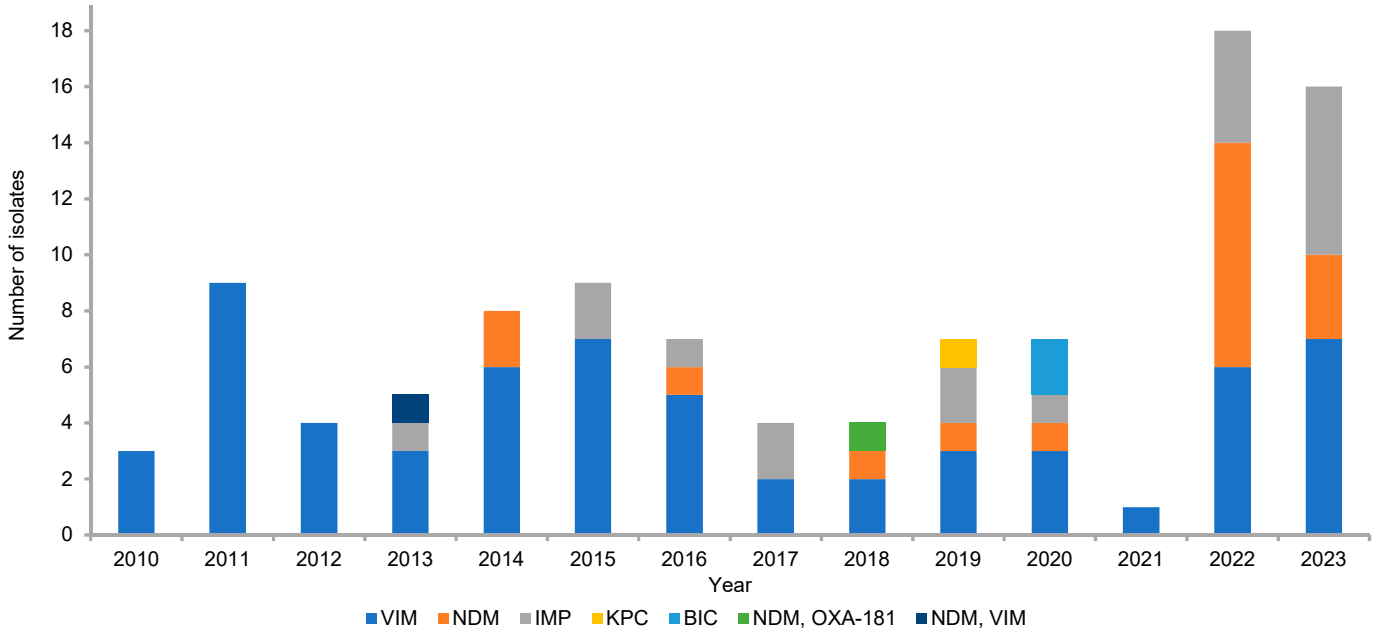


Figure 8.16 Carbapenemase-producing *Pseudomonas* spp. and enzymes identified, Denmark, 2010-2023 DANMAP 2023



CPO - Place of origin 2019-2023

If a patient is detected positive with CPO, the Clinical Departments or a clinical physician must report travel in the period of six months before day of sample. The CPO-patient will then be classified as a travel-associated CPO-patient. The Clinical Departments or a clinical physician can also report a CPO-patient to be colonized or infected in Denmark implicating that the patient has not been travelling prior to positive sample. In order to qualify the information regarding the origin of a colonization with CPO in a Danish patient, the reported CPO-data from 2019 to 2023 has been evaluated and categorized into four categories: 1) colonized or infected in Denmark, 2) part of Danish outbreak, 3) travel outside the Nordic countries, and 4) unknown (Figure 8.16).

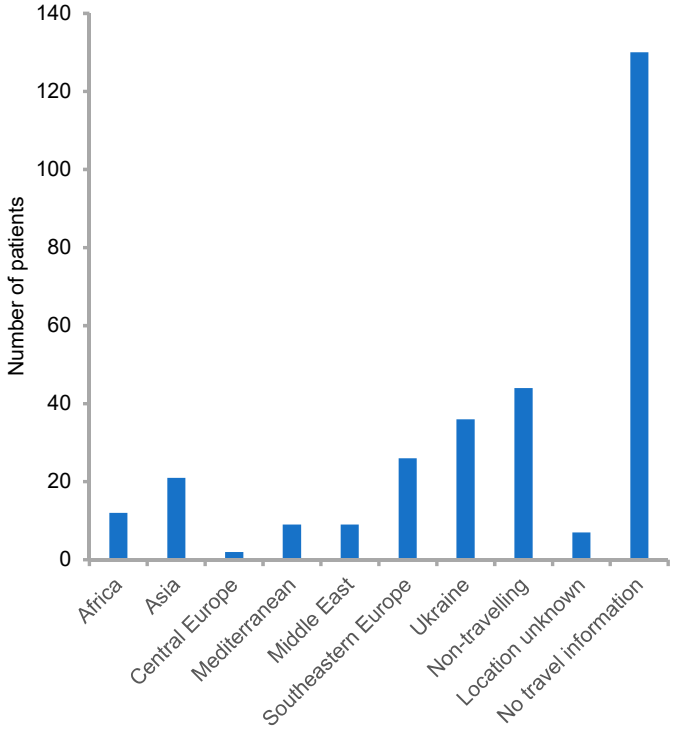
A CPO-patient can be affected by a Danish nosocomial outbreak and will be classified as an outbreak-patient. The presumed index patient (the first patient identified) in an outbreak will be registered according to possible travel information.

In 2023, 86 (29%) of 296 CPO cases with no relation to Danish domestic outbreaks reported travelling outside the Nordic countries, representing an increase compared to 2022, where 52 (19%) of 279 CPO positive cases had been travelling. The number of cases where no travel information is provided was high, 130 cases (44%).

The most frequent reported travel destinations in 2023 were Southeastern Europe (26), Asia (21), Middle East and Mediterranean (9 respectively) (Figure 8.17). The most reported single country travel destinations were Turkey (22), India (8), Pakistan and Thailand (5 respectively).

Due to the still ongoing war in Ukraine, a number of patients from Ukraine are still receiving care in the Danish healthcare system. In 2023, a total of 58 CPO isolates were collected from 36 patients from Ukraine. According to the Danish Health Authority, Denmark has received a total of 101 patients from Ukraine as part of medical evacuations in 2023. More than one isolate was included from individual patients, if the isolates belonged to different bacterial species and/or had different carbapenemases. Among the 58 CPO, 43 were CPE isolates, seven were *Acinetobacter* ssp. and eight were *Pseudomonas* spp. The findings show that the patients originating from Ukraine were colonized and/or infected by many CPO per patient [Stolberg et al. 2023, J Glob Antimicrob Resist. 34:15-17].

Figure 8.17 Place of origin of CPO, 2019-2023 DANMAP 2023



Outbreaks with CPO during 2023

In Denmark, outbreaks caused by CPO in healthcare settings are registered at SSI in a national database (KURS). At SSI, CPO isolates are routinely characterized by whole genome sequencing (WGS). Cluster analysis is conducted on all isolates using cgMLST to detect possible clustering between the CPO isolates. A genomic cluster is defined as at least two isolates (from different patients) belonging to the same cgMLST cluster (or having less than 15 alleles difference between the isolates). Due to the risk of long-term colonization of the patients, cluster analysis is carried out regardless of the time of isolation. When epidemiological investigations can establish a link (e.g., the patients had been at the same hospital ward at the same time) between at least two patients in a genomic cluster, the patients are classified as belonging to a "verified outbreak". When no epidemiological link can be established, the patients related to the genomic cluster are classified as part of a possible outbreak (Materials and methods, section 10.12).

In 2023, a total of 26 CPO outbreaks were registered compared to 17 CPO-outbreaks in 2022 (Table 8.17). In 18 of the outbreaks, it was possible to establish an epidemiological link between the majority of the patients. All, except for two of the outbreaks, epidemiological links were found in healthcare settings, caused by patients sharing the same ward or staying at the same hospital at the same time. Sixteen of the outbreaks have been ongoing for more than two years. Two outbreaks for more than ten years.

In total, 138 new patients were associated with outbreaks in Denmark in 2023. This is an increase compared to 2022 where 78 new patients were affected. Ten patients were part of more than one outbreak. Of the 26 outbreaks registered in 2023, eight new small clusters were identified involving two to five patients each. Five of the new clusters was identified in Region Zeealand, where the intensified screening regime for CPO also identified more CPO outbreaks than previous years.

Table 8.17 Outbreaks of carbapenemase-producing Enterobacterales (CPE) during 2023, n = 26, Denmark DANMAP 2023

Outbreak ID	Year	Patients total	Patients 2023	Carbapenemase	Type of Outbreak	Species (clonal spread)	Regions[1]	Status
Outbreaks of carbapenemase-producing Enterobacterales (CPE)								
41	2012 - 2023	108	15	NDM-1	Clonal/ plasmid	ST18 *C. freundii*	1 / 2 / 3 / 4 / 5	Verified
48	2013 - 2023	38	4	OXA-436/OXA-48	Clonal/ plasmid	ST90 *E. cloacae*/ ST22 *C. freundii*	1 / 4 / 5	Verified
21	2015 - 2023	96	12	NDM-5/OXA-181	Clonal	ST410 *E. coli*	1 /2 / 5	Verified
22	2015 - 2023	15	2	OXA-181	Clonal	ST440 *E. coli*	1 / 2	Verified
42	2015 - 2023	16	3	OXA-48	Clonal	ST65 *C. freundii*	1 / 3 / 5	Verified
47	2015 - 2023	12	1	VIM-2	Clonal	ST111 *P. aeruginosa*	2 / 3	Possible
1066	2017 - 2023	30	12	OXA-48	Clonal	ST91 *C. freundii*	1 / 5	Possible
1070	2017 - 2023	9	3	OXA-48	Clonal	*C. farmeri*	5	Verified
1081	2017 - 2023	6	1	NDM-5/OXA-48	Clonal	ST22 *C. freundii*	1 / 5	Possible
43	2019 - 2023	22	17	OXA-48	Clonal	ST323 *C. freundii*	1 / 5	Verified
1061	2020 - 2023	14	4	OXA-181	Clonal	ST22 *C. freundii*	2	Possible
1062	2020 - 2023	38	24	NDM-5	Clonal/ plasmid	ST79 *E. hormaechei*	National	Verified
1110	2020 - 2023	4	2	OXA-181	Clonal	ST116 *C. freundii*	1	Possible
1089	2021 - 2023	7	1	OXA-244	Clonal	ST131 *E. coli*	2	Verified
1132	2021 - 2023	10	2	NDM-1	Plasmid	*C. freundii*	1	Possible
1107	2022 - 2023	5	3	OXA-181	Clonal	ST636 *C. freundii*	5	Verified
1113	2022 - 2023	6	4	OXA-48	Clonal	ST22 *C. freundii*	5	Verified
1115	2022 - 2023	4	1	NDM-1	Clonal	ST2 *K. oxytoca*	3	Verified
1112	2023	5	5	OXA-244	Clonal	ST10 *E. coli*	1	Verified
1146	2023	5	4	OXA244	Clonal	ST13730 *E. coli*	1 / 5	Possible
1127	2023	3	3	NDM-1/OXA-181	Clonal	ST781 *E. ludwigii*	5	Verified
1133	2023	2	2	OXA-48	Clonal	ST124 *C. freundii*	5	Possible
1131	2023	4	4	OXA-181	Clonal	ST2 *K. oxytoca*	5	Verified
1135	2023	3	3	OXA-181	Clonal	ST36 *K. oxytoca*	5	Verified
1139	2023	4	4	OXA-181	Clonal	ST65 *C. freundii*	5	Verified
Outbreaks of carbapenemase-producing organisms (CPO)								
1129	2023	2	2	OXA-23	Clonal	ST2069 *A. baumannii*	1 / 5	Verified

[1] Regions will be listed as: 1) Capital Region, 2) Central Denmark Region, 3) North Denmark Region, 4) Southern Denmark Region and 5) Zealand Region

Outbreaks with CPO of special interest

The single outbreak affecting most new cases in 2023 was the unusual outbreak with the same unique epitype ST79 *Enterobacter hormaechei* (outbreak ID1062), caused by medical treatment with contaminated dicloxacillin capsules from the same producer. The outbreak was described in the DANMAP report 2022 (Textbox 8.1). Since September 2023, no new cases have been identified in this outbreak.

In 2019, two cases were identified as part of the same cluster of ST323 *Citrobacter freundii* (outbreak ID43). In 2021 and 2022, three more cases were identified as part of the cluster. In 2023, the intensified screening in Region Zealand has identified several new cases, which explains the increase in the number of cases in this outbreak. The screening strategy recommended in the CPO guideline is an important tool, together with the infection control measures (IPC) to control and stop an outbreak due to antimicrobial resistance (AMR).

The ST18 NDM-1-producing *Citrobacter freundii* outbreak (ID41), which started in 2012 in the North Denmark Region, continued in 2023 [Hammerum et al. 2016, J. Antimicrob. Agents, 71:3117-3124]. Since 2012, the outbreak has now spread to all five Danish regions. The main reason for the spread between regions is the movement of infected patients between regions. By the end of 2023, a total of 108 cases were involved in this outbreak. In 2023, 15 new cases were identified, a decrease from the previous year when 20 new cases were identified. None of the cases had a travel history.

Since 2015, another large outbreak (ID21) has been ongoing, primarily in two regions of the Zealand Region and in the Capital Region. The outbreak is associated with the spread of ST410 NDM-5/OXA-181 *E. coli*. [Roer et al. 2018, mSphere;3(4)]. By the end of 2023, a total of 96 cases have been identified as part of this outbreak. During 2023, 12 new cases were detected to be part of this outbreak. With the exception of the initial cases reported in 2015, who had been traveling to Egypt [Overballe-Petersen et al. 2018, Genome Announc.6(5): e01542-17] none of the cases had a history of travel.

Conclusion

During 2023 the numbers of CPO in Denmark continued to increase. The number of patients received from Ukraine are contributing to this increase, but the main contributing factor is a general increase in CPO at Danish hospitals and the increase in numbers of CPE in nosocomial outbreaks. Furthermore, intensified screening, particularly in one region, also contributed to the increase in CPE cases in 2023.

The national outbreak caused by medical treatment with Dicloxacillin capsules from the same producer also contributed to the number of new outbreak-patients in 2023.

The number of new nosocomial detected outbreaks in 2023 has increased since 2022. For the first time 10 patients were involved in more than one outbreak. The number of patients belonging to the largest outbreaks in hospital settings continued to increase. This highlight the importance to start early interventions with infection prevention control (IPC) in order to prevent further spread of CPO in hospitals and between hospitals.

Travel outside the Nordic countries is a contributing factor to the number of CPO isolates detected in Denmark. The most frequently reported travel destinations in 2023 were South Eastern Europe and Asia. The number of cases where no travel information is provided is high.

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 infection, which makes outbreak control difficult and have consequences for medical treatment.

Anette M. Hammerum, Lone Jannok Porsbo, Frank Hansen, Anne Kjerulf, Louise Roer, Mikkel Lindegaard, Brian Kristensen and Henrik Hasman
For further information: Henrik Hasman, henh@ssi.dk

8.3.3 Vancomycin-resistant/vancomycin-variable enterococci

Background

Enterococci are intrinsically resistant to a number of first-line antimicrobial agents, including cephalosporins, which increases the risk of enterococci being selected for in-hospital environments and emerging in patients that receive cephalosporins for other conditions. Most hospital-acquired *Enterococcus 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 within the last decade, both internationally as well as in Denmark. Newer antibiotics such as linezolid and daptomycin can be used for treatment of VRE, but both antimicrobial agents may lead to adverse events, and the development of resistance, particularly against linezolid, is relatively common and has also been reported against daptomycin. In recent years, isolates of phenotypically vancomycin-susceptible *E. faecium* have been described to harbor a *vanA*-gene complex, in various countries. These isolates are referred to as vancomycin-variable enterococci (VVE). It has been demonstrated that VVE retain the ability to become vancomycin resistant upon exposure to vancomycin [Patel 2018 – PLOS one], and are often associated with nosocomial outbreaks. However, VVE cannot be selectively cultured on vancomycin-containing media and can only be detected by molecular methods. In 2016, a new VVE clone belonging to ST1421- CT1134, displaying variable vancomycin susceptibility due to a deletion in the *vanX* gene, was detected. [TA Hansen *et al.*, J. Antimicrob. Agents, 2018, 73: 2936–2940]. The VVE clone has spread to all five Danish regions [Hammerum *et al.* Euro Surveill. 2024;29(23)].

Conclusively, VVE may be of clinical relevance and timely detection as well as continuous monitoring are critical to avoid treatment failure and further transmission.

Surveillance of VRE/VVE

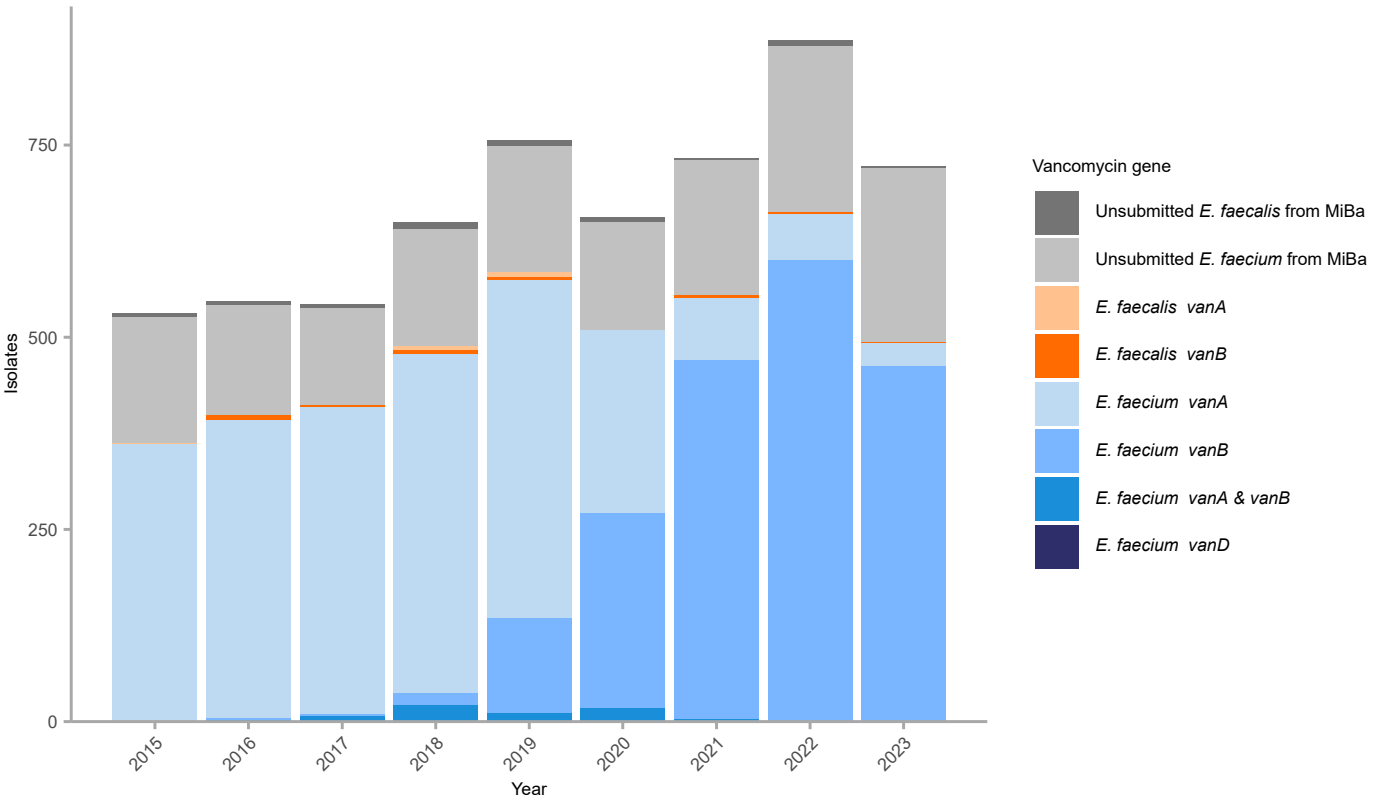
Since 2005, Danish departments of clinical microbiology (DCM) have voluntarily submitted clinical VRE (one VRE/VVE isolate per patient, isolated within 12 months) for species identification, genotyping and surveillance purposes to the Antimicrobial Resistance Reference Laboratory at Statens Serum Institut (SSI).

Since 2015, the received clinical VRE/VVE isolates have been analysed using whole-genome sequencing (WGS). The WGS data are used for in silico genotyping of isolate characteristics such as species identification, multilocus sequence typing (MLST), core genome sequence typing (cgMLST) and detection of van-genes and core locus MLST (cgMLST) used for clonal detection (see Section 10.13.3).

To determine any underreporting in the submissions of VRE isolates, the number of VRE/VVE submitted to SSI since 2015 were compared to data from clinical VRE reported by the DCM to MiBa (the Danish Microbiology Database). These isolates were not genotyped, yet they are included in Figure 8.18. This comparison showed that the number of submitted VRE/VVE isolates to SSI was not complete (Figure 8.18). The MiBa isolates were distributed evenly throughout the study period and it did not seem to be a systematic loss. In 2023, 494 VRE/VVE isolates were submitted to SSI, and were genotyped by WGS. Furthermore, 228 VRE/VVE isolates were identified in MiBa, which had not been submitted to SSI and hence have not been genotyped. This was a decrease compared to 2022, were 662 VRE/VVE isolates were sent to SSI, while 224 VRE/VVE isolates were identified in MiBa yet not submitted to SSI. (Figure 8.18).

Figure 8.18 Overview and distribution of vancomycin resistance genes in vancomycin-resistant isolates, Denmark, 2015-2023

DANMAP 2023



Of the 494 clinical VRE/VVE isolates sequenced in 2023, 31 were *vanA E. faecium*, 462 vanB *E. faecium*, and 1 *vanB E. faecalis* (Figure 8.18). Until 2020, *vanA E. faecium* were most common, but during the last years this has changed. In 2023, 93,7% of the *E. faecium* isolates had the *vanB* gene.

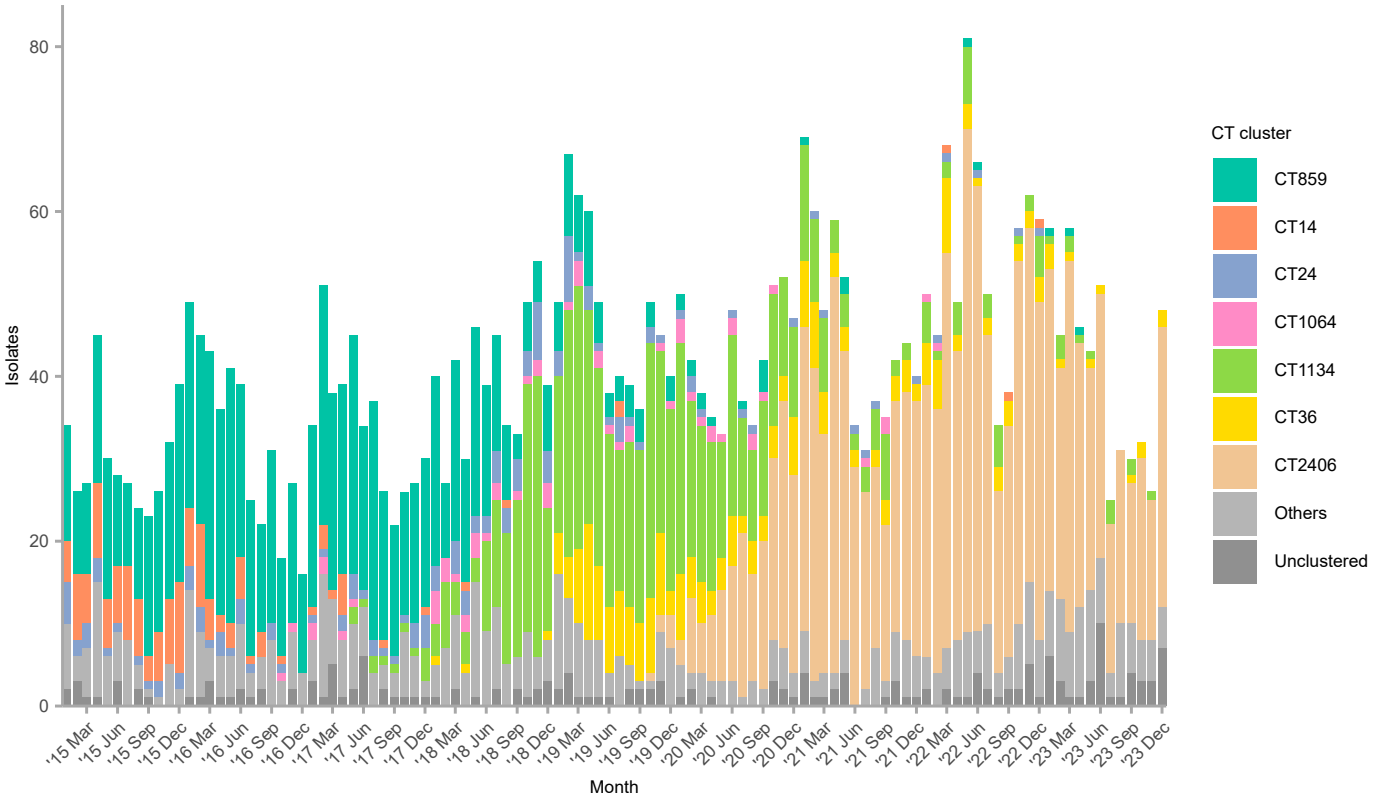
WGS-based cgMLST analysis was performed on the 494 *E. faecium* isolates using SeqSphere+ (Ridom). The 494 *E. faecium* isolates belonged to unique 113 CT clusters. When investigating the composition of sequence types (ST) and clonal types (CT) for *E. faecium*, we observed a clustering tendency between isolates, where ST's and CT's were diverging while the allelic differences were minimal within each cluster. MLST clusters were set up using the MLST algorithm of SeqSphere+, setting the maximal allelic distances to 20. The top seven complex type clusters were devised, based on clustering of the isolates. Each complex type cluster were named according to the ST and CT of the earliest observed isolate within each cluster. One clonal group (covering several different CTs but presumably originating from the same clone) were predominant: The ST80-CT2406 *vanB E. faecium* group containing 332 isolates (Table 8.18).

Table 8.18 Description of the most common types of *vanA* and/or *vanB Enterococcus faecium* according to MLST and cgMLST, Denmark, 2015-2023

DANMAP 2023

Complex type	2015		2016		2017		2018		2019		2020		2021		2022		2023		All years	
Cluster	(n = 361)		(n = 392)		(n = 409)		(n = 478)		(n = 574)		(n = 509)		(n = 551)		(n = 660)		(n = 493)		(n = 427)	
ST203-CT859	179	50%	249	64%	260	64%	153	32%	57	10%	12	2%	3	0,5%	2	0,3%	3	0,6%	918	21%
ST80-CT14	85	24%	37	9%	12	3%	2	0,4%	2	0,3%	0	0,0%	0	0%	3	0,5%	0	0%	141	3%
ST117-CT24	21	6%	18	5%	20	5%	39	8%	25	4%	8	2%	6	1%	5	0,8%	0	0%	142	3%
ST80-CT1064	0	0%	2	0,5%	6	1%	23	5%	12	2%	14	3%	3	0,5%	2	0,3%	0	0%	62	1%
ST1421-CT1134	0	0%	0	0%	13	3%	161	34%	285	50%	196	39%	63	11%	35	5%	14	3%	767	17%
ST117-CT36	0	0%	0	0%	0	0%	3	0,6%	94	16%	54	11%	43	8%	41	6%	12	2%	247	6%
ST80-CT2406	0	0%	0	0%	0	0%	0	0%	7	1%	178	35%	370	67%	476	72%	332	67%	1363	31%
Other clusters	63	17%	72	18%	74	18%	83	17%	73	13%	37	7%	45	8%	73	11%	89	18%	609	14%
Unclustered	13	4%	14	4%	24	6%	14	3%	19	3%	10	2%	18	3%	23	3%	43	9%	178	4%

Figure 8.19 Timeline of the clonal group prevalence in all sequenced VRE isolates. Clonal groups are named according to sequence type and clonal type of the earliest observed member, Denmark, 2015-2023 DANMAP 2023



From 2015 to 2023, seven *E. faecium* clusters dominated: ST80-CT14 *vanA*, ST117-CT24 *vanA*, ST203 -CT859 *vanA*, ST1421-CT1134 *vanA*, ST80-CT1064 *vanA-vanB*, ST117-CT36 *vanB* and ST80-CT2406 *vanB* [Hammerum et al. Euro Surveill. 2024;29(23)]. Figure 8.19 shows the monthly count of these seven clusters alongside the remaining clusters during the period 2015 to 2023.

We detected ST80-CT14 *vanA E. faecium* in all Danish Regions during 2015. On a national level, the numbers of ST80-CT14 *vanA E. faecium* decreased from 2016 to 2018. In 2022, only a few isolates ST80-CT14 *vanA E. faecium* were detected. ST117-CT24 *vanA E. faecium* was first detected in the Capital Region in January 2015. Between 2015 and 2022 it was detected in all five regions. ST203-CT859 *vanA E. faecium* was first detected in Denmark in December 2014. It was one of the dominating clones from 2015 through July 2018 and was detected in all five regions. In 2022, only a few isolates belonging to ST203-CT859 *vanA E. faecium* were detected. ST80-CT1064 *vanA-vanB E. faecium* was first detected in Central Denmark Region in October 2016. It spread to the North Jutland Region during April 2018. During 2019, a single case of ST80-CT1064 *vanA-vanB E. faecium* was detected. This clone disappeared during January 2022. *E. faecium* isolates belonging to ST1421-CT1134 *vanA E. faecium* (VVE cluster) were first detected in clinical samples in 2016. In 2018, 34% of the *E. faecium* isolates belonged to ST1421-CT1134, and they were mostly detected in The Capital Region. During 2019, ST1421-CT1134 *vanA E. faecium* was the most prevalent type (44%) but decreased during 2021 and 2022. ST117-CT36 *vanB E. faecium* was detected in January 2019 in Capital Region of Denmark. During 2019, ST117-CT36 vanB *E. faecium* was detected in all five Danish Regions. In October 2019, the first clinical ST80-CT2406 *vanB E. faecium* sample was detected in a patient hospitalised in the Capital Region. It spread further to other patients in the Capital Region in 2019. During 2020, this cluster was detected in all Danish regions except the Northern Region of Denmark but during 2021, it was also detected there. [Hammerum et al. Euro Surveill. 2020;25(32)]. Since 2022, the ST80-CT2406 have been and continues to be, the main dominant complex type cluster in all Danish regions. [Hammerum et al. Euro Surveill. 2024;29(23)].

Infection prevention and control guidelines for VRE

Acting in compliance with the national guidelines for infection prevention and control (published by National Centre for Infection Control at SSI) is key in preventing the spread of multidrug-resistant microorganisms, with correct hand hygiene and correct use of personal protective equipment (PPE) among the most important control measures [https://hygiejne. ssi.dk/NIRgenerelle]. The supplemental national guideline for infection prevention and control includes specific guidance on VRE/VVE and should be followed when examining and treating patients, residents and citizens with multidrug-resistant microorganisms [https://hygiejne.ssi.dk/NIRsupplerende]. Precautions include the use of PPE such as a disposable gown and gloves

and proper cleaning of the environment [https:// hygiejne.ssi.dk/NIRdesinfektion]. Moreover, the guidelines emphasise the importance of prescribing antibiotics with caution. According to the national supplemental infection prevention control (IPC) guideline, screening of a patient is recommended on admission to hospital if the patient: 1) is transmitted from a hospital outside the Nordic countries, 2) has been hospitalised outside the Nordic countries within the last 6 months, 3) has been hospitalised in wards in Denmark or another Nordic country with outbreaks of VRE within the last 6 months or 4) previously has been diagnosed with a VRE-infection or carrier state. Isolation is recommended in the national supplemental IPC-guideline in case of verification of VRE in the patient.

Conclusion

The number of VRE/VVE cases has decreased from 2022 to 2023 to a level that is comparable to 2021. While the decrease is a desired development, the level of VRE infections is still high. Therefore, more prevention strategies are required to prevent spread of VRE in the Danish health care system.

Anette M. Hammerum, Kasper Thystrup Karstensen, Louise Roer, Anne Kjerulf, Asja Kunøe and Henrik Hasman
For further information: Anette M. Hammerum, ama@ssi.dk

8.3.4 Detection of linezolid-vancomycin resistant enterococci

Background

Linezolid is an antimicrobial belonging to the oxazolidinones. Its indication of use are nosocomial pneumonia and complicated skin and soft tissue infections caused by Gram-positive bacteria. It belongs to the defined last line choices and should be used with caution, based on microbiological testing and only if other antibiotics are not available. In Denmark it is primarily used in combination treatments for patients with very complicated Gram-positive infections and as treatment against 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.

Particular interest has been paid to the transferable resistance genes and these are monitored in the Danish surveillance system due to a potential risk of a shared pool of resistance genes with enterococci stemming from animals that have been treated with pleuromutilines, a group of antimicrobials related to the oxazolidinones.

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-vancomycin-resistant Enterococci (LVRE)

In order to detect linezolid-resistant VRE isolates (LVRE), the LRE-Finder was implemented in the automated surveillance at SSI for detection of LRE mutations and LRE genes in genomes from VRE isolates submitted by the DCM directly for the national VRE Surveillance. During the period 2015 to 2023, no linezolid vancomycin resistant *E. faecalis* were detected, whereas 48 linezolid-vancomycin resistant *E. faecium* were identified.

In 2023, nine linezolid-vancomycin resistant *E. faecium* were identified. All nine LVRE *E. faecium* had the G2576T mutation and were all positive for *vanB* (Table 8.19).

Conclusion

As in previous years the numbers of LVRE have been low, however the findings are of concern as linezolid is important for the treatment of VRE. Often, only few antimicrobial agents are available for treatment of infections with LVRE.

Anette M. Hammerum, Kasper Thystrup Karstensen, Louise Roer, and Henrik Hasman
For further information: Anette M. Hammerum ama@ssi.dk

Table 8.19 Characterization of the nine linezolid vancomycin resistant enterococci (LVRE), 2023, Denmark DANMAP 2023

Linezolid genotype	Vancomycin gene	2015 (n = 1)	2016 (n = 2)	2017 (n = 0)	2018 (n = 2)	2019 (n = 5)	2020 (n = 4)	2021 (n = 10)	2022 (n = 15)	2023 (n = 9)	All years (n = 48)
G2576T	*vanA*	1	2	0	2	0	4	0	5	0	14
G2576T	*vanB*	0	0	0	0	1	0	9	10	9	29
G2576T & *optrA*	*vanA*	0	0	0	0	0	0	1	0	0	1
cfrB	*vanB*	0	0	0	0	1	0	0	0	0	1
optrA	*vanA*	0	0	0	0	3	0	0	0	0	3

8.3.5 *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.

Surveillance of *S. aureus* bacteraemia

The number of *S. aureus* bacteraemia cases were 2,571 in 2023 corresponding to 43 cases per 100,000 inhabitants. This is almost the same number as in 2022 (2,578). Thirty-nine (1.5%) of the bacteraemia cases were caused by MRSA. During the last decade this proportion has been between 1.5% and 2.9% (2014) and remains below most other European countries participating in EARS-Net [EARS-Net 2022]. LA-MRSA CC398 caused nine of the 39 MRSA bacteraemia cases. Within 30 days from the bacteraemia onset, 617 (24%) patients died (all-cause mortality). The mortality for the MRSA bacteraemia cases was 28%.

The antimicrobial susceptibility remained at the same level as the previous years for most agents (Table 8.20). Resistance to penicillin in 2023 was 68%, which confirms the decreasing trend since the beginning of the 1990s, where resistance to penicillin was around 86%. The highest frequency of resistance to antimicrobials other than penicillin was observed for fusidic acid (12%), erythromycin (9%) and clindamycin (8%).

Typing revealed a high diversity with 764 different *spa* types distributed in 30 different clonal complexes (CCs). The ten most prevalent *spa* types, representing 31% of the total number, are presented in Table 8.21 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 38 (1.5%) cases of which eight were MRSA. The 38 isolates with the PVL gene were distributed among 24 different *spa* types.

Surveillance of methicillin-resistant *S. aureus*

In 2023, 3,649 MRSA cases were detected (62 per 100,000 inhabitants), a 20% increase compared to 2022 (2,982; Figure 8.20a), reflecting the return to normality after covid-19. 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). Infections constituted 55% of the cases. The proportion of infections in the years 2014 to 2023 has varied between 38% to 55% (Figure 8.20b).

Table 8.20 Resistance (%) in isolates from *Staphylococcus aureus* bacteraemia cases 2014-2023, Denmark DANMAP 2023

	2014	2015	2016	2017	2018	2019	2020*	2021	2022	2023
Antimicrobial agent	%	%	%	%	%	%	%	%	%	%
Methicillin	2.9	1.5	2.1	2.2	1.6	2.1	1.6	1.6	1.9	1.5
Penicillin	77	71	71	72	72	72	72	69	68	68
Erythromycin	8	7	7	6	5	9	7	7	9	9
Clindamycin	8	7	6	5	4	8	7	7	8	8
Tetracycline	5	4	3	3	3	2	3	2	3	2
Fusidic acid	15	16	12	14	17	14	14	13	13	12
Rifampicin	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Moxifloxacin#	6	6	4	4	4	5	6	4	4	4
Kanamycin	2	3	1	1	2	<1	nt	nt	nt	nt
Linezolid	0	0	0	0	0	0	0	0	0	0
Mupirocin	<1	<1	0	<1	0	<1	<1	<1	<1	<1
Trimethoprim-sulfamethoxazole	1	<1	<1	<1	0	<1	<1	<1	<1	<1

Notes: nt = not tested. * From 2020 and onwards, resistance data was extracted from the Danish Microbiology database, MiBa. # Prior to 2021 testing for fluoroquinolone resistance was reported for norfloxacin

Table 8.21 The ten most prevalent *spa* types demonstrated in SAB and in non-LA-CC398 MRSA cases, Denmark 2023 DANMAP 2023

SAB			MRSA			
spa type	CC group	No. of cases	*spa* type	CC group	No. of cases	No. causing infections (%)
t127	CC1	125	t304	CC8	263	58
t084	CC15	116	t127	CC1	188	53
t091	CC7	87	t008	CC8	162	69
t230	CC45	80	t223	CC22	149	54
t002	CC5	76	t002	CC5	136	61
t021	CC30	69	t4549	CC8	129	83
t012	CC30	68	t005	CC22	84	69
t008	CC8	65	t688	CC5	82	70
t1451	CC398	61	t1476	CC8	62	63
t701	CC8	44	t272	CC121	47	77

CC = Clonal complex, SAB = *S. aureus* bacteraemia

Figure 8.20a Number of new MRSA cases 1994-2023, Denmark, with a three years moving average DANMAP 2023

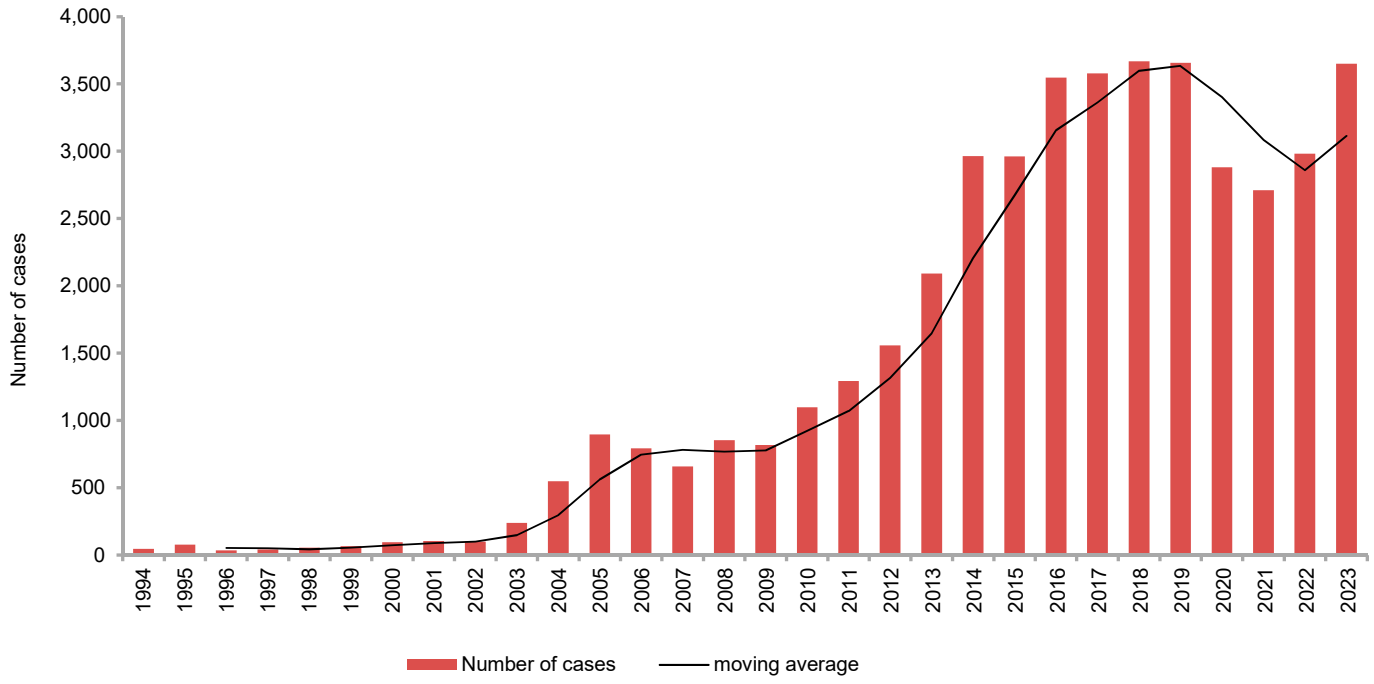
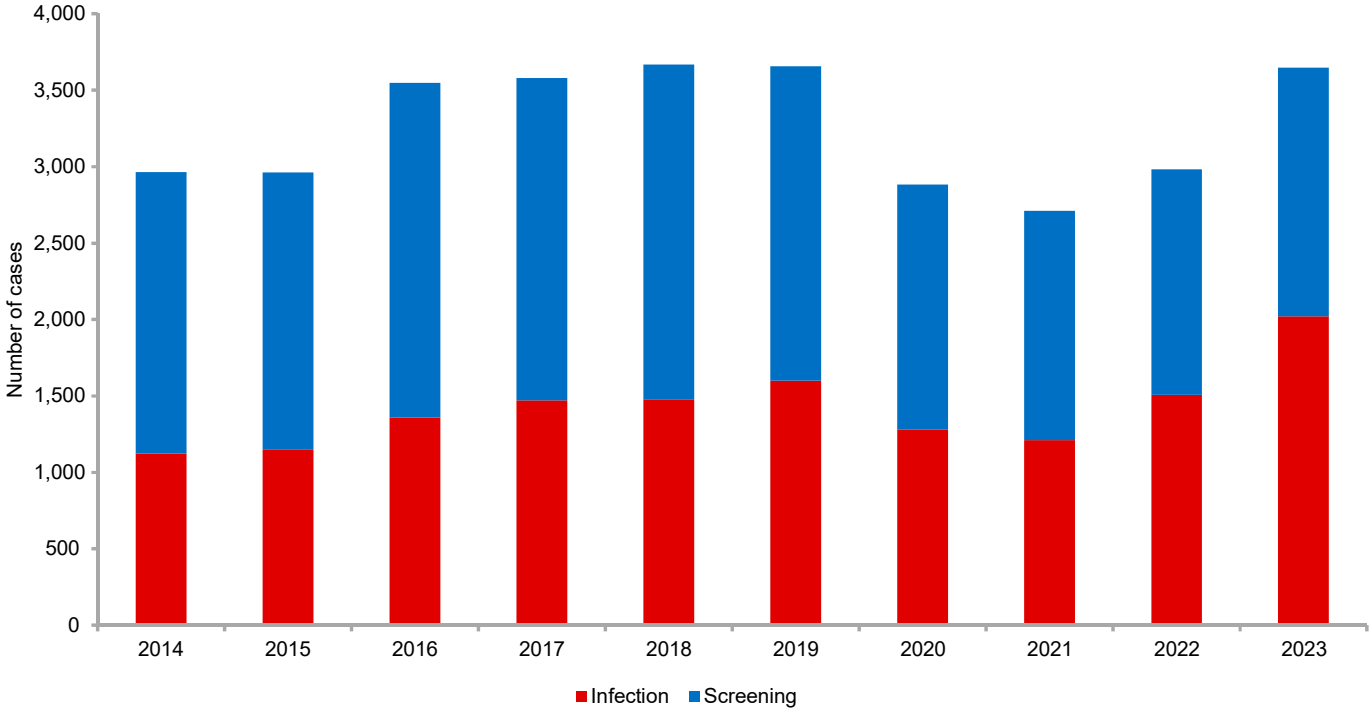


Figure 8.20b Number of new MRSA cases 2014-2023, Denmark, divided in infection and screening samples DANMAP 2023



CC398 cases constituted 25% (n = 897) of new MRSA cases, of which 856 belonged to the livestock-associated clone (LA-MRSA CC398) and the remaining 41 to a human adapted variant harbouring the PVL encoding genes. More LA-MRSA CC398 isolates (72%) were found in healthy carriers compared to MRSA of other types (45%), which likely reflects the active screening of patients with contact to livestock at admission to healthcare.

MRSA isolates carrying *mecC* were detected in 58 cases (1.6%). Forty-five of the cases (78%) had infections at the time of diagnosis. Three patients reported contact to hedgehogs, which recently has been shown to be a major environmental reservoir for *mecC* MRSA. One patient worked with livestock, one patient had a family member working with livestock, while the remaining 50 patients did not report any contact to livestock or other animals.

spa typing revealed 410 different strain types, not including isolates belonging to LA-CC398. Among the infections, 299 *spa* types were identified. The 10 dominating non-LA-CC398 *spa* types isolated in 2023 are listed in Table 8.21. They constituted 47% of the total number of non-LA-CC398 MRSA isolates. Table 8.21 does not list *spa* types in CC398, which for many years has been the dominating CC. *spa* types t304 and t223 have been among the five most prevalent *spa* types since 2016, when their increase was linked to the refugee crisis following the civil war in Syria. This year *spa* type t127 was the second most numerous. This type has been involved in several outbreaks in neonatal units in recent years.

The PVL encoding gene was detected in 26% of the infections and in 12% of the asymptomatic carriers and most often in relation to isolates with *spa* types t008 (n = 90), t005 (n = 73), t355 (n = 45), t034 (n = 32) and t021 (n = 31).

Forty-one MRSA outbreaks were registered at hospitals, nursing homes and other institutions. These outbreaks comprised a total of 199 cases of which 80 had an infection. Seven of the outbreaks occurred in neonatal departments, comprising a total of 48 cases. Additionally, eight outbreaks were registered in other hospital departments, comprising 18 patients and thirteen outbreaks were observed in nursing homes (counting a total of 29 residents).

The total number of cases and the number of cases presenting with infection according to epidemiological classification are shown in Table 8.22. Most of the cases (86%) were acquired in Denmark.

The trend of MRSA infections for 2014-2023 based on their epidemiological classification is shown in Figure 8.21. The number of infections in community-acquired cases increased in 2023 when compared to 2022, and the number of infections among imported cases were also higher. Importantly, number of infections among hospital-acquired MRSA remained low in 2023 (59 cases).

Resistance among MRSA isolates

Resistance data for non-LA-CC398 isolates are presented in Table 8.23. Resistance prevalences were similar to previous years, with relatively high resistance to erythromycin (32%), fusidic acid (26%), clindamycin (21%), tetracycline (21%) and moxifloxacin (24%), and low resistance (<1%-1) to trimethroprim-sulfamethoxazole, linezolid, mupirocin and rifampicin.

Table 8.22 Epidemiological classification of new MRSA cases, Denmark 2023 DANMAP 2023

Epidemiologic classification	Exposure	No. of cases (% of total)	No. (%) of cases with infections (% of cases in same epi. class)
Imported (IMP)		**573 (16)**	433 (76)
Hospital-acquired (HA)		**102 (3)**	44 (43)
Health-care associated, community onset (HACO)		**281 (8)**	
	with known exposure	43	27 (63)
	without known	238	215 (90)
Health care worker		**33 (1)**	15 (45)
Community-acquired (CA)		**1,799 (49)**	
	with known exposure	808	144 (18)
	without known	991	904 (91)
LA-MRSA CC398		**856 (23)**	
	with known exposure	691	119 (17)
	without known	165	117 (71)
Total		**3,649**	**2,020 (55)**

Note: Numbers shown in bold are totals

Figure 8.21 Number of MRSA infections according to epidemiological classification, 2014-2023, Denmark DANMAP 2023

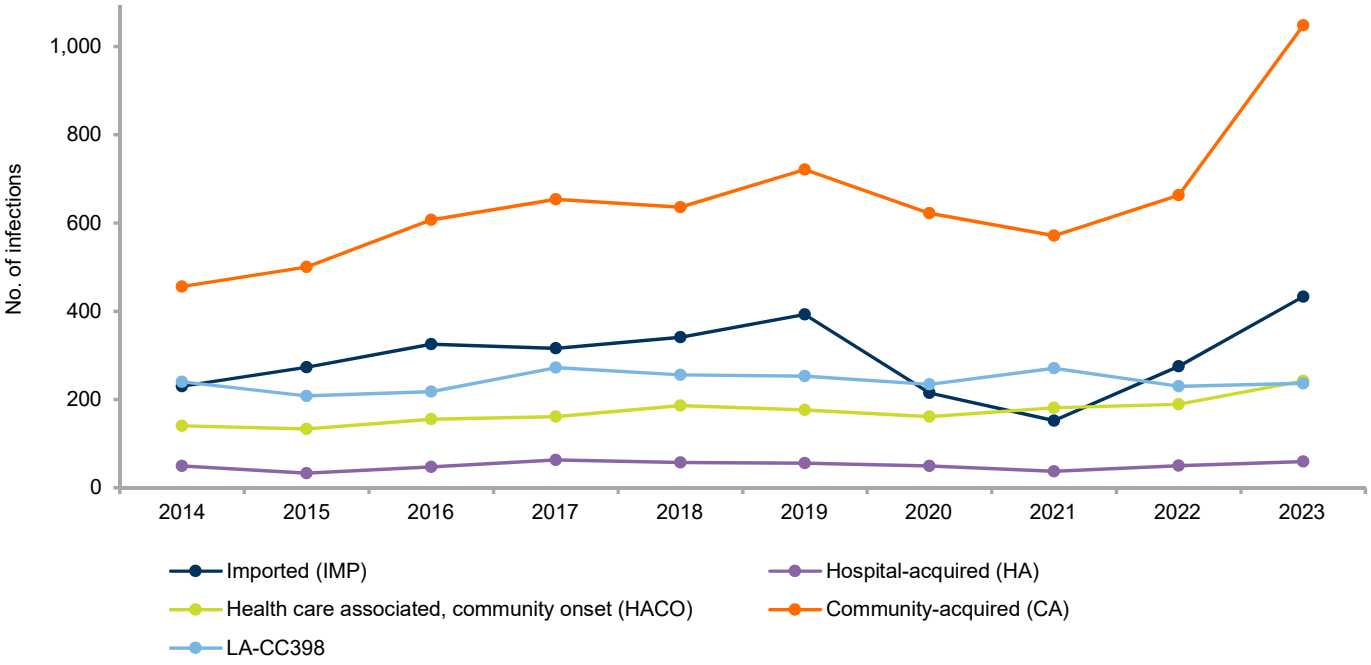


Table 8.23 Resistance (%) in non LA-CC398 MRSA isolates, 2014-2023, Denmark DANMAP 2023

	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023
Erythromycin	33	37	34	34	33	33	30	29	34	32
Clindamycin	23	29	25	27	28	23	22	19	21	21
Tetracycline	21	24	26	24	26	22	22	21	23	21
Fusidic acid	17	19	18	16	18	23	22	21	22	26
Rifampicin	<1	<1	1	1	1	<1	<1	<1	<1	1
Moxifloxacin#	27	21	19	20	21	21	17	19	23	24
Linezolid	<1	0	<1	0	<1	0	<1	<1	<1	<1
Mupirocin	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Trimethoprim-sulfamethoxazole	3	4	2	3	3	4	2	<1	1	1
Number of tested isolates	1,616	1,242	1,184	1,193	1,233	1,025	1,920*	1,520*	2,043*	2,696*

*Not all isolates were tested for all listed antimicrobials
Prior to 2021 testing for fluoroquinolone resistance was reported for norfloxacin

Conclusion

The number of *S. aureus* bacteraemia cases was 2,571 in 2023 and at the same level as in 2022. Of these, 39 cases (1.5%) were caused by methicillin-resistant *S. aureus* (MRSA) with nine being livestock-associated-MRSA (LA-MRSA). Resistance to penicillin continued to decrease and was 68% in 2023. There were 3,649 cases of MRSA from both screening (45% of cases) and infections (55%), which was a 20% increase compared to 2022. Forty-one MRSA outbreaks were registered at hospitals, nursing homes and other institutions for a total of 199 cases with 80 being infections.

Andreas Petersen, Tinna Urth and Anders Rhod Larsen
For further information: Anders Rhod Larsen, arl@ssi.dk

8.3.6 *Streptococcus pneumoniae*

Background

Streptococcus pneumoniae is known to cause various diseases that can be classified into two main groups: non-invasive and invasive diseases. Among these, invasive pneumococcal diseases (IPD) are considered the most severe, while non-invasive pneumococcal infections are more commonly observed. Pneumonia, which can be categorized as non-invasive or invasive, is estimated to be the leading cause of pneumococcal disease worldwide, affecting both children and adults. This is followed by the severe cases of the invasive disease's bacteraemia and meningitis. Pneumococcus often causes the non-invasive acute otitis media (AOM) in children, with more than half of Danish children estimated to experience this condition. AOM is frequently treated with antibiotics, although their effectiveness is uncertain. In addition to these well-known pneumococcal-related diseases, pneumococci are also associated with other common infections such as non-invasive sinusitis and bronchitis, as well as invasive diseases like endocarditis (infection of the heart valves), peritonitis, and septic arthritis.

The surveillance of pneumococci (*S. pneumoniae*) causing invasive disease in Denmark is conducted through mandatory submission of clinical isolates to Statens Serum Institut (SSI) from invasive cases. At SSI, the isolates are serotyped and tested for antimicrobial susceptibility. Any remaining cases without a submitted isolate are identified through MiBa.

Results

In Denmark, 635 cases of invasive pneumococcal disease (IPD) were registered in 2023 (Table 8.24). Pneumococci were mainly found in either blood (571) or cerebrospinal fluid (48). For 16 cases, pneumococci were found in other, normally sterile sites. Earlier, only IPD cases from blood and cerebrospinal fluid, were included in the DANMAP report. This year, we have included all IPD cases, in the report. Of the 635 IPD cases identified in MiBa, 597 isolates were received at the reference laboratory. Data for the 35 remaining cases, where isolates were not provided, were retrieved from MiBa and serotypes for these cases are unknown. Failure of submitting an isolate for serotyping was mainly due to non-viable isolates or diagnosis through PCR. In total, data for serotypes and antimicrobial susceptibility data for penicillin were available for six cases.

The IPD isolates belonged to 39 different serotypes. For the 616 cases with available penicillin susceptibility data, 580 isolates were susceptible to penicillin (94.0%), 30 isolates (4.9%) were classified as non-susceptible to penicillin. Of note, one IPD isolate obtained from cerebrospinal fluid was penicillin resistant with a MIC of 2 mg/L (serotype 9N).

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 8.22), 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. The predominant serotype in 2023 was serotype 3 (20.9%) (Table 8.24). All 133 serotype 3 isolates were susceptible to penicillin. The second and third most frequently isolated serotypes are serotype 8 (84 isolates in 2023) and serotype 22F (69 isolates in 2023), and these isolates were all fully sensitive to penicillin.

Conclusion

The level of penicillin non-susceptible IPD isolates in 2023 (6%) increased slightly compared to 2022 (5%). Both the great fluctuation in the prevalence of penicillin non-susceptible IPD isolates and the general IPD incidence in the years 2020 and 2021 is assumed to be an effect of the COVID-19 restrictions on the general prevalence of infectious diseases associated with respiratory droplet transmission. Such fluctuations have been observed in other countries as well [Shaw, et al., Lancet Digit Health. 2023 Sep;5(9):e582-e593].

Hans-Christian Slotved and Kurt Fuursted
For further information: Hans-Christian Slotved, hcs@ssi.dk or Kurt Fuursted, kfu@ssi.dk

Table 8.24 Number of invasive pneumococcal isolates (IPD) observed in Denmark, 2023 DANMAP 2023

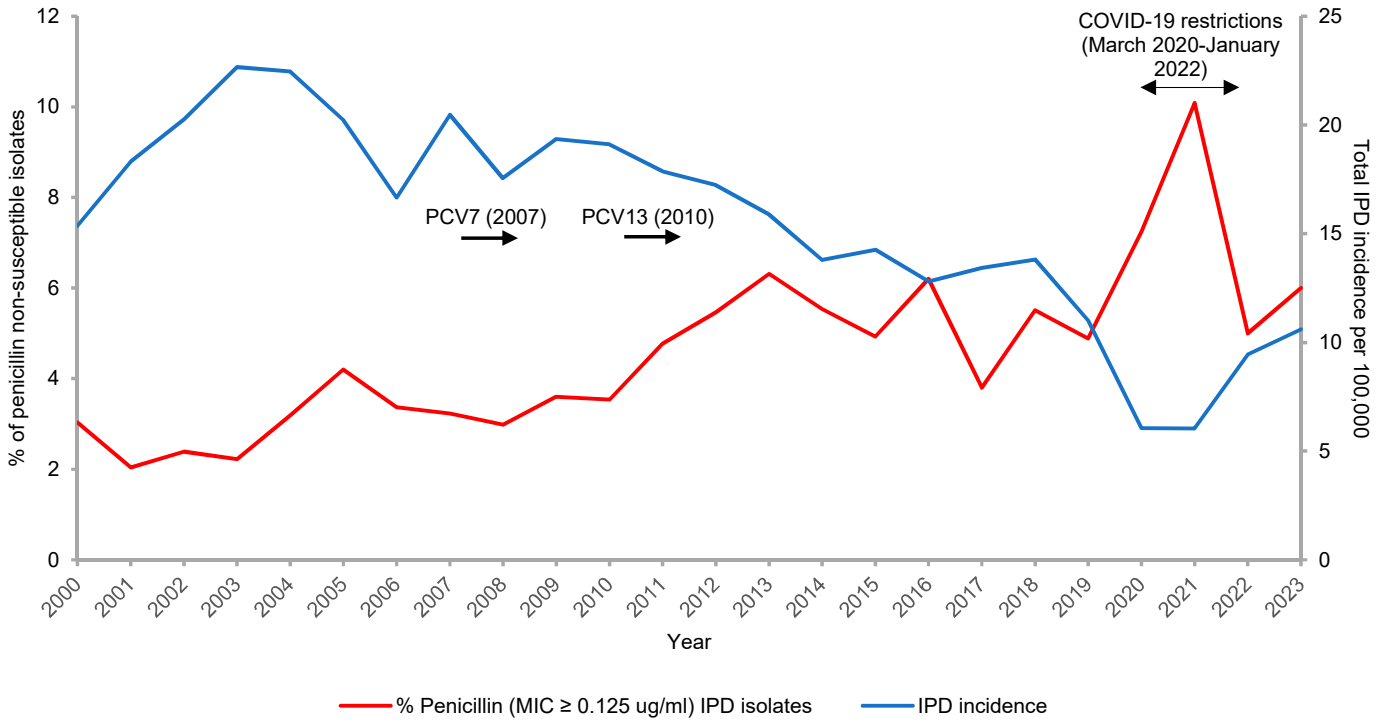
Serotype	Included in pneumococcal vaccines	N 2023	PEN-S	PEN-NON-S	Unknown	% Suscep
Unknown		38	18	1	19	*95%
4	PCV13, PCV15, PCV20, and PPV23	3	2	1	0	67%
6B	PCV13, PCV15, PCV20, and PPV23	1	0	1	0	0%
9V	PCV13, PCV15, PCV20, and PPV23	0	0	0	0	0%
14	PCV13, PCV15, PCV20, and PPV23	1	1	0	0	100%
18C	PCV13, PCV15, PCV20, and PPV23	0	0	0	0	0%
19F	PCV13, PCV15, PCV20, and PPV23	7	1	0	0	14%
23F	PCV13, PCV15, PCV20, and PPV23	1	1	0	0	100%
1	PCV13, PCV15, PCV20, and PPV23	0	0	0	0	0%
3	PCV13, PCV15, PCV20, and PPV23	133	133	0	0	100%
5	PCV13, PCV15, PCV20, and PPV23	0	0	0	0	0%
6A	PCV13, PCV15, and PCV20	2	1	1	0	50%
7F	PCV13, PCV15, PCV20, and PPV23	0	0	0	0	0%
19A	PCV13, PCV15, PCV20, and PPV23	17	15	2	0	88%
2	PPV23	0	0	0	0	0%
8	PCV20 and PPV23	84	84	0	0	100%
9N	PPV23	35	32	3	0	91%
10A	PCV20 and PPV23	17	17	0	0	100%
11A	PCV20 and PPV23	14	14	0	0	100%
12F	PCV20 and PPV23	6	6	0	0	100%
15B	PCV20 and PPV23	10	10	0	0	100%
17F	PPV23	4	1	3	0	25%
20	PPV23	5	5	0	0	100%
22F	PCV15, PCV20, and PPV23	69	69	0	0	100%
33F	PCV15, PCV20, and PPV23	7	7	0	0	100%
24F		29	28	1	0	97%
15A		19	15	4	0	79%
23A		17	16	1	0	94%
38		17	16	1	0	94%
35F		16	16	0	0	100%
7C		12	12	0	0	100%
23B		11	4	7	0	36%
35B		10	10	0	0	100%
15C		10	10	0	0	100%
16F		8	6	2	0	75%
31		8	8	0	0	100%
6C		5	5	0	0	100%
10B		5	5	0	0	100%
17A		3	2	1	0	67%
34		2	1	1	0	50%
21		2	2	0	0	100%
24B		2	2	0	0	100%
35D		2	2	0	0	100%
16A		1	1	0	0	100%
33C		1	1	0	0	100%
9A		1	1	0	0	100%
Sum		635	580	30	19	*94%

N = number of isolates, PEN-S = Penicillin susceptible, PEN-NON-S = Penicillin non-susceptible, % Suscep = percentage of IPD isolates susceptible to penicillin

* Calculations are based on available isolates

Figure 8.22 The incidence of invasive pneumococcal infection (IPD) and the percentage of penicillin non-susceptible IPD isolates in Denmark, 2000–2023. PCV-7 was introduced into the child vaccination program in 2007, and replaced with PCV13 in 2010

DANMAP 2023



8.3.7 Beta-haemolytic streptococci

Background

Beta-haemolytic streptococci (BHS) are classified 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 soft tissue infections, 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 blood, cerebrospinal fluid or other normally sterile anatomical sites) submitted from the departments of clinical microbiology (DCM) in 2022 to the Neisseria and Streptococcus Reference laboratory (NSR). This report includes only non-duplicate isolates, i.e. isolates obtained with an interval of more than 30 days. Isolates are received from all DCM in Denmark. It is voluntary to submit isolates of BHS.

Infections with BHS are usually treated with penicillins or macrolides. All submitted isolates of BHS 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 (http://www.eucast.org/) (version 13.0).

Results

In 2023, a total of 1,651 isolates from invasive cases were received. The number of isolates from unique invasive cases was 1,615, i.e. an increase of 39% compared to 2023 (991). The number of GAS isolates increased by a factor 3.7 compared to 2022, while the number in 2022 had increased by a factor 3.9 compared to 2021. For GBS these ratios were 1.2 and 1.1, respectively. For GCS they were 1.1 and 1.0, respectively, and for GGS they were 1.3 and 1.0.

Figure 8.23 shows the resistance findings for the years 2014 through 2023. All isolates were fully susceptible to penicillin. Comparing GAS in 2023 to 2022, the rates of erythromycin resistance, clindamycin resistance and inducible clindamycin resistance all decreased. For GBS, GCS and GGS these rates all remained nearly unchanged. The percentage of fully susceptible isolates compared to 2022 was increasing for GAS and remained virtually unchanged for the three other serogroups.

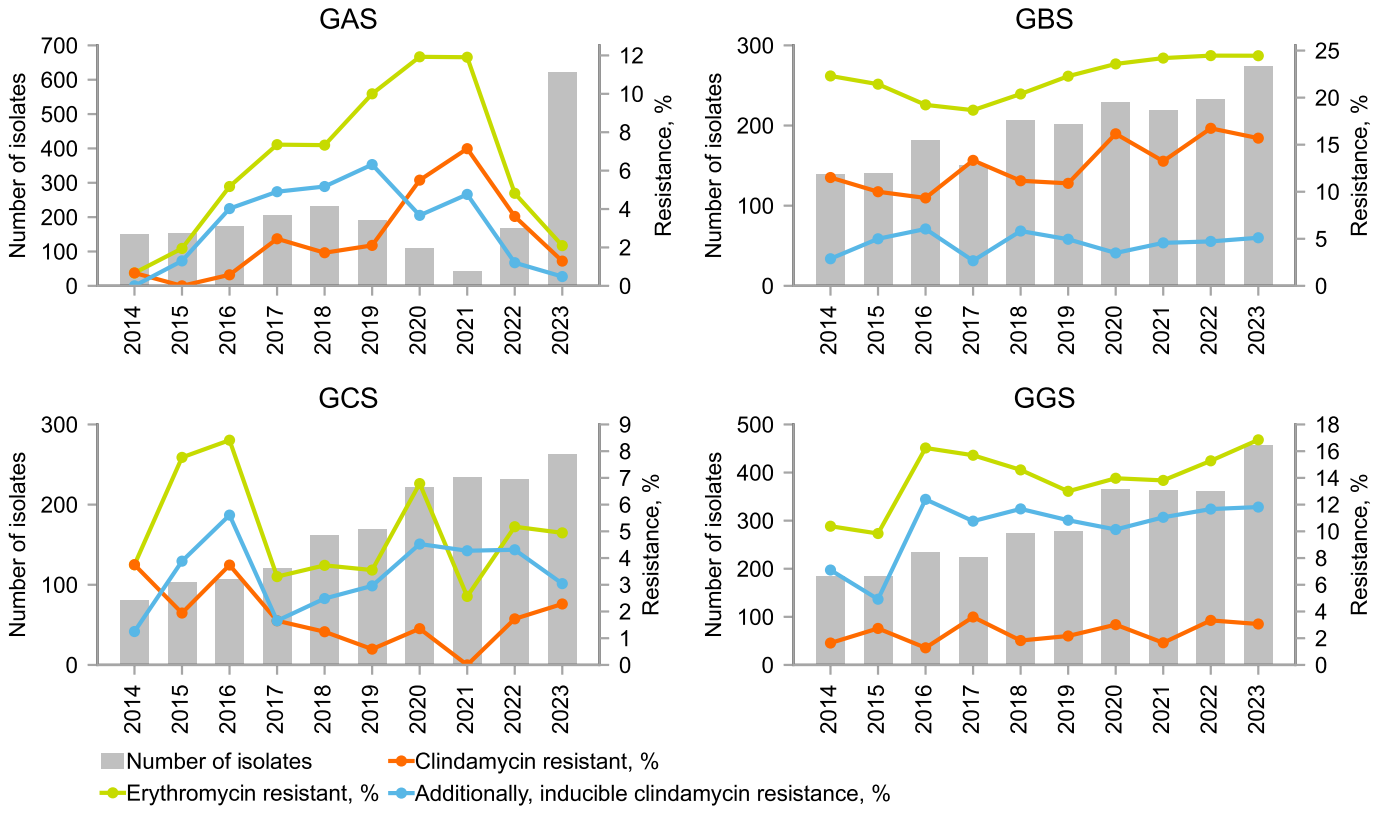
Comments and conclusions

The substantial increase from 2021 to 2022 and further in 2023 for GAS was part of the national as well as international surge of invasive and non-invasive GAS infections, including a high proportion of cases with a serious course. The increase in the number of submitted invasive GAS isolates is probably a consequence of discontinued COVID-19 related restrictions on upper respiratory tract colonization and airborne transmission of this species.

The increase cannot be explained by changes in the prevalence of antimicrobial resistance. All isolates were fully susceptible to penicillin. Comparing GAS in 2023 to 2022, the rates of erythromycin resistance, clindamycin resistance and inducible clindamycin resistance all decreased. For GBS, GCS and GGS these rates all remained nearly unchanged.

Steen Hoffmann and Hans-Christian Slotved
For further information: Steen Hoffmann, hof@ssi.dk

Figure 8.23 Invasive beta-haemolytic streptococci: Antimicrobial resistance testing results, Denmark, 2014-2023 DANMAP 2023



8.3.8 *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 ano-rectal specimens is usually due to contamination with vaginal secretion, while in men who have sex with men (MSM) it is 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 new-borns after transmission from an infected mother during labour and rarely in adults following direct or indirect inoculation.

Gonococcal Surveillance

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.

Both resistant and intermediary susceptible isolates were categorized as resistant in this report. However, isolates with intermediary susceptibility are non-existing according to EUCAST breakpoints for ceftriaxone and azithromycin and they are exceedingly rare regarding ciprofloxacin.

As part of NSR's participation in ECDC's surveillance (Euro-GASP) of sexually transmitted infections since 2009, 110 - 120 gonococcus isolates are collected consecutively each year in September-October 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 isolates received in 2023 were from urethra or cervix, while clinicians only rarely obtained specimens from rectum and pharynx. Occasionally, the NSR laboratory receives isolates from other anatomical sites, such as conjunctivae, joint fluid, blood, Bartholin's abscess, etc.

Repetitive detections of gonococci in a patient are considered to represent unique cases of gonorrhoea if separated by more than 21 days. The NSR laboratory received 2,653 isolates from 2,152 unique cases of gonorrhoea diagnosed in 2023 (1,350 males, 802 females). Only one isolate from each unique case is counted in this report.

The annual number of received isolates increased considerably from 2011 through 2017 (Figure 8.24). This was most likely due to 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 also to an increasing incidence of gonorrhoea, especially among young heterosexual persons, among whom an increasing proportion until 2016 were women. The proportion of female cases has decreased slightly since 2017, but it is still substantially higher than in the beginning of the observation period (2003-2016). A decrease in the annual number of isolates from unique cases was seen in 2018 followed by an increase in 2019-2021 and in particular from 2021 to 2023 [Pedersen et al., Euro Surveill. 2024 Feb;29(7):2400059]. It should be recalled, however, that many cases diagnosed by NAATs are not followed-up by culture.

The ciprofloxacin resistance rate was 45% in 2023 (40% in 2022 and 48% in 2021), (Figure 8.24). Only 0.9% of the isolates were classified as ciprofloxacin intermediate (MIC 0.047-0.064 mg/L).

The percentage of strains producing penicillinase was 14% (14% in 2022 and 23% in 2021). Previously, it has fluctuated between 22% in 2005 and 7% in 2016. Azithromycin resistance (MIC above the present ECOFF >1 mg/L) was found in 6% of the tested isolates (2.9% in 2022 and 2.8% in 2021). In 2018, azithromycin resistance was observed in 6% of the isolates and intermediary susceptibility in 4%. However, EUCAST breakpoints were changed as of January 1, 2019 leading to a greater proportion of strains being classified as sensitive, compared to previously, albeit only if used in conjunction with another effective agent.

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. As shown in Figure 8.25, the ceftriaxone MIC distribution changed from 2003 through 2009 with the highly susceptible isolates becoming gradually less frequent. However, since then, this change has completely reversed. In 2017, there was a single case among the submitted isolates with ceftriaxone MIC of 0.25 mg/L (i.e. resistant) and azithromycin MIC of 0.25 mg/L. Secondary cases were not reported and no strains with ceftriaxone resistance have been encountered among the submitted isolates since 2017.

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 p.o.) was recommended for the empiric treatment of gonorrhoea. This therapeutic regimen has gradually been abandoned during 2019 and onwards by most clinicians.

Ciprofloxacin (500 mg p.o. as a single dose) may still be used for treatment if the strain is fully susceptible.

Figure 8.24 Number of submitted gonococcus isolates from unique cases of *gonorrhoea* in males and females, and the percentage of isolates with ciprofloxacin resistance, azithromycin resistance, and penicillinase production, Denmark, 2003-2023 DANMAP 2023

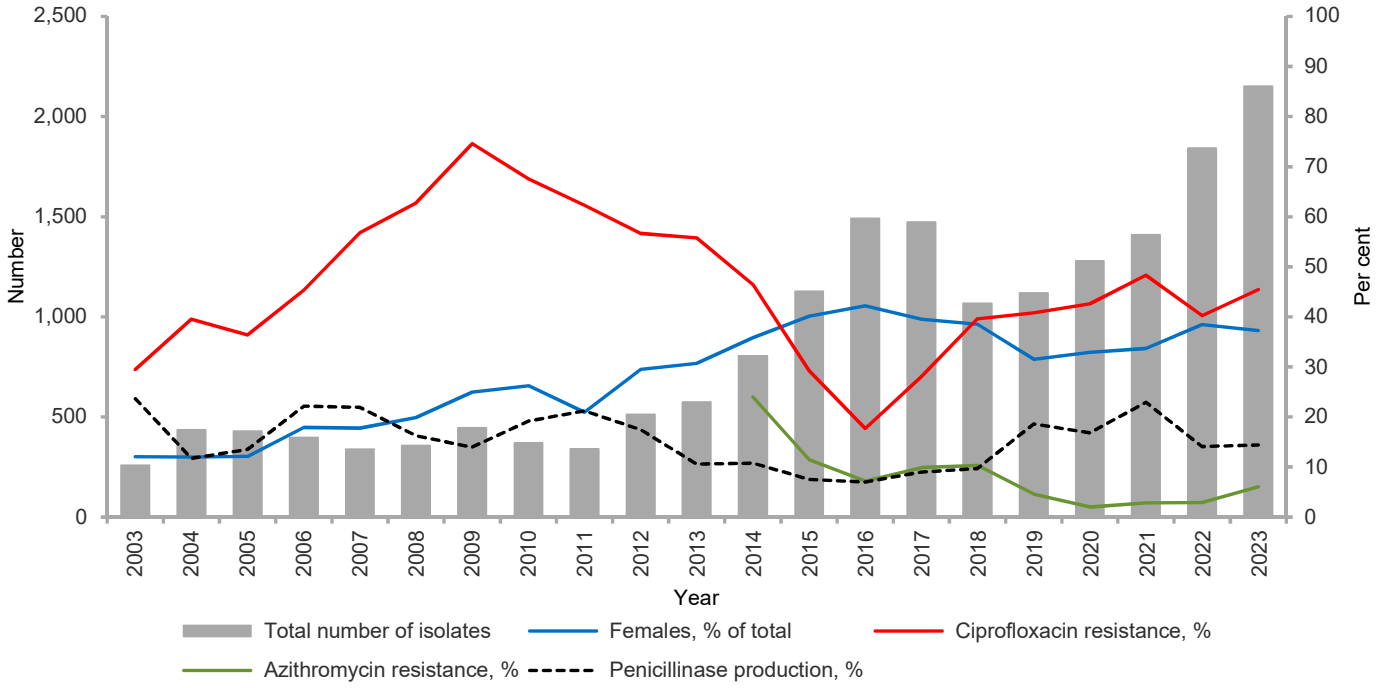
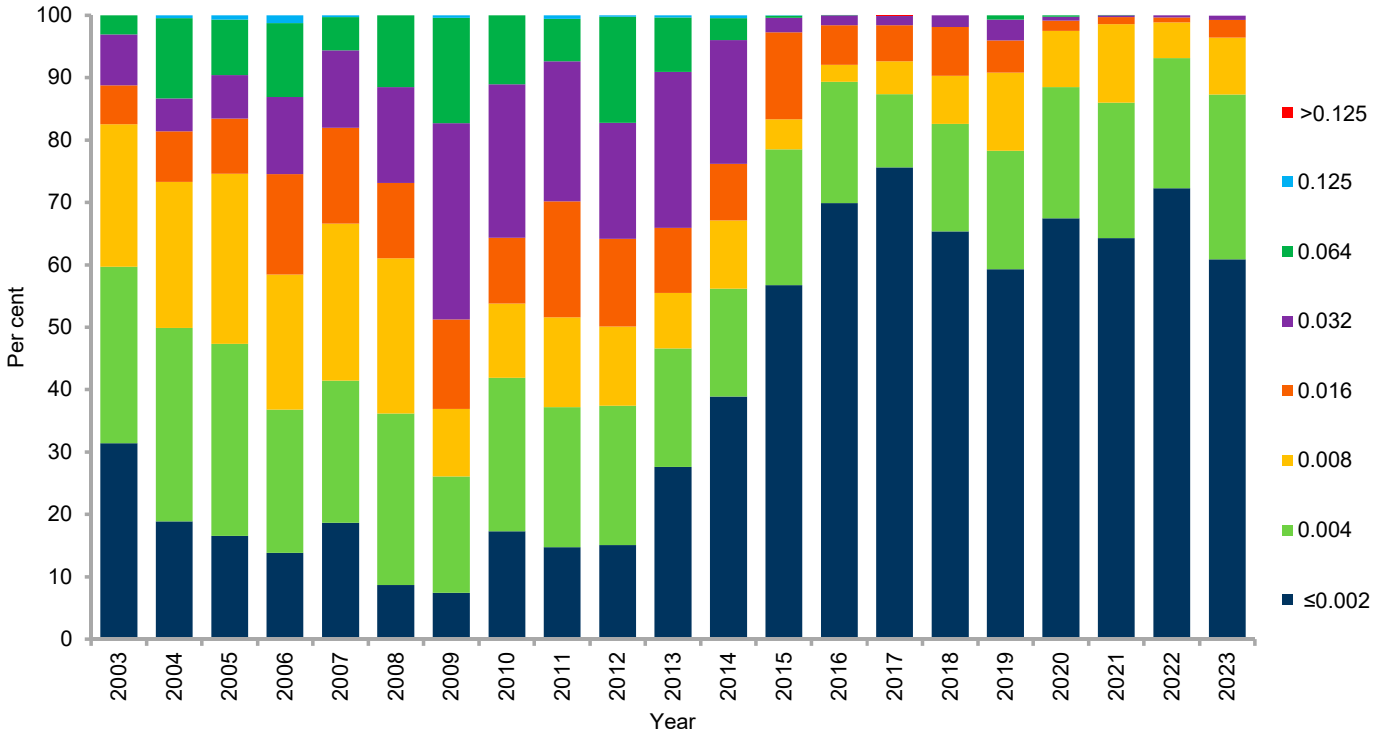


Figure 8.25. Distribution of ceftriaxone MIC (mg/L) values in gonococci, Denmark, 2003–2023 DANMAP 2023



Participation in Euro-GASP

In a subset of 128 isolates tested as part of the Danish participation in Euro-GASP, resistance against cefixime (MIC >0.125 mg/L) was 0% in 2023 (Table 8.25), like in 2022. Cefixime is an oral cephalosporin that has never been used in Denmark.

Resistance against tetracycline (MIC >0.5 mg/L) was 34%. Susceptibility testing against spectinomycin and gentamicin are only performed in selected years and was not carried out in 2023.

Resistance findings by gender and anatomical origin of the isolates

In males, the ciprofloxacin resistance rate was higher in anorectal isolates than in urogenital and pharyngeal isolates (Table 8.26).

The azithromycin resistance rate among males was higher in pharyngeal isolates than in anorectal and urogenital isolates, while in females it was close to the rate in anorectal isolates (Table 8.27).

Penicillinase production was demonstrated at a higher rate among anorectal isolates than among urogenital and pharyngeal isolates in males, while no differences were noted among isolates from females (Table 8.28).

Table 8.25 Distribution of 128 gonococcal isolates according to MIC values for cefixime and tetracycline; number of isolates

DANMAP 2023

	MIC values (mg/L)					
	≤0.016	0.032	0.064	0.125	0,25	0,5
Cefixime	106	16	6			
Tetracycline		1	8	14	12	50

Table 8.26 Ciprofloxacin resistance rates by gender and anatomical origin of the isolates DANMAP 2023

	Males		Females		Total	
	No. resistant / total no.	% resistant	No. resistant / total no.	% resistant	No. resistant / total no.	% resistant
Urogenital	525 / 1,067	49	214 / 683	31	739 / 1,750	42
Anorectal	115 / 151	76	5 / 22	23	120 / 173	69
Pharynx	70 / 120	58	41 / 97	42	111 / 217	51
Blood	1 / 1	100	0 / 0	-	1 / 1	100
Eye	5 / 6	83	0 / 0	-	5 / 6	83
Other or unknown	2 / 5	40	0 / 0	-	2 / 5	40
Total	718 / 1,350	55	260 / 802	32	978 / 2,152	45

Table 8.27 Azithromycin resistance rates by gender and anatomical origin of the isolates DANMAP 2023

	Males		Females		Total	
	No. resistant / total no.	% resistant	No. resistant / total no.	% resistant	No. resistant / total no.	% resistant
Urogenital	57 / 1.067	5.3	38 / 683	5.6	95 / 1750	5.4
Anorectal	6 / 151	4	3 / 22	13.6	9 / 173	5.2
Pharynx	12 / 120	10	12 / 97	12.4	24 / 217	11.1
Blood	1 / 1	0	0 / 0	-	0 / 1	0
Eye	1 / 1	0	0 / 0	-	0 / 6	0
Other or unknown	0 / 5	0	0 / 0	-	0 / 5	0
Total	77 / 1,350	5.7	53 / 802	6.6	128 / 2,152	5.9

Table 8.28 Penicillinase production by gender and anatomical origin of the isolate DANMAP 2023

	Males		Females		Total	
	No. resistant / total no.	% resistant	No. resistant / total no.	% resistant	No. resistant / total no.	% resistant
Urogenital	173 / 1,067	16	62 / 683	9	235 / 1,750	13
Anorectal	45 / 151	30	2 / 22	9	47 / 173	27
Pharynx	15 / 120	13	9 / 97	9	24 / 217	11
Blood	0 / 1	0	0 / 0	-	0 / 1	0
Eye	2 / 6	33	0 / 0	-	2 / 6	33
Unknown	2 / 5	40	0 / 0	-	2 / 5	40
Total	237 / 1,350	18	73 / 802	9	128 / 2,152	6

Conclusions

The ciprofloxacin resistance rate was higher in 2023 (45%) than in 2022 (40%) but lower than in 2021 (48%) and the ceftriaxone MIC distribution remained without notable changes. Although resistance problems among gonococci are still not present in Denmark, the 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

8.3.9 *Haemophilus influenzae*

Background

Haemophilus influenzae is part of the normal upper respiratory tract flora, with colonization varying by age. *H. influenzae* frequently causes upper and lower respiratory infections, while invasive infections are relatively rare and primarily occur in young children and elderly individuals, particularly those with underlying conditions like chronic obstructive pulmonary disease or cancer. Bacteremia/sepsis is the predominant form (80-90%), while meningitis is observed in approximately 10% of cases. *H. influenzae* is categorized into six capsular serotypes (a, b, c, d, e, and f), also known as Hia, Hib, Hic, Hid, Hie, and Hif, and there are noncapsular (non-typeable, NTHi) strains as well. The introduction of the polysaccharide type b vaccine in 1993 significantly reduced the incidence of systemic infection caused by *H. influenzae* type b (Hib) isolates, as the vaccine specifically protects against Hib. Antimicrobial resistance in *H. influenzae* is an increasing problem globally, especially within beta-lactam antibiotics. The molecular antibiotic resistance in *H. influenzae* is complex, where both beta-lactamase production and alterations in penicillin binding proteins (PBPs), particularly PBP3, encoded by the *ftsI* gene, contribute to resistance to betalactam antibiotics.

Surveillance of *Haemophilus influenzae*

The surveillance of *H. influenzae* causing invasive disease in Denmark involves submitting clinical isolates to Statens Serum Institut (SSI). Cases without a submitted isolate are identified through MiBa. At SSI, the isolates are only subjected to whole-genome sequencing to extract the serotype, sequence type, and beta-lactam resistance genes (beta-lactamase genes and mutations in the *ftsI* gene encoding penicillin-binding protein 3). Phenotypic beta-lactam susceptibility is retrieved through MiBa, if available.

Results

In 2023, a total of 123 cases of invasive *H. influenzae* were registered. The majority of cases were in blood (117) or cerebrospinal fluid (4), and two isolates from other normally sterile sites. The age and serotype distribution of the submitted isolates can be seen in Figure 8.26. Invasive *H. influenzae* infections are most commonly observed in the elderly.

Figure 8.26 The distribution of age and serotype of *H. influenzae* in Denmark, 2023 DANMAP 2023

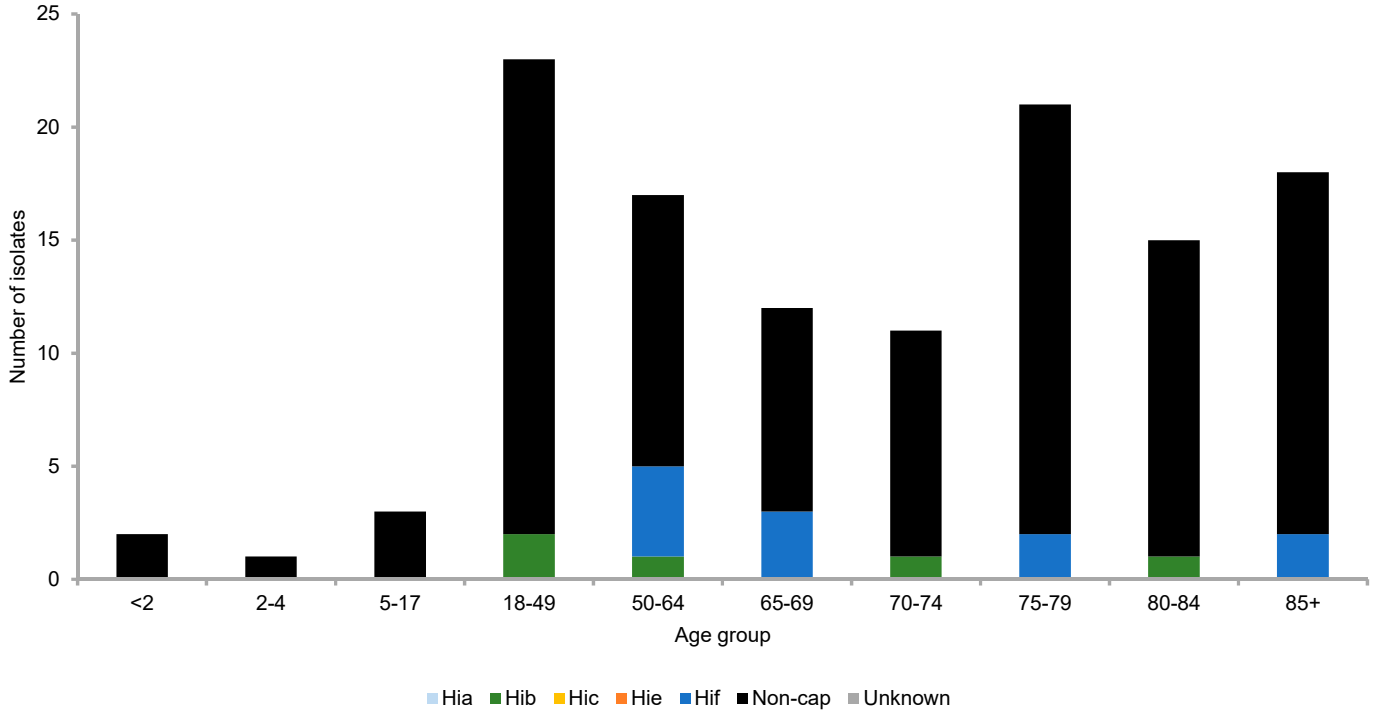


Table 8.29 Sequence type and clonal complex found among the *H. influenzae* isolates with a capsule DANMAP 2023

ST	Clonal complex	A	B	C	D	E	F	Noncap
6	ST-6 complex	0	5	0	0	0	0	1
124	ST-124 complex	0	0	0	0	0	11	0

Table 8.30 Phenotypic resistance against penicillin/ampicillin in *Haemophilus influenzae* DANMAP 2023

Sensitivity	Number (%)
Resistant	40 (33)
Susceptible	80 (67)
Total	120

Table 8.31 Phenotypic resistance against penicillin/ampicillin vs. beta-lactam resistance mechanism in *H. influenzae* DANMAP 2023

Beta-lactam resistance mechanism	Pen/ampi resistant	Pen/ampi susceptible	Total (%)
BLNAS	1	79	80 (67)
BLPAR	20	0	20 (17)
BLNAR	19	1	20 (17)
BLPACR	0	0	0 (0)
Total	40	80	120

Non-capsular *H. influenzae* is still the most commonly tested type (87%), with Hif being the most common serotype (9%), followed by Hib (4%).

Sequence type (ST) and clonal complex (CC) were linked to the serotype of *H. influenzae* (Table 8.29). Thus, the MLST type can indicate the correct identification of the genotype because the capsular isolates appear to be part of only a few clonal complexes. This was not the case for the non-capsular *H. influenzae* isolates which had 53 different STs, including five novel STs.

Data on both molecular and phenotypic antimicrobial susceptibility were available for 120 *H. influenzae* isolates. Of these, 33,3% were penicillin/ampicillin-resistant (Table 8.30). Twenty isolates were tested positive for TEM beta-lactamase genes (all were *TEM-1*). BLNAR-defining mutations in the *ftsI* gene were found in 20 isolates (18 had the N526K mutation and two had the R517H mutation). The most common *ftsI* type were IIb (9), followed by IId (6), IIa (3) and III-like (2).

A comparison between phenotypic beta-lactam susceptibility and beta-lactam resistance mechanisms (BLNAS=beta-lactamase-negative ampicillin-susceptible; BLPAR=beta-lactamase positive ampicillin-resistant; BLNAR=beta-lactamase negative ampicillin-resistant; BLPACR=beta-lactamase-positive amoxicillin-clavulanic acid-resistant) can be found in Table 8.31. A 98% concordance was observed between phenotypic susceptibility testing and molecular resistance gene detection: one isolate with no beta-lactam resistance markers was tested penicillin/ampicillin resistant, and one isolate with a BLNAR resistance mechanism was tested penicillin/ampicillin susceptible.

Only three *H. influenzae* isolates had other resistance genes (aminoglycoside-modifying enzymes).

Conclusions

The number of invasive *H. influenzae* cases in 2023 were 123 comparable to 118 cases in 2022 and 101 cases in 2021. The majority of isolates are still of the noncapsular type (87%) while Hif was the most common serotype (9%). Resistance towards penicillin and ampicillin was 33% with 17% BLNAR and 17% BLPAR (none were observed with a BLPACR resistance mechanism).

Hans-Christian Slotved and Kurt Fuursted
For further information: Hans-Christian Slotved, hcs@ssi.dk or Kurt Fuursted, kfu@ssi.dk

8.3.10 Meningococci

Background

Neisseria meningitidis (meningococci) are capable of causing invasive meningococcal disease (IMD), usually meningitis or septicaemia or both, which may be life threatening and may cause permanent neurological sequelae. Asymptomatic throat carriage is frequent and only rarely leads to invasive disease. IMD is usually treated with intravenous penicillin or ceftriaxone. Single-dose ciprofloxacin or rarely rifampicin may be used for prophylaxis to close contacts to patients with IMD.

Meningococci are classified into serological groups (A, B, C, W, X, Y, Z, and 29E) according to antigenic properties of the polysaccharide capsule. In Denmark, serogroups B and C are the most frequent ones in most years, although serogroup W was quite frequent in 2016 through 2019. Vaccination against meningococci has never been part of the Danish childhood immunization programme but is used for prophylaxis to close contacts to patients with IMD. Specific vaccines exist for group B and combined vaccines for groups A, C, W, and Y.

This report presents data on antimicrobial resistance in non-duplicate invasive isolates (i.e. usually from blood, cerebrospinal fluid) submitted from the departments of clinical microbiology (DCM) during 2012-2023 to the Neisseria and Streptococcus Reference laboratory (NSR). Isolates are received from all DCM in Denmark. Until November 1, 2023

it was voluntary to submit isolates of meningococci, but the coverage rate is estimated to be 100% when compared to the mandatory clinical notification system. The two surveillance systems continuously supplement each other.

Results

During the years 2012 through 2019, 18 to 52 isolates were received annually. In 2020, where restrictions to counteract the spread of COVID-19 were implemented, the number was 14, decreasing to 8 and 11, respectively, in 2021 and 2022. Figure 8.27 shows the number of isolates of groups B, C, W, and Y received during 2012-2023. Because of low numbers the following have been omitted: One isolate of group 29E (2017), three isolates of group X (2016, 2019 and 2023), and two isolates which were non-groupable (2019 and 2023). The susceptibility pattern of these four isolates did not show any specific deviations from the remaining findings and are therefore not further included in the present report.

All isolates were susceptible to ceftriaxone (MIC ≤0.125 mg/L), Figure 8.28.

All isolates were susceptible to ciprofloxacin (MIC ≤0.016 mg/L), Figure 8.29. Isolates of serogroup W (none in 2023) and Y (11 in 2023) tended to have slightly lower levels of ciprofloxacin MIC values than isolates of serogroup B and C.

In total, 95% of the isolates during the study period were susceptible to penicillin (MIC ≤0.25 mg/L), Figure 8.30. Eight isolates of serogroup B including one from 2023 and six isolates of serogroup C were penicillin-resistant (MIC >0,25 mg/L), (Table 8.32).

Nearly all isolates during the study period were susceptible to rifampicin (MIC ≤0.25 mg/L), Figure 8.31. Only one (from 2017) was resistant (MIC = 32 mg/L).

Figure 8.27 Number and serogroup of meningococcal isolates received during 2012-2023 DANMAP 2023

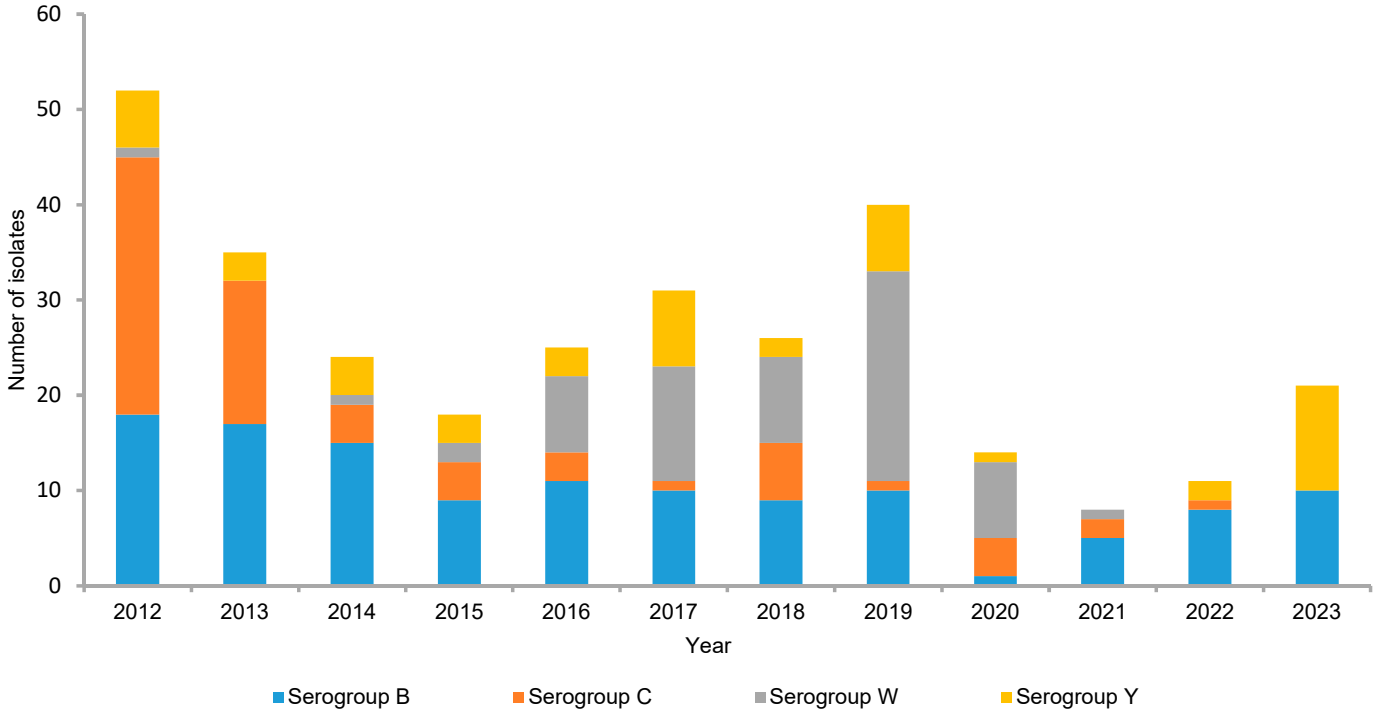


Figure 8.28 Distribution of ceftriaxone MIC values (mg/L) by serogroup, 2012-2023 DANMAP 2023

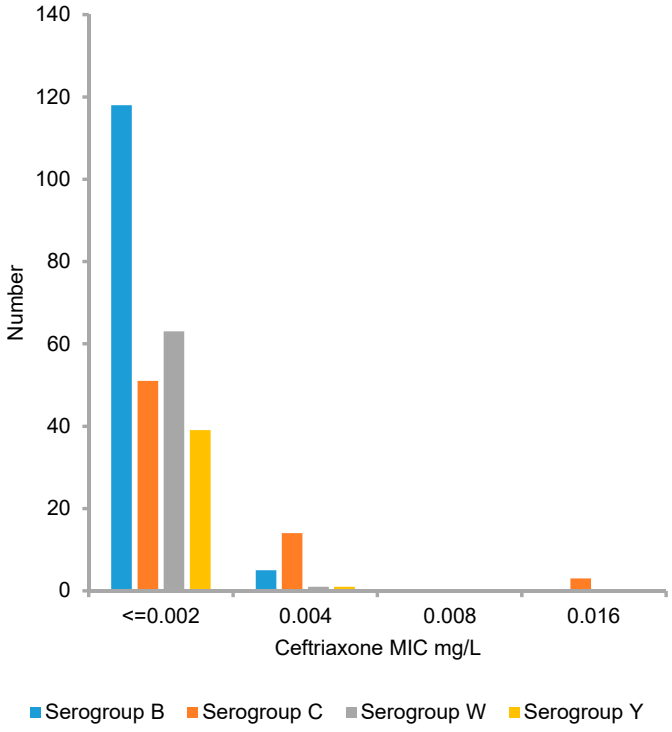


Figure 8.29 Distribution of ciprofloxacin MIC values (mg/L) by serogroup, 2012-2023 DANMAP 2023

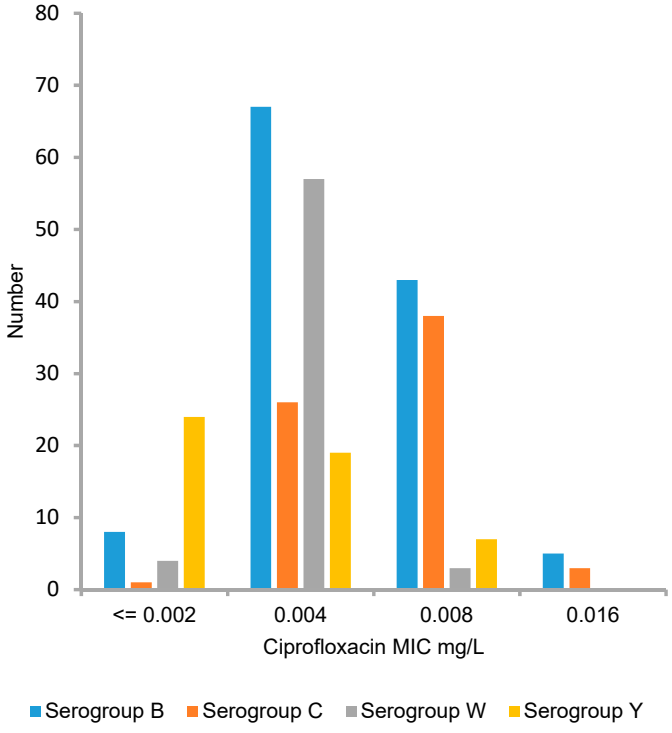


Figure 8.30 Distribution of penicillin MIC values (mg/L) by serogroup, 2012-2023 DANMAP 2023

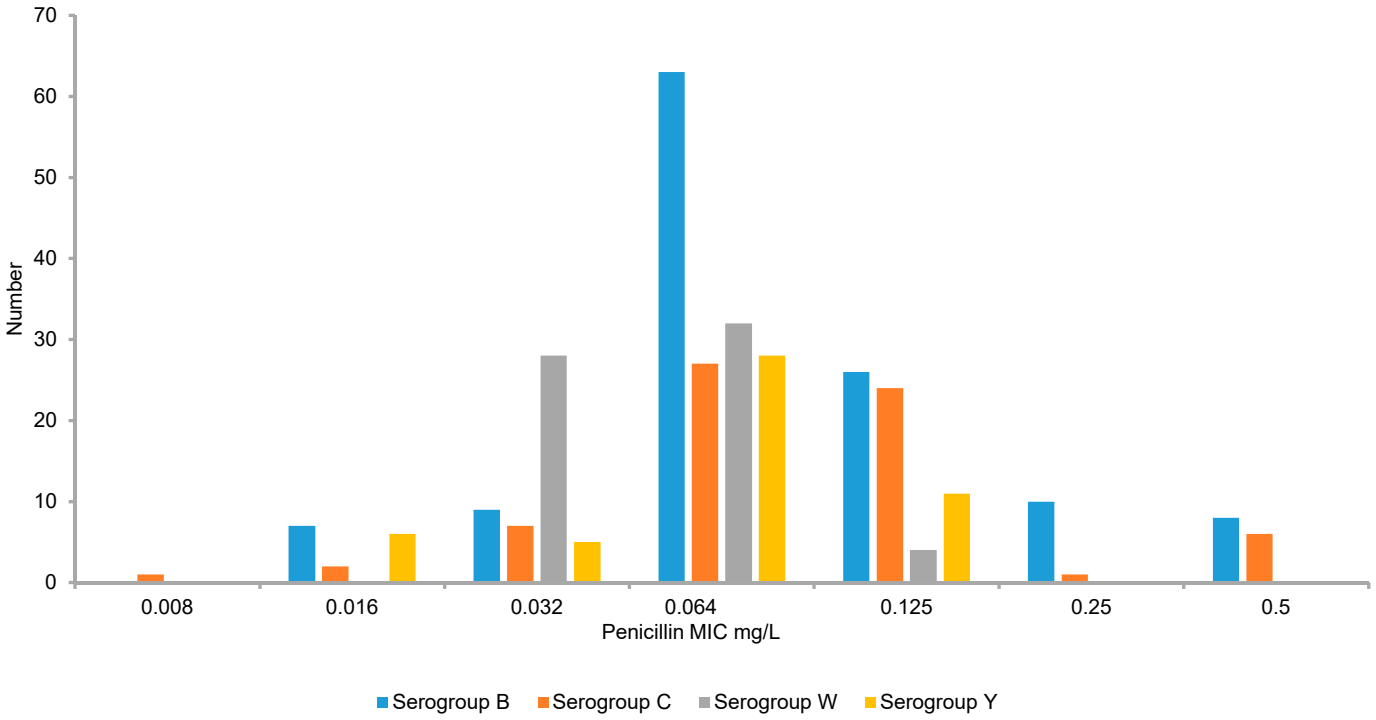
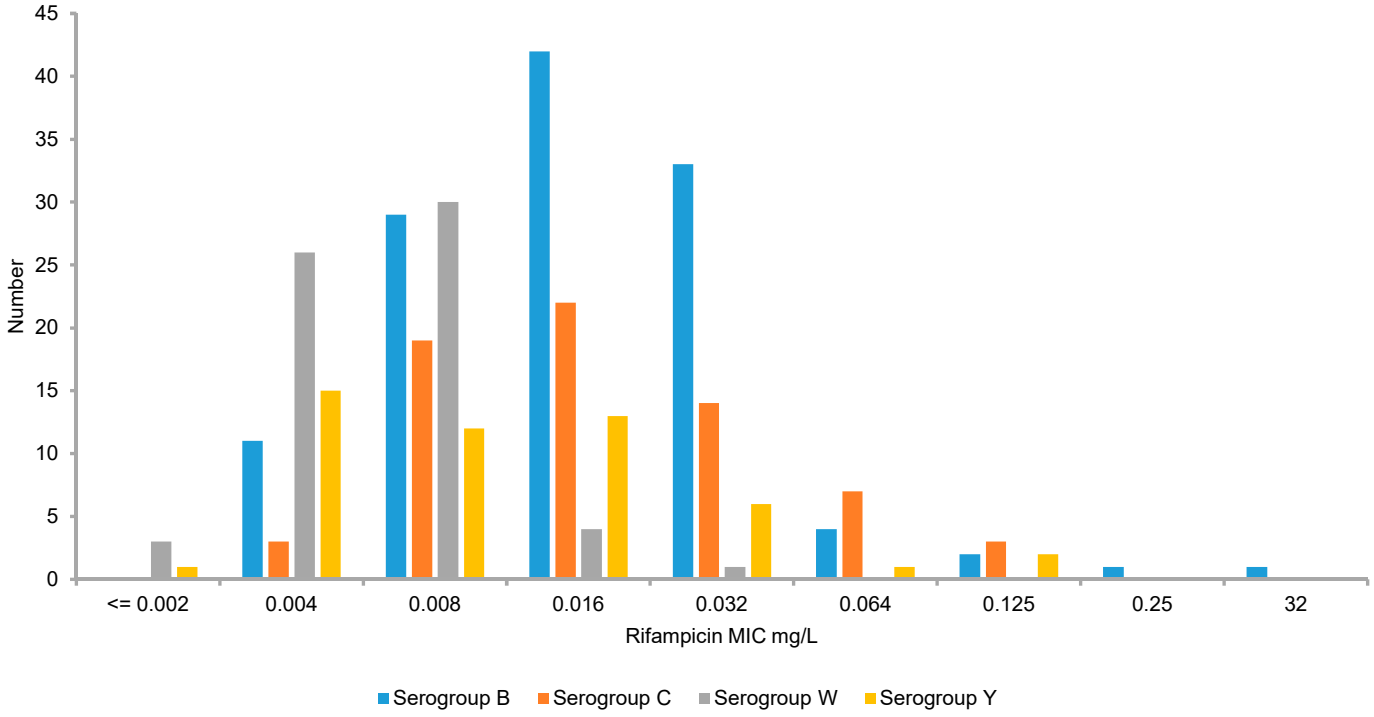


Table 8.32 Number of penicillin-resistant meningococci (MIC = 0.5 mg/L), serogroups B and C, 2012-2023 DANMAP 2023

	2013	2014	2016	2017	2018	2020	2022	2023
Serogroup B	1	2	1	2			1	1
Serogroup C		1			2	3		

Figure 8.31 Distribution of rifampicin MIC values (mg/L) by serogroup, 2012-2023 DANMAP 2023



Comments and conclusions

The substantial decrease of received meningococcal isolates during and following 2020 are most likely due to the social restrictions implemented in April 2020 because of COVID-19. Likewise, the modest increase during 2023 probably represent the influence of the lifting of the restrictions which have enabled more respiratory transmission than during the preceding 2-3 years.

The antibiotic susceptibility patterns of meningococci do not reveal any imposing threats of resistance.

During the first five months of 2024 a total of 13 cases of IMD have been diagnosed in Denmark (not described in this report).

Steen Hoffmann
For further information: Steen Hoffmann, hof@ssi.dk

Textbox 8.1

Danish surveillance of azole resistant *Aspergillus fumigatus* from clinical samples – a 4-year update

Azole resistance in *Aspergillus fumigatus* (AR*Af*) has increased worldwide. The most efficacious and the only orally available drug class is the azoles [1]. A significant and growing proportion of resistance has emerged in the environment due to the widespread use of azoles in agri- and horticulture. This can lead to infections in azole naïve patients, where resistance may not be anticipated. This "environmental" resistance is characterised by a combination of a sequence duplication in the promoter region of the *cyp51A* gene coupled with one or several point mutation(s) [2,3]. The most common mechanism is TR_{34}/L98H, which causes pan-azole resistance, whereas TR_{46}/Y121F/T289A results in high voriconazole and isavuconazole MICs. Delayed appropriate treatment for invasive aspergillosis caused by these isolates has been associated with significantly increased mortality. Consequently, an international expert group has advocated for the use of alternative empiric treatment when the local resistance rate exceeds 10% [2,4].

A voluntary Danish Nationwide surveillance of azole resistance in clinical *A. fumigatus* isolates was initiated in October 2018 [5]. Clinical isolates of *A. fumigatus* were susceptibility tested using the EUCAST method and azole resistant isolates were *cyp51A* sequenced. Repeat isolates from individual patients were excluded if ≤30 days apart.

During the first four years, a total of 3,455 isolates from 2,543 patients were included. Overall, 5.8% (201/3455) of isolates were resistant to at least one azole (between 5.2% to 5.4% for the individual azoles). In 3.0% (105/3455) of isolates, resistance was caused by environmental mechanisms (Table 1). Of the 137 patients with AR*Af*, 58.4% (80/137) harboured isolates with environmental resistance mechanisms, whereas a plethora of other alterations were detected among the remaining (Figure 1). Resistant isolates without Cyp51A alterations were also identified (Table 1, Figure 1). TR_{46} variants were exclusively found in year three. Almost half of the patients with AR*Af* (45% (62/137)) also harboured susceptible isolates the same year. This underscores the importance of sequential testing to detect isolates with different susceptibility patterns over time.

Increased resistance over the four-year period was not found (Chi square time trend p>0.5) but continued surveillance over time is required as resistance may fluctuate and can increase only gradually [6,7] (Figure 2). In the Netherlands, resistance rates have increased (from 7.6% in 2013 to 14.7% in 2018), whereas resistance rates in Denmark remain below the 10% threshold. *A. fumigatus* is abundant in the environment and is acquired breathing ambient air. A Danish environmental study from 2020-2022 demonstrated ARA*f* in 4.2% of 4,538 environmental *A. fumigatus* isolates. Resistance was mainly caused by TR_{34} (79.4%) and TR_{46} (11.3%) genotypes, the last of which was only detected in 2021-2022 [8]. This is slightly higher than the environmental resistance rate in patients, suggesting a future increase in resistant infections may be expected, and it correlates with the fact that TR_{46} was only detected in patients from the third surveillance year.

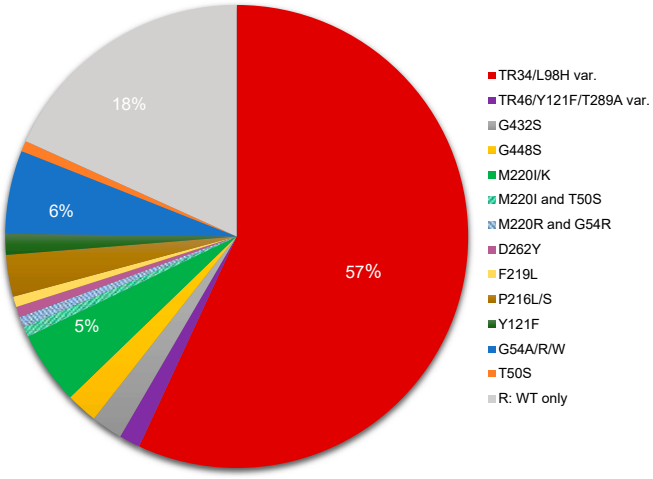
Dual use of same-class active agents in both clinical drugs and environmental pesticides also poses a risk to future drugs under development that could be used to treat azole resistant infections [9]. Regulatory efforts and continued surveillance are still needed.

Table 1 Yearly number of isolates and patients DANMAP 2023

	Quarter and year of surveillance									
	1 Q4 2018-Q3 2019		2 Q4 2019-Q3 2020		3 Q4 2020-Q3 2021		4 Q4 2021-Q3 2022		All Q4 2018-Q3 2022	
	*N**	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*
Patients	675		562		688		618		2,543	
Isolates	978		843		883		751		3,455	
Susceptible	922	94.3%	782	92.8%	832	94.2%	718	95.6%	3,254	94.2%
Resistant	56	5.7%	61	7.2%	51	5.8%	33	4.4%	201	5.8%
R env.	35	3.6%	21	2.5%	32	3.6%	17	2.3%	105	3.0%
R other Cyp51A	14	1.4%	21	2.5%	14	1.6%	11	1.5%	60	1.7%
R non-Cyp51A**	7	0.7%	19	2.3%	5	0.6%	5	0.7%	36	1.0%

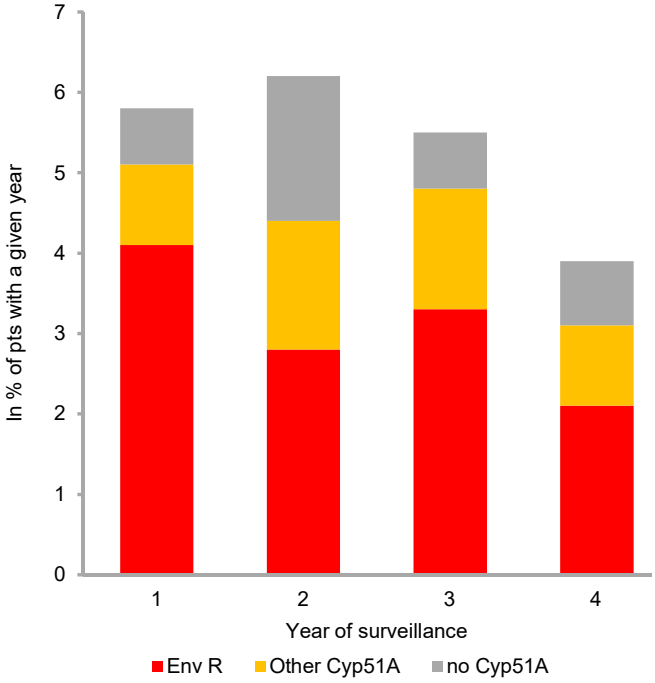
R env.: Resistance due to alterations of environmental type. *For year 1, isolates that had not been sequenced (five isolates/three patients) but were phenotypically similar to same-patient isolates with *cyp51A* mutations were counted as the given mutation. **For year 2, one resistant isolate had an N248K mutation which is not linked to azole resistance

Figure 1 Cyp51A alterations found in 137 patients with azole resistance DANMAP 2023



R: WT denotes patients with resistant isolates without demonstrated *cyp51A* mutations. Var: TR_{34}/L98H and TR_{46}/Y121F/T289A isolates may have additional alterations (not shown). Some patients harbouring isolates with *cyp51A* mutations also have susceptible or resistant isolates without mutations within the same year (not detailed). The resistant isolate with an N248K alteration is shown under R:WT

Figure 2 Resistance on a patient level over the four-year period DANMAP 2023



Karen Marie Thyssen Astvad, Rasmus Krøger Hare, Karin Meinike Jørgensen, Nissrine Abou-Chakra, Jan Berg Gertsen, Lise Kristensen, Flemming Schønning Rosenvinge, Lisbeth Lützen, Ea Sofie Marmolin, Bent Løwe Røder, Sofia Sulim, Michael Pedersen, Jette Bangsborg, Raluca Datcu, Turid Snekloth Søndergaard and Maiken Cavling Arendrup
For further information: Karen Astvad, kaas@ssi.dk

Data has in part been presented as a poster P2917 at the ESCMID Global 2024 in Barcelona, Spain.

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Textbox 8.2

Increasing rates of drug resistance in *Mycobacterium tuberculosis* isolates in Denmark

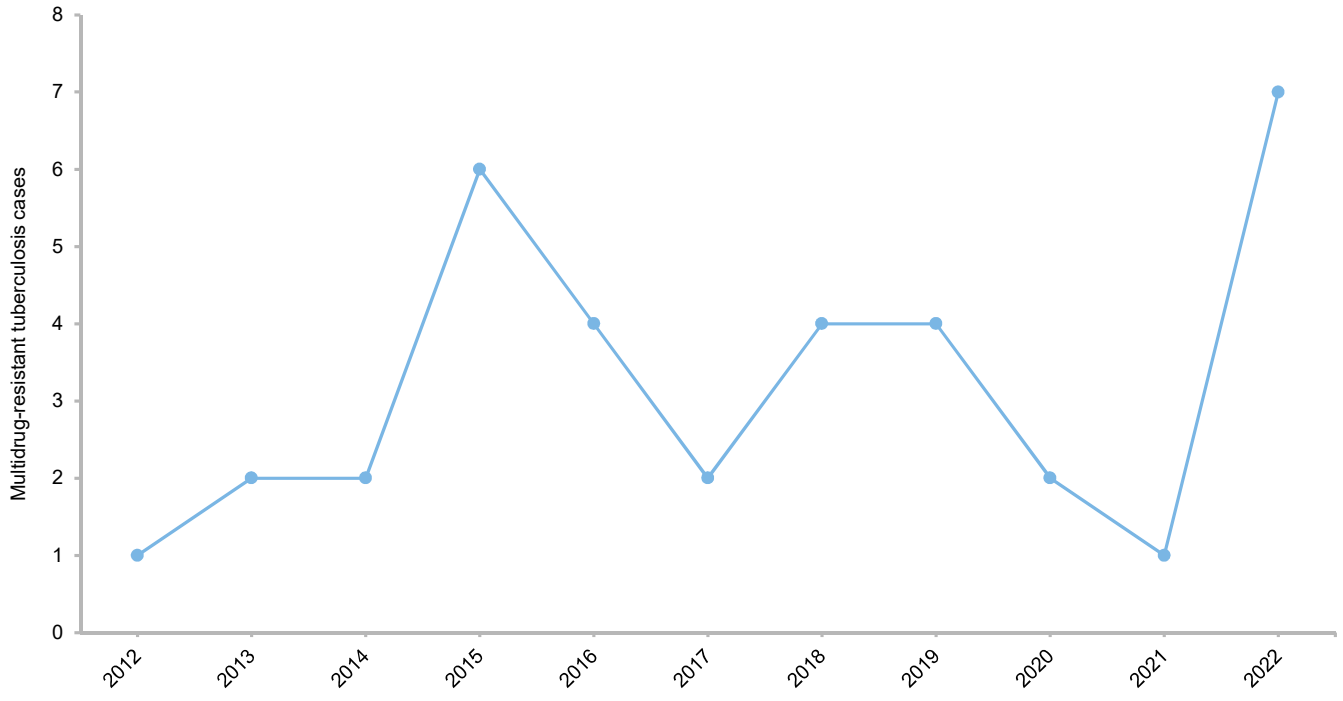
Background

In recent years, the global incidence of tuberculosis (TB) has declined. However, the emergence of drug-resistant Mycobacterium tuberculosis (Mtb) strains poses an urgent challenge to TB control in many parts of the world. It is estimated that only two out of five people with drug-resistant TB obtain the proper treatment (https://www.who.int/news-room/fact-sheets/detail/tuberculosis). Multidrug-resistant (MDR) TB is caused by Mtb resistant to at least rifampin (RIF) and isoniazid (INH), the two most important drugs for TB treatment. Extensively-drug-resistant (XDR) TB is a form of MDR-TB with additional resistance to any of the fluoroquinolones (e.g. moxifloxacin) and at least one other group A drug (bedaquiline or linezolid). Mtb drug resistance is caused by spontaneous gene mutations during bacterial multiplication, which confer resistance to the drugs at different frequencies. Exposure to a single drug or suboptimal drug concentrations provides a selective environment favouring drug-resistant bacteria and the development of MDR/XDR-TB [2]. For many years, MDR-TB was seen in 2-3 cases a year in Denmark, with a rise in 2015 with five cases in a Danish outbreak[1], even though the majority of MDR/XDR TB in Denmark is seen among immigrants.

Methods

In Denmark, all clinical samples from suspected TB patients are cultured at the International Reference Laboratory of Mycobacteriology, Statens Serum Institut (SSI). Fluorescent microscopy of acid-fast auramine-rhodamine stained samples and for every new patient, an Mtb PCR including genotypic results for the two most critical first-line drugs RIF and INH. Subsequently, the samples are cultured in liquid and solid media. If the culture is positive for Mtb, resistance detection and subtyping are performed by WGS. If any drug resistance mutations are present, phenotypic susceptibility testing is performed by the modified proportion method in liquid media in MGIT 320/960 Systems (BD).

Figure 1 MDR-TB cases microbiologically verified in Denmark from 2012-2022 DANMAP 2023



Results

Seven MDR-TB cases were detected in Denmark in 2022 among 232 notified cases, corresponding to 3% MDR-TB total (Fig. 1). Additionally six cases (4%) were isoniazid mono-resistant.

Discussion

The 2022 drug-resistance figures for Mtb are the highest ever registered in Denmark with seven laboratory-confirmed MDR-TB cases plus, to the knowledge of the laboratory, at least five instances of immigrants entering Denmark with MDR-TB. Although the total figures are small, we must be prepared for an increasing number of future MDR/XDR-TB cases in Denmark. It is vital to remind clinicians to be aware of drug-resistant tuberculosis and to send all specimens for culturing, among others, to secure sensitive, fast and correct susceptibility testing performed through a combination of phenotypic- and genotypic test methods. The sensitivity of genotypic tests to detect RIF and INH resistance mutations is high. Therefore, susceptibility towards these drugs can be trusted based on the absence of resistance-conferring mutations[2]. Compared with detecting resistance-conferring mutations to the first-line drugs, the knowledge of which mutations are causing resistance to second-line drugs is lower. Hence, the more time-consuming phenotypic susceptibility testing is still essential for identifying the correct regime.

Dorte Bek Folkvardsen and Erik Svensson, International Reference Laboratory of Mycobacteriology, SSI
For further information: Dorte Bek Folkvardsen dbe@ssi.dk

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[2] Kurtzhals ML, Norman A, Svensson E, Lillebaek T, Folkvardsen DB. Applying whole genome sequencing to predict phenotypic drug resistance in Mycobacterium tuberculosis: leveraging 20 years of nationwide data from Denmark 2024.

Textbox 8.3

First results from antimicrobial resistance monitoring in *Shigella* spp. in Denmark

Shigella is an enteroinvasive bacterium causing shigellosis, a gastrointestinal infection in humans characterized by diarrhea, abdominal pain and general malaise.

The genus *Shigella* includes four species, *S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei*. The predominating *Shigella* species in Danish patients are *S. sonnei* and *S. flexneri*. Infections occur through fecally contaminated food or drinking water and are therefore particularly associated with poor sanitation and poor food hygiene, but may also occur via direct contact, e.g. among children in day care or in adults through sexual contact. Shigellosis is a self-limiting infection that typically lasts five to seven days. In more severe cases or in case of prolonged diarrhea the recommended antibiotic is azithromycin. Infections caused by *Shigella* or Enteroinvasive *Escherichia coli* (EIEC) are mandatory reportable in Denmark.

In 2023, 717 cases of EIEC were registered in Denmark. The primary PCR-based diagnostics do not distinguish between *Shigella* spp. and EIEC, but the species is determined for the subset of cases where an isolate is referred to the reference laboratory at SSI.

In 2023, the reference laboratory at SSI received 92 *S. sonnei* and 30 *S. flexneri* isolates from Danish patients. Travelling abroad before onset of disease was recorded for 58% (53/92) of the *S. sonnei* and 37% (11/30) of the *S. flexneri* cases. The remaining cases were not travel related or lacked information about travel status. The isolates were tested for antimicrobial susceptibility by determination of the minimal inhibitory concentration (MIC) and interpreted using epidemiological cut off values. Further 67 *S. sonnei* and 14 *S. flexneri* isolates were subjected to whole-genome sequencing (WGS).

The results of the phenotypic susceptibility testing are presented in Table 1.

Table 1 Resistance (%) in *S. sonnei* and *S. flexneri* isolates from Danish patients in 2023 DANMAP 2023

Antimicrobial agent	*S. sonnei*	*S. flexneri*
Amikacin	0	0
Ampicillin	67	90
Azithromycin	30	23
Cefotaxime	62	10
Ceftazidime	20	3
Chloramphenicol	0	63
Ciprofloxacin	84	50
Colistin	0	0
Gentamicin	0	3
Meropenem	0	0
Nalidixic acid	47	33
Sulfamethoxazole	83	50
Tetracycline	77	87
Tigecycline	0	0
Trimethoprim	100	77
Fully sensitive (%)	0	3
Number of isolates	92	30

Most *Shigella* isolates were resistant to a number of different antimicrobials. Azithromycin resistance levels of 30% and 23%, respectively, were observed in *S. sonnei* and *S. flexneri*, and the WGS data showed that all sequenced resistant strains harbored the *mph(A)* gene.

The levels of quinolone resistance were notably high, 84% and 50% respectively for *S. sonnei* and *S. flexneri*, and it is noteworthy that the levels of fluoroquinolone resistance were higher than the levels of nalidixic acid resistance. The sequence analysis showed that *Shigella* frequently harbors *qnr* gene variants on plasmids.

Resistance towards 3rd generation cephalosporins are commonly observed in *Shigella*. Cefotaxime resistance was observed in 62% of the *S. sonnei* isolates and in 10% of the *S. flexneri* isolates. Sixteen of 61 patients who were infected with a cefotaxime resistant *Shigella* had no history of travel. Cefotaxime resistance was in most cases mediated by the *blaCTX15* gene, but *blaCTX-M-27* and *blaCTX-M-231* were also identified.

Carbapenemase (meropenem) resistance was not observed in any of the tested isolates and the levels of aminoglycoside, amikacin and gentamicin resistance was low. Colistin resistance was not observed.

Chloramphenicol resistance was frequent in *S. flexneri* of which 62% of the isolates were resistant, whereas chloramphenicol resistance was rare in *S. sonnei*.

The levels of resistance observed in the Danish *Shigella* isolates were generally in concordance with the levels that are reported internationally.

Jeppe Boel
For further information: Jeppe Boel, jebl@ssi.dk

9

RESISTANCE IN ANIMAL PATHOGENS

9. Resistance in animal pathogens

Highlights

Surveillance of antimicrobial resistance in 2023 focused on pathogenic bacteria from pigs and included results obtained through antimicrobial susceptibility testing (AST) and/or whole genome sequencing (WGS) of isolates belonging to *Actinobacillus pleuropneumoniae* (AST and WGS), *Bordetella bronchiseptica* (AST and WGS), *Clostridium perfringens* (WGS), *Erysipelothrix rhusiopathiae* (WGS), haemolytic and non-haemolytic *Escherichia coli* (AST and WGS), *Glaesserella parasuis* (WGS), *Klebsiella pneumoniae* (AST and WGS), *Salmonella enterica* (AST and WGS), *Staphylococcus hyicus* (AST and WGS) and *Streptococcus suis* (AST and WGS).

Most pathogenic bacteria isolated from pigs in 2023 displayed similar frequencies of phenotypic resistance as in 2022 (1-year period) and 2018 (5-year period). However, nine pathogen-drug combinations were associated with significantly increased resistance, whereas one was associated with a significantly decreased frequency.

The increased frequency of neomycin resistance in haemolytic *E. coli* (52.3%) is concerning because it is one of only few drugs recommended in Denmark as first choice for treating *E. coli*-associated post-weaning diarrhea. The rapid increase in neomycin resistance coincided with increased use of neomycin in weaners.

The increased frequency of gentamicin resistance in haemolytic *E. coli* (35.2%) is also concerning because it is considered critically important for human medicine by the World Health Organization.

WGS-based detection of resistance mechanisms (genes and point mutations) in pathogenic bacteria isolated from pigs in 2023 showed that 22 pathogen-resistance mechanism combinations were associated with significantly increased frequencies when compared to 2022 (1-year period) and 2021 (2-year period), whereas one was associated with a significantly decreased frequency.

Resistance towards carbapenems, 3rd, 4th and 5th generation cephalosporins, oxazolidinones and polymyxins remained at a low level.

The observed concordance between AST results and WGS-based detection of resistance mechanisms was 99.6% for *A. pleuropneumoniae*, 79.6% for *B. bronchiseptica*, 94.2% for haemolytic *E. coli*, 94.3% for non-haemolytic *E. coli*, 76.0% for *K. pneumoniae*, 97.3% for *S. enterica*, 91.5% for *S. hyicus* and 94.5% for *S. suis*.

9.1 Introduction

Antimicrobial susceptibility testing (AST) and surveillance of antimicrobial resistance (AMR) in pathogenic bacteria from pigs, including *Actinobacillus pleuropneumoniae*, haemolytic *Escherichia coli* and *Streptococcus suis*, have been part of the DANMAP programme since 2015. In 2020, the Danish Veterinary and Food Administration (DVFA) asked the Danish Veterinary Consortium (DK-VET) to investigate whether it would be possible to implement whole genome sequencing (WGS) in the surveillance of AMR in pathogenic bacteria from food-producing animals as a basis to detect resistance mechanisms (genes and point mutations). WGS-based AMR surveillance in pathogenic bacteria from pigs commenced in January 2021 and included AST and/or WGS of isolates belonging to *A. pleuropneumoniae* (AST and WGS) *Bordetella bronchiseptica* (AST and WGS), *Clostridium perfringens* (WGS), *Erysipelothrix rhusiopathiae* (WGS), haemolytic and non-haemolytic *E. coli* (AST and WGS), *Glaesserella parasuis* (WGS), *Klebsiella pneumoniae* (AST and WGS), *Salmonella enterica* (AST and WGS), *Staphylococcus hyicus* (AST and WGS) and *S. suis* (AST and WGS), which were identified in clinical samples submitted by veterinarians to the Veterinary Laboratory, The Danish Agriculture and Food Council.

9.2 Temporal trends of AMR in pathogenic bacteria from pigs

The Veterinary Laboratory performed AST of isolates belonging to *A. pleuropneumoniae*, *B. bronchiseptic*a, haemolytic and non-haemolytic *E. coli*, *K. pneumoniae*, *S. enterica*, *S. hyicus* and *S. suis*. Table 9.1 shows the frequencies of resistant isolates in 2023, while all results from 2016-2023 can be found on DK-VET's homepage (https://www.vetssi.dk/). Data are based on epidemiological cut-offs (ECOFFs) and clinical breakpoints when ECOFFs are unavailable (https://www.vetssi.dk/).

Table 9.1 Phenotypic antimicrobial resistance among pathogenic bacteria from pigs, Denmark, 2023 DANMAP 2023

Antimicrobial agent	Ap	Bb	H-Ec	NH-Ec	Kp	Se	Sh	Ss
	R (%)	R (%)	R (%)	R (%)	R (%)	R (%)	R (%)	R (%)
Amoxicillin	0.0%	ND	73.8%	77.4%	ND	78.0%	100.0%*	ND
Amoxicillin-clavulanic acid	ND	ND	14.1%	12.6%	0.0%	4.9%	ND	ND
Cefpodoxime	ND	ND	4.7%	0.5%	ND	ND	ND	ND
Cefquinome	ND	ND	ND	ND	3.8%	ND	ND	ND
Ceftiofur	1.3%	ND	ND	ND	ND	ND	ND	ND
Colistin	ND	ND	0.3%	0.5%	0.0%	ND	ND	ND
Doxycyclin	0.0%	ND	53.0%	58.4%	34.6%	85.4%	ND	40.4%
Enrofloxacin	2.6%	ND	12.8%	4.7%	ND	ND	0.0%*	0.0%
Florfenicol	0.0%	3.1%	20.8%	28.9%	26.9%	39.0%	25.0%*	0.0%
Gentamicin	ND	ND	35.2%	18.4%	11.5%	43.9%	ND	ND
Lincomycin	ND	ND	ND	ND	ND	ND	100.0%*	ND
Neomycin	ND	ND	52.3%	18.9%	15.4%	ND	ND	ND
Penicillin	0.0%	ND	ND	ND	ND	ND	100.0%*	2.1%
Spectinomycin	ND	ND	68.8%	41.6%	ND	65.9%	ND	ND
Streptomycin	ND	ND	82.9%	76.3%	ND	82.9%	ND	ND
Tetracycline	ND	ND	71.1%	71.6%	34.6%	85.4%	ND	ND
Tiamulin	0.0%	ND	ND	ND	ND	ND	100.0%*	ND
Tildipirosin	0.0%	0.0%	ND	ND	ND	ND	ND	ND
Tilmicosin	0.0%	ND	ND	ND	ND	ND	37.5%*	ND
Trimethoprim-sulfamethoxazole	0.0%	ND	58.4%	72.6%	50.0%	46.3%	62.5%*	15.6%
Tulathromycin	0.0%	6.3%	ND	ND	ND	ND	ND	ND
Tylosin	ND	ND	ND	ND	ND	ND	37.5%*	ND

Data are based on epidemiological cut-offs (ECOFFs) and clinical breakpoints when ECOFFs are unavailable (https://www.vetssi.dk/)
Percentages based on small sample sizes (n<20) are indicated by asterisks and should be interpreted with caution.
Abbreviations: Ap, *Actinobacillus pleuropneumoniae*; Bb, *Bordetella bronchiseptica*; H-Ec, haemolytic *Escherichia coli*; NH-Ec, non-haemolytic *Escherichia coli*; Kp, *Klebsiella pneumoniae*; Se, *Salmonella enterica*; Sh, *Staphylococcus hyicus*; Ss, *Streptococcus suis,* R, resistant; ND, not determined

Most pathogenic bacteria isolated from pigs in 2023 displayed similar frequencies of phenotypic resistance as in 2022 (1-year period) and 2018 (5-year period). However, nine pathogen-drug combinations were associated with significantly increased resistance, whereas one was associated with significantly decreased resistance. Table 9.2 and Figure 9.1 show all significant changes in phenotypic resistance over a 1-year period (2023 vs. 2022) and a 5-year period (2023 vs. 2018).

Haemolytic *E. coli* displayed significantly increased resistance to florfenicol, gentamicin, neomycin, spectinomycin, streptomycin and tetracycline (Figure 9.1).

The increased frequency of neomycin resistance in haemolytic *E. coli* (52.3%) is worrisome because it is one of only a few drugs recommended in Denmark as first choice for treating *E. coli*-associated post-weaning diarrhea. Furthermore, haemolytic *E. coli* also displayed medium to high frequencies of resistance to the other first-choice drugs, including amoxicillin-clavulanic acid (14.1%), spectinomycin (68.8%), trimethoprim-sulfamethoxazole (58.4%) and streptomycin (82.9%). *E. coli* isolates from 2023 were not tested for susceptibility to the remaining first-choice drug ampicillin, but it should be noted that we observed a high frequency of ampicillin resistance in haemolytic *E. coli* from 2022 (60.9%). The rapid increase in neomycin resistance coincided with increased use of neomycin in weaners (Figure 9.2) following two recent decisions to restrict the use of alternative drugs in pigs: 1) the Danish Yellow Card initiative to reduce the use of colistin in 2016 and 2) the European Union-wide ban of medicinal zinc in 2022.

The increasing frequency of gentamicin resistance in haemolytic *E. coli* (35.2%) is also worrisome because it is considered critically important for human medicine by the World Health Organization.

Non-haemolytic *E. coli* displayed significantly increased resistance to florfenicol and significantly decreased resistance to amoxicillin-clavulanic acid, while *S. enterica* and *S. hyicus* displayed significantly increased resistance to gentamicin and tiamulin, respectively. However, the results for *S. hyicus* are based on <20 isolates and should be interpreted with caution (Figure 9.1).

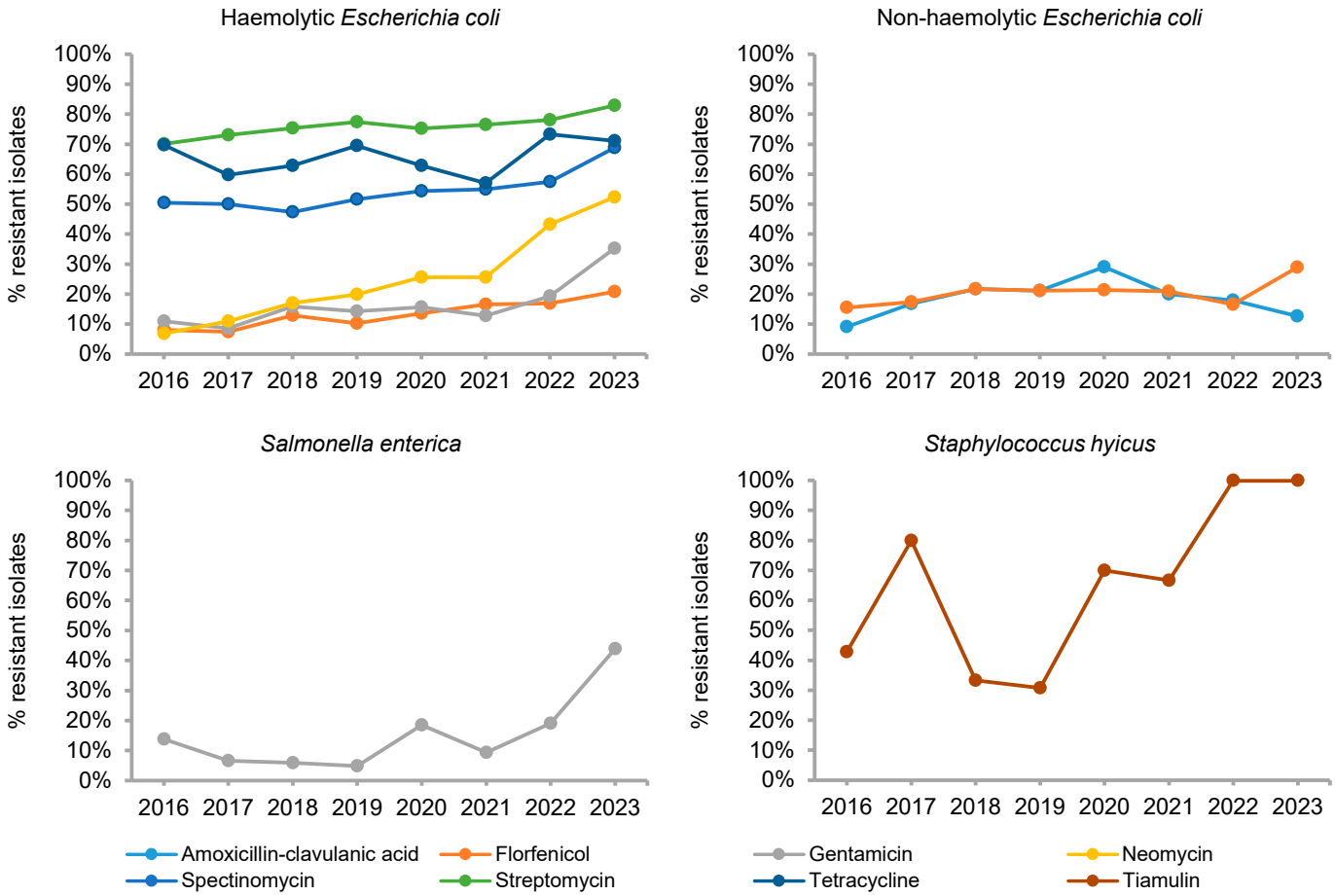
Table 9.2 Statistically significant temporal changes in antimicrobial resistance phenotypes among pathogenic bacteria from pigs, Denmark, 2023 vs. 2022 and 2023 vs. 2018

DANMAP 2023

Pathogen	Antimicrobial agent	2016 R (%)	2017 R (%)	2018 R (%)	2019 R (%)	2020 R (%)	2021 R (%)	2022 R (%)	2023 R (%)	2023 vs. 2022 P value	2023 vs. 2018 P value
H-Ec	Florfenicol	8.1%	7.4%	12.9%	10.3%	13.6%	16.5%	16.9%	20.8%	0.2489	0.0136
	Gentamicin	10.9%	8.6%	15.9%	14.3%	15.6%	12.8%	19.3%	35.2%	0.0000	0.0000
	Neomycin	6.9%	10.9%	17.0%	19.8%	25.6%	25.6%	43.2%	52.3%	0.0270	0.0000
	Spectinomycin	50.5%	50.0%	47.3%	51.6%	54.4%	55.0%	57.4%	68.8%	0.0050	0.0000
	Streptomycin	70.1%	73.0%	75.4%	77.4%	75.2%	76.4%	78.0%	82.9%	0.1479	0.0365
	Tetracycline	69.8%	59.8%	62.9%	69.4%	62.8%	57.0%	73.3%	71.1%	0.5831	0.0388
NH-Ec	Amoxicillin-clavulanic acid	9.1%	16.8%	21.7%	21.1%	29.1%	20.0%	18.0%	12.6%	0.2102	0.0264
	Florfenicol	15.5%	17.3%	21.7%	21.1%	21.3%	20.9%	16.5%	28.9%	0.0090	0.1209
Se	Gentamicin	13.8%	6.6%	5.9%	4.8%	18.5%	9.4%	19.0%	43.9%	0.0186	0.0000
Sh	Tiamulin	42.9%*	80.0%*	33.3%*	30.8*%	70.0%*	66.7%*	100.0%*	100.0%*	1.0000	0.0090

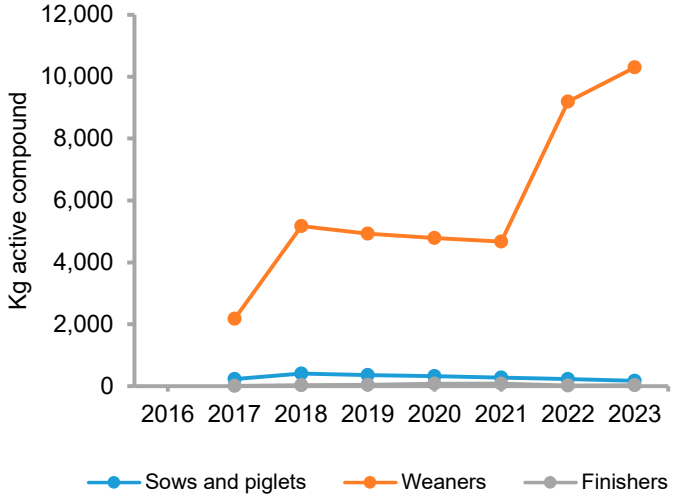
Antimicrobial resistance phenotypes that remained at the same level during 2022-2023 and 2018-2023 were excluded (https://www.vetssi.dk/)
Percentages based on small sample sizes (n<20) are indicated by asterisks and should be interpreted with caution
Abbreviations: H-Ec, haemolytic *Escherichia coli*; NH-Ec, non-haemolytic *Escherichia coli*; Se, *Salmonella enterica*; Sh, *Staphylococcus hyicus;* R, resistant

Figure 9.1 Statistically significant temporal changes in antimicrobial resistance phenotypes among pathogenic bacteria from pigs, Denmark, 2023 vs. 2022 and 2023 vs. 2018
DANMAP 2023



The percentages of tiamulin-resistant *Staphylococcus hyicus* isolates are based on small sample sizes (n<20) and should therefore be interpreted with caution

Figure 9.2 Neomycin use in pigs by age group, Denmark, 2016-2023
DANMAP 2023



9.3 WGS-based detection of resistance mechanisms

A randomly selected subset of *A. pleuropneumoniae* (n=284), *B. bronchiseptica* (n=83), *C. perfringens* (n=222), *E. rhusiopathiae* (n=2), haemolytic *E. coli* (n=303), non-haemolytic *E. coli* (n=214), *G. parasuis* (n=122), *K. pneumoniae* (n=52), *S. enterica* (n=89), *S. hyicus* (n=17) and *S. suis* (n=355) isolates from 2021-2023 was subjected to WGS. Table 9.3 shows all significant changes in resistance mechanisms over a 1-year period (2023 vs. 2022) and a 2-year period (2023 vs. 2021), while a full list of resistance mechanisms detected in isolates from 2021-2023 can be found on DK-VET's homepage (https://www.vetssi.dk/).

Most pathogenic bacteria isolated from pigs in 2023 displayed similar frequencies of resistance mechanisms as in 2022 and 2021. However, 22 pathogen-resistance mechanism combinations were associated with significantly increased frequencies, whereas one was associated with a significantly decreased frequency.

In 2023, *aph(3')-Ia* encoding resistance to neomycin was present in 62.9% of the haemolytic *E. coli* isolates and in 14.3% of the non-haemolytic *E. coli* isolates. No other resistance genes known to confer resistance to neomycin were detected in *E. coli*. Interestingly, *aph(3')-Ia* was also present in 8.3% of the *K. pneumoniae* isolates from 2023, and in 38.7% of the *S. enterica* isolates from 2023, respectively. In addition, *aph(3')-Ia* was present in 2.0% of the *G. parasuis* isolates from 2021 but absent in *G. parasuis* isolates from 2022 and 2023.

Table 9.3 Statistically significant temporal changes in resistance mechanisms identified through whole genome sequencing of pathogenic bacteria from pigs, Denmark, 2023 vs. 2022 and 2023 vs. 2021 DANMAP 2023

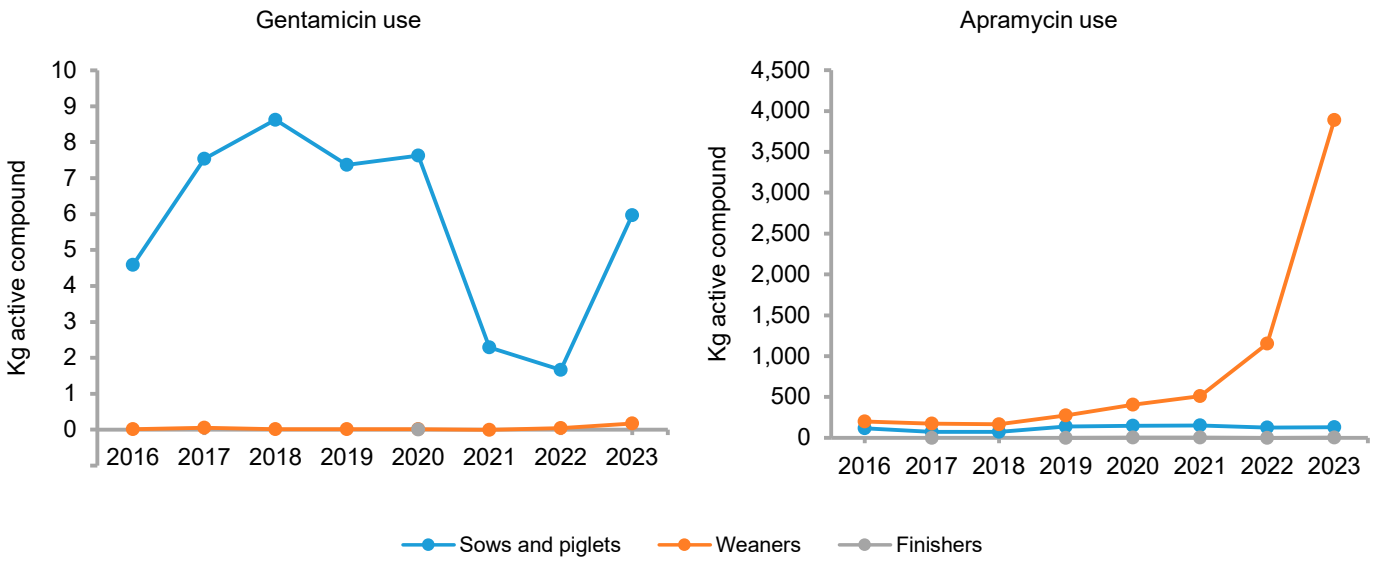
Pathogen	Resistance gene/mutation	Class	Phenotype	2021	2022	2023	2023 vs. 2022	2023 vs. 2021
				Presence (%)	Presence (%)	Presence (%)	P value	P value
Cp	*ant(6)-Ib*	Aminoglycoside	Streptomycin	73.8%	85.7%	87.8%	0.80	0.03
	tet(44)	Tetracycline	Doxycycline, Tetracycline, Minocycline	73.8%	85.7%	87.8%	0.7989	0.0298
H-Ec	*aac(3)-IV*	Aminoglycoside	Apramycin, Gentamicin, Tobramycin	9.6%	21.8%	25.8%	0.5081	0.0037
	aph(3')-Ia	Aminoglycoside	Neomycin, Kanamycin, Lividomycin, Paromomycin, Ribostamycin	30.8%	34.5%	62.9%	0.0001	0.0000
	aph(4)-Ia	Aminoglycoside	Hygromycin	9.6%	20.0%	21.3%	0.8611	0.0269
	$bla_{TEM-127}$	Beta-lactam	Amoxicillin, Ampicillin, Cephalothin, Piperacillin, Ticarcillin	0.0%	0.9%	4.5%	0.1749	0.0436
	cmlA1	Amphenicol	Chloramphenicol	14.4%	7.3%	18.0%	0.0279	0.5580
	mef(C)	Macrolide	Erythromycin	0.0%	0.0%	4.5%	0.0385	0.0436
	mph(G)	Macrolide	Erythromycin	0.0%	0.0%	4.5%	0.0385	0.0436
	tet(B)	Tetracycline	Doxycycline, Tetracycline, Minocycline	21.2%	35.5%	38.2%	0.7677	0.0110
NH-Ec	*floR*	Amphenicol	Chloramphenicol, Florfenicol	22.8%	11.5%	28.6%	0.0264	0.4669
	lnu(F)	Lincosamide	Lincomycin	5.4%	0.0%	8.6%	0.0375	0.5334
	mef(C)	Macrolide	Erythromycin	3.3%	1.9%	12.9%	0.0426	0.0315
	mph(B)	Macrolide	Erythromycin, Spiramycin, Telithromycin	8.7%	7.7%	0.0%	0.0308	0.0104
	mph(G)	Macrolide	Erythromycin	3.3%	1.9%	12.9%	0.0426	0.0315
Kp	*aph(3'')-Ib*	Aminoglycoside	Streptomycin	8.3%	37.5%	54.2%	0.3487	0.0111
	aph(6)-Id	Aminoglycoside	Streptomycin	8.3%	37.5%	54.2%	0.3487	0.0111
Se	*aac(3)-IV*	Aminoglycoside	Apramycin, Gentamicin, Tobramycin	0.0%	7.7%	25.8%	0.0509	0.0177
	aph(4)-Ia	Aminoglycoside	Hygromycin	0.0%	7.7%	25.8%	0.0509	0.0177
	bla_{TEM-1B}	Beta-lactam	Amoxicillin, Ampicillin, Cephalothin, Piperacillin, Ticarcillin	63.2%	59.0%	87.1%	0.0155	0.0776
	sul1	Folate pathway antagonist	Sulfamethoxazole	31.6%	20.5%	51.6%	0.0107	0.2420
	tet(B)	Tetracycline	Doxycycline, Tetracycline, Minocycline	47.4%	59.0%	77.4%	0.1285	0.0371
Ss	*erm*(B)	Macrolide, Lincosamide, Streptogramin B	Erythromycin, Lincomycin, Clindamycin, Quinupristin, Pristinamycin IA, Virginiamycin S	58.3%	64.8%	74.3%	0.1272	0.0201

Abbreviations: Cp, *Clostridium perfringens*; H-Ec, haemolytic *Escherichia coli*; NH-Ec, non-haemolytic *Escherichia coli*; Kp, *Klebsiella pneumoniae*; Se, *Salmonella enterica*; Ss, *Streptococcus suis*

aac(3)-IV encoding resistance to gentamicin was present in 25.8% of the haemolytic *E. coli* isolates, in 7.1% of the non-haemolytic *E. coli* isolates, in 12.5% of the *K. pneumoniae* isolates and in 25.8% of the *S. enterica* isolates. We also identified three less frequent gentamicin resistance genes in haemolytic *E. coli*, including *aac(3)-IId* (6.7%), *aac(3)-IVa* (1.1%) *and ant(2")-Ia* (2.2%). The use of gentamicin in pigs is negligible and cannot explain the rapidly increasing resistance to this drug (Figure 9.3). Interestingly, tthree of the four gentamicin resistance genes found in haemolytic *E. coli, aac(3)-IId, aac(3)-IV and aac(3)-IVa,* also confer resistance to apramycin, which is increasingly used in weaners (Figure 9.3). These observations suggest a causal relationship between increased use of apramycin and increased resistance to gentamicin in haemolytic *E. coli.*

As in previous years, WGS demonstrated a low level of resistance mechanisms towards carbapenems, 3rd, 4th and 5th generation cephalosporins, oxazolidinones and polymyxins.

Figure 9.3 Gentamicin and apramycin use in pigs by age group, Denmark, 2016-2023 DANMAP 2023



9.4 WGS-based prediction of AMR

WGS-based prediction of AMR was assessed by determining the concordance, sensitivity, specificity, positive predictive value, negative predictive value, major error rate and very major error rate between the results obtained through AST and WGS using the genotype-to-phenotype translations in the ResFinder 4.1 database. Table 9.4 shows aggregated results, while results for all pathogen-drug combinations can be found on DK-VET's homepage (https://www.vetssi.dk/). The observed concordance was 99.6% for *A. pleuropneumoniae,* 79.6% for *B. bronchiseptica,* 94.2% for haemolytic *E. coli,* 94.3% for non-haemolytic *E. coli,* 76.0% for *K. pneumoniae,* 97.3% for *S. enterica,* 91.5% for *S. hyicus* and 94.5% for *S. suis.*

Table 9.4 Diagnostic performance of ResFinder 4.1 as an antimicrobial resistance prediction tool for pathogenic bacteria from pigs, Denmark, 2021-2023 DANMAP 2023

Pathogen	Antimicrobial agent	P+/G+	P-/G-	G+/P-	G-/P+	Concordance	Sensitivity	Specificity	PPV	NPV	ME rate	VME rate
Ap	All	8	2,810	2	8	99.6	50.0	99.9	80.0	99.7	0.1	50.0
Bb	All	3	176	1	45	79.6	6.3	99.4	75.0	79.6	0.6	93.8
H-Ec	All	1,403	2,492	162	80	94.2	94.6	93.9	89.6	96.9	6.1	5.4
NH-Ec	All	917	1,836	108	57	94.3	94.1	94.4	89.5	97.0	5.6	5.9
Kp	All	65	311	101	18	76.0	78.3	75.5	39.2	94.5	24.5	21.7
Se	All	374	450	13	10	97.3	97.4	97.2	96.6	97.8	2.8	2.6
Sh	All	44	63	1	9	91.5	83.0	98.4	97.8	87.5	1.6	17.0
Ss	All	229	1,581	4	102	94.5	69.2	99.7	98.3	93.9	0.3	30.8

Data are based on epidemiological cut-offs (ECOFFs) and clinical breakpoints when ECOFFs are unavailable (https://www.vetssi.dk/).
Abbreviations: Ap, *Actinobacillus pleuropneumoniae*; Bb, *Bordetella bronchiseptica*; H-Ec, haemolytic *Escherichia coli*; NH-Ec, non-haemolytic *Escherichia coli*; Kp, *Klebsiella pneumoniae*; Se, *Salmonella enterica*; Sh, *Staphylococcus hyicus*; Ss, *Streptococcus suis*; P+, resistant phenotype; P-, susceptible phenotype; G+, resistant gentotype; G-, susceptible gentotype; PPV, positive predictive value; NPV, negative predictive value; ME, major error; VME, very major error

9.5 Conclusions and perspectives

Haemolytic *E. coli* displayed significantly increased resistance to florfenicol, gentamicin, neomycin, spectinomycin, streptomycin and tetracycline, while non-haemolytic *E. coli* displayed significantly increased resistance to florfenicol and significantly decreased resistance to amoxicillin-clavulanic acid. In addition, *S. enterica* and *S. hyicus* displayed significantly increased resistance to gentamicin and tiamulin, respectively.

The increasing frequency of neomycin and gentamicin resistance in haemolytic *E. coli* is worrisome and should be monitored closely in the coming years. Of note, our interpretation of neomycin resistance was based on ECOFFs, which do not necessarily indicate whether a drug is clinically active. Future studies should therefore seek to establish animal-specific clinical breakpoints to antimicrobial agents of veterinary importance by considering what happens to the drug within a specific animal and body site (pharmacokinetics).

WGS demonstrated that resistance towards carbapenems, 3rd, 4th and 5th generation cephalosporins, oxazolidinones and polymyxins remained at a low level.

WGS-based prediction of AMR using the genotype-to-phenotype translations in the ResFinder 4.1 database seems to be a promising tool for pathogenic bacteria from pigs. However, it is not always possible to compare AST and WGS results due to the lack of ECOFFs and clinical breakpoints for many antimicrobial agents of veterinary importance, and due to limited knowledge on mechanisms conferring resistance to these drugs. In addition, ResFinder 4.1 does not always list all the phenotypes for a given resistance mechanism. For example, it predicts that *aac(3)-IV* confers resistance to gentamicin and tobramycin but not to apramycin, even though this gene is known to encode resistance to apramycin. Finally, the ResFinder 4.1 genotype-to-phenotype translation scheme for point mutations in *K. pneumoniae* is under development and the phenotypes are currently based on antimicrobial classes rather than agents, which might explain some of its poor performance in this species. Closing these gaps could substantially improve the usefulness of WGS for AMR prediction and surveillance in pathogenic bacteria from animals. WGS is also a useful tool for monitoring resistance mechanisms in pathogenic bacteria, for which AST is unavailable, and for tracing the spread of specific resistance genes and pathogenic bacteria within and between animal and human populations.

Lina M. Cavaco, Mikkel Lindegaard, Ute W. Sönksen, Pia T. Hansen, Svend Haugegaard, Charlotte M. Salomonsen, Peter Damborg and Jesper Larsen
For further information: Jesper Larsen, jrl@ssi.dk

10

MATERIALS AND METHODS

10. Materials and methods

10.1 General information

For the DANMAP 2023 report, population sizes and geographical data were obtained from Statistics Denmark [www.dst.dk] and data on the number of 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 for analyses of AMR trends. An overview of all antimicrobial agents registered for humans and animals in Denmark is presented in Table 2.4.

10.2 Data on antimicrobial consumption in animals

10.2.1 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. This monopoly was suspended in April 2007, and since then private companies have been able to obtain license to sell prescribed veterinary medicinal products for animals, if they adhere to the same guidelines that apply to pharmacies. A pharmacy or licensed company either sells the medicine to veterinarians for use in their practice or for resale to farmers or sells the medicine directly to the animal owner upon presentation of a prescription.

Data on all sales of veterinary prescription medicine from pharmacies, private companies, feed mills and veterinarians are sent electronically to a central database, VetStat, which is hosted by the Danish Veterinary and Food Administration. 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 their invoice system. Electronic registration of the sales at pharmacies is linked to the billing process and stock accounts at the pharmacy. This ensures a very detailed data of high quality. Data are transferred daily from pharmacies to The Register of Medicinal Product Statistics at The Danish Health Authority and to VetStat.

In addition, data on coccidiostatics as feed additives (non-prescription) and antimicrobial growth promoters (not used since 2000) are also collected by VetStat, providing an almost complete register of all antimicrobial agents used for animals in Denmark since 2000. In very rare instances, medicine is prescribed on special license, i.e. medicines not approved for marketing in Denmark. These are not included in VetStat data.

VetStat contains detailed information about source (veterinarian/pharmacy/feed mill) 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 sector [www.whocc.no]. The data presented in DANMAP 2023 were extracted from VetStat on 1 July 2024.

10.2.2 Methods

In DANMAP, we report use of antimicrobials in different animal populations. As a first step, the amount of antimicrobial agents used in animals is measured in kg active compound. This enables an overall crude comparison of consumption among different animal species and between the veterinary and human sectors.

Furthermore, a more detailed comparison of antimicrobial use is performed, taking into account 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, and sows (including piglets and boars) and exclude antimicrobials dispensed as tablets, products for topical use, intramammaries and gynaecologicals.

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 principles 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 2023, only the live biomass for pigs and cattle 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]. The size of the breeding animals (sows and boars) 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-gender categories.

Treatment proportion - DAPD

The treatment proportion is a statistical measure for AMU in animal populations, calculated as the annual number of DADDs administered in the population, divided by the estimated total population live biomass. For a single animal, the mg active compound (e.g. the number of DADDs) given in a daily treatment depends on the body weight. The treatment proportions, therefore, also represents the proportion of animals treated daily with an average maintenance- dose of a particular antimicrobial agent. These 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 DID, defined daily dose per 1,000 inhabitants per day (DID), used in pharmaco-epidemiology for the human sector, see Section 10.8.2.

In 2023, DAPD calculations were carried out for pigs and cattle.

For example, the antimicrobial use per pig produced is calculated as:

$$DAPD = \frac{DADD_{sows} + DADD_{weaners} + DADD_{finishers}}{\Sigma biomassdays}$$

Where DADDs, DADDw, and DADDf are amounts of antimicrobial agents used in finishers, weaners, and sows (including piglets and boars).

10.3 Collection of bacterial isolates from animals and meat

In DANMAP, samples originate both from the EU harmonised monitoring of antimicrobial resistance in zoonotic and commensal bacteria, and the national *Salmonella* surveillance programs. Since 2014, most isolates available for DANMAP have been collected in accordance with the EU harmonised monitoring, according to Decision 2013/652/EU. This Decision was repealed by Decision 2020/1729/EU, applied from 1 January 2021. With the aim to ensure continuity in assessing future trends in antimicrobial resistance, the new Implementing Decision includes adaptations of food categories to be sampled, sampling design to be followed, bacterial species to be tested and the analytical methods to be used.

EU harmonized monitoring from 2021 to 2027 shall cover *Salmonella* spp., *Campylobacter coli*, *Campylobacter jejuni*, indicator commensal *Escherichia coli*, ESBL-, AmpC- or carbapenemase- producing *Salmonella* spp. and *E. coli*, and may cover *Enterococcus faecalis* and *Enterococcus faecium*. Previously, monitoring of *Campylobacter coli* was voluntary. For the monitoring of *Salmonella* in poultry, it is now possible to report only samples collected within the national control programme in poultry farms, while the monitoring of *Salmonella* in fattening pigs at slaughter is still required for most countries, including Denmark, due to the inexistence of an implemented national surveillance programme which has been approved at EU level.

Additionally to monitoring of fresh meat at retail, the present EU legislation requires monitoring of indicator *E. coli* and ESBL-, AmpC- and CP-producing *E. coli* on fresh imported meat sampled at border control posts, and the fresh meat categories to be monitored include turkey, both at retail and at the border.

Decision 2020/1729/EU further allows the use of whole genome sequencing as an alternative method for the specific monitoring of ESBL-, AmpC- or CP-producing *E. coli* or for further testing of indicator *E. coli* and *Salmonella* showing resistance to cefotaxime, ceftazidime or meropenem.

The legislation continues to require mandatory sampling of broilers and fattening turkeys and meat thereof in even years (2022, 2024, 2026), and sampling of fattening pigs and cattle <1 year, and meat thereof in odd years (2021, 2023, 2025, 2027). In Denmark, fattening turkeys are not sampled at slaughter as part of the EU harmonised monitoring, because the national production of turkey meat is below 10.000 tonnes per year.

10.3.1 Animals

In 2023, most of the sampling for DANMAP was allocated to the mandatory sampling of caeca from fattening pigs and cattle <1 year at slaughter, and additional sampling of caeca from broilers was also carried out.

Caecal samples from healthy broilers, cattle (<1 year) and pigs were collected by meat inspection staff at the slaughterhouses. Samples were collected throughout the year, in major Danish slaughterhouses slaughtering conventionally produced chicken, pigs and cattle.

Sampling was stratified per slaughterhouse by allocating the number of samples from domestically produced animals per slaughterhouse, proportionally to the annual throughput of the slaughterhouse. For broiler flocks, ten intact caeca were pooled into one sample. For pigs and cattle, samples contained 30-100 g caecal material from a single animal.

All samples were processed by the Danish Veterinary and Food Administration's (DVFA) laboratory in Ringsted or by a DVFA-approved private laboratory. Samples from all three animal species were examined for indicator *E. coli, Campylobacter jejuni and Campylobacter coli.*

Furthermore, pig and cattle samples were also examined for ESBL/AmpC/carbapenemase-producing *E. coli*, and samples from pigs were examined for *Enterococcus faecalis and Enterococcus faecium* (Table 10.1).

Pathogenic bacteria from pigs reported in 2023 comprised *Actinobacillus pleuropneumoniae* (AST and WGS), *Bordetella bronchiseptica* (AST and WGS), *Clostridium perfringens* (WGS), *Erysipelothrix rhusiopathiae* (WGS), haemolytic and non-haemolytic *Escherichia coli* (AST and WGS), *Glaesserella parasuis* (WGS), *Klebsiella pneumoniae* (AST and WGS), *Salmonella enterica* (AST and WGS), *Staphylococcus hyicus* (AST and WGS) and *Streptococcus suis* (AST and WGS) isolates identified in clinical samples submitted by veterinarians to the Veterinary Laboratory, The Danish Agriculture and Food Council.

10.3.2 Meat

In 2023, ESBL/AmpC/carbapenemase-producing *E. coli* were isolated from packages of fresh, chilled pork and beef meat collected in Danish wholesale and retail outlets and at border control posts. These samples were collected throughout the year by DVFA officers (Table 10.1). Products with added saltwater or other types of marinade as well as minced meat were not included. Packages of meat were selected without preselecting by country of origin, as requested for the harmonised EU monitoring.

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.

Salmonella isolates from domestically produced pork and beef originated from the national control programme at the slaughterhouses (Table 10.1). Carcasses were swabbed in four designated areas (covering 4 x 100 cm^2) after min. 12 hours of chilling. All samples were processed at DVFA-approved Industry laboratories and isolates were sent to the DVFA laboratory.

10.4 Microbiological methods – isolates from animals and meat

10.4.1 *Salmonella*

Salmonella from pork not originating from the national *Salmonella* surveillance program were isolated at DVFA in accordance with the methods issued by the NMKL [NMKL No. 187, 2007] and in accordance with Annex D, ISO 6579-1 [ISO6579-1:2017]. Serotyping of those isolates was performed at DVFA 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.

Salmonella from carcasses originating from the national *Salmonella* surveillance program were isolated and serotyped according to the White-Kauffmann-Le Minor scheme at DVFA-approved Industry laboratories.

10.4.2 *Campylobacter*

Campylobacter from broiler and cattle caeca was isolated and identified according to the methods issued by the NMKL [NMKL No. 119, 2007] with modifications, with pre-enrichment in Bolton broth, and followed by species-determination by BAX® rtPCR assay (Hygiena, BAX® System PCR Assays for Campylobacter). Only one *Campylobacter* isolate per broiler flock or cattle or pig herd was selected for antimicrobial susceptibility testing.

Table 10.1 Legislative and voluntary sampling plans under national control programmes and EU harmonised monitoring that contributed with isolates to DANMAP 2023 DANMAP 2023

Bacteria	Origin of isolates	Legislative reporting frequency (2020/1729/EU)	Number of tested and positive samples in 2023
Campylobacter spp.	Caecal samples from broilers[a]	Even years	168 flocks (64 positive)
	Caecal samples from cattle <1 yr[a]	Odd years	225 animals (201 positive)
	Caecal samples from fattening pigs[a]	Odd years	296 animals (160 positive)
Enterococcus spp.	Caecal samples from fattening pigs[b]	Odd years	492 animals (169 positive)
Indicator *E. coli*	Caecal samples from broilers	Even years	129 flocks (125 positive)
	Caecal samples from fattening pigs	Odd years	176 animals (173 positive)
	Caecal samples from cattle <1 yr	Odd years	172 animals (169 positive)
Specific monitoring of ESBL/AmpC and carbapenemase-producing *E. coli*	Caecal samples from fattening pigs	Odd years	294 animals (54 positive)[c]
	Caecal samples from cattle <1 yr	Odd years	309 animals (13 positive)[c]
	Fresh pork meat at BCPs	Odd years	3 units (0 positive)
	Fresh beef meat at BCPs	Odd years	6 units (0 positive)
	Fresh pork meat at retail (Danish)	Odd years	209 units (5 positive)[c]
	Fresh pork meat at retail (Imported)	Odd years	110 units (9 positive)[c]
	Fresh beef meat at retail (Danish)	Odd years	120 units (1 positive)[c]
	Fresh beef meat at retail (Imported)	Odd years	192 units (8 positive)[c]
	WGS data for collected ESBL/AmpC isolates	Odd years	90 isolates[d]
Salmonella spp.	Caecal samples from fattening pigs	Odd years	772 animals (85 positive)[f]
	Caecal samples from cattle <1 yr	Odd years	301 animals (3 positive)[f]
	Carcase swabs from fattening pigs[e]	Odd years	3448 units (67 positive)[f]
	Carcase swabs from cattle <1 yr[e]	Odd years	883 units (3 positive)[f]

a) Broilers: *C. jejuni* (n=44), *C. coli* (n=12), unspecified (n=5) ; Cattle <1 yr: *C. jejuni* (n=175), *C. coli* (n=8), unspecified (n=13); Fattening pigs: *C. jejuni* (n=1), *C. coli* (n=128), unspecified (n=24)
b) Fattening pigs: *E. faecalis* (n=87), *E. faecium* (n=85)
c) Positive for ESBL/AmpC-producing *E. coli* and negative for carbapenemase-producing *E. coli*
d) 90 isolates from the positive samples were sequenced (54 from fattening pigs, 13 from cattle <1 yr, 5 from Danish pork meat, 9 from imported pork meat, 1 from Danish beef meat and 8 from imported beef meat)
e) Carcass swab samples are part of the national *Salmonella* surveillance program and are classified in DANMAP as meat of domestic origin. Samples collected at slaughterhouses slaughtering more than 30,000 pigs or 7,500 cattle are analysed in pools of 5 individual samples. The total number of animals tested and the total number of positives refer to individual pooled samples
f) Fattening pigs: *S.* Derby (n=42), *S.* 4,[5],12:i:- (n=31), *S.* Typhimurium (n=6), other serotypes or unspecified (n=6); Cattle <1 yr: *S.* Dublin (n=2), *S.* Agona (n=1); Pork meat (carcass): *S.* Derby (n=23), *S.* 4,[5],12:i:- (n=18), *S.* Typhimurium (n=12), other serotypes or unspecified (n=14); Beef meat (carcass): *S.* Dublin (n=2), unspecified (n=1)

10.4.3 *Escherichia coli*

Indicator *E. coli* from broilers, pigs and cattle was isolated by direct spread 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. The specific isolation of ESBL/AmpC or carbapenemase-producing *E. coli* from pork and beef meat and caecal samples of pigs and cattle <1 year occurred within 96 h after sample collection, applying the current EURL-AR laboratory protocol [https://www.eurl-ar.eu/protocols.aspx]. Carbapenemase-producing *E. coli* screening was done with ChromID CARBA and ChromID OXA-48 plates. ESBL/AmpC-producing *E. coli* screening was done with MCA cefotaxime plates. All presumptive ESBL/AmpC or carbapenemase producing *E. coli* isolates were sequenced by WGS using the Illumina MiSeq platform (paired-end sequencing 2x250). Only one ESBL/AmpC-producing *E. coli* isolate per herd and meat sample was selected for antimicrobial susceptibility testing.

10.4.4 Enterococci

Indicator enterococci were isolated from pig caeca by adding 2 ml buffered peptone water to the content of a cotton swab, after which 100 µl were inoculated onto Slanetz agar and incubated for 48 h at 41,5 °C. Presumptive *E. faecium/E. faecalis* were identified by real-time PCR assay. When present, only one *E. faecalis* isolate per herd was selected for antimicrobial susceptibility testing.

10.5 Susceptibility testing – isolates from animals and meat

Antimicrobial susceptibility testing of *Salmonella*, *Campylobacter* and *E. coli* was carried out by Minimum Inhibitory Concentration (MIC) determination, using broth microdilution by Sensititre (Trek Diagnostic Systems Ltd.). Inoculation and incubation procedures were performed in accordance with the CLSI guidelines [Clinical and Laboratory Standards Institute, USA] and the European standard [ISO 20776-1:2020]. The isolates were tested for antimicrobial susceptibility in accordance with the Decision 2020/1729/EU about the EU harmonised monitoring of antimicrobial resistance.

The quality control strains used were: *E. coli* ATCC 25922, *E. faecalis* ATCC 29212, *C. jejuni* ATCC 33560 and *P. aeruginosa* ATCC 27853. Isolates from animals and meat were tested for antimicrobial susceptibility at the DVFA laboratory in Ringsted, which is accredited by DANAK (the national body for accreditation).

Antimicrobial susceptibility testing of pathogenic bacteria from pigs was performed at the Veterinary Laboratory, The Danish Agriculture and Food Council. In brief, MICs were determined by broth microdilution using customised Sensititre panels according to CLSI standards. The analysis is accredited by DANAK.

10.6 Whole genome sequencing – isolates from animals and meat

In addition to *Salmonella* serotyping performed by sequencing, whole genome sequencing (WGS) and in silico bioinformatics 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 using the ResFinder application v. 4.4.2 and the ResFinder database v. 2.2.1.

WGS of pathogenic bacteria from pigs was performed on Illumina platforms at Statens Serum Institut. Acquired resistance genes and point mutations were detected by mapping sequence reads against the ResFinder 4.1 database [Bortolaia *et al.* 2020. J. Antimicrob. Chemother 75(12):3491-3500) using the k-mer alignment (KMA) tool 1.3 (Clausen *et al.* 2018. BMC Bioinformatics 19(1):397], setting both length match and similarity match to 0.9 and excluding hits with less than 10X coverage.

10.7 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 sent 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.

For the samples processed at the Veterinary Laboratory, The Danish Agriculture and Food Council, sampling details and laboratory results were stored in the information management system used at the Veterinary Laboratory. Following internal validation and anonymisation, data were sent to DK-VET (Excel sheets). At DK-VET, data were harmonised and one isolate per epidemiological unit was selected for reporting.

Table 10.2 Interpretation criteriae for MIC-testing by EUCAST- and EFSA-provided epidemiological cut-off values (ECOFFs)

DANMAP 2023

Antimicrobial agent	*Salmonella*	*E. coli*	*E. faecalis*	*C. jejuni*	*C. coli*
	ECOFF µg/ml	ECOFF µg/ml	ECOFF µg/ml	ECOFF µg/ml	ECOFF µg/ml
Amikacin	>4	>8	Not tested	Not tested	Not tested
Ampicillin	>8	>8	>4	Not tested	Not tested
Azithromycin	>16	>16 [a]	Not tested	Not tested	Not tested
Cefepime	>0.125 [a]	>0.125 [a]	Not tested	Not tested	Not tested
Cefotaxime	>0.5	>0.25	Not tested	Not tested	Not tested
Cefotaxime-clavulanic acid	>0.5 [a]	>0.25	Not tested	Not tested	Not tested
Cefoxitin	>8	>8	Not tested	Not tested	Not tested
Ceftazidime	>2	>0.5	Not tested	Not tested	Not tested
Ceftazidime-clavulanic acid	>2 [a]	>0.5	Not tested	Not tested	Not tested
Chloramphenicol	>16	>16	>32	>16 [d]	>16 [d]
Ciprofloxacin	>0.064	>0.064	>4	>0.5	>0.5
Colistin	>2 [a] [b]	>2	Not tested	Not tested	Not tested
Daptomycin	Not tested	Not tested	>4	Not tested	Not tested
Ertapenem	>0.064 [a]	>0.064 [a]	Not tested	>0.5 [a] [d]	>0.5 [a] [d]
Erythromycin	Not tested	Not tested	>4	>4	>8
Gentamicin	>2	>2	>64	>2 [a]	>2 [a]
Imipenem	>1	>0.5	Not tested	Not tested	Not tested
Linezolid	Not tested	Not tested	>4	Not tested	Not tested
Meropenem	>0.125 [a]	>0.125	Not tested	Not tested	Not tested
Nalidixic acid	>8	>8	Not tested	Not tested	Not tested
Quinopristin-dalfopristin	Not tested	Not tested	>1 [a] [c]	Not tested	Not tested
Sulfamethoxazole	>256 [a]	>64 [a]	Not tested	Not tested	Not tested
Teicoplanin	Not tested	Not tested	>2	Not tested	Not tested
Temocillin	>16 [a]	>16	Not tested	Not tested	Not tested
Tetracycline	>8	>8	>4	>1	>2
Tigecycline	>0.5 [a]	>0.5	>0.25	Not tested	Not tested
Trimethoprim	>2	>2	Not tested	Not tested	Not tested
Vancomycin	Not tested	Not tested	>4	Not tested	Not tested

EUCAST epidemiological cut-off values (ECOFFs) and ECOFFs provided by EFSA for EU harmonized reporting

a) ECOFF as provided by EFSA [EFSA Supporting publication 2023:EN-7826]

b) For colistin, a tentative ECOFF of 16 µg/ml for *Salmonella* Dublin is established by EUCAST. The same ECOFF is used in DANMAP to interpret results of *Salmonella* Enteritidis. Both serotypes belong to the O-group (O:1, 9,12), which has been associated with increased MIC for colistin [https://www.doi.org/10.1089/fpd.2011.1015]

c) For quinopristin-dalfopristin, ECOFF only applies for *E. faecium*. ECOFF >1 for *E. faecalis* (intrinsically resistant to quinopristin-dalfopristin) is used only for the purpose of EU harmonized reporting

d) In 2021, chloramphenicol and ertapenem were introduced in the test panel for *Campylobacter* spp.

Table 10.3 Definitions of antimicrobial classes for calculation of multidrug-resistance in *Salmonella* and indicator *E. coli*
DANMAP 2023

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
Polymyxins	Colistin
Aminoglycosides	Gentamicin and/or amikacin
Carbapenems	Meropenem
Sulfonamides	Sulfamethoxazole
Tetracyclines	Tetracycline
Glycylcyclines	Tigecycline
Trimethoprim	Trimethoprim

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; The aminoglycoside antimicrobial amikacin has been introduced in the test panel in 2021

10.7.1 Interpretation of MIC values

MIC values were retained as continuous variables, from which binary variables (resistant/sensitive) were created using the relevant cut-off. Since 2007, MIC results have been interpreted using EUCAST epidemiological cut-off (ECOFF) values, with a few exceptions, as described in Table 10.2. An isolate is considered multidrug-resistant if resistant to three or more of the antimicrobial classes defined in Table 10.3 and fully sensitive if susceptible to all antimicrobial agents included in the test panel.

For pathogenic bacteria from pigs, MIC values were interpreted with ECOFFs (1st choice) or tentative ECOFFs (2nd choice) established by EUCAST. When ECOFFs were unavailable, interpretation was based on CLSI-approved animal-specific or human clinical breakpoints (3rd and 4th choice, respectively) (available at https://www.vetssi.dk/).

10.7.2 ESBL/AmpC phenotypes

Classification of CP-, ESBL- or AmpC-producing phenotypes was done according to the scheme provided by EFSA. [EFSA 2023. EFSA Journal 21(3):7867].

1. ESBL phenotype if cefotaxime and/or 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);
2. AmpC phenotype if cefotaxime and/or 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);
3. ESBL-AmpC phenotype if cefotaxime and/or 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. CP phenotype if meropenem MIC >0.12 µg/ml;
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.

10.7.3 Statistical tests

Significance tests of differences between proportions of resistant isolates were calculated using Chi-square, or Fisher's Exact Tests as appropriate depending on sample size. Significance tests for trends in rates of resistance were performed by applying the Cochran-Armitage test, using the DescTools R package version 0.99.45. One-sided tests were chosen because of preliminary expected trend directions. A significance level of 0.05 was considered in all significance tests.

Analyses were done using R statistical software version 4.4.1 [R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org].

10.8 Data on antimicrobial consumption in humans

10.8.1 Data registration

Annual data on antimicrobial consumption in Denmark has been provided to DANMAP by the Register of Medicinal Product Statistics at the Danish Health Data Authority every year since 1997. Since 2020, DANMAP also reports monthly antimicrobial consumption data to allow analysis of the impact of the Covid-19 pandemic on antimicrobial consumption in humans since 2020.

Until 2012, data from hospitals on certain infusion substances such as cephalosporins, carbapenems and trimethoprim were obtained by DANMAP directly from hospital pharmacies. Since 2013, all data from hospitals are reported to and provided to DANMAP by The Register of Medicinal Product Statistics at the Danish Health Data Authority.

Reports of human antimicrobial consumption in Denmark existed already before 1997. These were prepared by 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 pharmacies. These reports became less reliable over time since there was an increasing amount of parallel imported drugs from the late 1980s, which were not covered by MEDIF/MEFA.

In the primary sector, all antibacterial agents for human use are prescription-only medicines. Sales are reported by pharmacies using a code relating to the defined package. The code includes information on the active drug, the brand name of the product, formulation, size and number of packages. The report also includes age, gender and regional residence of the patient. Since 2004, the sales registration has included a code for indication of the prescription as well. However, clinical indications provided for the treatment of infectious diseases were often quite unspecific ("against infection"). Since 2016, the use of more specific indication codes has increased following the implementation of electronic prescribing via the "common medicine card" (fælles medicinkortet, FMK), a digital pharmacy platform which is mandatory to be used by all medical doctors. In 2023, indication codes were available for 94% of prescriptions, but specific indication codes still only accounted for 75%.

For hospitals, reporting is based on deliveries 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 five Danish Regions. Amgros is responsible for harmonisation of prices and for ensuring deliveries to all hospitals and works 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 hospital making precise calculations of the consumption on specialty level difficult. In DANMAP, reporting of data on hospital consumption is therefore kept at national and regional level. In case of production failures and shortages in delivery of specific products, the hospitals have to apply for special delivery through the Danish Medicines Agency (Figure A5.2 in web annex). These special deliveries are reported separately to DANMAP through the hospital pharmacies. An example is the shortages in delivery 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 2023, 126.490 DDD (3%) of the total antimicrobial consumption were special deliveries. Data on consumption at patient level are available at some hospitals and have so far been used in local quality assurance only but have not been available to DANMAP.

10.8.2 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 3% of the antimicrobial consumption at hospitals in Denmark.

The present report includes data on the consumption of "antibacterials for systemic use", or group J01, of the 2023 update of the Anatomical Therapeutic Chemical (ATC) classification, in primary healthcare and in hospitals as well as consumption of 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.

10.8.3 DDD

Defined daily dose is the assumed average maintenance dose per day for a drug used for it's 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 [https://www.whocc.no/atc_ddd_index/].

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) (Table 10.5). From DANMAP 2018, the new DDD values were applied and all tables and figures were updated ten years back.

10.8.4 DBD

DDD/100 occupied bed-days. The number of occupied bed-days are calculated as the exact duration of a hospital stay in hours divided by 24 hours. 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 are still being validated and may be adjusted in the future. This has to be kept in mind when data are interpreted.

10.8.5 DAD

DDD/100 admissions. One admission is registered whenever a patient is admitted to a specific hospital for ≥12 hours. If a patient is transferred between wards within 4 hours, it will not count as a new admission. 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 are still being validated and may be adjusted in the future. This has to be kept in mind when data are interpreted.

10.8.6 Antimicrobial consumption for elderly living in long care facilities

Data from the Care Home Register were combined with data from the Danish Civil Registration System (CPR) and with data from the Register of Medicinal Product Statistics in order to determine the antimicrobial consumption for elderly people living in care homes and for elderly people living in their own homes.

10.9 *Salmonella* and *Campylobacter* isolates from humans

10.9.1 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.

10.9.2 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.

10.9.3 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.

Table 10.5 New DDDs assigned by WHO Collaborating Centre per January 2019 DANMAP 2023

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

10.9.4 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.

10.10 *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter* species, *Enterococcus faecium* and *Enterococcus faecalis* isolates from humans

10.10.1 Data source

The surveillance of invasive isolates of *E. coli, K. pneumoniae, E. faecalis* and *faecium, P. aeruginosa* and A. spp. and urine isolates of *E. coli* and *K. pneumoniae* are all based on data from routine diagnostics at the ten Departments of Clinical Microbiology (DCMs) in Denmark. All data were extracted directly from the Danish Microbiology Database (MiBa) [https://miba.ssi.dk]. Before 2018, data were reported by the individual DCM to SSI. A description of MiBa and the usage and validation of MiBa-data is given in Textbox 8.1 in DANMAP 2018 [www.danmap.org].

10.10.2 Microbiological methods

All microbiological analyses including species identification, susceptibility testing and interpretation of test results, were performed by the DCM. Since November 2015, all Danish DCMs used the EUCAST terminology with the EUCAST clinical breakpoints and the EUCAST methods for roughly all species. Few exceptions exist at some DCMs where local rules were applied to 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 individual DCMs and may influence interpretation results. This was commented on when necessary in the affected sections.

To be included in resistance surveillance more than 75% of respective isolates need to be antimicrobial susceptibility tested for a given antibiotic, if not stated otherwise. Data of antimicrobial susceptibility testing was mainly performed by disc diffusion. The presented data consist of the registered interpretation results, performed by the respective DCM, based on the S-I-R system. In addition, zone diameters have also been registered since 2015 and will be commented upon in specific cases.

Urine specimen taken in primary health care are also being tested at DCMs except for some samples taken by GPs in the Capital Region of Denmark that are being tested at a private laboratory.

All enterococci isolates reported as VRE in MiBa (based on PCR results for *vanA/B* genes) were reported as vancomycin-resistant independent of the actual zone/MIC result. It was not distinguished between VRE and VVE (vancomycin-variable enterococci). High-level resistance to gentamicin was defined using EUCAST break points (MIC >128 mg/L and/or zone diameters <8 mm) for MIC and/or zone diameters reported in MiBa. Gentamicin MIC and/or zone diameters were routinely reported by three DCMs in 2020.

10.10.3 Data handling

Cases and susceptibility results were extracted from MiBa and analysed in Python 3.8.10.

The case definition has been harmonised with the definition used 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 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 patients at hospitals or primary healthcare settings.

10.11 ESBL-producing bacterial isolates from humans

10.11.1 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.

10.11.2 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.

10.11.3 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/src/master/] 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.food.dtu.dk/]. Possible clonal clusters were detected using the SeqSphere+ (Ridom) software to call cgMLST types (*E. coli* scheme).

10.12 CPO isolates from humans

10.12.1 Data source

Historically, Danish DCMs have submitted carbapenem-resistant isolates for verification and genotyping on a voluntary basis to the Antimicrobial Resistance Reference Laboratory at the Statens Serum Institut. Since 5 September 2018, notification of CPO has been mandatory in Denmark. For outbreak investi-gation Data from The National Patient Register (LPR), informa-tion gathered at the hospitals and information of residence from the Danish Civil Registration System (CPR) has been included in the analysis for this report.

10.12.2 Microbiological methods

All submitted isolates (originating both from screening and clinical samples) predicted to carry a carbapenemase based on initial phenotypic tests were analysed using WGS. More than one isolate from the same patient was only included in the dataset if the isolates belonged to different bacterial species and/or if isolates within the same species harboured different carbapenemases.

10.12.3 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 investigations, identified clonal clusters were linked with patient data like time and place of hospitalization and place of residence. Identification of isolates from two or more persons (cases) sharing the same unique genotype was defined as an outbreak. An outbreak was defined as a verified outbreak if 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 lived at 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 providing an epidemiological link between two or more of the cases becomes available. Both, possible and verified outbreaks, are registered in the CPO-outbreak database KURS (coordinated outbreak registration).

Outbreak investigations of a cluster of cases are closed when no new cases have been reported within 6 months after the last reported case, but can be reopened, if new cases are being detected.

10.13 VRE isolates from humans

10.13.1 Data source

Danish DCMs are submitting VRE for species identification, genotyping and surveillance on a voluntary basis to the Antimicrobial Resistance Reference Laboratory at Statens Serum Institut.

10.13.2 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.

10.13.3 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.

10.14 Invasive *Streptococcus pneumoniae* isolates from humans

10.14.1 Data source

Invasive pneumococcal disease is a notifiable disease in Denmark and it is mandatory to submit all invasive isolates of *S. pneumoniae* for serotyping and susceptibility testing to the Neisseria and Streptococci Reference Laboratory at Statens Serum Institut. For cases of invasive pneumococcal disease, where isolates from blood/spinal fluid could not be submitted, identification and registration of cases is conducted by extracting the required information from the Danish Microbiology Database (MiBa).

10.14.2 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 and Ply 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.

10.14.3 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, SSI Diagnostica, Denmark). Breakpoints were according to EUCAST Clinical Breakpoint Tables v. 11.0. 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.11.0). For cases, where an isolate was not received at the reference laboratory, susceptibility data could often be found in MiBa.

10.14.4 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.

10.15 Isolates of beta-haemolytic streptococci of groups A, B, C, and G from invasive infections in humans

10.15.1 Data source

All invasive isolates of beta-haemolytic streptococci (BHS) (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.

10.15.2 Microbiological methods

Identification of streptococcal group was performed by latex agglutination (Streptococcal Grouping Reagent, Oxoid, Denmark). Genomic DNA was extracted using an enzymatic pre-lysis step before automated purification on MagNA Pure 96 DNA Small Volume Kit (Roche Diagnostics). 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 (Il- lumina) 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.

For Group A Streptococcus (GAS), isolates were *emm* typed by performing a BLAST search to all published *emm* types by CDC [https://www.cdc.gov/streplab/protocol-emm-type.html]. For Group B Streptococcus (GBS), all isolates were serotyped by latex agglutination test and, if needed, confirmed using Lancefield tests. In addition, blasting of capsular sequencing was used for identification of genotypes. No additional identification tests were performed for isolates from Group C or G.

10.15.3 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, Den- mark) on Mueller-Hinton agar (Mueller-Hinton plate, 5% blood, SSI Diagnostica, Denmark). Isolates were also tested for inducible clindamycin resistance. For non-susceptible streptococci the MIC was determined with ETEST (Biomérieux), with erythromycin or clindamycin on Mueller-Hinton agar. The breakpoints used were as defined by the EUCAST (EUCAST v. 14.0 Breakpoint Tables).

Isolates that were either resistant or susceptible to increased exposure were categorised together as resistant.

10.15.4 Data handling

A case of invasive BHS disease was defined as the isolation of BHS from a normally sterile site (e.g., blood, cerebrospinal fluid, synovial fluid, pleural fluid, ascites, and tissue obtained during surgery). 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 (*emm*-type or GBS serotype) if the group was identical on both occasions.

Only one isolate from each unique case of BHS infection was included in the DANMAP report.

10.16 Invasive *Haemophilus influenzae* isolates from humans

10.16.1 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".

10.16.2 Microbiological methods

At SSI, the received isolates were analysed by whole-genome sequencing, from which serotype and biotype were extracted.

10.16.3 Susceptibility testing

Susceptibility data for the 2023 isolates were retrieved from MiBa. In cases where a series of isolates from the same episode developed non-susceptibility over time, the most non-susceptible profile was used for the analysis in DANMAP. In addition, for isolates received at SSI, whole-genome sequencing data was analysed for the presence of beta-lactamase encoding plasmids TEM-1 and ROB-1 as well as for the presence of mutations in the *ftsI* gene that encodes for penicillin-binding protein 3 (PBP3).

10.16.4 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.

10.17 *Staphylococcus aureus* including MRSA isolates from humans

10.17.1 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.

10.17.2 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).

10.17.3 Susceptibility testing

Data on antimicrobial susceptibility was extracted from MiBa.

10.17.4 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.

10.18 Gonococcal isolates

10.18.1 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.

10.18.2 Microbiological methods

The bacteriological identification of the received isolates was performed by MALDI-TOF.

10.18.3 Susceptibility testing

For all isolates the MICs of azithromycin, ceftriaxon and ciprofloxacin were determined with ETEST (Biomérieux) on chocolate agar incubated at 35 °C in 5% CO_2. The breakpoints used were those defined by EUCAST (EUCAST v. 14.0 Breakpoint Tables). A cefinase disc technique was used to examine the isolates for beta-lactamase production.

The breakpoints for azithromycin were changed as of January 1, 2019 (EUCAST version 9.0) (S: MIC ≤1 mg/L; R: MIC >1 mg/L) and it was advised that 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.

In addition to the above, the MIC of cefixime was determined for 117 consecutive isolates as part of an ECDC project on gonococcal antimicrobial resistance (Euro-GASP). The breakpoints used were those defined by EUCAST (EUCAST v. 14.0 Breakpoint Tables).

10.18.4 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.

11

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
CA	Community-acquired
CC	Clonal complex
CDI	Clostridium difficile infections
CHR	Central Husbandry Register
CPE	Carbapenemase producing Enterobacterales/Enterobacteriaceae
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
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/1,000 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*
PCR	Polymerase chain reaction
PHC	Primary health care
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 [https://www.whocc.no/atc_ddd_index/].

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).

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 a hospital accounts for the exact length of the hospital stay. This corresponds to the actual hours at hospital divided by 24 hours.

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/1,000 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 depends 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).

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