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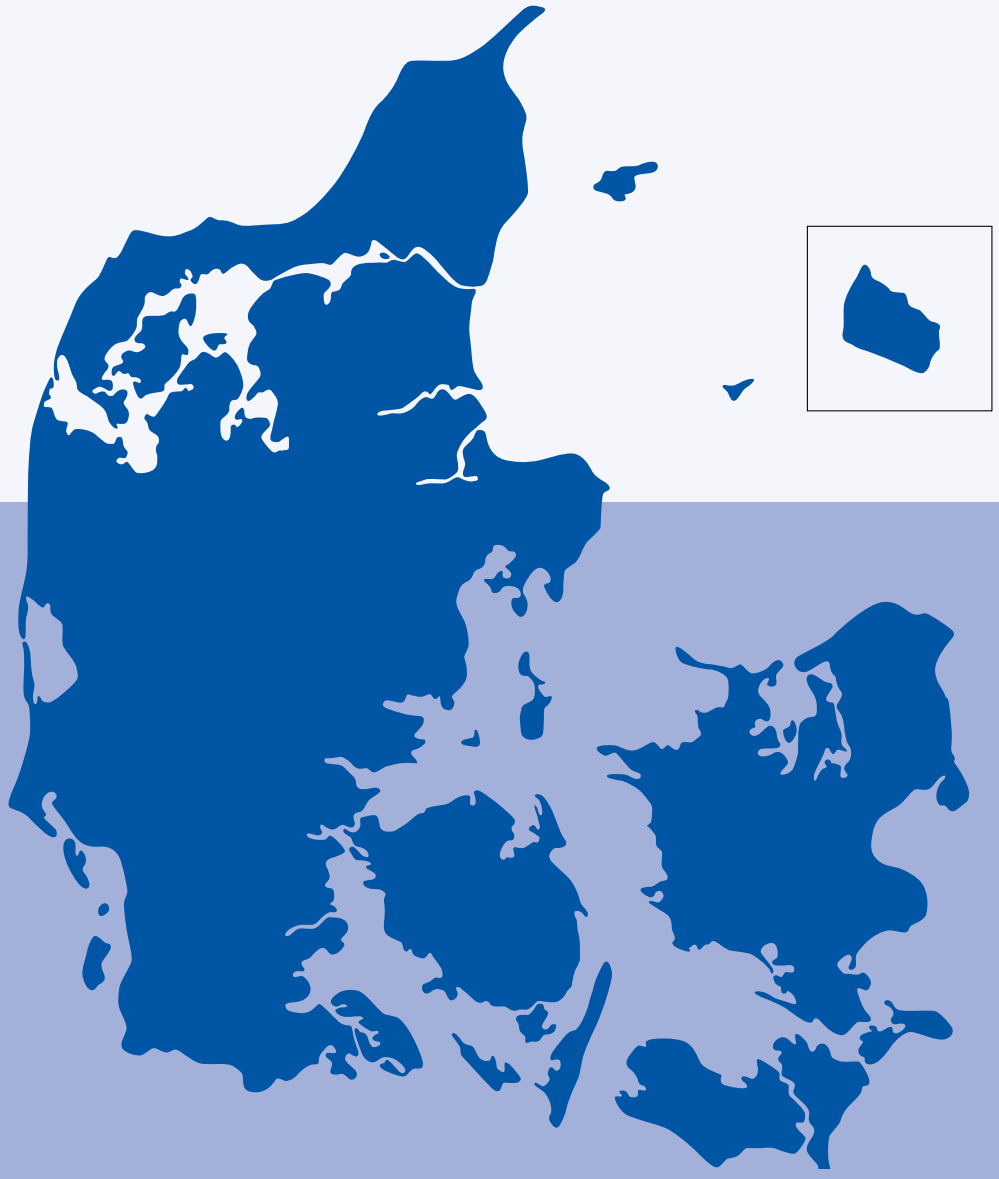
STATENS SERUM INSTITUT

DANMAP 2021

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



Statens Serum Institut
National Food Institute, Technical University of Denmark

DANMAP 2021

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

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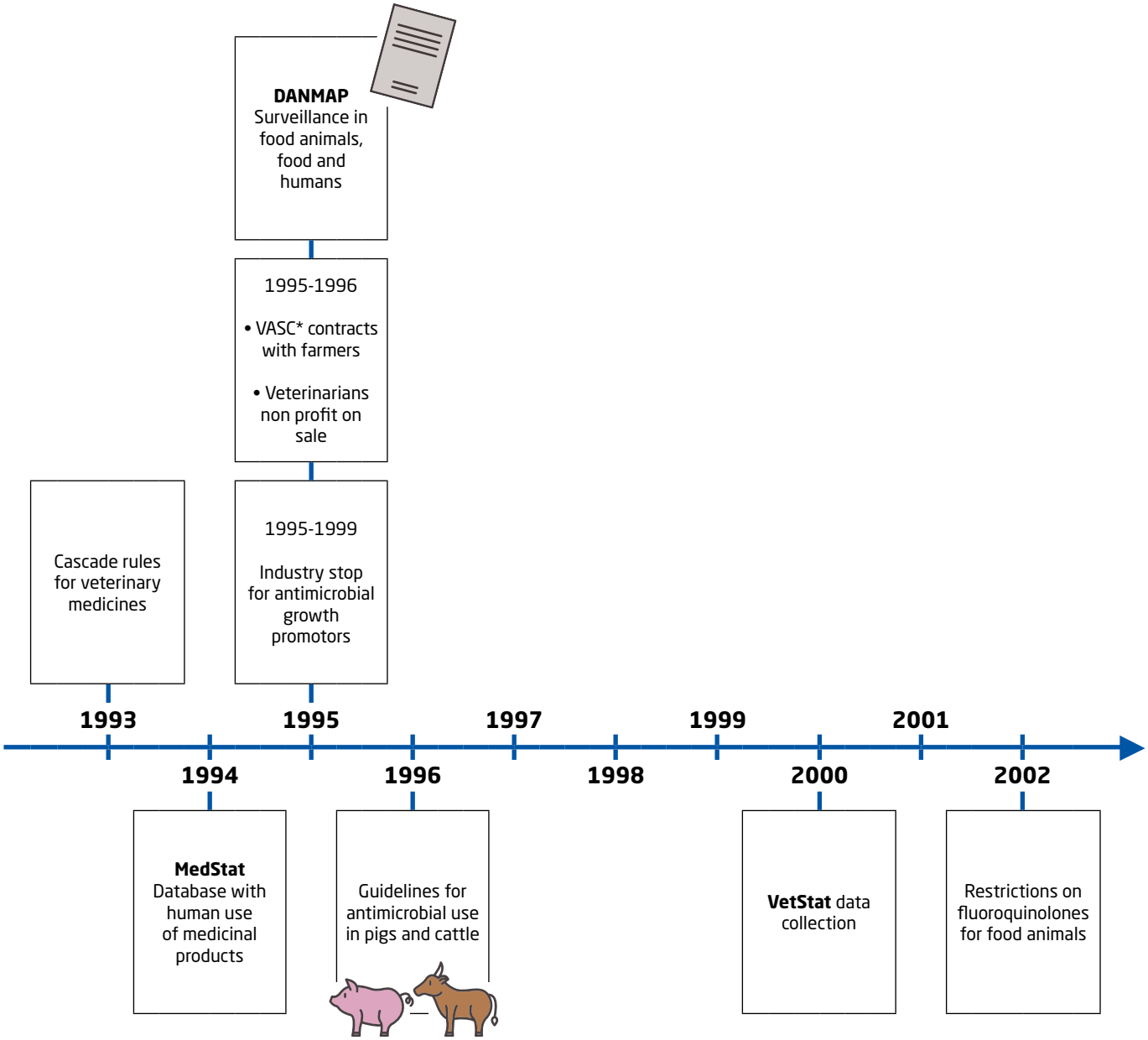
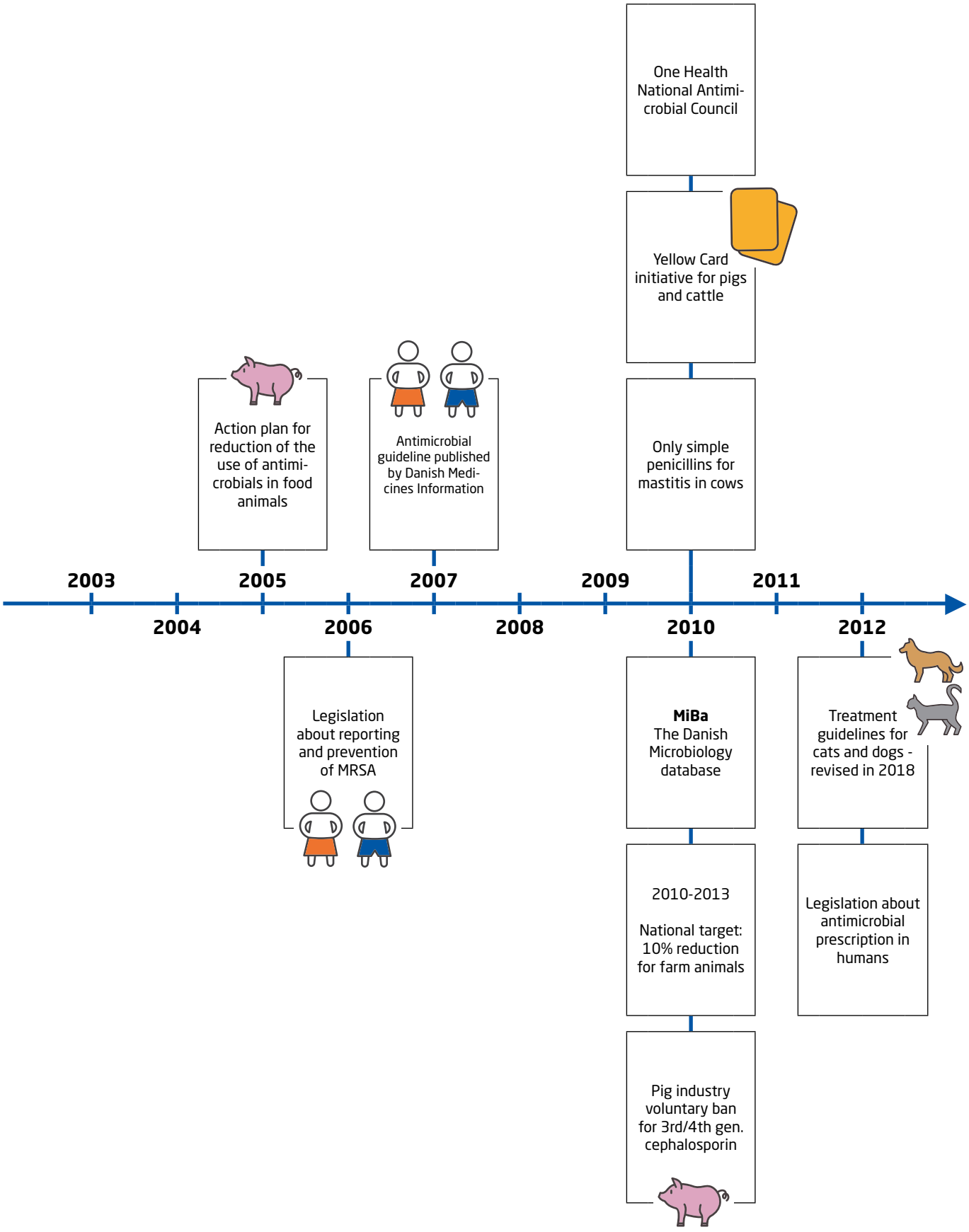


* Veterinary Advisory Service contracts


2003
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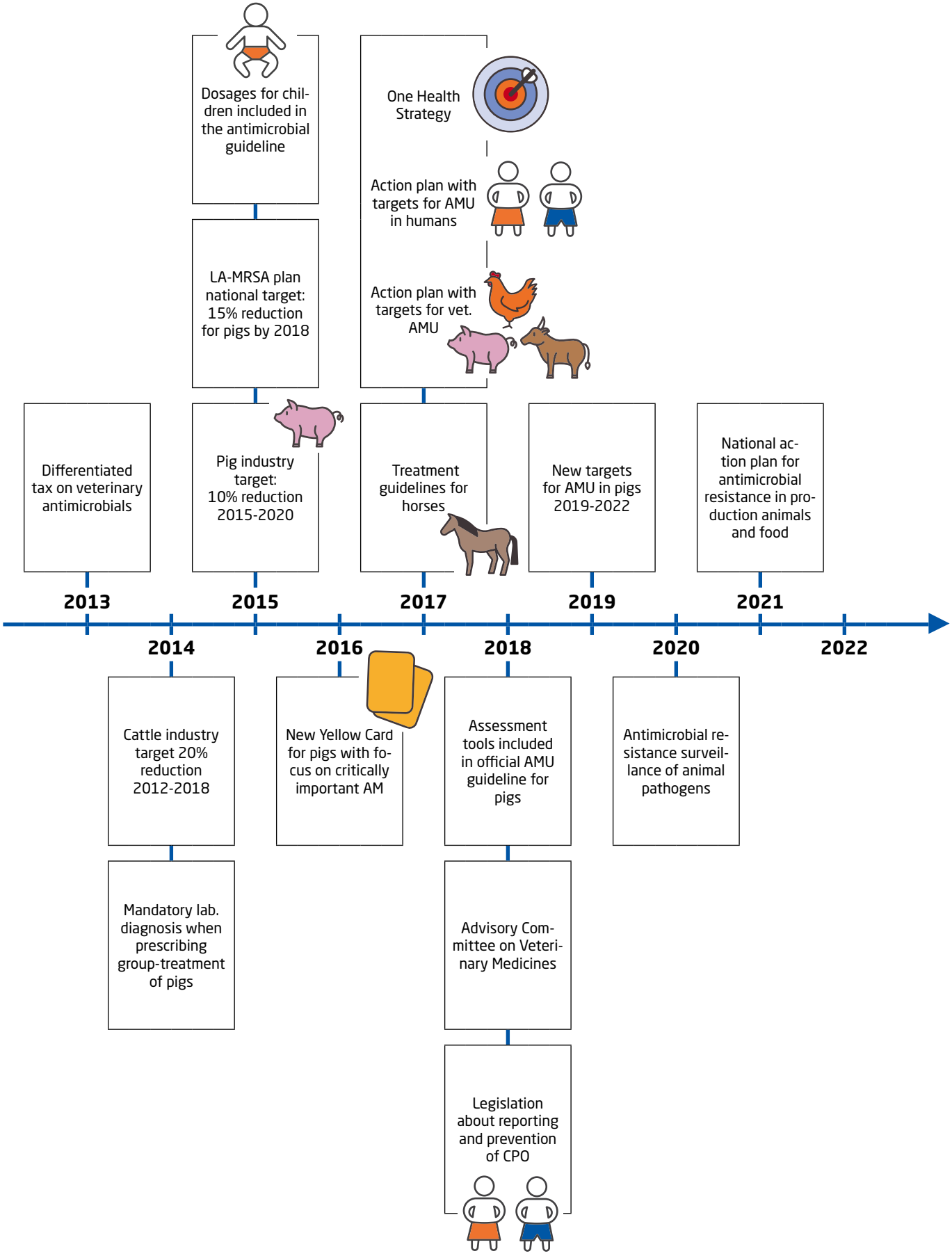
2012
Action plan for reduction of the use of antimicrobials in food animals
Antimicrobial guideline published by Danish Medicines Information
Legislation about reporting and prevention of MRSA
One Health National Antimicrobial Council
Yellow Card initiative for pigs and cattle
Only simple penicillins 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
Legislation about antimicrobial prescription in humans

continued ... Timeline of DANMAP



1. Editorial

The antimicrobial resistance (AMR) challenge requires a continued integrated One Health approach

Antibiotic resistance is acknowledged as one of the biggest threats to global health and food security, and the WHO has described AMR as a slow tsunami which threatens to undo a century of medical progress. Over the past decades, many resources have been dedicated to monitor, research, and manage the problem, but resistance to essential medicines to treat infections continues to spread.

DANMAP – The Danish Integrated Antimicrobial Resistance Monitoring and Research Programme – is internationally recognized for its quality, durability, and comprehensiveness. The term 'integration' in the title of the programme is therefore noteworthy. It highlights that the "architects" behind the programme were visionary and applied a One Health approach from the very beginning in the early nineties. Both the animal and the human sectors were involved in designing the programme and with research to fill the knowledge gaps as well as providing the data needed for action. Today, the need for a One Health approach to combat AMR is even more relevant, and perhaps even more challenging, than it was when the DANMAP programme was established. In the future, a much broader perspective of One Health will be relevant including the environment, the animal reservoirs, the food system and human cases in order to prevent antimicrobial resistance.

The results generated through the DANMAP programme have been used to develop measures to prevent increased occurrence of AMR through antimicrobial stewardship and the establishment of a national strategy and action plans to prevent AMR in animals and humans.

Solutions to big societal challenges call for cross-disciplinary actions. Our institutions, the National Food Institute at Technical University of Denmark (DTU), and Statens Serum Institute have worked with One Health for many years. To us, One Health means taking a task or subject beyond the established research groups or boundaries and addressing it across silos and sectors in a collaborative spirit and manner. The DANMAP programme is just one good example of such collaboration in Denmark.

Based on comprehensive AMR monitoring systems in northern and western European countries, it has become clear that exchange of AMR across sectors and populations does occur, e.g., livestock-associated MRSA, but varies for different pathogens and resistance genes. Still, the magnitude of the exchange of AMR between different reservoirs is less well understood and was, until recently, based on estimates and assumptions derived from the different monitoring systems in different sectors. It has therefore been difficult to accurately assess magnitude and impact of the spill-over of AMR from one population to another. New methodologies in diagnostics, but also using digital tools available for data analysis and data visualization, open new doors to understanding and describing AMR problems. In time, they will also make it easier to carry out risk assessments and to design mitigation programmes addressing AMR.

We are proud of the DANMAP programme. As can be seen in this annual report 2022, challenges in the prevention of AMR development and transmission still raise several scientific questions. DTU and SSI will continue to apply a One Health approach both in terms of collaborative research and risk assessments nationally and internationally as well as in developing digital open-source opportunities. That way, our two organisations and Denmark will continue contributing to the international prevention of AMR. We need to continue focusing on coordinated actions and ambitious plans leading to the conquer of the global AMR challenge.

The COVID-19 pandemic clearly demonstrated the consequences of the failure to invest in preparedness, and the impact it has on all sectors. However, the pandemic also demonstrated how quickly the global community can develop new vaccines and medicine by collaborating across countries and sectors in a true One Health spirit. Preventing the spread of antimicrobial resistance also calls for a global, multisectorial investment and effort.

Christine Nellemann, director of the National Food Institute, DTU
Henrik Ullum, CEO of Statens Serum Institut

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 and discussing the interpretation of the results
- The Danish Veterinary and Food Administration, for collecting and transmitting data on antimicrobial resistance in food and animal samples. Furthermore, we would like to thank the staff of the Food and Feed Safety Division for discussing the interpretation of the data
- The Danish Veterinary and Food Administration, for collecting and transmitting data on veterinary consumption of antimicrobial agents from VetStat, financing data collection and providing data for interpretation. Furthermore, we would like to thank the staff of the Animal Welfare and Veterinary Medicine Division for introducing the New Vetstat database and discussing interpretation of the data
- The Danish Veterinary Consortium (DK-VET) for their contributions on AMR in animals pathogens
- The Danish Agriculture and Food Council, Kjellerup, for their contributions on AMR in animal pathogens.
- The Department of Medication Statistics and Research Support at the Danish Health Data Authority (formerly the Danish Medicines Agency and SSI) for collecting and transmitting data on veterinary consumption of antimicrobial agents from the pharmacies
- The Danish Agriculture and Food Council for collaboration regarding the estimation of live biomass of production animals
- Colleagues at the National Food Institute, DTU, from the research group for Foodborne Pathogens and Epidemiology, and the research group for Genomic Epidemiology, for valuable discussions on many topics related to the report

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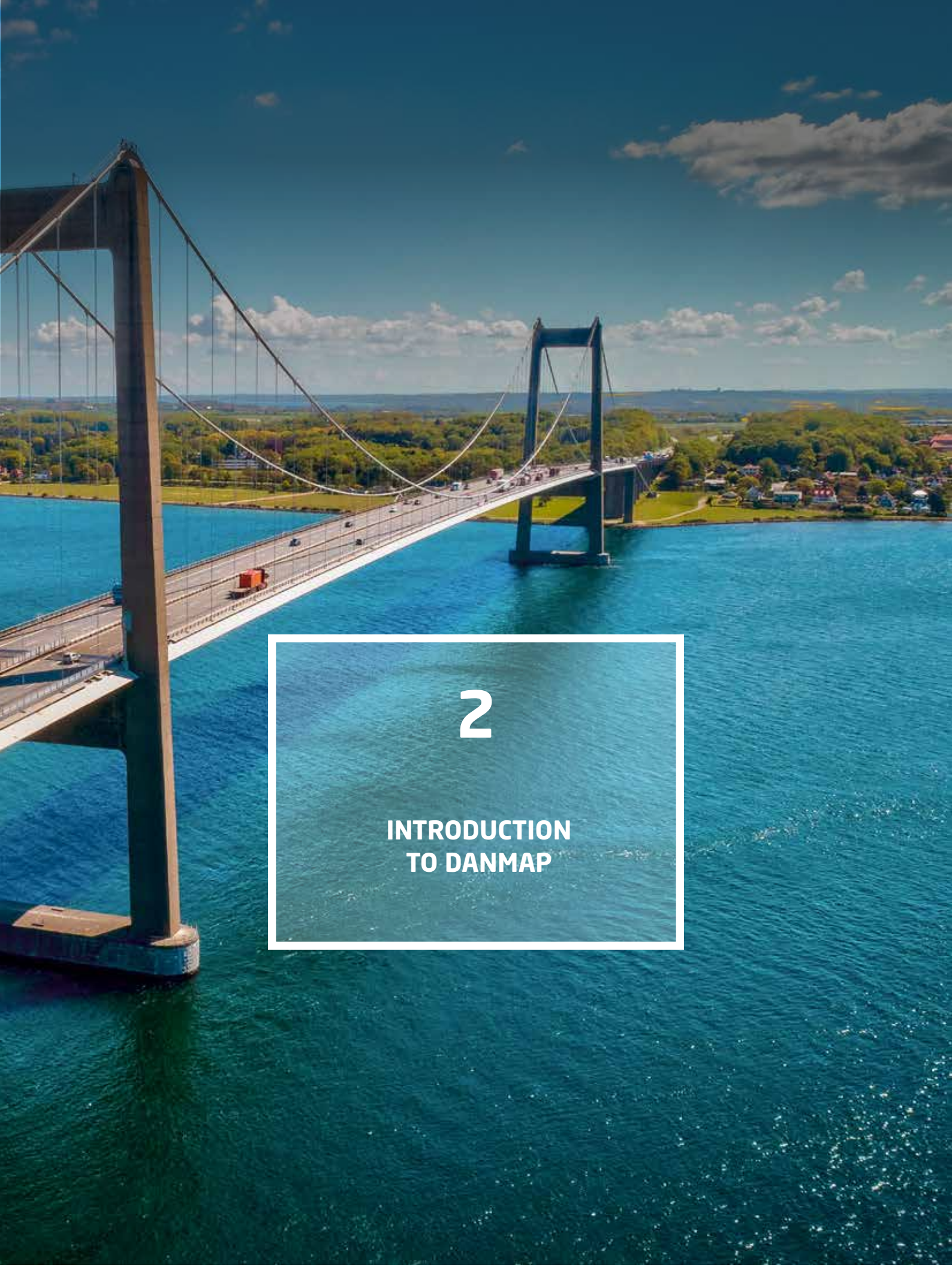
- The Departments of Clinical Microbiology and the DANRES group - Danish Study Group for Antimicrobial Resistance Surveillance - for providing data on resistance in bacteria from human clinical samples and discussing many of the topics included in the report
- The staff of the Neisseria and Streptococcus 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 animals and humans
- To carry out surveillance of the occurrence of antimicrobial resistance in bacteria isolated from food animals, food of animal origin (e.g. meat) and humans
- To identify areas for further research e.g. transmission or regarding possible associations between antimicrobial consumption and antimicrobial resistance
- To deliver data to veterinarians, medical doctors and other health professionals for the development of antibiotic guidelines for treatment
- To act as a knowledge base for authorities, academia and politicians when performing risk assessment and management, thus supporting decision making in the prevention and control of resistant bacterial infections

Since 2020, DANMAP also covers the COVID-19 pandemic's impact on antimicrobial use and resistance in Denmark.

This year, 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 organisation.

However, much of the design of the programme, including participation of the human laboratories and referral of strains is based on a voluntary principle.

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

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

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

Three categories of bacteria are always included in DANMAP:

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

The National Food Institute 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. 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 ensuring the registers, which the current surveillance system relies upon.

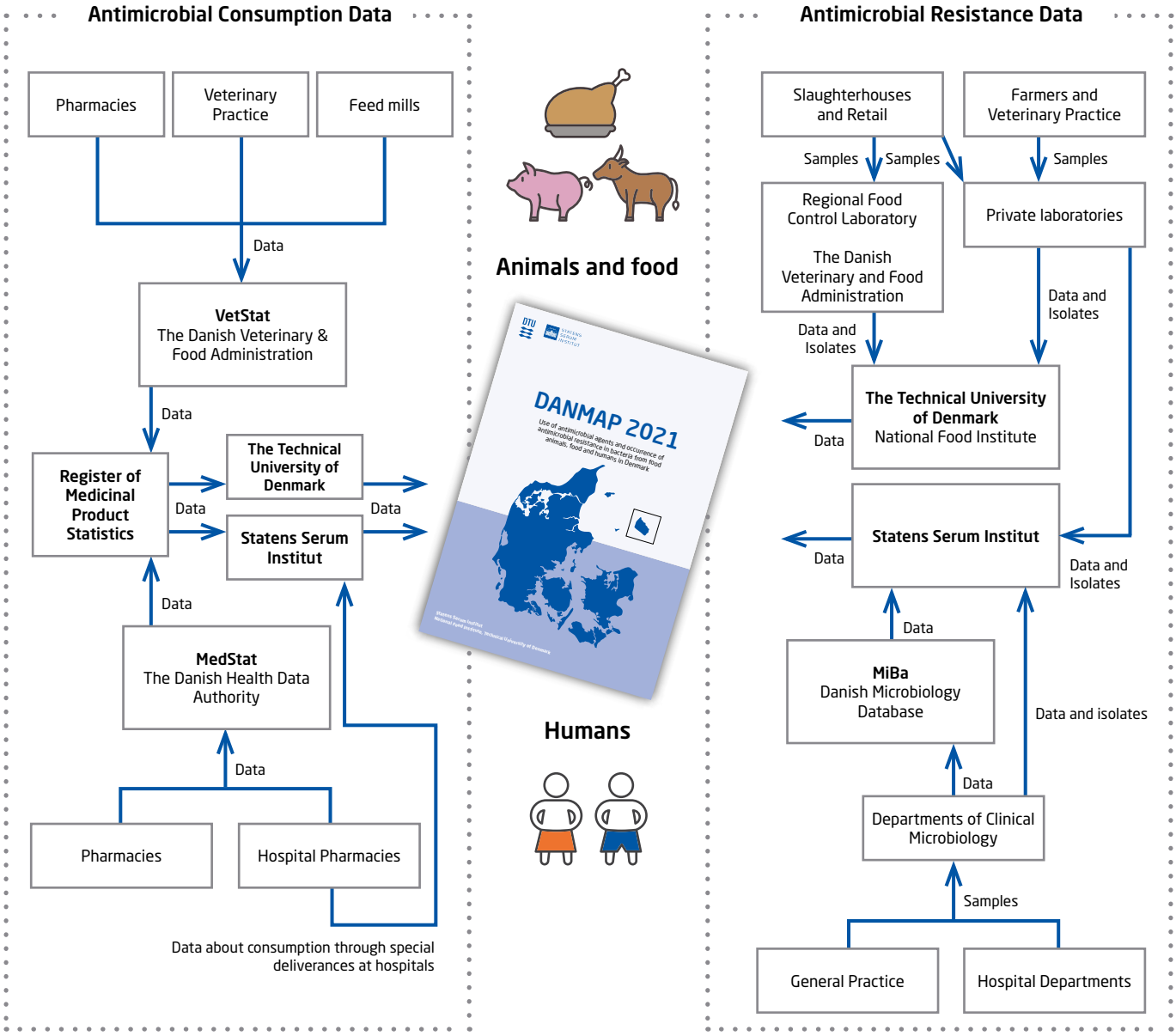
For further information on the development and history of DANMAP, please read chapter 2, "DANMAP – A 20 year perspective" in DANMAP 2015.

Organisation and data flow

Since 1995, a main purpose of DANMAP has been to monitor the entire chain from farm to fork to patient. The organisation and collection of DANMAP data and the interdisciplinary collaboration between sectors and organisations is presented in Figure 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 a 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 of the DANMAP collaboration regarding data and data flow DANMAP 2021



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). In 2021, WGS was extensively performed on selections of single isolates. These isolates were analysed for clonal relationship, as well as antimicrobial resistance genotypes (including ESBL, AmpC and CPO), and the presence of mobile elements such as plasmids. When specific clones carrying the same antimicrobial resistance genes are found in both food and human isolates, genomic data analysis such as core genome multilocus sequence typing (cgMLST) and single nucleotide polymorphism (SNP) calling, are used to examine possible transmission between the reservoirs. The choice of the methods in surveying different bacteria and infections is described in more detail in the different chapters and sections of the report.

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 2021

North Denmark Region

No. of inhabitants	590,439
No. of inhabitants/km^2	75
No. of inhabitants/GP	2,086

Central Denmark Region

No. of inhabitants	1,332,048
No. of inhabitants/km^2	102
No. of inhabitants/GP	1,668

Capital Region of Denmark

No. of inhabitants	1,855,084
No. of inhabitants/km^2	724
No. of inhabitants/GP	1,804

Region Zealand

No. of inhabitants	838,840
No. of inhabitants/km^2	116
No. of inhabitants/GP	1,969

Region of Southern Denmark

No. of inhabitants	1,223,634
No. of inhabitants/km^2	100
No. of inhabitants/GP	1,579

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 26 years, the human population in Denmark has increased from approximately 5.2 million inhabitants in 1995 to 5.8 million in 2021 [www.dst.dk]. Simultaneously, the average age has increased gradually. In 2021, the national average age was 42 years. The population and the respective regional distribution, in 2021, is presented in Figure 2.2, while regional differences and changes in age are presented in Figure 2.3.

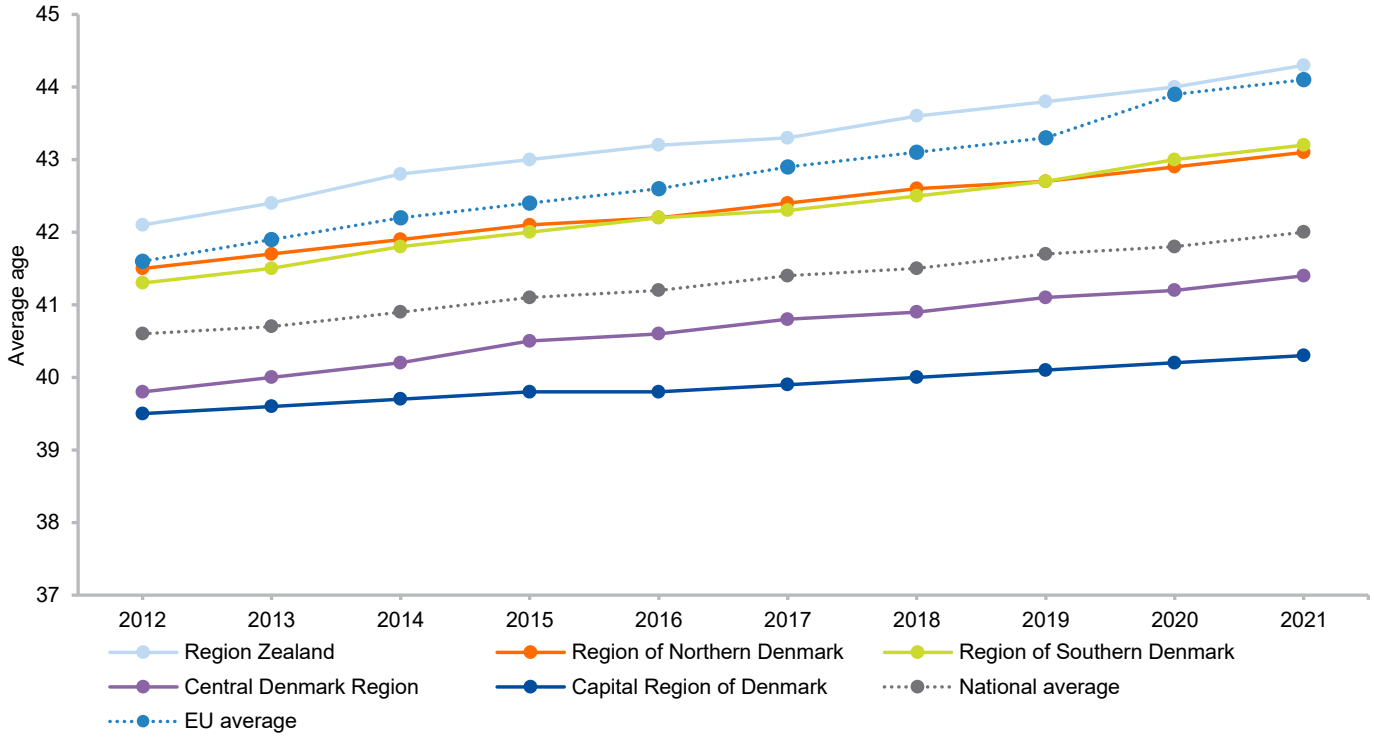
In Denmark, microbiological analyses are carried out by ten hospital departments of clinical microbiology (DCMs) situated at the main regional hospitals, Figure 2.2. The analyses performed cover all samples from public hospitals and most samples from general practitioners (GPs). In addition, some GPs perform culturing of urinary samples from their patients. In the Capital Region of Denmark one private laboratory also performs additional analyses for the GPs.

The activity in general practice during 2020-2021 differed from 2019. Figure 2.4 shows the number of consultations in general practice per thousand inhabitants from 2012-2021. The number of consultations per 1,000 inhabitants was 8.5% higher in 2020 compared to 2019.

Data on regional and national health care activity at hospitals in 2012 and 2021 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 2021, the number of admissions at Danish somatic hospitals was registered to be 700,710 and the number of bed-days was registered to be 3,008,588. From 2012-2021, the number of bed-days decreased by 20%, the number of admissions decreased by 9% whereas the Danish population grew by 5%.

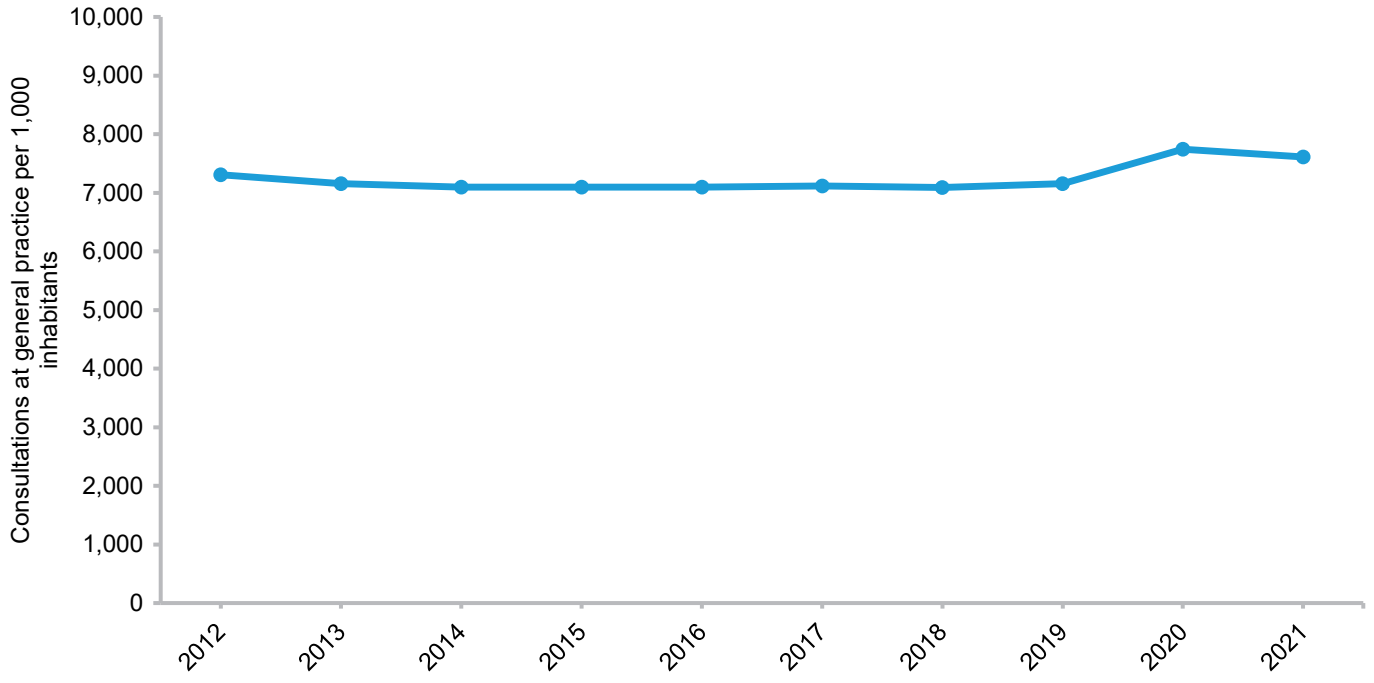
Chapter 5 presents more details about how COVID-19 affected the activity in the Danish health care system.

Figure 2.3 Changes in average age, Denmark and EU, 2012-2021 DANMAP 2021



Data source: Statistics Denmark and Eurostat

Figure 2.4 Number of consultations per 1,000 inhabitants in general practice, Denmark, 2012-2021 DANMAP 2021



Data source: The National Health Insurance Service Registry and Register of Health Insurance Service Providers

Table 2.1 Activity at Danish hospitals, 2012 and 2021 DANMAP 2021

Region	Number of bed-days in somatic hospitals		Number of admission to somatic hospitals		Population	
	2012	2021	2012	2021	2012	2021
Capital Region of Denmark	1,319,091	989,881	256,018	236,274	1,714,589	1,855,084
Zealand Region	530,253	450,163	105,569	102,675	817,907	838,840
Region of Southern Denmark	763,342	608,401	163,469	144,536	1,201,342	1,223,634
Central Denmark Region	765,332	630,091	170,625	145,795	1,266,682	1,332,048
North Denmark Region	399,655	330,053	77,504	71,430	579,996	590,439
Denmark	3,777,674	3,008,588	773,185	700,710	5,580,516	5,840,045

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

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

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

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

Table 2.2 Production (1,000 heads) of food animals, Denmark DANMAP 2021

	Pigs		Cattle		Poultry	
Year	Total	Exported[a]	Slaughter cattle	Dairy cows	Broilers	Turkeys[b]
2012	29047	9562	539	580	111080	1103
2013	28996	9864	551	574	117315	692
2014	30002	11120	556	563	115497	595
2015	30874	12133	513	561	114238	598
2016	31660	13280	540	571	120685	834
2017	31662	14173	509	570	117602	601
2018	32571	14449	533	575	122268	642
2019	31694	14897	518	567	123976	661
2020	32025	14736	500	567	120508	684
2021	32811	14258	506	564	118431	467

Source: Statistics Denmark (www.dst.dk). Export data for poultry from Statistics Denmark (personal communication).
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

Table 2.3 Production (mill kg) of meat, milk and fish , Denmark DANMAP 2021

						Farmed fish[b]	
Year	Pork	Beef	Broiler meat[a]	Turkey meat	Milk	Land based	Marine net ponds
2012	1902	138	168	12	4928	44	14
2013	1896	140	177	8	5025	48	15
2014	1924	143	174	9	5113	47	14
2015	1954	135	172	9	5278	52	16
2016	1943	142	182	10	5376	49	12
2017	1896	135	178	7	5478	51	14
2018	1967	141	185	10	5615	53	14
2019	1870	137	187	8	5615	53	14
2020	1955	132	195	8	5666	54	14
2021	2082	134	144	6	5644	-	-

Source: Statistics Denmark (www.dst.dk). Export data for poultry and average weight after slaughter from Statistics Denmark (personal communication)
a) In 2021, a final slaughtered weight of 1.21 kg per broiler produced and 12.02 kg per turkey produced was estimated
b) The numbers for 2021 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 2021

ATC / ATCvet codes[a]	Therapeutic group	Antimicrobial agents within the therapeutic groups	
		Animals	Humans
J01AA / QJ01AA, QJ51AA	Tetracyclines	Chlortetracycline, doxycycline, oxytetracycline	Doxycycline, lymecycline, tetracycline, tigecycline
QJ01BA	Amphenicols	Florfenicol	
J01CA / QJ01CA	Penicillins with extended spectrum	Ampicillin, amoxicillin	Ampicillin, pivampicillin, amoxicillin, pivmecillinam, mecillinam
J01CE / QJ01CE	Beta-lactamase sensitive penicillins	Benzylpenicillin, phenoxymethylpenicillin, procaine penicillin, penethamate hydroiodide	Benzylpenicillin, phenoxymethylpenicillin
J01CF / QJ51CF	Beta-lactamase resistant penicillins	Cloxacillin, nafcillin	Dicloxacillin, 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	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		Trimethoprim
J01EB / QJ01EQ	Short-acting sulfonamides	Sulfadimidine	Sulfamethizole
J01EE / QJ01EW	Comb.of sulfonamides and trimethoprim, incl. derivatives	Sulfadiazine/trimethoprim, sulfadoxine/trimethoprim, sulfamethoxasol/trimethoprim	Sulfamethoxazole/trimethoprim
J01FA / QJ01FA	Macrolides	Spiramycin, tylosin, tilmicosin, tylvalosintartrat, tulathromycin, gamithromycin, tildiprocin	Erythromycine, roxithromycine, clarithromycine, azithromycine
J01FF / QJ01FF	Lincosamides	Clindamycin, lincomycin	Clindamycin
QJ01XX [b]	Streptogramins	(Virginiamycin)	
J01GB / QJ01RA, QA07AA	Aminoglycosides	Streptomycin, dihydrostreptomycin, gentamicin, neomycin, apramycin	Tobramycin, gentamicin, amikacin
J01MA / QJ01MA	Fluoroquinolones	Enrofloxacin, marbofloxacin, difloxacin, ibafloxacin, pradofloxacin	Ciprofloxacin, levofloxacin, moxifloxacin
QJ01MB	Other quinolones	Oxolinic acid	
QJ01MQ [b]	Quinoxalines	(Carbadox, olaquindox)	
J01XA, A07AA / Not in ATCvet [b,c]	Glycopeptides	(Avoparcin)	Vancomycin, teicoplanin
J01XB / QA07AA [b]	Polypeptides (incl. polymyxins)	Colistin, bacitracin	Colistin
J01XC	Steroid antibacterials		Fusidic acid
J01XD, P01AB [c]	Imidazole derivatives		Metronidazole
J01XE	Nitrofurane derivatives		Nitrofurantoin
J01XX / QJ01FF	Other antibacterials	Spectinomycin	Methenamine, linezolid, daptomycin, 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 – NEW PERSPECTIVES

3. One Health AMR – new perspectives

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.

DANMAP has always been considered an integrated research and surveillance programme, but with integration taking place at the decision-making and implementation level rather than at the level of data management. Hence, data are stored in separate databases by the animal- and human sectors, although interpretation of results is done in cooperation. Moreover, integration happens when deciding which common indicators to monitor (i.e. indicator *E. coli* and enterococci), and when discussing the findings and their use as a basis for recommendations and treatment guidelines among different participants of the programme. Furthermore, development and definition of strategies and action plans to reduce AMR on both sides are also done in collaboration in the working- and steering-group, where actors and stakeholders, respectively, from the participating institutions are represented.

However, there has always been the wish to get a more in depth understanding of the potential relationship between the veterinary, food-producing and human sector, concerning antimicrobial usage (AMU) and development of antimicrobial resistance (AMR). To be able to foresee if changes in one sector will have a potentially significant impact on the other sector, it requires knowledge of the possible 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 sector.

At the EU level, an attempt to perform cross-analysis has been made since 2015 in the JIACRA reports [ECDC/EFSA/EMA 2015. EFSA Journal 13(1):4006], despite the additional challenge of jointly analysing data collected in different countries. At the national level, even in a country such as Denmark with a long-established detailed monitoring system based on stable data delivery of high quality, 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.

This chapter is a first attempt to cross-analyse antimicrobial resistance data from monitoring in livestock animals and humans in Denmark. First, it compares the phenotypic resistance profile of indicator *E. coli* strains recovered from the caecum of healthy animals at slaughter with the profile of *E. coli* strains isolated from the urine of UTI patients at primary health care. These two datasets represent the closest representation of *E. coli* occurrence in the animal and human populations at the community level. Ideally, a dataset based on samples from healthy humans should be used for these analyses, presenting the actual pool of bacteria and resistance genes circulating in the human population. However isolates of such cases are rarely available. Next, it maps the frequency of MLST sequence types and resistance genes of ESBL *E. coli* isolates recovered from livestock animals and meat and from humans with bloodstream infections. Such analysis is a visual demonstration of possible relationships between isolates of both origins, and can help identifying strains that may require more targeted genomic analyses to further investigate on possible transmission between human and animal reservoirs and vice-versa.

An integrated surveillance system is constituted by different levels and elements that regularly need to be evaluated (Aenishaenslin et al. 2021. *Front. Vet. Sci.* 8:611931). Textbox 3.1 in this chapter shows the first attempt to evaluate the level of One Health in DANMAP, by assessing to what extent AMR and AMU surveillance are integrated and facilitate the optimal impact of the program on the animal and human sectors.

3.2 Phenotypic resistance in indicator and clinical *E. coli* from livestock animals and clinical *E. coli* from UTI patients in primary health care

A zoonotic link between urinary tract infection (UTI) *E. coli* and *E. coli* from production animals and meat has been previously shown in Denmark by detecting clonal relatedness by PFGE between UTI *E. coli* from human patients and *E. coli* from broilers, pork meat and broiler meat [Jakobsen et al. 2012. *Eur J Clin Microbiol Infect Dis.* 31(6):1121-1129]. Another study [Jakobsen et al. 2011. *J Med Microbiol.* 60(10):1502-1511], compared a strain collection of *E. coli* phylogroup B2 (phylogenetic group of extraintestinal pathogenic *E. coli* (ExPEC) most often causing UTI) recovered from UTI patients, community-dwelling humans, broiler meat, pork, Danish broilers, and Danish pigs using DNA microarray analysis. That study showed a comparable frequency of virulence genes, but a varying frequency of resistance genes among all isolate origins. A recent study in Poland also found similar genetic background for virulence factors, and the same phylogenetic groups among avian and human ExPEC, including UTI strains [Sarowska et al. 2019. *Gut Pathog.* Feb 21;11:10], while in the USA, a study comparing *E. coli* isolated from fecal samples of healthy humans with those from UTI patients by MLST showed that the primary reservoir of UTI strains may reside outside of the human intestine [Matsui et al. 2020. *Microbiologyopen* 9(6):1225-1233].

Despite numerous findings suggesting a zoonotic link, the challenge of establishing the foodborne origin of a UTI *E. coli* remains, due to the lag between colonization of a human patient with a foodborne *E. coli* strain and the development of a UTI infection. Depending on the length of that lag, the antimicrobial resistance (AMR) profile of a UTI strain acquired by zoonotic transmission may resemble the AMR profile found at the primary animal reservoir or deviate from it. This study compares the AMR profiles obtained by antimicrobial susceptibility testing of UTI *E. coli* strains recovered from humans and strains recovered from animals, in Denmark.

We gathered a dataset containing a total of 1,370 *E. coli* isolates collected in Denmark in 2021, which are among the data reported in chapters 6, 7 and 8 of the present DANMAP report. Data included 399 isolates recovered from animals and 971 isolates recovered from humans. Isolates recovered from animals included indicator *E. coli* from caecal content collected at slaughter from healthy- broilers (34), calves <1 year of age (109) and fattening pigs (65), as well as isolates of hemolytic- (103) and non-hemolytic (88) *E. coli* recovered from clinical samples (of various kinds) collected from sick pigs. The 971 clinical isolates from humans were recovered from UTI patients at primary health care, and represent a random sub-sample of the total number of *E. coli* isolates recovered from such patients in 2021 (n= 99,077). Sub-sampling was performed in order to achieve a better balance in terms of dataset composition regarding the origin of the isolates.

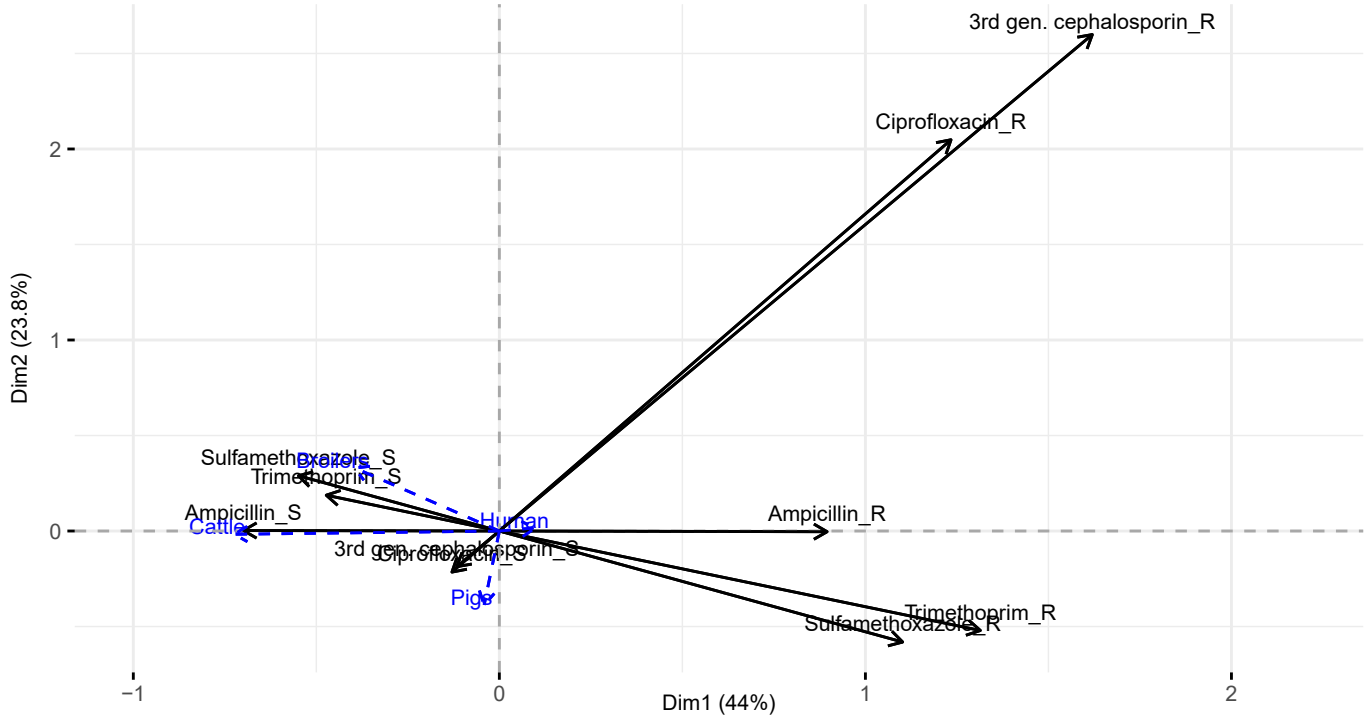
For each isolate, the data contained binary results (sensitive/resistant) of antimicrobial susceptibility testing for a selection of antibiotics that overlapped between the antibiotic panels tested in both sectors. This selection included ampicillin, ciprofloxacin, sulfamethoxazole, trimethoprim and a 3rd generation cephalosporin (cefotaxime and cefotaxime/cefpodoxim for isolates of animal and human origin, respectively). Additionally, a nominal variable indicated the origin of the isolate.

The analysis was performed in two independent steps. First, by including all isolates from humans and all indicator *E. coli* from animals. Next, by including all isolates recovered from humans and all isolates recovered from pigs, including indicator and clinical isolates.

A multiple correspondence analysis (MCA) was performed in order to assess the clustering patterns of resistance and sensitivity to different antibiotics and of different isolate origins. Analyses were performed using R statistical software version 3.6.1 [R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org]. MCA was performed with the R package *FactoMineR* version 2.4, and MCA loading plots produced with the R package *factoextra* version 1.0.7. Loading plots show how strongly each individual antibiotic resistance or sensitivity influences a principal dimension (*Dim1 and Dim2*), and the isolate origin was set as a supplementary variable to illustrate the clustering of observations of different origins.

Figure 3.1 Multiple Correspondence Analysis with indicator *E. coli* recovered from healthy animals at slaughter and *E. coli* recovered from UTI human patients

DANMAP 2021



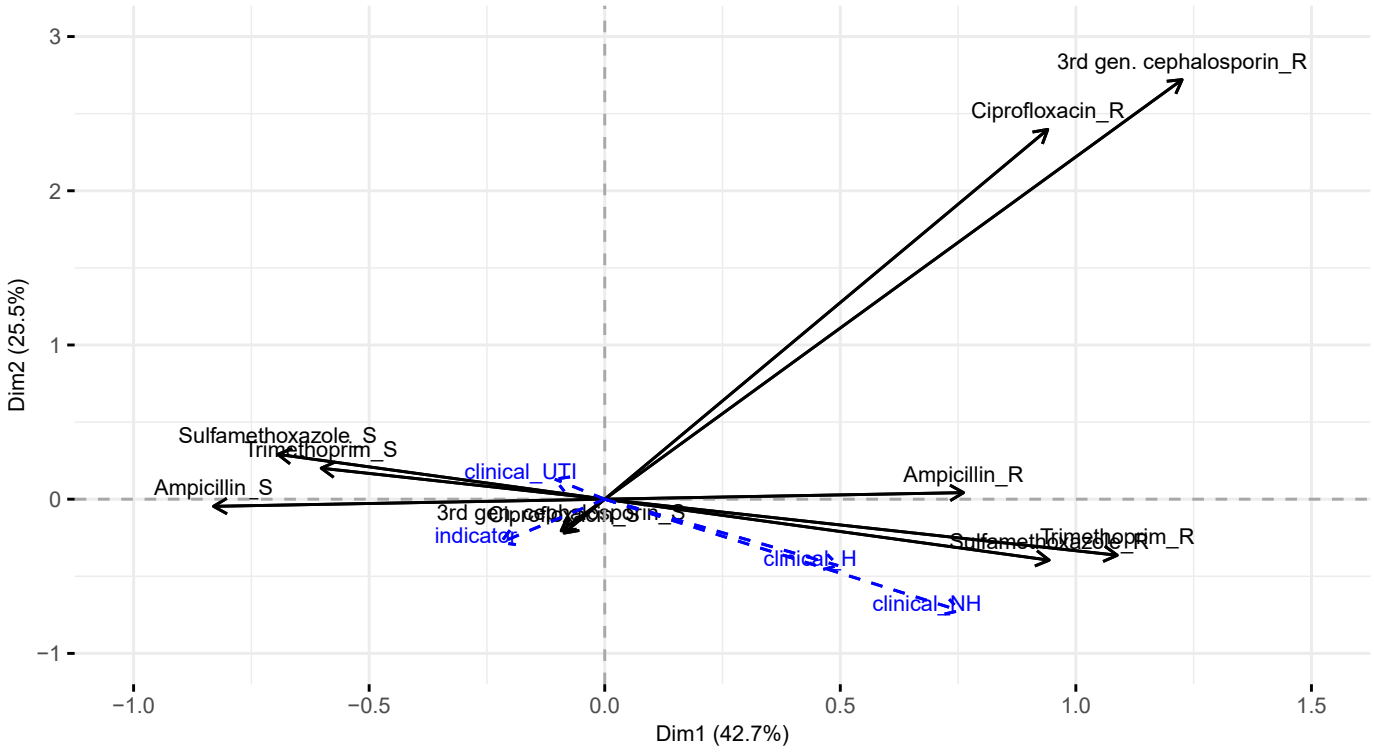
The loading plot shows the coordinates of the explanatory variables (phenotypic resistance profile to five different antibiotics) and the clustering of samples of different origins (Human, Broilers, Cattle and Pigs) in the ordination space of the two first principal dimensions (Dim1 and Dim2) These two dimensions jointly explain a total of 67.8% of variation in the data.

The *dimdesc* function from package *FactoMineR* was used to statistically assess the relationship of *DIM1 and DIM2* with the different variables.The first MCA analysis (Figure 3.1) showed a clear separation along *Dim1* between resistance and sensitivity to all antimicrobials, and some degree of separation between sample origins. All antibiotics showed a significant relation (R^2) with *Dim1*, with resistance (*R*) and sensitivity (*S*) clustering on the positive and negative coordinate space, respectively. Sample origin showed a low but significant R^2 in relation to *Dim1* (R^2 = 0.06), including all classes except *Pigs*. Observations of indicator *E. coli* from cattle and broilers located on the negative coordinate space, while UTI isolates from humans clustered on the positive space. This shows that UTI *E. coli* and indicator *E. coli* from broilers and cattle have dissimilar resistance profiles, with the first presenting overall higher levels of resistance and the latter overall higher levels of sensitivity. *Dim2* was significantly associated with all antibiotics except ampicillin, as well as to the sample origin *Pigs* (R2 = 0.01). Antibiotic variables separated along *Dim2*, by clustering resistance to 3rd generation cephalosporins and ciprofloxacin on the positive coordinate space and resistance to trimethoprim and sulfamethoxazole, and observations from pigs, on the negative space. Trimethoprim and sulfamethoxazole are antibiotics commonly used in the treatment of pigs, thus it is not surprising that resistance to these antibiotics are associated with isolates of such origin. However, in humans the usage of both trimethoprim and sulfamethoxazole has decreased markedly over the last decade and for both drugs the consumption in individuals under the age of 50 is nearing zero. Both antibiotics are used in the treatment of UTI, sulfamethoxazole formerly known as a drug to be used primarily by young women for non-recurring uncomplicated UTI, trimethoprim to be used primarily for treatment of uncomplicated UTI in the elderly above 65 years. The low levels of resistance towards trimethoprim and sulfamethoxazole in clinical human samples in the plot clearly mirror these low trends in usage among humans in Denmark.

Of no surprise is the resistance towards ciprofloxacin and 3rd generation cephalosporins, which primarily presents itself associated with human samples. Although usage of ciprofloxacin is restricted and 3rd generation cephalosporins were never introduced into the human primary sector, the human health sector is still the main area where usage of these two drugs takes place.

Similarly to the first analysis, the second MCA (Figure 3.2) also showed a clear separation along *Dim1* between resistance and sensitivity to all antimicrobials, with resistance (*R*) and sensitivity (*S*) clustering on the positive and negative coordinate space, respectively. Furthermore, this MCA separated along *Dim1* clinical isolates from pigs (hemolytic and non-hemolytic strains) from UTI isolates from humans (significant R^2 = 0.07 for sample origin, excluding the class *indicator E. coli* from pigs), with the first clustering towards resistance and the

Figure 3.2 Multiple Correspondence Analysis with indicator *E. coli* recovered from healthy pigs at slaughter, clinical haemolytic and non-haemolytic *E. coli* recovered from sick pigs and *E. coli* recovered from UTI human patients DANMAP 2021



The loading plot shows the coordinates of the explanatory variables (phenotypic resistance profile to five different antibiotics) and the clustering of samples of different origins (clinical_UTI, indicator, clinical_H and clinical_NH) in the ordination space of the two first principal dimensions (Dim1 and Dim2). These two dimensions jointly explain a total of 68.2% of variation in the data.

latter towards sensitivity. This suggests that clinical *E. coli* isolates recovered from pigs, which include isolates from several types of infection, have overall higher levels of resistance than UTI *E. coli* from humans. The second principal dimension showed again a significant association to all antibiotics except ampicillin, with clustering of resistance to 3rd generation cephalosporins and ciprofloxacin on the positive coordinate space and resistance to trimethoprim and sulfamethoxazole on the negative space. Additionally, *Dim2* showed a significant R^2 for sample origin (R^2 = 0.07), especially for the classes *clinical_UTI* (R^2= 0.23) and *clinical_NH* (non-haemolytic clinical isolates from pigs) (R^2 = -0.20). This result suggests a higher occurrence of resistance to the critically important antibiotics among human UTI isolates, compared to a higher occurrence of resistance to sulfamethoxazole and trimethoprim among non-haemolytic clinical isolates from pigs.

While several studies, using different microbiological methods, have found indication of a possible zoonotic transmission of *E. coli* from livestock animals to UTI patients, the resistance profiles of *E. coli* strains recovered from the different populations have shown less overlap. Using antimicrobial susceptibility results, the present findings did not indicate a zoonotic link and are in agreement with previous studies by suggesting that antimicrobial resistance of *E. coli* strains at the animal and human UTI patient levels are most likely led by antimicrobial consumption within each population. Further studies are needed in Denmark in order to investigate the association of antimicrobial consumption and phenotypic resistance within and across populations.

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3.3 Genotypic comparison of ESBL *E. coli* from livestock animals, meat and human bloodstream infections

There has been decreasing numbers of extended spectrum beta-lactamase *E. coli* (ESBL Ec) bloodstream infections (BSIs) in humans in Denmark since 2019 (see section 8.3.1), and a significant reduction in ESBL Ec has been observed in Danish broilers and broiler meat. Mughini-Gras, et al. [Mughini-Gras, et al. 2019. *Lancet Planet Health* 3(8):e357-e369] found that the primary source of community acquired ESBL Ec was through human-to-human transmission, although transmission to and from non-human sources was also evident. Other studies [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 transmissions, underlining the importance of surveying the possibility of zoonotic transfer of resistance from animals to humans.

The objective was to compare the multilocus sequence types (MLST) and ESBL-genes between humans, production animals and meat to identify any major overlaps between sectors – indicating a zoonotic link or transmission of resistance genes.

We gathered a dataset of 1,457 ESBL isolates from humans and animals from 2018 through 2021. The 884 human isolates were clinical isolates from infections sent voluntarily to the SSI reference laboratory for antibiotic resistance from the departments of clinical microbiology. The animal isolates (broiler meat: 196, broilers: 82, cattle: 51, beef: 40, pigs: 156 and pork: 48) stem from the mandatory screening programme from healthy animals and meat products. See chapter 7 and 8.3.1 for more information.

Each isolate has been sequenced as part of the surveillance and the multilocus sequence type and ESBL-gene were extracted from the sequences. All data handling was done in Python 3.8.10 and the plotly package version 5.9.0 was used to make the Sankey diagram. For the purposes of this report, only flows of five or more isolates are shown on the Sankey diagram.

Limited overlap was found in both STs and ESBL genes from humans vs. animals and food (Figure 3.3). In accordance with formerly observed overlaps (see DANMAP 2015, Textbox 7.3). ST23 was found in both humans and pigs. Likewise, ST38 was found in both humans and broiler meat. For both sequence types the ESBL genes detected differed between human and animal strains. The pig isolates from ST23 harboured C-42T mutations, whereas the human isolates harboured CTX-M-14. The broiler meat isolates from ST38 were of the CMY-2-kind, whereas the human isolates carried mainly CTX-M-14 and CTX-M-14b. Only CMY-2 and CTX-M-1 were found in both humans and food production animals or food, but not in high abundance. ST131 was responsible for roughly 50% of the ESBL-bacteraemia cases in humans, usually accompanied by a CTX-M-15 gene. ST2040 was found exclusively in broilers or broiler meat and only carried the CMY-2-gene. In general, sequence types seem to associate with species, whereas there is more variation with combinations of sequence types and ESBL-genes.

In the DANMAP 2018 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 a clonal relationship. A One Health compartmental analysis over a three-year period from Réunion [Miltgen, et al. 2022. *J Antimicrob Chemother* 77(5):1254-1262] investigated transmission of ESBL-Ec from humans, animals and environment to human colonization and infection. The study found little evidence of transmission and suggested that focus should be primarily on preventing human-to-human transmission. Conclusively, it remains challenging to find clear evidence of zoonotic transmission of ESBL Ec, even though the animal and food sectors are

potential reservoirs and possibly have a role in the introduction of ESBL Ec into the human sector, as detailed by Mughini-Gras, et al., 2019. Thus, it remains important to monitor the occurrence of ESBL Ec in humans and animals, as part of an integrated antimicrobial resistance surveillance program.

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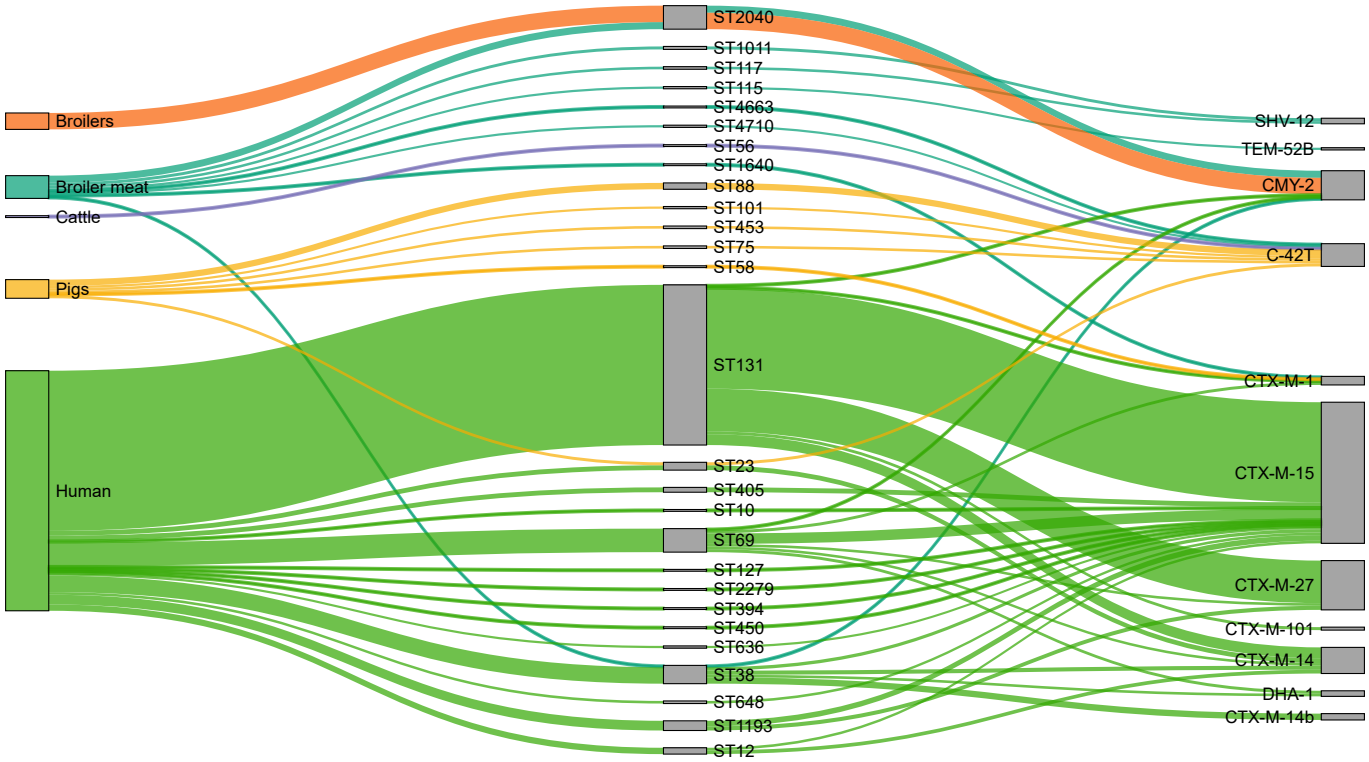
Figure 3.3 A Sankey diagram comprised of 803 ESBL-isolates from humans, animals and food showing the relationship between the isolates' source, sequence type and ESBL-gene. The flows between nodes are coded according to source. Only flows of five or more isolates are shown to limit clutter. An interactive version of this figure without filtering can be found on www.danmap.org

DANMAP 2021



Concluding remarks

This new One Health chapter presents recent work DANMAP has done towards integrated analyses of surveillance data for antimicrobial resistance (AMR) from the human and animal/food sectors.

Two studies analyse phenotypic and genotypic AMR resistance profiles using national data for *E. coli* isolates from humans and livestock animals/meat, respectively. The comparison of phenotypic resistance profiles of indicator *E. coli* from livestock animals with those of urine isolates from UTI patients in primary health care suggests that resistance patterns found in *E. coli* isolated from human urine are associated with patients' AMU rather than with AMR patterns found in isolates from livestock animals, which again are driven by AMU in livestock production. Genomic analysis of ESBL *E. coli* isolates from livestock animals, meat and human bloodstream infections also suggests limited overlap between the sources with regards to sequence type and ESBL-genes. These findings seem to indicate that efforts to prevent transmission of AMR *E. coli* infections between sectors are currently successful in Denmark but warrant continued monitoring. Extension of these analyses to other pathogens should be explored.

As a recent evaluation of DANMAP (Textbox 3.1) also shows, the One Health approach has been a pillar of AMR and AMU surveillance in Denmark since the start of the programme and is based on high quality data and strong stakeholder engagement. In order to further strengthen preparedness and detect AMR outbreaks and transmission across sectors, more timely and routine comparison of surveillance data from both the human and animal sectors could be explored. New schemes, such as the surveillance of AMR in pathogenic bacteria from livestock (see Chapter 6), could facilitate such initiatives.

Furhtermore, it should be discussed how environmental aspects to spread of AMR through a highly intensified food production system could be investigated and included.

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Textbox 3.1

Evaluating the "Onehealth-ness" of DANMAP

Background

According to the One Health High-Level Expert Panel (OHHLEP) of the World Health Organization (WHO), "One Health (OH) is an integrated, unifying approach that aims to sustainably balance and optimize the health of people, animals, and ecosystems. It recognizes that the health of humans, domestic and wild animals, plants, and the wider environment (including ecosystems) are closely linked and interdependent". One Health has always been a pillar of the DANMAP program and in 2017 with the release of the Danish National One Health Strategy Against Antibiotic Resistance, this position has been reaffirmed.

Given the sheer complexity of designing and operating a multi-sectorial national-scale hazard surveillance program, the need to evaluate the "Onehealth-ness" of the Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP) has been recognized. Evaluation using the newly developed OH-EpiCap tool was performed to identify components that could be further improved in future developments of the DANMAP program.

DANMAP was evaluated considering the spread of AMR as monitored in clinical isolates from humans and indicator bacteria from food producing animals as the main hazard under surveillance and the prevention of potential risks connected to AMR that might affect both humans and animals as the objective of the program. It is necessary to clarify that in this evaluation, we tried to assess the surveillance components of the program as a whole, except for the management/execution part, which was focused on the animal sector. OH-EpiCap is under development by the MATRIX consortium, which is funded by the One Health European Joint Programme (https://onehealthejp.eu/). This textbox reports a preliminary work that will later be further developed and published by the authors. In the coming scientific paper, an overview of the OH-EpiCap tool will be presented and evaluated by using case studies from different countries as part of the CoEvalAMR network (https://coevalamr.fp7-risksur.eu/).

Materials and Methods

The OH-EpiCap aims to facilitate the identification of opportunities to improve the "Onehealth-ness" of collaborations in the surveillance of a hazard. The OH-EpiCap tool is composed of three thematic domains (Dimensions), each with four different targets that are segmented into four questions, for a total of 48 standardized indicators, which are briefly presented in Table 1. These questions are intended to be answered using a semi-quantitative scale from 1 to 4, with 4 representing the ideal scenario, in most cases.

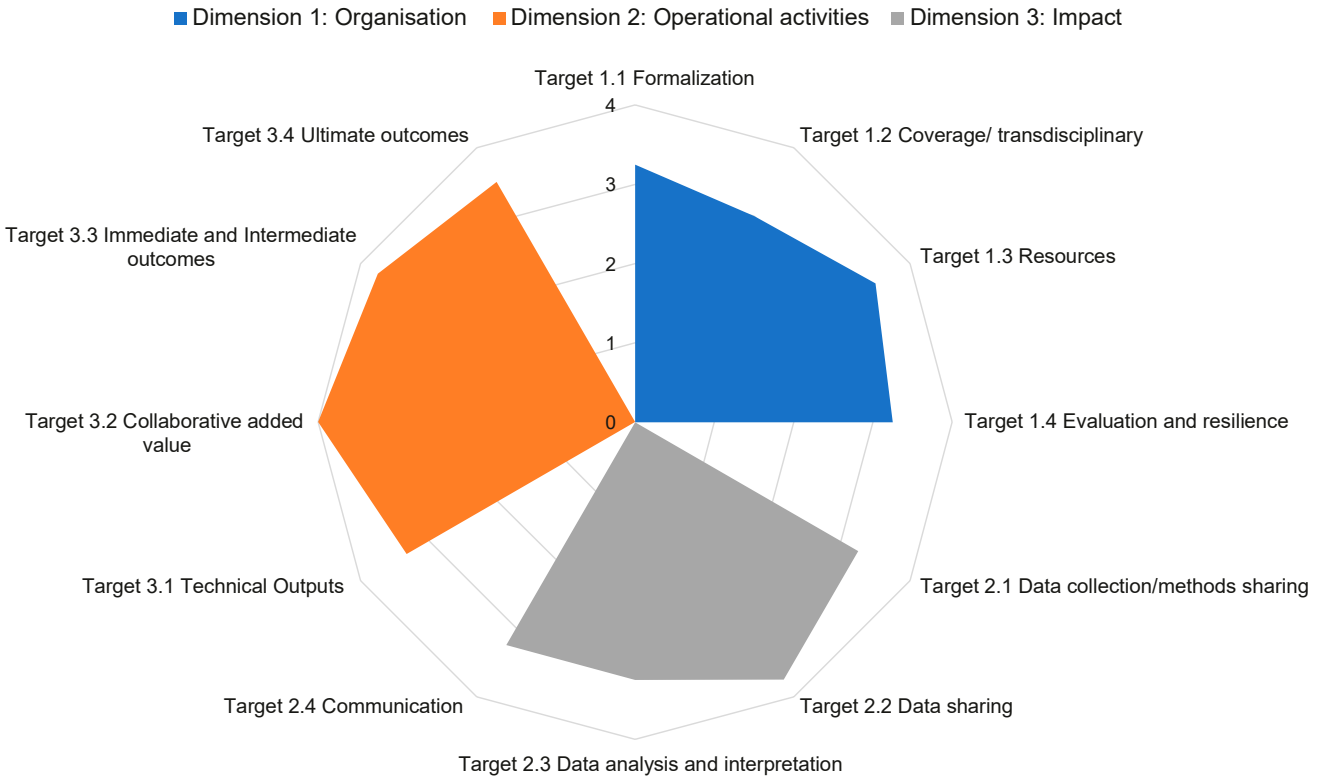
Table 1 Dimensions, targets and topics evaluated by the OH-EpiCap tool. (Rephrased after the flyer OH-EpiCap: evaluation tool for One Health epidemiological surveillance capacities and capabilities, available at: https://onehealthejp.eu/) DANMAP 2021

Dimension 1: Organisation			
Target 1.1 Formalisation: common aim, support documentations, shared leadership, and definition of roles/composition of coordination committees.	**Target 1.2 Coverage:** inclusion of all relevant actors, disciplines, sectors, geography, populations, and related hazards.	**Target 1.3 Resources:** budget and human resources, program training, and sharing of resources.	**Target 1.4 Evaluation and resilience:** internal and external evaluations, development/ implementation of corrective measures, and adaptability to change.
Dimension 2: Operational activities			
Target 2.1 Data collection and methods sharing: multisectoral collaboration in the design of surveillance protocols and data collection, harmonization of laboratory techniques and data warehousing.	**Target 2.2 Data sharing:** data sharing agreements, assessment of data quality, usefulness of shared data, and the compliance of data with the FAIR (findability, accessibility, interoperability, reusability principle.	**Target 2.3 Data analysis and interpretation:** multisectoral integration for data analysis, sharing of analysis techniques, sharing of scientific expertise, and harmonization of indicators.	**Target 2.4 Communication:** internal and external communication, dissemination to decision-makers, and information sharing in case of suspicion/ particular events.
Dimension 3: Impact			
Target 3.1 Technical outputs: timely detection of emergence, epidemiological knowledge improvement, increased effectiveness of surveillance, and reduction of operational costs.	**Target 3.2 Collaborative added value:** strengthening of the OH team and network, international collaboration, and common strategy (road map) design.	**Target 3.3 Immediate and intermediate outcomes:** advocacy, awareness, preparedness, and interventions based on the information generated.	**Target 3.4 Ultimate outcomes:** research opportunities, policy changes and behavioural changes and better health outcomes.

continued ... Textbox 3.1

The first version of the OH-EpiCap was filled in at a meeting between representatives from the DANMAP management, academia, and the Danish livestock industry. The answers given were sent to other relevant stakeholders, who agreed and commented on the answers given.

Figure 1: Average score of DANMAP in the target areas covered by the OH-EpiCap tool DANMAP 2021



Results and Discussion

A graphical outcome of the OH-EpiCap evaluation of DANMAP can be seen in Figure 1. The program scored highly in every dimension, averaging above 3 in all target areas, demonstrating a high level of "Onehealth-ness". Overall, when filling in the OH-EpiCap tool a conservative approach was chosen. Hence, when in doubt between two options, a lower score was chosen to raise awareness and promote discussion around those target areas. The following points were identified as relevant for discussion.

Dimension 1: Organisation

Main points of critique identified in the interview were three regarding organisation: 1. All relevant supporting documentation to DANMAP should preferably be compiled and shared at one common digital point, increasing the public accessibility. 2. The steering committee does not include all the sectors that potentially are relevant to OH surveillance, as the environment is not represented. 3. All actively engaged sectors are represented in the program's coordination group. However, more representatives from the livestock industry and the environmental sector could reinforce the OH approach.

Previous national research performed around the shift of the sequel have led to the non-inclusion of environmental data into the surveillance programme. Since then, animal production and hospital activity have markedly intensified, and AMR potentially spreading through wastewater or manure have been mentioned as possible critical observation points for future inclusion into AMR surveillance. The needed data, methodologies, and analyses are currently being considered. Over time, the program has evolved, adapting to new challenges, and optimizing content and processes, some of it following the conduction of regular internal evaluations. Yet, evaluations could have been performed using a more standardized methodology, easing the implementation of corrective measures proposed in a timelier way.

For the current aims of the program, economic and human resources are sufficient and sustainable. However, to investigate emerging issues, adapt and include them in the surveillance program, an extension of the budget would be required to get more staffing resources and analytical means. This is also the case for addition of new components to the surveillance program, e.g. environmental aspects.

Dimension 2: Operational activities
Harmonisation of indicators across sectors and methodology for sampling the animal population for AMR surveillance could possibly be improved but would inevitably come with budget challenges. Joint multi-sectorial analysis could potentially be improved in the future. Transparency, communication, and accessibility of data need to be constantly evaluated, given that these change when the methodology changes or the aim of the program is altered. Investment in further training of professionals responsible for prescribing and using antimicrobials could also be considered.

Dimension 3: Impact
OH surveillance has always been the basis of DANMAP, therefore questions regarding the added value of adapting to a OH response were not considered relevant. Given the more than 25 years of the program, its impact on epidemiological knowledge are clear and have guided sector interventions and policy changes. In Denmark, there is a strong will of working collaboratively, OH networks are well-functioning and the level of awareness among the stakeholders is very high, even if translation into action could be further improved.

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Textbox 3.2

Update from International Centre for Antimicrobial Resistance Solutions (ICARS)

ICARS offers tailored support to low-ressource settings for the development and implementation of context-specific AMR solutions, contributing to each country's National Action Plan and presenting an opportunity for scale-up, cross-country, and cross-regional learning.

Why ICARS?

While much research has revealed successful solutions for tackling AMR, there is still a critical gap in translating this evidence into action. Even though many countries have developed AMR National Action Plans (NAPs), in resource poor settings the challenge remains for how best to prioritise and implement interventions to reduce AMR. Furthermore, the growing rates of drug-resistant microorganisms detected in animals, humans and the environment are evidence that a siloed approach is not enough, and global efforts to address AMR should span the One Health spectrum.

What does ICARS do?

Using simultaneous top-down and bottom-up approaches, ICARS partners with low-and middle-income ministries and their local research institutions, to co-develop cost-effective solutions to tackle AMR. Each solution, informed by intervention and implementation research, is tailored to tackle a local AMR challenge and advance NAP implementation. To deliver long lasting change and to avoid duplication, ICARS collaborates with a range of stakeholders to build on existing national and international initiatives, and aiming to boost investments and efforts across sectors.

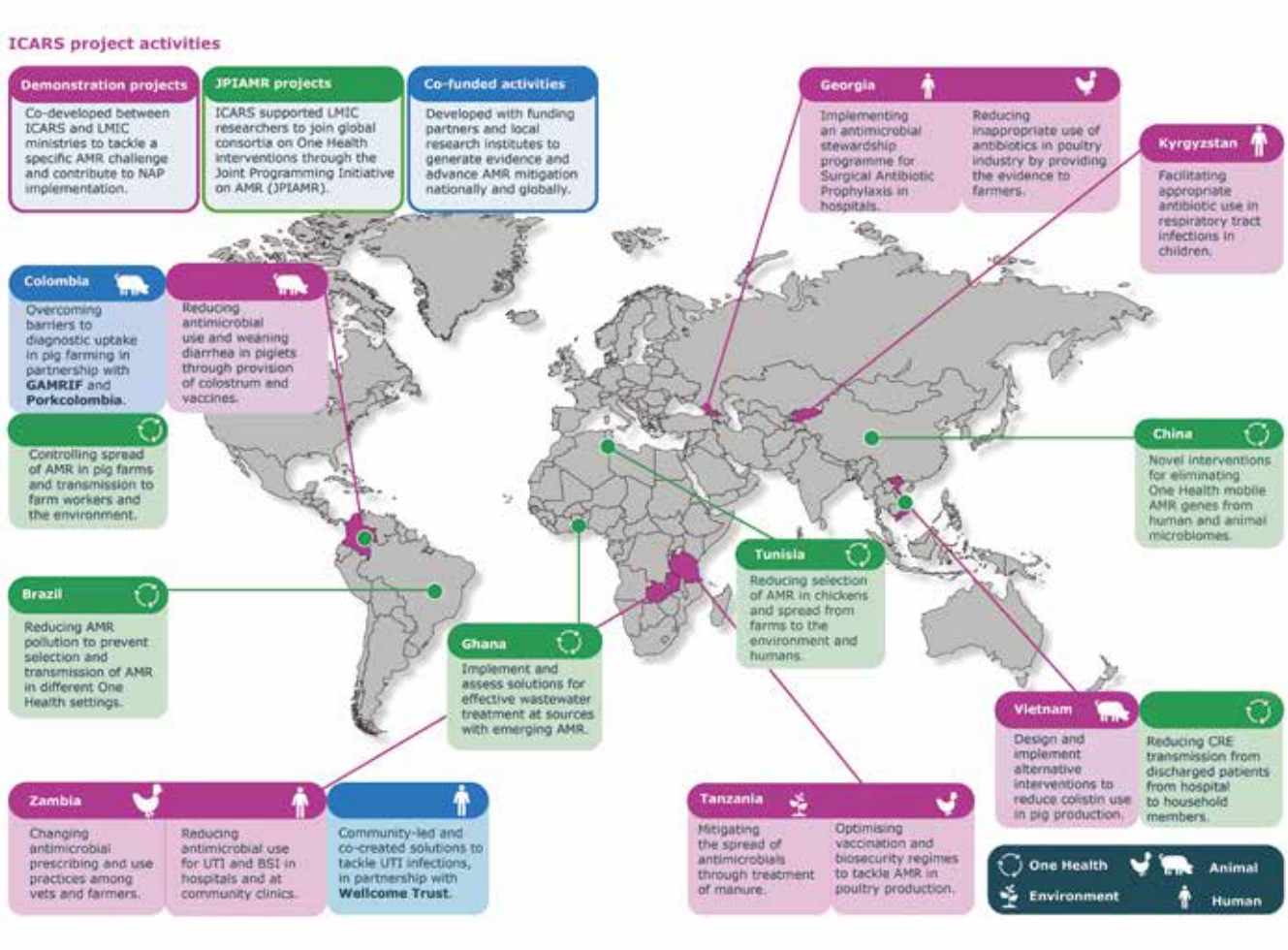
The ICARS strategy is based on the following interconnected strategic pillars:

- Pillar 1: Develop and test context-specific solutions for AMR mitigation
- Pillar 2: Support the translation and uptake of existing evidence and innovation into policies, programmes and practice
- Pillar 3: Advocate for context-specific, country-owned AMR mitigation solutions
- Pillar 4: Support targeted capacity and capability building
- Cross cutting pillar: A trustworthy partner and platform for delivering country-owned AMR solutions

ICARS research project portfolio

In 2021, ICARS began implementing its first two demonstration projects in Georgia and Vietnam, in the human and animal sector respectively. In 2022 a further seven demonstration projects across Latin America, Africa and Asia the One Health spectrum have been agreed upon and signed. This milestone follows exchange between ICARS research advisors, country ministries and local research institutions, discussing and developing a range of interventions that tackle specific AMR challenges.

In addition to co-developing projects with country ministries, ICARS has also begun collaborations with national and international organisations on a range of supporting actitivities to tackle AMR. For example, last year ICARS and the UK Global AMR Innovation Fund (GAMRIF) joined hands to foster and support an innovative project led by Porkcolombia to improve uptake of disease diagnostics at pig farms in Colombia. With funding from ICARS and GAMRIF, interdisciplinary experts on the ground are assessing the challenges and opportunities around the uptake of diagnostic veterinary services in Colombia, and their potential impact on the reduction of antibiotic use in farming. Results from the pilot (published later this year) will serve as an example for other countries to consider how to improve access to veterinary diagnostics to improve sanitary status of farms, increase understanding of disease occurrence and provide a variety of information on herd health and the different pathogens that cause swine diseases.



We are also working in partnership with multiple stakeholders including the World Health Organisation (WHO), the Global AMR R&D Hub, The British Society for Antimicrobial Chemotherapy (BSAC), the International Livestock Research Institute (ILRI) and ReAct on a range of activities that strengthen existing demonstration projects or compliment other in-country initiatives.

Governance

Initiated by the Danish government in 2019, ICARS became an independent organisation in 2021 governed by an international Board of Directors and informed by a Technical Advisory Forum. Guided by its Board, ICARS aims to partner with countries, foundations and organisations to advance drug-resistant infections mitigation in LMICs. ICARS is also supported by Funding and Mission Partners, who provide financial and in-kind contributions to advance ICARS' agenda. In 2022, Zambia and India joined ICARS as Mission Partners, a commitment that demonstrates their support to the vision and mission of ICARS and a commitment to work actively to promote the AMR agenda in their national context.

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Textbox 3.3

Emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) in the pre-antibiotic era: the role of hedgehogs and antibiotic-producing dermatophytes

The discovery of antibiotics more than 80 years ago instigated an era of drug innovation and implementation in human and animal health. Clinical use of these antibiotics were all followed by the rapid emergence of antibiotic resistance genes in clinical isolates of many common pathogens. This history has led to the generally accepted view that antibiotic resistance in pathogenic bacteria is a modern phenomenon driven by our use of antibiotics.

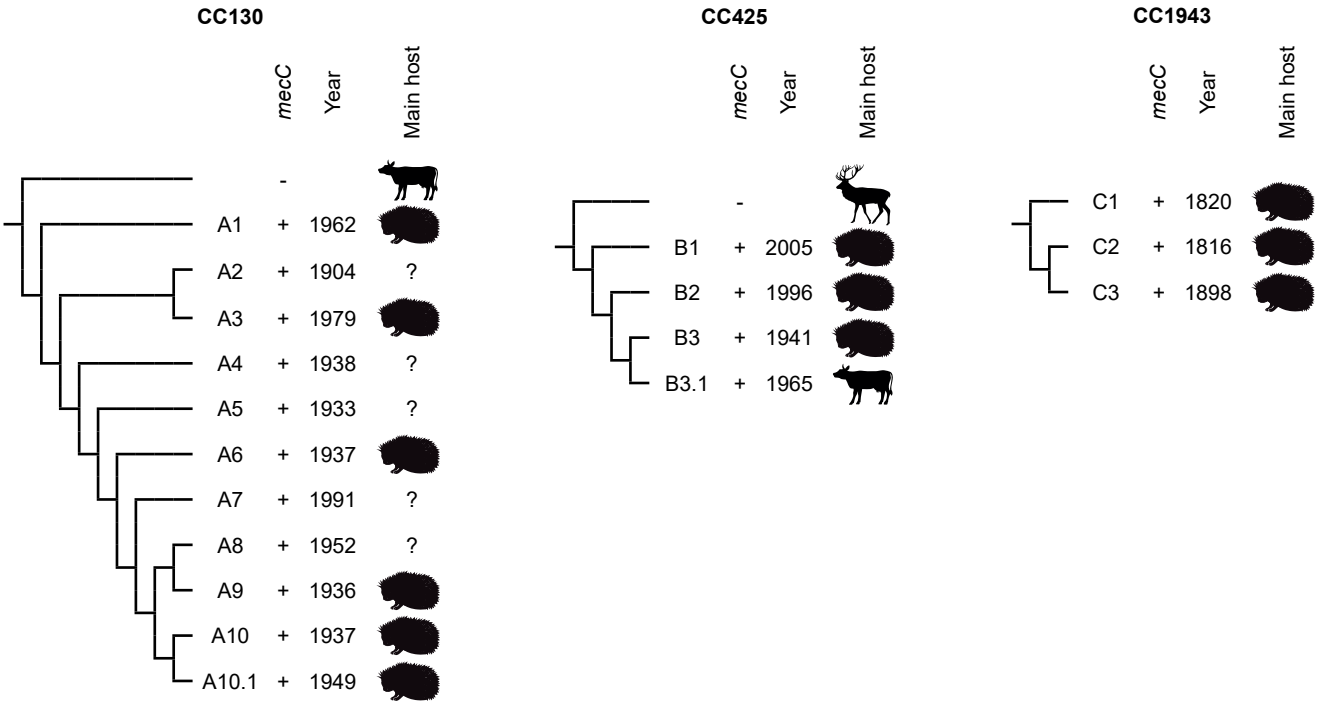
Methicillin-resistant *Staphylococcus aureus* (MRSA) was first identified in patients in 1960 just one year after the introduction of the penicillinase-stable penicillin methicillin as a therapeutic option against the rapid emergence of penicillinase-producing – and hence penicillin-resistant – *S. aureus* isolates during the 1940s and 1950s. Methicillin resistance has subsequently emerged in many *S. aureus* clones around the world as a result of horizontal acquisition of the *mecA* gene from other staphylococci, both in hospital and community settings as well as food animals such as pigs and cattle. Two recent reports from Denmark and Sweden have shown that hedgehogs are frequent carriers of particular lineages of MRSA carrying the *mecC* gene, which is a homologue of the *mecA* gene. These clones account for 3% of all human MRSA infections in Denmark and differ from other MRSA clones by being susceptible to almost all non-β-lactam antibiotics.

The detection of *mecC* carrying MRSA clones in hedgehogs prompted us to investigate the hypothesis that the emergence of these bacteria was initially driven by natural selection rather than by clinical use of antibiotics. For this purpose, we sequenced 1,127 *mecC*-MRSA and MSSA isolates belonging to CC130, CC425, and CC1943, which represent the most successful *mecC*-MRSA clones.

Bioinformatics analyses (e.g., identification of antimicrobial resistance genes and host-specific genetic markers) and construction of time-scaled phylogenies, showed that the *mecC*-MRSA isolates belonged to 16 monophyletic lineages, most of which contained hedgehog isolates, and that the earliest *mecC*-MRSA lineages appeared in European hedgehogs in the early 1800s well before the first antibiotics became widely available as therapeutic options in human and veterinary medicine in the 1940s (Figure 1).

In addition, the analyses revealed that most *mecC*-MRSA isolates from humans and food animals originate from local hedgehog reservoirs (Figure 1). Interestingly, the *mecC*-MRSA CC130 and *mecC*-MRSA CC425 lineages appear to have evolved from two distinct methicillin-susceptible *S. aureus* (MSSA) populations circulating in ruminants and wildlife (Figure 1). Analyses of the hedgehog dermatophyte *Trichophyton erinacei* revealed that it produces penicillin-like antibiotics, which provide a natural selective environment where MRSA isolates have an advantage over MSSA isolates. Our findings thus suggest that methicillin-resistance is an acquired phenotype associated with adaptation to hedgehogs. These results underscore that there are no boundaries between natural, agricultural, and human ecosystems and it is therefore necessary to look at antibiotic resistance in a One Health perspective.

Figure 1 Population structure of *S. aureus* CC130, CC425 and CC1943 DANMAP 2021



The *mecC*-MRSA isolates could be divided into 16 lineages (A1 to A10, B1 to B3, and C1 to C3). A10.1 and B3.1 refer to a Danish *mecC*-MRSA CC130 sublineage from Jutland and to a British *mecC*-MRSA CC425 sublineage carrying a genetic marker of ruminant adaptation

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References

[1] Larsen et al. Emergence of methicillin resistance predates the clinical use of antibiotics. Nature 602, 135-141 (2022)

Textbox 3.4

Clostridioides difficile – investigating genetic overlap between human and animal strains

Clostridioides difficile is a spore-forming, strictly anaerobic bacterium that is widely disseminated in a broad range of domestic and wild animal species. It shows pronounced intra- and inter-species differences in prevalence and clinical relevance, ranging from being a commensal of the gut in some and causing gastrointestinal diseases in others. In humans especially younger individuals may be asymptomatic carriers, while vulnerable recently hospitalised and/or antibiotic treated patients will be at a much higher risk of being infected with the hyper-virulent types, particularly of strains carrying the binary toxin. Since 2009, *C. difficile* of the toxin-producing "ST1 (PCR ribotype 027)" isolated from hospitalised patients is mandatory to report and national surveillance of hypervirulent strains is carried out by the Department for Foodborne Infections at Statens Serum Institut. Since 2016, a sentinel surveillance of all toxin-positive clinical samples or isolates has been performed in a collaborative effort between the clinical departments of microbiology and SSI. Samples are collected and sent during one month in spring and one month in autumn. This ensures a more real and unselected image of the type distribution in the Danish reservoir and serves to discover and record emergence of unusual types.

Recently, the numbers of *C. difficile* infections (CDI) not associated with hospitalisation have increased, indicating changing epidemiology of community associated (CA) transmissions. International studies have shown that some types are more prevalent among CA infection, in particular ST11, which has also been found in farmed animals and is considered a human hypervirulent and multidrug resistant type. In Denmark, there are approximately 5000 CDI cases each year and ST11 is the second most common type accounting for 8-10% of all human CDI cases (See Figure 1 for population structure of Danish human types).

In order to investigate possible genetic overlap between human and animal *C. difficile*, a total of 330 pig fecal samples were collected from 14 different organic farms in 2020 and cultured for *C. difficile*. Thirty-three (10%) were *C. difficile* positive and among those 21 were ST11 originating from 6 pig farms. Additionally, 184 conventional slaughterhouse pig fecal samples (2021) were cultured and among those we found seven STs (ST6, ST8, ST9, ST13, ST16, ST44 and ST45), none of them being related to human isolates.

All ST11 isolates were whole genome sequenced and sequences were compared to 390 human ST11 isolates from 2020-2021 by core genome/whole genome MLST (cgMLST/wgMLST) and single nucleotide polymorphism (SNP), while the presence of antimicrobial resistance genes (ARG) were investigated by AMRFinder (https://www.ridom.de/u/NCBI_AMRFinderPlus.html). Eighteen out of 21 veterinary ST11 were 0-2 wgMLST different from human isolates indicating either direct transmission or a shared intermediate reservoir.

The 18 pig isolates were part of three different human clusters (≤3 SNP) representing ca. 30% of human isolates, indicating that the animal clones are common among human CDI. Two clusters were ST11(RT078) (Figure 2) and one was ST11(RT066). ARG profile within each human/animal cluster, confirmed similarity and important potential of resistance (not confirmed be phenotype), (Table 1).

This study confirms previous findings, where genetic overlap was found among human and animal *C. difficile* ST11. However, the study also indicates that ST11 is restricted to certain farmed animal environments as none was found in conventional slaughter pigs. Therefore, more data are needed to pinpoint specific routes of transmission and future studies should include investigation of more samples from food, animal and environmental sources, along with geographical location of patients and farms from where similar strains are obtained.

Figure 1 Population structure obtained by minimum spanning tree of core-genome MLST (cgMLST) (BioNumerics, 1999 alleles) of major Danish clinical *C. difficile* sequence types (STs) (colored) derived from WGS data on 2788 isolates obtained from the national sentinel surveillance 2018-2022 (all toxigenic isolates collected one month in spring and fall from all Danish Dept. of clinical Microbiology). Two different ST11, i.e. RT078 and RT066 are circled on figure DANMAP 2021

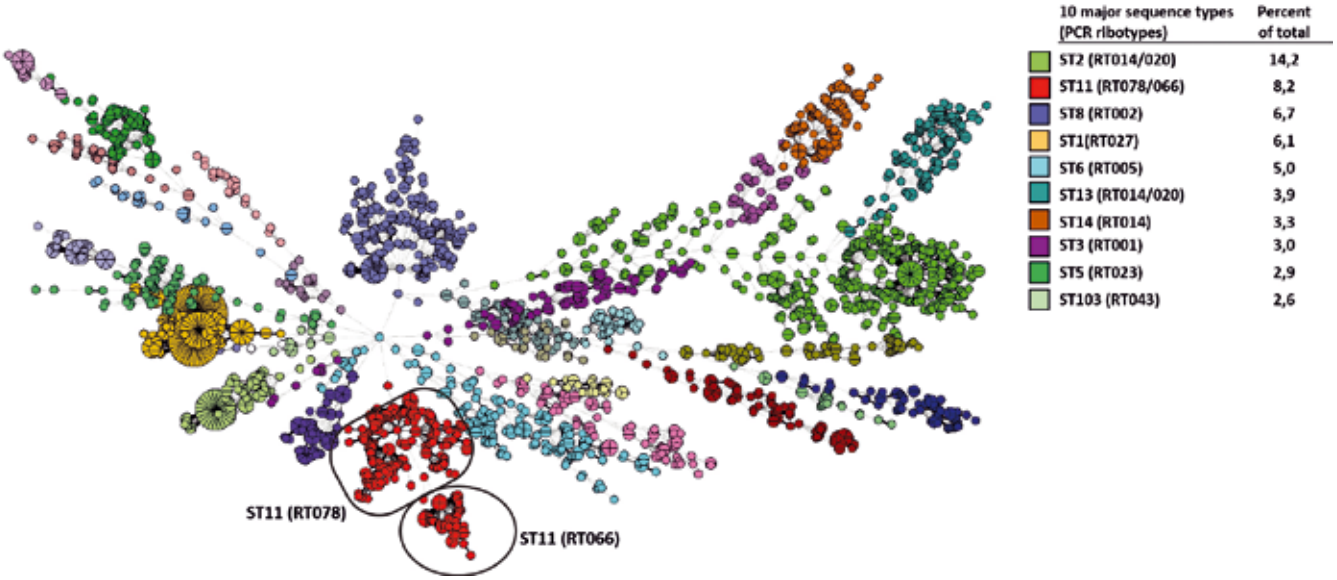
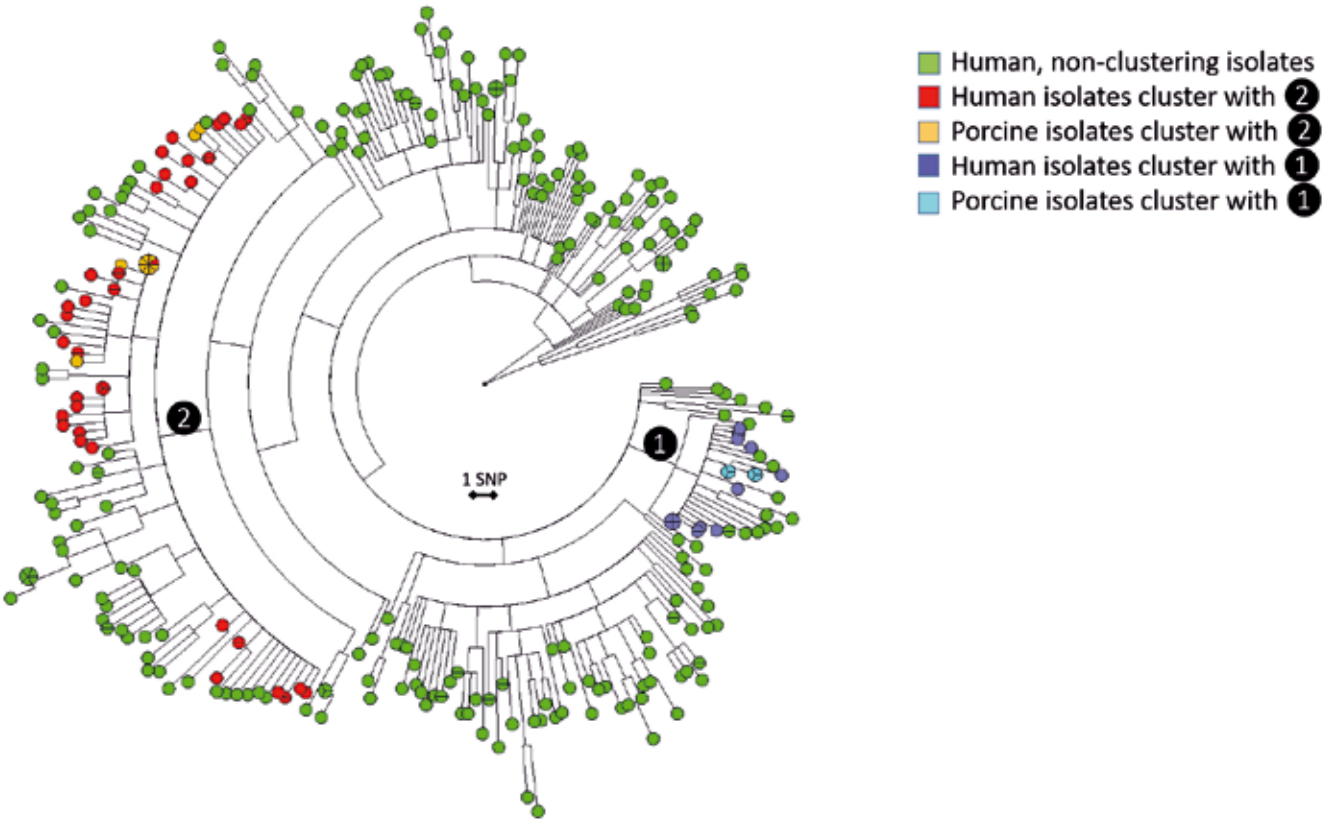


Figure 2 SNP tree (1047 SNPs) containing 308 clinical isolates from 2020-2021 (ST11, RT078) and 17 porcine isolates (2020). The two highlighted clusters are defined as ≤3 SNP differences DANMAP 2021



continued ... Textbox 3.4

Table 1 Characteristics of the three different ST11 veterinary-human clusters. Cluster 1 and 2 of ST11(RT078) are shown in Figure 2
DANMAP 2021

Cluster # (ST/RT)	No. porcine isolates (Origin)	No. human isolates (% of total human)	Major ARGs
❶ (Figure 2) (ST11/RT078)	6 (Farm #1, 2)	14 (4.5%)	β-lactam: *blaCDD* Fluoroquinolone: *gyrB* (S366V+S416A)
❷ (Figure 2) (ST11/RT078)	11 (Farm #2, 6, 8, 9, 10)	35 (11.4%)	β-lactam: *blaCDD* Fluoroquinolone: *gyrB* (S366V+S416A) Aminoglycoside: *ant(6), aph(3), sat4* Tetracycline: *tet(M)*
❸ (ST11/RT066)	1 (Farm #8)	12 (14%)	β-lactam: *blaCDD* Fluoroquinolone: *gyrB* (S366V+S416A)

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4

ANTIMICROBIAL CONSUMPTION IN ANIMALS

4. Antimicrobial consumption in animals

Highlights: For this edition of DANMAP, data on antimicrobial use was extracted from the **new VetStat database**, launched June 2021 by the Danish Veterinary and Food Administration. The database includes all veterinary prescription medicines and is an important tool for monitoring and benchmarking of veterinary antimicrobial use. The new database applies a different method for registering medicinal products than the former VetStat, which has impacted absolute values for kg active compounds, but not impacted trends over time (Textbox 4.1).

In 2021, the **total use** of antimicrobials in animals amounted to 88.0 tonnes of active compound. The **pig sector** used approximately 82% of all veterinary-prescribed antimicrobials, equal to 72.2 tonnes active compound. Calculated in treatment proportions, an estimated 2.7% (27 DAPD) of all pigs, on average, received antimicrobial treatment per day in 2021. The highest treatment proportions were observed in the treatment of weaners, (9%, corresponding to 9.1 DAPD). In sows and piglets, the treatment proportions remained at 1% to 2%, (corresponding to 19 DAPD in sows and piglets and 16 DAPD in finishers, respectively).

Over time, and particularly as a response to the enforcement of the Yellow Card initiative, the antimicrobial classes used in the treatment of pigs have changed notably. Critically important antimicrobials have been phased out. The use of tetracyclines has decreased significantly over the last decade (9.6 DAPD in 2012 to 4.1 DAPD in 2021). During the same period, noticeable but smaller, increases in the use of macrolides (6.4 DAPD in 2012 to 7.3 DAPD in 2021), aminoglycosides (1.5 DAPD in 2012 to 2.7 DAPD in 2021) and penicillins (4.8 DAPD in 2012 to 5.7 DAPD in 2021) have occurred.

In 2021, antimicrobials prescribed for **cattle** amounted to 9.4 tonnes. Approximately two thirds were used to treat older cattle (>1 year). Over the past decade, the antimicrobial use has decreased for older cattle (>1 year), from 3.7 DAPD in 2012 to 2.9 DAPD in 2021, while it has increased from approximately 4.8 DAPD to 6.6 DAPD in young cattle. Also in cattle, the changes in use of antimicrobial classes are noticeable, i.e. more penicillins and macrolides are used for treatment of young cattle and more penicillins for intramammary treatment.

The antimicrobial use in **poultry** was at lowest level (1.2 tonnes) since 2013. The decrease was driven particularly by lower use of tetracyclines in broilers and turkeys; tetracyclines are typically prescribed for respiratory disease.

In 2021, cephalosporins were prescribed mainly for **pets and horses (77 kg)** or as intramammaries for **cattle (51 kg)**. Furthermore, 3rd and 4th generation cephalosporins (1 Kg) were prescribed solely for pets and horses, while fluoroquinolones (15 kg) were prescribed almost exclusively for pets.

4.1 Introduction

Since the early 1990s, there has been increased political and public focus on the use of antimicrobial agents in the Danish animal production. The DANMAP programme began monitoring the national use of antimicrobial agents in humans and animals in 1995. Changes in use, such as the phasing out of antimicrobial agents for growth promotion and a number of other initiatives, including voluntary bans on the use of cephalosporins in the pig and cattle production, as well as regulatory legislation regarding therapeutic use is presented in th timeline figure on page 6.

Figure 4.1 presents the total use of antimicrobials in animals and humans since 1990 and 1997, respectively. In addition to the mentioned changes in patterns of antimicrobial use, increases in and intensification of pig production have had a significant impact on the overall use over time.

The observed decrease in antimicrobial consumption after 1994 was likely the result of 1) limitation of veterinary practitioners' profit from sales of medicine; 2) implementation of Veterinary Advisory Service contracts (VASCs) with regular visits from the veterinarian to promote preventive veterinary strategies and optimize antimicrobial use; and 3) enforcement of the so-called "cascade rule" [Order (DK) 142/1993], limiting the use of (cheaper) extemporaneously produced medicines.

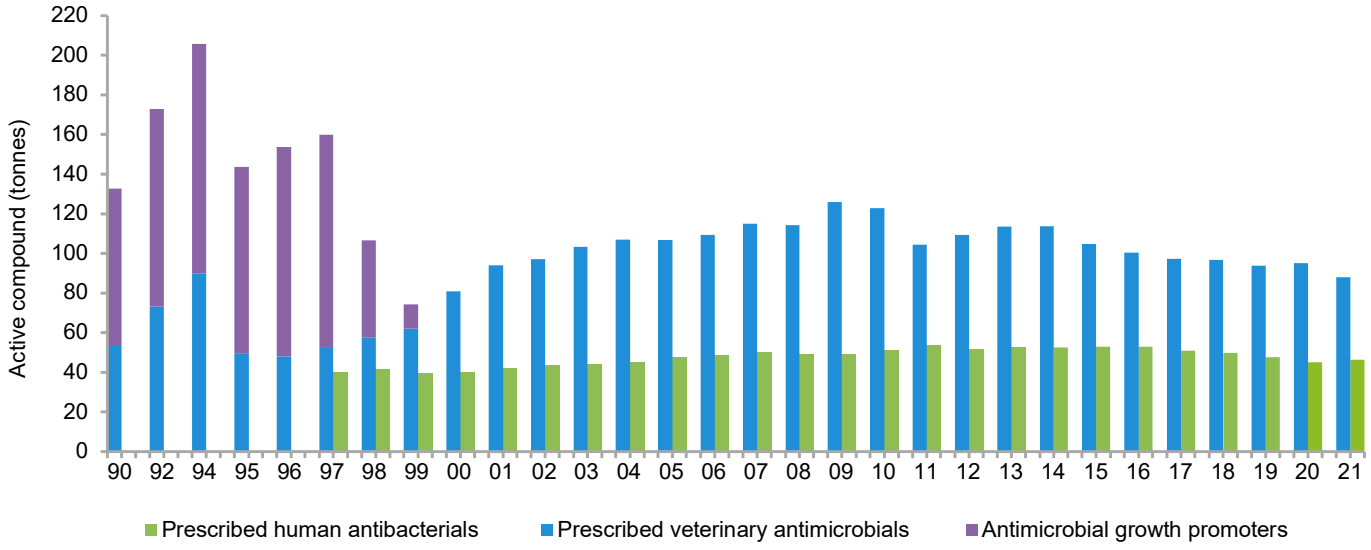
Other important interventions were the restriction on the use of fluoroquinolones in production animals through legislation implemented in 2002 and 2003, and the voluntary ban on the use of cephalosporins in the pig industry in 2010, followed by a similar initiative in the dairy cattle industry in 2014.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

The Yellow Card initiative was established in 2010, introducing monitoring of antimicrobial use at farm level and specifying threshold values for individual farms to enable taking legal action against pig farmers with particularly high antimicrobial use per pig [DANMAP 2010]. As a result, a distinct decrease in consumption was observed between 2010 to 2011. In 2016, the Yellow Card initiative was revised, adding multiplication factors to adjust the consumption of certain antimicrobials. Fluoroquinolones, cephalosporins and colistin (added in 2017) were given the highest multiplication factor of 10. Tetracyclines were multiplied by 1.2, and the factor was further increased to 1.5 in 2017 [DANMAP 2017].

Through the years Denmark has had different specific goals to reduce antimicrobial use in animals: Initially, a 10% reduction of antimicrobial use in farm 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 use in pigs from 2015 to 2018.

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

Figure 4.1 Prescribed antimicrobial agents for humans and all animal species, tonnes of active compound, Denmark DANMAP 2021



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 2021, consumption data were extracted from the new VetStat on 24 August 2022

Together with the strategy, two national action plans to reduce AMR were introduced, setting specific targets to further reduce the antimicrobial use for both humans and animals. As part of the political agreement on the veterinary strategy 2018-2021 (Veterinærforlig III), an Advisory Committee on Veterinary Medicines was established in 2018. In 2019, a new national target was determined for an 8% reduction in the use of antimicrobials in the pig sector by 2022. In 2021, a new action plan was launched, setting goals for the coming years.

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

Also, to reduce the need for disposal of excess antimicrobials, veterinarians and pharmacies were permitted to split packages of veterinary medicine as from 1 January 2019 [Order (DK) 1655/2018]. This initiative also enhances quality of surveillance by reducing the difference between amounts of antimicrobials prescribed and amounts used.

Official treatment guidelines for pigs and cattle have been available since 1996. The guidelines provide specific recommendations for selection of the most appropriate antimicrobial treatment of all common infectious diseases in the major production animal species. Since 2005, the Danish Veterinary and Food Administration (DVFA) has updated the guidelines in collaboration with stakeholders and technical experts. In 2010 new dynamic evidence-based treatment guidelines for pigs were launched [DANMAP 2010, www.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 at the Technical University of Denmark. A revision of the guidelines was published in 2018. Similarly, DVA published treatment guidelines for use of antimicrobials in horses in 2017.

4.1.1 Data sources

In Denmark, antimicrobials are available by prescription only, and data on antimicrobial use have been collected in Denmark since 1990. Since 2001, data on all medicines prescribed for use in animals, including vaccines, antimicrobial growth promoters (no longer permitted) and coccidiostatic agents (non-prescription) have been recorded in the national database VetStat, which is hosted and maintained by DVFA.

In June 2021, DVFA launched a new and updated platform for VetStat, (Textbox 4.1). For the 2021 DANMAP report, data were extracted from the new VetStat (extraction date 24 August 2022). Data included all antimicrobials (ATCvet QJ01) registered for use in animals. Antimicrobials for use in humans (ATC J01), which are sometimes prescribed for pets, were not included. Data were extracted for the years 2004-2021 and analysed and interpreted for DANMAP by the National Food Institute, Technical University of Denmark. All totals have been recalculated using the new VetStat from 2004 to 2021.

4.1.2 Methods

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

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

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

The **Defined animal daily dose (DADD)** is the average maintenance dose per day for the main indication of a drug per animal species. DADD is not defined at product level but for each antimicrobial agent, administration route, and animal species as mg active compound per kg live animal. DADDs were defined specifically for use in DANMAP based on current knowledge about averaging animal size and weight. They may vary from the prescribed daily dose, or the recommended dosage in the summaries of product characteristics (SPC) or in the VetStat database.

The **Proportion of population in treatment per day (DAPD)** is used to describe trends in the antimicrobial use in animals, where possible. The DAPDs are statistical measures that provide rough estimates of the proportion of animals treated daily with a particular antimicrobial agent. For example, 10 DAPD means that an estimated 1% of the animal population, on average, receives a certain treatment on a given day (see section 9.2). In principle, DAPD as a metric is analogous to human DID (defined daily dose per 1,000 inhabitants per day, see section 9.8), the metric used to measure antimicrobial consumption in the human sector.

Large differences in DAPDs between age groups affect the DAPD of the total animal population, and trends are influenced by changes in population structure. As an example, increased export of live pigs just after weaning could lead to a measured increase in DAPD in the total pig population since the exported pigs were only in the country during the short period when the treatment proportion was highest. Approximately 44% of the pigs produced in 2021 were exported as live pigs at approximately 30 kg (Table 3.1). In comparison, this percentage was approximately 33% in 2012.

In DANMAP 2021, treatment proportions were calculated for pigs and cattle.

4.2 Total antimicrobial consumption in animals

Together with data extraction from the new VetStat, the criteria for allocating antimicrobial use to the different animal species and age groups were revised. This affected the calculated amounts for antimicrobials used per species while the overall trends in consumption remain the same.

The total use of antimicrobial agents in all animals amounted to 88.0 tonnes active compound, corresponding to a 7% (-6,967 kg) decrease compared to 2020 (Figure 4.1 and Table 4.1). Similar to previous years, the 2021 antimicrobial use in pigs, cattle and poultry comprised approximately 82%, 10%, and 1%, of the total antimicrobial use in animals, respectively, (Figure 4.2).

The pig industry is the main driver of antimicrobial usage in animals in Denmark, due to the magnitude of the production. Cattle and pigs comprise almost equal proportions of the total live biomass. However, the vast proportion of cattle biomass consists of dairy cows, which have very low consumption of antimicrobial agents compared with growing animals.

The overall use of kg active compound was 57% lower in 2021 compared to 1994. A major part of this reduction can be explained by the discontinued use of growth promoters from 1994 to 1999.

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

Figure 4.2 Distribution antimicrobial consumption in main animal species, tonnes, 2021, Denmark DANMAP 2021

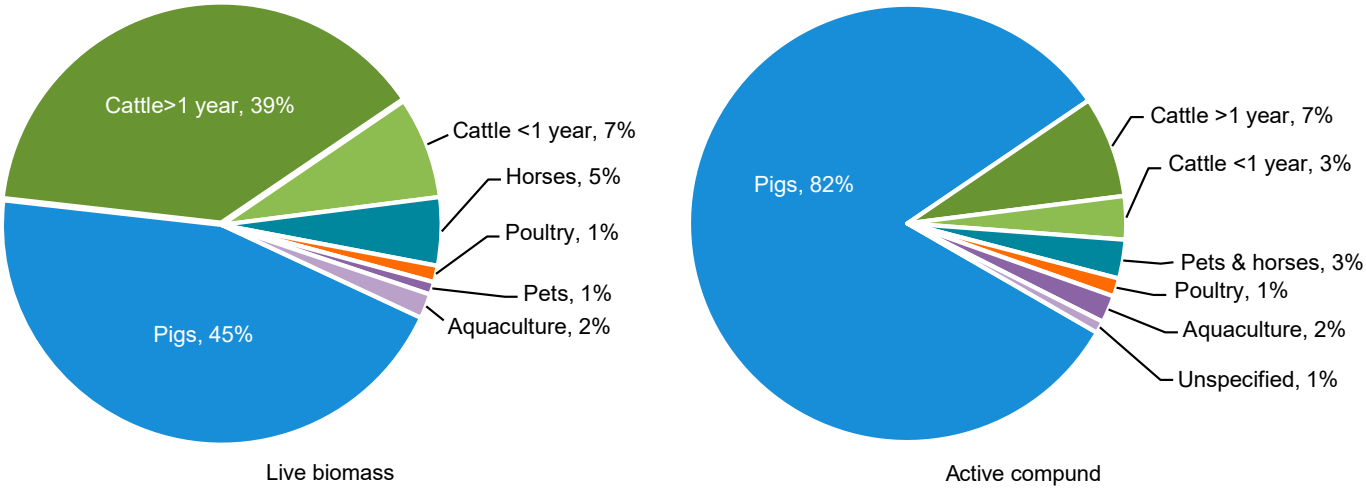


Table 4.1 Antimicrobial agents sold (kg active compound) by animal species and age group, Denmark DANMAP 2021

	Aminoglycosides	Amphenicols	Cephalosporins[a]	Fluoroquinolones	Lincosamides	Macrolides	Other Ab[b]	Other quinolones	Penicillins, b-lactamase sensitive	Penicillins, others	Pleuromutilins	Sulfonamides and trim-ethoprim	Tetracyclines	2020	2021
Pigs	10126	725	0	0	2145	12364	<1	0	12467	9357	8844	4921	11397	74382	72345
Sows, piglets, gilts and boars	2231	392	0	0	467	538	<1	0	6283	3060	1371	4042	1134	19553	19518
Weaners (=<30kg)	7710	308	0	0	988	8325	0	0	1786	5403	2830	781	7441	37221	35572
Finishers and polts	185	25	0	0	689	3500	<1	0	4399	894	4643	98	2823	17608	17256
Cattle	1094	950	51	0	7	220	<1	0	4865	576	0	453	1209	10069	9425
Intramammaries	26	0	51	0	5	0	0	0	248	155	0	0	0	541	485
Cows, bulls, heifers and steers (>24 months)	232	18	<1	0	<1	65	<1	0	3875	306	0	365	670	6292	5531
Calves (<12 months)	621	915	<1	0	<1	152	<1	0	476	106	0	86	483	2998	2838
Young cattle (12-24 months)	215	18	0	0	<1	3	0	0	266	10	0	2	57	239	571
Poultry	53	0	0	0	25	169	0	0	112	188	<1	33	616	2458	1196
Poultry	17	0	0	0	8	62	0	0	20	70	0	13	339	2458	529
Broilers	27	0	0	0	14	105	0	0	0	26	0	17	147	0	337
Layer hens	0	0	0	0	0	0	0	0	49	0	<1	0	17	0	67
Turkeys	9	0	0	0	3	0	0	0	43	85	0	0	103	0	243
Other poultry	0	0	0	0	0	2	0	0	0	7	<1	3	9	0	21
Other production animals	<1	296	<1	<1	<1	2	<1	366	<1	20	0	1089	15	4469	1788
Aquaculture	0	296	0	<1	<1	0	0	366	<1	20	0	1089	<1	1965	1771
Fur animals	0	0	0	0	0	0	<1	0	0	0	0	<1	1	2504	1
Other	<1	<1	<1	<1	<1	2	<1	0	<1	<1	0	<1	13	<1	15
Companion animals	14	1	77	15	74	2	59	0	17	552	<1	1659	40	2446	2520
Horses	<1	0	<1	<1	<1	<1	<1	0	5	<1	0	105	2	120	113
Pets	3	<1	28	5	19	2	14	0	11	100	<1	271	24	478	485
Pets or Horses	11	<1	49	10	55	0	45	0	<1	452	0	1282	14	1848	1922
Unspecified[c]	116	ND[d]	<1	2	9	5	4	0	366	110	3	20	143	1188	772
Total	11403	1967	129	17	2259	12761	63	366	17828	10802	8847	8174	13420	95012	88045

Note: Data for 2021 were extracted from the new VetStat on 24 August 2022
Combination products are split into active compounds
a) In 2021, 3rd and 4th generation cephalosporins were only used in pets (1 kg)
b) Including other antibacterials, dermatologicals, opthamologicals, tologicals, and quinolones
c) Including data with no information on animal species/age group, or mismatch between animal species and age group
d) ND: Not determined. Negative number (-5kg) due to double-reporting of the same product by veterinarians and pharmacies

4.3 Antimicrobial consumption by animal species

4.3.1 Antimicrobial consumption in pigs

Most antimicrobials in animals are used in the pig production. The **total antimicrobial consumption** in pigs was 72.3 tonnes of active compound, which was 2,037 kg less than in 2020 (Table 4.1).

The national MRSA action plan aimed to reduce the antimicrobial use in pigs by 15% in 2018 compared to 2014. This goal was reached not reached in 2018, but in 2019 the antimicrobial use had been reduced by 16%. A revised action plan with new targets were agreed upon in 2019. The new action plan set new targets: antimicrobial use in the pig production should decrease by 2% each year from 2019-2022 (in total 8%) compared to the 2018 level. Even with the decrease observed in 2021 the targets have not been achieved in either 2020 or 2021.

The **treatment proportion** (DAPD) of the total population reflects the trends in selection pressure within the population. In the pig population, the treatment proportion is much higher in weaners than in finishers and sows. DAPDs in the whole pig population and by age group are presented in Figures 4.3 and 4.4, and DADDs are shown in the supplementary material at www.DANMAP.org.

Historically, DAPD increased from 2004 to 2009, followed by a clear decrease in 2010 and 2011 with introduction of the Yellow Card initiative. Since 2013, a overall deccreasing trends in treatment proportions have been observed (Figure 4.3).

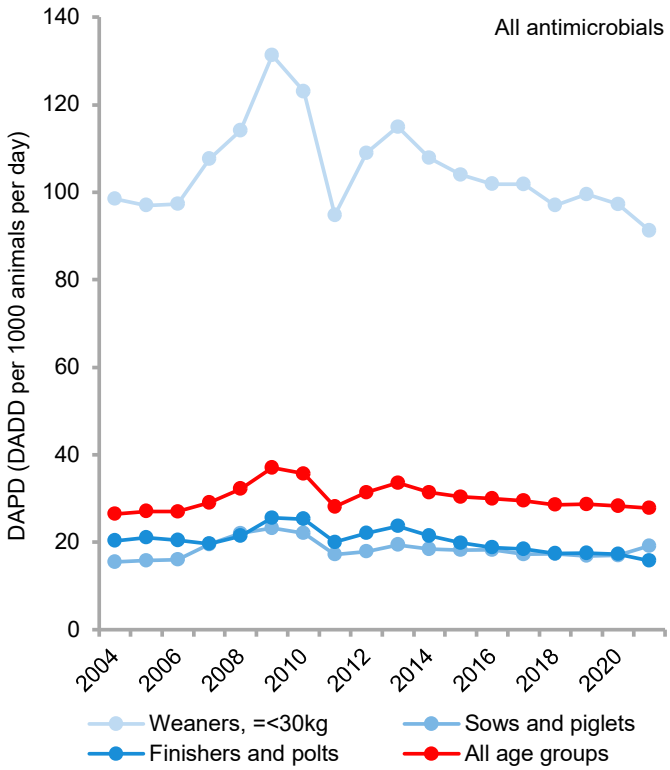
Even though there was a clear decrease in the antimicrobial use in pigs when inspecting crude consumption data (Table 4.1), the changes in the overall treatment proportion are more subtle and vary between age groups and antimicrobial classes (Figure 4.3 and 4.4). Similar to previous years, on a given day in 2021, approximately 2% of sows and piglets and finishers, and approximately 9% of weaners were treated with antimicrobials.

Tetracyclines have been some of the most commonly used antimicrobials in the Danish pig production, especially for treatment of gastrointestinal disease in weaners and finishers, usually administered orally. The overall use of tetracyclines has decreased since 2013, and in 2021 the treatment proportion was at the lowest level registered in the last 16 years, with the most marked changes following the recent adjustments to the Yellow Card initiative (Figure 4.4).

The proportion of weaners treated with tetracycline on any given day has decreased from approximately 4% (45 DAPD) in 2010 to less than 2% (18 DAPD) in 2021. In contrast, the use of other antimicrobial agents has increased, particularly the use of aminoglycosides (mainly neomycin), macrolides, and b-lactamase sensitive penicillins (Figures 4.3 and 4.4). Similar shifts between antimicrobial classes have been observed also in the other age groups.

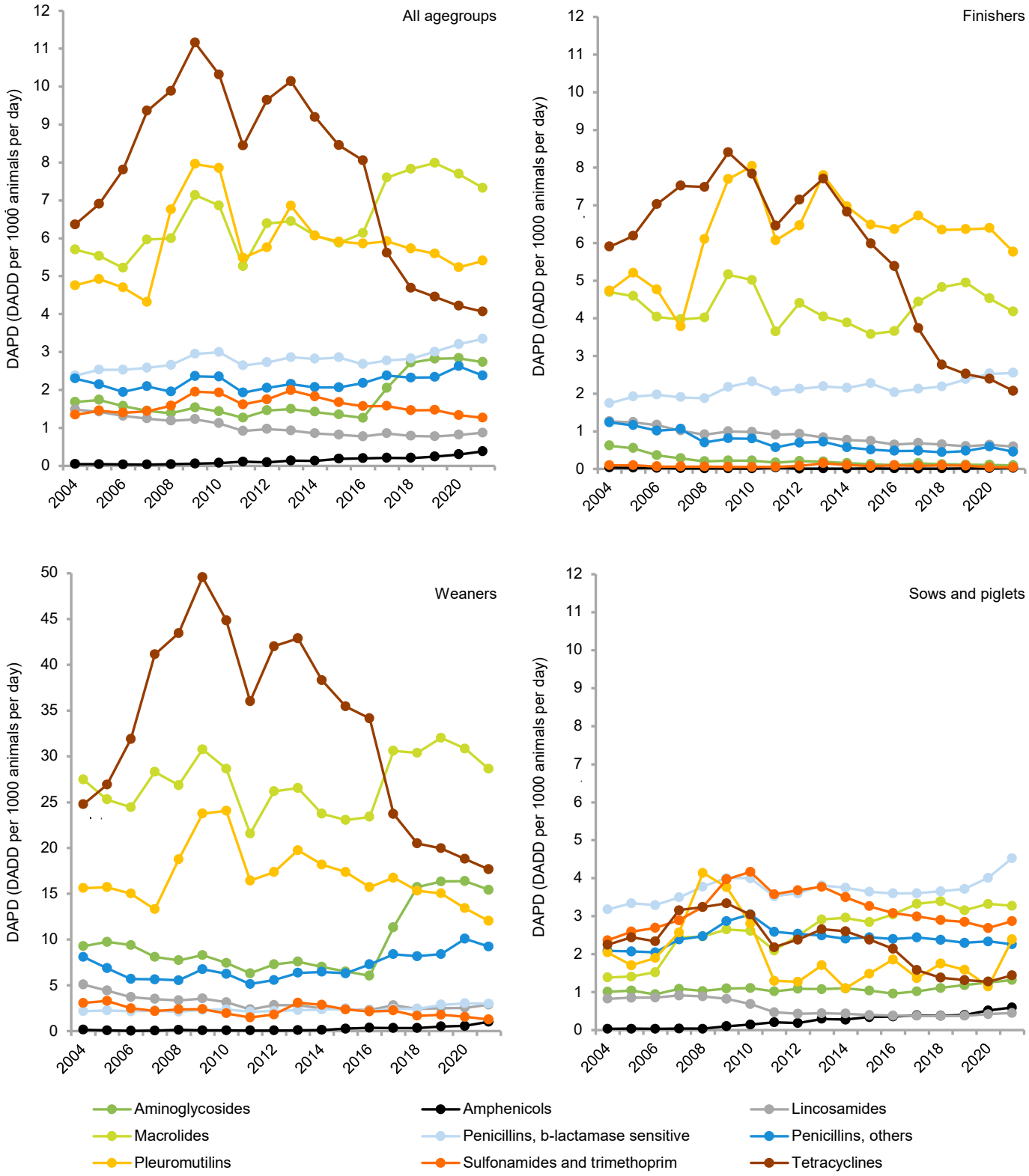
No use of the critically important antimicrobial agents fluoroquinolones and 3rd and 4th generation cephalosporins was registered for use in pigs in 2021 (Table 4.1).

Figure 4.3 Total antimicrobial use in the pig production, DAPD, Denmark DANMAP 2021



"Sows and piglets" includes treatment in boars. DAPDs are calculated as the number of standard doses for one kg animal divided by the estimated live biomass in the age group of the total population (in tonnes)

Figure 4.4 Antimicrobial use in the total pig production and in each age group, DAPD, Denmark DANMAP 2021



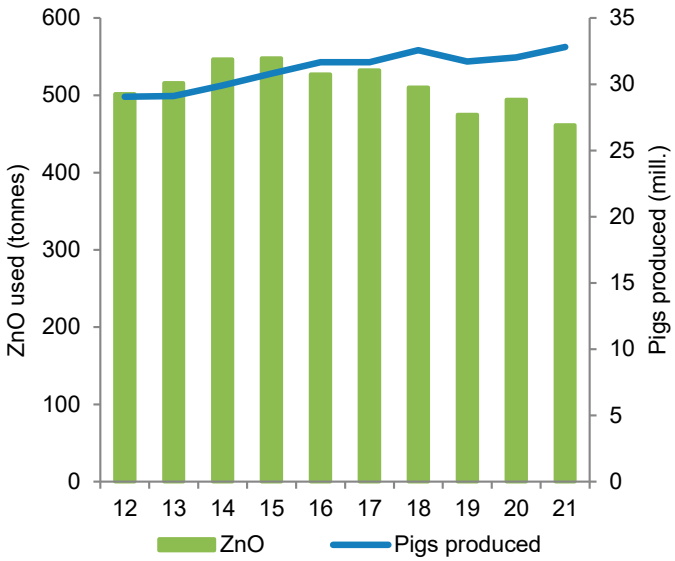
DAPDs are calculated as the number of standard doses for one kg animal divided by the estimated live biomass in the age group or the total population (in tonnes)
The age group “sows and piglets” includes treatment in boars, where boars constitute 4-5% of the estimated live biomass for the age group

Use of medical zinc

Monitoring the use of medical zinc is relevant because its use may select for antimicrobial resistance in some bacteria, including MRSA. Medical zinc, in the form of zinc oxide, is prescribed to piglets after weaning to prevent or treat diarrhoea, and approximately 90% of prescribed zinc oxide is used in weaners. The use of zinc oxide for Danish pigs peaked at 548 tonnes in 2015 (Figure 4.5).

In 2017, the European Commission announced an EU-wide withdrawal of medical zinc for pigs effective from June 2022. In 2016, the Danish pig industry launched an action plan to help pig producers reduce the use of medical zinc. An updated action plan followed in 2018, and a number of research projects are on-going and web tools to help producers reduce the use, have been developed. The use of medical zinc is shown Figure 4.5. In 2021, the pig sector used approximately 462 tonnes of medical zinc. Overall, the use has decreased since 2015 and in 2021 it was 16% (equivalent to 87 tonnes) lower than in 2015 but remains at a level, far from the target of zero use by June 2022.

Figure 4.5 Usage (in tonnes) of medical zinc - zinc oxide (ZnO) and zinc (Zn) - in the pig production, Denmark DANMAP 2021



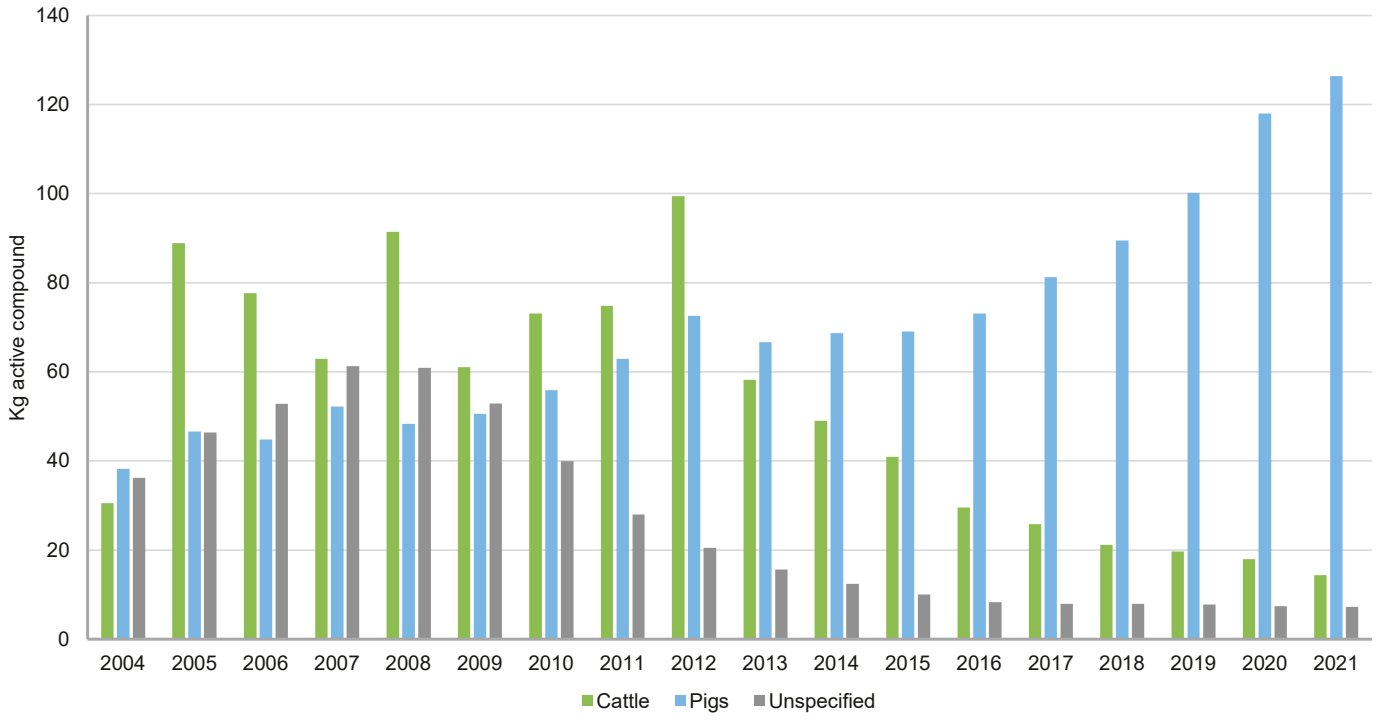
Note: The most commonly used product is zinc oxide (ZnO) which contains 80% zinc and which is largely insoluble in water

Topical use of chlortetracycline

For some antimicrobials, DAPD's are not calculated due to the way they are administered. This applies to products used topically as spray or ointment, for example chlortetracycline. Figure 4.6 shows the trends for topical use of chlortetracycline in pigs and cattle.

The overall use of chlortetracycline for topical administration peaked in 2008. Since 2012 a steady decrease has been observed in cattle, while the use in pigs has inceased steadily since 2016.

Figure 4.6 Usage (kg) of chlortetracycline used for topical use in pigs and cattle, Denmark DANMAP 2021



4.3.2 Antimicrobial consumption in cattle

Legislation-supported thresholds for antimicrobial use in cattle have been in place since 2011. In 2021, approximately 9.4 tonnes were recorded for use in cattle, of which 485 kg were used for intramammary therapeutic or dry-cow treatment. Around 70% of the antimicrobials used for cattle, were used to treat adult cattle (Table 4.1). The production of veal and beef has remained relatively stable over the past 5-10 years, while the production of milk has increased (Table 3.1).

Measured in kg active compound, there has been a gradual decrease in the overall use of antimicrobials for systemic treatment in adult cattle over the past decade. The consumption was 6% lower in 2021 compared to 2017 and 21% lower than in 2012. Measured in treatment proportion, the use for adult cattle has been approximately 2-3 DAPD since 2012, and in 2021 it was 2.3 DAPD, compared to 3.1 DAPD in 2012.

The main indication for systemic treatment in adult cattle was mastitis, and beta-lactamase sensitive penicillin accounted for 61% of the antimicrobials used in this age group (Figures 4.7 and 4.8).

In 2021 a total of 2,838 kg was used in young cattle. The treatment proportion was more than twice as high as in the older cattle (6.6 DAPD in 2021). The main indication for systemic treatment in calves is respiratory disease followed by joint/limb and gastrointestinal infections

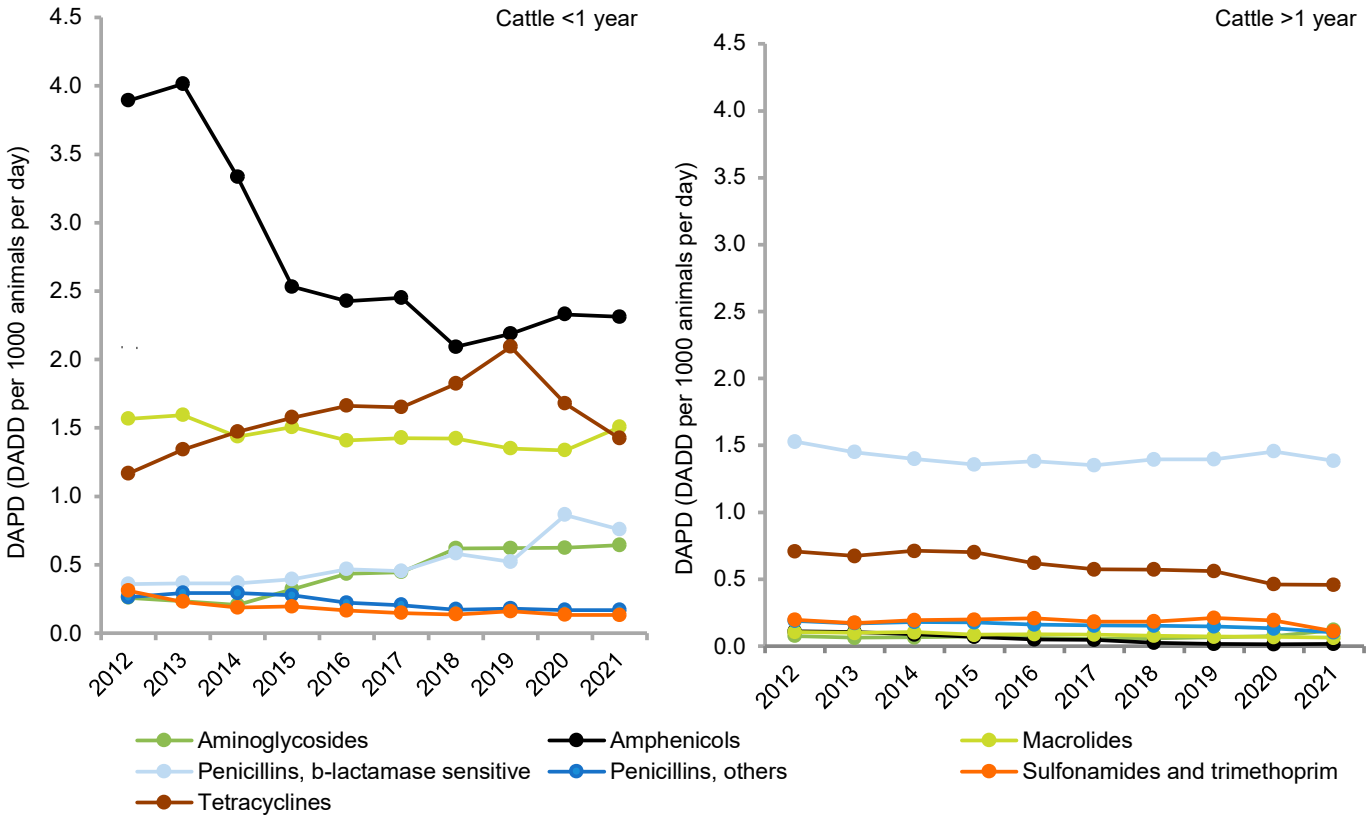
In calves and young cattle, the overall treatment proportion increased from 4.8 DAPD in 2012 to 6.6 DAPD in 2021. The use of amphenicols (florfenicol) has decreased, while the use of tetracyclines and b-lactamase penicillins have increased somewhat. Amphenicols remain the most frequently prescribed antimicrobial class (33%), followed by macrolides (22%) and tetracyclines (20%).

The use of fluoroquinolones in cattle has been close to zero for the last decade. Fluoroquinolones are only prescribed in food-producing animals as a last-line drug, based on microbiological analysis and susceptibility testing in an accredited laboratory. Use of fluoroquinolones in food-producing animals is also notifiable to the DVFA. No fluoroquinolones were registered for use in cattle in 2021.

In 2014, the cattle industry began to phase out the use of 3rd and 4th generation cephalosporins used for systemic treatment (orally and parenterally), resulting in a significant drop in 2015, and the annual usage stabilised at approximately 10 kg. In 2019, the cattle industry implemented a voluntary ban on use of 3rd and 4th generation cephalosporins in all cattle. No use of these agents was registered in 2020 or in 2021.

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

DANMAP 2021



DAPDs are calculated as the number of standard doses for one kg animal divided by the estimated live biomass in the age group (in tonnes)
Amphenicols - the treatment proportions of amphenicols in young cattle differ from previous reports, due to missing data in the old VetStat

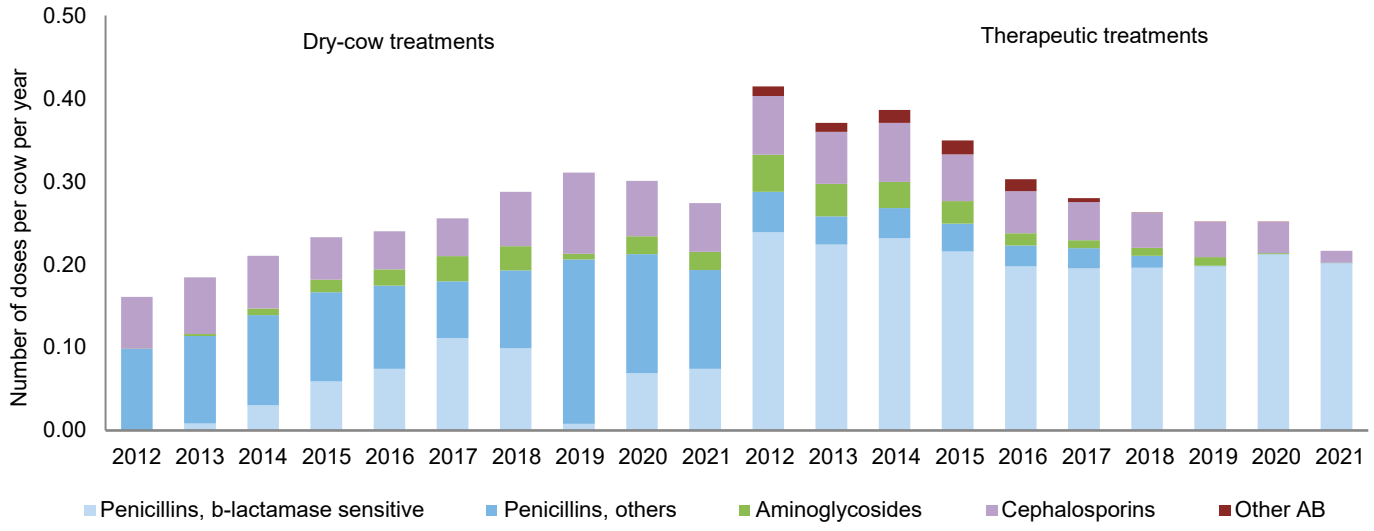
The board of Danish dairy and beef producers' strategy for good udder health has aimed at a 20% reduction in use of antimicrobials for treatment of mastitis and other cattle diseases (compared to 2012) as well as a lowering of geometric mean bulk tank cell counts to 150,000 by the year 2020. The dairy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

In 2021 the overall antimicrobial use in cattle was 21% lower than in 2012 and the the bulk tank cell counts were historically low, at 192,000 in February 2021.

Most antimicrobials administered parenterally in cattle are used in dairy cows, primarily to treat mastitis. The use of intramammary treatment, measured in doses per cow per year, is shown in Figure 4.8. The use of beta-lactamase sensitive penicillins has increased, whereas the use of 1st generation cephalosporins has decreased.

In 2019, there was a noticeable shift in treatments. The use of the beta-lactamase sensitive benzylpenicillin decreased, while the use of the extended spectrum penicillins, especially cloxacillin, increased. This shift was caused by a product shortage, where the only beta-lactamase sensitive benzylpenicillin product for dry-cow treatment was unavailable for longer periods of 2019, and extended spectrum penicillins, especially products containing cloxacillin, had to be used instead [Personal communication; Michael Farre, Danish Agriculture and Food Council]. In 2020 and 2021 the use shifted again towards beta-lactamase sensitive penicillins (Figure 4.8).

Figure 4.8 Use of antimicrobial agents for intramammary application in cattle, DAPD, Denmark DANMAP 2021



4.3.3 Antimicrobial consumption in poultry

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

Previously, VetStat has not allowed for easy differentiation of antimicrobial use in the 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.

In 2021, the usage was at its lowest since 2013 (Table 4.2), and less than half the amount used in 2020. The decrease was driven particularly by lower use in broilers and in turkeys [personal communication, Mie Nielsen Blom, Danish Agriculture and Food Council]. Decreases were seen mainly for tetracyclines prescribed for respiratory disease. For the past decade, cephalosporins have not been used in the poultry industry, and the use of fluoroquinolones has been close to zero. Colistin has not been used since 2016.

Table 4.2 Use of antimicrobial agents in poultry, kg active compound, Denmark DANMAP 2021

	Aminoglycosides	Amphenicols	Fluoroquinolones	Lincosamides	Macrolides	Other Ab[a]	Penicillins, b-lactamase sensitive	Penicillins, others	Pleuromutilins	Sulfonamides and trimethoprim	Tetracyclines	Total
2012	28	5	<1	14	278	3	32	180	8	23	150	722
2013	36	9	<1	18	293	1	172	220	4	62	488	1303
2014	21	9	<1	10	400	2	133	374	<1	83	604	1636
2015	258	4	1	129	133	9	204	566	<1	446	817	2569
2016	60	5	<1	24	176	8	265	258	<1	111	765	1671
2017	65	5	<1	32	245	1	356	335	<1	85	487	1610
2018	51	0	<1	25	195	0	358	243	<1	37	521	1430
2019	55	<1	<1	27	275	0	368	234	<1	64	694	1719
2020	58	0	<1	29	157	0	334	237	<1	55	1588	2458
2021	53	0	0	25	169	0	112	188	<1	33	616	1196

Data for 2021 were extracted from VetStat on 24 August 2022
a) Other AB include other quinolones and polymyxins

4.3.4 Antimicrobial consumption in aquaculture and companion animals

Aquaculture

Antimicrobial consumption in aquaculture is partly driven by the summer temperatures because bacterial diseases are more likely to occur when temperatures are high. However, in recent years the industry has also been challenged by disease outbreaks that have affected the use antimicrobials. The aquaculture industry continued focus on developing improved vaccination strategies to reduce the risk of diseases that may require antibiotic treatment. In 2021, the antimicrobial use decreased compared to previous years and was at the same level as in 2017 (Table 4.3).

Table 4.3 Use of antimicrobial agents for aquaculture, kg active compound, Denmark DANMAP 2021

	Amphenicols	Other Ab[a]	Other quinolones	Penicillins, others	Tetracyclines	Hovedtotal
2012	162	1	539	8	18	2920
2013	180	<1	943	10	2	3414
2014	297	0	1678	10	0	5117
2015	311	0	1005	5	<1	2976
2016	314	<1	893	14	<1	2307
2017	350	<1	637	35	<1	1702
2018	323	<1	899	52	<1	3568
2019	293	0	447	44	22	2526
2020	341	<1	565	27	1	1965
2021	296	<1	366	20	<1	1771

Data for 2021 were extracted from VetStat on 24 August 2022
a) Other AB include aminoglycosides and lincosamides

Mainly three compounds are used to treat bacterial infections in aquaculture: sulfonamide/trimethoprim (61%), 1st generation quinolones (21%), and amphenicols (17%) (Table 4.3). The new VetStat also includes new categories for aquaculture e.g. salmon and sturgeon. This will enable more detailed analyses of usage data for aquaculture in the coming years.

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 use registered for companion animals (pets and horses). This table includes the category "unspecified", which are products typically used for companion animals, but where the animal species was not registered.

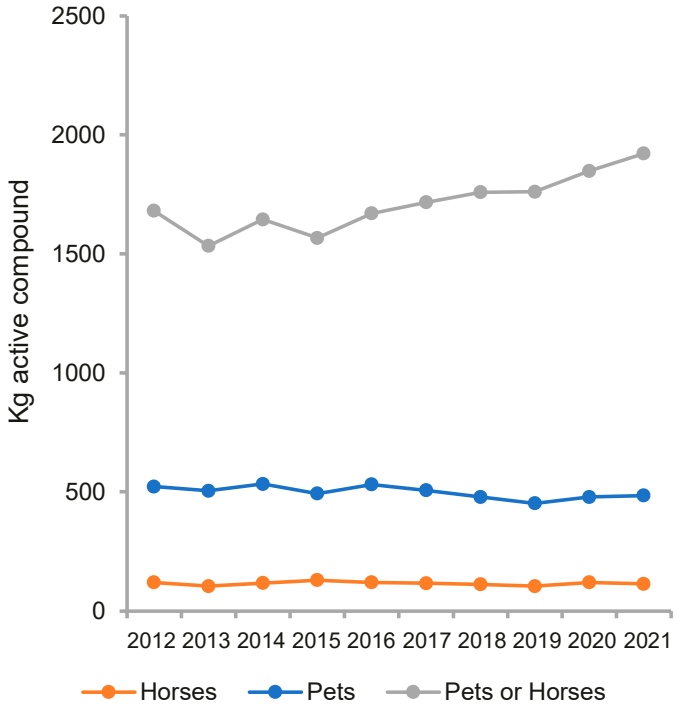
The total amount of antimicrobials estimated for use in companion animals was 2,520 kg in 2021 (Table 4.4, Figure 4.8), which represents a 3% increase compared to 2020 and an 8% increase compared to 2017. For most of this, the animal species treated was not specified. Furthermore, the total amount of antimicrobials for companion animals is somewhat underestimated, since any products registered for use in humans, but prescribed for animals, has not been included.

Table 4.4 Estimated use of antimicrobial agents for horses and pets, kg active compound, Denmark DANMAP 2021

	Aminoglycosides	Amphenicols	Cephalosporins	Fluoroquinolones	Lincosamides	Macrolides	Other Ab[a]	Penicillins, b-lactamase sensitive	Penicillins, others	Pleuromutilins	Sulfonamides and trimethoprim	Tetracyclines	Total
Horses													
2012	<1	1	<1	<1	0	0	<1	8	<1	0	105	3	119
2013	1	2	<1	<1	0	0	<1	8	<1	0	87	5	104
2014	1	<1	<1	<1	0	<1	<1	9	<1	0	98	7	116
2015	3	<1	<1	<1	<1	<1	<1	7	<1	0	114	5	130
2016	<1	<1	<1	<1	0	0	<1	5	<1	0	108	5	120
2017	<1	<1	<1	<1	0	0	<1	5	<1	0	106	3	116
2018	<1	<1	<1	0	0	<1	<1	6	<1	0	101	4	111
2019	<1	0	<1	<1	0	<1	<1	5	<1	0	94	4	104
2020	<1	0	<1	<1	0	0	<1	4	<1	0	111	4	120
2021	<1	0	<1	<1	<1	<1	<1	5	<1	0	105	2	113
Pets													
2012	4	<1	79	5	17	6	6	9	110	<1	264	21	522
2013	4	<1	75	5	17	3	6	8	114	1	252	19	505
2014	6	<1	81	5	19	5	8	12	122	<1	261	13	533
2015	5	4	62	6	22	3	7	13	123	2	226	20	493
2016	3	3	55	5	22	2	7	10	131	<1	269	21	531
2017	4	<1	42	5	18	2	8	9	126	<1	272	19	507
2018	4	<1	36	5	18	2	15	10	114	<1	253	21	478
2019	4	<1	32	4	17	7	15	10	108	<1	237	15	451
2020	4	<1	31	5	19	4	18	13	103	<1	262	18	478
2021	3	<1	28	5	19	2	21	11	100	<1	271	24	485
Unspecified													
2012	21	<1	194	9	52	<1	28	1	424	<1	937	16	1681
2013	18	<1	155	9	47	<1	26	1	416	0	844	17	1534
2014	18	<1	132	8	50	0	27	2	420	0	968	20	1645
2015	14	<1	96	9	46	<1	26	1	414	0	945	16	1567
2016	15	<1	82	10	48	<1	26	2	456	0	1014	16	1670
2017	15	<1	69	9	48	<1	28	2	459	0	1071	15	1716
2018	14	1	61	10	44	0	35	2	443	0	1136	13	1759
2019	14	<1	61	10	47	<1	37	2	435	0	1139	16	1761
2020	10	<1	57	11	50	0	40	3	441	0	1221	16	1848
2021	11	<1	49	10	55	0	47	<1	452	0	1282	14	1922

Data for were extracted from the new VetStat 24 August 2022
The estimates include all antimicrobial agents registered, by the either the pharmacy or veterinarians for use in horses, pets, as well as products typically used for companion animals, but without a specified animal species (unspecified)
a) Other AB include other antibacterials (27%), other otologicals (52%), pleuromutilins, polymyxins and sulphonamides (plain)

Figure 4.9 Estimated use of antimicrobial agents for horses and pets, kg active compound, Denmark DANMAP 2021



Data for were extracted from the new VetStat 24th August 2022
The estimates include all antimicrobial agents registered, by the either the pharmacy or veterinarians for use in horses, pets, as well as products typically used for companion animals, but without a specified animal species (unspecified)

A large proportion of antimicrobials used for dogs and cats are prescribed for the treatment of chronic or recurrent disease, mainly dermatitis. Due to the close contact between owners and their pets, repeated use of critically important antimicrobials may pose a risk to the owners, and the use of these antimicrobials is monitored carefully. Since the treatment guidelines by DVA were published in November 2012, the use of cephalosporins has been reduced by 72% (-195 kg).

The use of fluoroquinolones in companion animals was 15 kg and represented 90% of all fluoroquinolones used in all animals in 2021. Similarly, the pets accounted for 60% of all the cephalosporins used in animals (Tables 4.1 and 4.4). In 2021, 3rd and 4th generation cephalosporins were only used for treatment of pets in the amount of 1 kg active compound (only products registered for veterinary use).

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Textbox 4.1

The new VetStat

In June 2021, the Danish Veterinary and Food Administration launched the new and updated VetStat data platform. VetStat is the Danish database collecting use data on veterinary prescription medicines. VetStat also includes the register of authorized Danish veterinarians, their practices and their Health Advisory Service Contracts.

Data regarding use of antimicrobials, vaccines and medical zinc oxide are extracted from the VetStat database for annual reporting in the DANMAP report. At the Danish Veterinary and Food Administration, VetStat serves as an important tool in the continuous monitoring of use of veterinary prescription medicines, and data are used as the foundation of multiple initiatives, such as identifying future focus areas, the yellow card initiative and for inspection purposes, just to mention a few.

VetStat was originally launched in 2000. Since then, technology has taken major steps and the new VetStat contains an array of updates and new functions. Key functionalities related to the collection of antimicrobial use data are updated and include a changed way in which products are registered in the database and a new validation tool. These two major changes are described in the following paragraphs. All the new features and updated design can be found at www.vetstat.fvst.dk.

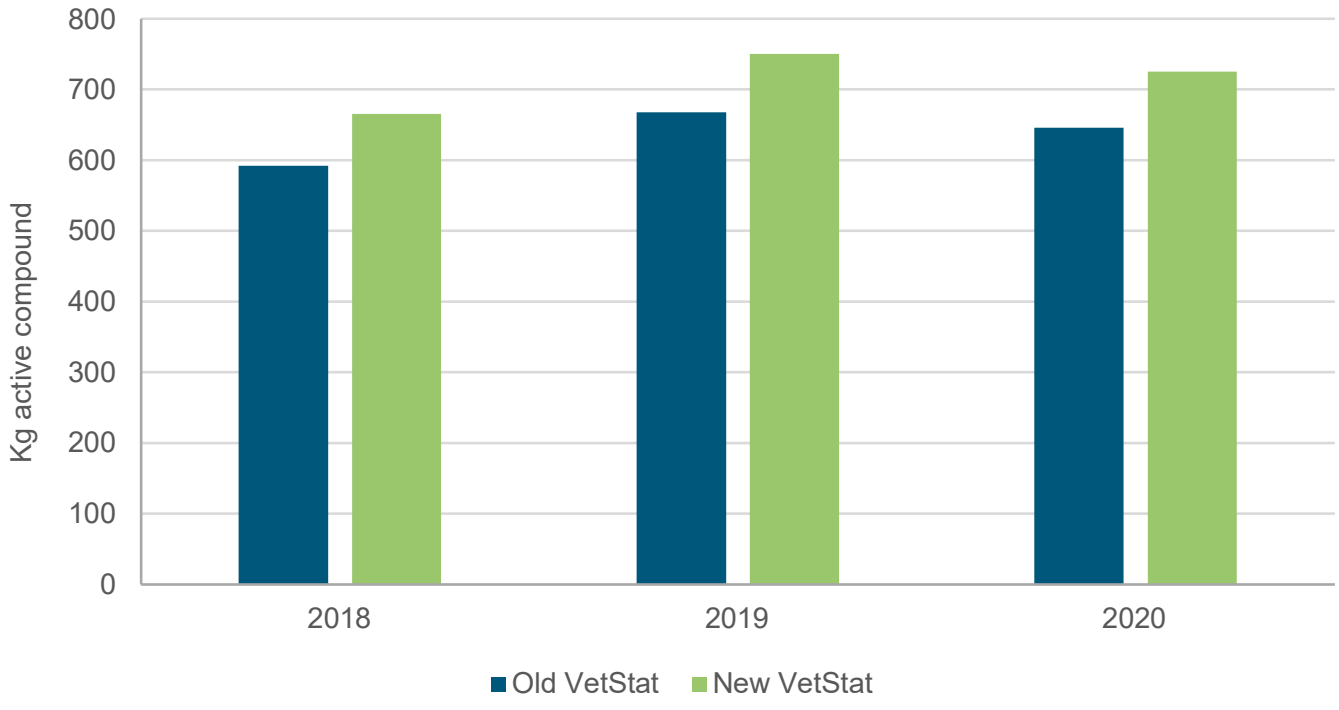
Changes in registration of products

To make registration of veterinary medicinal products less time consuming and to align to the way human medicinal products are registered, new VetStat was designed to automatically transfer information regarding medicinal products licensed in Denmark from Medicinpriser (the Danish Medicinal Register) to VetStat.

In the old VetStat each product was registered with the concentration of the base formulation of the active substance, if available. In Medicinpriser, many products are registered with the concentration of the salt formulation of the active compound. This shift in registration method affects the total amount of active compound calculated but does not represent an actual change in medicine used. All VetStat data from 2000 until today has been updated according to this new methodology.

Figure 1 Kg active compound used of Doxylin Vet. in 2018 – 2020, calculated using old and new VetStat data; the number of packages is the same

DANMAP 2021



continued ... Textbox 4.1

An example showing an actual consequence of the change is presented in Figure 1. In the old VetStat, Doxylin Vet. was registered with 445 mg/g and in the new VetStat Doxylin Vet. is registered with 500 mg/g. The figure shows the total amount of kg active compound calculated using data from old and new VetStat.

Although certain products have an altered registered concentration in the new VetStat, all data have been recalculated based on this, and the political target on antibiotic consumption in pigs and the total antibiotic consumption has not been negatively affected by this shift.

New validation tool

Ensuring data quality and validity were key focus points in developing the new VetStat. Pharmacists are obligated to keep records of all prescriptions distributed and prescription medicines supplied. Data validation on each record VetStat receives ensures that the records hold valid information in all fields. If not, the records are sent to either the veterinarian, the feed mill or the VetStat-administration for correction of errors. Pharmacy generated errors are, in most cases, forwarded to the veterinarian. The veterinarians are responsible for the prescription and therefore responsible for the records reported to VetStat by the pharmacy. Veterinarians and feed mills are obligated to correct errors within a certain time.

In the first six months following the launch of the new VetStat, the Danish Veterinary and Food Administration received close to 1.4 mill records of which approximately 300,000 included one or more errors (Table 1). This was unexpected and shows that even with a long history of collecting prescription data, the task of ensuring data quality is not easy. Some types of errors were due to 'too strict' validation rules, but most of the errors were due to deficient reporting and lack of understanding of the later use of the data. It demonstrates, that clear communication between the system owner (the Danish Veterinary and Food Administration) and the data reporter (veterinarian, feed mill and pharmacy) is highly important for success. Both in the early stage of the development, and in the period up towards the launch of a new system. It is of great importance to ensure a mutual understanding of the task, the different responsibilities and the necessity for valid data. Judged by the amount of errors, this task has not been lifted to complete success yet, and the Danish Veterinary and Food Administration is currently focused on finding, correcting and stopping errors from arising.

Table 1. Common errors seen in the first six months after the launch of the new VetStat DANMAP 2021

Product information	Veterinarian and practice	Herd and farm	Animal information
Product is no longer on the market	ID of veterinarian does not exist	Herd ID does not exist	Animal species does not exist on the farm
Amount is too big or small compared to the pack size	ID of practice is identical to ID of veterinarian		Age group does not correspond to animal species
Product is not known in the system			

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Textbox 4.2

New legislation on antibiotic treatment of mastitis

With effect from 1 June 2021, the requirements for antibiotic treatment of mastitis were amended. The changes were requested by the industry due to increased knowledge on mastitis microbiology, increased technological diagnostic capability and a wish to target and minimize the antibiotic consumption. The aim of the amendments is to ensure prudent use of antibiotics and up-to-date and consistent quality of diagnostic work in private veterinary practices. The adjustments were based on recommendations from a working group on mastitis with participation from The Danish Veterinary Association, Danish Agriculture & Food Council, SEGES Innovation and the Technical University of Denmark.

The main changes:

- A milk sample must be taken prior to antibiotic treatment of mastitis. The sample must be examined by a veterinarian or at an external qualified laboratory as soon as possible and no later than 7 days after initiation of the antibiotic treatment. No later than 7 days after the result of the examination is available, the veterinarian must report the result to the industry-owned register "Kvægdatabasen" ("the cattle database").
- Antibiotic treatment of mastitis is allowed only with simple penicillins. However, the veterinarian may extraordinarily prescribe other antibiotics, and only if the veterinarian personally initiates the treatment.
- In herds covered by an advanced Veterinary Advisory Service Contract the veterinarian may, in herds with a herd diagnosis of mastitis, for a period of up to 12 months deviate from the requirement of using simple penicillins only. However, this is limited to cases where resistance to simple penicillins has been demonstrated within the past 6 months in at least 5 cows diagnosed with the same mastitis-pathogen, and where penicillin resistance has been documented with a minimal inhibitory concentration (MIC) test.
- Dry cow treatment, i.e. antibiotic treatment of dairy cows at the introduction of the dry period, is allowed without bacteriological examination of a milk sample, if the cow, within the last 4 months prior to the dry period, has had a somatic cell count above 200,000 cells/mL in two examinations with at least 3 weeks interval.
- Treatment with 3rd and 4th generation cephalosporins is allowed only if a MIC test documents resistance to other antibiotics. The MIC test must be performed in a laboratory using an accredited method. This latter change is not limited to mastitis but applies to any treatment of production animals with 3rd or 4th generation cephalosporins.

Legislation
The requirements for antibiotic treatment of mastitis can be found in:

- Executive order no 927 of 21 June 2022 on animal owner's use of veterinary medicine etc.
- Executive order no 2542 of 15 December 2021 on veterinarians' use, dispense and prescription etc. of veterinary medicine
- Executive order no 992 of 25 May 2021 on veterinary advisory service contracts for cattle herds

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5

ANTIMICROBIAL CONSUMPTION IN HUMANS

5. Antimicrobial consumption in humans

Highlights

Total antimicrobial consumption in Denmark was 14.71 DID in 2021 which is the same as in 2020 but 18% lower than 10 years ago in 2012 (17.98 DID). This seems to show that the lower levels of consumption observed during the COVID-19 pandemic in 2020 continued. However, analysis of monthly antimicrobial consumption data showed that consumption increased from August 2021, i.e. following the lifting of almost all COVID-19-related restrictions, to similar levels seen in corresponding months in 2018 and 2019.

In primary health care, total antimicrobial consumption was 12.86 DID in 2021, similar to 2020 (12.83 DID) and 20% lower than a decade ago in 2012 (16.03 DID). Penicillins constituted 64% of the consumption and the group of penicillins with extended spectrum was the most used (25% of total consumption). After a significant drop in prescriptions for respiratory infections in 2020, numbers started to increase from August 2021 to levels seen in pre-pandemic years.

Analysis of consumption by age groups showed a 16% higher consumption of antimicrobials in 0-4 year olds in 2021 compared to 2020 following a 38% reduction from 2019 to 2020. Among 15-24 year olds, consumption also increased (6%) in 2021 after significant decreases observed from 2019 to 2020. However, the consumption of antimicrobials among 5-14 year olds continued to decrease (11% lower in 2021 than in 2020). In older age groups, total consumption showed only minor changes (max. 2%) between 2020 and 2021.

Consumption in hospital care measured in DID (i.e. not accounting for hospital activity) was 1.81 DID in 2021, similar to 2020 (1.82 DID) and 3% lower than a decade ago in 2012 (1.86 DID). When measuring in DDD per 100 bed-days (DBD), the consumption in 2021 (124.01 DBD) was similar to 2020 (124.75 DBD) but 25% higher than a decade ago in 2012 (99.54 DBD).

The National Action Plan on the reduction of antibiotics in humans, 2016-2020, was extended to 2021 by the Danish Ministry of Health due to the significant impact of the COVID-19 pandemic on healthcare provision. **Goal 1** (reduction of total consumption in primary care to 350 prescriptions/1,000 inhabitants) was already achieved in 2020. Even though the consumption increased from 329 prescriptions/1,000 inhabitants in 2020 to 333 prescriptions/1,000 inhabitants in 2021, it stayed below the goal's threshold. **Goal 2** (a share of 36% of beta-lactamase sensitive penicillins out of the total consumption in primary care) has not been achieved. Their share was 28% in 2021. **Goal 3** (10% reduction of consumption of critically important antimicrobials [cephalosporins, fluoroquinolones and carbapenems] compared to 2016) was achieved in 2021. Consumption of critically important antimicrobials in hospitals fell by 11% from 2016 to 2021.

5.1 Introduction

In Denmark, only medical doctors, veterinarians and dentists are authorized to prescribe antimicrobials and sale is restricted to licensed pharmacies who have exclusive right to sell prescription-only medicines. 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 hospital sector since 1997.

Registration of medicines consumption in the primary care sector is based on sales from pharmacies to individuals and private clinics. Sales data contain information on the ATC code, formulation, package size and number of packages sold. They also include an identifier of the prescriber and the patient's age, gender and address. Since 2004, the Register of Medicinal Product Statistics also includes the indication for prescribing. All systemic antimicrobials are prescription-only medicines and with no over-the-counter sale. This allows for a very detailed and close to 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 have been 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. However, in Figure 5.1 the complete antimicrobial consumption in Denmark including data from all healthcare providers, both public and private sectors is included.

An upgraded version of The National Patient Register, implemented during 2019, changed the definitions of bed-days and other hospital activity measures, thus affected the calculations of the consumption of antimicrobials at hospitals; see new definitions in paragraph 9.8 in Chapter 9 'Materials and methods'.

Ongoing reorganization of the Danish healthcare system has led to functions being reassigned from hospital ambulatory care to smaller health units, rehabilitation centres and general practitioners. The resulting changes in activity across the healthcare sector, for example number of bed-days in hospitals, location of prescribers etc. need to be taken into account when interpreting antimicrobial consumption surveillance data and changes in consumption over time.

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. Additional antimicrobials included are metronidazole (ATC code P01AB01) and for hospitals vancomycin (ATC code A07AA09). Their consumption has been included in DANMAP since 2014. Consumption of tuberculostica, antifungal drugs and antiviral are not included in this chapter.

In January 2019, the WHO Collaborating Centre for Drug Statistics Methodology introduced new DDD values for some commonly used antimicrobials. These changes were implemented in the DANMAP 2018 report and data recalculated retrospectively. Therefore, figures in the present report are not directly comparable to figures included in reports preceding the DANMAP 2018 report.

Changes in consumption within the last decade have often followed initiatives calling for more prudent use of antibiotics due to concerns about increases seen in the past. In 2012, the National Antibiotic Council, a national cross-sectoral mechanism, was established following decisions on a national AMR strategy in 2010 that featured improved monitoring of and research in antimicrobial resistance and antibiotic use in humans and animals. In the following years, many different initiatives regarding proper use of antibiotics were undertaken on both sides; the `HAPPY AUDIT´ study on better diagnostics guiding antibiotic prescribing by general practitioners, updated antibiotic guidelines issued by different medical associations, and since 2013, annual - except for in the pandemic years 2020 and 2021 - antibiotic awareness campaigns aimed at the public were launched by the Ministry of Health.

The COVID-19 pandemic had significant impact on the Danish healthcare system, thus DANMAP started to analyse antimicrobial consumption data by month to provide more granular information on the impact of COVID-19 infection waves on prescribing.

5.2 Total antimicrobial consumption in the Danish healthcare system

During the first five years of systematic registration from 1996 to 2000, the consumption of systemic antimicrobials in Denmark showed no significant trends and consumption was estimated between 13 and 14 Defined Daily Dose per 1,000 inhabitants per day (DID). Calculations back then were based on slightly differently reported data and are thus not fully comparable to later years (consumption 20 years back was 14.66 DID, Figure 5.1). In the following years, between 2001 and 2011, consumption of antimicrobials increased steadily and peaked at a total of 18.95 DID in 2011 (not shown). Since 2011, consumption has decreased markedly (Figure 5.1).

In 2021, total consumption of antimicrobials was 14.71 DID (including all public and private healthcare facilities), which is similar to the consumption in 2020 (14.71 DID) but 18.2% lower than the consumption 10 years ago, i.e. in 2012 (17.98 DID) (Figure 5.1). In 2021, primary sector accounted for 12.86 DID, the somatic hospital sector for 1.81 DID whereas psychiatry, private hospital and unspecified use accounted for 0.04 DID. The total consumption in 2021 corresponded to 45,080 kg active compound consumed.

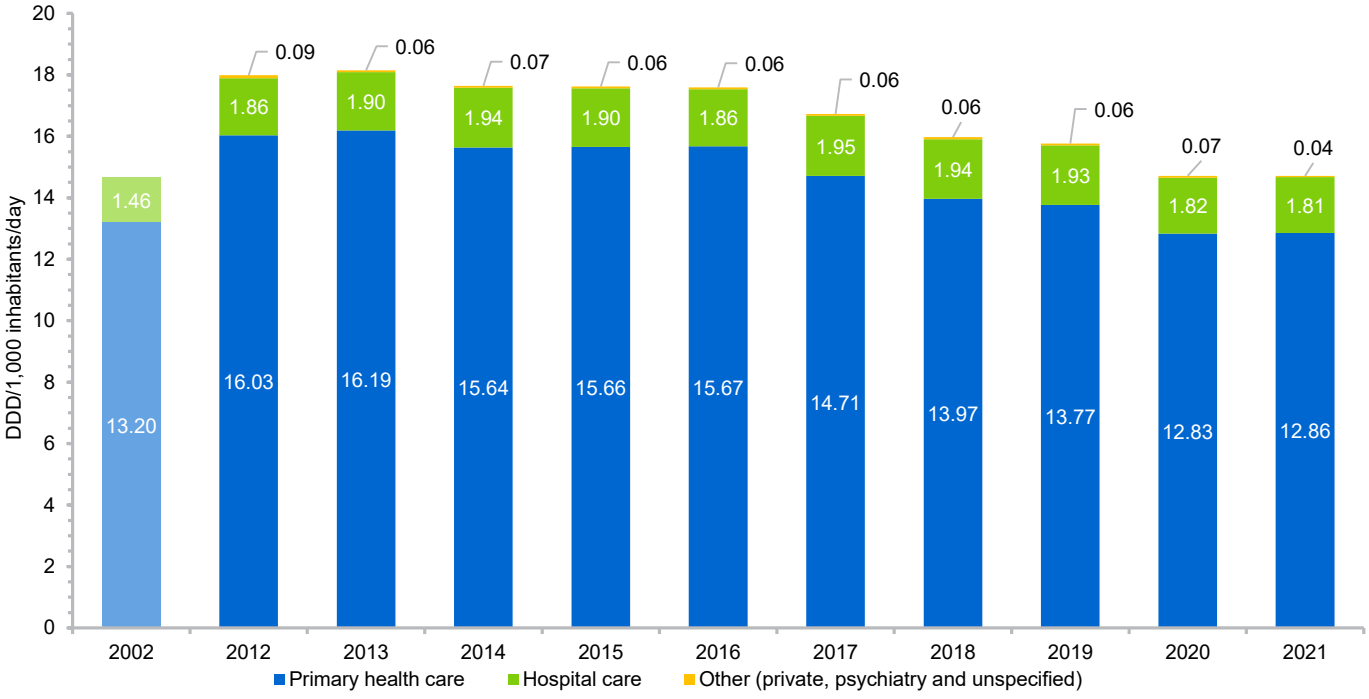
The decrease in total antimicrobial consumption since 2012 in Denmark has mainly been driven by reduced prescribing in primary health care, which accounted for 87% in 2021. Measured in DID and not adjusted for hospital activity, antimicrobial consumption at hospitals fluctuated over the years; increased from 2012 to 2014 and from 2016-2017. Since 2017, the consumption decreased, with a higher decrease from 2019 to 2020, probably due to the COVID-19 pandemic. In 2021, the consumption was the same as in 2020 (Figure 5.1). Thus, the share of total consumption in hospitals increased from 10.4% in 2012 to 12.3% in 2021.

The main antimicrobial drug classes and their consumption in primary health care and somatic hospitals are presented in Figure 5.2. Most notable are high use of beta-lactams in both health sectors and low use of cephalosporins/aminoglycosides as well as no use of carbapenems in primary health care.

Consumption of antimicrobials in primary health care and somatic hospitals in the five Danish health regions is presented in Figure 5.3. The consumption per 1,000 inhabitants showed marked decreases for all five regions in the primary sector since 2017. In 2021, the consumption was similar to 2020 (minor changes of max. 2%). Capital Region and Region Zealand, two neighbouring regions, showed highest total consumptions of 14.48 DID and 15.58 DID, respectively, in 2021, whereas Central Region of Denmark had the lowest total consumption of 13.41 DID.

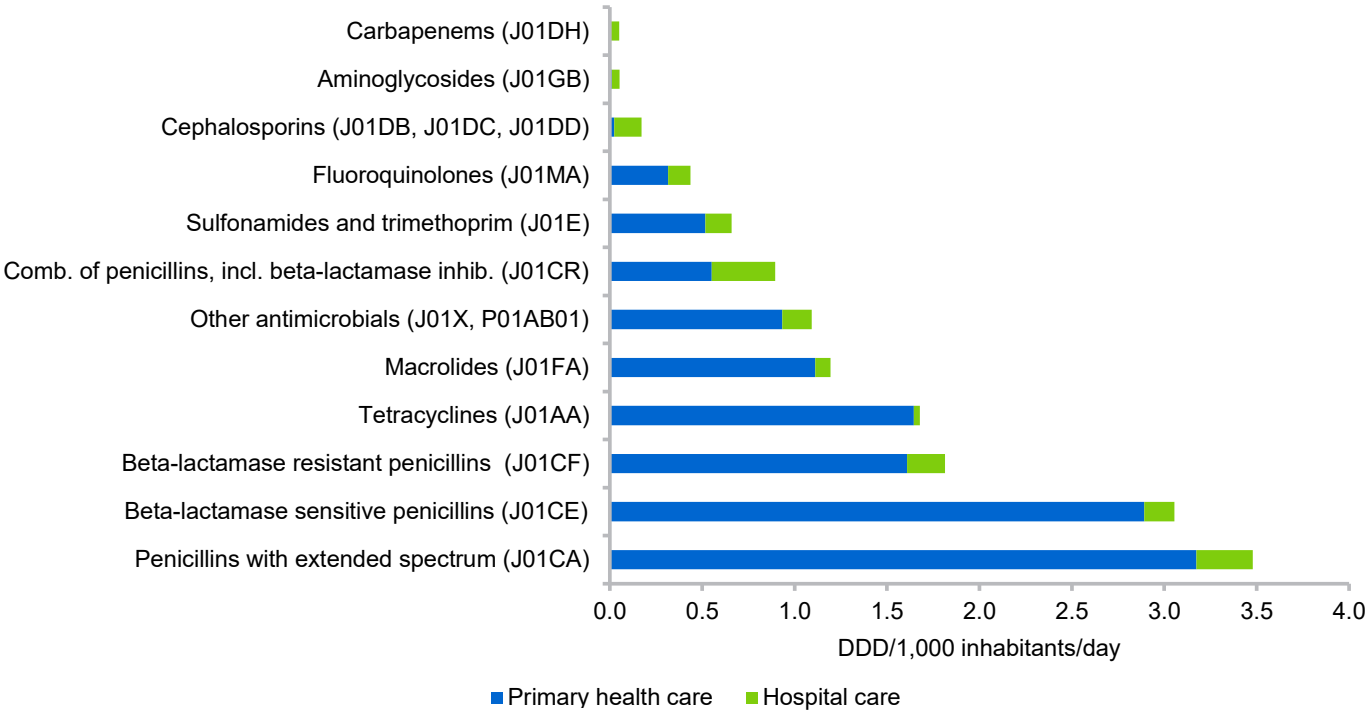
Figure 5.1 Total consumption of systemic antimicrobial agents in humans, DDD per 1,000 inhabitants per day, Denmark, 2002 and 2012-2021

DANMAP 2021



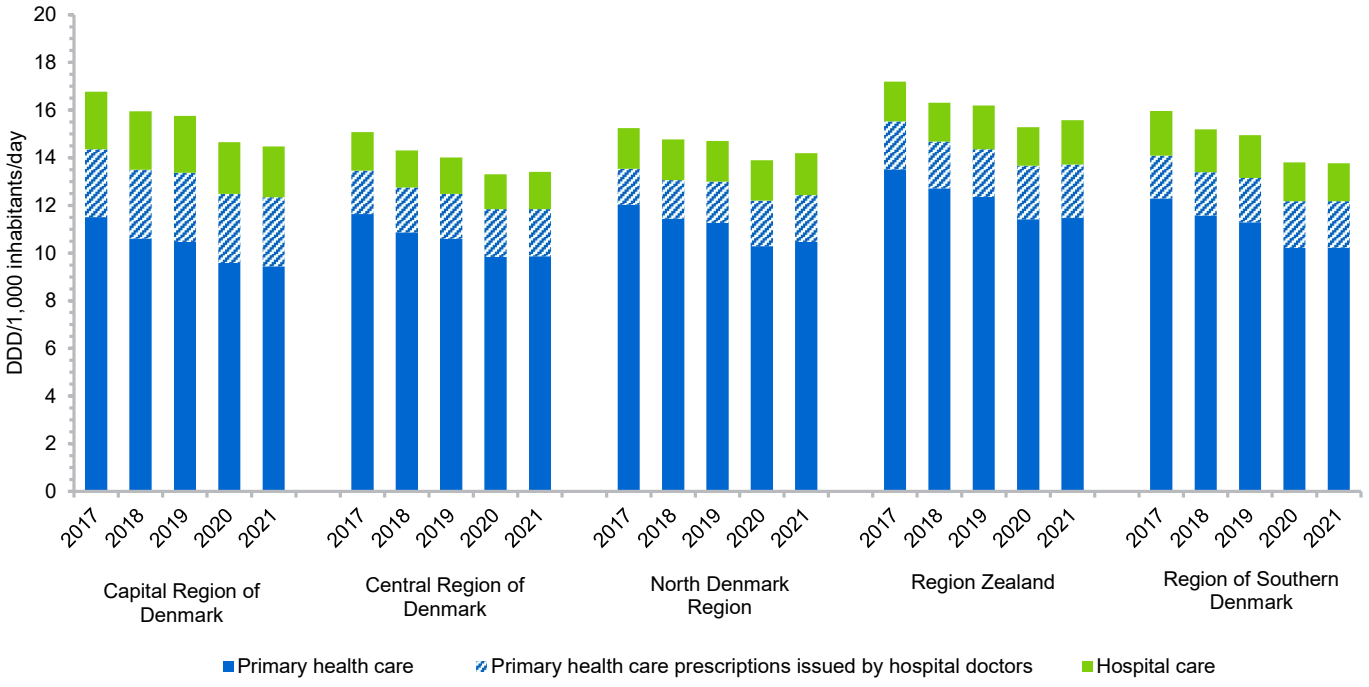
Data: Total sales in Denmark
Data source: Register of Medicinal Product Statistics and 2022 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 5.2 Distribution of main antimicrobial classes used for humans in primary and hospital care, DDD per 1,000 inhabitants per day, Denmark, 2021
DANMAP 2021



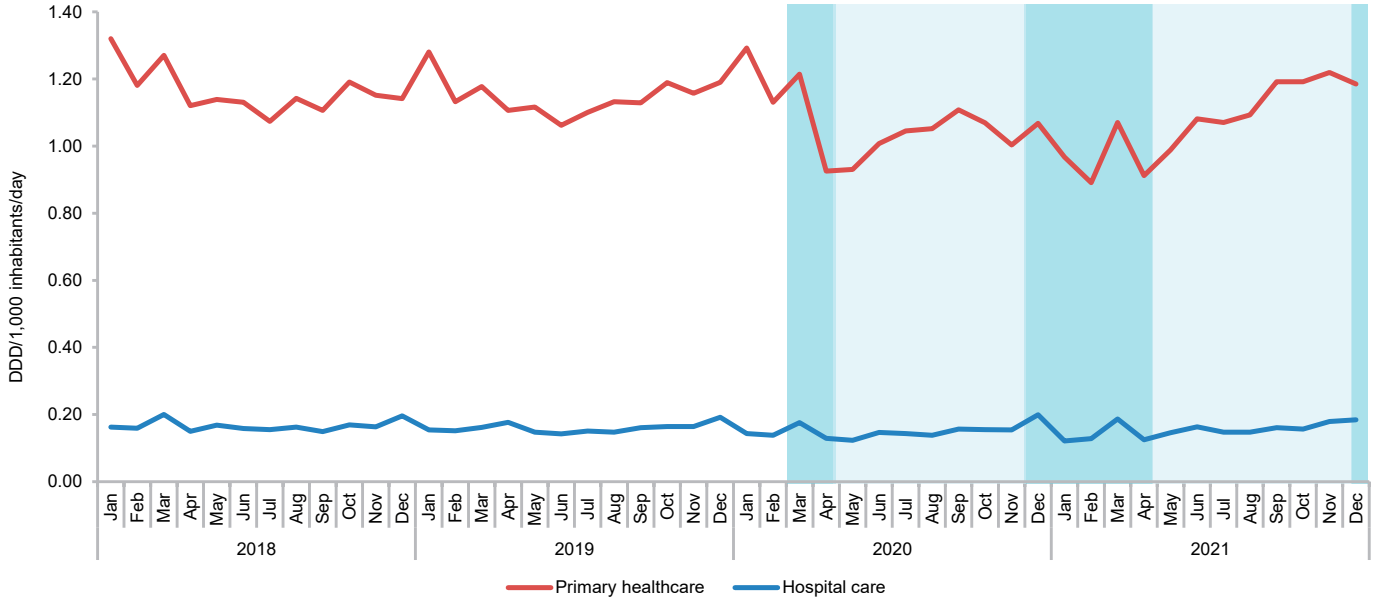
Data: Registered sales to individuals and consumption at somatic hospitals
Data source: Register of Medicinal Product Statistics and 2022 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 5.3 Consumption of systemic antimicrobial agents in primary health care and at somatic hospitals, DDD per 1,000 inhabitants per day, by Danish region, 2017-2021
DANMAP 2021



Data: Registered sales to individuals and consumption at somatic hospitals
Data source: Register of Medicinal Product Statistics and 2022 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 5.4 Total consumption of systemic antimicrobial agents in humans per month in primary health care and at hospitals, DDD per 1,000 inhabitants per day, Denmark, 2018-2021 DANMAP 2021



COVID-19 restrictions in place
Fewer restrictions in place
Data: Total sales in Denmark
Data source: Register of Medicinal Product Statistics and 2022 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Monthly data for antimicrobial consumption from 2018 to 2021 show the effect of the COVID-19 pandemic with its changes in healthcare delivery, infection rates and social life, Figure 5.4.

Total antimicrobial consumption in primary care measured in DID (Figure 5.4) was lower from April 2020 until May 2021 when compared to previous years but increased with the lifting of restriction in the summer of 2021. More detailed analysis of the monthly primary care consumption data can be found in Section 5.3 Primary health care.

In April 2020, total hospital consumption total hospital consumption measured in DID also fell below levels seen in previous years. However, levels returned to similar levels as measured in 2018/19 from August 2021. More detailed analysis of the monthly hospital consumption data can be found in Section 5.4 Hospital care.

For information on population size and hospital activity by health region, see Figure 2.2 and Table 2.1 in Chapter 2 'Introduction'.

A comparison of the total antimicrobial consumption in both the human and the animal sector is shown in Figure 4.1 in Chapter 4 'Antimicrobial consumption in animals'.

5.3 Primary health care

In the following sections, the consumption of antimicrobials in primary health care is described by using 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.

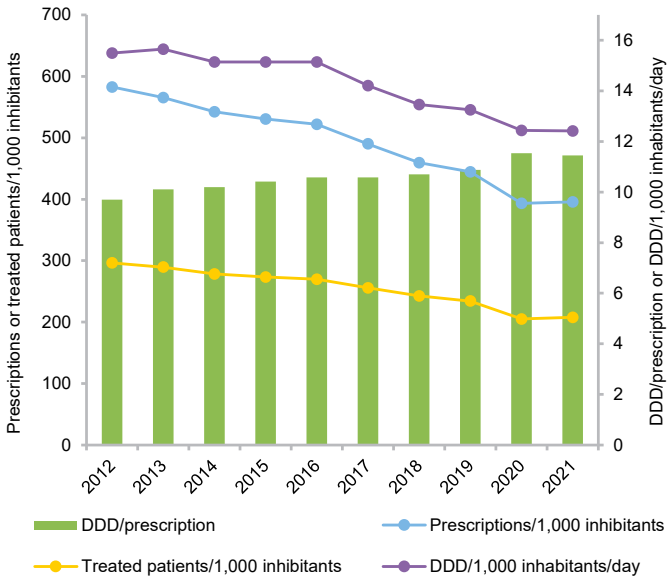
5.3.1 Overall antimicrobial consumption

The comparison of trends over time for different indicators of consumption in 2021 showed only minor changes compared to previous years (maximum 1% increase or decrease, Figure 5.5). In 2021, the average DDD/prescription was 11.5, almost similar to 2020, but 18% higher compared to the 9.7 DDD per prescription in 2012. The total number of prescriptions was 396 per 1,000 inhabitants, a 32% reduction from the 583 prescriptions per 1,000 inhabitants in 2012. In 2021, the total number of patients treated was 208 per 1,000 inhabitants (Table 5.3). In comparison, the number was 296 treated patients per 1,000 inhabitants in 2012. Thus, the number of treated patients and prescriptions has decreased over the decade, probably due to raising awareness among prescribers and the public. Doses per prescription have increased, probably due to switch to antibiotics that contribute with more DDDs, e.g. the switch to pivmecillinam as drug of choice in the treatment of human urinary tract infections.

Figure 5.6 shows the number of prescriptions per 1,000 inhabitants at municipality level in 2016 and 2021, respectively. In 2021, the consumption ranged from 328 to 526 prescriptions per 1,000 inhabitants. Five years earlier, in 2016, the range was 434-727 prescriptions per 1,000 inhabitants.

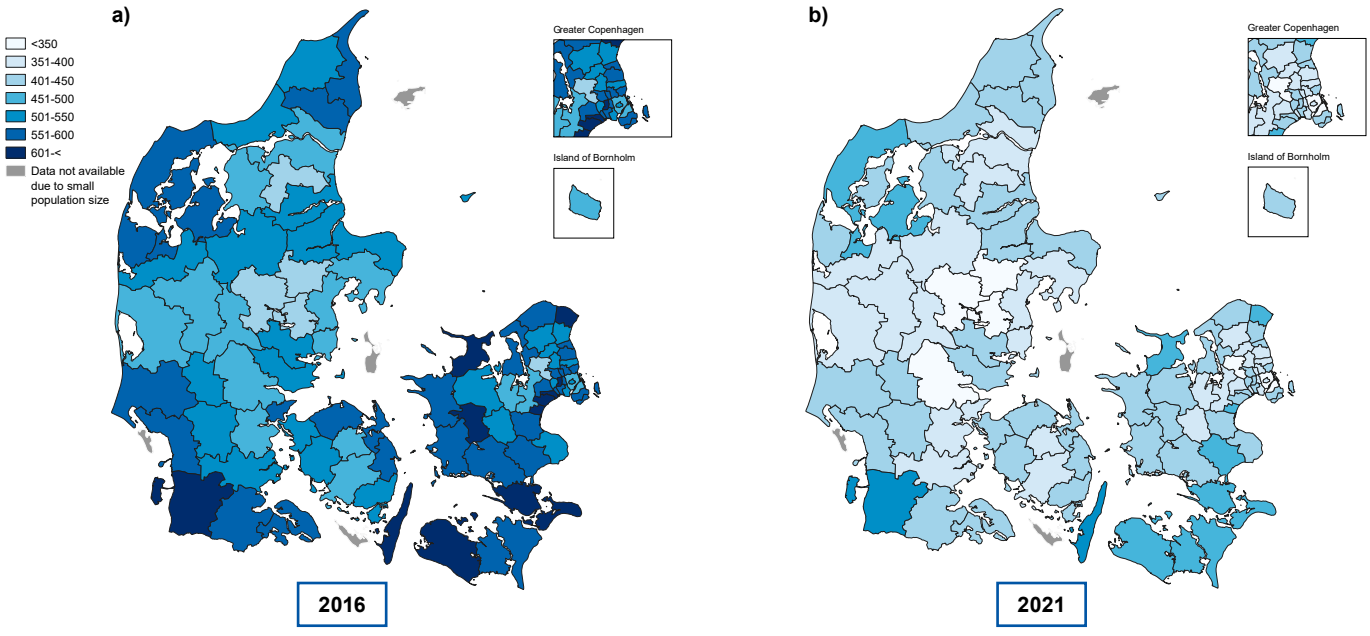
Of note is that all municipalities have reduced their prescribing activities in the shown period. Demographic differences might impact the range of prescribing.

Figure 5.5 Trends in consumption of systemic antimicrobial agents in primary health care using four different measurements, Denmark, 2012-2021 DANMAP 2021



Data: Registered sales to individuals
Data source: Register of Medicinal Product Statistics and 2022 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 5.6 Number of prescriptions from primary health care per 1,000 inhabitants in Danish municipalities in a) 2016 and b) 2021 DANMAP 2021



Data: Registered sales to individuals
Data source: Register of Medicinal Product Statistics and 2022 edition of the Anatomical Therapeutic Chemical (ATC) classification system

5.3.2 Consumption of antimicrobial groups

For decades, beta-lactamase sensitive penicillins were the most used antimicrobials in primary health care in Denmark. In 2021, Beta-lactamase sensitive penicillins accounted for 2.89 DID (23%), penicillins with extended spectrum for 3.17 DID (25%), beta-lactamase resistant penicillins for 1.61 DID (13%). 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 8.22 DID (64%) of antimicrobials consumed in primary health care in 2021. Tetracyclines accounted for 1.64 DID (13%) and macrolides for 1.11 DID (9%). Fluoroquinolones accounted for 0.32 DID (2.5%), which is the lowest in a decade. A decade ago, in 2012, the four groups of penicillins accounted for 9.62 DID, equally corresponding to 60% of the total consumption, yet beta-lactamase sensitive penicillins accounted for 29%, penicillins with extended spectrum for 21%, beta-lactamase resistant penicillins for only 8%, while macrolides accounted for 14% (DANMAP 2012). For most other antimicrobial groups, the proportion of total consumption did not change notably.

During the pandemic years 2020 and 2021, penicillins with extended spectrum became the most used antimicrobial group (Figure 5.7).

Other beta-lactams such as cephalosporins, monobactams and carbapenems were either used at extremely low level or are restricted to hospital use only.

Penicillins

From 2012 to 2021, consumption of beta-lactamase sensitive penicillins decreased by 38% (from 4.68 DID in 2012), while beta-lactamase resistant penicillins increased by 33% (1.21 DID in 2012) (Figure 5.7). For penicillins with extended spectrum consumption increased during the first years of the decade, but has since levelled off. Combination penicillins increased continuously from their introduction to the Danish market in 2009 until 2015 (0.95 DID), showed no changes in 2016 and since declined, accounting for 0.55 DID in 2021. The increases described for the penicillins with extended spectrum are primarily due to increases in the consumption of pivmecillinam which accounted for 77% of this antimicrobial class in 2021 (Figure 5.8). Over the decade pivmecillinam increased by 24% from 1.96 DID in 2012 to 2.43 DID in 2021. In the same time period pivampicillin decreased by 82% from 0.29 DID to 0.05 DID and amoxicillin decreased by 9.9% from 0.75 DID to 0.67 DID (Figure 5.8). Consumption of amoxicillin fluctuated within the decade, decreasing from 2011 to 2016 (0.61 DID), increasing from 2016-2019 by 12%, decreasing from 2019-2020 by 11% and increasing from 2020-2021 by 9.6% (Figure 5.8). Increases in the use of pivmecillinam were related to changed recommendations for the treatment of urinary tract infections, while the decreased use of pivampicillin followed increased resistance towards ampicillin in *E. coli* (see section 8.2.1.) and use of amoxicillin followed recommendations on a more prudent use of antimicrobials in young children.

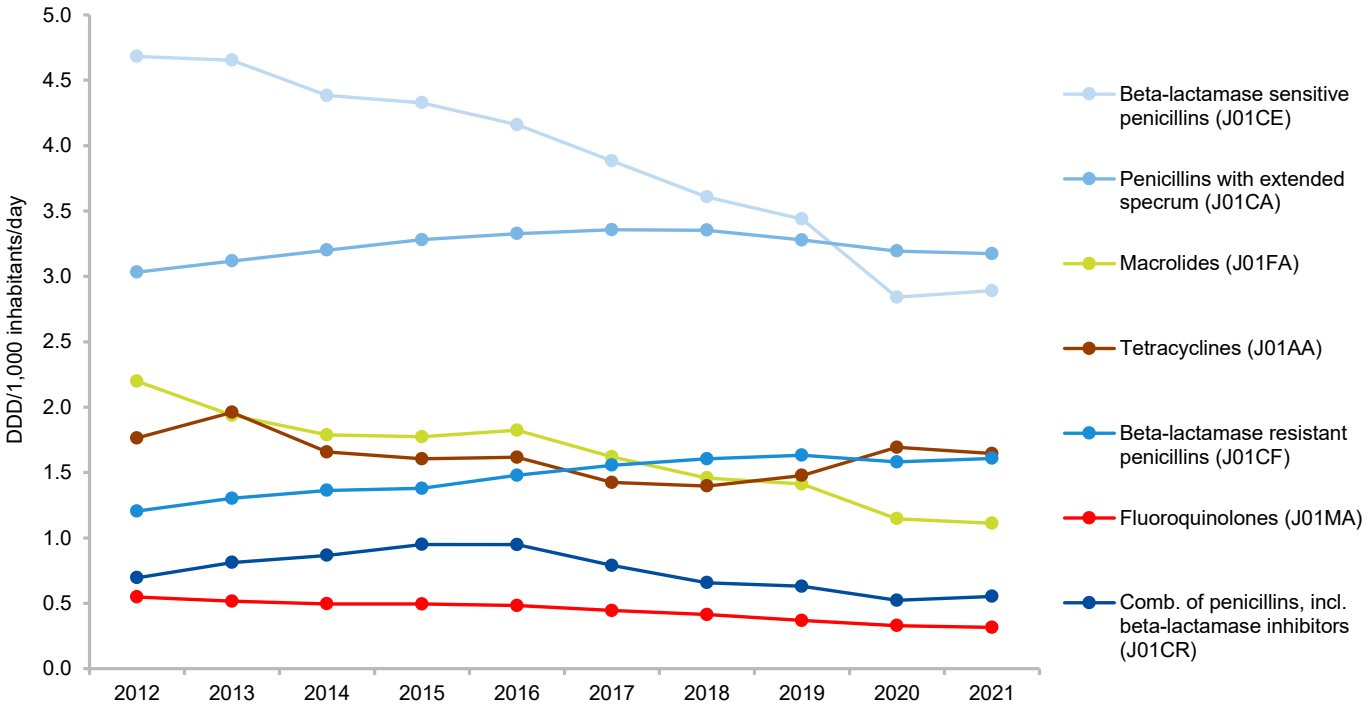
Table 5.1 Consumption of antimicrobial agents for systemic use in primary health care, DDD per 1,000 inhabitants per day, Denmark, 2002 and 2012-2021

DANMAP 2021

ATC group	Therapeutic group	Year										
		2002	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021
J01AA	Tetracyclines	1.05	1.76	1.96	1.66	1.60	1.62	1.42	1.40	1.48	1.69	1.64
J01CA	Penicillins with extended spectrum	2.17	3.03	3.12	3.20	3.28	3.33	3.36	3.35	3.28	3.19	3.17
J01CE	Beta-lactamase sensitive penicillins	5.06	4.68	4.65	4.38	4.33	4.16	3.88	3.61	3.44	2.84	2.89
J01CF	Beta-lactamase resistant penicillins	0.78	1.21	1.30	1.36	1.38	1.48	1.56	1.60	1.63	1.58	1.61
J01CR	Combinations of penicillins, including beta-lactamase inhibitors	0.03	0.70	0.81	0.87	0.95	0.95	0.79	0.66	0.63	0.52	0.55
J01D	Cephalosporins and other betalactam antibiotics	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
J01EA	Trimethoprim and derivates	0.36	0.52	0.53	0.55	0.56	0.56	0.56	0.53	0.45	0.43	0.42
J01EB	Short-acting sulfonamides	0.36	0.22	0.22	0.21	0.18	0.16	0.15	0.14	0.13	0.11	0.09
J01EE	Combination of sulfonamides and trimethoprim, including derivates	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
J01FA	Macrolides	2.18	2.20	1.94	1.79	1.77	1.82	1.62	1.46	1.41	1.15	1.11
J01FF	Lincosamides	0.01	0.04	0.05	0.05	0.05	0.06	0.06	0.06	0.06	0.07	0.07
J01GB	Aminoglycosides	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
J01MA	Fluroquinolones	0.18	0.55	0.52	0.50	0.49	0.48	0.44	0.41	0.37	0.33	0.32
J01XC	Steroid antibacterials (combination fusidic acid)	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00
J01XE	Nitrofuran derivates (nitrofurantoin)	0.41	0.50	0.49	0.48	0.45	0.43	0.26	0.15	0.27	0.27	0.28
J01XX	Other antibacerials (metheamine >99%)	0.34	0.25	0.24	0.24	0.25	0.27	0.28	0.29	0.32	0.34	0.39
J01XD and P01AB01	Nitroimidazole derivates (metronidazole)	0.18	0.28	0.28	0.28	0.28	0.28	0.25	0.24	0.24	0.23	0.24
J01 and P01AB01	Antibacterial agents for systemic use (total)	13.20	16.03	16.19	15.64	15.66	15.67	14.71	13.97	13.77	12.83	12.86

Data: Registered sales to individuals and clinics
Data source: Register of Medicinal Product Statistics and 2022 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 5.7 Consumption of leading antimicrobial groups for systemic use in primary health care, DDD per 1,000 inhabitants per day, Denmark, 2012-2021
DANMAP 2021



Data: Registered sales to individuals and clinics
Data source: Register of Medicinal Product Statistics and 2022 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Table 5.2 Number of prescriptions per 1,000 inhabitants for leading antimicrobial agents in primary health care, Denmark, 2002 and 2012-2021
DANMAP 2021

ATC group	Therapeutic group	Year										
		2002	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021
J01AA	Tetracyclines	18.66	22.56	22.89	20.00	17.90	17.18	15.89	14.63	15.11	20.19	18.25
J01CA	Penicillins with extended spectrum	100.11	115.91	114.30	113.83	113.53	113.16	114.37	114.31	112.19	105.93	107.97
J01CE	Beta-lactamase sensitive penicillins	228.77	186.91	180.54	170.70	163.09	157.13	148.52	136.81	128.77	104.07	107.28
J01CF	Beta-lactamase resistant penicillins	34.01	40.42	41.25	41.04	40.81	41.87	41.87	43.35	43.16	42.87	43.17
J01CR	Combinations of penicillins, including betalctamase inhibitors	1.45	24.71	28.01	29.02	30.73	31.13	27.09	23.71	23.07	19.14	20.36
J01E	Sulphonamides and trimethoprim	53.18	43.86	43.53	41.51	38.39	36.41	34.29	31.74	28.14	25.59	23.07
J01FA	Macrolides	90.89	85.89	74.51	68.01	68.00	68.85	60.00	52.64	50.71	33.66	33.80
J01MA	Fluoroquinolones	9.85	22.14	20.65	19.67	19.50	18.74	17.37	15.97	13.99	12.07	11.41
J01X	Other antibacterials	13.36	18.03	17.41	16.73	16.28	15.82	10.18	6.76	10.29	10.62	10.70
P01AB01	Nitroimidazole derivatives (metronidazole)	13.38	19.68	19.26	19.06	19.15	18.63	17.26	16.31	15.78	15.62	16.00
J01 and P01AB01	Antibacterial agents for systemic use (total)	564.83	582.80	565.26	542.53	530.56	522.19	490.08	459.39	444.53	393.34	395.78

Data: Registered sales to individuals
Data source: Register of Medicinal Product Statistics and 2022 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Table 5.3 Number of treated patients per 1,000 inhabitants for leading antimicrobial agents in primary health care, Denmark, 2002 and 2012-2021

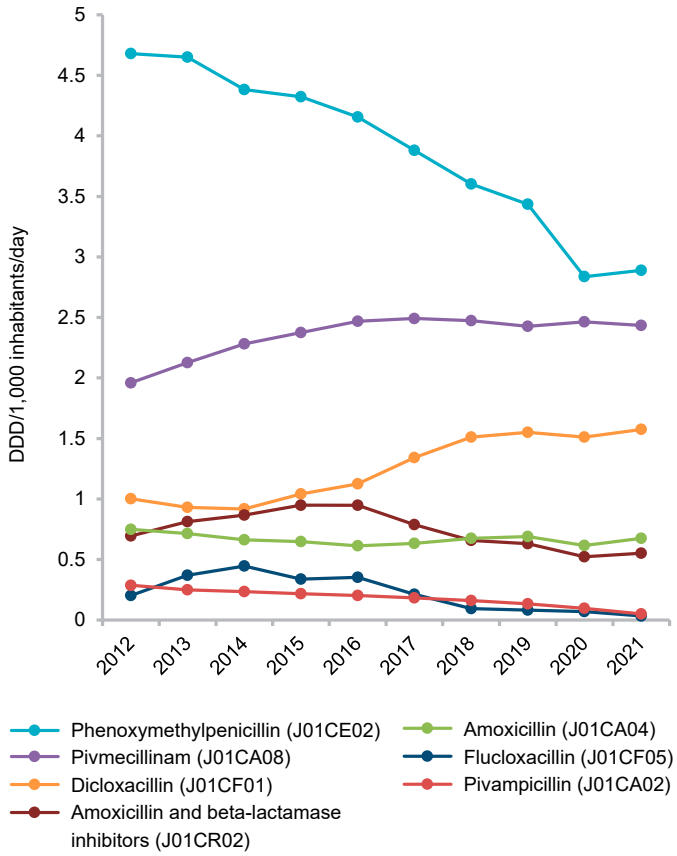
DANMAP 2021

ATC group	Therapeutic group	Year										
		2002	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021
J01AA	Tetracyclines	11.49	13.53	13.86	12.20	11.32	11.04	10.35	9.69	10.10	14.43	12.99
J01CA	Penicillins with extended spectrum	69.14	77.31	76.10	75.32	74.87	74.05	74.04	73.56	71.97	67.14	68.61
J01CE	Beta-lactamase sensitive penicillins	173.43	145.53	142.19	134.79	130.06	125.69	119.32	110.90	104.70	84.93	87.70
J01CF	Beta-lactamase resistant penicillins	24.09	28.51	29.07	29.24	28.85	29.70	29.96	31.10	31.06	30.52	30.89
J01CR	Combinations of penicillins, including betalctamase inhibitors	0.99	17.32	19.71	20.52	22.03	22.17	19.89	17.73	17.33	14.43	15.50
J01E	Sulphonamides and trimethoprim	36.32	26.48	26.16	24.65	22.45	21.17	19.87	18.42	16.63	15.04	13.66
J01FA	Macrolides	66.89	64.73	56.16	51.38	51.75	53.21	46.01	40.11	38.45	25.13	24.97
J01MA	Fluoroquinolones	7.67	17.25	16.04	15.30	15.04	14.37	13.36	12.26	10.74	9.01	8.52
J01X	Other antibacterials	6.99	7.54	7.48	7.16	7.35	7.47	5.01	3.62	5.66	5.80	5.95
P01AB01	Nitroimidazole derivatives (metronidazole)	11.57	16.86	16.51	16.31	16.47	16.03	14.84	14.05	13.57	13.36	13.77
J01 and P01AB01	Antibacterial agents for systemic use (total)	305.27	296.40	289.54	278.62	273.49	269.72	255.72	242.55	234.34	205.27	207.85

Data: Registered sales to individuals
Data source: Register of Medicinal Product Statistics and 2022 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 5.8 Consumption of main penicillins in the primary health care, DDD per 1,000 inhabitants per day, Denmark, 2012-2021

DANMAP 2021



Data: Registered sales to individuals and clinics
Data source: Register of Medicinal Product Statistics and 2022 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Tetracyclines and macrolides

In 2021, tetracycline consumption in primary health care was 1.64 DID, corresponding to 13% of the total consumption, whereas macrolides accounted for 1.11 DID (9%) (Figure 5.7). During the last decade, the consumption of tetracyclines decreased from 1.76 DID in 2012 (6.7%). However, in 2020 the consumption increased again compared to 2019, 1.48 DID. Macrolides decreased from 2.20 DID in 2012 (-49%) and showed an additional marked reduction from 1.41 DID in 2019 to 1.15 DID in 2020 (-18.8%). These changes in tetracycline and macrolide consumption may reflect compliance with the new guideline for chlamydia treatment issued by the Danish Dermatological Society in 2019 (Table 5.4). The guideline recommends doxycycline as first-line treatment instead of the previously recommended azithromycin. The treatment recommendation was changed due to concerns in Denmark about increasing azithromycin-resistance in *Mycoplasma genitalium*, a frequent co-infection in patients with chlamydial infections.

The most common indications for tetracycline prescriptions in primary health care are shown in Table 5.4.

5.3.3 Prescribing patterns by medical specialties

Interregional differences in the levels of prescribing have been described in DANMAP since 2017 (Table 5.5). In general, the Danish population is relatively homogenous and health care is of standardized quality, which, combined with several initiatives to educate GPs in proper 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 behavioural differences between urban and rural populations.

General practitioners can monitor their own prescribing levels through ordiprax+, an online dashboard with personal access which visualises prescribing data and enables comparisons with other practices on regional level (DANMAP 2020 Textbox 5.2).

Table 5.4 Percentage distribution of tetracyclines consumption by clinical indication code, Denmark, 2017-2021 DANMAP 2021

Indication written on the prescription	Year				
	2017	2018	2019	2020	2021
Against acne	48.4	52.1	52.5	48.9	54.3
Against Chlamydia and mycoplasma infection	0.5	0.8	1.2	11.3	8.7
Against Borrelia infection	2.5	3.9	4.2	2.7	2.4
Against pelvic inflammatory disease	1.7	1.8	1.8	2.0	1.9
Against skin and soft tissue infection	1.1	1.5	1.5	1.5	1.7
Unspecified indications	41.8	35.6	34.3	28.7	26.2
Other indications	4.0	4.2	4.4	4.9	4.8

Data: Registered sales to individuals
Data source: Register of Medicinal Product Statistics and 2022 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Table 5.5 Consumption of antimicrobial agents for systemic use in primary health care at regional level, Denmark, 2017-2021 DANMAP 2021

Region	Indicator	Year				
		2017	2018	2019	2020	2021
Capital Region	DDD/1000 inhabitants/day	14.36	13.49	13.37	12.49	12.34
	Prescriptions/1000 inhabitants	489	453	441	382	378
Region Zealand	DDD/1000 inhabitants/day	15.53	14.68	14.36	13.66	13.72
	Prescriptions/1000 inhabitants	539	501	482	436	440
Region of Southern Denmark	DDD/1000 inhabitants/day	14.09	13.39	13.16	12.17	12.18
	Prescriptions/1000 inhabitants	497	470	455	401	405
Central Denmark Region	DDD/1000 inhabitants/day	13.46	12.76	12.49	11.84	11.85
	Prescriptions/1000 inhabitants	458	431	417	374	380
North Denmark Region	DDD/1000 inhabitants/day	13.54	13.07	13.00	12.21	12.43
	Prescriptions/1000 inhabitants	472	452	436	390	400
Denmark (total)	DDD/1000 inhabitants/day	14.21	13.46	13.25	12.43	12.42
	Prescriptions/1000 inhabitants	490	459	445	393	396

Data: Registered sales to individuals
Data source: Register of Medicinal Product Statistics and 2022 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Prescribing trends in primary health care also clearly differ by type of doctor. An overview of the numbers of prescriptions by the different specialists, including hospital doctors issuing prescriptions for patients in ambulatory care, can be found in Table 5.6. It shows a decrease for both 2020 and 2021 in the numbers of prescriptions by all medical specialties except for hospital doctors. The most marked reduction can be seen for general practitioners, especially when comparing 2020 and 2021 to 2019 before the COVID-19 pandemic (-15% reduction). Prescriptions by hospital doctors have increased due to ongoing restructuring of the healthcare system. In 2021, hospital doctors accounted for 63 prescriptions per 1,000 inhabitants (16% of the antimicrobials sold at pharmacies). In 2008, it was 38 prescriptions per 1,000 inhabitants (corresponding to 6% of sales) (data not shown).

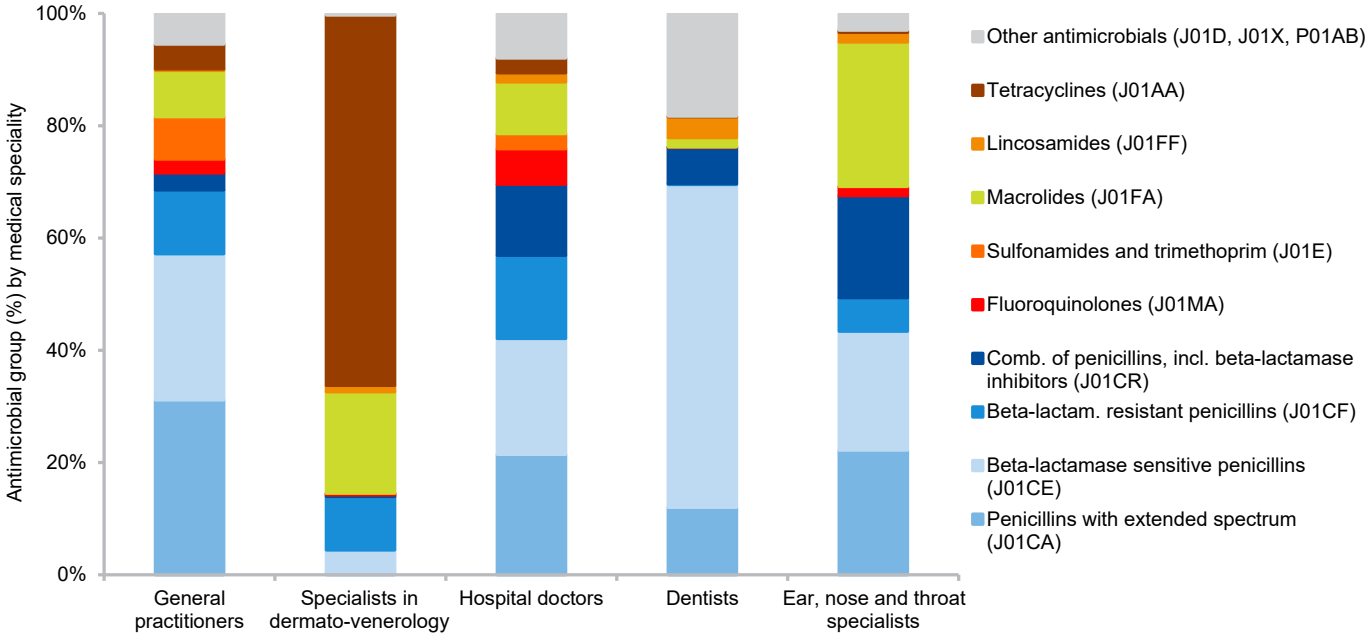
Table 5.6 Number of prescriptions per 1,000 inhabitants for different doctor types, Denmark, 2017-2021 DANMAP 2021

Doctor type	Year				
	2017	2018	2019	2020	2021
General practitioners	368.6	341.5	326.8	280.2	278.8
Ear nose throat specialists	8.9	8.4	7.8	6.1	6.9
Specialists in dermato-venerology	5.9	5.2	5.4	5.3	5.0
Hospital doctors	62.6	62.8	63.0	64.5	63.4
Dentists	29.1	27.8	28.8	25.6	28.9

Data: Registered sales to individuals
Data source: Register of Medicinal Product Statistics and 2022 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 5.9 shows the main antimicrobial groups prescribed by medical specialty in primary health care in 2021, including hospital doctors issuing prescriptions for patients in ambulatory care. In 2021, 66% of antimicrobial prescriptions from specialists in dermato-venerology were tetracyclines, which are indicated for treatment of severe acne and sexually transmitted chlamydia/mycoplasma infections (see section 'Tetracyclines and macrolides'). More than half (57%) of all prescriptions by dentists were narrow-spectrum beta-lactamase sensitive penicillins reflecting adherence to the recommended first-line treatment for common dental infections in the primary health care.

Figure 5.9 Antimicrobial groups prescribed by main medical specialities, primary health care, Denmark, 2021 DANMAP 2021

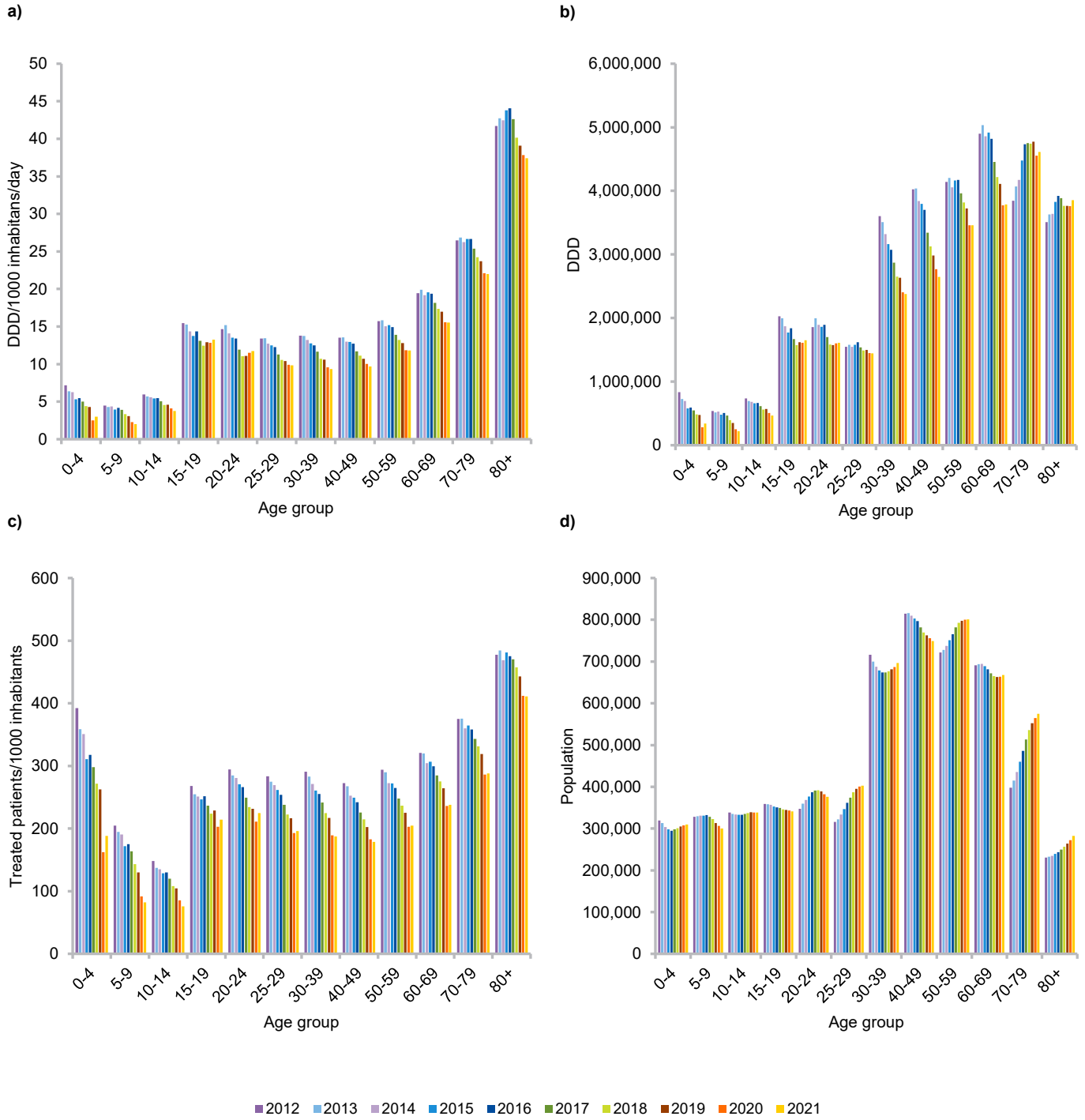


Data: Registered sales to individuals
Data source: Register of Medicinal Product Statistics and 2022 edition of the Anatomical Therapeutic Chemical (ATC) classification system

5.3.4 Antimicrobial consumption by age

Figure 5.10a-c presents consumption of antimicrobials by age group based on different denominators: Figure 5.10a presents consumption in DDD per 1,000 inhabitants per day, Figure 5.10b in crude DDD, i.e. not corrected for population size. Figure 5.10c presents the number of patients treated per 1,000 inhabitants. Figure 5.10d presents population size by age group. All figures show data from 2012 to 2021. Children and adolescents are presented in five-year age groups, while adults are shown in 10-year age groups.

Figure 5.10a-d 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, 2012-2021 DANMAP 2021



Data: Registered sales to individuals
Data source: Register of Medicinal Product Statistics, 2022 edition of the Anatomical Therapeutic Chemical (ATC) classification system and Statistics Denmark

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 2021, there were 141 treated patients per 1,000 inhabitants, receiving 225 prescriptions per 1,000 inhabitants. In 2020, the corresponding numbers were 136 treated patients and 212 prescriptions per 1,000 inhabitants (increase by 3% and 6% between 2020 and 2021, respectively). Since 2012, the consumption decreased from 252 treated patients per 1,000 inhabitants and 432 prescriptions per 1,000 inhabitants (reduction by 44% and 48% from 2012 to 2021, respectively).

Consumption in the 0-4 year olds. Consumption of antimicrobial agents in the youngest age group decreased by 52% from 2012 (392 treated patients per 1,000 inhabitants) to 2021 (188 treated patients per 1,000 inhabitants). After the 38% decrease observed from 2019 to 2020, the consumption increased by 16% from 2020 (162 treated patients per 1,000 inhabitants) to 2021 (Figure 5.11a). The increase from 2020 to 2021 may be due to fewer COVID-19 related restrictions in 2021. The antimicrobials used also changed during the last decade, but penicillins with extended spectrum were still the main antimicrobial agents used to treat children between 0-4 years in 2021 (100 patients per 1,000 inhabitants). In 2020, beta-lactamase sensitive penicillins were the most prescribed (78 patients per 1,000 inhabitants) (Figure 5.11a).

Consumption in the 5-9 year olds. In 2021, 82 patients per 1,000 inhabitants of 5-9 years were treated with antimicrobial agents (Figure 5.10c). This is 60% lower than 2012 (205 patients per 1,000 inhabitants) and 10% lower than 2020 (92 patients per 1,000 inhabitants). Thus, the consumption continued to decrease after the substantial decrease of 30% observed from 2019 to the pandemic year 2020. The distribution of the antimicrobials used to treat 5-9 year olds did not change markedly over the last decade (Figure 5.11b), and beta-lactamase sensitive penicillins remained the main antimicrobial agent used (47 patients per 1,000 inhabitants).

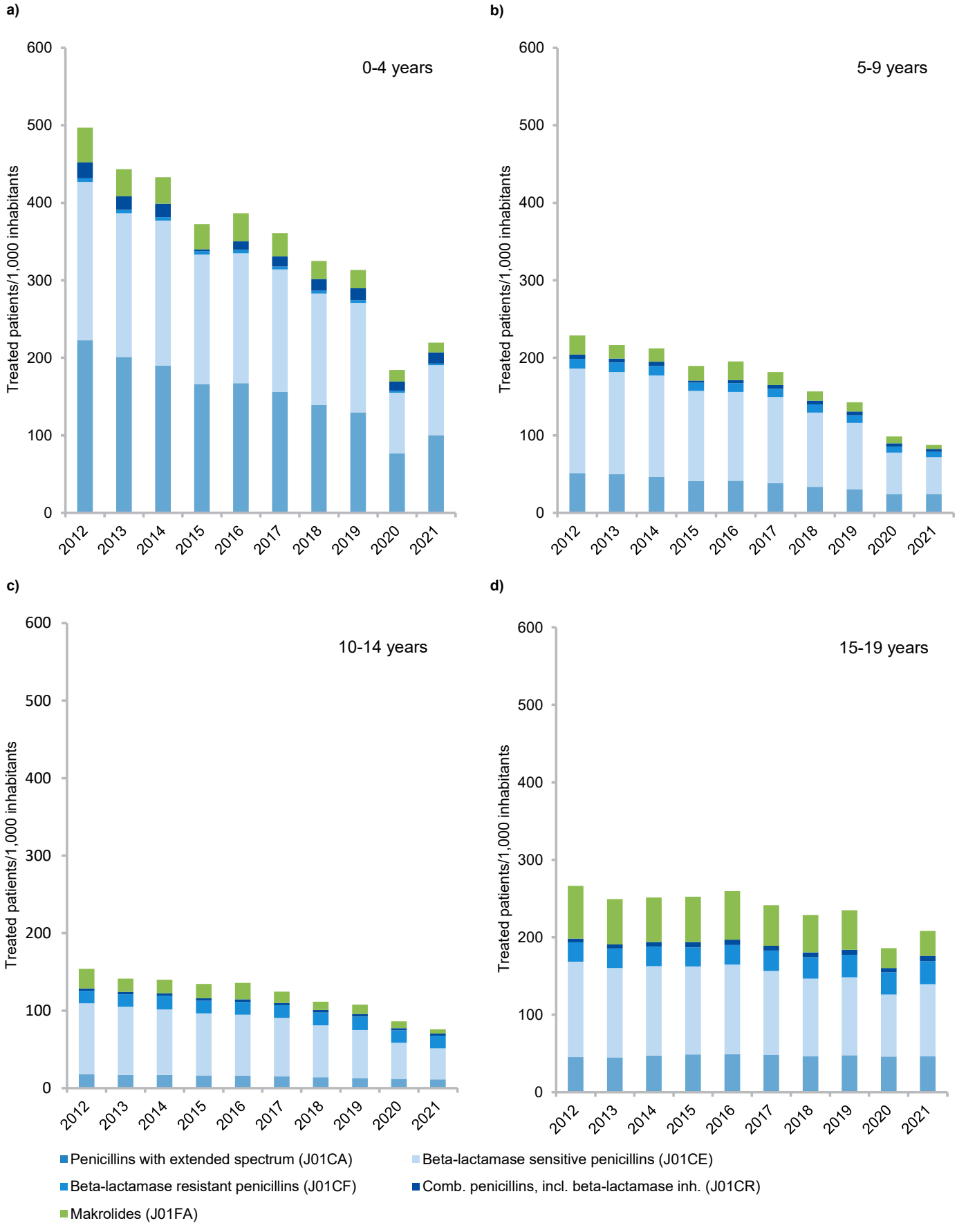
Consumption in the 10-14 year olds. In 2021, the total consumption of antimicrobial agents (76 patients per 1,000 inhabitants) was 49% lower than a decade ago (148 patients per 1,000 inhabitants) and 11% lower than 2020 (85 patients per 1,000 inhabitants) (Figure 5.10c). The first year of COVID-19, 2020, showed a 19% reduction compared to 2019. Beta-lactamase sensitive penicillins remained the main antimicrobial agent even with continuous reduction in consumption the last decade (Figure 5.11c).

Consumption in the 15-19 year olds. Consumption of antimicrobial agents in older teenagers increased in 2021 with 6%; thus, the 11% decrease observed in 2020 did not continue (Figure 5.10a-c). In 2020, 203 patients per 1,000 inhabitants were treated with antimicrobial agents, whereas 214 patients per 1,000 inhabitants were treated in 2021. Over the last decade, the consumption decreased by 20%, driven by a 53% decrease of macrolides and a 24% decrease in beta-lactamase sensitive penicillins (Figure 5.11d).

Macrolides play an important role in the treatment of bacterial respiratory tract infections in children and adolescents. Macrolides were also used as first-line treatment for chlamydia infections until the recent change in guidance (already described in DANMAP2020) which may be the reason for the relatively high consumption of macrolides in the 15-19 year olds. However, penicillins are the most used antimicrobial agents for children and adolescents, constituting between 63% and 90% of all antimicrobials prescribed depending on age group (Figure 5.11).

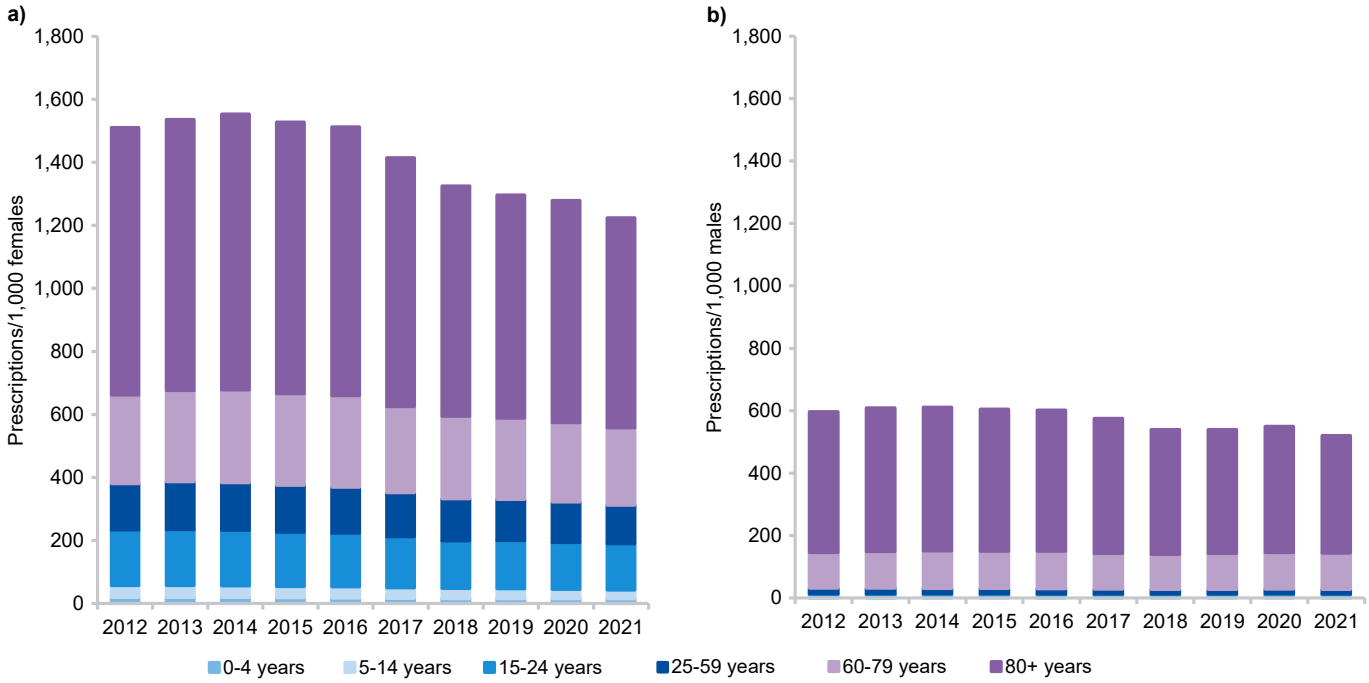
Figure 5.11 Consumption of five main antimicrobial agents for children/adolescents by age group, a) 0-4 years, b) 5-9 years, c) 10-14 years and d) 15-19 years, Denmark, 2012-2021

DANMAP 2021



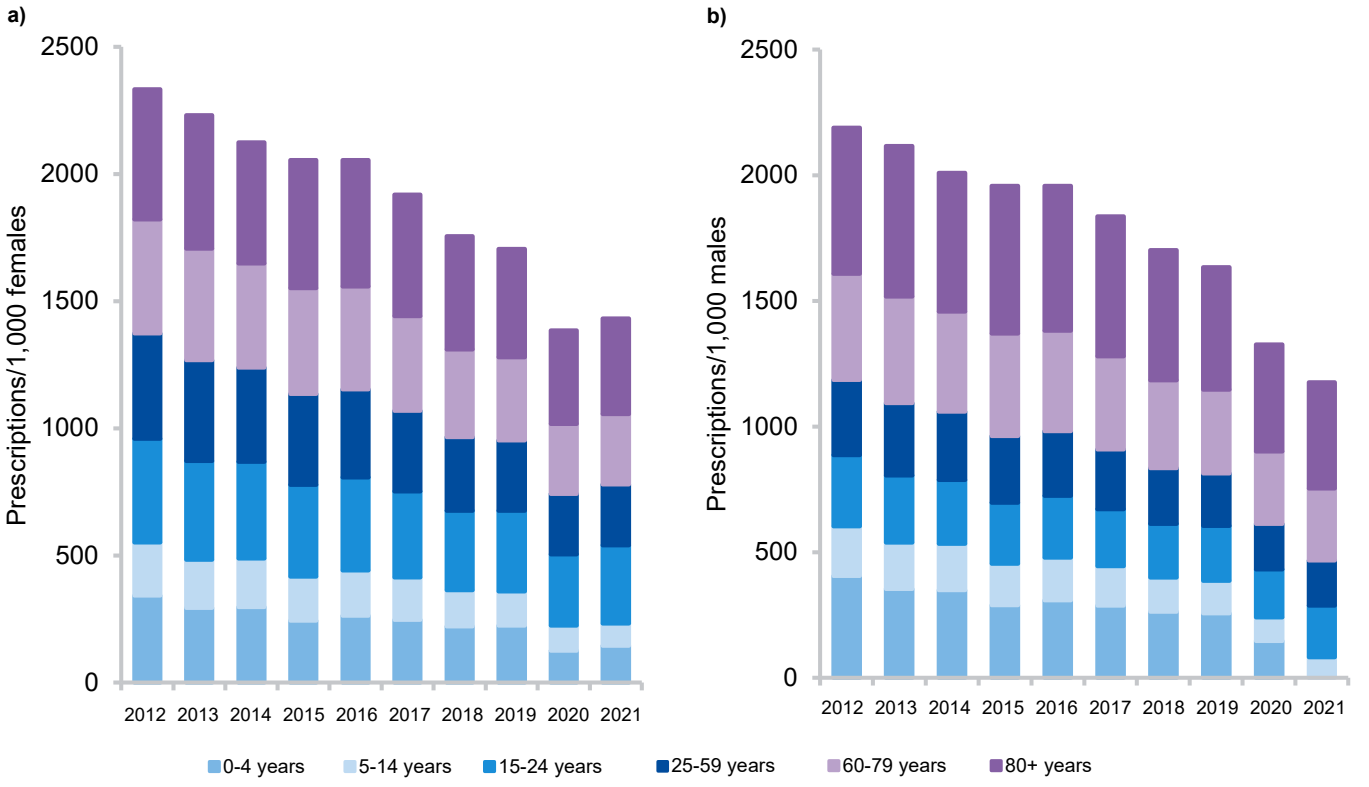
Data: Registered sales to individuals
Data source: Register of Medicinal Product Statistics and 2022 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 5.12 Consumption of antimicrobials primarily used against urinary tract infections* in primary health care for a) females and b) males, prescriptions per 1,000 inhabitants, Denmark, 2012-2021 DANMAP 2021



*Pivmecillinam, sulfonamides, trimethoprim and nitrofurantoin
Data: Registered sales to individuals
Data source: Register of Medicinal Product Statistics and 2022 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 5.13 Consumption of antimicrobials primarily used for respiratory and skin infections* in primary health care for a) females and b) males, prescriptions per 1,000 inhabitants, Denmark, 2012-2021 DANMAP 2021



*Penicillins (beta-lactamase sensitive, beta-lactamase resistant and combination penicillins), macrolides and tetracyclines
Data: Registered sales to individuals
Data source: Register of Medicinal Product Statistics and 2022 edition of the Anatomical Therapeutic Chemical (ATC) classification system

5.3.5 Antimicrobial consumption by gender

Differences in antimicrobial consumption between genders are well known. From 2012 to 2021, the number of treated females (all age groups) decreased by 28% from 346 to 248 per 1,000 inhabitants per year and the number of treated males by 32% from 246 to 167 per 1,000 inhabitants per year. In general, females receive more treatment - a trend driven by higher incidence of urinary tract infections and different healthcare-seeking behaviour. 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 5.12). The reduction in consumption of these antimicrobials was primary driven by fewer prescriptions for elderly women (80+ years), who are the most frequently treated (666 prescriptions per 1,000 females).

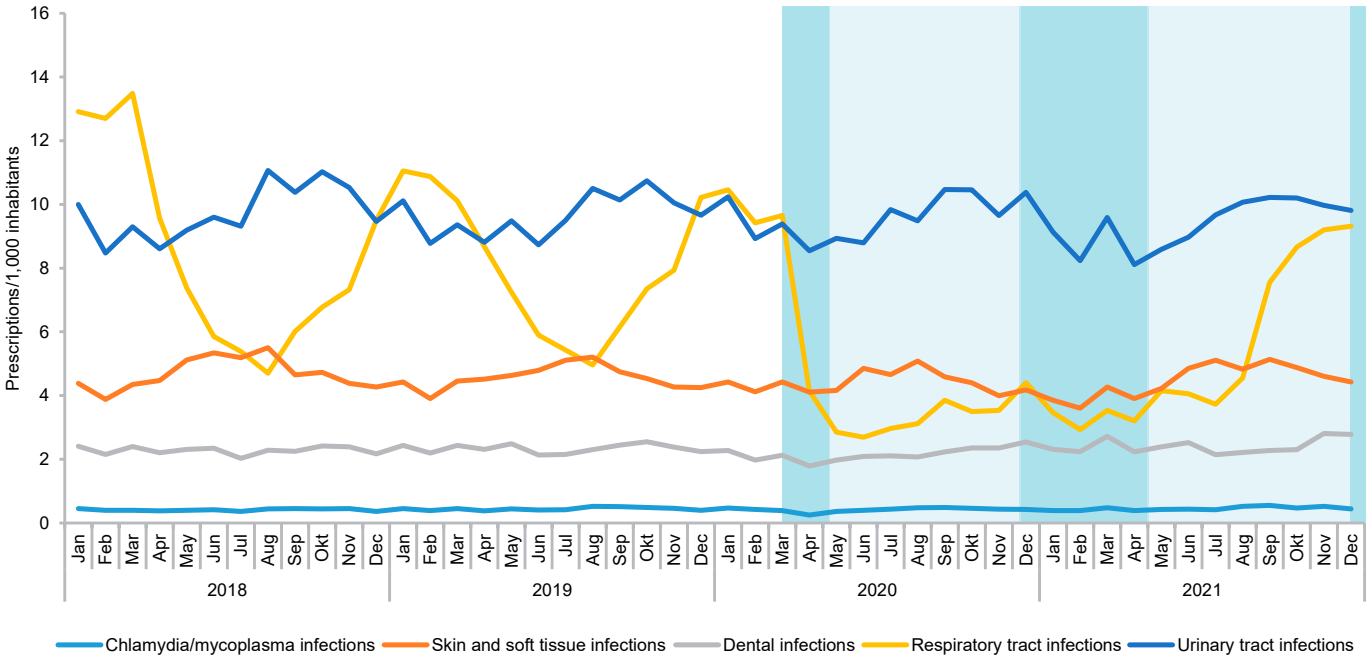
Also for antimicrobials used to treat respiratory tract and skin infections (tetracyclines, penicillins and macrolides) the differences in consumption between genders are substantial (Figure 5.13). These differences may be due to different healthcare-seeking behaviour more than higher incidence of infection. The only exception is among 0-4 year olds, where boys are treated more frequently than girls (145 prescriptions/1,000 girls versus 166 prescriptions per/1,000 boys in 2021).

5.3.6 Monthly consumption data by indication

The main indications provided by prescribers in primary health care for treatment with antimicrobials are upper/lower respiratory tract, urinary tract infections, skin & soft tissue infections and oral infections. In 2020, consumption of antimicrobials prescribed for treatment of respiratory tract infections showed slightly lower levels compared to previous years until March followed by a sharp drop and lower levels from April onwards most likely reflecting the impact of restriction measures due to COVID-19 on the incidence of respiratory tract infections (Figure 5.14). However, from August 2021 the consumption increased to similar levels as in the corresponding pre-pandemic months in 2019. Antimicrobials were also less prescribed for dental infections following the national lockdown in March 2020 but the number of prescriptions returned to similar levels as in previous years after easing of restrictions in the early summer months of 2020. Antimicrobials prescribed for skin and soft tissue infections were on a similar level in 2020 and in 2021 as in the two previous years, thus did not seem to be affected by the COVID-19 pandemic.

Prescriptions for chlamydia/mycoplasma infections were lower at the beginning of the first lockdown in 2020 compared to 2018 and 2019. The number of chlamydia infections captured by SSI's surveillance (Overvågning i tal, grafer og kort https://statistik.ssi.dk/) also document low numbers during the national lockdown between March and May 2020.

Figure 5.14 Monthly consumption of systemic antimicrobials by indication in primary health care, prescriptions per 1,000 inhabitants, Denmark, 2018-2021
DANMAP 2021



Data: Registered sales to individuals
Data source: Register of Medicinal Product Statistics and 2022 edition of the Anatomical Therapeutic Chemical (ATC) classification system

5.4 Hospital care

Sales of systemic antimicrobials (ATC code J01, P01AB01 and A07AA09) from all Danish hospital pharmacies in 2021, excluding private hospitals and psychiatric departments (approximately 2-3% of the total hospital consumption), are shown in Table 5.7. Antimicrobial consumption data are presented as DDD per 100 occupied bed-days (DBD) to account for hospital activity. 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 2023.

Changes in hospital activity, for example due to earlier discharge of patients from hospitals, i.e. decreased numbers of bed-days, and increasing ambulatory care functions in the community as well as in care homes, need to be taken into account when interpreting antimicrobial consumption trends in hospitals (see Table 2.1 in Chapter 2 'Introduction').

5.4.1 Antimicrobial consumption at public somatic hospitals accounting for hospital activity

In 2021, the consumption of antimicrobial agents at somatic hospitals was 124.01 DBD. This is similar to 2020 (124.75 DBD) and 25% higher than a decade ago (99.54 DBD in 2012) (Table 5.7).

Table 5.7 Consumption of antimicrobial agents for systemic use in somatic hospitals, DDD per 100 bed-days, Denmark, 2012-2021

DANMAP 2021

ATC group	Therapeutic group	Year									
		2012	2013	2014	2015	2016	2017	2018	2019	2020	2021
J01AA	Tetracyclines	1.80	1.71	1.78	2.00	2.43	2.18	2.78	3.66	3.12	3.24
J01CA	Penicillins with extended spectrum	14.44	14.98	14.71	15.62	16.75	16.87	17.98	18.70	20.28	20.45
J01CE	Beta-lactamase sensitive penicillins	10.79	10.94	10.07	10.04	10.61	10.88	12.17	11.40	11.49	10.70
J01CF	Beta-lactamase resistant penicillins	9.50	10.21	10.05	10.25	10.81	10.69	12.24	13.06	14.05	14.12
J01CR	Comb. of penicillins. incl. beta-lactamase inhibitors	10.77	12.70	13.81	16.20	17.41	14.89	19.27	20.12	22.18	23.58
J01DB	First-generation cephalosporins	0.14	0.13	0.07	0.05	0.05	0.04	0.04	0.03	0.04	0.03
J01DC	Second-generation cephalosporins	15.46	14.28	12.29	11.20	10.68	11.78	10.53	9.45	9.29	8.81
J01DD	Third-generation cephalosporins	1.20	1.26	1.08	1.15	1.19	1.42	1.40	1.39	1.38	1.38
J01DF	Monobactams	0.17	0.16	0.07	0.03	0.01	0.01	0.01	0.01	0.01	0.01
J01DH	Carbapenems	2.98	3.24	3.57	3.22	3.12	3.07	3.27	3.45	3.75	3.60
J01EA	Trimethoprim and derivatives	0.42	0.44	0.51	0.44	0.43	0.44	0.51	0.46	0.52	0.49
J01EB	Short-acting sulfonamides	0.20	0.19	0.15	0.13	0.12	0.11	0.12	0.10	0.07	0.07
J01EE	Comb. of sulfonamides and trimethoprim. incl. derivatives	4.36	5.12	5.23	5.77	6.20	5.97	7.01	7.76	8.40	9.26
J01FA	Macrolides	3.92	3.81	3.94	4.81	5.43	6.09	7.33	7.83	7.06	5.62
J01FF	Lincosamides	0.70	0.74	0.70	0.63	0.72	0.69	0.89	0.86	0.83	0.79
J01GB	Aminoglycosides	2.44	2.50	2.21	2.39	2.25	2.38	2.51	2.84	2.94	2.79
J01MA	Fluoroquinolones	9.70	10.03	9.33	9.17	8.66	7.69	8.19	7.89	8.11	8.36
J01XA	Glycopeptides	1.45	1.53	1.25	1.28	1.26	1.40	1.48	1.55	1.73	1.74
J01XB	Polymyxins	0.26	0.31	0.24	0.21	0.22	0.21	0.27	0.26	0.27	0.27
J01XC	Steroid antibacterials (fusidic acid)	0.26	0.26	0.25	0.18	0.13	0.07	0.07	0.07	0.06	0.07
J01XD	Imidazole derivatives	4.54	4.76	4.78	4.66	5.22	4.96	5.06	4.78	4.92	4.55
J01XE	Nitrofuran derivatives (nitrofurantoin)	0.38	0.39	0.34	0.30	0.27	0.27	0.31	0.33	0.40	0.36
J01XX05	Methenamine	0.09	0.08	0.06	0.10	0.09	0.08	0.12	0.09	0.10	0.13
J01XX08	Linezolid	0.36	0.41	0.37	0.48	0.42	0.40	0.61	0.62	0.57	0.58
J01XX09	Daptomycin	0.02	0.03	0.06	0.04	0.06	0.09	0.17	0.08	0.11	0.14
P01AB01	Metronidazole	2.65	2.61	2.13	2.22	2.52	2.17	2.28	2.23	2.30	2.21
A07AA09	Vancomycin	0.54	0.57	0.56	0.52	0.56	0.55	0.58	0.63	0.77	0.67
J01, P01AB01, A07AA09	Antibacterial agents for systemic use, including metronidazole and vancomycin (total)	99.54	103.41	99.59	103.09	107.64	105.40	117.20	119.65	124.75	124.01

Data: Consumption at somatic hospitals
Data source: Register of Medicinal Product Statistics, 2022 edition of the Anatomical Therapeutic Chemical (ATC) classification system and The National Patient Register

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 68.85 DBD, corresponding to 56% of the total consumption of antimicrobials at somatic hospitals in Denmark in 2021. The consumption of combinations of penicillins, including beta-lactamase inhibitors, continued to increase (119% higher than in 2012) and accounted for 23.58 DBD, making it the largest group consumed in 2021 (19%). Penicillins with extended spectrum also increased markedly over the last decade (42% higher in 2021 compared to 2012) and were the second largest group consumed at Danish hospitals with 20.45 DBD (17%). Beta-lactamase sensitive penicillins accounted for 10.70 DBD (8.7%) and beta-lactamase resistant penicillins for 14.12 DBD (11.4%).

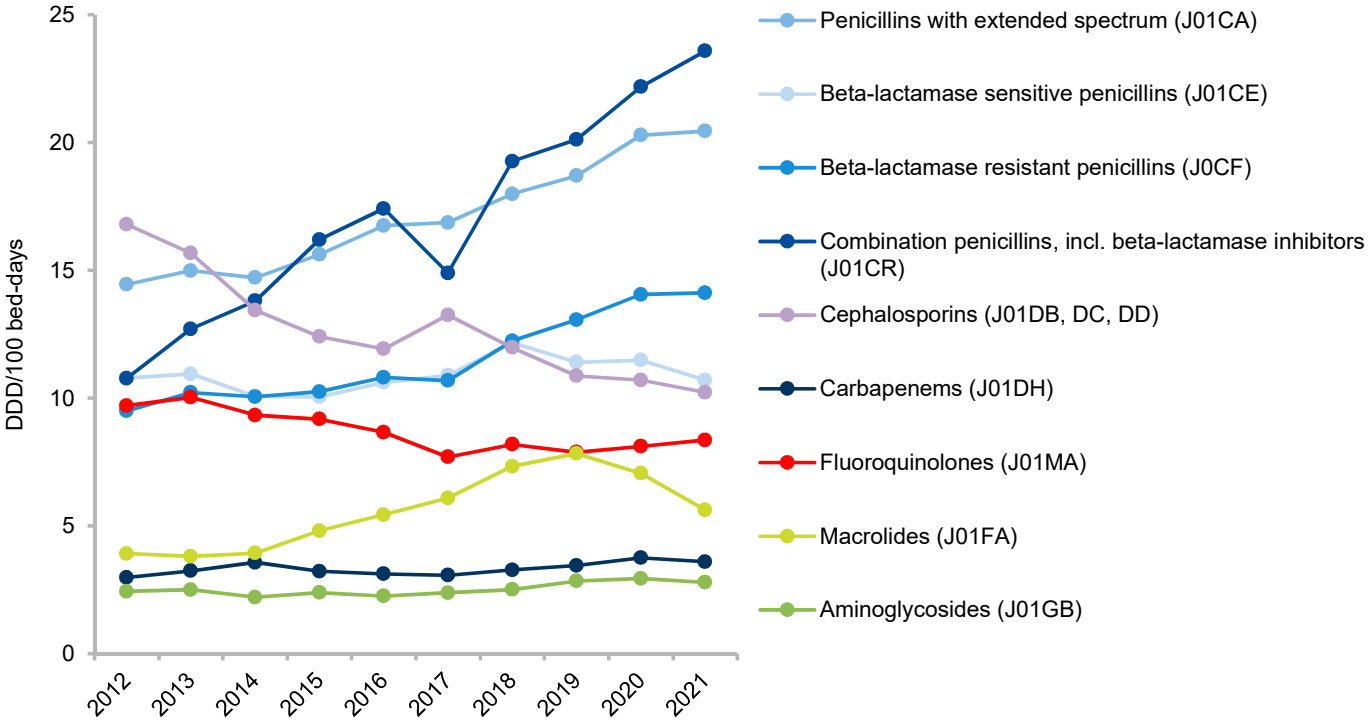
Tetracycline consumption increased during the past decade from 1.80 DBD in 2012 to 3.66 in 2019 but has fallen slightly since to 3.24 DBD in 2021. Consumption of combinations of sulfonamides and trimethoprim increased from 4.36 DBD in 2012 to 9.26 DBD in 2021, a total increase of 112% over the decade. Macrolide consumption continuously increased between 2012 and 2019 but has since decreased to 5.62 DBD in 2021. Consumption of carbapenems increased over the last decade from 2.98 in 2012 DBD to 3.60 DBD in 2021 (Table 5.7, Figures 5.15 and 5.16).

Linezolid consumption decreased to 0.58 DBD in 2021 after it peaked in 2019 (0.62 DBD). Over the past decade, the consumption of linezolid increased by 62% (0.36 DBD in 2012). Consumption of daptomycin peaked in 2018 (0.17 DBD), decreased in 2019 (0.08 DBD) and increased again in 2021 (0.14 DBD) (Table 5.8). 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* (Section 8.3.3 and 8.3.4, Chapter 8 'Resistance in human pathogens').

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 5.8). Consumption estimated in DAD showed similar trends compared to trends measured in DBD between 2012 and 2021.

Figure 5.15 Consumption at somatic hospitals by leading groups of antimicrobial agents, DDD per 100 bed-days, Denmark, 2012-2021

DANMAP 2021



Data: Consumption at somatic hospitals
Data source: Register of Medicinal Product Statistics, 2022 edition of the Anatomical Therapeutic Chemical (ATC) classification system and The National Patient Register

Table 5.8 Consumption of antimicrobial agents for systemic use in somatic hospitals, DDD per 100 admissions, Denmark, 2012-2021

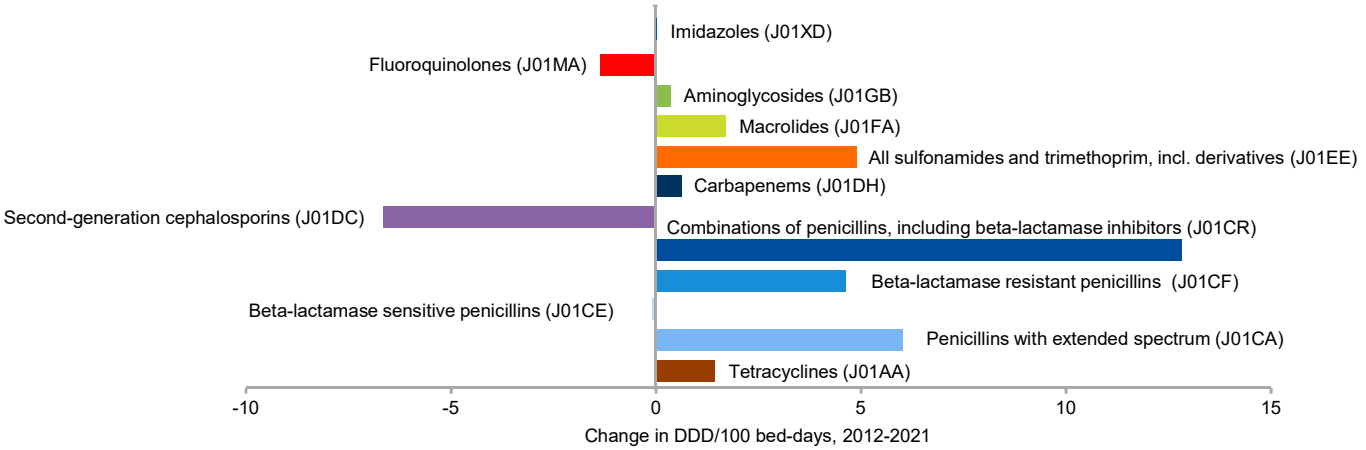
DANMAP 2021

ATC group	Therapeutic group	Year									
		2012	2013	2014	2015	2016	2017	2018	2019	2020	2021
J01AA	Tetracyclines	8.79	8.28	9.00	9.86	11.35	10.96	12.53	16.20	13.40	13.91
J01CA	Penicillins with extended spectrum	70.56	72.48	74.24	76.87	78.32	84.67	81.17	82.69	87.00	87.79
J01CE	Beta-lactamase sensitive penicillins	52.71	52.95	50.80	49.42	49.64	54.62	54.93	50.44	49.27	45.96
J01CF	Beta-lactamase resistant penicillins	46.42	49.42	50.71	50.46	50.56	53.64	55.22	57.77	60.28	60.63
J01CR	Comb. of penicillins. incl. beta-lactamase inhibitors	52.62	61.45	69.67	79.72	81.44	74.74	86.95	88.96	95.14	101.24
J01DB	First-generation cephalosporins	0.68	0.63	0.34	0.24	0.23	0.22	0.20	0.14	0.16	0.15
J01DC	Second-generation cephalosporins	75.55	69.10	62.00	55.15	49.96	59.15	47.53	41.79	39.84	37.83
J01DD	Third-generation cephalosporins	5.84	6.09	5.45	5.65	5.57	7.13	6.33	6.13	5.91	5.91
J01DF	Monobactams	0.82	0.80	0.35	0.15	0.06	0.04	0.03	0.05	0.04	0.03
J01DH	Carbapenems	14.57	15.69	18.02	15.84	14.60	15.40	14.78	15.26	16.09	15.44
J01EA	Trimethoprim and derivatives	2.04	2.13	2.55	2.16	2.02	2.22	2.30	2.05	2.22	2.09
J01EB	Short-acting sulfonamides	1.00	0.91	0.78	0.65	0.54	0.55	0.53	0.45	0.31	0.31
J01EE	Comb. of sulfonamides and trimethoprim. incl. derivatives	21.31	24.75	26.38	28.40	28.99	29.99	31.62	34.31	36.03	39.76
J01FA	Macrolides	19.14	18.41	19.86	23.67	25.42	30.57	33.09	34.63	30.26	24.15
J01FF	Lincosamides	3.42	3.60	3.53	3.10	3.38	3.46	4.02	3.82	3.57	3.38
J01GB	Aminoglycosides	11.91	12.12	11.15	11.74	10.54	11.95	11.32	12.56	12.61	11.97
J01MA	Fluoroquinolones	47.42	48.52	47.06	45.15	40.50	38.62	36.95	34.87	34.77	35.87
J01XA	Glycopeptides	7.09	7.39	6.29	6.29	5.87	7.02	6.69	6.87	7.41	7.45
J01XB	Polymyxins	1.27	1.51	1.22	1.05	1.05	1.03	1.20	1.13	1.18	1.16
J01XC	Steroid antibacterials (fusidic acid)	1.28	1.25	1.25	0.89	0.61	0.36	0.33	0.29	0.26	0.29
J01XD	Imidazole derivatives	22.18	23.02	24.13	22.94	24.41	24.90	22.86	21.15	21.11	19.55
J01XE	Nitrofuran derivatives (nitrofurantoin)	1.84	1.91	1.72	1.46	1.28	1.36	1.42	1.45	1.73	1.53
J01XX05	Methenamine	0.46	0.41	0.30	0.48	0.43	0.38	0.55	0.40	0.45	0.56
J01XX08	Linezolid	1.75	2.00	1.85	2.38	1.97	1.99	2.76	2.73	2.42	2.49
J01XX09	Daptomycin	0.10	0.13	0.30	0.21	0.27	0.44	0.75	0.33	0.48	0.61
P01AB01	Metronidazole	12.96	12.64	10.77	10.91	11.79	10.91	10.27	9.86	9.86	9.50
A07AA09	Vancomycin	2.64	2.78	2.84	2.55	2.63	2.79	2.61	2.80	3.30	2.88
J01, P01AB01, A07AA09	Antibacterial agents for systemic use, incl. metronidazole and vancomycin (total)	486.36	500.35	502.56	507.37	503.44	529.09	528.94	529.15	535.08	532.44

Data: Consumption at somatic hospitals
Data source: Register of Medicinal Product Statistics, 2022 edition of the Anatomical Therapeutic Chemical (ATC) classification system and The National Patient Register

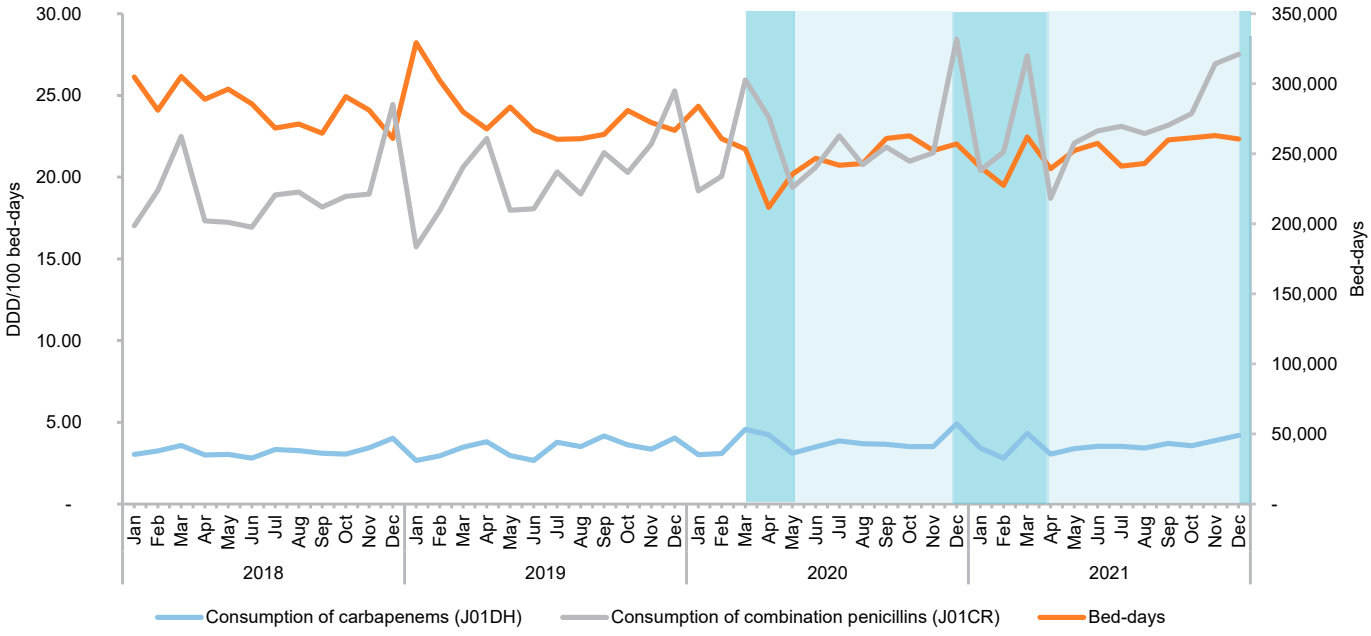
Monthly consumption of the main antimicrobial groups for treatment of critically ill patients at hospitals as well as the monthly number of bed-days for 2018 to 2021 are shown in Figures 5.17. Usage levels of carbapenems (ertapenem, imipenem, meropenem) and penicillin/beta-lactamase inhibitor combinations (amoxicillin/clavulanic acid, piperacillin/tazobactam) per bed-day were high during the COVID-19-related lockdowns in Denmark in 2020 and 2021. This reflects most likely changes in hospital activity and in case mix in hospitals during these periods.

Figure 5.16 Changes in the consumption of leading groups of antimicrobial agents at somatic hospitals, DDD per 100 bed-days, Denmark, 2012-2021 DANMAP 2021



Data: Consumption at somatic hospitals
Data source: Register of Medicinal Product Statistics, 2022 edition of the Anatomical Therapeutic Chemical (ATC) classification system and The National Patient Register

Figure 5.17 Consumption of key antimicrobials used for treatment of seriously ill patients in hospital, DDD per 100 bed-days, Denmark, 2018-2021 DANMAP 2021



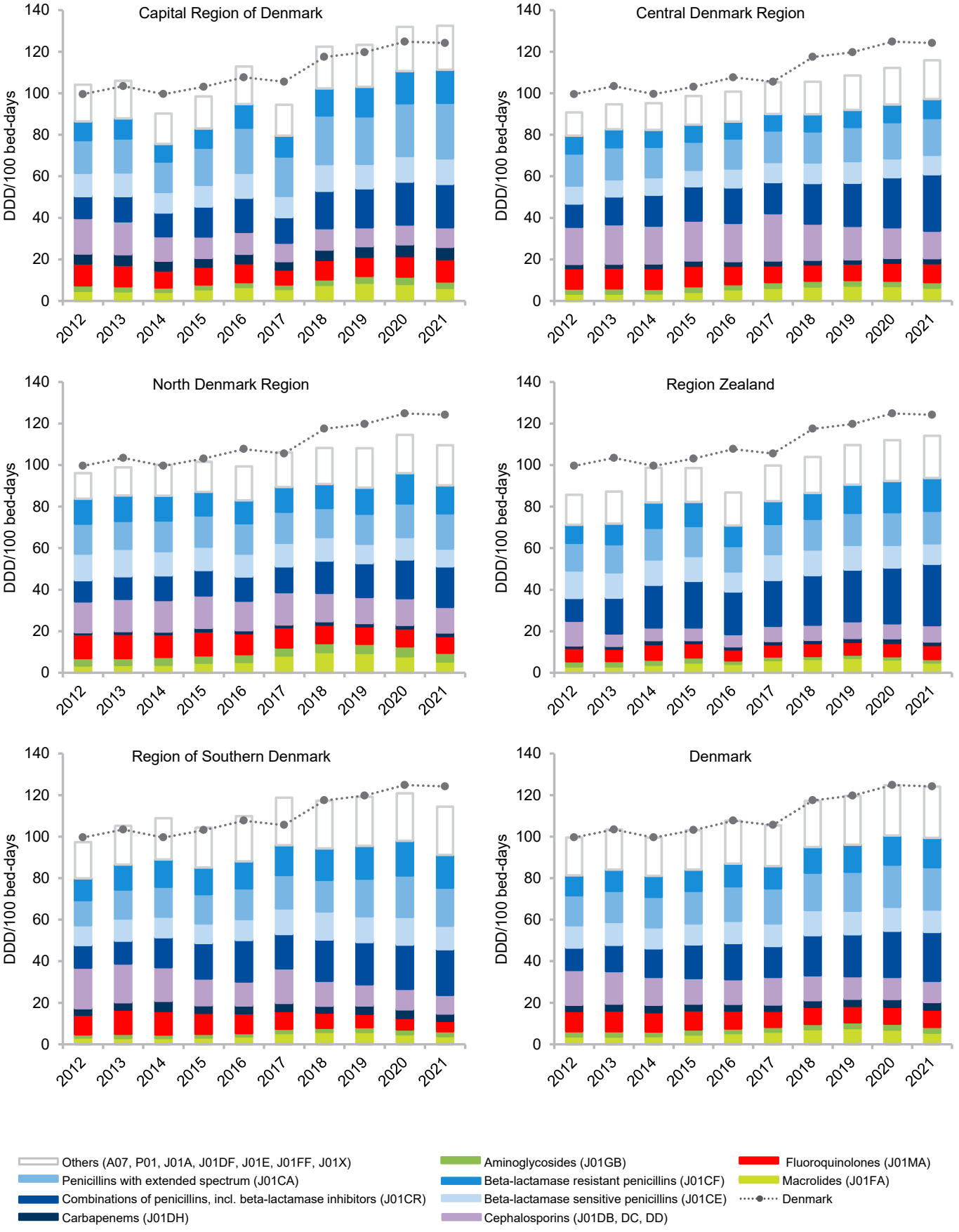
COVID-19 restrictions in place
Fewer restrictions in place
Data: Consumption at somatic hospitals
Data source: Register of Medicinal Product Statistics, 2022 edition of the Anatomical Therapeutic Chemical (ATC) classification system and The National Patient Register

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 5.18. The Capital Region of Denmark shows the highest level of consumption when compared to the other regions in 2021. It is also notable that consumption increased for each region between 2019 and 2020 when measured in DBD (Figure 5.18) but decreased over the same period when measured in DID (Figure 5.19). This reflects that hospital activity changed during the COVID-19 pandemic and more antimicrobials were used in relation to hospital patients' bed-days. In 2021, consumption trends varied by region when compared with the preceding year 2020.

Figure 5.18 Consumption of antimicrobial agents for systemic use in the five health regions, DDD per 100 bed-days, Denmark, 2012-2021

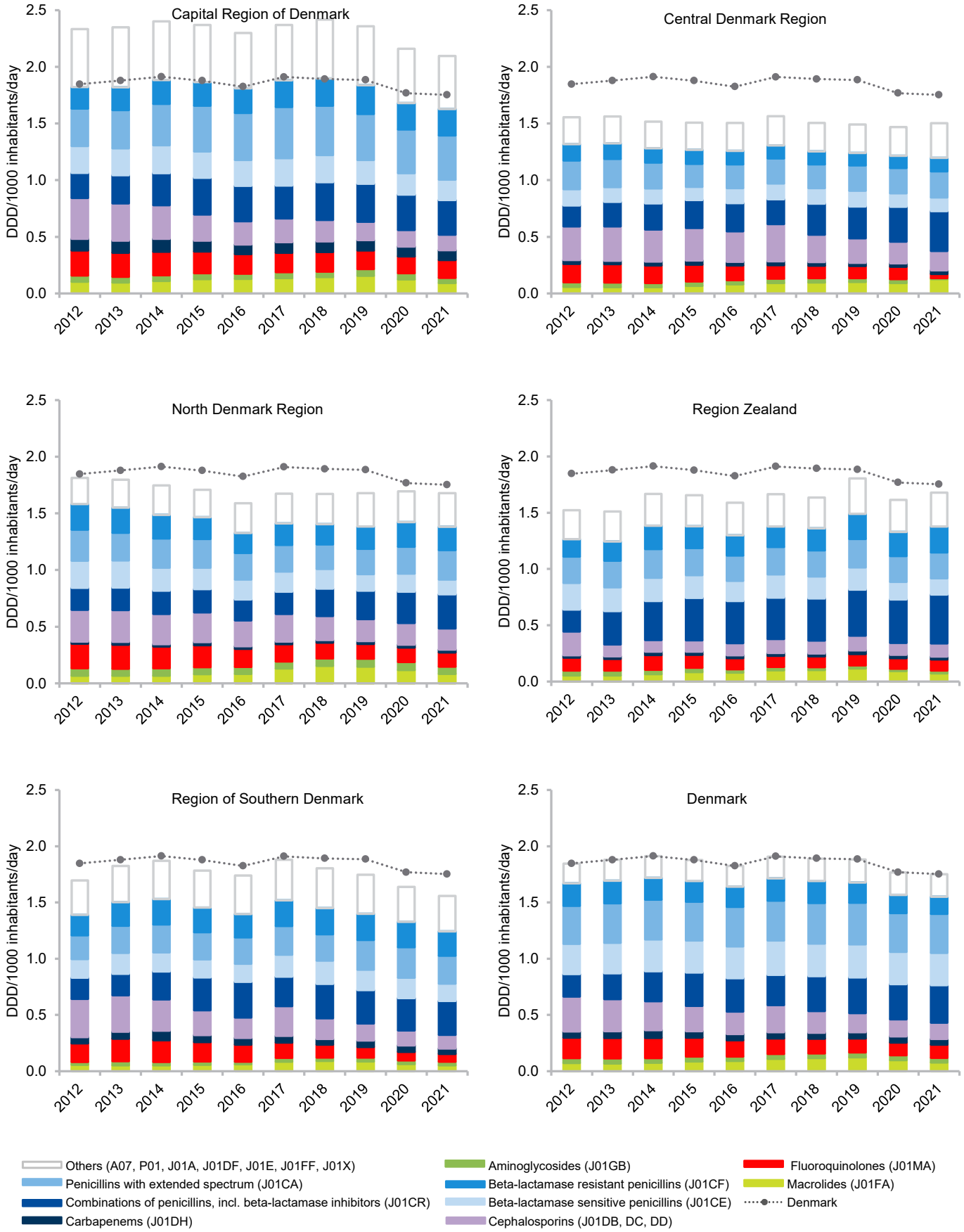
DANMAP 2021



Data: Consumption at somatic hospitals
Data source: Register of Medicinal Product Statistics, 2022 edition of the Anatomical Therapeutic Chemical (ATC) classification system and The National Patient Register

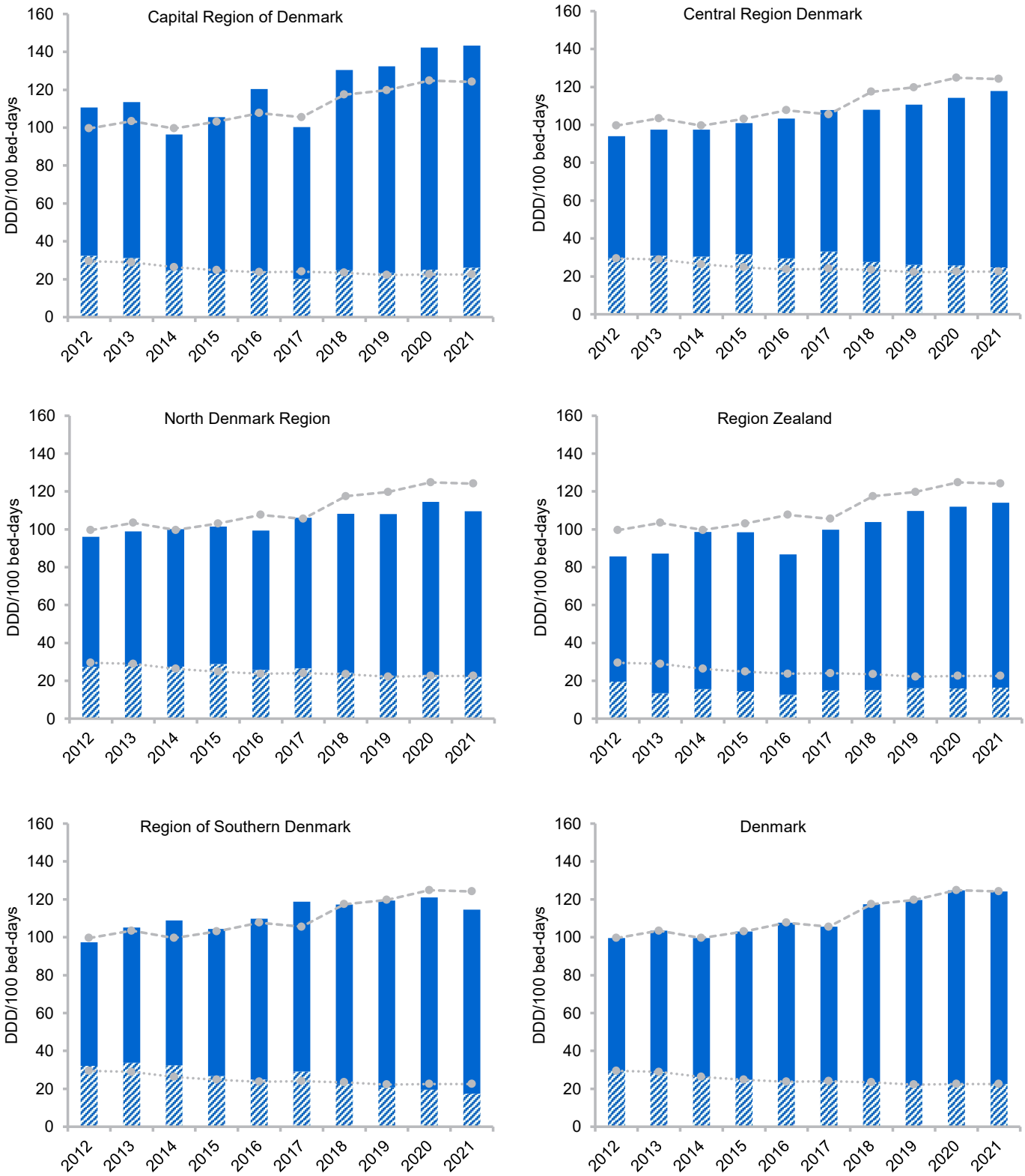
Figure 5.19 Consumption of antimicrobial agents for systemic use in the five health regions, DDD per 1,000 inhabitants per day, Denmark, 2012-2021

DANMAP 2021



Data: Consumption at somatic hospitals
Data source: Register of Medicinal Product Statistics, 2022 edition of the Anatomical Therapeutic Chemical (ATC) classification system and The National Patient Register

Figure 5.20 Consumption of antimicrobials of special critical interest (cephalosporins, fluoroquinolones and carbapenems) and all other antimicrobials in the five health regions, DDD per 100 bed-days, Denmark, 2012-2021 DANMAP 2021



Data: Consumption at somatic hospitals
Data source: Register of Medicinal Product Statistics, 2022 edition of the Anatomical Therapeutic Chemical (ATC) classification system and The National Patient Register

5.4.4 Changes in the consumption of antimicrobials of special critical interest

In Denmark, cephalosporins, fluoroquinolones and carbapenems have been defined as antimicrobials of special critical interest due to their resistance potential and their reserved use for treatment of severe infections. In 2021, the antimicrobials of special critical interest constituted 18% of the total consumption at somatic hospitals compared to 30% in 2012 (Table 5.7, Figure 5.20).

In 2021, cephalosporins accounted for 8.3%, fluoroquinolones for 6.8% and carbapenems for 2.9% of the total antimicrobial consumption in somatic hospitals in Denmark. The consumption trends for antimicrobials of special critical interest and all other antimicrobials are presented at regional and national level from 2012 to 2021 in Figure 5.20.

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

Additional tables and figures can be found in Supplementary Material on the DANMAP webpage (www.danmap.org).

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Textbox 5.1

National Action Plan on the reduction of antibiotics in humans, 2017-2021

In 2017, the Danish Ministry of Food, Agriculture and Fishery and the Danish Ministry of Health published a national One Health AMR Strategy. The content of the strategy were developed by a cross-sectoral AMR expert working group from the Danish Veterinary and Food Administration, the Danish Health Authority and Statens Serum Institut, supported by members of the National Antibiotic Council. Together with the strategy, two action plans were issued to support the overarching goals of the strategy. The Danish Ministry of Health issued a National Action Plan on the reduction of antibiotics in humans July 2017, building on existing work on proper use of antibiotics initiated by the Danish College of General Practitioners and the "National Quality Team" goals from 2016 for antibiotic use at Danish somatic hospitals.

Both strategy and action plan are available at the Danish Ministry of Health's homepage at www.SUM.dk. In the light of the CO-VID-19 pandemic, the Ministry of Health decided to extend the action plan until 2021 acknowledging the significant impact the pandemic had on healthcare provision and to allow the primary health care and hospital sectors more time for achieving the goals.

The National Action Plan has set three measurable goals:

- The first goal targets an overall reduction in antimicrobial consumption in primary health care, aiming at an average of 350 redeemed prescriptions per 1,000 inhabitants by 2020 (prescriptions issued by general practitioners, medical specialists and dentists). Starting point were 462 redeemed prescriptions per 1,000 inhabitants in 2016 (Figure 1). In 2021, the number of prescriptions in primary healthcare had been reduced to 333 prescriptions per 1,000 inhabitants. Already in 2016, some of the general practitioners prescribed less than 350 prescriptions per 1,000 inhabitants (DANMAP 2017).
- The second goal aims to increase the share of beta-lactamase sensitive penicillins used in primary health care to 36% by 2020, thus emphasizing the importance of beta-lactamase sensitive penicillins as the continued drug of choice in many common infections (prescriptions issued by general practitioners, medical specialists and dentists). The proportion of beta-lactamase sensitive penicillins remained unchanged (approximately 31%) between 2016 and 2019 (Figure 2). In 2021, the proportion decreased to 28%. At the time of halfway evaluation in 2019 it was discussed to change this goal slightly and include other groups of narrow spectrum penicillins in the aspired share.

Figure 1 Prescribing trends in the primary sector, prescriptions per 1,000 inhabitants, Denmark DANMAP 2021

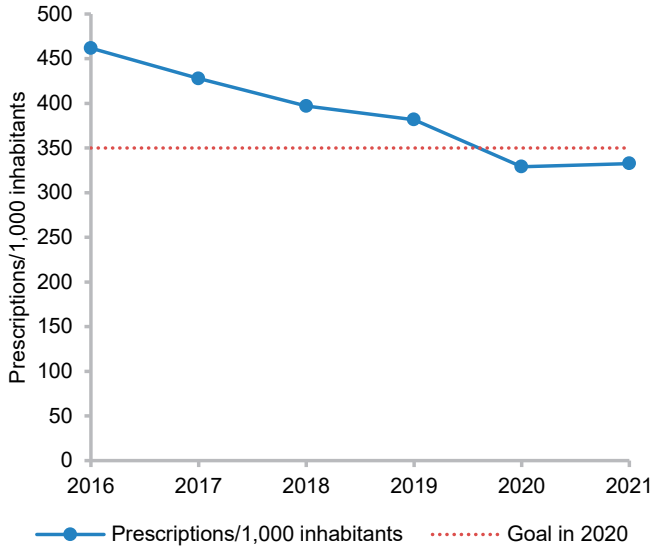
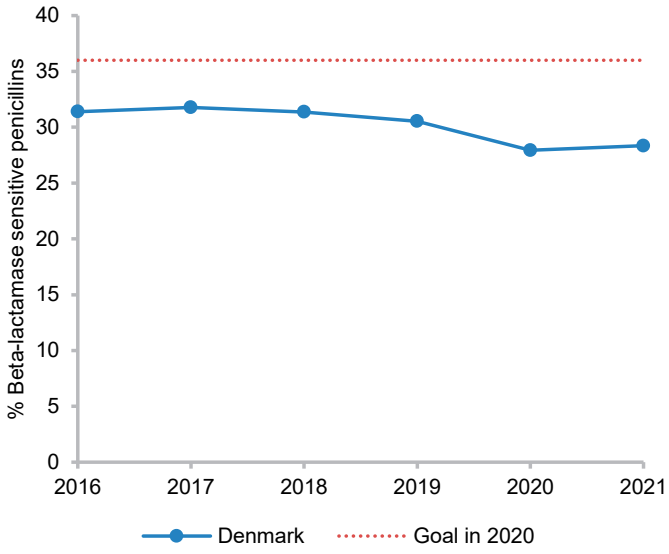


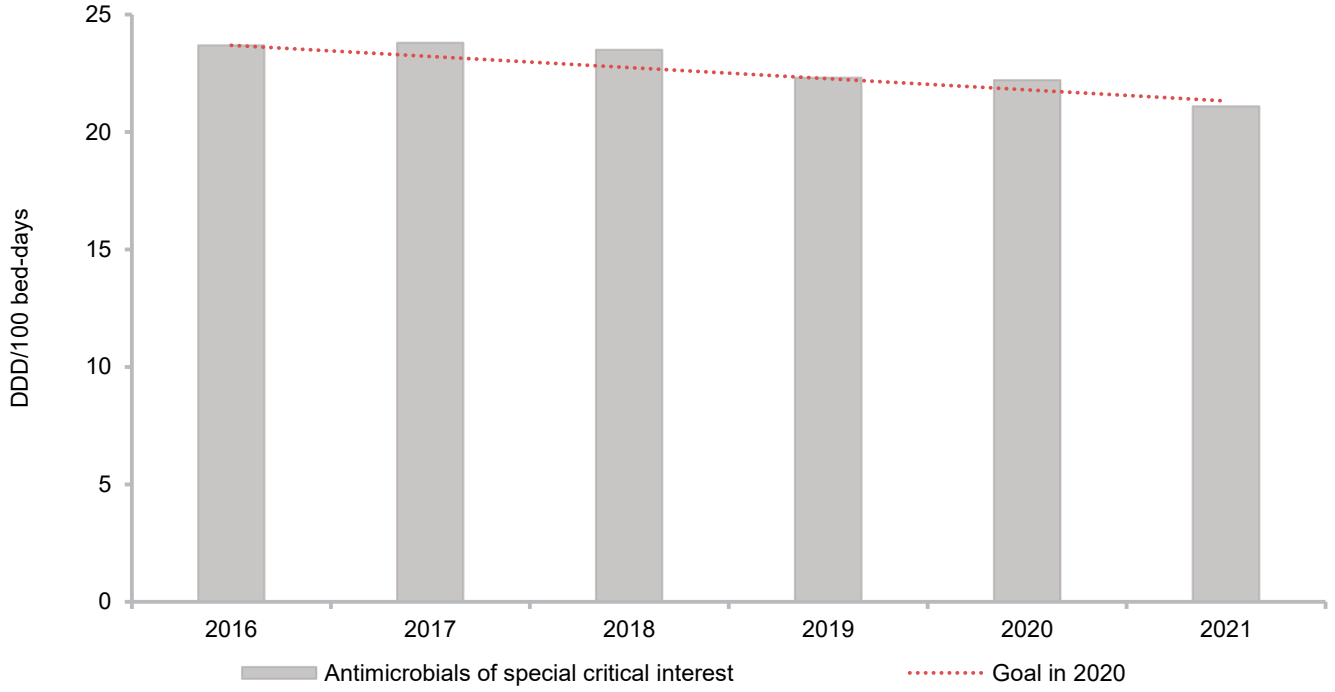
Figure 2 Share of beta-lactamase sensitive penicillins out of total consumption of antimicrobials, primary sector, Denmark DANMAP 2021



Data: Registered sales to individuals
Data source: Register of Medicinal Product Statistics and 2022 edition of the Anatomical Therapeutic Chemical (ATC) classification system

- **The third goal** aims to achieve a 10% reduction in the consumption of the three antimicrobials of special critical interest (cephalosporins, fluoroquinolones and carbapenems) at hospitals between 2016 and 2020, measured in DBD. The consumption of antimicrobials of special critical interest at hospitals decreased by 11.0% from 23.7 DBD in 2016 to 21.1 DBD in 2021 (Figure 3). At regional level, the consumption decreased in hospitals in the Capital Region of Denmark (-3.4%), Central Denmark Region (-21.2%), North Denmark Region (-12.8%) and Region of Southern Denmark (-28.6%). The level of consumption of antimicrobials of special critical interest increased in hospitals in the Region Zealand (+35.3%) between 2016 and 2021; however, the consumption in Region Zealand remained lower than the consumption levels in the other regions.

Figure 3 Consumption of antimicrobials of special critical interest on regional level incl. 10% reduction goal, DDD per 100 bed-days, Denmark
DANMAP 2021



Data: Registered sales to individuals
Data source: Register of Medicinal Product Statistics and 2022 edition of the Anatomical Therapeutic Chemical (ATC) classification system

The changes in usage and consumption of antibiotics observed for 2020 and 2021 are most likely linked to decreased numbers of respiratory infections in the community due to lockdowns and changes in hospital activity during the COVID-19 pandemic years rather than enforcement of initiatives supporting the goals of the National Action Plan. Of note is that the mandate and tasks of the National Antibiotic Council ran out in 2020 and were not renewed.

The Danish Food Administration issued an extended version of their National Action Plan in 2021 and an updated version with adjusted and new targets for the animal production and agriculture is expected for 2023.

The targets and the achieved results of the National Action Plan will be evaluated by the Ministry of Health in 2022-2023, which will include considering new targets for future improvement of human consumption of antimicrobials. It is expected that these will align with a renewal of the National AMR strategy and include goals for infection prevention and control or mitigation of AMR at hospitals and between different parts of the human health sector.

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Textbox 5.2

Antimicrobial consumption for elderly living in care homes

Background: During 2018-2020, The Danish Health Data Authority established a Care Home Register upon request from The Ministry of Health. The Care Home Register enables comparison of antimicrobial consumption for elderly residents living in care homes with those living in their own homes. In 2020, 935 care homes were included in the register with approximately 38,700 residents. In comparison, 1.12 million citizens above 65 years were living in their own homes.

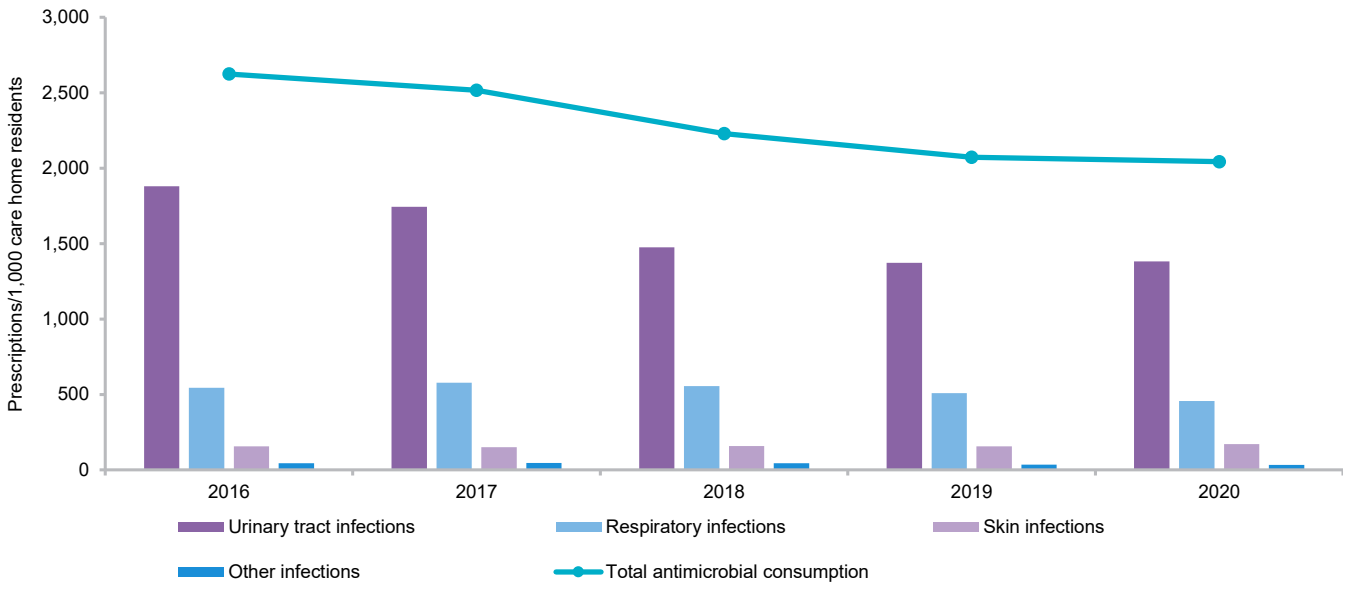
Definitions:

- Elderly people living in care homes: residents aged 65 years and above with registered address at a care home.
- Elderly people living in their own homes: residents aged 65 years and above with registered address outside a care home. This population is standardised by age and gender to obtain a similar distribution to the population in care homes.
- Included antimicrobials: ATC codes J01 and P01AB01 prescribed for patients aged 65 years and above in primary health care.
  a. Antimicrobials for urinary tract infections: pivmecillinam, nitrofurantoin, sulfonamides, trimethoprim, ciprofloxacin, pivampicillin, methenamin and other beta-lactams.
  b. Antimicrobials for respiratory infections: penicillin V/G, amoxicillin, moxifloxacin, penicillin with beta-lactamase inhibitor and macrolides except erythromycin.
  c. Antimicrobials for skin infections: beta-lactamase resistant penicillins, lincosamines, erythromycin and fucidic acid.
  d. Antimicrobials for other infections: Other antimicrobials not included in the above.

Methods: Data from the Care Home Register were combined with data from the Danish Civil Registration System (CPR) and 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.

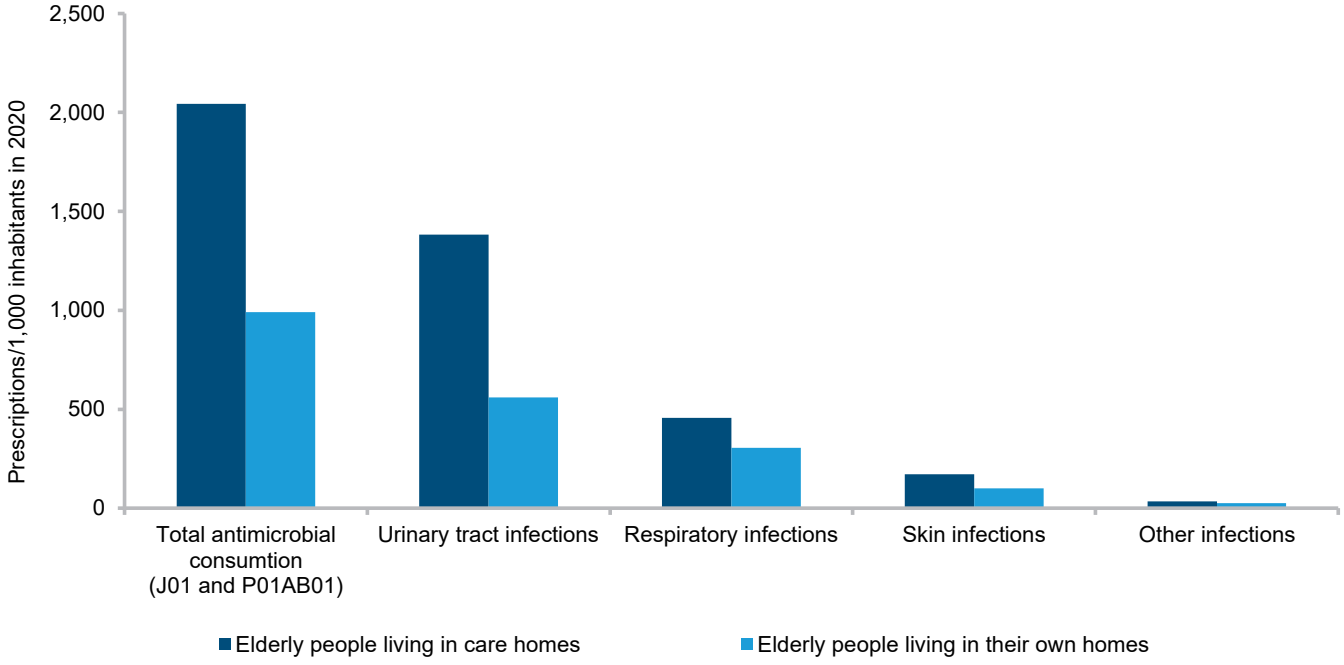
Results: In 2020, the total consumption of antimicrobials was 2,043 prescriptions per 1,000 care home residents (Figure 1). In 2016, the consumption was 2,624 prescriptions per 1,000 care home residents, corresponding to a 23% decrease. This reduction has been mainly driven by fewer prescriptions for urinary tract infections. However, prescriptions for skin infections in elderly care home residents increased by 10% between 2016 and 2020.

Figure 1 Consumption of antimicrobials (J01 and P01AB01) in primary health care for elderly people living in care homes, Denmark, 2016-2020
DANMAP 2021



Total antimicrobial consumption for elderly residents living in care homes was notably higher than consumption for elderly residents living in their own homes in 2020. This difference is also shown when looking at consumption by the main indications for antimicrobial prescribing in primary health care (Figure 2).

Figure 2 Consumption of antimicrobials (J01 and P01AB01) in primary health care for elderly living in care homes and for elderly living in their own homes, Denmark, 2020
DANMAP 2021



The biggest difference is observed in the treatment of urinary tract infections which is 147% higher for elderly residents living in care homes in 2020 (1382 prescriptions/1,000 inhabitants versus 559 prescriptions/1,000 inhabitants).

Conclusion: The observed higher antimicrobial consumption level for elderly residents living in care homes is expected, since this population is usually frailer compared to residents who are able to live in their own homes. However, since the antimicrobial consumption for elderly living in their own homes is standardized for age and gender, the observed differences call for further investigation.

The establishment of the Care Home Register allows close surveillance of antimicrobial consumption in one of the most fragile populations in society to ensure high quality treatment of infections and thereby prevent emergence of antimicrobial resistant pathogens. The Ministry of Health has announced that the upcoming new health strategy from 2023 will include improved focus on health services at care homes.

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Textbox 5.3

Consumption of antimicrobials in the Faroe Islands

Background: The Faroe Islands (FI) consist of 18 islands inhabited by approximately 52,000 inhabitants, ca. 22,000 of whom live in the capital Torshavn. The main hospital (Landssjukrahusid, LS, with 120 beds), is located in Torshavn, and there are two smaller hospitals in Klaksvik (22 beds) and Suduroy (22 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 LS were supplied by the Chief Pharmaceutical Office. Data on somatic bed-days were obtained from LS.

Antimicrobial consumption at Landssjukrahusid: The total antimicrobial consumption was 72.66 DDD/100 bed-days (DBD), a 16% decrease compared to 2020, but a 7% increase compared to 2019 (68.19 DBD) and 4% increase compared to 2016 (69.77 DBD). Special attention to three broad-spectrum antimicrobials, cephalosporins, carbapenems and fluoroquinolones is still required: In 2021, the consumption of cefuroxime was 14.75 DBD (20% of the total LS antimicrobial consumption), whereas it was 17.67 DBD in 2020 and 13.16 DBD in 2019. Likewise, quinolone use increased in 2020 (6.39 DBD) and decreased in 2021 (4.69 DBD), which is still to be considered as too high. For carbapenems, the corresponding data were 2.58 DBD in 2021, 2.24 DBD in 2020, and 2.26 DBD in 2019, but only 1.90 DBD in 2016 (Figure 1).

Altogether, an increase in many antimicrobials was observed for 2020 (Table 1), most remarkable for macrolides, quinolones and tetracyclines. However, the consumption of macrolides and quinolones decreased in 2021. The rather large fluctuations need further investigation and focus, but could possibly be a consequence of the COVID-19 pandemic.

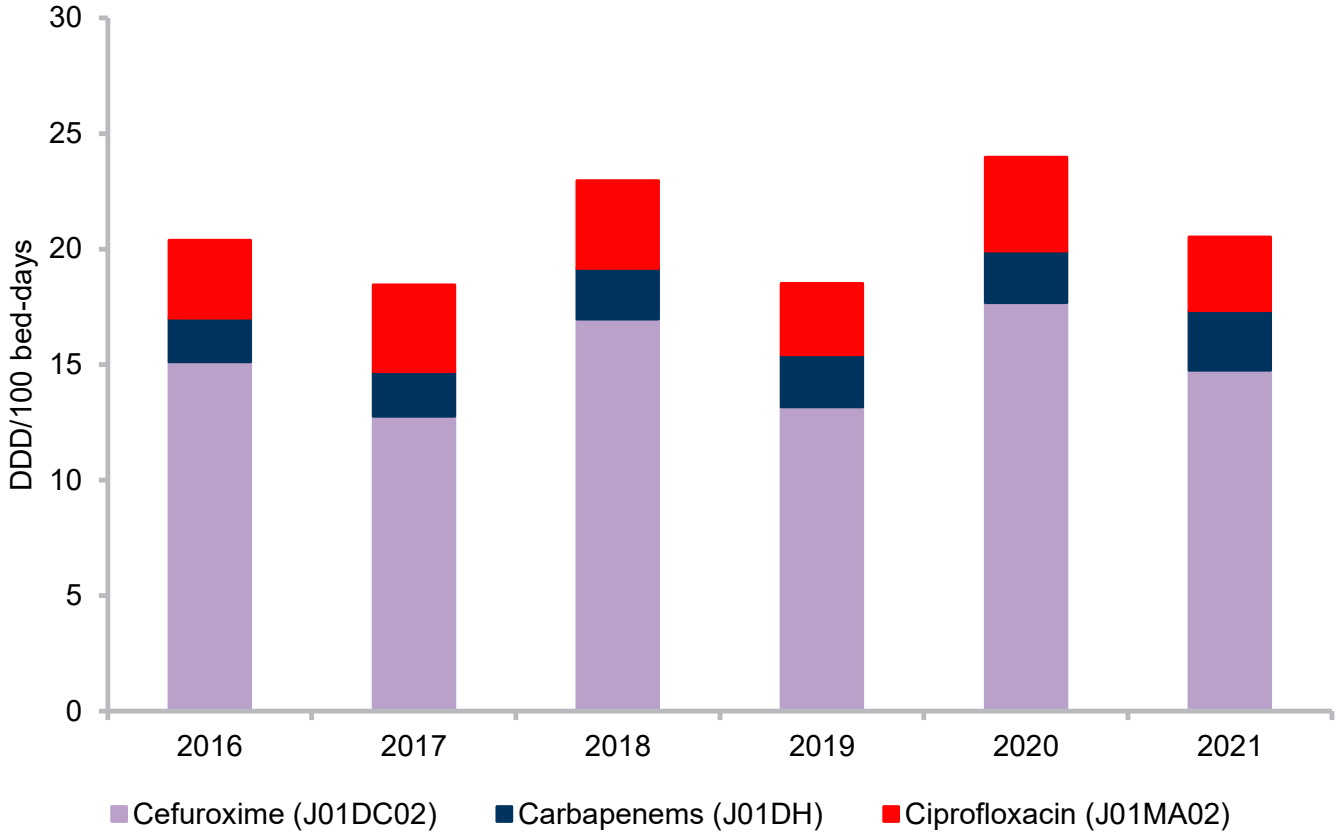
One encouraging observation, however, is the steady increase (7%) in the use of beta-lactamase resistant penicillins, from 5.73 DBD in 2019 to 6.15 DBD in 2021.

Table 1 Consumption of selected antimicrobials, DDD per 100 bed-days, Landssjukrahusid, 2016-2021 DANMAP 2021

	Year					
	2016	2017	2018	2019	2020	2021
Beta-lactamase sensitive penicillins (J01CE)	10.85	9.56	9.46	9.55	9.12	8.41
Beta-lactamase resistant penicillins (J01CF)	4.48	6.64	4.03	5.73	5.68	6.15
Ampicillin (J01CA01)	2.25	1.61	2.31	2.55	2.07	2.43
Mecillinam (J01CA11)	4.4	5.34	2.61	5.33	5.68	1.8
Ampicillin incl. beta-lactamase inhibitor (J01CR01)	2.05	2.88	2.41	2.37	1.72	2.8
Piperacillin incl. beta-lactamase inhibitor (J01CR05)	0.37	0.29	0.28	0.66	1.06	0.99
Cefuroxime (J01DC02)	15.12	12.75	16.98	13.16	17.67	14.75
Carbapenems (J01DH)	1.9	1.94	2.19	2.26	2.24	2.58
Aminoglycosides (J01GB)	2.1	1.33	1.58	2.25	2.46	2.62
Quinolones (J01MA)	5.22	4.94	5.38	4.28	6.39	4.69
Metronidazol (J01XD01, P01AB01)	7.59	5.91	6.15	7.03	7.81	6.49
Macrolides (J01FA)	3.09	1.81	1.23	1.44	7.02	2.21
Tetracyclines (J01AA)	0.87	0.4	1.23	1.09	3.9	5.59
Other	9.5	8.7	9.59	10.51	13.61	11.16
Total	69.77	64.1	65.42	68.19	86.41	72.66

Figure 1 Consumption of selected critically important antimicrobials, DDD per 100 bed-days, Landssjukrahusid, 2016-2021

DANMAP 2021



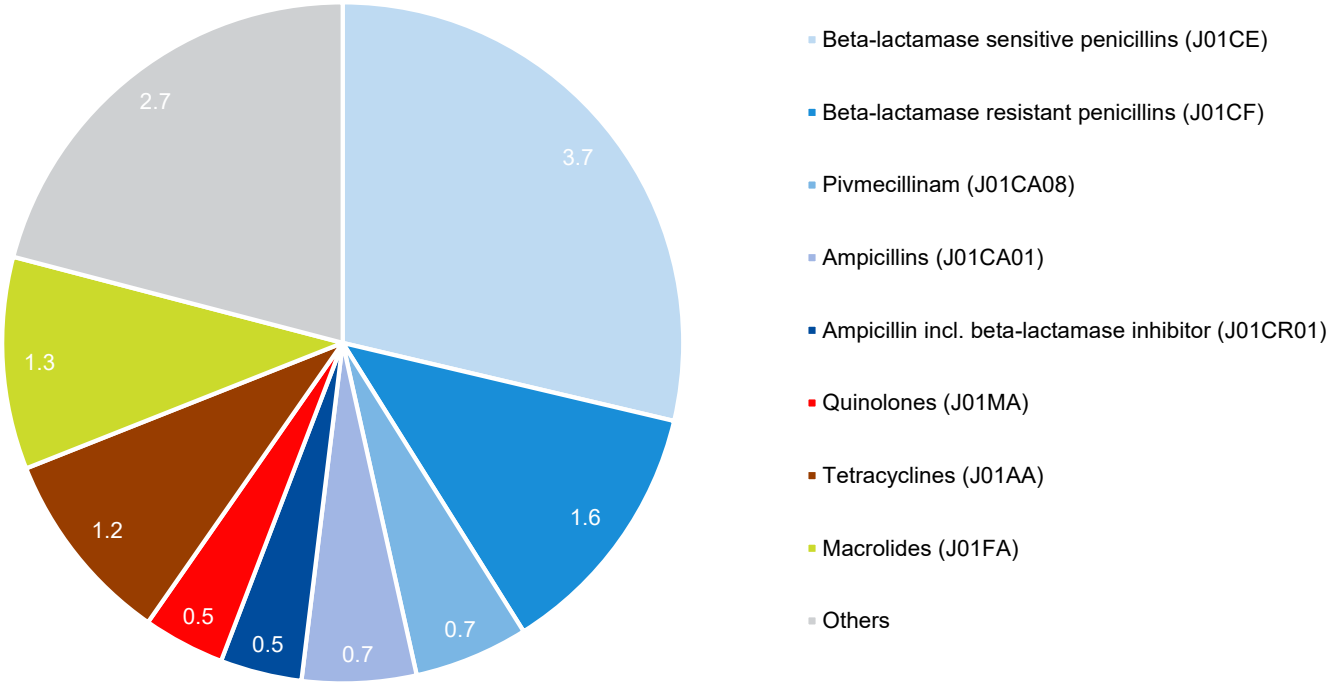
Antimicrobial consumption in primary healthcare: In 2021, the total antimicrobial consumption in primary healthcare was 12.90 DDD/1,000 inhabitants/day (DID) – representing an 11% increase compared to 2020, but a decrease compared to the preceding years (range 13.41-13.50 DID in 2016-2019). The distribution of antimicrobial consumption is shown in Figure 2. Contrary to the consumption at LS, the use of many antimicrobials decreased in 2020, and increased again in 2021, although not reaching the 2019-level, e.g. beta-lactamase sensitive penicillins consumption was 4.21 DID in 2019, 3.24 DID in 2020, and 3.72 DID in 2021. Macrolide consumption was 1.46 DID in 2019, 1.08 DID in 2020 and 1.28 DID in 2021.

Remarkable were also the changes in antimicrobials commonly used for prophylaxis or treatment of urinary tract infections. Ciprofloxacin use increased from 3.65 DID in 2020 to 4.17 DID in 2021 (14%). The use of sulfamethizol decreased (12%) from 0.46 DID in 2019 to 0.40 in 2021, and sulfamethoxazole with trimethoprim increased from 0.03 DID in 2019 to 0.06 DID in 2021. The use of methenamin increased (45%) from 0.51 DID to 0.75 DID in the same time period.

continued ... Textbox 5.3

Figure 2 Consumption of antimicrobials in primary healthcare, DDD per 1,000 inhabitants per day, Faroe Islands, 2021

DANMAP 2021



Conclusion: In 2021, the antimicrobial consumption at LS for most antimicrobials decreased somewhat from an earlier increased 2020-level, whereas the tendency was the opposite in primary healthcare. The reasons for this need to be further investigated in order to further implement antibiotic stewardship. 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 cefuroxime, carbapenems and tetracycline.

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6

RESISTANCE IN ZOONOTIC BACTERIA AND ANIMAL PATHOGENS

6. Resistance in zoonotic bacteria and animal pathogens

Highlights: In 2021 macrolide (erythromycin) resistance in ***Campylobacter*** *jejuni* was less than one percent in human isolates and resistance was not detected in cattle and broiler isolates.

Fluoroquinolone (ciprofloxacin) resistance remained common in *C. jejuni* isolates from human cases (52%), broilers (32%) and cattle (25%). The decrease observed in isolates from broilers in 2020 continued in 2021.

The level of azithromycin resistance in ***Salmonella*** Typhimurium was less than 1% in human isolates, while one of 39 isolates from Danish pork showed resistance. Unlike previous years, where azithromycin resistance was not detected in isolates from pigs, in 2021, three of 34 isolates of *S.* Typhimurium from pigs were azithromycin resistant.

Fluoroquinolones may be used for treatment of human *Salmonella* infections and resistance to fluoroquinolones is monitored using ciprofloxacin. Ciprofloxacin resistance was observed in 7% of *S.* Typhimurium isolates from domestically acquired infections and in 3 of 13 travel-associated cases. The resistance level in domestic isolates in 2021 was higher than the level reported in 2019 and 2020, where 4% and 1% resistance, were reported, respectively. Historically, ciprofloxacin resistance has predominantly been observed in isolates from travel-associated cases. Fluoroquinolone resistance has not been recorded in *S.* Typhimurium from Danish pigs and pork since 2010 and 2007, respectively.

Resistance to the critically important 3rd generation cephalosporins and carbapenems is rare in *S.* Typhimurium. In 2021 cephalosporin resistance was observed in one human isolate and no carbapenem resistance was observed. In line with the previous years, these resistances were not observed in *S.* Typhimurium isolates from Danish pigs and pork.

Pathogenic bacteria from pigs, including *Actinobacillus pleuropneumoniae*, haemolytic *Escherichia coli* and *Streptococcus suis*, displayed similar levels of resistance as in previous years.

There was a high concordance between antimicrobial susceptibility testing results and presence/absence of corresponding resistance genes/point mutations in pathogenic bacteria from pigs (100% for *A. pleuropneumoniae*, 96% for haemolytic *E. coli* and 90% for *S. suis*).

Resistance to the critically important 3rd generation cephalosporin cefotaxime was identified in 7% of the haemolytic *E. coli* isolates from pigs and was in all cases associated with presence of either the $bla_{\text{CTX-M-1}}$ gene or point mutations in the *ampC* promoter.

The *optrA* gene, which has been associated with resistance to oxazolidinones (e.g. linezolid used in human medicine) and phenicols (e.g. florfenicol used in veterinary medicine), was identified in 2% of the *S. suis* isolates from pigs.

A point mutation in the *pmrB* gene associated with resistance to colistin was identified in 14% of the haemolytic *E. coli* isolates from pigs, but only one of the 15 isolates with this point mutation was phenotypically resistant to colistin.

6.1 Resistance in zoonotic bacteria

6.1.1 Introduction to resistance in zoonotic bacteria

Zoonoses are infectious diseases that are transmitted between animals and humans, either through direct contact with animals or indirectly by contaminated food, water, or the environment. A description of the trends and sources of zoonoses in Denmark and of national surveillance and control programmes can be found in Annual Report on Zoonoses in Denmark 2021 [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 animal meat.

In Denmark, antimicrobials are generally not recommended for treatment of diarrhoea in human patients unless there is prolonged duration of disease or the patient is severely ill. If treatment is required, macrolides (azithromycin) are recommended for treatment of *Campylobacter* infections. For *Salmonella* infections no specific recommendations regarding antibiotic treatment exist for the primary sector. For infections treated in hospitals, intravenous ceftriaxon is recommended for septic patients and per oral azithromycin for less severe cases. For prolonged or recurrent infections, combination therapy will be used with ciprofloxacin or sulfamethoxazol and trimethoprim added. The Register of Medicinal Product Statistics at the Danish Health Data Authority does not register the specific pathogen that was treated with antimicrobials.

Macrolides are often used for treatment of infections in food producing animals in Denmark, especially pigs. Fluoroquinolones are not used in production animals, whereas there is limited use of 2nd generation and no 3rd or 4th generation cephalosporins in cattle. The use of antimicrobials in the Danish poultry sector is low and limited to a few antimicrobial classes, primarily tetracyclines (Table 4.1).

In humans, monitoring of antimicrobial resistance is performed on clinical isolates of *Salmonella* and for *Campylobacter jejuni* a geographically stratified selection of isolates is subjected to susceptibility testing. The testing is performed in accordance with the ECDC recommendations (Materials and methods, section 9.9). Travel histories of the patients are collected, when possible.

Campylobacter and *Salmonella* non-clinical isolates were obtained from healthy animals at slaughter (caecal samples). In addition, *Salmonella* isolates were obtained from fresh retail meat and from carcasses at slaughter. *C. jejuni* was isolated from broilers and cattle, and *C. coli* was isolated from pigs (Table 9.1).

In 2021, the phenotypic susceptibility testing of *Campylobacter* and *Salmonella* from humans, animals and meat was done in accordance with the new Commission Implementing Decision 2020/1729/EU of 17 November 2020 on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria. This has caused changes in the antimicrobial test panels compared to those used previously for the DANMAP report. Ertapenem (carbapenem) and chloramphenicol have replaced nalidixic acid and streptomycin in the *Campylobacter* test panel, and amikacin (aminoglycoside) has been included on the *Salmonella* test panel. Further details about Decision 2020/1729/EU are available in materials and methods, section 9.5.

6.1.2 *Campylobacter*

A total of 363 human *C. jejuni* isolates were susceptibility tested. The isolates represented 285 domestically acquired infections, 29 travel-associated infections and 49 infections of unknown origin; 32 outbreak related isolates from three different outbreaks were included.

A selection of *C. jejuni* isolates, including 31 recovered from broilers and 177 recovered from cattle, and a selection of 123 *C. coli* isolates recovered from pigs were also susceptibility tested.

Resistance in *Campylobacter jejuni*

The levels of antimicrobial resistance in *C. jejuni* isolates from Danish broilers, cattle and humans are presented in Table 6.1. Resistance to ciprofloxacin and tetracycline is common in isolates from humans, broilers, and cattle, whereas resistance to chloramphenicol, erythromycin and gentamicin s rarely observed. Ertapenem was included as a new antimicrobial in the *Campylobacter* panel in 2021. Ertapenem resistance was found in 4% and 14% of isolates from domestically and travel-associated human isolates, respectively, whereas no ertapenem resistance was observed in isolates from Danish broilers or cattle.

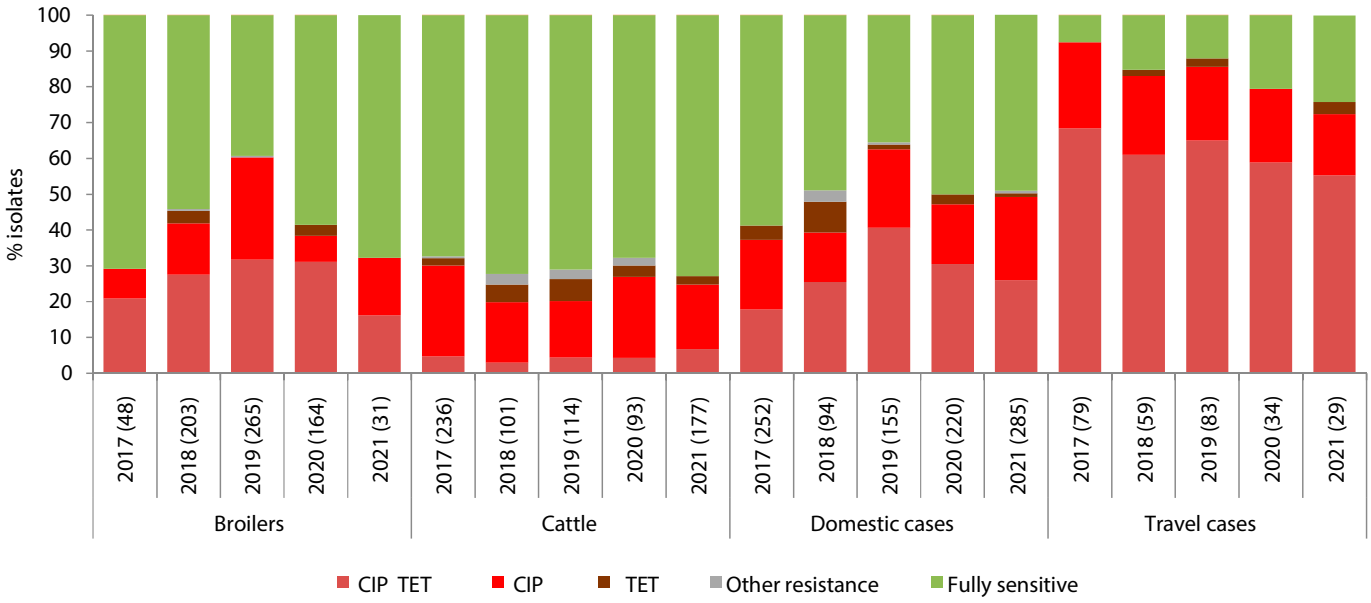
In 2021, 68% of *C. jejuni* from broilers, 73% from cattle and 50% from domestic human cases were sensitive to all antimicrobials tested. The percentage of fully sensitive *C. jejuni* isolates from domestic human infections was similar to the previous years, as the increase in antimicrobial resistant cases observed for 2019 was primarily due to a specific ciprofloxacin and tetracycline resistant clone that caused several outbreaks. As in previous years, the resistance levels were higher in infections from travel-associated cases compared to domestically acquired infections. The percentage of fully sensitive *C. jejuni* isolates from broilers continued to increase in 2021, while the percentage of fully sensitive isolates from cattle has remained relatively stabile in the past five years (Figure 6.1).

In 2021, the percentage of *C. jejuni* isolates from broilers with resistance to only ciprofloxacin was 16% as was the percentage of combined resistance to ciprofloxacin and tetracycline. Also domestically acquired human cases showed unchanged levels (24%). Resistance to only tetracycline and resistance to antimicrobials other than ciprofloxacin or tetracycline remained rare in isolates from animals and humans in 2021.

Macrolide resistance in *Campylobacter* is monitored using erythromycin. Among the human *C. jejuni* infections, erythromycin resistance was observed in one isolate in 2021. Macrolide resistance is rarely observed in isolates from domestically acquired infections and in travel-associated cases in the last three years the level of macrolide resistance has not exceeded 4%. The levels of erythromycin resistance in *C. jejuni* isolates from Danish broilers and cattle have also remained low between 0% and 2% of resistant isolates per year (Table 6.1, Figure 6.2). The low prevalence of macrolide resistance in animal and food isolates thus remained very close to the limit of detection by the current sampling scheme and resistant isolates were only captured sporadically.

As in previous years, the occurrence of resistance to ciprofloxacin or tetracycline in 2021 was higher in travel-associated isolates (72% and 59%, respectively) than in isolates from domestically acquired infections (49% and 27%, respectively), and it was higher in human clinical isolates than in isolates from broilers and cattle (Figure 6.2).

Figure 6.1 Distribution (%) of AMR profiles in *Campylobacter jejuni* from broilers, cattle and human cases, Denmark DANMAP 2021



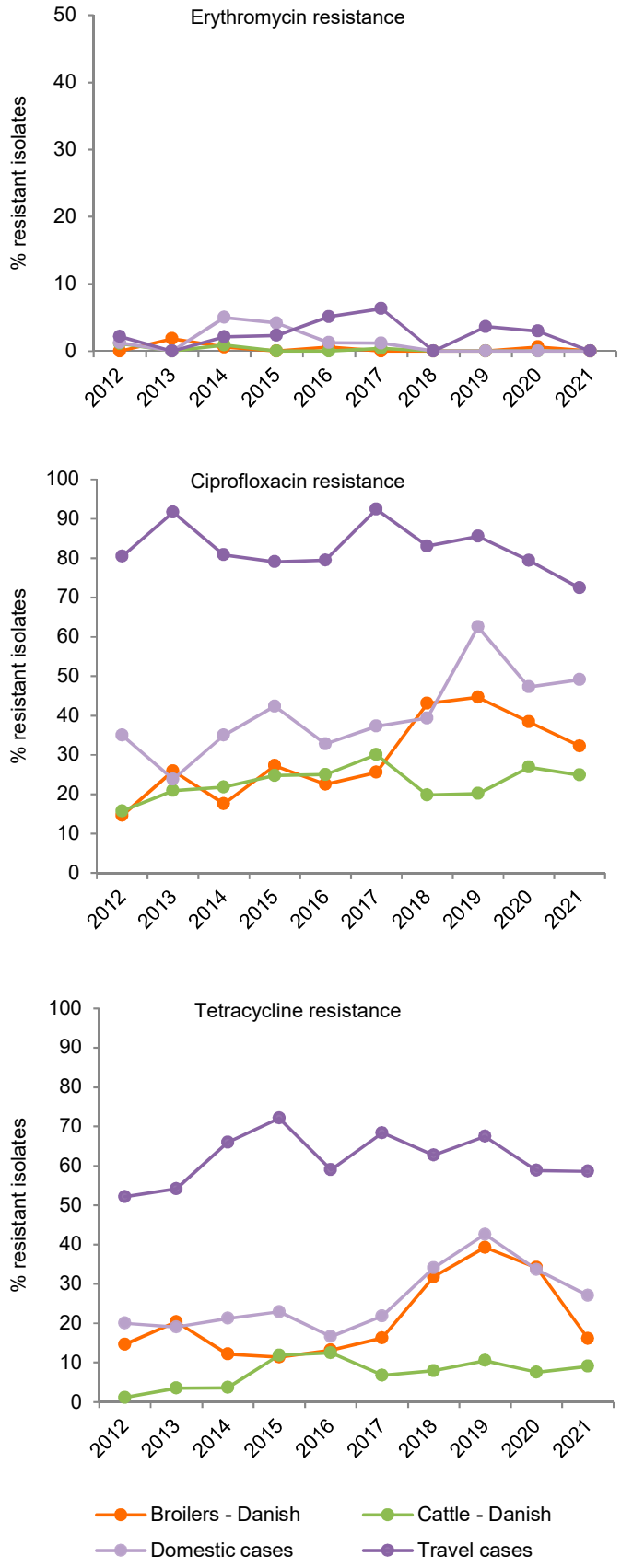
The number of isolates included each year is shown in parentheses. A human isolate is categorized as domestically acquired if the patient did not travel outside Denmark one week prior to the onset of disease. CIP: all isolates with ciprofloxacin resistance, but without tetracycline resistance. TET: all isolates with tetracycline resistance, but without ciprofloxacin resistance. CIP/TET: all isolates with ciprofloxacin and tetracycline resistance. Other resistance: all isolates with neither ciprofloxacin nor tetracycline resistance. CIP/TET, CIP and TET isolates may be resistant to other antimicrobials in the test panel (Table 6.1)

Table 6.1 Resistance (%) in *Campylobacter jejuni* isolates from broilers, cattle and human cases, Denmark DANMAP 2021

	Broilers	Cattle	Human			
	Danish	Danish	Domestically acquired	Travel abroad reported	Unknown origin	Total
Antimicrobial agent	%	%	%	%	%	%
Chloramphenicol	0	0	0	0	0	0
Ciprofloxacin	32	25	49	72	57	52
Ertapenem	0	0	4	14	6	5
Erythromycin	0	0	0	0	2	<1
Gentamicin	0	0	0	0	0	0
Tetracycline	16	9	27	59	31	30
Fully sensitive (%)	68	73	49	24	41	46
Number of isolates	31	177	285	29	49	363

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

Figure 6.2 Erythromycin, ciprofloxacin and tetracycline resistance (%) among *Campylobacter jejuni* from broilers, cattle and human cases, Denmark DANMAP 2021



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

Over the last decade, and until 2019, ciprofloxacin resistance has overall increased in *C. jejuni* from Danish broilers. This increase discontinued first in 2020, and further in 2021, (Figure 6.2) and coincided with the trend in the levels of tetracycline resistance and the combined resistance to tetracyclines and fluoroquinolones (Figure 6.1). This points towards other mechanisms rather than direct use being a main cause of resistance development and spread.

Gentamicin resistant isolates were not observed in 2021 among human, cattle and broiler isolates. During the last 10 years of monitoring, gentamicin resistance has been low or absent among *C. jejuni* from human isolates and has not been observed among *C. jejuni* from broilers and cattle. In 2021, resistance to chloramphenicol or ertapenem was not observed among *C. jejuni* isolates from broilers and cattle. In isolates from humans, ertapenem resistance was observed in 4% and 14% of isolates from domestic- and travel-associated cases, respectively (Table 6.1).

Resistance in *Campylobacter coli*

Gentamicin and chloramphenicol resistance were not observed in *C. coli* isolates from pigs in 2021. Resistance to erythromycin and to ertapenem were detected in few isolates, 6% (seven isolates) and <1% (one isolate), respectively. Twenty percent (24 isolates) and 26% (32 isolates) of the pig *C. coli* isolates were resistant against ciprofloxacin and tetracycline, respectively.

6.1.3 *Salmonella*

DANMAP focuses on resistance in *Salmonella* Typhimurium, as this serotype is present among human clinical isolates and in isolates from food-producing animals. Clonal dissemination plays an important role for the occurrence of antimicrobial resistance among *S.* Typhimurium. The global dissemination of genomic islands conferring resistance to ampicillin, sulfonamide and tetracycline (the ASuT multidrug-resistance profile) among *S.* Typhimurium and its monophasic variants continues to contribute to a high level of multidrug-resistance among isolates from animals and humans. In Denmark, *S.* Typhimurium isolates from humans and production animals often have ASuT resistance. However, these antimicrobials are only rarely used for treatment of salmonellosis in humans, and thus the public health impact of ASuT multidrug-resistance may be of less direct importance than resistance to more commonly used antimicrobials in human medicine such as macrolides, fluoroquinolones and cephalosporins. In DANMAP, *S.* Typhimurium includes the monophasic variants with antigenic formulas S. 4, [5],12:i:-, unless otherwise stated.

Resistance in *S.* Typhimurium

In 2021, a total of 205 human isolates of *S.* Typhimurium were susceptibility tested, including 117 diphasic and 88 monophasic variants. The diphasic variants were dominated by ST36 (73 isolates) and ST19 (36 isolates). All monophasic isolates (human and animal origin) were of sequence type (ST) 34 (91 isolates). Only five other ST types were identified. Sixty-nine isolates were associated with three outbreaks that encompassed 49 isolates (ST36, fully sensitive), nine isolates (ST36, fully sensitive) and 11 isolates (ST34, tetracycline resistant) respectively. Thirteen isolates were from travel-associated cases. This number is low compared to the pre-COVID 19, and hence data for travel-associated isolates are not presented independently in 2021.

Fifty isolates from pork (39 from Danish and 11 from imported meat) and 33 isolates from pigs were recovered and susceptibility tested in 2021. Isolates from Danish pork included 16 diphasic and 23 monophasic variants and the pig isolates included 7 diphasic and 26 monophasic variants. AMR results from imported pork are not reported due to the low number of isolates.

Table 6.2 Resistance (%) in *Salmonella* Typhimurium isolates from pigs, domestic pork and humans, Denmark DANMAP 2021

	Pigs	Pork	Human	
	Danish	Danish	Domestically acquired	Total
Antimicrobial agent	%	%	%	%
Amikacin	0	0	1	1
Ampicillin	79	79	45	43
Azithromycin	9	3	0	<1%
Cefotaxime	0	0	1	<1%
Ceftazidime	0	0	1	<1%
Chloramphenicol	9	21	5	5
Ciprofloxacin	0	0	7	6
Colistin	0	0	0	<1%
Gentamicin	3	0	1	1
Meropenem	0	0	0	0
Nalidixic acid	0	0	5	5
Sulfonamide	82	79	45	43
Tetracycline	88	67	51	51
Tigecycline	9	3	3	3
Trimethoprim	18	31	5	4
Fully sensitive (%)	3	15	46	47
Number of isolates	33	39	147	205

Includes isolates verified as monophasic variants of *S.* Typhimurium with antigenic formulas s. 4,[5],12:i:-. Isolates of 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 (Materials and methods, Table 9.3)

The resistance data for *S.* Typhimurium for 15 antimicrobials are presented in Table 6.2 for pig, domestic pork, and human isolates. The level of resistance towards ampicillin, sulfonamide and tetracycline was higher in animal and food isolates than in isolates from humans. The level of fully sensitive human isolates was 47%, compared to 3% and 15% in pigs and Danish pork, respectively.

Figure 6.3 presents the relative distribution in percent of AMR profiles for *S.* Typhimurium from pigs, domestic pork and human domestic cases. For domestic human cases the level of fully sensitive *S.* Typhimurium isolates increased from 2020 to 2021. For 2021, two outbreaks encompassed 49 and nine fully sensitive strains, respectively. The majority of ASuT resistant isolates were ST34, that encompassed 39 of the 41 isolates with exclusive resistance towards ASuT.

Most of the *S.* Typhimurium isolates recovered from pigs and pork were resistant to several antimicrobials. Only 15% of the Danish pork isolates and 3% of the isolates from pigs were fully sensitive to all tested antimicrobials in 2021, (Table 6.2). In 2021, multidrug-resistance among *S.* Typhimurium from Danish pork was similar to 2020, and as in previous years the ASuT phenotype was the most frequent resistance profile (Figure 6.3).

Fluroroquinolones may be used for treatment of human *Salmonella* infections and resistance is monitored using ciprofloxacin. Ciprofloxacin resistance was observed in 7% of the isolates from domestically acquired infections and in three of 13 isolates from travel associated cases. The resistance in domestic isolates in 2021 was higher than in 2019 and 2020, (4% and 1%, respectively). Historically, ciprofloxacin resistance has predominantly been observed in isolates from travel-associated cases (Figure 6.4).

During the last ten years, ciprofloxacin resistance in *S.* Typhimurium from Danish pigs and pork has been rare. In 2021, no ciprofloxacin resistance was found in pork and pig isolates.

Since 2014, macrolide resistance in *Salmonella* has been monitored using azithromycin. Azithromycin is used for treatment of human *Salmonella* infections in Denmark. Resistance to azithromycin in *S.* Typhimurium has been low in human isolates and in 2021 resistance was only found in one isolate (<1%). In contrast, a higher level of azithromycin resistance was detected in pigs in 2021 compared to previous years, with three of 34 (9%) *S.* Typhimurium isolates from domestic pigs showing resistance (Figure 6.4). There is no obvious explanation for the observed increase in the prevalence of azithromycin resistance in pigs, and the trend will be closely monitored in the coming years.

Gentamicin resistance in domestically acquired human isolates has been low and stable over the last years, and in 2021, only two *S.* Typhimurium isolates (1%) were resistant to gentamicin. The resistance to gentamicin in isolates from pigs and pork returned to low levels, similar to those observed before the peak in 2019, with no observed gentamicin resistance in domestic pork and 3% resistant isolates among domestic pigs, respectively (Figure 6.4).

Among human isolates, the level of resistance towards 3rd generation cephalosporins was low, and the combination of cefotaxime and ceftazidime resistance was only found in one domestic isolate. Meropenem resistance was not observed among human isolates. As in previous years, none of the *S.* Typhimurium isolates from pigs and pork were resistant to third generation cephalosporins or to meropenem (Table 6.2).

Resistance to tigecycline and colistin in *S.* Typhimurium is rare in Denmark. In 2021, one human isolate was recorded as colistin resistant, and as in previous years, no colistin resistance was found among pig and pork isolates. Resistance towards tigecycline was observed in 3% of the human isolates and in 3% and 9% of the pork and pig isolates, respectively (Table 6.2). The results for pig isolates represent a slight increase compared to previous years, and are caused to some extent by the change in the adopted epidemiological cut-off for the interpretation of tigecycline resistance in *Salmonella*, which changed from >1 µg/ml to >0.5 µg/ml in 2021 (Materials and methods, Table 9.2). When interpreted with the previous cut-off, the prevalence of tigecycline resistance in isolates from pigs was comparable to previous observations (3%).

Resistance to amikacin, the new antimicrobial on the test panel in 2021, was observed in 1% of the human *S.* Typhimurium isolates whereas all isolates from pigs and pork were sensitive (Table 6.2).

The steady reduction in use of tetracycline in pig production observed since 2014 (Chapter 4, Table 4.1) was still not reflected in the levels of resistance in *S.* Typhimurium from pigs. While isolates from Danish pork showed a lower level of tetracycline resistance, (67% in 2021 compared to 77% in 2020), resistance in *S.* Typhimurium from pigs increased to 87% (80% in 2020), (Table 6.2).

Resistance in other *Salmonella* serotypes

A total of 27 human isolates of *S.* Dublin were susceptibility tested. *S.* Dublin is intrinsically resistant to colistin. All isolates, except one, were fully susceptible. The non-susceptible strain was resistant to ampicillin, azithromycin, chloramphenicol, sulfonamide and trimethoprim.

Among samples from pigs and pork, next to *S.* Typhimurium, the most common serotype detected was *S.* Derby. *S.* Derby was recovered from 55 pig samples and 34 Danish pork samples.

Figure 6.3 Relative distribution (%) of multidrug-resistant, resistant and fully sensitive *S.* Typhimurium from pigs, domestic pork and human cases, Denmark

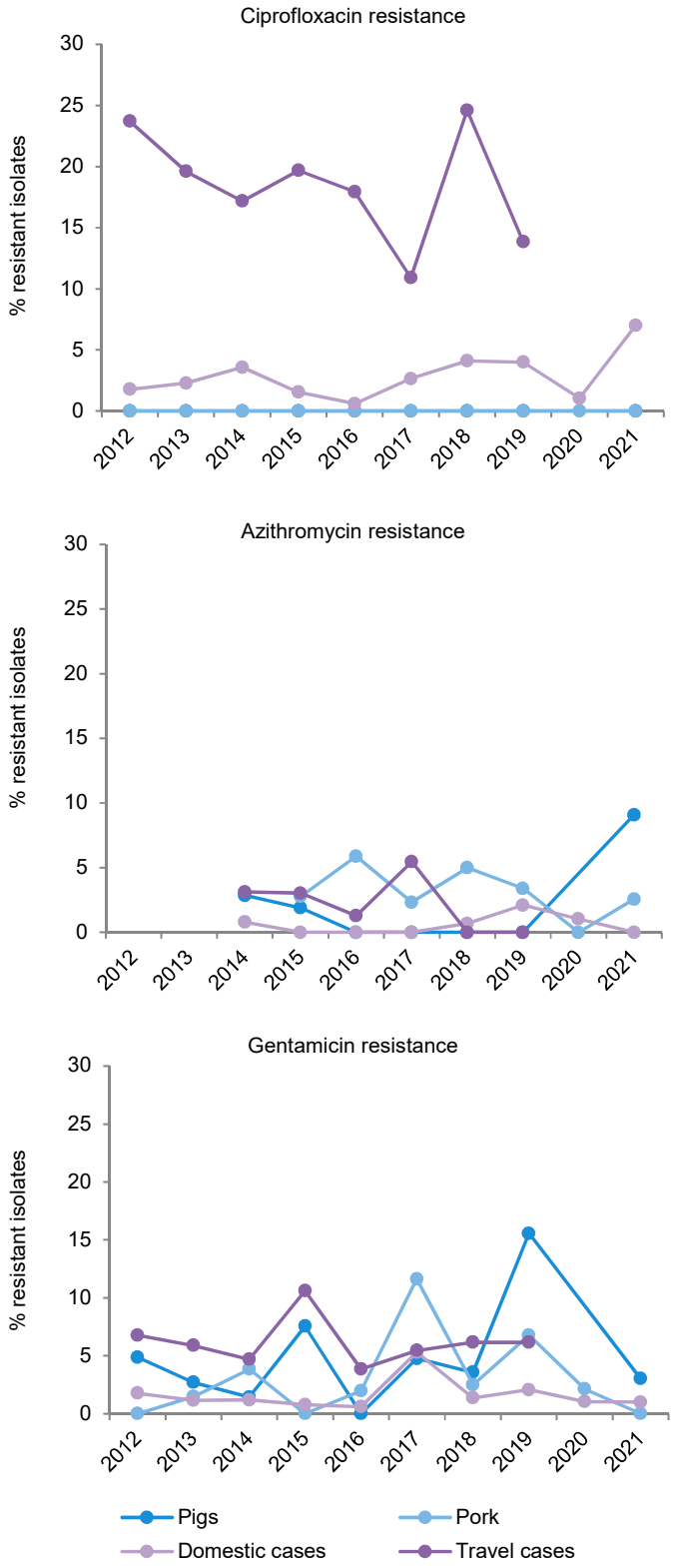
DANMAP 2021

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

a) No data

b) Distribution not shown due to low number of isolates (N<15)

Figure 6.4 Ciprofloxacin, azithromycin and gentamicin resistance (%) among *S.* Typhimurium from pigs, domestic pork and human cases, Denmark DANMAP 2021



Includes isolates verified as monophasic variants of *S.* Typhimurium with antigenic formulas s. 4,[5],12:i:-. An isolate is categorised as domestically acquired if the patient did not travel outside Denmark one week prior to the onset of disease. Due to the low number (N<15), AMR in isolates from travel-associated cases are not shown separately for 2020 and 2021

The resistance levels in *S.* Derby are generally lower than in *S.* Typhimurium, and in 2021, 39 of the 55 *S.* Derby isolates from pigs (71%) and 25 of the 34 isolates from pork (74%) were sensitive to all tested antimicrobials. Resistance to ampicillin, sulfonamide and trimethoprim in isolates from Danish pork has overall increased after 2013, but with decreasing trends for 2021. Resistance levels for 2021 are presented in Table 6.3.

Resistance to critically important antimicrobials remained rare in 2021 in isolates from pigs and was not observed in isolates from domestic pork.

Table 6.3 Resistance (%) in *Salmonella* Derby isolates from domestic pigs and pork, Denmark DANMAP 2021

	Pigs	Pork
Antimicrobial agent	Danish %	Danish %
Amikacin	0	0
Ampicillin	9	15
Azithromycin	2	0
Cefotaxime	0	0
Ceftazidime	0	0
Chloramphenicol	4	12
Ciprofloxacin	0	0
Colistin	2	0
Gentamicin	2	0
Meropenem	0	0
Nalidixic acid	0	0
Sulfonamide	18	15
Tetracycline	15	21
Tigecycline	0	0
Trimethoprim	13	6
Fully sensitive (%)	71	74
Number of isolates	34	55

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 (Table 9.3)

Additional tables can be found in Supplementary Material on the DANMAP webpage (www.danmap.org).

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6.2 Resistance in pathogenic bacteria from pigs

Phenotypic susceptibility testing and surveillance of 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 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 genes and point mutations. WGS-based AMR surveillance in pathogenic bacteria from pigs commenced in January 2021 and included antimicrobial susceptibility testing (AST) and WGS of isolates belonging to *A. pleuropneumoniae*, haemolytic *E. coli* and *S. suis*, which were identified in clinical samples submitted by veterinarians to the Veterinary Laboratory, The Danish Agriculture and Food Council.

6.2.1 Temporal trends of AMR in pathogenic bacteria from pigs

Figures 6.5, 6.6 and 6.7 illustrate the temporal trends of resistance in *A. pleuropneumoniae*, haemolytic *E. coli* and *S. suis* isolates recovered from pigs during 2016-2021 (one isolate/organism/clinical submission), whereas Table 6.4 shows the proportion of resistant isolates in 2021.

As in previous years, *A. pleuropneumoniae* displayed very little or no resistance in 2021. None of the isolates were resistant to ceftiofur (3rd generation cephalosporin), florfenicol (phenicol), tiamulin (pleuromutilin), tilmicosin (macrolide), or trimethoprim-sulfamethoxazole (folate pathway inhibitor). Four and one percent of the isolates were resistant to tetracycline and ampicillin, respectively.

In 2021, the proportion of resistant haemolytic *E. coli* isolates was at a level similar to previous years, ranging from less than 1% for ceftiofur and colistin to 76% for streptomycin. Interestingly, 9% of the isolates were resistant to ciprofloxacin in 2021, despite the fact that usage of fluoroquinolones in the Danish pig production system has been close to zero since 2003. The small proportion of isolates displaying resistance to colistin is consistent with the low levels of resistance to this drug in previous years. The reintroduction of neomycin for treatment of gastrointestinal infections in pigs in 2017 might, at least in part, explain the increasing levels of resistance to this drug between 2016 (7%) and 2020 (26%), but this trend seems to have levelled off in 2021. Nonetheless, neomycin usage and resistance should be monitored closely in the coming years, since it is one of few drugs recommended in Denmark as first choice for treating *E. coli* diarrhoea in nursery pigs. The overall lowered usage of tetracycline due to the Yellow Card initiative might be responsible for the recent decrease in tetracycline resistance.

S. suis also displayed similar levels of resistance as in previous years, ranging from no resistance against chloramphenicol to 74% resistance against tetracycline.

Figure 6.5 Resistance (%) in *Actinobacillus pleuropneumoniae*, Denmark DANMAP 2021

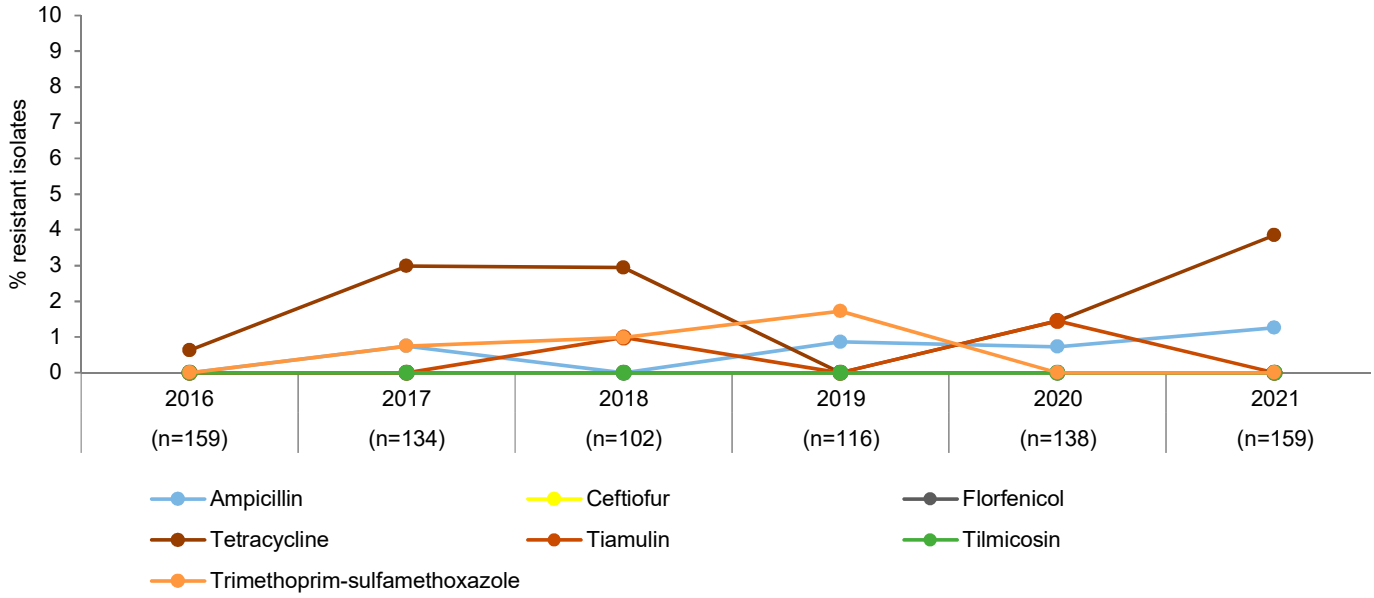

Figure 6.6 Resistance (%) in haemolytic *Escherichia coli*, Denmark DANMAP 2021

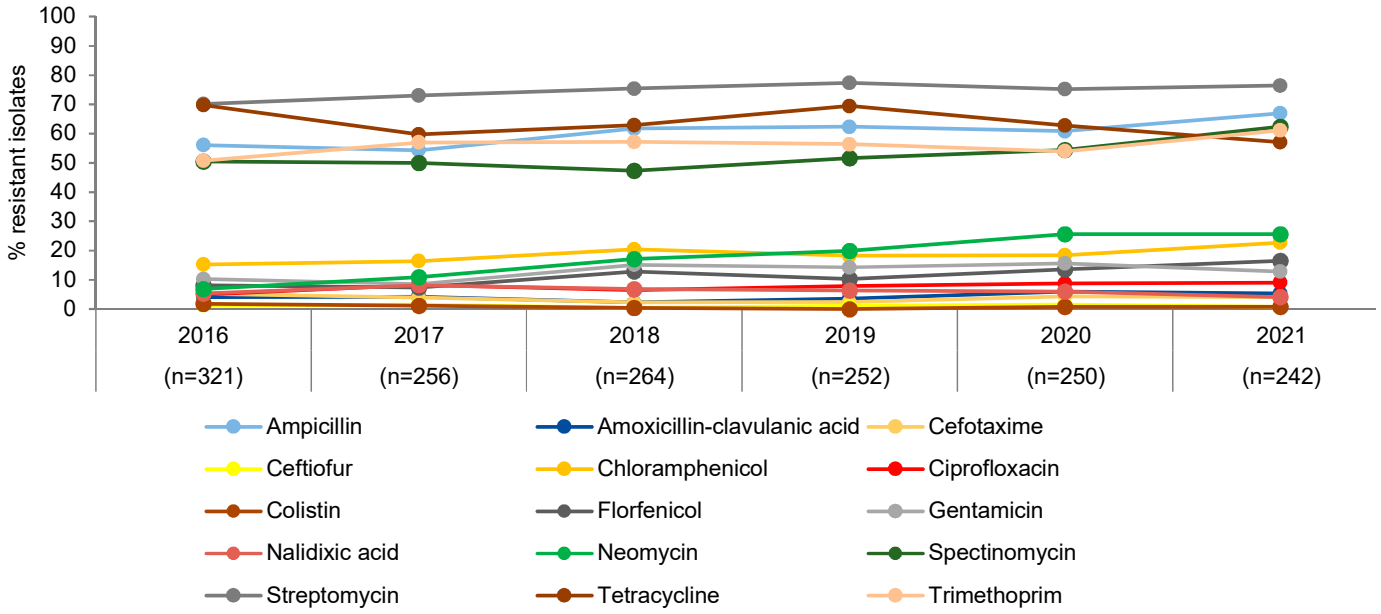

Figure 6.7 Phenotypic antimicrobial resistance in *Streptococcus suis* from pigs, Denmark DANMAP 2021

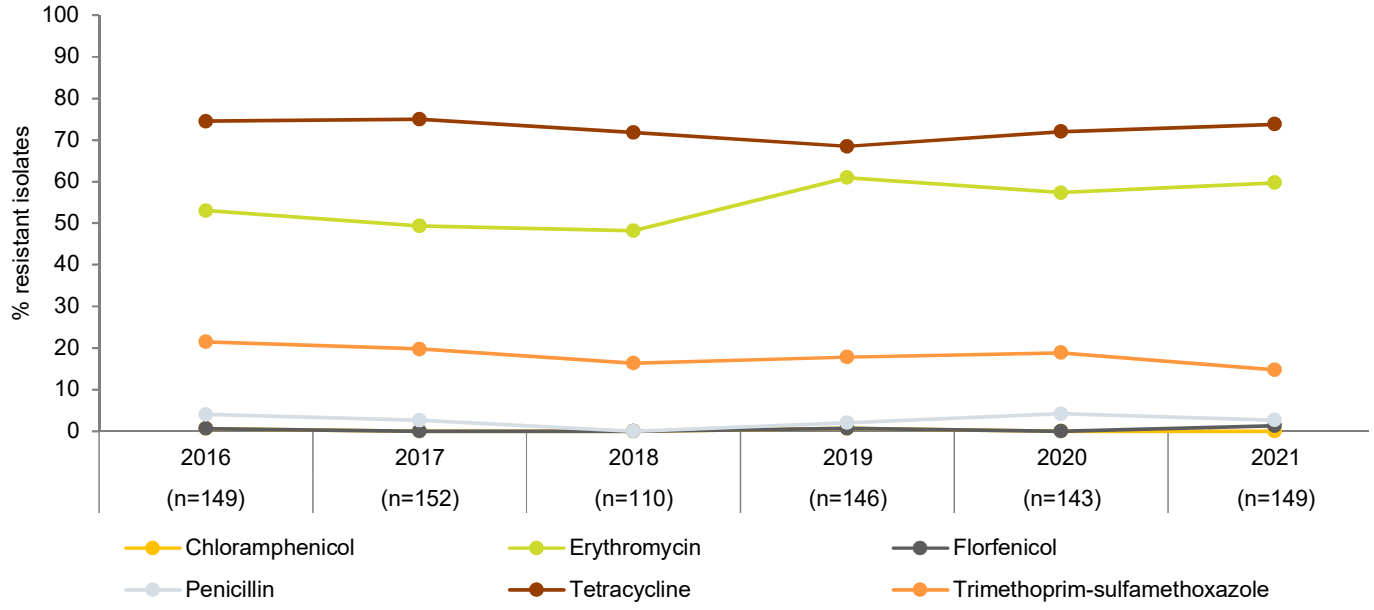

Table 6.4 Phenotypic antimicrobial resistance among pathogenic bacteria from pigs, Denmark DANMAP 2021

Antimicrobial agent	*Actinobacillus pleuropneumoniae* non-wild-type/R (%)	*Escherichia coli* non-wild-type/R (%)	*Streptococcus suis* non-wild-type/R (%)
Ampicillin	1.3	66.9	Not determined
Amoxicillin-clavulanic acid	Not determined	5.4	Not determined
Cefotaxime	Not determined	4.1	Not determined
Ceftiofur	0.0	0.8	Not determined
Chloramphenicol	Not determined	22.7	0.0
Ciprofloxacin	Not determined	9.1	Not determined
Colistin	Not determined	0.8	Not determined
Erythromycin	Not determined	Not determined	59.7
Florfenicol	0.0	16.5	1.3
Gentamicin	Not determined	12.8	Not determined
Nalidixic acid	Not determined	4.1	Not determined
Neomycin	Not determined	25.6	Not determined
Penicillin	Not determined	Not determined	2.7
Spectinomycin	Not determined	62.4	Not determined
Streptomycin	Not determined	76.4	Not determined
Tetracycline	3.8	57.0	73.8
Tiamulin	0.0	Not determined	Not determined
Tilmicosin	0.0	Not determined	Not determined
Trimethoprim	Not determined	61.2	Not determined
Trimethoprim-sulfamethoxazole	0.0	Not determined	14.8

Data are based on ECOFFs and clinical breakpoints when ECOFFs are unavailable (Materials and methods, Table 9.4)
Abbreviations: ECOFF, epidemiological cut-off; R, resistant

6.2.2 WGS-based detection of resistance genes and point mutations

A randomly selected subset of *A. pleuropneumoniae* (128 out of 159), haemolytic *E. coli* (104 out of 242) and *S. suis* (108 out of 149) isolates recovered from pigs during 2021 was subjected to WGS. Table 6.5 provides a list of the detected resistance genes and point mutations in *A. pleuropneumoniae* (2 genes), haemolytic *E. coli* (48 genes and 6 point mutations) and *S. suis* (12 genes).

Some isolates harboured genes/point mutations associated with resistance towards antimicrobials considered critically important for human medicine by the World Health Organization. For example, cefotaxime resistance was identified in 7% of the haemolytic *E. coli* isolates and was always associated with presence of either the extended-spectrum β-lactamase (ESBL)-encoding $bla_{CTX\text{-}M\text{-}1}$ gene or point mutations in the *ampC* promoter. In addition, 14% of the haemolytic *E. coli* isolates harboured a point mutation in the *pmrB* gene associated with resistance to colistin. However, only one of the 15 isolates with this point mutation was phenotypically resistant to colistin.

Two percent of the *S. suis* isolates harboured the *optrA* gene, which has been associated with resistance to oxazolidinones, including linezolid that is critically important for human medicine, and to phenicols such as florfenicol that is used only in veterinary medicine. As expected, the two *optrA*-positive *S. suis* isolates were resistant to florfenicol. Linezolid is not part of the customised Sensititre panel used for this species at the Veterinary Laboratory, and there are currently no ECOFFs or clinical breakpoints available for *S. suis*. It was therefore not possible to determine whether the *optrA* gene was also associated with decreased susceptibility to linezolid.

6.2.3 WGS-based prediction of AMR

Table 6.6 shows 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 6.5).

The observed concordance was 100% for *A. pleuropneumoniae*, 96% for haemolytic *E. coli* and 90% for *S. suis*. We observed a total of 134 discrepancies, including 67 for haemolytic *E. coli* and 67 for *S. suis*. Most of the discrepancies in haemolytic *E. coli* were due to presence of genes/point mutations associated with resistance to colistin, spectinomycin and streptomycin in phenotypically susceptible isolates, whereas the majority of discrepancies in *S. suis* were due to the absence of resistance genes and point mutations in isolates that were phenotypically resistant to tetracycline and trimethoprim-sulfamethoxazole.

Table 6.5 Antimicrobial resistance genes and mutations identified through whole-genome sequencing of pathogenic bacteria from pigs, Denmark DANMAP 2021

Bacterial species	Gene/mutation	Class	Phenotype	Presence (%)
Actinobacillus pleuropneumoniae	*bla*$_{ROB-1}$	β-lactam	Penicillin, amoxicillin, ampicillin	0.8
	tet(B)	Tetracycline	Doxycycline, tetracycline, minocycline	3.1
Haemolytic *Escherichia coli*	*aac(3)-IId*	Aminoglycoside	Apramycin, gentamicin, tobramycin, dibekacin, netilmicin, sisomicin	1.9
	aac(3)-IV	Aminoglycoside	Gentamicin, tobramycin	9.6
	aadA1	Aminoglycoside	Spectinomycin, streptomycin	41.3
	aadA11	Aminoglycoside	Spectinomycin, streptomycin	1.0
	aadA12	Aminoglycoside	Spectinomycin, streptomycin	17.3
	aadA13	Aminoglycoside	Spectinomycin, streptomycin	1.9
	aadA2	Aminoglycoside	Spectinomycin, streptomycin	16.3
	aadA22	Aminoglycoside	Spectinomycin, streptomycin	1.0
	aadA3	Aminoglycoside	Spectinomycin, streptomycin	2.9
	aadA5	Aminoglycoside	Spectinomycin, streptomycin	1.0
	ant(2'')-Ia	Aminoglycoside	Gentamicin, tobramycin	1.0
	ant(3'')-Ia	Aminoglycoside	Streptomycin	26.0
	aph(3'')-Ib	Aminoglycoside	Streptomycin	47.1
	aph(3')-Ia	Aminoglycoside	Neomycin, kanamycin, lividomycin, paromomycin, ribostamycin	30.8
	aph(4)-Ia	Aminoglycoside	Hygromycin	9.6
	aph(6)-Id	Aminoglycoside	Streptomycin	45.2
	bla$_{CTX-M-1}$	β-lactam	Amoxicillin, ampicillin, aztreonam, cefepime, cefotaxime, ceftazidime, ceftriaxone, piperacillin, ticarcillin	1.0
	bla$_{TEM-1A}$	β-lactam	Amoxicillin, ampicillin, cephalothin, piperacillin, ticarcillin	1.0
	bla$_{TEM-1B}$	β-lactam	Amoxicillin, ampicillin, cephalothin, piperacillin, ticarcillin	62.5
	bla$_{TEM-1C}$	β-lactam	Amoxicillin, ampicillin, cephalothin, piperacillin, ticarcillin	1.0
	bla$_{TEM-30}$	β-lactam	Amoxicillin, amoxicillin/clavulanic acid, ampicillin, ampicillin/clavulanic acid, piperacillin, piperacillin/tazobactam, ticarcillin, ticarcillin/clavulanic acid	1.9
	catA1	Amphenicol	Chloramphenicol	1.9
	cmlA1	Amphenicol	Chloramphenicol	14.4
	dfrA1	Folate pathway antagonist	Trimethoprim	31.7
	dfrA12	Folate pathway antagonist	Trimethoprim	7.7
	dfrA14	Folate pathway antagonist	Trimethoprim	5.8
	dfrA17	Folate pathway antagonist	Trimethoprim	1.0
	dfrA32	Folate pathway antagonist	Trimethoprim	1.0
	dfrA36	Folate pathway antagonist	Trimethoprim	1.0
	dfrA5	Folate pathway antagonist	Trimethoprim	5.8
	ere(A)	Macrolide	Erythromycin	1.0
	erm(B)	Macrolide, lincosamide, streptogramin B	Erythromycin, lincomycin, clindamycin, quinupristin, pristinamycin IA, virginiamycin S	13.5
	floR	Amphenicol	Chloramphenicol, florfenicol	16.3
	lnu(F)	Lincosamide	Lincomycin	1.0
	lnu(G)	Lincosamide	Lincomycin	2.9
	mef(B)	Macrolide	Erythromycin, azithromycin	1.0
	mph(A)	Macrolide	Erythromycin, azithromycin, spiramycin, telithromycin	15.4
	mph(B)	Macrolide	Erythromycin, spiramycin, telithromycin	2.9
	qnrS1	Quinolone	Ciprofloxacin	5.8
	sitABCD	Peroxide	Hydrogen peroxide	11.5
	sul1	Folate pathway antagonist	Sulfamethoxazole	45.2
	sul2	Folate pathway antagonist	Sulfamethoxazole	38.5
	sul3	Folate pathway antagonist	Sulfamethoxazole	14.4

continued ... Table 6.5 Antimicrobial resistance genes and mutations identified through whole-genome sequencing of pathogenic bacteria from pigs, Denmark DANMAP 2021

Bacterial species	Gene/mutation	Class	Phenotype	Presence (%)
	tet(A)	Tetracycline	Doxycycline, tetracycline	39.4
	tet(B)	Tetracycline	Doxycycline, tetracycline, minocycline	21.2
	tet(C)	Tetracycline	Doxycycline, tetracycline	1.0
	tet(G)	Tetracycline	Doxycycline, tetracycline	1.0
	tet(M)	Tetracycline	Doxycycline, tetracycline, minocycline	1.0
	16S (rrsB) A523C		Streptomycin	1.0
	ampC promoter T-32A,G,C		Ampicillin, ampicillin/clavulanic acid, amoxicillin, amoxicillin/clavulanic acid, cefixime, cefotaxime, cefoxitin, ceftazidime, piperacillin	1.9
	ampC promoter C-42T,A,G		Ampicillin, ampicillin/clavulanic acid, amoxicillin, amoxicillin/clavulanic acid, cefixime, cefotaxime, cefoxitin, ceftazidime, piperacillin	3.8
	gyrA S83L,W,A,V		Nalidixic acid, ciprofloxacin	2.9
	parE I529L		Nalidixic acid, ciprofloxacin	3.8
	pmrB V161G		Colistin	14.4
Streptococcus suis	*ant(6)-Ia*	Aminoglycoside	Streptomycin	19.4
	ant(6)-Ib	Aminoglycoside	Streptomycin	0.9
	aph(3')-III	Aminoglycoside	Kanamycin, amikacin, neomycin, butirosin, isepamicin, lividomycin, paromomycin, ribostamycin	7.4
	erm(47)	Macrolide, lincosamide, streptogramin B	Erythromycin, lincomycin, clindamycin, quinupristin, pristinamycin IA, virginiamycin S	1.9
	erm(B)	Macrolide, lincosamide, streptogramin B	Erythromycin, lincomycin, clindamycin, quinupristin, pristinamycin IA, virginiamycin S	57.4
	lnu(B)	Lincosamide	Lincomycin, clindamycin	25.9
	lsa(E)	Lincosamide, streptogramin A, pleuromutilin	Lincomycin, clindamycin, dalfopristin, pristinamycin IIA, virginiamycin M, tiamulin	25.9
	mef(A)	Macrolide	Erythromycin, azithromycin	4.6
	msr(D)	Macrolide, streptogramin B	Erythromycin, azithromycin, telithromycin, quinupristin, pristinamycin IA, virginiamycin S	3.7
	optrA	Oxazolidinone, amphenicol	Linezolid, chloramphenicol, florfenicol	1.9
	tet(M)	Tetracycline	Doxycycline, tetracycline, minocycline	7.4
	tet(O)	Tetracycline	Doxycycline, tetracycline, minocycline	30.6

Table 6.6 Diagnostic performance of ResFinder 4.1 as an antimicrobial resistance prediction tool for pathogenic bacteria from pigs, Denmark

DANMAP 2021

Bacterial species	Antimicrobial agent	ECOFF/clinical breakpoint	Cut-off, non-wild-type/R > (µg/ml)	P+/G+	P-/G-	G+/P-	G-/P+	Concordance	Sensitivity	Specificity	PPV	NPV	ME rate	VME rate
Actinobacillus pleuropneumoniae	Ampicillin	ECOFF	0.5 (a)	1.00	127.00	0.00	0	100.0	100.0	100.0	100.0	100.0	0.0	0.0
	Ceftiofur	Clinical breakpoint	4.00	0.00	128.00	0.00	0	100.0	NA	100.0	NA	100.0	0.0	NA
	Florfenicol	Clinical breakpoint	4.00	0.00	128.00	0.00	0	100.0	NA	100.0	NA	100.0	0.0	NA
	Tetracycline	Clinical breakpoint	1.00	4.00	124.00	0.00	0	100.0	100.0	100.0	100.0	100.0	0.0	0.0
	Tiamulin	Clinical breakpoint	16.00	0.00	128.00	0.00	0	100.0	NA	100.0	NA	100.0	0.0	NA
	Tilmicosin	Clinical breakpoint	16.00	0.00	128.00	0.00	0	100.0	NA	100.0	NA	100.0	0.0	NA
	Trimethoprim-sulfamethoxazole	ECOFF	0.125	0.00	128.00	0.00	0	100.0	NA	100.0	NA	100.0	0.0	NA
	Total	Not applicable	Not applicable	5.00	891.00	0.00	0	100.0	100.0	100.0	100.0	100.0	0.0	0.0
Haemolytic *Escherichia coli*	Ampicillin	ECOFF	8.00	71.00	32.00	0.00	1	99.0	98.6	100.0	100.0	97.0	0.0	1.4
	Amoxicillin-clavulanic acid	Clinical breakpoint	16/8 (b)	8.00	95.00	0.00	1	99.0	88.9	100.0	100.0	99.0	0.0	11.1
	Cefotaxime	ECOFF	0.25	7.00	97.00	0.00	0	100.0	100.0	100.0	100.0	100.0	0.0	0.0
	Ceftiofur	ECOFF	1.00	0.00	103.00	0.00	1	99.0	0.0	100.0	NA	99.0	0.0	100.0
	Chloramphenicol	ECOFF	16.00	25.00	76.00	3.00	0	97.1	100.0	96.2	89.3	100.0	3.8	0.0
	Ciprofloxacin	ECOFF	0.064	9.00	91.00	4.00	0	96.2	100.0	95.8	69.2	100.0	4.2	0.0
	Colistin	ECOFF	2.00	1.00	89.00	14.00	0	86.5	100.0	86.4	6.7	100.0	13.6	0.0
	Florfenicol	ECOFF	16.00	17.00	87.00	0.00	0	100.0	100.0	100.0	100.0	100.0	0.0	0.0
	Gentamicin	ECOFF	2.00	13.00	91.00	0.00	0	100.0	100.0	100.0	100.0	100.0	0.0	0.0
	Nalidixic acid	ECOFF	8.00	3.00	97.00	4.00	0	96.2	100.0	96.0	42.9	100.0	4.0	0.0
	Neomycin	ECOFF	8.00	31.00	72.00	1.00	0	99.0	100.0	98.6	96.9	100.0	1.4	0.0
	Spectinomycin	ECOFF	64.00	57.00	33.00	11.00	3	86.5	95.0	75.0	83.8	91.7	25.0	5.0
	Streptomycin	ECOFF	16.00	76.00	15.00	11.00	2	87.5	97.4	57.7	87.4	88.2	42.3	2.6
	Tetracycline	ECOFF	8.00	60.00	41.00	3.00	0	97.1	100.0	93.2	95.2	100.0	6.8	0.0
	Trimethoprim	ECOFF	2.00	52.00	44.00	0.00	8	92.3	86.7	100.0	100.0	84.6	0.0	13.3
Streptococcus suis	Chloramphenicol	Clinical breakpoint	8 (b)	0.00	106.00	2.00	0	98.1	NA	98.1	0.0	100.0	1.9	NA
	Erythromycin	ECOFF	0.25 (a)	62.00	44.00	0.00	2	98.1	96.9	100.0	100.0	95.7	0.0	3.1
	Florfenicol	Clinical breakpoint	4.00	2.00	106.00	0.00	0	100.0	100.0	100.0	100.0	100.0	0.0	0.0
	Penicillin	Clinical breakpoint	0.5	0.00	104.00	0.00	4	96.3	0	100.0	NA	96.3	0.0	100.0
	Tetracycline	Clinical breakpoint	1.00	37.00	22.00	0.00	39	60.2	48.7	100.0	100.0	36.1	0.0	51.3
	Trimethoprim-sulfamethoxazole	ECOFF	0.25 (a)	0.00	88.00	0.00	20	81.5	0	100.0	NA	81.5	0.0	100.0
	Total	Not applicable	Not applicable	101.00	470.00	2.00	65	89.5	60.8	99.6	98.1	87.9	0.4	39.2

Data are based on ECOFFs and clinical breakpoints when ECOFFs are unavailable (Materials and methods, Table 9.4)
a) Tentative ECOFF
b) Human breakpoint
Abbreviations: ECOFF, epidemiological cut-off; P, phenotype; G, genotype; PPV, positive predictive value; NPV, negative predictive value; ME, major error; VME; very major error

6.2.4 Perspectives for AMR surveillance in animal pathogens

These results show that WGS is a promising tool for surveillance of AMR in pigs. However, the potential use of WGS for clinical decision-making in terms of selection of antimicrobials for treatment needs further investigation. Phylogenetic analysis of the WGS data are currently underway to determine if and how resistance genes and pathogenic bacteria spread within the Danish pig production system and between this potential zoonotic reservoir and humans. In addition to the results presented above, similar data for *Bordetella bronchiseptica*, *Clostridium perfringens*, *Erysipelothrix rhusiopathiae*, non-haemolytic *E. coli*, *Haemophilus parasuis*, *Klebsiella pneumoniae*, *Salmonella enterica*, and *Staphylococcus hyicus* will be made available on DK-VET's homepage (https://www.vetssi.dk/overvaagning/overvaagningsprogrammer/overvaagning-af-resistens-i-patogene-bakterier-fra-husdyr-i-danmark).

Additional tables can be found in Supplementary Material on the DANMAP webpage (www.danmap.org).

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Textbox 6.1

Surveillance of antimicrobial resistance in clinical pathogens of animal origin – an update in Denmark and at the European level through EARS-Vet

In DANMAP 2020, we described how the Danish Veterinary Consortium (DK-VET) was planning future monitoring of antimicrobial resistance (AMR) in clinical isolates from food animals (see Textbox 6.2 in DANMAP 2020). In brief, the idea was to focus on AMR data from a subset of animal species, for which data of sufficient quality exist, and to sequence the genome of up to 700 isolates annually. Adding sequencing to traditional phenotypic data is expected to bring a new dimension to existing surveillance, allowing for a more in-depth analysis of e.g. the presence and dispersion of resistance and virulence genes. In DANMAP 2021, we present, for the first time, a combination of phenotypic and sequence-based analysis of veterinary clinical isolates, focusing on porcine *Escherichia coli*, *Streptococcus suis* and *Actinobacillus pleuropneumoniae* (see chapter 6, section 6.2). Further use of sequencing data, e.g. for tracking dissemination of outbreak clones will be conducted.

Internationally, cross-European AMR surveillance in animal pathogens is being planned with the establishment of the European Antimicrobial Resistance Surveillance Network in Veterinary Medicine (EARS-Vet). The idea for this network has arisen from a complete lack of harmonized European surveillance of AMR in veterinary clinical isolates. Consequently, it has been difficult to keep track of what data exist in Europe and to compare AMR trends across countries. Establishing EARS-Vet seeks to overcome these issues, and data may also be used for other purposes such as advise policy making, monitoring the effect of AMR interventions, evaluating marketing authorizations of antimicrobials, and supporting antimicrobial stewardship initiatives such as development of treatment guidelines.

Although sustainable funding to run such a network has yet to be established, three articles on EARS-Vet have already been published in international journals. These articles explain the background and idea of the network [1], the scope of future work including drug/bug combinations to include [2] and describe the existing national AMR surveillance systems for veterinary pathogens in Europe [3]. Furthermore, a pilot study has been launched in 2022, where national stakeholders submit AMR surveillance data for the period 2016-2020 to a central coordinator. From Denmark, AMR data on pig and companion animal pathogens have been submitted from the SEGES Diagnostic laboratory and University of Copenhagen, respectively. The pilot study will be used to assess how AMR data from different sources can be organized, analyzed, and compared, and in broader terms to prove the relevance of EARS-Vet for future surveillance.

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7

RESISTANCE IN INDICATOR BACTERIA

7. Resistance in indicator bacteria

Highlights: Over the last 5-year monitoring period, there has been no statistically significant increasing or decreasing trends in the prevalence of fully sensitive **indicator *E. coli*** isolates from broilers, cattle or pigs. Nonetheless, fluctuations were observed from 2020 to 2021, with an increase in fully sensitive *E. coli* from broilers (64% in 2021) and a decrease in fully sensitive *E. coli* from pigs and cattle (43% in pigs and 87% in cattle in 2021).

As in previous years, no colistin, meropenem or tigecycline resistance was detected in indicator *E. coli*. Resistance to ciprofloxacin continued to be low in *E. coli* from cattle and pigs and levelled out in *E. coli* from broilers at a prevalence similar to 2020 (16%). Azithromycin resistance was detected in few isolates from pigs (3%).

Compared to 2020, resistance to ampicillin, sulfamethoxazole and tetracycline decreased in *E. coli* isolates from broilers and increased in isolates from cattle and pigs. An increase was also observed in the prevalence of isolates from pigs resistant to chloramphenicol and trimethoprim. While the relative occurrence of multidrug-resistant indicator *E. coli* from broilers and cattle was similar to the previous year, it increased among isolates from pigs. Combined resistance to ampicillin, sulfamethoxazole and tetracycline (ASuT) continued to be overall the most common multidrug-resistance profile.

The observed occurrence of ***E. coli* producing extended-spectrum cephalosporinases (ESC)**, obtained through selective procedures, decreased in Danish pigs and imported pork, but continued to increase in isolates from imported beef. Importantly, as in previous years, samples examined for carbapenemase-producing (CP) *E. coli* (including OXA-48) were found negative.

The phenotypic and genotypic resistance profiles of ESC-producing *E. coli* were mostly in agreement, however 17 isolates revealed both ESBL and AmpC enzymes encoding genes, even though susceptibility testing did not show combined ESBL and AmpC resistance.

Among the AmpC-producing isolates, resistance was mainly conferred by upregulated AmpC promotor C-42T mutations. T-32A AmpC mutation, and plasmid-mediated AmpC enzymes CMY-2 and DHA-1 were also sporadically observed. Among the ESBL-producing isolates, 25 different ESBL genes were detected, with most variation among isolates from pigs. Overall, the most commonly observed ESBL encoding genes were CTX-M-1 and TEM-1B.

In 2021, 21% of ***E. faecalis*** isolated from pigs were fully sensitive. No resistance to ampicillin, linezolid, teicoplanin, tigecycline, vancomycin, ciprofloxacin or daptomycin was detected in any of the isolates. Resistance to all other antibiotics in the test panel decreased in 2021 compared to 2019. Resistance to tetracycline, erythromycin and chloramphenicol continued to be the most common.

7.1 Introduction

Escherichia coli and *Enterococcus* spp. are included in the DANMAP programme to monitor occurrence of antimicrobial resistance 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 production of extended-spectrum beta-lactamases (ESBLs) and AmpC beta-lactamases (AmpCs) is among the fastest spreading antimicrobial resistances in both humans and production animals worldwide. Some studies have suggested a zoonotic transmission of ESBL/AmpC-producing *E. coli* [Roer et al 2019. J Antimicrob Chemother 74(3):557; Mughini-Gras et al 2019. Lancet Planet Health, 3(8):e357-e369], while others found no evidence of transmission between animals and the general human population [Dorado-Garcia et al 2018, J Antimicrob Chemother; 73: 339–347; Findlay et al 2020, Applied and Environmental Microbiology 87(1): e01842-20]. The zoonotic nature of ESBL/AmpC-producing *E. coli* isolated in Denmark from humans, animals and meat was addressed in Chapter 3.

Carbapenemase-producing *Enterobacteriaceae* (CPE) are a great threat to human health, due to the importance of carbapenems as last-line antimicrobial drugs for treatment of infections caused by multidrug-resistant Gram-negative bacteria. In recent years, CPE have been increasingly detected in production animals in EU and there is hence the concern that animals might become a CPE reservoir in the future [EFSA/ECDC 2022. EFSA Journal 2022;20(3):7209].

Isolation and antimicrobial susceptibility testing of indicator *E. coli*, extended-spectrum cephalosporinase (ESC) and carbapenemase (CP) producing *E. coli* and indicator enterococci are performed in accordance with the EU harmonised monitoring of antimicrobial resistance [Decision 2020/1729/EU]. In 2021, isolates were obtained from randomly selected caecal samples collected from healthy animals at slaughter. Additionally, for the specific monitoring of ESC- and CP-producing *E. coli*, fresh meat was collected at retail and at border control posts. Details on sampling, analysis, susceptibility testing and interpretations are presented in Chapter 9.

7.2 Indicator *Escherichia coli*

E. coli isolates were obtained from 94% of caecal samples from broilers (132/141), 95% of samples from pigs (172/182) and 98% of samples from cattle (165/168). Indicator *E. coli* isolates were obtained with a non-selective isolation procedure. Results obtained by selective procedures for specific monitoring of ESC- 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, cattle or pigs during the past five years of monitoring (Figure 7.2), although for isolates from pigs the result was close to significant (= 0.057). Nevertheless, the percentage of broiler isolates susceptible to all antimicrobials in the test panel increased from 58% in 2020 to 64% in 2021, while a lower percentage of cattle and pig isolates were fully sensitive in 2021 (87% and 43%, respectively) (Table 7.1).

Compared to 2020, only minor fluctuations (≤ 1%) in occurrence of resistance were observed for most antimicrobials in the test panel, with few exceptions. Resistance to ampicillin and sulfamethoxazole decreased in broilers (from 20% to 16%). On the contrary, the percentage of *E. coli* resistant to ampicillin, sulfamethoxazole or tetracycline increased in 2021 in isolates from cattle and pigs, by magnitudes of 2% to 8%. Additionally, an increase in the prevalence of isolates resistant to chloramphenicol (by 7%) and trimethoprim (by 6%) was also detected in pigs in 2021 (Table 7.1).

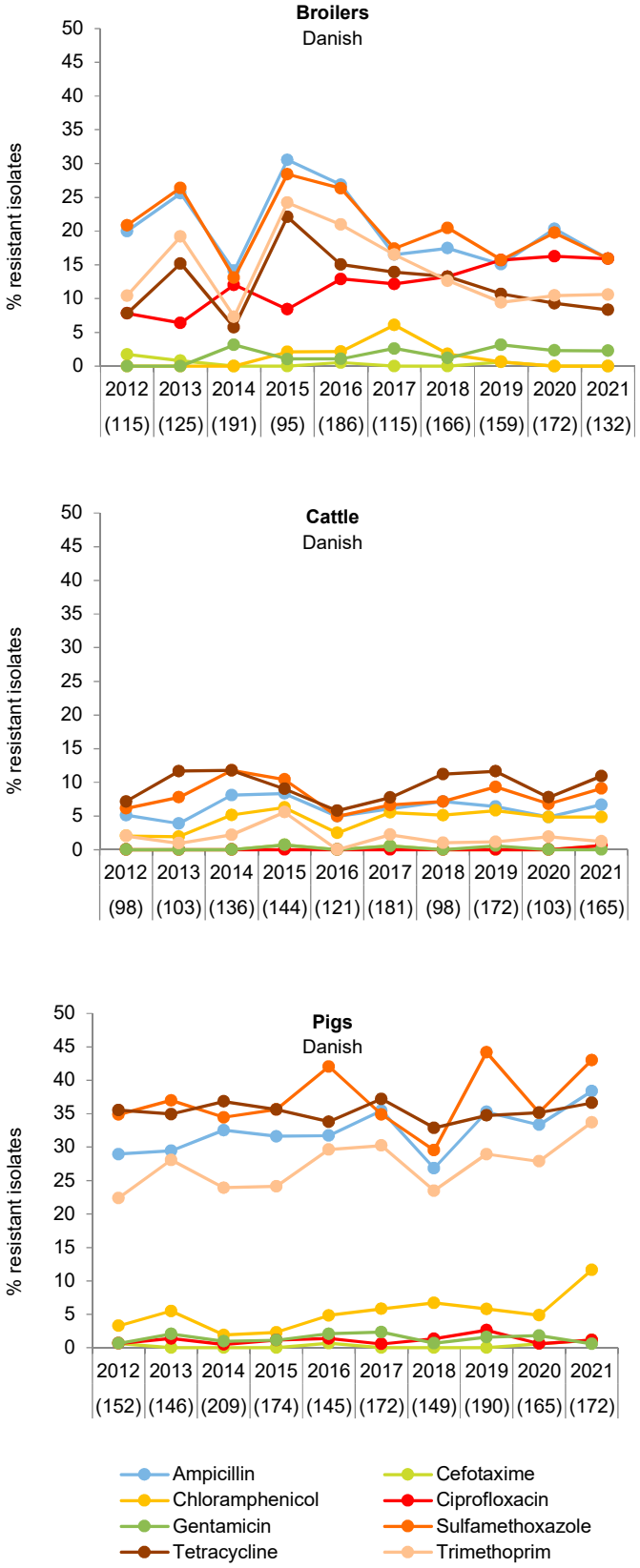
As in previous years, no colistin, meropenem or tigecycline resistance was detected among the isolates. Azithromycin resistance was detected in 3% of the isolates from pigs, and resistance to 3rd generation cephalosporins was detected in few isolates from cattle and pigs using non-selective methods (Table 7.1).

Table 7.1 Resistance (%) in *Escherichia coli* isolates from broilers, cattle and pigs, Denmark DANMAP 2021

	Broilers	Cattle	Pigs
Antimicrobial agent	Danish %	Danish %	Danish %
Amikacin	0	0	0
Ampicillin	16	7	38
Azithromycin	0	0	3
Cefotaxime	0	<1	1
Ceftazidime	0	<1	1
Chloramphenicol	0	5	12
Ciprofloxacin	16	<1	1
Colistin	0	0	0
Gentamicin	2	0	1
Meropenem	0	0	0
Nalidixic acid	14	0	1
Sulfamethoxazole	16	9	43
Tetracycline	8	11	37
Tigecycline	0	0	0
Trimethoprim	11	1	34
Fully sensitive (%)	64	87	43
Number of isolates	132	165	172

An isolate is considered fully sensitive if susceptible to all antimicrobial agents included in the test panel (Materials and methods, Table 9.3)

Figure 7.1 Resistance (%) among *Escherichia coli* isolates from broilers, cattle and pigs, Denmark DANMAP 2021



The number of isolates included each year is shown in parentheses

Resistance to fluoroquinolones continues to be low in *E. coli* from cattle and pigs, and remained at levels similar to 2020 in broiler isolates, with 16% resistant to ciprofloxacin and 14% resistant to nalidixic acid. Also among isolates from broilers, resistance to ampicillin, sulfamethoxazole or tetracycline decreased compared to the previous year (Figure 7.1).

The proportions of multidrug-resistant *E. coli* isolates were approximately the same as in 2020 in broilers and cattle, and increased in pigs. Combined resistance to ampicillin, sulfonamide (sulfamethoxazole) and tetracycline (ASuT) was observed approximately in half of the multidrug-resistant isolates from broilers and in more than half of the multidrug-resistant isolates from cattle and pigs (Figure 7.2).

Among indicator *E. coli* isolated with a non-selective procedure, one ESBL-producing isolate was found in cattle and two in pigs (Table 7.1).

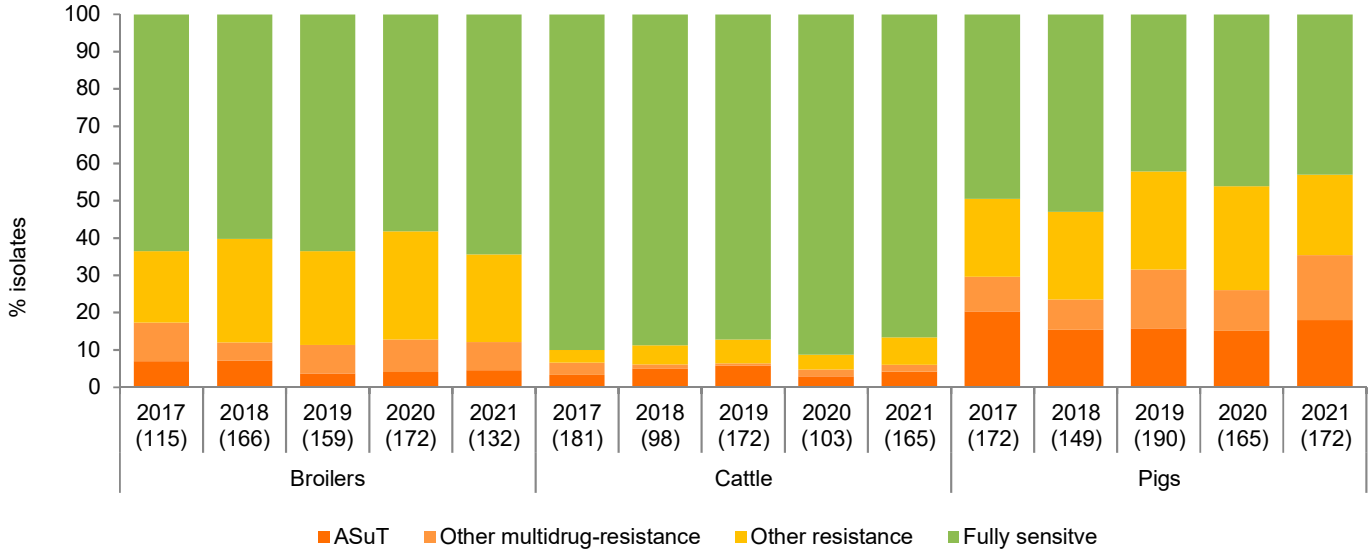
7.2.2 Perspectives

At the EU level, full sensitivity in indicator *E. coli* isolated from pigs and cattle has not changed significantly between 2015 and 2019. Significant decreasing trends in antimicrobial resistance have been observed for some countries in isolates from pigs and from cattle, but not for Denmark. Also at the EU level, full sensitivity in indicator *E. coli* from broilers has significantly increased between 2014 and 2019, however at the individual country level, Denmark and three other countries showed a significant decrease, i.e. an increase in the occurrence of resistance [EFSA/ ECDC 2022,EFSA Journal 2022;20(3):7209].

In DANMAP 2021, no significant increasing or decreasing trends were observed in the occurrence of fully sensitive indicator *E. coli* recovered from broilers, cattle or pigs in the 5-year period between 2017 and 2021. It is possible that different trends are observed when different monitoring periods are included in the analysis, or different statistical methods are applied. Also, significant changes might be missed when trends are assessed with annual data. In the future, it would be desirable to transition to trend analysis in Denmark using monthly or weekly monitoring records.

Furthermore, at the EU level, a clear negative association has been determined between the probability of full sensitivity in indicator *E. coli* and the overall consumption of antimicrobials by food-producing animals [EFSA/ECDC/EMA 2021, JIACRA III, DOI 10.2900/056892]. That analysis was based on monitoring data of total antimicrobial use and percentage of full sensitivity in isolates from broilers, turkeys, pigs and veal calves, and from more than 20 countries, with a large variation in consumption and resistance levels among them. In the future, the association between antimicrobial use and full sensitivity of indicator *E. coli* should be assessed separately for broilers, pigs and cattle, specifically for Denmark.

Figure 7.2 Relative distributions (%) of fully sensitive, resistant and multidrug-resistant *Escherichia coli* isolates from broilers, cattle and pigs, Denmark DANMAP 2021



The number of isolates included each year is shown in the parentheses. An isolate is considered fully sensitive if susceptible to all antimicrobial agents in the test panel, and multidrug-resistant if resistant to 3 or more of the antimicrobial classes included in the test panel (Materials and methods, Table 9.3). ASuT is the multidrug-resistance to ampicillin, sulfonamide and tetracycline

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

In 2021, caecal samples collected from fattening pigs and from cattle at slaughter, and packages of fresh, chilled pork and beef collected from Danish wholesale and retail outlets were monitored for the presence of extended-spectrum cephalosporinase (ESC)- and carbapenemase (CP)-producing *E. coli*. In accordance with the harmonised EU monitoring, packages of meat were collected at retail without pre-selecting by country of origin, hence including both imported and domestically-produced products. Of the samples randomly collected at retail, 9% of pork and 54% of beef were imported products. According to the new Decision 2020/1729/EU, fresh, frozen pork and beef meat were also randomly collected at border control posts and monitored for ESC- and CP-producing *E. coli*.

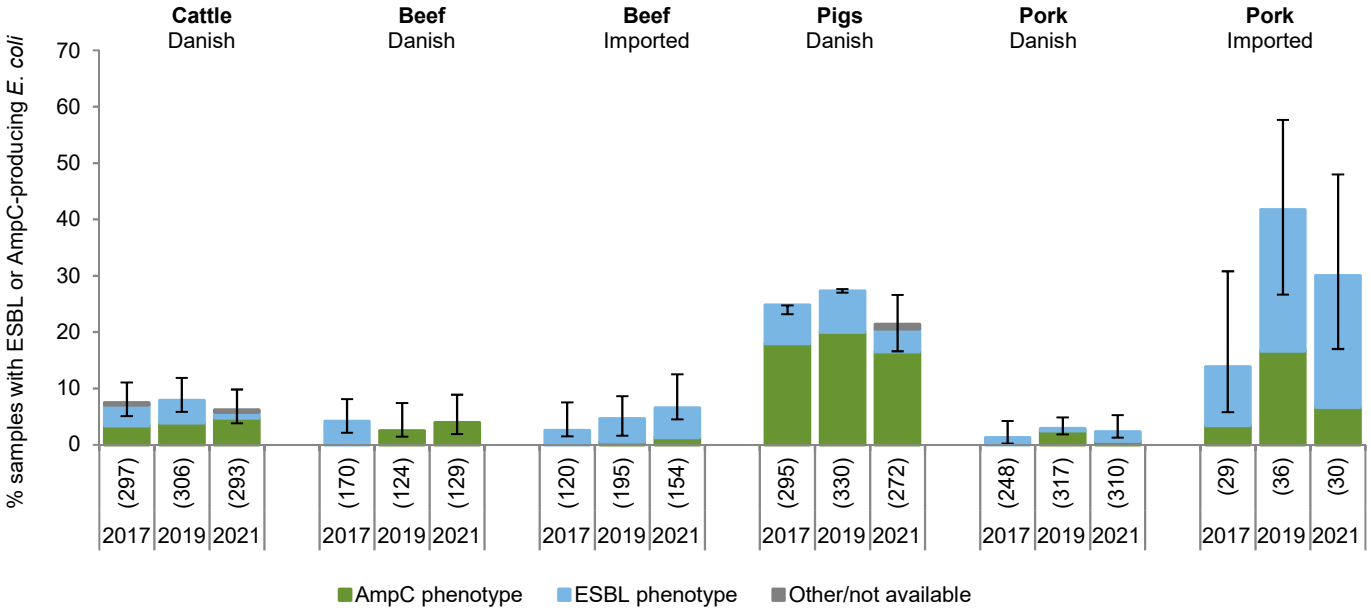
Using selective procedures, ESBL/AmpC-producing isolates were obtained from cattle (18/293 samples), beef (Danish: 5/129 samples, imported: 10/154 samples), pigs (58/272 samples) and pork (Danish: 7/310 samples, imported: 9/30 samples). The selective procedures for detection of CP-producing *E. coli* (including oxacillinase-producing OXA-48-like enzymes), recovered no isolates (Table 7.2). ESBL- or AmpC-producing *E. coli* was not detected in samples of imported pork (N=3) or imported beef (N=3) collected at border control posts.

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

Following selective enrichment, *E. coli* resistant to 3rd generation cephalosporins (cefotaxime and/or ceftazidime) were obtained from 6% (CI 95%: 4-10%) of samples from cattle, 4% (CI 95%: 2-9%) of samples from Danish beef, 6% (CI 95%: 4-12%) of samples from imported beef, 21% (CI 95%: 17-27%) of samples from pigs, 2% (CI 95%: 1-5%) of samples from Danish pork and 30% (CI 95%: 17-48%) of samples from imported pork (Figure 7.3). Compared to 2019, in 2021 the most recognizable changes in the prevalence of ESBL/AmpC-producing *E. coli* were the decrease among samples from Danish pigs and imported pork. The prevalence among samples from imported beef continued the increase observed in 2019 (Figure 7.3). In 2021, the relative frequency of ESBL-producing and AmpC-producing phenotypes remained mostly unchanged in comparison to 2019. The only exception were isolates from imported pork, which showed higher prevalence of ESBL- and lower prevalence of AmpC-producing *E. coli*. In 2021, among isolates from Danish and imported beef different phenotypes dominated – AmpC- and ESBL-producing *E. coli*, respectively (Figure 7.3).

All recovered ESBL/AmpC-producing isolates were resistant to 3rd generation cephalosporins (cefotaxime and ceftazidime) as well as to ampicillin. Resistance to 4th generation cephalosporins (cefepime) was found at higher prevalence in imported beef (90%) compared to Danish beef (40%) and in more than 80% of all isolates recovered from pork (Table 7.2). These levels of resistance to cefepime represent an increase in comparison with what was observed in 2019.

Figure 7.3 Occurrence (%) of samples with phenotypic ESBL- or AmpC-producing *E. coli* from animals and meat recovered by selective enrichment, Denmark DANMAP 2021



Number of samples tested per year is presented in the parentheses. Classification of ESBL and AmpC phenotypes is based on the results of antimicrobial susceptibility testing (Materials and methods, section 9.7.2). Confidence intervals for total proportion of samples positive for a phenotype calculated as 95% binomial proportions presenting Wilson intervals. Other/not available does not include any ESBL- and AmpC-producing *E. coli*, as this phenotype was not detected. The total number of samples of imported beef includes 151 samples collected at retail and 3 samples collected at border control posts (BCPs). The total number of samples of imported pork includes 27 samples collected at retail and 3 samples collected at BCPs

During the same period, the observed resistance to quinolones (ciprofloxacin) has also increased in ESBL/AmpC-producing *E. coli* from pigs (2% to 7%) and from Danish and imported pork (11% to 29% and 0% to 22%, respectively), and decreased in cattle (16% to 11%) and in imported beef (56% to 30%). No resistance to colistin, meropenem or imipenem was observed in the specific monitoring of ESC-producing *E. coli* in 2021, however ertapenem resistance was observed in one out of ten isolates (10%) from imported beef. In 2021, azithromycin resistance was observed in isolates from imported beef (10%) and imported pork (22%), and resistances to tigecycline and to temocillin were observed in 2% of isolates from Danish pigs (Table 7.2).

The genetic basis for ESBL- and AmpC enzymes was detected in most isolates recovered by selective enrichment. The detected enzymes corresponded to the phenotypes derived from the susceptibility testing for the majority of the isolates. In 17 isolates (1 from cattle, imported beef and imported pork, and 14 from pigs), whole genome sequencing revealed both ESBL and AmpC encoding genes, even though the susceptibility testing did not show the corresponding phenotype (Tables 7.2 and 7.3).

Among the AmpC-producing isolates recovered in 2021, resistance was mainly conferred by upregulated AmpC promotor C-42T mutations, and one T-32A mutation was detected in a cattle isolate. The CMY-2 plasmid-mediated AmpC enzyme was detected in two isolates from imported beef and in one from imported pork, while the DHA-1 plasmid-mediated AmpC was detected in a single isolate recovered from imported pork.

Among all ESBL-producing isolates, 25 different ESBL genes were detected, of which 10 occurred as the only encoding gene (CTX-M-1, CTX-M-8, CTX-M-14, CTX-M-15, CTX-M-32, CTX-M-55, CTX-M-65, OXA-1, OXA-10 and SHV-12). Overall, the most commonly observed ESBL encoding genes were CTX-M-1 and TEM-1B, the latter mostly among isolates from pigs (Table 7.3). Among isolates that harboured both ESBL and AmpC encoding genes, upregulated AmpC promotor C-42T mutation was detected together with ESBL genes in one isolate from cattle and from imported pork and in 14 isolates from pigs. ESBL- and AmpC genotypes due to the AmpC plasmid-mediated CMY-2 combined with ESBL encoding genes was observed in one isolate from imported beef and one isolate from imported pork. The ESBL encoding gene most often present in ESBL and AmpC genotypes was TEM-1B.

7.3.2 Perspectives

At EU level, there is a large variation in the prevalence of ESC-producing *E. coli* recovered from animals and meat. In 2019, prevalence ranged between 2% and 99% in pigs, 1% and 71% in cattle, and 0% and 24% in pork and beef [EFSA/ ECDC 2022,EFSA Journal 2022;20(3):7209]. With the prevalence levels observed in 2021, Denmark continues to be among the countries with lower occurrence of ESC-producing *E. coli*.

The zoonotic transmission of ESC-producing *E. coli* continues to be investigated, with studies presenting different conclusions. In Chapter 3, ESBL-producing *E. coli* isolates from animals and meat and the isolates from human bloodstream infections, collected in Denmark between 2018 and 2021, were compared and very limited coincidence of combination of MLSTs and ESBL encoding genes was identified.

Still no carbapenemase-producing *E. coli* were detected in the 1188 samples tested in 2021. This was the first year of mandatory monitoring of CP-producing *E. coli* according to Decision 2020/1729/EU. Previously, in voluntary monitoring at EU level, isolates have been sporadically detected in several countries and across monitored animal species. The emergence of this important type of resistance in livestock populations in Europe will only be possible to assess after a few years of harmonized monitoring.

Table 7.2 Resistance (%) and beta-lactam resistance phenotype distributions (%) in ESC-producing *Escherichia coli* from animals and meat recovered by selective enrichment, Denmark DANMAP 2021

	Cattle	Beef		Pigs	Pork	
Antimicrobial agent	Danish %	Danish %	Import %	Danish %	Danish %	Import %
Amikacin	0	0	0	0	0	0
Ampicillin	100	100	100	100	100	100
Azithromycin	0	0	10	7	0	22
Cefepime	33	40	90	43	86	89
Cefotaxime	100	100	100	100	100	100
Cefotaxime/clavulanic acid	83	100	20	81	29	22
Cefoxitin	78	100	20	76	29	22
Ceftazidime	100	100	100	100	100	100
Ceftazidime/clavulanic acid	83	100	20	81	29	22
Chloramphenicol	11	0	40	17	0	22
Ciprofloxacin	11	0	30	7	29	22
Colistin	0	0	0	0	0	0
Ertapenem	0	0	10	0	0	0
Gentamicin	0	0	10	7	0	11
Imipenem	0	0	0	0	0	0
Meropenem	0	0	0	0	0	0
Nalidixic acid	0	0	30	2	14	11
Sulfamethoxazole	61	20	70	55	57	44
Temocillin	0	0	0	2	0	0
Tetracycline	56	20	70	55	29	44
Tigecycline	0	0	0	2	0	0
Trimethoprim	11	20	60	38	57	67
Number of AmpC phenotypes	14	5	2	45	2	2
Number of ESBL phenotypes	3	0	8	11	5	7
Number of other phenotypes	1	0	0	2	0	0
Number of ESC isolates	18	5	10	58	7	9
Number of CP isolates	0	0	0	0	0	0
Number of samples	293	129	154	272	310	30

Classification of CP-, ESBL- and AmpC-producing phenotypes is based on the results of antimicrobial susceptibility testing (Materials and methods, section 9.7.2). AmpC, ESBL and other phenotypes indicate the percentage of ESC-producing *Escherichia coli* isolates (Number of ESC isolates) expressing each specific phenotype. Other phenotypes does not include any combined ESBL- and AmpC-producing *E. coli*, as this phenotype was not detected. The total number of samples of imported beef includes 151 samples collected at retail and 3 samples collected at border control posts (BCPs). The total number of samples of imported pork includes 27 samples collected at retail and 3 samples collected at BCPs

Table 7.3 Number of ESBL and AmpC enzymes detected in ESC-producing *E. coli* isolates from animals and meat recovered by selective enrichment, Denmark DANMAP 2021

	Cattle	Beef		Pigs	Pork	
Enzymes	Danish %	Danish %	Import %	Danish %	Danish %	Import %
CTX-M-1	1	1	5	3	3	2
CTX-M-14	1				1	1
CTX-M-15	1		1	2		
CTX-M-55				1		1
CTX-M-65				1		
CTX-M-8				1		
CTX-M-32			1			1
OXA-1			3			
OXA-10				1		
SHV-12				1		
TEM-112				1		
TEM-146				1		
TEM-148				1		
TEM-17				1		
TEM-176	1					
TEM-189				1		
TEM-196				1		
TEM-1A				6		
TEM-1B	1		4	14	2	2
TEM-1C				1		1
TEM-1D				1		
TEM-209				1		
TEM-219				1		
TEM-32				1		
TEM-82				2		
CMY-2			2			1
DHA-1						1
Chromossomal AmpC	14	4		39	2	
Number of AmpC genotypes	14	4	1	24	2	1
Number of ESBL genotypes	3	0	8	9	4	5
Number of AmpC+ESBL genotypes	1	0	1	14	0	1
Not available	1	1	0	11	1	2
Number (%) positive samples	18 (6%)	5 (4%)	10 (6%)	58 (21%)	7 (2%)	9 (30%)
Number of tested samples	293	129	154	272	310	30

Number (%) positive samples are isolates recovered by selective enrichment methods for specific monitoring of ESC-producing *E. coli*. ESBL/AmpC enzymes were determined by whole genome sequencing (WGS) of the recovered isolates (Materials and methods, section 9.6). Not available refers to isolates without available WGS results

7.4 Indicator Enterococci

Enterococci were obtained from 171 (35%) of the 484 faecal samples taken from healthy pigs at slaughter, and antimicrobial susceptibility testing was performed on *E. faecalis*, when present (N=81).

7.4.1 Enterococci from pigs

Overall, 21% of the *E. faecalis* isolates from pigs were susceptible to all antimicrobials in the test panel (excluding resistance to quinupristin/dalfopristin).

Similar to observations in 2019, the last year of *E. faecalis* monitoring in pigs, no resistance towards ampicillin, linezolid, teicoplanin, tigecycline or vancomycin was observed in any *E. faecalis* isolates. Resistance to all other antibiotics in the test panel decreased in 2021, and resistance to ciprofloxacin and daptomycin was not observed (Table 7.4). Resistance to tetracycline, erythromycin and chloramphenicol continued to be the most common, but also decreased in 2021 to 75%, 51% and 23%, respectively. Resistance to gentamicin decreased from 11% in 2019 to 6% (5 isolates) in 2021 (Figure 7.4).

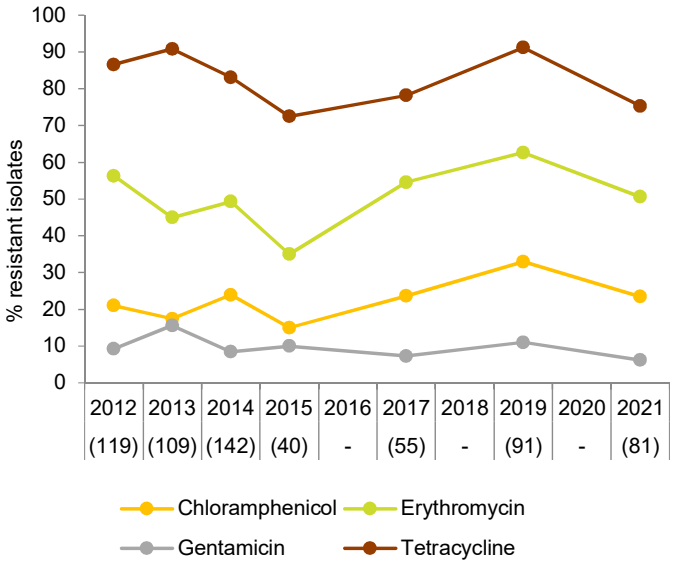
Among the resistant *E. faecalis* (N=64), four different resistance profiles were observed. Combined resistance to tetracycline and erythromycin was most common (42 isolates), however multidrug resistance was also observed, with 34% of the resistant isolates (N=22) presenting resistance to three antimicrobials or more. All multidrug resistant isolates were resistant to tetracycline and erythromycin, and the most common multidrug resistance profile included additional resistance to chloramphenicol (17 isolates), followed by the profile with additional resistance to gentamicin (3 isolates). Two isolates were resistant to all four antimicrobials.

Table 7.4 Resistance (%) in *Enterococcus faecalis* isolates from pigs, Denmark DANMAP 2021

Antimicrobial agent	*Enterococcus faecalis* %
Ampicillin	0
Chloramphenicol	23
Ciprofloxacin	0
Daptomycin	0
Erythromycin	51
Gentamicin	6
Linezolid	0
Teicoplanin	0
Tetracycline	75
Tigecycline	0
Vancomycin	0
Fully sensitive (%)	21
Number of isolates	81

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

Figure 7.4 Resistance (%) among *Enterococcus faecalis* isolates from pigs, Denmark DANMAP 2021



Number of isolates included each year is presented in the parentheses

7.4.2 Perspectives

Enterococci are commensal bacteria in the intestine in both animals and humans, however, both *E. faecalis* and *E. faecium* can cause human disease. In 2021, *E. faecalis* isolates recovered from pigs and cattle exhibited no resistance to linezolid, tigecycline, teicoplanin or vancomycin (Table 7.4). These antimicrobials are critically important to human medicine and are considered last resort compounds to treat severe infections caused by Gram positive bacteria.

In humans, an increase in infections caused by invasive enterococci, was observed for the first time after 2018, with a 10% proportion of invasive vancomycin-resistant *E. faecium* isolates reported in MiBa in 2021 [Chapter 8, Figure 8.11].

The monitoring of *E. faecium* from animals has not been constant in DANMAP, which hampers the comparison with the invasive isolates that have been increasingly recovered from humans.

Additional tables can be found in Supplementary Material on the DANMAP webpage (www.danmap.org).

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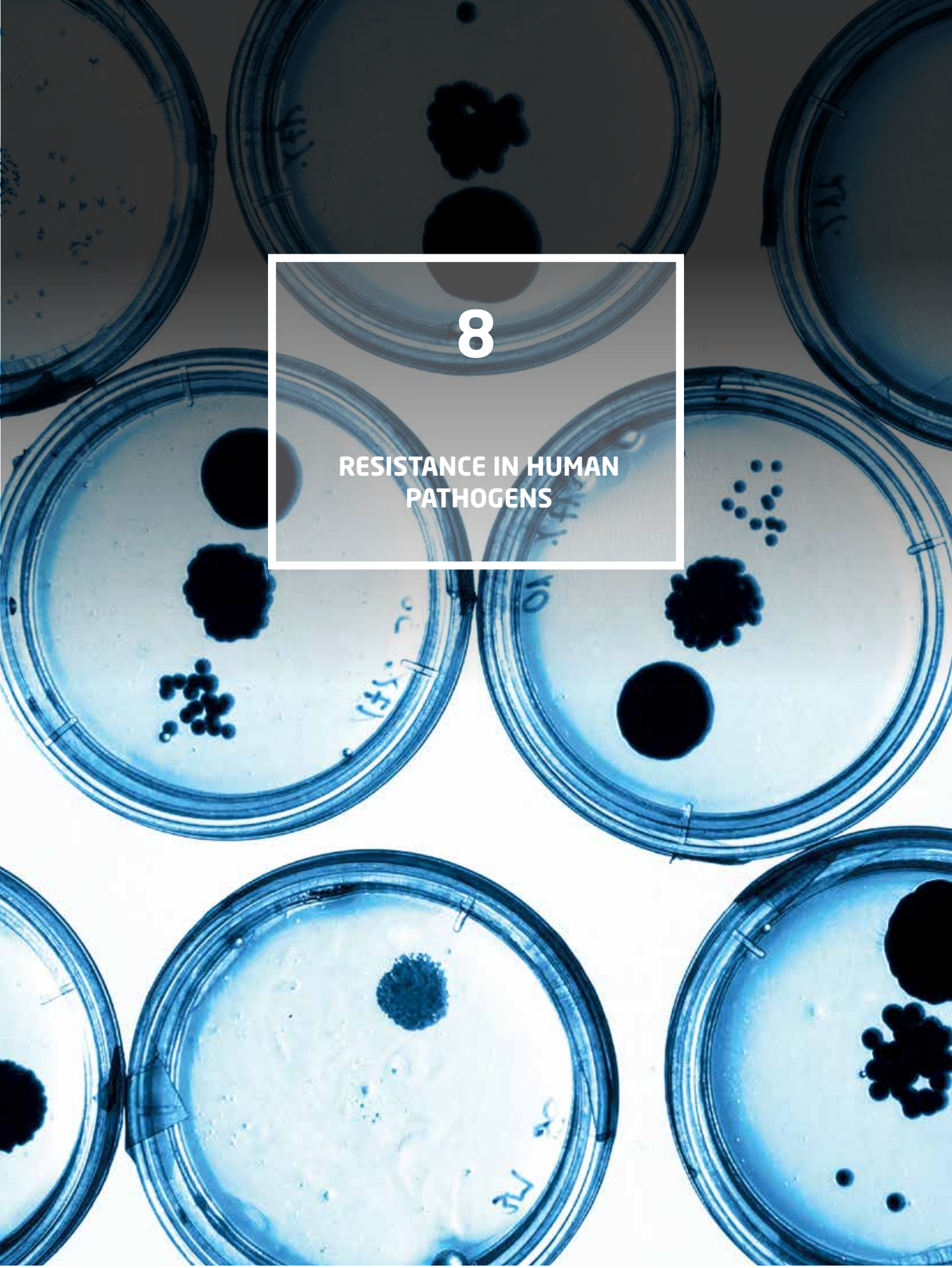


8

RESISTANCE IN HUMAN PATHOGENS

8. Resistance in human pathogens

Highlights

Invasive bacterial infections. The total number of invasive infections (blood or cerebrospinal fluid isolates) caused by the surveyed bacteria has been increasing steadily over the past ten years and 12,244 cases were reported in 2021. *Escherichia coli* caused about 49% of bacteraemias with *Staphylococcus* aureus being the second most causative organism with 20%. While uncommon, an increase of invasive infections with *Acinetobacter* spp. of 47% was observed as compared to 2020. The cause of this increase is unknown. *Klebsiella pneumoniae* and *Streptococcus pneumoniae* were the only species with decreasing invasive infections, both in absolute numbers and per capita.

Escherichia coli. The numbers of invasive and urinary tract *E. coli* infections continue the increasing tendency observed over the past ten years. However, resistance levels in *E. coli* are decreasing with the notable exception of piperacillin-tazobactam resistance that has increased over the last four years. Carbapenem-resistance is still very low, but increasing numbers of isolates are observed. The number of ESBL- and/or pAmpC-positive *E. coli* isolates decreased by 28% from 2020. CTX-M-15 remained the most prevalent ESBL enzyme (46%) followed by CTX-M-27 (21%).

Klebsiella pneumoniae. Resistance in *K. pneumoniae* has been decreasing over the last ten years. Notably, in 2021, a large decrease in resistance was observed for all surveyed antibiotics for isolates from invasive infections, including carbapenems, 3rd generation cephalosporin and piperacillin-tazobactam.

Carbapenemase-Producing Organisms/Enterobacterales (CPO/CPE). In 2021, 16% more CPOs were identified compared to 2020. The primary sources of this increase were more Danish outbreak-related cases (131% increase since 2019) - and travel-related cases coinciding with the easing of COVID-19 restrictions. There were 15 ongoing outbreaks registered. The most commonly found carbapenemases are of the NDM- and OXA-48-groups.

Enterococci. A small increase in the number of invasive infections with *Enterococcus faecium* and *Enterococcus faecalis* was reported. The percent of vancomycin-resistant *E. faecium* isolates increased to 10.2% after being stable at 9.4% since 2018. A clonal shift in vancomycin-resistant *E. faecium* has been observed recently where the clonal group ST80-CT2406 has seemingly outcompeted the clonal group ST1421-CT1134. Additionally, a shift towards the *vanB*-gene from the *vanA*-gene has been observed starting in 2018. The *vanB*-gene is now found in the vast majority of vancomycin-resistant *E. faecium* isolates.

Beta-haemolytic streptococci. During the COVID-19 pandemic, the number of submitted group A streptococci isolates has dropped considerably, while the number of the three other serotypes (group B, C and G) have either increased or remained virtually the same. All isolates were fully susceptible to penicillin.

Staphylococcus aureus. The number of *S. aureus* bacteraemias (SABs) has increased continuously over the past ten years and was 2,506 in 2021, a 75% and 4.8% increase compared to 2012 and 2020, respectively. There were 40 cases of bacteraemia with methicillin-resistant *S. aureus* (MRSA) comprising 1.6% of SABs, a number that has remained relatively stable in recent times. Meanwhile, the number of MRSA regardless of clinical status (infection or colonisation) dropped during the COVID-19 pandemic, presumably due to related restrictions. The all-cause 30-day mortality for SABs was 22% and 20% for the MRSA-related cases.

8.1 Introduction

The Danish national surveillance programme of antimicrobial resistance in human clinical bacteria collects data on routine diagnostics from all of Denmark. Data stem from the ten Departments of Clinical Microbiology (GPs), situated at main acute care hospitals geographically dispersed across the five health regions of 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).

Data coverage is high and includes microbiology data from all hospitals and the majority of general practitioners, thus covering a close to complete proportion of microbiological analyses performed in Denmark.

Table 8.1 Summary of species/types, sampling and sources of isolates for national resistance surveillance in humans, Denmark, 2021

DANMAP 2021

Routine diagnostics from all 10 DCMs in Denmark. All data are directly identified and extracted in MiBa	
Species	**Sampling**
Escherichia coli *Klebsiella pneumoniae*	First isolate per patient per year from blood or cerebrospinal fluid, from urine in hospitalised patients and from urine from primary 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	**Sampling**
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 exceptional 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	**Sampling**
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 normally more isolates are received, but for the statistics each patient is counted only once

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

A systematic national surveillance is performed by extracting 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 DCMs. MiBa thus simultaneously delivers real time patient data to the DCMs 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 DCMs, beginning with data from just two DCMs in 1995, but quickly expanding. Since 2015, all DCM have contributed to the program resulting in a 100% coverage of hospitalised patients and – since the DCMs 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://miba.ssi.dk/Service/English.aspx] 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 *Acineto-*

bacter species. Also included are results from 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 9.10 in Chapter 9.

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 voluntary programme includes day-to-day referral of vancomycin resistant enterococci and monthly or periodically reporting of ESBL-positive invasive *E. coli* and *K. pneumoniae* as well as invasive beta-haemolytic streptococci.

The mandatory programme includes invasive *Streptococcus pneumoniae* and *Haemophilus influenzae* serotype b (Hib), *Neisseria gonorrhoeae* from all clinical samples, methicillin-resistant *S. aureus* (MRSA) and carbapenemase-producing organisms (CPO) from clinical and screening samples, (Table 8.1).

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

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 in Denmark for the major bacterial species included in the surveillance programs for both DANMAP and EARS-Net from 2012 to 2021 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 2012 and 2021).

Between 2012 and 2021, the number of registered individual invasive cases increased by 38% from 8,893 to 12,244 cases in Denmark. *E. coli*: 3,925 to 5,981 cases (52% increase), *S. aureus*: 1,431 to 2,506 (75%) and *K. pneumoniae* 948 to 1,336 (41%).

The only species with an overall decreasing number of cases during the past 10 years was *S. pneumoniae*. The drop observed since 2018 (798 cases) has continued and the number of cases in 2021 was 333, a 62% decrease.

Figure 8.1 Number of invasive cases for bacterial species under surveillance, Denmark, 2012-2021 DANMAP 2021

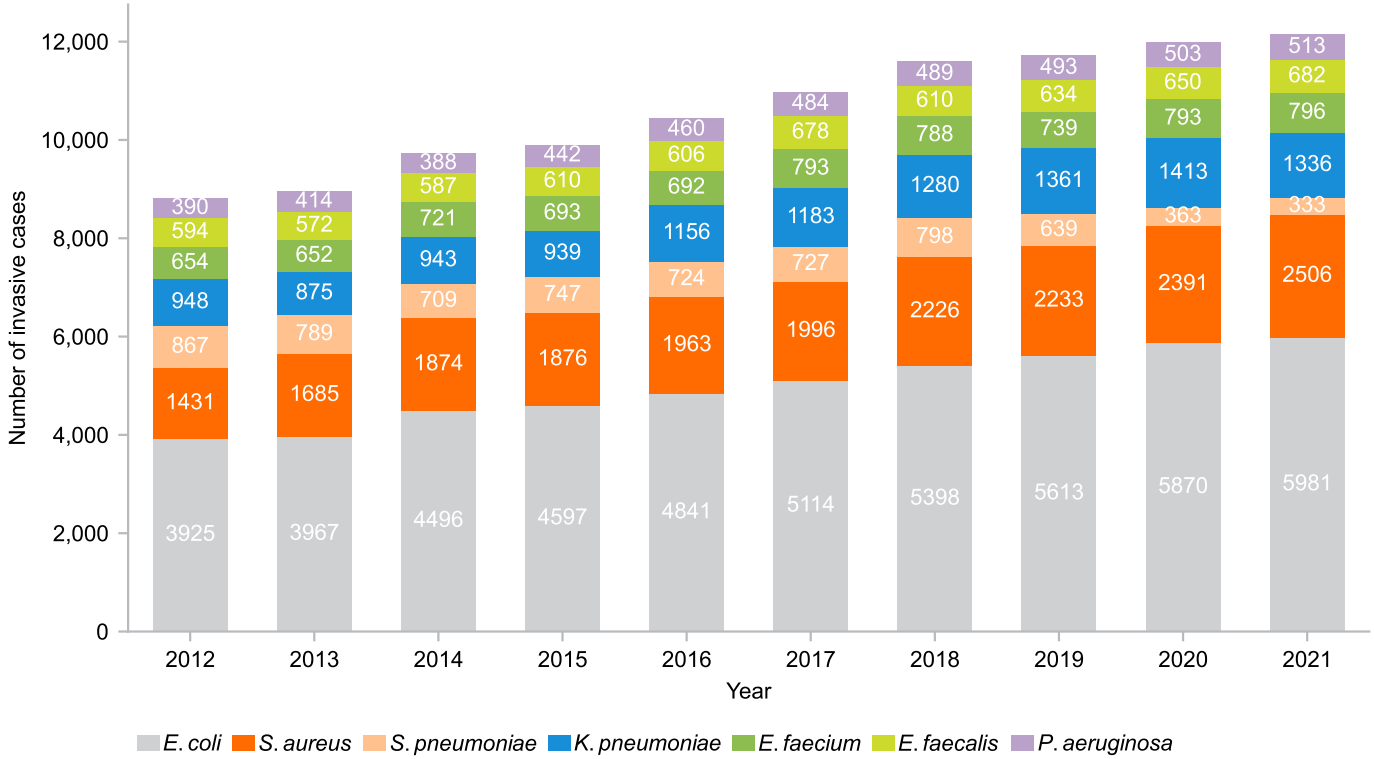


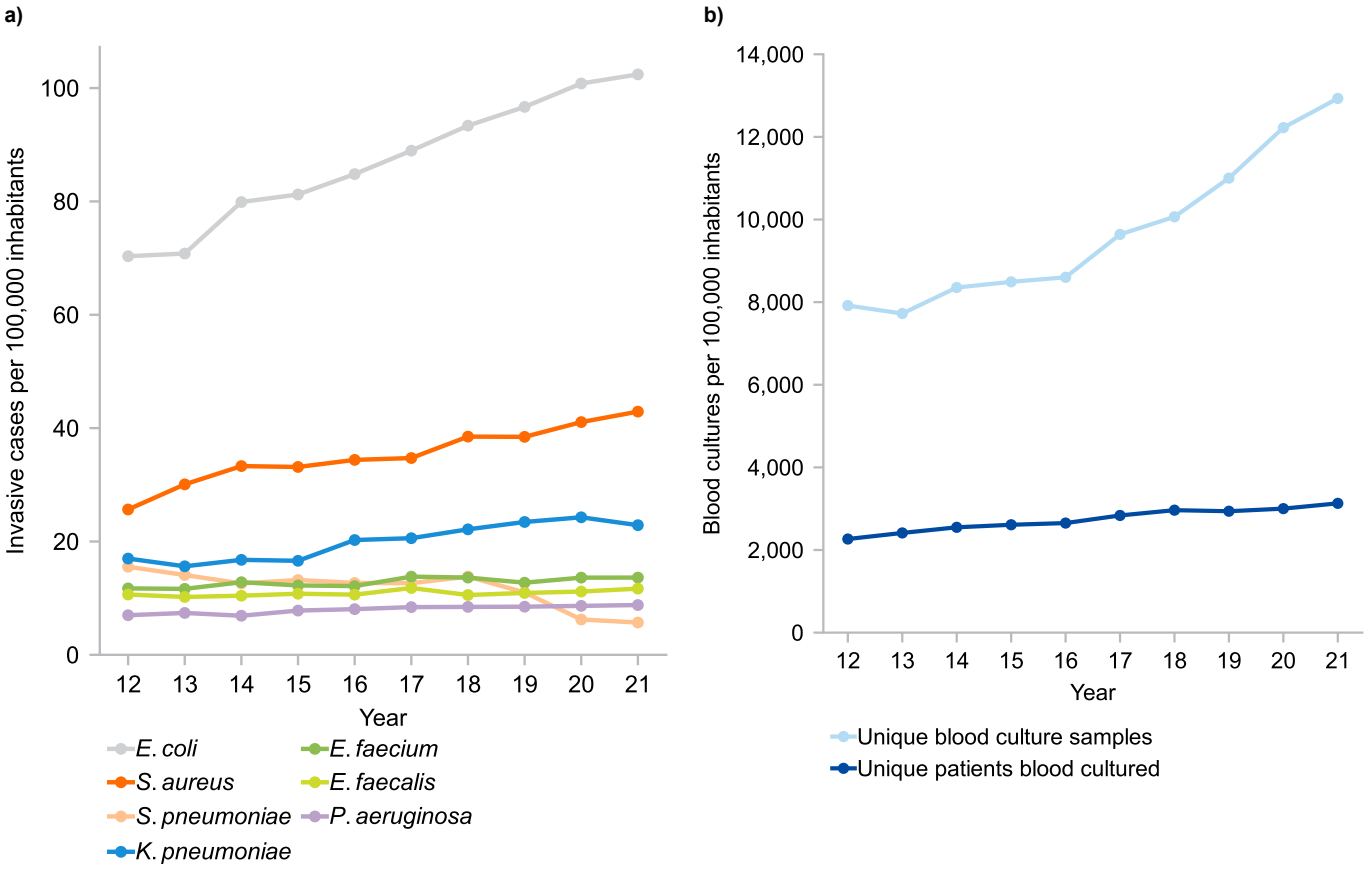
Figure 8.2a shows the number of invasive cases per 100,000 inhabitants in Denmark per year from 2012 to 2021.

During this period, the Danish population increased by 4.7% (from 5,580,516 inhabitants in 2012 to 5,840,045 inhabitants in 2021). 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 a 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 2,266 patients per 100,000 inhabitants in 2012 to 3,129 patients per 100,000 inhabitants in 2021 (an increase of 38%). The total number of blood samples (unique samples in MiBa) taken per 100,000 inhabitants increased even more (63%). Thus, on average a higher number of patients have an even higher number of blood cultures taken each year.

Changes in hospital workflow (reduced number of bed-days), healthcare-related infections, improved diagnostics and increased numbers of elderly at risk of infections have probably affected the increased number of total infections.

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

Figure 8.2 a) Invasive cases and b) blood cultures taken per 100,000 inhabitants, Denmark, 2012-2021 DANMAP 2021



8.2 Results from MiBa data surveillance

8.2.1 *Escherichia coli*

Escherichia coli (*E. 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 well as in urine samples from hospitals and primary health care (see details in later paragraphs). Figures 8.3a and 8.3b show the total annual number of invasive isolates and proportion of resistant isolates, respectively, between 2012 and 2021. The percentages of isolates resistant to key antimicrobials are presented in Figure 8.3c.

Invasive cases from hospital patients

In 2021, a total of 5,981 individual patients with invasive *E. coli* isolates were identified in MiBa. Antimicrobial susceptibility test results for key antimicrobials were also extracted for these isolates (See Chapter 9 'Material and methods').

As mentioned in the introduction, an increase in the number of invasive *E. coli* cases was observed over the past decade corresponding to 70.3 cases and 102.4 cases per 100,000 inhabitants respectively (a 46% increase, data not shown).

In 2021, 8.7% of invasive *E. coli* isolates were resistant to cefuroxime. This level has been stable (range 8.6-10%) over the last decade. A minor EUCAST breakpoint change in 2017 influenced data from 2017 compared to data from 2016. For 3rd generation cephalosporins, the percentage of resistant isolates was 5.4% with a decreasing tendency the past decade.

The ciprofloxacin resistance for invasive *E. coli* was 10.3% in 2021, and ranged from 10.3% to 14% in the period 2012-2021. The increased ciprofloxacin resistance rate in 2017 compared to 2016 mainly reflected a relatively large EUCAST breakpoints change in 2017. Ciprofloxacin breakpoints were changed (lowered) once again combined with the introduction of the Area of Technical Uncertainty (ATU) in the EUCAST clinical breakpoint table v. 9.0 in 2019. Therefore, trends for ciprofloxacin resistance should be interpreted with caution. Piperacillin-tazobactam resistance has gradually increased from 4.1% of invasive *E. coli* isolates being reported as resistant in 2012 to 6.0% in 2021. Gentamicin resistance was 4.3% in 2021 and resistance rates have declined over the last decade (Figure 8.3b and 8.3c).

The number of carbapenem resistant invasive *E. coli* isolates remained low in 2021 with three isolates categorised carbapenem-resistant and two isolates categorised "susceptible, increased exposure" (as per new EUCAST definition of previous intermediate category applicable from January 2019 [www.eucast.org/newsiandr]). The occurrence of multidrug-resistant (combined resistance to 3rd generation cephalosporins, ciprofloxacin and gentamicin) invasive *E. coli* remained low at around 1.1% (Table 8.3).

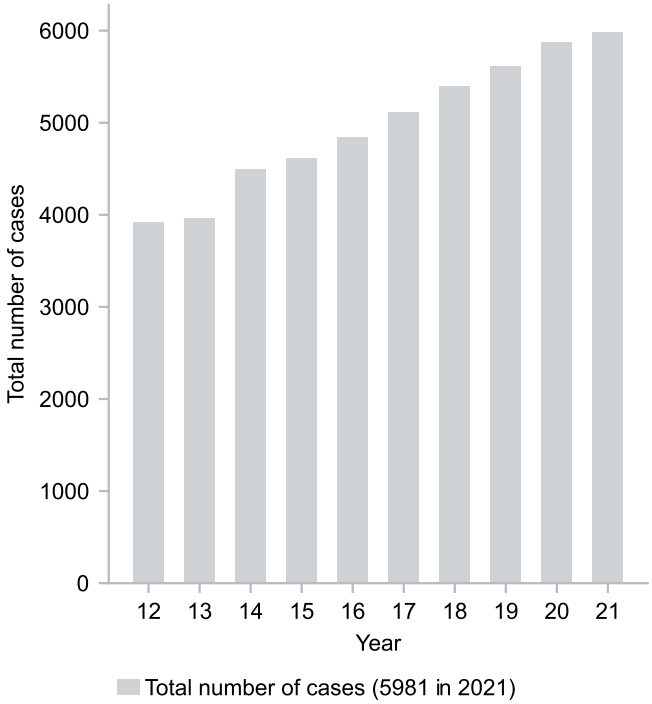
Table 8.2 *Escherichia coli*. Resistance (%) in isolates from humans, Denmark, 2021 DANMAP 2021

	Invasive isolates, hospitals %	Urine isolates, hospitals %	Urine isolates, primary health care %
Ampicillin	41	39	34
Mecillinam	13	6.9	4.6
Piperacillin-tazobactam	6.0	4.5	4.1 (1)
Amoxicillin/clavulanic acid	25	12	4.6 (3)
Sulfonamide		28 (1)	25
Trimethoprim		21	19
Nitrofuratoin		0.9 (5)	0.8
Gentamicin	4.3	4.2	3.3 (2)
Ciprofloxacin	10	8.7	6.9
Cefuroxime	8.7	6.7	5.0 (3)
3rd generation cephalosporins	5.4	5.8	4.4
Carbapenem	0.0	0.0	0.0 (1)
Max. number of isolates tested for resistance to the presented antibiotics	5975	49914	99018

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

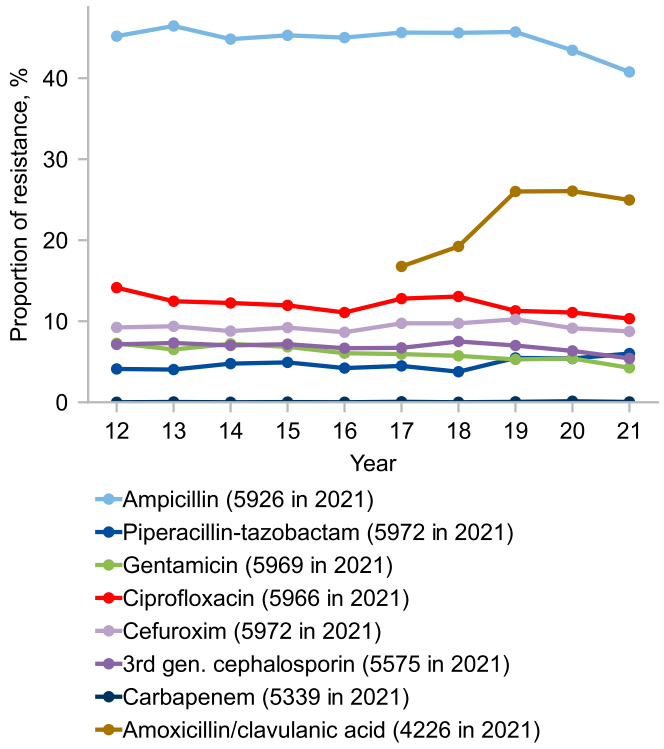
Figure 8.3 Invasive *Escherichia coli* isolates from humans: a) annual number of isolates from unique cases, b) proportion of resistant isolates and c) table of resistance percentages, Denmark, 2012-2021 DANMAP 2021

a)



Numbers in parantheses indicate number of isolates tested

b)



c)

Substance	Percent resistant invasive *E. coli* isolates									
	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021
Ampicillin	45.2	46.4	44.8	45.3	45	45.6	45.5	45.7	43.4	40.7
Piperacillin-tazobactam	4.1	4	4.8	4.9	4.2	4.5	3.8	5.5	5.4	6.0
Gentamicin	7.3	6.5	7.2	6.8	6.1	6	5.7	5.3	5.4	4.3
Ciprofloxacin	14.1	12.5	12.3	12	11.1	12.8	13	11.3	11.1	10.3
Cefuroxime	9.2	9.4	8.8	9.2	8.6	9.7	9.8	10.2	9.2	8.7
3rd gen.cephalosporins	7.1	7.3	7	7.2	6.7	6.7	7.5	7.0	6.3	5.4
Carbapenem	0	0	0	0	0	0.1	0	0.1	0.1	0.0
Total number of isolates	3922	3967	4492	4618	4841	5114	5398	5613	5870	5981

Table 8.3 *Escherichia coli*. Combined resistance to 3rd generation cephalosporins, ciprofloxacin, and gentamicin (multidrug-resistance) in invasive isolates from humans, Denmark, 2014-2021 DANMAP 2021

	2014 % (N)	2015 % (N)	2016 % (N)	2017 % (N)	2018 % (N)	2019 % (N)	2020 % (N)	2021 % (N)
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)
Percentage (no.) of isolates tested for multidrug-resistance	90 (4039)	88 (4071)	98 (4763)	95 (4883)	93 (4997)	94 (5259)	93 (5470)	93 (5564)
Total number of invasive isolates	4495	4614	4841	5114	5398	5613	5870	5981

Urinary cases from hospitals

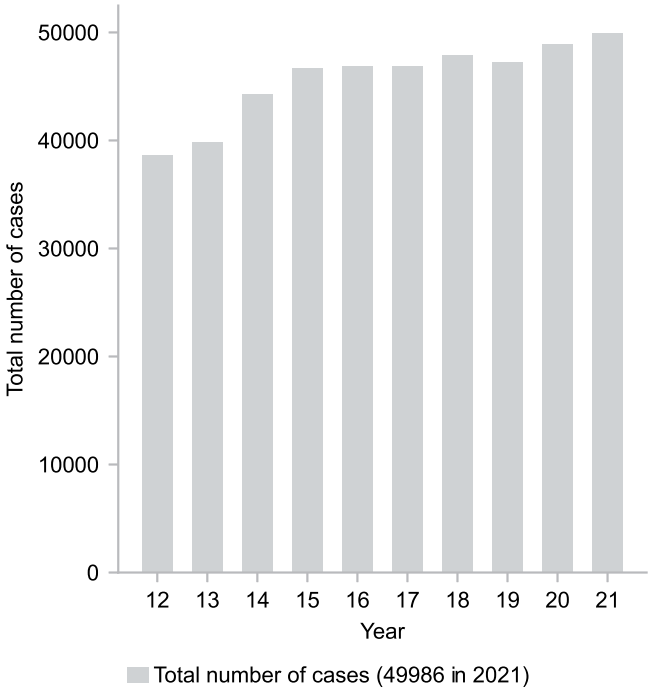
In 2021, 49,986 individual hospital patients had *E. coli* isolated from urine samples, a 37% and 2.1% increase compared to 2012 and 2020, respectively.

Interpretation results from antimicrobial susceptibility test (AST) data on *E. coli* urine isolates were available from all DCMs for ampicillin, mecillinam and ciprofloxacin. In addition, susceptibility test results were reported for: piperacillin-tazobactam (nine DCMs), gentamicin (nine DCMs), cefuroxime (eight DCMs), trimethoprim (eight DCMs), 3rd generation cephalosporins (eight DCMs), amoxicillin/clavulanic acid (seven DCMs), carbapenem (six DCMs), nitrofurantoin (five DCMs) and sulphonamide (four DCMs).

Summarized antimicrobial susceptibility results for *E. coli* isolates (invasive and urine specimen) are shown in Table 8.2. In Figure 8.4, resistance for *E. coli* urine isolates from hospitalised patients are shown for 2012-2021.

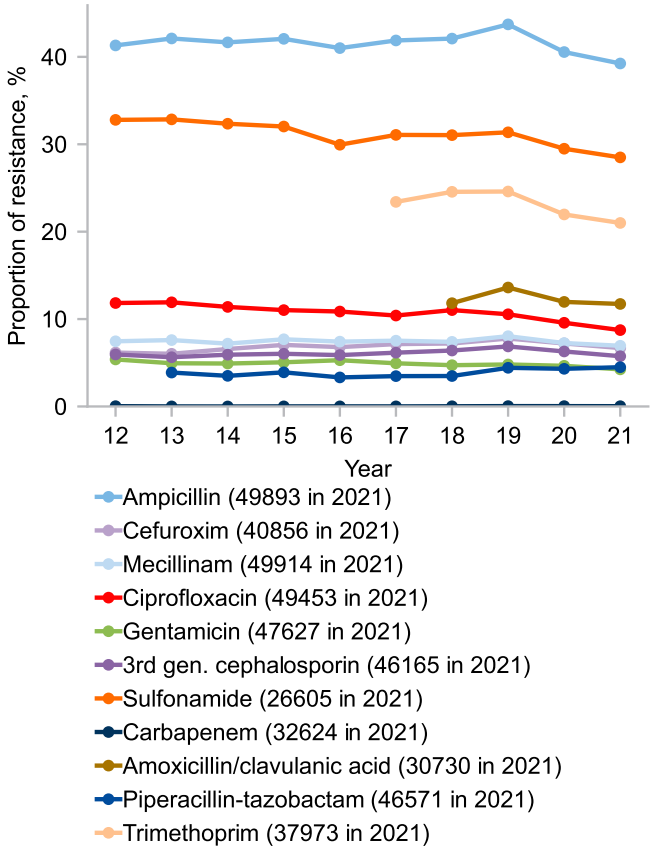
Figure 8.4 Urine *Escherichia coli* isolates from humans (hospitals): a) annual number of isolates from unique cases, b) proportion of resistant isolates and c) table of resistance percentages, Denmark, 2012-2021 DANMAP 2021

a)



Numbers in parantheses indicate number of isolates tested

b)



c)

Substance	Percent resistant *E. coli* isolates from hospital urine									
	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021
Ampicillin	41.3	42.1	41.7	42.1	41	41.9	42.1	43.7	40.6	39.2
Mecillinam	7.5	7.6	7.2	7.7	7.4	7.5	7.4	8.1	7.3	6.9
Piperacillin-tazobactam		3.9	3.5	3.9	3.3	3.7	3.5	4.4	4.3	4.5
Sulfonamide	32.8	32.8*	32.3*	32	34.9	31.1	31*	31.4*	29.4*	28.5*
Gentamicin	5.4	4.9	4.9	5.1	5.3	4.9	4.7	4.6	4.6	4.2
Ciprofloxacin	11.8	11.9	11.4	11	10.9	10.4	11	10.6	9.6	8.7
Cefuroxime	6.2	6	6.6	7	6.8	7.1	7.2	7.8	7.2	6.7
3rd gen. cephalosporins	5.9	5.6	5.9	6.0	5.9	6.2	6.4	6.9	6.3	5.8
Carbapenem			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total number of isolates	38610	39820	44288	46723	46865	46884	47914	47235	48962	49986

* Indicates less than 6 DCMs reported rutine susceptibility testing

As can be seen in Figure 8.4c, a decrease in ciprofloxacin and gentamicin resistance was observed for the past five and ten years. However, 3rd generation cephalosporins and cefuroxime resistance had an increasing trend from 2013, but has decreased since 2020.

In 2021, 20 carbapenem-resistant and 10 carbapenem-susceptible, increased exposure *E. coli* urine isolates from hospitalised patients were reported, compared to 22 and 17 isolates in 2020, respectively.

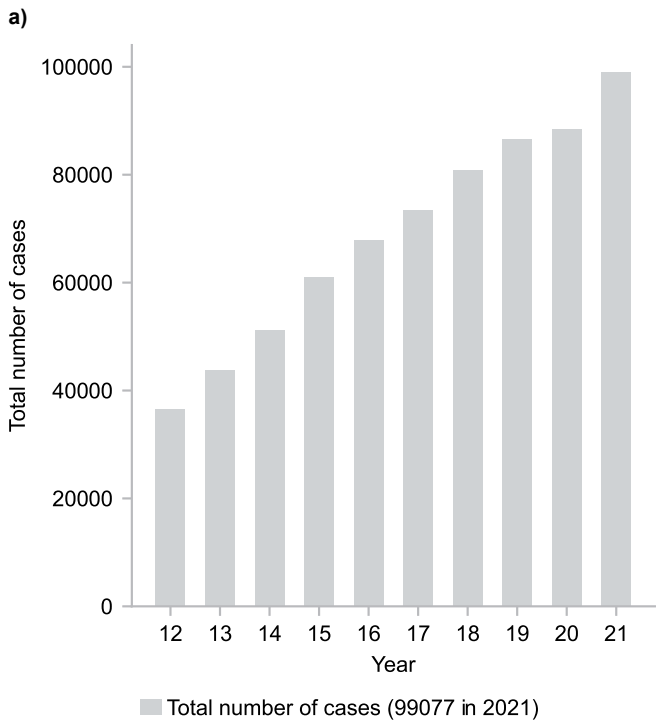
Urinary cases from primary health care

In 2021, 99,077 unique patients in primary health care had *E. coli* isolated from urine samples, a 171% and 12% increase compared to 2012 and 2020, respectively.

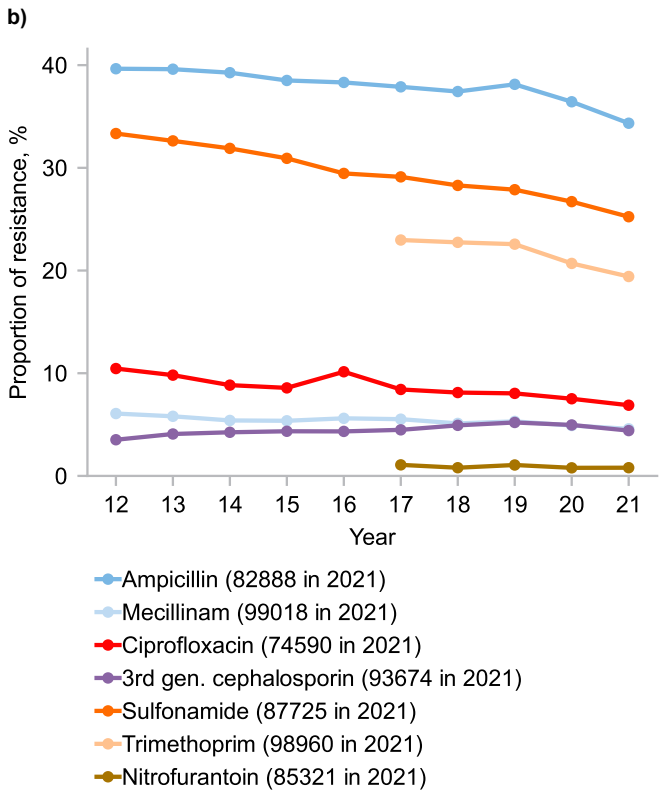
Susceptibility results for all tested antimicrobials are shown in Table 8.2. In Figure 8.5, resistance for *E. coli* urine isolates from primary health care are shown for the past decade.

A continuous slow increase in resistance to 3rd generation cephalosporins was observed between 2012 and 2019. However, since 2019 it has decreased by 0.8%-points. Additionally, marked declines for resistance towards ampicillin, mecillinam, sulphonamide and ciprofloxacin were observed over the same period (Figure 8.5).

Figure 8.5 Urine *Escherichia coli* isolates from humans (primary health care): a) annual number of isolates from unique cases, b) proportion of resistant isolates and c) table of resistance percentages, Denmark, 2012-2021 DANMAP 2021



Numbers in parantheses indicate number of isolates tested



c)

Substance	Percent resistant *E. coli* isolates from primary health care urine									
	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021
Ampicillin	39.7	39.6	39.3	38.5	38.3	37.9	37.4	38.1	36.5	34.3
Mecillinam	6.1	5.8	5.2	5.4	5.6	5.5	5.1	5.3	4.9	4.6
Sulfonamide	33.3	32.6	31.9	30.9	29.5	29.1	28.3	27.9	26.7	25.2
Ciprofloxacin	10.5	9.8	8.8	8.6	10.1	8.4	8.1	8	7.5	6.9
3rd gen. cephalosporins	3.5	4.1	4.2	4.3	4.3	4.5	4.9	5.2	5	4.4
Total number of isolates	36475	43770	51272	61083	67798	73497	80851	86508	88462	99077

Conclusion

A steady increase in the number of invasive and urinary tract infections (from hospital and primary health care) caused by *E. coli* has been observed since 2012. Around 34-41% of *E. coli* urine isolates are ampicillin resistant and 7-10% are ciprofloxacin resistant. Proportion of resistance to cefuroxime and 3rd generation cephalosporins for invasive *E. coli* decreased slightly from 9.2% to 8.7% and 6.2% to 5.4% from 2020 to 2021, respectively, following the trend from the previous year. Resistance to carbapenems in invasive *E. coli* remains low.

8.2.2 *Klebsiella pneumonia*

Klebsiella pneumoniae (*K. pneumoniae*) is commonly found in the environment and in the human intestinal tract and can also colonise the respiratory tract in humans, especially in hospitalised patients. *K. pneumoniae* may cause urinary tract infections, severe pneumonia or blood stream infections – the latter especially in patients with indwelling devices - and may cause nosocomial outbreaks. *K. pneumoniae* frequently displays plasmid-mediated resistance mechanisms, which are transferrable to other organisms.

The percentage of resistance in invasive *K. pneumoniae* isolates for each key antimicrobial is presented in Table 8.4. Figures 8.6a and 8.6b show total annual numbers of invasive isolates and percentages of resistance in invasive isolates, respectively, between 2012 and 2021. The proportions of isolates resistant to key antimicrobials are presented in Figure 8.6c.

Invasive cases from hospitals

In 2021, a total of 1,336 unique patients were registered in MiBa with invasive *K. pneumoniae* isolates.

As for invasive *E. coli* cases, a continuous increase in the number of invasive *K. pneumoniae* cases was observed over the last decade, from 948 cases in 2012 to 1336 cases in 2021. This corresponds to 16.4 and 24.3 cases per 100,000 inhabitants, respectively (a 48.3% increase). At the same time, the total number of blood cultures taken increased by 63% per 100.000 inhabitants (commented on in subsection 8.1.3).

Resistance in invasive *K. pneumoniae* isolates has decreased over the past 10 years for gentamicin, ciprofloxacin, cefuroxime and 3rd generation cephalosporins. For more details, see Figure 8.6b and 8.6c. The increased proportion of ciprofloxacin-resistant isolates in 2017 when compared to 2016 mainly reflects a change in interpretation of S-I-R due to implementation of new EUCAST breakpoints for ciprofloxacin in most Danish DCMs in January 2017. Ciprofloxacin breakpoints changed (lowered) once again combined with the introduction of the "Area of Technical Uncertainty" (ATU) in the EUCAST clinical breakpoint table v. 9.0 in 2019. Therefore, trends for ciprofloxacin resistance should be interpreted with caution. Resistance for piperacillin-tazobactam should also be interpreted with caution. EUCAST breakpoints did not change since 2012 but an ATU for piperacillin-tazobactam was introduced in 2019.

Carbapenem resistance in invasive *K. pneumoniae* is very low (6 resistant isolates out of 1313 tested [0.5%] in 2021). The increase shown in Figure 8.6b and 8.6c in 2020 is mainly due to hospital outbreaks (see Section 8.3.2 Carbapenemase-producing bacteria in Denmark, DANMAP 2020). The level of multidrug-resistant (combined resistance to 3rd generation cephalosporins, ciprofloxacin and gentamicin) invasive *K. pneumoniae* was 1% in 2021 (Table 8.5). One invasive *K. pneumoniae* isolate was registered resistant to colistin. Susceptibility to colistin is not routinely tested, though.

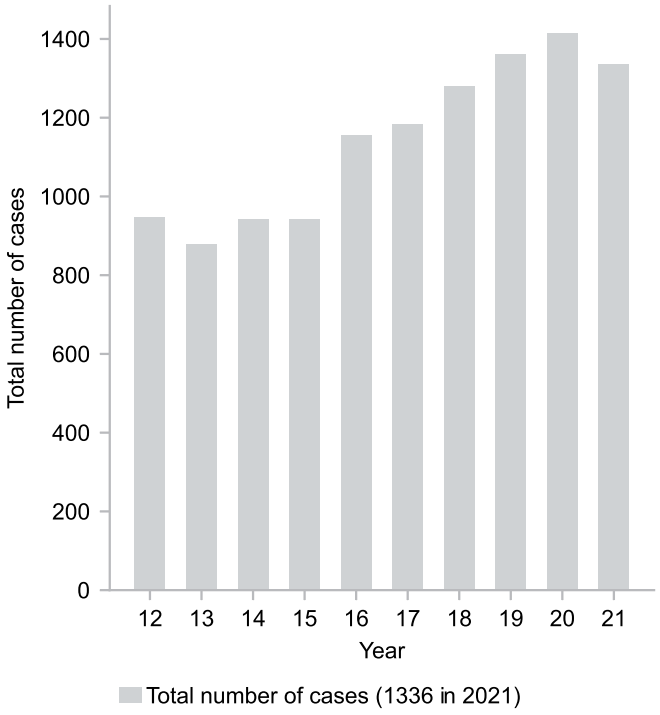
Table 8.4 *Klebsiella pneumoniae*. Resistance (%) in isolates from humans, Denmark, 2021 DANMAP 2021

Substance	Invasive isolates, hospitals %	Urine isolates, hospitals %	Urine isolates, primary health care %
Mecillinam	12	11	8.6
Piperacillin/tazobactam	7.5	8.7	5.7 (1)
Amoxicillin/clavulanic acid	14	8.8	3.7 (3)
Sulfonamide		15.1 (4)	13.8
Trimethoprim		15.3	13.9
Nitrofuratoin		32 (5)	28.9
Gentamicin	2.1	2.4	1.7 (2)
Ciprofloxacin	6.7	6.6	4.9
Cefuroxime	7.9	8.4	4.4 (3)
3rd generation cephalosporins	4.9	5.4	3.7
Carbapenem	0.5	0.1	0.0 (1)
Max. number of isolates tested for resistance to the presented antibiotics	1335	7672	10187

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

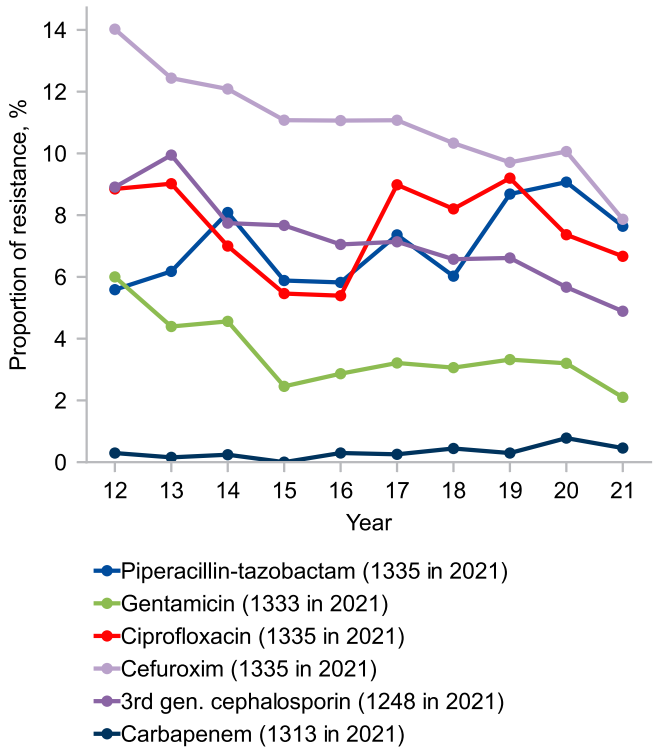
Figure 8.6 Invasive *Klebsiella pneumoniae* isolates from humans: a) annual number of isolates from unique cases, b) proportion of resistant isolates and c) table of resistance percentages, Denmark, 2012-2021 DANMAP 2021

a)



Numbers in parantheses indicate number of isolates tested

b)



c)

Substance	Percent resistant invasive *K. pneumoniae* isolates									
	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021
Piperacillin/tazobactam	5.6	6.2	8.1	5.9	5.8	7.4	6.1	8.7	9.1	7.5
Gentamicin	6.0	4.4	4.6	2.5	2.9	3.2	3.1	3.3	3.2	2.1
Ciprofloxacin	8.8	9.4	7	5.5	5.4	9.0	8.1	9.2	7.4	6.7
Cefuroxime	14.0	12.4	12.1	11.1	11.1	11.1	10.3	9.7	10.1	7.9
3rd gen. cephalosporins	8.9	9.9	7.7	7.7	7.1	7.1	6.6	6.6	5.7	4.9
Carbapenem	0.3	0.2	0.2	0	0.3	0.3	0.5	0.3	0.8	0.5
Total number of isolates	948	875	943	939	1156	1183	1280	1361	1413	1336

Table 8.5 *K. pneumoniae*. Combined resistance to 3rd generation cephalosporins, ciprofloxacin, and gentamicin (multidrug-resistance) in invasive isolates from humans, Denmark, 2014-2021 DANMAP 2021

	2014 % (N)	2015 % (N)	2016 % (N)	2017 % (N)	2018 % (N)	2019 % (N)	2020 % (N)	2021 % (N)
Resistance	3.0 (26)	1.1 (9)	1.6 (18)	2.4 (27)	1.7 (20)	2.4 (30)	1.5 (19)	1.0 (13)
Percentage (no.) of isolates tested for combined resistance (multiresistance)	91 (859)	89 (840)	98 (1131)	95 (1122)	93 (1188)	94 (1275)	93 (1308)	93 (1248)
Total number of invasive isolates	943	943	1156	1183	1280	1361	1413	1336

Urinary cases from hospitals

In 2021, 7,701 unique hospital patients had *K. pneumoniae* isolated from urine samples in Denmark. This represents a 29% increase and 1.4% decrease compared to 2012 and 2020, respectively.

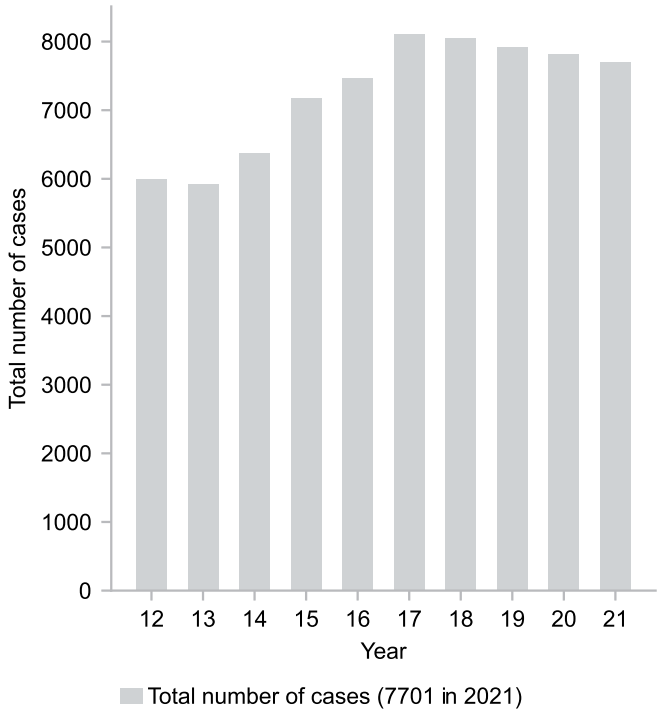
Summarized antibiotic-specific susceptibility results for *K. pneumoniae* isolates (invasive and urine specimen) are shown in Table 8.4. In Figure 8.7, resistance for *K. pneumoniae* urine isolates from hospitals is shown for 2012-2021.

Similar to the changes seen in invasive *K. pneumoniae* isolates, resistance in urine isolates from hospitals has decreased over the past 10 years for gentamicin, ciprofloxacin, cefuroxime and 3rd generation cephalosporins. The increase in resistance to mecillinam observed in 2017 and 2018 has been followed by a marked decrease to the current 10.6% (Figure 8.7). Amoxicillin/clavulanic acid resistance has only been reported since 2018 in urinary isolates from hospitals, due to less than six DCMs testing isolates previously. The proportion of resistance decreased since then.

In 2021, there were 13 carbapenem-resistant and eight carbapenem-susceptible, increased exposure *K. pneumoniae* urine isolates from hospital patients reported in MiBa, respectively, compared to 15 and seven isolates in 2020, respectively.

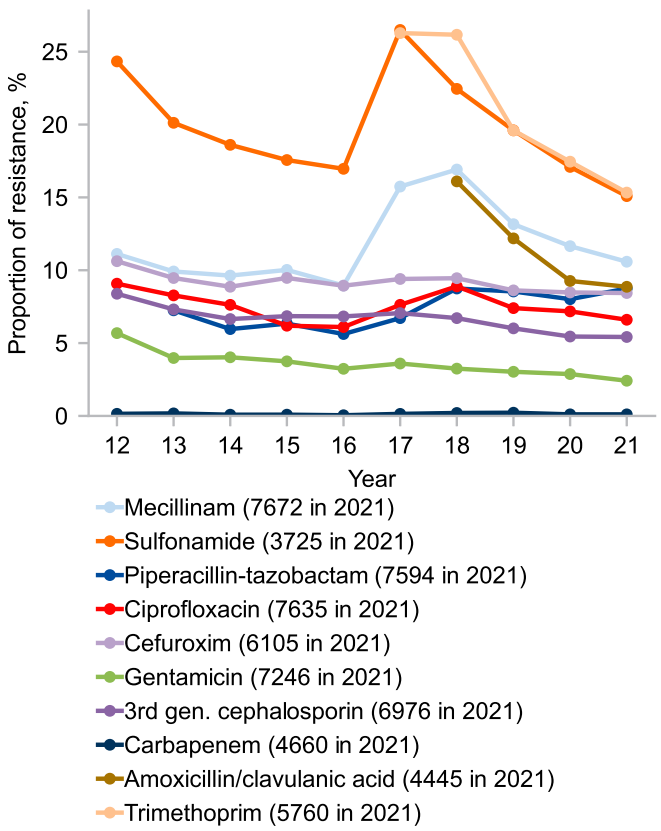
Figure 8.7 Urine *Klebsiella pneumoniae* isolates from humans (hospitals): a) annual number of isolates from unique cases, b) proportion of resistant isolates and c) table of resistance percentages, Denmark, 2012-2021 DANMAP 2021

a)



Numbers in parantheses indicate number of isolates tested

b)



c)

Substance	Resistance in *K. pneumoniae* urine isolates from hospitals									
	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021
Mecillinam	11.1	9.9	9.6	10	8.9	15.7	16.9	13.2	11.7	10.6
Piperacillin/tazobactam		7.2	6	6.3	5.6	6.7	8.7	8.5	8	8.7
Gentamicin	5.7	4	4	3.7	3.2	3.6	3.2	3	2.9	2.4
Ciprofloxacin	9.1	8.3	7.6	6.2	6.1	7.6	8.9	7.4	7.2	7.2
Cefuroxime	10.6	9.5	8.9	9.5	9.1	9.4	9.5	8.6	8.5	8.4
3rd gen. cephalosporins	8.4	7.3	6.6	6.8	6.8	7.1	6.7	6	5.4	5.4
Total number of isolates	5990	5919	6372	7175	7467	8106	8047	7926	7814	7701

Urinary cases from primary health care

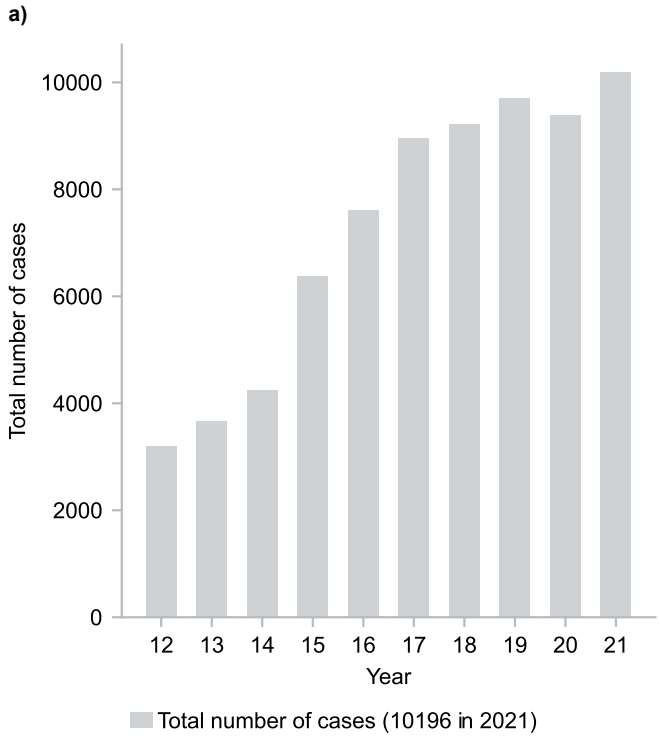
In 2021, 10,196 unique patients in primary health care had *K. pneumoniae* isolated from urine samples, a 218% and 8.6% increase compared to 2012 and 2020, respectively.

As for the results from invasive isolates and isolates from hospital urine samples susceptibility results for all tested antimicrobials are shown in Table 8.4. In Figure 8.8, resistance for *K. pneumoniae* urine isolates from primary health care is shown for the past decade.

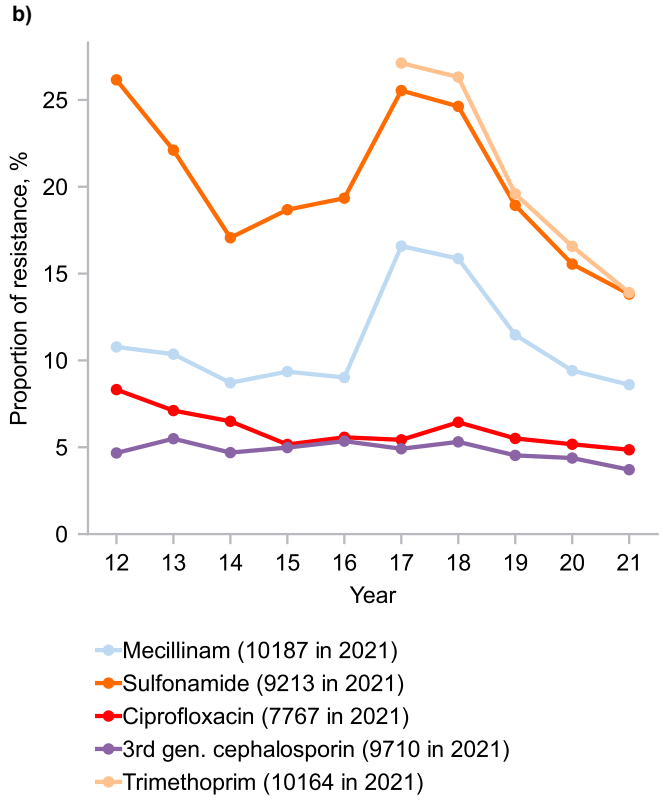
Following a sharp increase in 2017, resistance to mecillinam and sulfonamides/trimethoprim has since decreased. Additionaly, resistance to ciprofloxacin and 3rd generation cephalosporins has also continued to decline (Figure 8.8).

Three carbapenem-resistant isolates and one carbapenem-susceptible, increased exposure isolate were registered in 2021 compared to four and one in 2020 respectively. However, susceptibility to carbapenems is only routinely reported to MiBa by one DCM.

Figure 8.8 Urine *Klebsiella pneumoniae* isolates from humans (primary health care): a) annual number of isolates from unique cases, b) proportion of resistant isolates and c) table of resistance percentages, Denmark, 2012-2021 DANMAP 2021



Numbers in parantheses indicate number of isolates tested



c)

Substance	Percent resistant *K. pneumoniae* isolates from primary health care urine									
	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021
Mecillinam	10.6	10.4	8.8	9.4	9	16.6	15.9	11.5	9.4	8.6
Sulfonamide	25.7	22.1	17.1	18.7	19.3	25.5	24.6	18.9	15.5	13.8
Ciprofloxacin	7.8	7.1	6.5	5.2	5.6	5.4	6.4	5.5	5.2	4.9
3rd gen. cephalosporins	4.7	5.5	4.7	5.0	5.4	4.9	5.3	4.5	4.4	3.7
Total number of isolates	3203	3669	4246	6372	7615	8948	9227	9696	9387	10196

Conclusion

The general trend for *K. pneumoniae* in all three specimen types (blood/cerebrospinal fluid, urine [hospital/primary health care]) has been a decrease in resistance for important antimicrobials (cephalosporins, gentamicin and ciprofloxacin) over the last ten years. Following a sharp increase in mecillinam and sulfonamide/trimethoprim resistance in urine samples from hospitals and primary care in 2017 and 2018, the proportion of resistant *K. pneumoniae* isolates has since been decreasing. The carbapenem resistance in *K. pneumoniae* continues to be very low (<1%).

8.2.3 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is an opportunistic pathogen, which can colonise the lung, urinary tract, burn wounds, superficial wounds and can cause invasive infections. The pathogen is capable of biofilm formation and has the ability to colonise medical devices (e.g. indwelling catheters and implants) and in most 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. The case fatality rate in these patients is high. *P. aeruginosa* is a clinically significant pathogen that exhibits intrinsic resistance to various antimicrobial agents, including β-lactam antibiotics, through chromosomal gene mutations and has the ability to acquire of β-lactamases (extended-spectrum β-lactamases (ESBLs) and carbapenemases (especially class B carbapenemases or metallo-β-lactamases [MBLs]) by horizontal transmission. The antimicrobial classes, which can be used for treatment include: fluoroquinolones, aminoglycosides (tobramycin, gentamicin and amikacin), beta-lactams (piperacillin-tazobactam, ceftazidime and carbapenems) and colistin. New antibiotic combinations with beta-lactamase inhibitors, such as aztreonam-avibactam and ceftolozane-tazobactam, may be used in serious cases of Gram-negative bacterial infections including MBL-producers.

Invasive cases from hospital patients

In 2021, a total of 513 unique patients with invasive *P. aeruginosa* isolates were registered in Denmark. Number of cases over the last decade are presented in Figure 8.9.

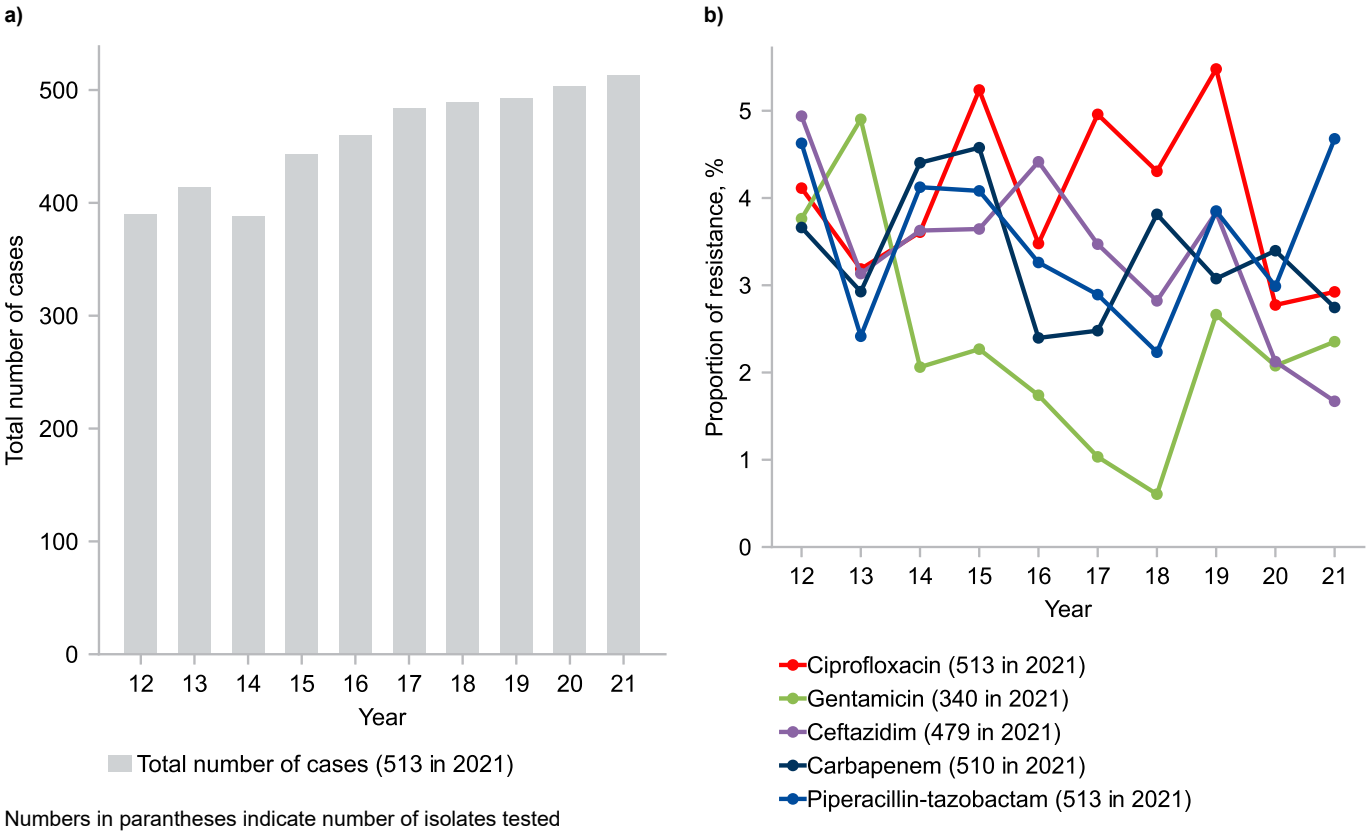
The highest proportion of resistance was reported for ciprofloxacin ranging from 2.8 - 5.4% over the past 10 years. In 2021, meropenem resistance was reported in 2.7% of the cases, and only 1.6% of the cases were resistant to three or more of the five antimicrobials under surveillance.

Conclusion

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

Figure 8.9 Invasive *Pseudomonas aeruginosa* isolates from humans: a) annual number of isolates from unique cases and b) proportion of resistant isolates, Denmark, 2012-2021

DANMAP 2021



Numbers in parantheses indicate number of isolates tested

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 colonize the skin and wounds but may also cause hospital-acquired infections such as central line-associated bloodstream infections, nosocomial pneumonia, UTIs and wound infections. Many of the different subspecies are phenotypically alike, and thus identification at species level can be difficult. Species belonging to the *A. baumannii* group are considered as the most clinically important. *Acinetobacter* spp. is intrinsically resistant to a broad range of antimicrobials mediated by a low membrane permeability and constitutive expression of various efflux systems. The antimicrobial classes that are recommended for treatment, include fluoroquinolones, aminoglycosides, carbapenems and colistin.

Invasive cases from hospitals

In 2021, a total of 97 unique patients with invasive *Acinetobacter* species were reported. Data are presented in Table 8.6 and in Figure 8.10.

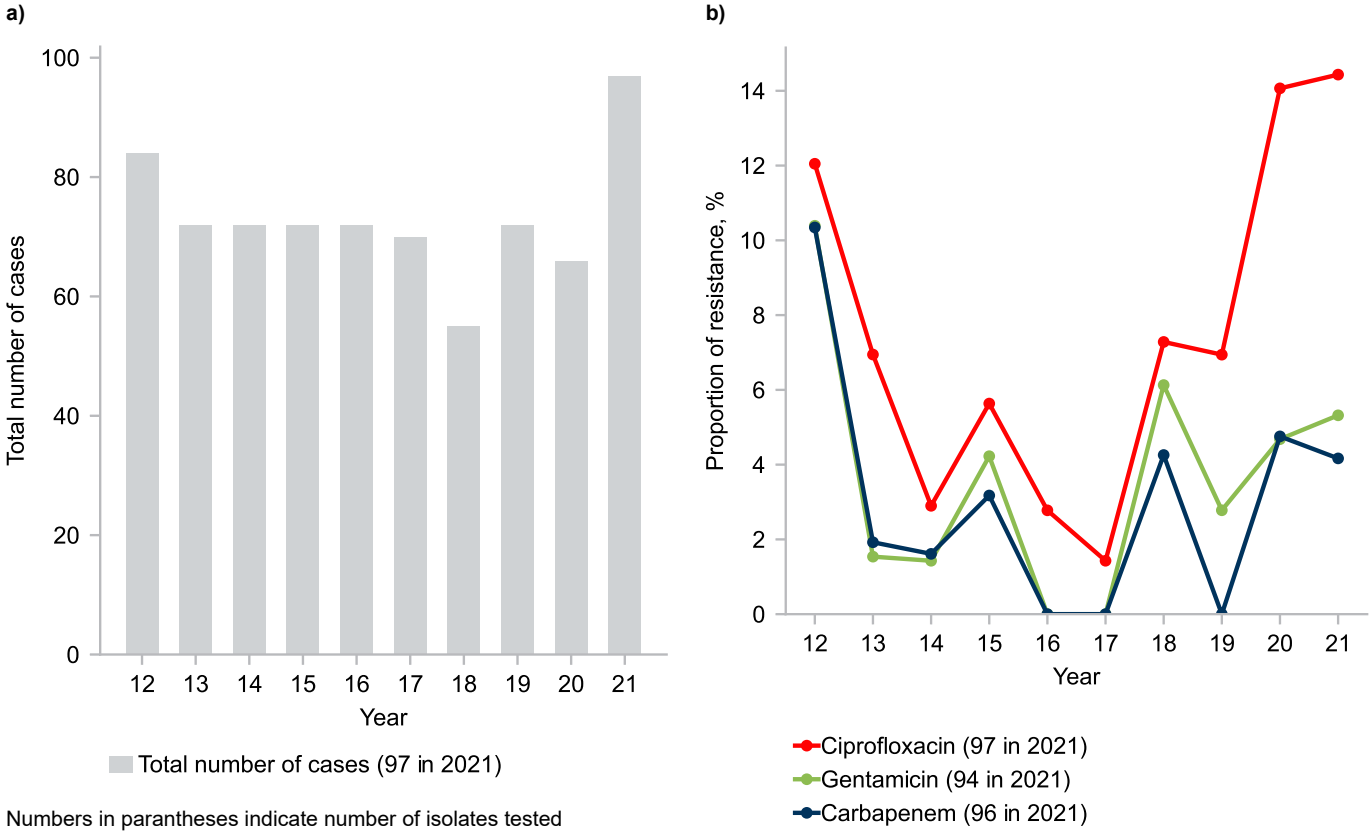
The number of invasive *Acinetobacter* spp. cases in 2021 was markedly higher than the numbers in previous years and increased by 47% compared to 2020. Four of the 97 isolates identified in 2020 were resistant to meropenem, 14 were resistant to ciprofloxacin and five were resistant to gentamicin. Four isolates had combined resistance to ciprofloxacin and gentamicin and none were reported resistant to colistin, however, susceptibility to colistin, is not routinely tested.

Table 8.6 *Acinetobacter* spp. tested and resistant invasive isolates, Denmark, 2012-2021 DANMAP 2021

	2012		2013		2014		2015		2016		2017		2018		2019		2020		2021	
	res.	n	res.	n	res.	n	res.	n	res.	n	res.	n	res.	n	res.	n	res.	n	res.	n
Ciprofloxacin	10	83	5	72	2	69	4	71	2	72	1	70	4	55	5	72	9	64	14	97
Gentamicin	8	77	1	65	1	70	3	71	0	70	0	70	3	49	2	72	3	64	5	94
Meropenem	6	58	1	52	1	62	3	68	0	69	0	67	2	47	0	72	3	63	4	96
Total number of invasive isolates	84		72		72		71		72		70		55		72		66		97	

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

Figure 8.10 Invasive *Acinetobacter* spp. isolates from humans: a) annual number of isolates from unique cases and b) proportion of resistant isolates, Denmark, 2012-2021 DANMAP 2021



Numbers in parantheses indicate number of isolates tested

Conclusion

The number of invasive *Acinetobacter* spp. saw a marked increase compared to previous years; however, the proportion of invasive isolates resistant to key antimicrobials remained low in Denmark.

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, bacteraemia and infective endocarditis. Enterococci are intrinsically resistant to many groups of antimicrobials and thereby have a selective advantage in e.g. hospitalised patients under antibiotic treatment, which can lead to colonization or infection. The source of hospital infection is often associated with invasive medical devices.

Treatment of enterococcal infections may be challenging. Combination therapy based on a synergistic effect of beta-lactam antibiotics (penicillin/ampicillin) and aminoglycoside (most often gentamicin) or glycopeptide (vancomycin) is indicated in cases of complicated infection (e.g. endocarditis). In cases of high-level gentamicin resistance, combination of ampicillin and ceftriaxone may be used for treatment. The vast majority of *E. faecium* are ampicillin-resistant, and therefore most infections are treated with vancomycin. Antimicrobials such as linezolid and daptomycin can be used for treatment of the multidrug-resistant vancomycin-resistant enterococci (VRE).

Invasive cases from hospitals

In 2021, 682 unique patients with invasive *E. faecalis* isolates and 796 unique patients with invasive *E. faecium* isolates were reported in MiBa.

The proportion of resistant invasive *E. faecalis/faecium* isolates in 2021 are presented for each key antimicrobial in Table 8.7. In Figure 8.11, total numbers of invasive isolates of *E. faecalis* and *E. faecium* and the respective percentages of vancomycin resistance are shown for 2012 to 2021.

The total number of invasive cases caused by *E. faecalis* and *E. faecium* increased by 32% from 2012 to 2021.

A continuous high proportion of ampicillin resistance in invasive *E. faecium* has been observed with proportions of resistant isolates ranging between 92% and 95% since 2010. In 2002, the resistance rate was reported to be 65%. The proportion of invasive vancomycin resistant *E. faecium* isolates has increased to 10.2% in 2021 after having stabilised at around 9% in the previous past three years.

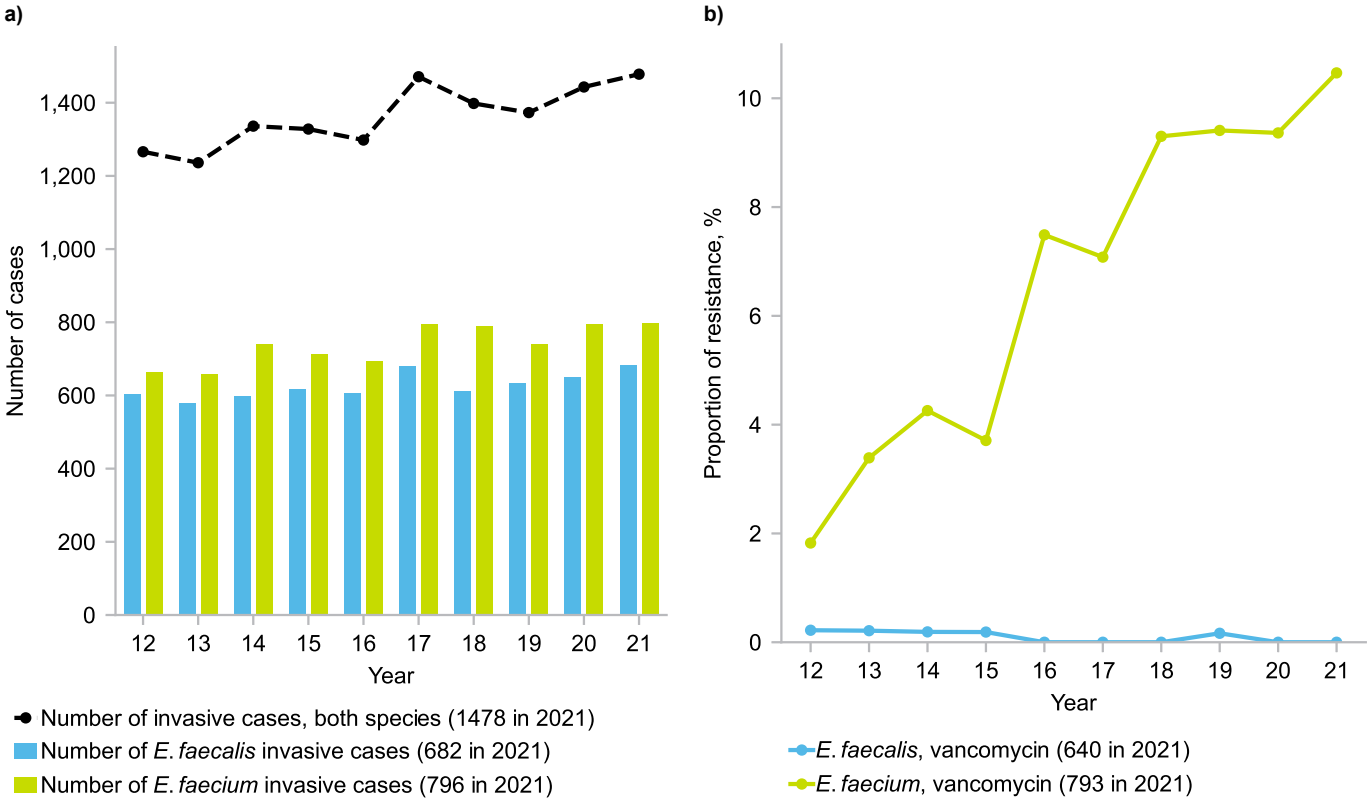
During 2021, four invasive isolates of *E. faecalis* and two invasive isolates of *E. faecium* from six unique patients were reported linezolid resistant (Table 8.7). In 2020, the numbers were six *E. faecalis* and three *E. faecium*. The two linezolid resistant invasive *E. faecium* isolates identified in MiBa in 2021 were also reported resistant towards vancomycin.

Table 8.7 Enterococci. Resistance (%) in invasive isolates from humans, 2021 DANMAP 2021

	E. faecalis	*E. faecium*	Number of included isolates (number of DCM)	
	%	%	*E. faecalis*	*E. faecium*
Ampicillin	0.3	92.5	678 (10)	791 (10)
Vancomycin	0	10.2	640 (9)	793 (10)
Linezolid	0.6	0.3	512 (6)	630 (6)
Teicoplanin	0.4	1.5	236 (2)	269 (2)
Tigecycline	0	0	92 (1)	134 (1)

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

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, 2012-2021 DANMAP 2021



Numbers in parantheses indicate number of isolates tested

Conclusion

An increase in invasive enterococci has been observed since 2002 (Figure 8.1.1. DANMAP 2015). The overall increase was combined with an increase in ampicillin resistance (65% in 2002 and >90% since 2010) and vancomycin resistance in *E. faecium* up to 2018. Up until 2020, the proportion of vancomycin resistance remained stable, but has since increased to 10.2% in 2021.

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

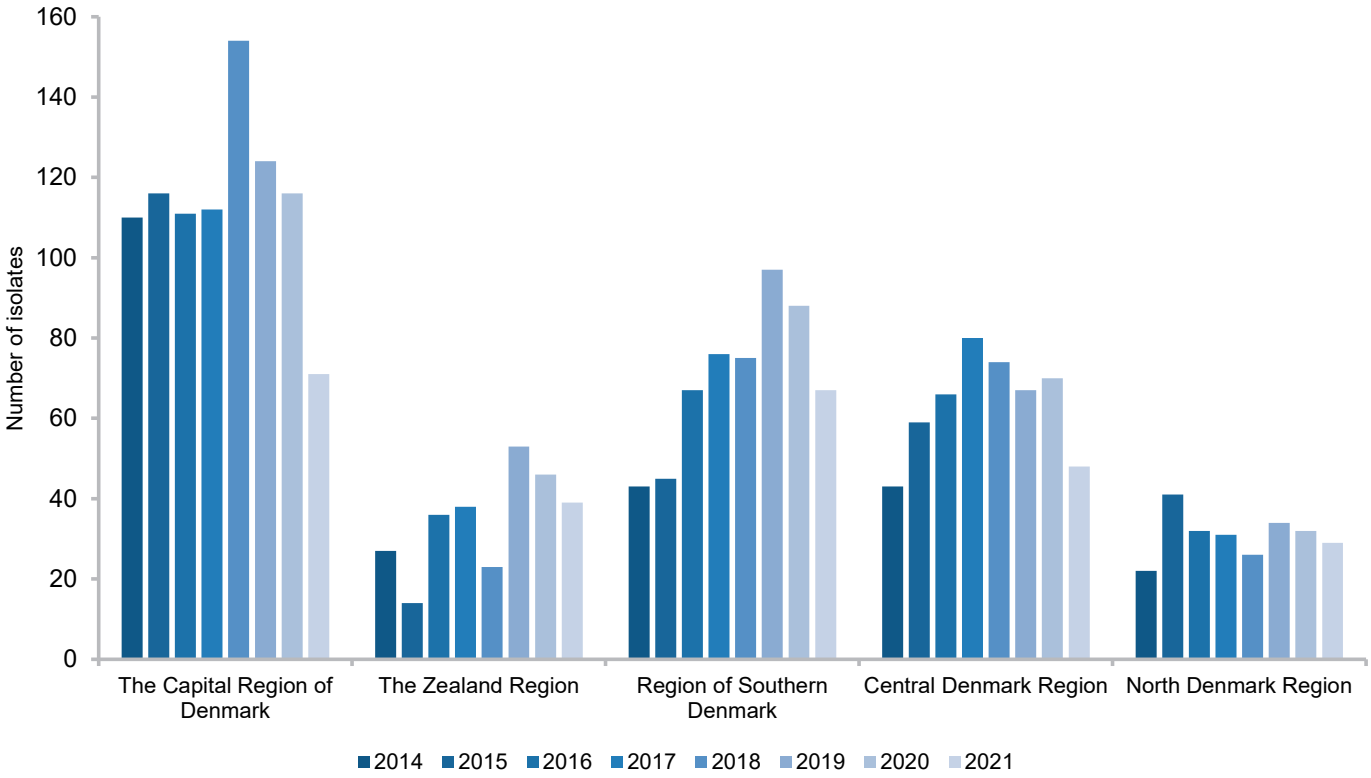
At SSI, the 3GC-R *Ec*'s collected in Denmark through 2021, were phenotypically tested for ESBL-production. ESBL- and/or pAmpC-positive isolates from January, March, May, July, September, and November were subjected to whole-genome sequencing and characterized according to Multilocus Sequence Types (MLSTs) and the encoding ESBL-, pAmpC- and carbapenemase genes.

Results

In 2021, a total of 254 *E. coli* isolates from unique patients, were identified with phenotypic test, as ESBL and/or AmpC positive isolates. Demographic data was available for all 254 *E. coli* isolates in 2021; 147 (58%) of the patients were men compared to 190 (54%) in 2020, and 107 (42%) were women compared to 162 (46%) in 2020. The median age at diagnosis was 74 years, ranging from below one year to 97 years.

The regional distribution of the 254 isolates with ESBL- and AmpC phenotype was compared to data from previous years (Table 8.8 and Figure 8.12).

Figure 8.12 ESBL/pAmpC producing *E. coli* from bloodstream infections by region, Denmark, 2014-2021 DANMAP 2021



Following the overall decreasing trend of reported cases of ESBL/pAmpC *E. coli* in bloodstream infections observed from 2019 to 2020 (6%), the number decreased a further 28% from 2020 to 2021 (from 352 to 254 isolates), roughly corresponding to the level of 2014 (245 isolates). Decreasing numbers were observed in all five regions, most notably in The Capital Region, with the number of reported cases decreasing from 116 cases in 2020 to 71 cases in 2021.

Whole genome sequencing data were obtained from 130 *E. coli* isolates (as only isolates from every second month were sequenced). Genes encoding ESBL and/or pAmpC were detected in all isolates (4 of which also having carbapenemase encoding genes detected).

In 2021, 17 different genes associated with ESBL-, and pAmpC enzymes were detected among the 130 sequenced isolates (Table 8.9). As in previous years, CTX-M-15 was the most prevalent enzyme, remaining relatively stable in occurrence at 46% in 2021, compared to 50% in 2020.

In addition, four carbapenemase producers were observed during 2021 among the 130 whole genome sequenced blood infection isolates (3%); one NDM- and three OXA-48-group producing isolates of which two isolates (one NDM-5- and one OXA-244 producer) belonged to known outbreaks while the remaining two isolates (an OXA-48- and an OXA-244 producer) occurred sporadically.

Table 8.8 Distribution of ESBL and Carbapenemase producing *E. coli* from bloodstream infections, Denmark, 2014-2021 DANMAP 2021

	DANMAP 2014	DANMAP 2015	DANMAP 2016	DANMAP 2017	DANMAP 2018	DANMAP 2019	DANMAP 2020	DANMAP 2021
Region	Numbers	Numbers	Numbers	Numbers	Numbers	Numbers	Numbers	Numbers
The Capital Region of Denmark	110	116	111	112	154	124	116	71
The Zealand Region	27	14	36	38	23	53	46	39
Region of Southern Denmark	43	45	67	76	75	97	88	67
Central Denmark Region	43	59	66	80	74	67	70	48
North Denmark Region	22	41	32	31	26	34	32	29
Total Numbers	245	275	312	337	352	375	352	254

Table 8.9 Most common ESBL enzymes and carbapenemases detected in *E. coli* from bloodstream infections, Denmark 2014-2021 DANMAP 2021

	DANMAP 2014		DANMAP 2015		DANMAP 2016		DANMAP 2017		DANMAP 2018		DANMAP 2019		DANMAP 2020		DANMAP 2021	
Enzyme	Number	%	Number	%	Number	%	Number	%	Number	%	Number*	%	Number*	%	Number*	%
blaCTX-M-1	10	4%	7	3%	8	3%	17	5%	25	7%	8	4%	7	3%	6	4%
blaCTX-M-101	12	5%	15	5%	14	4%	9	3%	4	1%	2	1%	2	1%	0	0%
blaCTX-M-14	38	16%	33	12%	40	13%	48	14%	31	9%	33	17%	15	7%	12	9%
blaCTX-M-14b	5	2%	5	2%	9	3%	3	1%	10	3%	3	2%	4	2%	0	0%
blaCTX-M-15	121	49%	139	51%	157	50%	164	49%	200	57%	82	43%	100	50%	63	46%
blaCTX-M-27	25	10%	33	12%	44	14%	52	15%	53	15%	37	19%	36	18%	29	21%
blaCTX-M-3	4	2%	4	1%	7	2%	8	2%	5	1%	4	2%	1	0%	3	2%
blaCTX-M-55	8	3%	14	5%	6	2%	13	4%	4	1%	8	4%	4	2%	5	4%
blaCMY-2	10	4%	6	2%	10	3%	7	2%	6	2%	5	3%	5	2%	2	1%
blaDHA-1	0	-	3	1%	5	2%	6	2%	10	3%	4	2%	7	3%	3	2%
blaSHV-12	2	1%	5	2%	5	2%	3	1%	4	1%	2	1%	5	2%	3	2%
Other CMY variants	4	2%	10	4%	3	1%	3	1%	3	1%	5	3%	0	0%	1	1%
Other ESBL enzymes	12	5%	8	3%	17	5%	10	3%	10	3%	3	2%	8	4%	6	4%
Carbapenemase enzymes	3	1%	3	1%	1	<1%	1	<1%	5	1%	0	0%	7	3%	4	3%

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

Table 8.10 Distribution of MLSTs in *E. coli* from bloodstream infections, Denmark, 2014-2021 DANMAP 2021

	DANMAP 2014		DANMAP 2015		DANMAP 2016		DANMAP 2017		DANMAP 2018		DANMAP 2019		DANMAP 2020		DANMAP 2021	
MLST	Number	%	Number	%	Number	%	Number	%	Number	%	Number*	%	Number*	%	Number*	%
ST131	124	51%	135	49%	177	57%	175	52%	189	54%	93	47%	89	46%	64	49%
ST38	18	7%	23	8%	21	7%	23	7%	22	6%	13	7%	8	4%	1	1%
ST405	13	5%	12	4%	7	2%	9	3%	9	3%	0	0%	4	2%	5	4%
ST410	4	2%	11	4%	6	2%	6	2%	3	1%	2	1%	4	2%	0	0%
ST69	10	4%	10	4%	16	5%	20	6%	27	8%	14	7%	20	10%	7	5%
ST648	7	3%	10	4%	5	2%	8	2%	6	2%	4	2%	0	0%	1	1%
ST12	5	2%	9	3%	14	4%	6	2%	5	1%	5	3%	2	1%	5	4%
ST88	2	1%	1	0%	0	0%	5	1%	2	1%	2	1%	2	1%	0	0%
ST1193	2	1%	5	2%	10	3%	7	2%	8	2%	6	3%	9	5%	9	7%
ST10	0	0%	6	2%	2	1%	4	1%	7	2%	5	3%	1	1%	3	2%
ST23	1	0%	0	0%	2	1%	3	1%	1	0%	11	6%	3	2%	0	0%
ST73	3	1%	2	1%	4	1%	2	1%	6	2%	4	2%	8	4%	1	1%
Other STs[1]	56	23%	51	19%	48	15%	69	20%	69	20%	38	19%	43	22%	34	26%

[1] Found in less than 2% in 2021
* Numbers based on sequenced data from odd months

In 2021, the 130 whole genome sequenced *E. coli* isolates belonged to 39 different MLSTs, with the most common sequence type (ST) being ST131 (49%), followed by ST1193 (7%) and ST69 (5%) (Table 8.10).

The proportion of ST23 isolates followed the decreasing trend observed from 6% in 2019 to 2% in 2020 ($p=0.03$, $\chi 2$-test), as no isolates belonging to this ST were detected during 2021. Besides this, no significant changes of the distribution of MLSTs were observed in 2021 (Table 8.10).

Among the 64 *E. coli* isolates belonging to ST131, CTX-M-15 (56%) was the most common enzyme, followed by CTX-M-27 (31%), and CTX-M-14 (6%). The distribution of ESBL and/or pAmpC enzymes observed within ST131 remained stable in 2021.

Conclusion
In 2021, the number of ESBL- and/or AmpC positive isolates continued to decrease from 352 to 254 isolates (28% decrease). CTX-M-15 remained by far the most prevalent ESBL enzyme in Danish *E. coli* from bloodstream infections in 2021. In isolates belonging to ST131, the relative distribution of ESBL/pAmpC enzymes was stable in 2021 compared to 2020.

In 2021, 4 carbapenemase producers were observed among the 130 whole genome sequenced ESBL- and/or pAmpC blood infection isolates.

With the exception of the further decrease of isolates belonging to ST23, the relative distribution of sequence types for the 130 whole genome sequences isolates were similar to the distributions in the previous years; the worldwide disseminated ST131 clone was still strongly represented in 2021 (49%).

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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 with 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 (DCMs) 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 2021, 277 CPOs were identified from 242 patients compared with 238 CPO isolates from 207 patients in 2020, an increase of 16%. More than one isolate from the same patient was included if the isolates belonged to different bacterial species and/or if the isolates harboured different car-

bapenemases. Eleven out of all CPOs were from bloodstream infections compared to seventeen out of all CPOs in 2020.

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 2021, 251 CPE isolates were reported from 221 patients compared to 209 CPE from 183 patients in 2020 resulting in a 20% increase compared to 2020. In 2021, 14 of the 246 CPE isolates produced both NDM and OXA-48 group enzymes, 170 produced OXA-48-like enzymes and 62 were NDM-producing. Furthermore, three KPC-, one VIM- and one CTX-M-33-producing isolate(s) were identified (Figure 8.13).

Carbapenemase-producing *Acinetobacter* spp.

In 2021, 25 carbapenemase-producing *Acinetobacter* spp. isolates were reported from 25 patients, compared to 22 isolates from 22 patients in 2020. Eleven of the patients had been travelling abroad prior to identification of the carbapenemase-producing *Acinetobacter* spp. In 2021, 23 carbapenemase-producing *Acinetobacter baumannii* with the following enzymes were identified: OXA-23 (15), OXA-72 (6), OXA-72/OXA-23 (1) and NDM-1/OXA-23 (1). Furthermore, one NDM-1-producing *Acinetobacter pittii* and one OXA-945-producing *Acinetobacter bereziniae* were identified.

Carbapenemase-producing *Pseudomonas* spp.

In 2021, only one carbapenemase-producing *Pseudomonas* spp. isolate was reported compared to seven isolates in 2019 and 2020, respectively. The isolate reported in 2021 was a VIM-2-producing *Pseudomonas stutzeri* from a patient with unknown travel history.

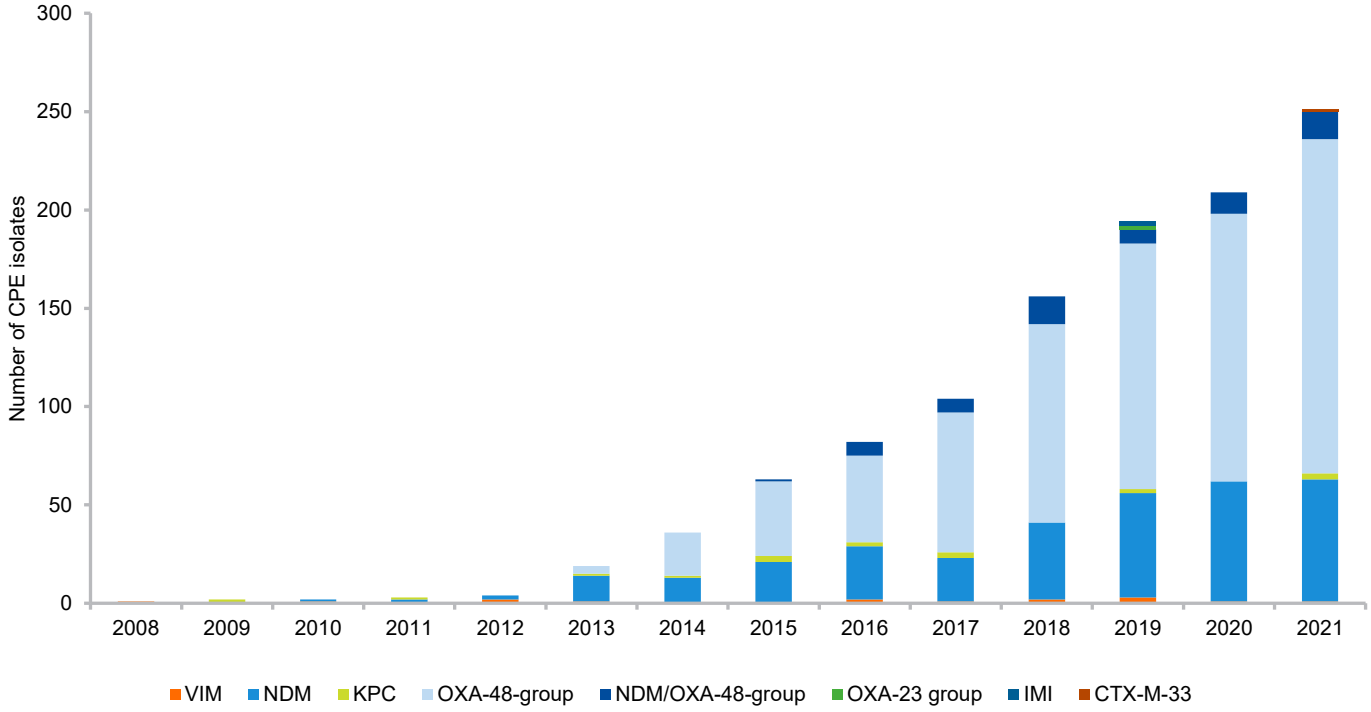
CPO - Place of origin 2019 - 2021

The Clinical Departments or a clinical physician can report travel in the period of six month before the colonisation is detected, and the CPO-patient will be classified as a travel-associated CPO-patient. In order to qualify the information regarding the origin of a colonisation with CPO in a Danish patient, the reported CPO-data from 2019 to 2021 has been evaluated and categorised into four categories: 1) Denmark, 2) Danish outbreak, 3) travel outside the Nordic countries, and 4) unknown.

The Clinical Departments or a clinical physician can report a CPO-patient to be colonised in Denmark implicating that the patient has not been travelling prior to colonisation. A CPO-patient can be affected by a Danish nosocomial outbreak and will be classified as an outbreak-patient. For some patients, no information is reported and the classification for place of origin will be unknown. The index patient (the first patient) in an outbreak will be registered according to travel information.

Since 2019, the number of CPO-patients who could be classified as a nosocomial outbreak-patient has increased by 131%. The COVID-19 pandemic situation with reduced travel activity has affected the number of travel-associated CPO-cases, especially in 2020.

Figure 8.13 Numbers of carbapenemase-producing Enterobacterales (CPE), Denmark, 2008-2021 DANMAP 2021



In 2021, the reported travel data show that 45 of 242 CPO-positive persons (19%) reported travelling outside the Nordic countries. The most frequent reported travel destinations in 2021 were Middle East (9), South Europe (5), Mediterranean (3), and Asia (3).

In 2020, due to COVID-19, travel abroad decreased, which in turn affected the number of travel-associated CPO-infections resulting in a much lower proportion of all CPO infections. This situation has probably also affected the number of travel-associated cases in 2021. In 2019, 80 of 187 CPO-positive persons (43%) reported travelling outside the Nordic countries. The most frequent reported travel destinations in 2019 were Egypt (8), Greece (7), Thailand (7) India (6), Spain (6) and Turkey (6).

Outbreaks with CPO during 2021

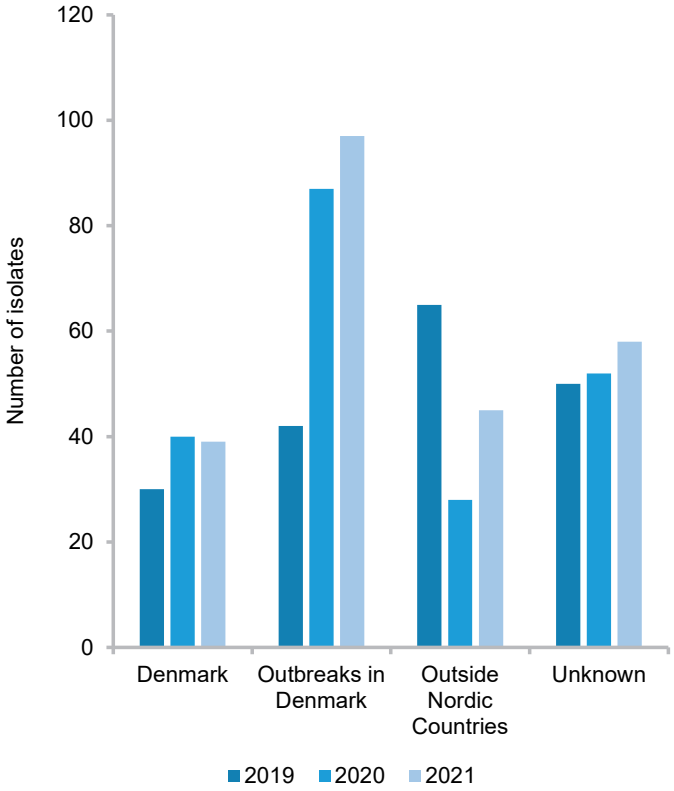
In Denmark, outbreaks caused by CPO in healthcare settings are registered at SSI in the national database (KURS). At SSI, CPO isolates are routinely characterised by whole genome sequencing (WGS). Cluster analysis is conducted on all isolates using cgMLST to detect possible clustering between the CPO 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 colonisation 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 9.12).

In 2021, a total of 15 CPO-outbreaks were registered compared to 20 CPO-outbreaks in 2020. In twelve of the outbreaks, it was possible to establish an epidemiological link between the patients. All epidemiological links were found in healthcare settings, caused by patients sharing the same ward or hospital. Seven of the fifteen outbreaks had been ongoing for more than two years and one up to ten years, meaning that new patients had been identified as belonging to the same cgMLST cluster as found in the previous years.

The decrease in the number of outbreaks from 2020 can be explained by the fact that several smaller outbreaks in 2020 did not cause further transmission in 2021.

In total, 77 new patients were affected in 2021 by these outbreaks. Of the fifteen outbreaks registered in 2021, five were new outbreak clusters, representing 16 patients. In four of these outbreaks, epidemiological investigations showed that the patients had been at the same hospital ward at the same time (Table 8.11).

Figure 8.14 Place of origin of CPO, 2019-2021 DANMAP 2021



Outbreaks with CPO of interest

The ST18 NDM-1-producing *Citrobacter freundii* outbreak (ID41), which started in 2012 in the North Denmark Region, continued in 2021 [Hammerum et al. 2016, J. Antimicrob. Agents, 71:3117-3124]. Since 2012, the outbreak has spread to four out of five Danish Regions. Until the end of 2021, 73 hospitalised patients have been involved in this outbreak. In all, 21 new patients were identified in 2021, an increase of 40% patients and of which 15 had been hospitalised in the North Denmark Region. None of these new outbreak-cases had a prior history of travel.

Since 2015, another large outbreak (ID21) has been ongoing in the Zealand Region and in the Capital Region with spread of ST410 NDM-5/OXA-181 *E. coli*. [Roer et al. 2018, mSphere;3(4).]. By the end of 2021, 72 patients in total have been involved in this outbreak. During 2021, twelve new patients were affected by this outbreak, and all were identified on Zealand. Apart from the first reported patient in 2015, who had been traveling to Egypt [Overballe-Petersen et al. 2018, Genome Announc.6(5): e01542-17] none of the patients have a prior history of travel.

Infection prevention and control guidelines for CPO

The Danish Health Authority issued the first national guideline on prevention the spread of CPO in 2018 (www.sst.dk/da/udgivelser/2018). The guideline presents the national health authorities' statutory recommendations and the national strategic framework for preventing the spread of CPO. The

main purpose of the guideline is to maintain a low prevalence of disease caused by CPO associated with certain high-risk situations as previously diagnosed with CPO, household-like contact with person tested positive for CPO, travelling outside the Nordic countries with admission to a hospital or clinic and/or receiving antibiotic treatment during the stay. Individual risk factors for acquiring colonization or infection with CPO are older age, immunosuppression, antibiotic treatment, invasive devices etc. The guideline emphasizes the importance of all health care staff complying with the national guidelines for infection prevention and control, and for all doctors to prescribe antibiotics with caution.

Acting in compliance with the national CPO guideline and the national guidelines for infection prevention and control (published by National Center for Infection Control at SSI) is key in preventing the spread of multidrug-resistant microorganisms with correct hand hygiene and use of personal protective equipment (PPE) among the most important control measures. 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 [https://hygiejne.ssi.dk/retningslinjer/infektionshygiejniske-retningslinjer-for-cpo].

It is noteworthy that patients are known to be carriers of CPE for several years (in some of the Danish outbreaks more than five years) with no treatment for CPE carrier state available. As a result, compliance with guidelines is extremely important in order to prevent further spread of CPO.

Under infection control

In relation to outbreaks at hospitals, environmental spread of CPO has occurred through bath drains and toilets. After intensive cleaning and disinfection of these parts it has in some cases been possible to stop an outbreak. This emphasizes the importance of more frequent cleaning and disinfection in an outbreak situation and the importance of well-functioning drains in general.

Table 8.11 Outbreaks of carbapenemase-producing Enterobacterales (CPE) and carbapenemase-producing organisms (CPO) during 2021, n=15, Denmark

DANMAP 2021

Outbreak ID	Year	Patients total	Patients 2021	Carbapenemase	Type of Outbreak	Species (clonal spread)	Regions	Status
Outbreaks of carbapenemase-producing Enterobacterales (CPE)								
41	2012-2021	73	21	NDM-1	Clonal/ plasmid	ST18 *C. freundii*	Capital Region/Central Denmark Region/North Denmark Region/ Southern Denmark Region	Verified
48	2013-2021	30	7	OXA-436/OXA-48	Clonal/ plasmid	ST90 *E. cloacae*/ ST22 *C. freundii*	Capital Region/Southern Denmark Region/Zealand Region	Verified
21	2015-2021	72	12	NDM-5/OXA-181	Clonal	ST410 *E. coli*	Capital Region/Central Denmark Region/Zealand Region	Verified
22	2015- 2021	10	4	OXA-181	Clonal	ST440 *E. coli*	Capital Region/Central Denmark Region	Possible
42	2015-2021	12	3	OXA-48	Clonal	ST65 *C. freundii*	Capital Region/North Denmark Region/Zealand Region	Verified
33	2016-2021	25	2	OXA-232	Clonal	ST231 *K. pneumoniae*	Central Denmark Region	Verified
1061	2020-2021	6	3	OXA-181	Clonal	ST22 *C. freundii*	Central Denmark Region	Possible
1068	2020-2021	9	7	OXA-48	Clonal	ST18 *C. freundii*	Capital Region	Verified
1052*	2020-2021	3	2	NDM-1	Clonal	ST18 *C. freundii*	Central Denmark Region	Possible
1070*	2017-2021	6	3	OXA-48	Clonal	*C. farmeri*	Zealand Region	Verified
1075*	2021	2	2	NDM-1/OXA-48	Clonal	ST23 *K. pneumoniae*	Capital Region	Verified
1076*	2021	2	2	OXA-48	Clonal	ST233 *K. aerogenes*	Zealand Region	Verified
Outbreaks of carbapenemase-producing organisms (CPO)								
1058	2020-2021	13	2	OXA-23	Clonal	ST195 *A. baumannii*	Capital Region	Verified
1067	2020-2021	6	4	OXA-23	Clonal	ST195, ST1816 *A. baumannii*	South Denmark Region	Verified
1074*	2021	3	3	OXA-72	Clonal	ST1757 *A. baumannii*	Capital Region	Verified

* Outbreak clusters identified in 2021

Conclusion

The occurrence of carbapenemase-producing bacteria in Denmark continued to increase throughout 2021. Fewer new nosocomial outbreaks were emerging in 2021, but the number of patients belonging to the two largest outbreaks in hospital settings continued to increase, which is a worrisome trend, as these larger outbreaks have a tendency to be long-lasting and spread across hospitals and regions. The spread of CPO 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.

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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 2015 and 2016, sporadic VVE isolates with different genetic background in relation to concurrent VRE-outbreaks were detected in the Capital Region of Denmark, [Hammerum *et al.* Euro Surveill. 2020;25(18)]. In 2016, a new VVE clone belonging to ST1421- CT1134, 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]. 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 (DCMs) have voluntarily submitted clinical VRE (one VRE 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). This has allowed investigation of biomarkers and clonal changes in Denmark, and shown an increase in clinical VRE isolates since 2013 (see Figure 8.15).

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 finding of *van*-genes (see Section 9.13.3).

To determine any underreporting in the submissions, the number of VRE/VVE submitted to SSI since 2016 were compared to data from clinical VRE reported by the DCMs to MiBa (the Danish Microbiology Database). This comparison showed that the number of submitted VRE/VVE isolates was not complete (Figure 8.15). In 2021, 569 VRE/VVE isolates were submitted to SSI, and were genotyped by WGS. 164 VRE/VVE isolates were identified in MiBa, which had not been submitted to SSI and hence have not been genotyped. This number has been increasing since 2019.

In 2021, a total of 733 VRE/VVE isolates were collected from patients, of which one patient was infected by both vancomycin resistant *E. faecium* and *E. faecalis*. This is an increase since 2020, where a total of 656 VRE/VVE isolates were collected (Figure 8.15).

Of the 569 clinical VRE/VVE isolates sequenced in 2021, 83 were *vanA E. faecium*, 479 *vanB E. faecium*, 3 *vanA/vanB E. faecium*, 1 *vanA E. faecalis*, and 3 *vanB E. faecalis* (Figure 8.15). WGS-based cgMLST analysis was performed on the 565 *E. faecium* isolates using SeqSphere+ (Ridom). The 565 *E. faecium* isolates were subdivided into 85 CTs. Two clonal groups (covering several different CTs but presumably originating from the same clone) were predominant: The ST80-CT2406 *vanB E. faecium* group containing 356 isolates and the VVE-type ST1421-CT1134 *vanA E. faecium* group containing 43 isolates (Table 8.12). 85% of the sequenced VRE *E. faecium* isolates were *vanB* positive. This is a substantial change from 2018, where the majority of the VRE *E. faecium* isolates were *vanA* positive.

Figure 8.15 Overview and distribution of vancomycin resistance genes in vancomycin resistant isolates DANMAP 2021

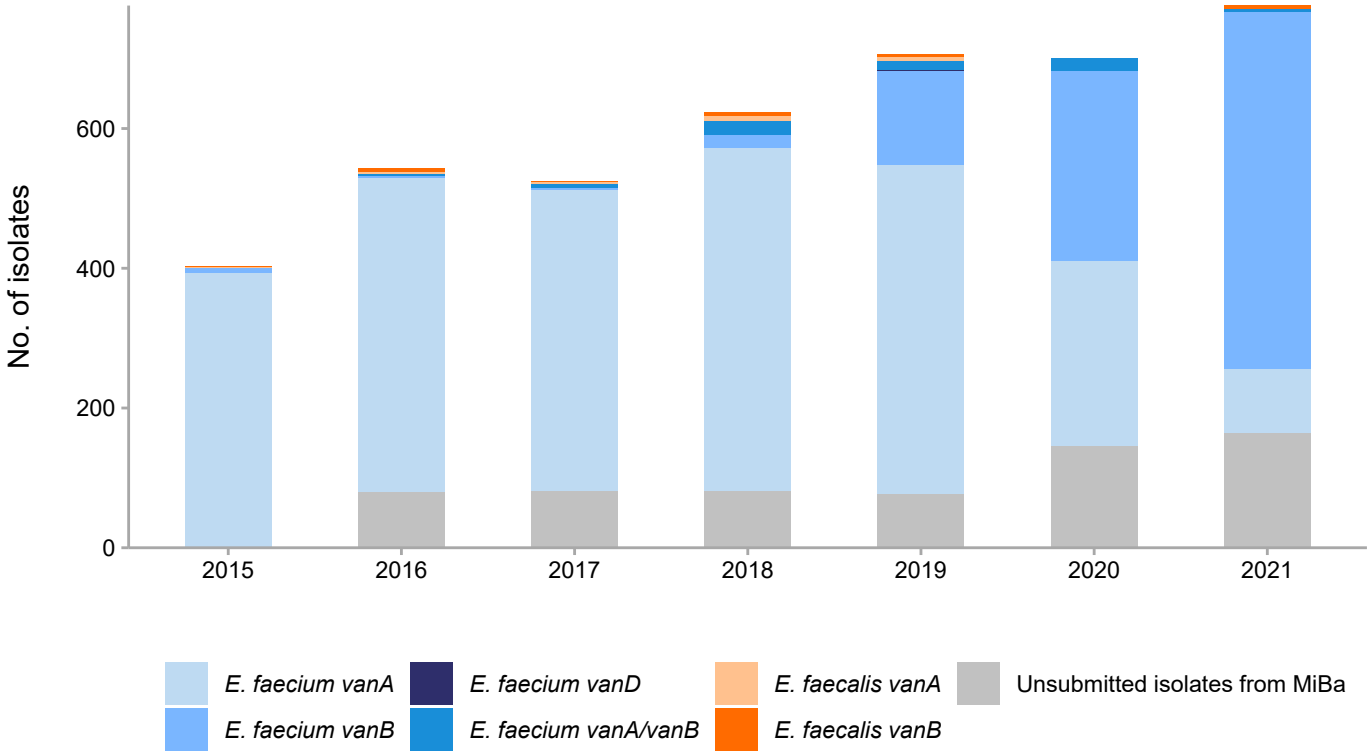


Table 8.12 Description of the most common types of *vanA* and/or *vanB Enterococcus faecium* according to MLST and cgMLST, 2015-2021, Denmark DANMAP 2021

Types[a]	2015		2016		2017		2018		2019		2020		2021		All years
	(n = 375)		(n = 435)		(n = 426)		(n = 518)		(n = 584)		(n = 519)		(n = 565)		Total
ST117-CT24 group[b]	24	6%	19	4%	20	5%	38	7%	26	4%	8	2%	7	1%	143
ST80-CT14 group[c]	82	22%	39	9%	16	4%	3	1%	3	1%	1	0%	0	0%	144
ST203-CT859 group[d]	184	49%	273	63%	265	62%	156	30%	57	10%	12	2%	3	1%	950
ST1421-CT1134 group[e]	0	0%	1	0%	12	3%	167	32%	285	49%	197	38%	63	11%	725
ST80-CT1064 group[f]	0	0%	2	0%	7	2%	23	4%	12	2%	13	3%	3	1%	60
ST117-CT36 group[g]	0	0%	0	0%	0	0%	3	1%	95	16%	56	11%	43	8%	197
ST80-CT2406 group[h]	0	0%	0	0%	0	0%	0	0%	7	1%	174	34%	356	63%	537
Other types	85	23%	101	23%	106	25%	128	25%	99	17%	58	11%	90	16%	807

a) ST, sequence type (MLST); CT, cluster type (cgMLST)
b) CT24,CT875,CT1180,CT1487,CT1834,CT2456,CT6018
c) CT14,CT869,CT1530,CT1797,CT2019
d) CT859,CT1051,CT1507,CT1688,CT2257,CT2758,CT5973
e) CT1134,CT1749,CT1854,CT2545,CT2911,CT3379,CT5936,CT6048
f) CT1064,CT2496,CT6123,CT6520
g) CT36,CT991,CT1526,CT2531,CT2659,CT2979
h) CT2406,CT2946,CT2949,CT3024,CT3234,CT4189,CT4835,CT5120,CT5143,CT5166,CT5211,CT5215,CT5928,CT5972,CT5974, CT5999, CT6117,CT6132,CT6253,CT6254,CT6417,CT6435,CT6435,CT6436,CT6494,CT6507,CT6531,CT6547,CT6598,CT6610

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. MST clusters were set up using the MST algorithm of SeqSphere+, setting the maximal allelic distances to 20. A total of seven clonal groups were devised, based on clustering of the isolates. Each clonal group were named according to the ST and CT of the earliest observed isolate within each cluster. Three clonal groups was conspicuous in the sense of being predominant for a limited time period (see Table 8.12 and Figure 8.16).

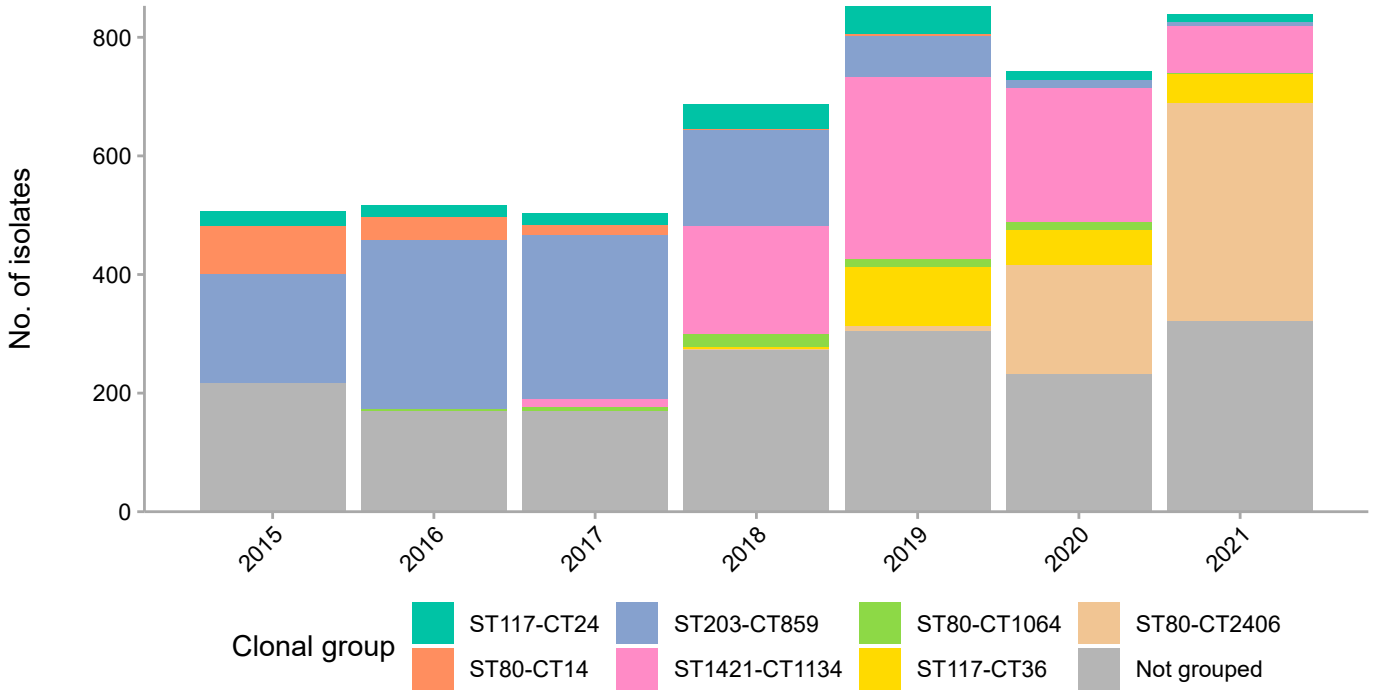
During 2015-2017, the ST203-CT859 *vanA E. faecium* clonal group was the most prevalent clone. It has since decreased in prevalence and in 2021 only 1% of the VRE/VVE *E. faecium* isolates belonged to ST203-CT859.

In 2017, testing for the presence of *vanA/vanB* genes by use of PCR in phenotypically vancomycin-susceptible *E. faecium* isolates from blood cultures was introduced in the DCMs in the Capital Region as a mean of detecting possible VVE [Hammerum *et al.* Euro Surveill. 2020;25(18)]. At that time, the ST1421-CT1134 clonal group only constituted 3% of the total clinical *E. faecium* isolates. This clone was initially only detected from clinical samples from the Capital Region, yet in 2018 and 2019 its prevalence increased to 32% and 49%, respectively, and it was now observed in the Capital Region and Zealand region [Hammerum *et al.* Euro Surveill. 2020;25(18)]. It has since been found in all regions of Denmark. While the ST1421-CT1134 *vanA E. faecium* VVE clone became the predominant clone throughout 2019 and 2020, it was overtaken by the ST80-CT2406 clone in 2021 where it decreased to 11%. When consulting the trends throughout the different clonal groups since 2015 shown in Table 8.12, this decrease could be an early premonition for the termination of the ST1421-CT1134 *vanA E. faecium* VVE clone in the Danish hospitals in the near future.

The earliest detection of the ST80-CT2406 *vanB E. faecium* clone in Denmark was in 2019, where it was present in 1% of the isolates. By 2020 and 2021, it increased to ~28% and ~41% respectively. The clone was detected in all of the five Regions, with the majority detected in the Capital Region (84%). Two DCMs in the Capital Region report a changed procedure for detecting the *vanB* positive *E. faecium* and clone-specific PCR methods have been developed to increase the detection of the clone. It is the most frequently occurring clone of the ST80-CT2406 clonal group, and the group itself is the most diversified clonal group, spanning 20 different CT clones. Since its detection, the ST80-CT2406 clonal group has increased from 34% to 63% of all clinical isolates, thus accounting for the majority of the *van*-positive *E. faecium* detected in 2021. Detailed in Figure 8.16.

Notably during 2019, the proportion of *vanB E. faecium* isolates within the ST117-CT36 clonal group increased to 16% of all detected VRE isolates. This increase was related to several introductions into Denmark from hospitals abroad, and was likewise scattered between the Danish regions due to patient transfer. It has since decreased and in 2021 the ST117-CT36 clonal group only constituted 8% of all clinical *E. faecium* isolates (Table 8.12 and Figure 8.16).

Figure 8.16 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
DANMAP 2021



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 (MDRO), 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 if the patient is transmitted from a hospital if 1) hospitalised outside the Nordic countries within the last 6 months, 2) hospitalised in Denmark or another Nordic country within the last 6 months with outbreaks of VRE or 3) the patient previously has been diagnosed with a MDRO-infection or carrier state. Isolation is recommended in the national supplemental IPC-guideline in case of verification of VRE in the patient.

Conclusion

Compared to the number of VRE/VVE cases in 2019 and 2020 in Denmark, the number of cases increased again in 2021. Focus on hygiene during the coronavirus disease 2019 (COVID-19) pandemic in 2020, may have affected the incidence of clinical VRE isolates. One study performed by the University of Copenhagen at the hospital of Bispebjerg, found a 10-fold decrease in vancomycin-resistant *Enterococcus faecium* in outbreak patients at Bispebjerg Hospital, when comparing the first 5 months of the COVID-19 pandemic with the corresponding period in 2019. The decrease in the clinical VRE incidences was reported to be mostly due to infection control bundle strategies (ICBSs) which were set up to curb the spread of COVID-19 [Gisselø et al., Microb Drug Res. 2022;28(1)]. An overall corresponding decrease was not found on a national level.

One change observed during the COVID-19 pandemic, was the emergence of the relatively new *vanB* ST80-CT2406 group. Whether this change in *E. faecium* clonality is part of the observed fluctuations between different VRE clones over time or related to the implementation of ICBSs, is yet to be determined. Yet it remains clear that more prevention strategies are required to break the annual trend of increasing occurrence of VRE in the Danish health care system.

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8.3.4 Linezolid-resistant/linezolid-vancomycin-resistant enterococci

Background

Linezolid is an antimicrobial belonging to the oxazolidinons. Its indications 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 oxazolidinons.

In 2018, a web tool for detection of the 23S rRNA mutations (G2576T and G2505A), and the *optrA, cfr, cfr(B)* and *poxtA* genes from whole genome sequences from enterococci was developed [Hasman *et al.,* J. Antimicrob. Chemother., 2019, 74:1473-1476].

Surveillance of linezolid-resistant enterococci (LRE)

Danish departments of clinical microbiology (DCMs) have, on a voluntary basis, submitted LRE for surveillance to the National Reference Laboratory for Antimicrobial Resistance at Statens Serum Institut (SSI). During 2015-2020, 8 linezolid-resistant *E. faecium* isolates and 17 linezolid-resistant *E. faecalis* isolates were sent to SSI (only one isolate per patient was included).

Table 8.13 Characterisation of linezolid-resistant enterococci (LRE) and linezolid-vancomycin-resistant enterococci (LVRE) submitted to SSI, Denmark, 2021 DANMAP 2021

	No. of isolates	Species	Linezolid resistance mechanism	Vancomycin resistance gene
LRE	1	*E. faecalis*	*optrA*	none
LVRE	1	*E. faecium*	*optrA*	*vanA*
	13	*E. faecium*	G2576T	*vanB*

In 2021, one linezolid-resistant *E. faecalis* isolate was sent to SSI. WGS data from the LRE isolates were investigated for 23S rRNA mutations (G2576T and G2505A), and the *optrA*, *cfr*, *cfr(B)* and *poxtA* genes using the LRE-Finder (https://cge.cbs.dtu.dk/services/LRE-Finder/).

The *E. faecalis* was positive for *optrA* (Table 8.13).

Surveillance of linezolid-vancomycin-resistant Enterococci (LVRE)

In order to detect linezolid-resistant VRE isolates (LVRE), the LRE-Finder was also implemented in the automated surveillance at SSI for detection of LRE mutations and LRE genes in genomes from VRE isolates submitted for the national VRE Surveillance DCMs directly. During the period 2015-2020, no linezolid vancomycin resistant *E. faecalis* were detected, whereas 19 linezolid-vancomycin resistant *E. faecium* were identified.

In 2021, 14 linezolid-vancomycin resistant *E. faecium* were identified. All 14 linezolid resistant *E. faecium* isolates had the G2576T mutation, one of these were positive for both the vanA and *optrA* gene and 13 were positive for *vanB* (Table 8.13).

Conclusion

As in previous years the numbers of LRE and LVRE are low, however the findings are of concern, as linezolid is important for the treatment of VRE. Often, only one antimicrobial agent is available for treatment of infections with LVRE.

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8.3.5 *Streptococcus pneumoniae*

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

In Denmark, 353 cases of invasive pneumococcal disease (IPD) were registered in 2021. The cases were mainly from pneumococci found in either blood (303) or cerebrospinal fluid (40). For ten cases, pneumococci had been found in other, normally sterile sites, but data from these are by tradition not included in this report. Of the 343 cases of bacteraemia and meningitis identified in MiBa, 324 isolates were received at the reference laboratory. Data for the nineteen 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 found to be due to non-viable isolates or diagnosis through PCR. Antimicrobial susceptibility data for the nineteen cases were retrieved, when available, through MiBa. In total, serotypes were available for 324 cases and antimicrobial susceptibility data for both penicillin and erythromycin were available for 337 cases.

The 343 isolates from blood or cerebrospinal fluid belonged to 33 different serotypes. For the 337 cases with available susceptibility data, 296 were susceptible to both penicillin and erythromycin (87.8%). For penicillin, 303 were susceptible (89.9%), 32 (9.5%) were classified as 'susceptible increased exposure' and two isolates (0.6%) were classified as resistant. In total, 10.1% were non-wildtype with respect to susceptibility to penicillin. For erythromycin, 320 isolates were susceptible (95.0%), and 17 isolates (5.0%) were resistant. Ten isolates (3.0%) were non-wildtype for both antimicrobials. The two isolates that were resistant to penicillin were two different serotypes (9N and 19F) and one of them (serotype 19F) was also resistant to erythromycin.

Antimicrobial susceptibility in pneumococci is highly correlated to serotypes (Table 8.14), and variations in susceptibility patterns through the years often reflects changes in circulating serotypes. The pneumococcal vaccine introduced in the childhood vaccination programme in 2007 has had a profound effect on the serotypes that are causing invasive pneumococcal disease in Denmark (Figure 8.17), as it is clear that IPD caused by the seven serotypes included in the vaccine (PCV7 vaccine from 2007) and later 13 serotypes (PCV13 vaccine from 2010) has decreased markedly. The predominant serotype in 2021 was serotype 8 (22.5%), of which all invasive isolates isolated in 2021, 2020, 2019 and 2018 were fully sensitive to both penicillin and erythromycin. The second and third most frequently isolated serotypes are serotype 3 (36 isolates in 2021) and serotype 22F (23 isolates in 2021), and these isolates were also fully sensitive to both penicillin and erythromycin.

Table 8.14 Number of invasive isolates and distribution of resistance in the most common serotypes of pneumococci, Denmark, 2018-2021

DANMAP 2021

Serotype	N 2021	PEN-S ERY-S	PEN-S ERY-R	PEN-I ERY-S	PEN-I ERY-R	PEN-R ERY-S	PEN-R ERY-R	Unk	% S-S	N (% S-S) 2020	N (% S-S) 2019	N (% S-S) 2018
8	73	73							100%	83 (100%)	162 (100%)	194 (100%)
3	36	36							100%	37 (97%)	69 (100%)	70 (97%)
22F	23	23							100%	25 (100%)	49 (96%)	69 (100%)
23B	21	4		17					19%	12 (33%)	18 (50%)	14 (7%)
35F	18	18							100%	9 (100%)	9 (89%)	14 (100%)
11A	14	13		1					93%	10 (80%)	17 (88%)	19 (95%)
9N	12	10		1		1			83%	17 (94%)	28 (96%)	62 (98%)
16F	12	12							100%	3 (100%)	17 (94%)	19 (84%)
15A	10	6			4				60%	9 (67%)	15 (93%)	25 (76%)
35B	10	10							100%	11 (82%)	13 (85%)	15 (100%)
7C	9	9							100%	8 (100%)	8 (100%)	6 (83%)
15B	9	9							100%	9 (100%)	8 (100%)	9 (100%)
24F	8	8							100%	11 (100%)	15 (67%)	17 (76%)
10A	6	6							100%	12 (100%)	9 (100%)	15 (100%)
10B	6	6							100%	3 (100%)	9 (100%)	9 (100%)
19A	6	6							100%	9 (78%)	8 (50%)	11 (82%)
19F	6	3	1	1			1		50%	3 (67%)	6 (100%)	7 (71%)
12F	5	5							100%	22 (100%)	44 (95%)	55 (100%)
17F	5	2		2	1				40%	8 (38%)	8 (63%)	12 (50%)
23A	5	4			1				80%	12 (100%)	14 (100%)	13 (100%)
33F	5	2	3						40%	5 (80%)	13 (77%)	17 (88%)
15C	4	3			1				75%	3 (100%)	11 (82%)	4 (100%)
31	4	4							100%	6 (100%)	6 (100%)	15 (100%)
17A	3	2	1						67%	1 (100%)	3 (100%)	5 (100%)
Other	33	22	2	1	2			6	81%	36 (84%)	64 (91%)	91 (88%)
Sum	343	296	7	23	9	1	1	6	88%	364 (91%)	623 (93%)	787 (93%)

N = number of isolates, PEN = penicillin, ERY = erythromycin, % S-S = percentage of isolates that were susceptible to both penicillin and erythromycin

Figure 8.17 Non-wild type (%) in *Streptococcus pneumoniae* blood and spinal fluid isolates from humans, Denmark, 1993-2021

DANMAP 2021

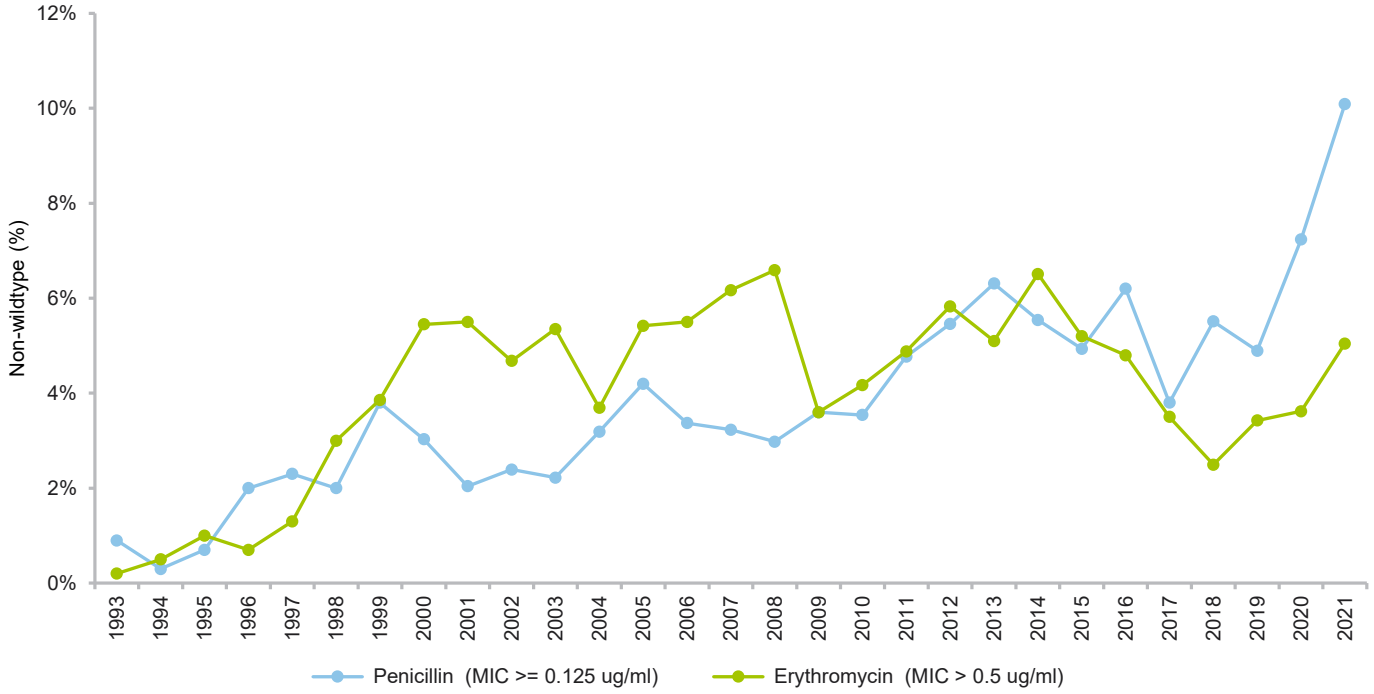
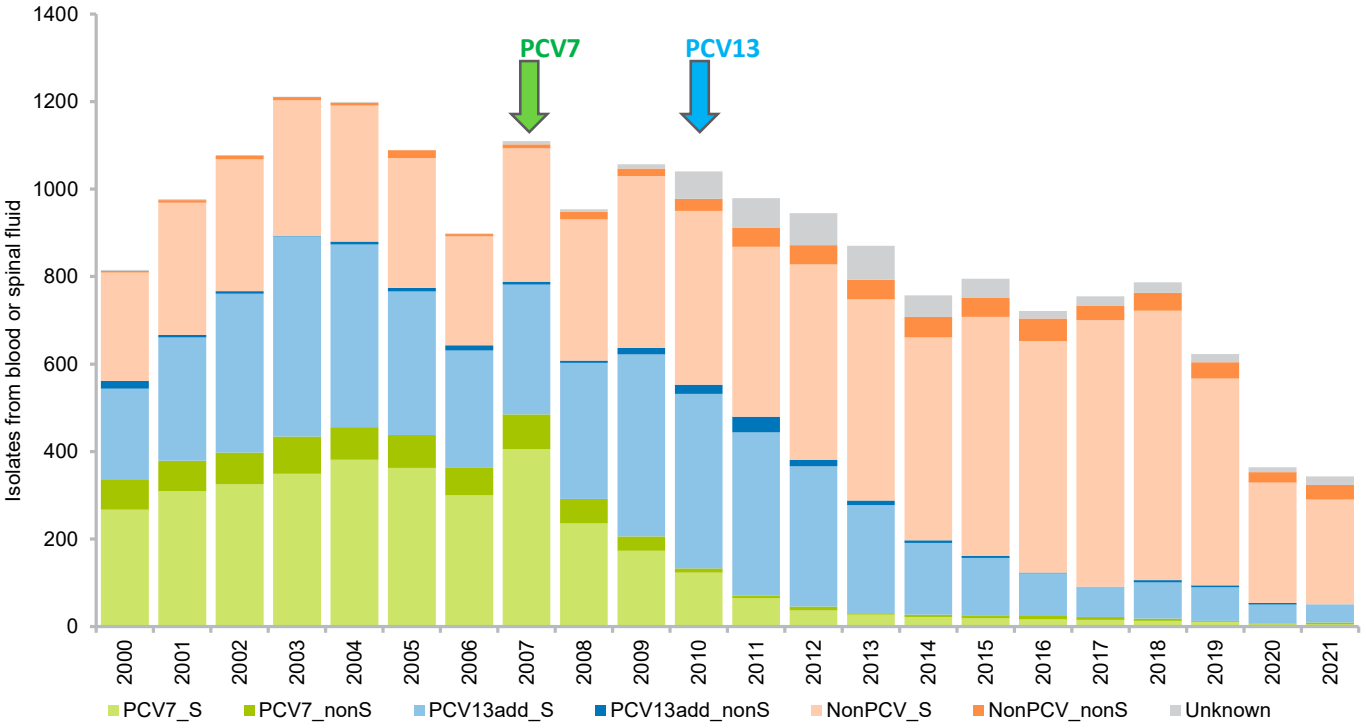


Figure 8.18 ***Streptococcus pneumoniae*** **blood and spinal fluid isolates from humans. Vaccine serotypes and non-vaccine serotypes in combination with susceptibility to penicillin and erythromycin, Denmark, 2000-2021** DANMAP 2021



Comparing the obtained results of antimicrobial susceptibility of IPD in Denmark in 2021 to the data reported in 2020 from other Scandinavian and neighboring countries, the levels of penicillin non-wild type reported by WHO/ECDC [https://www.ecdc.europa.eu/en/publications-data/antimicrobial-resistance-surveillance-europe-2022-2020-data] were: Sweden (8.5%), Norway (7.4%), Finland (11.5%), Germany (6.1%) and UK (7.4%), and the levels of erythromycin resistance were: Sweden (6.6%), Norway (5.1%), Finland (11.8%), Germany (7.2%) and UK (6.0%). Thus, the proportion of non-wild type isolates of invasive pneumococci in Denmark 2021 were slightly higher for most of these five other countries (2020) with respect to penicillin, and lower with respect to erythromycin resistance.

Conclusion

For penicillin, the level of non-wildtype in 2021 was noticeably higher than in 2020 and 2019 (10.1% compared to 7.2% and 4.9%). For erythromycin, the level of non-wildtype in 2021 was also higher than the levels in 2020 and 2019 (5.0% compared to 3.6% and 3.4%, Figure 8.18). Antimicrobial susceptibility in *S. pneumoniae* is highly correlated with serotypes, and the distribution of serotypes varies between the years, in part because of effects of vaccines. Currently, the dominant non-wildtype invasive isolates of *S. pneumoniae* are serotypes that are not contained in the PCV13 vaccine Figure 8.18. The number of cases of invasive pneumococcal disease in the years 2021 and 2020 were markedly lower than in previous years. This is assumed to be an effect of the COVID-19 restrictions on the general prevalence of infectious diseases associated with respiratory droplet transmission. Such decreases have been seen in other countries as well [Brueggemann, et al., 2021, Lancet Digit. Health, 3(6)].

More information on the surveillance of invasive pneumococcal disease in Denmark can be found on the SSI homepage (EPI-NEWS, No 10-2020, https://en.ssi.dk/news/epi-news/2020/no-10---2020.

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8.3.6 Beta-haemolytic streptococci

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 in 2021 to the Neisseria and Streptococcus Reference laboratory. This report includes only non-duplicate isolates, i.e. isolates obtained with an interval of more than 30 days. Isolates are received from all DCMs 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 group A, B, C, and G were therefore tested for susceptibility to penicillin, erythromycin, and clindamycin as well as inducible clindamycin resistance.

Figure 8.19 shows the resistance findings for the years 2013 through 2021. In 2021, the number of submitted isolates from unique cases was 857, a decrease of 7% compared to 2020 (924). Corresponding changes for individual serogroups were: GAS, - 61%; GBS, - 4%; GCS, + 6%; and GGS, - 1%.

The substantial and continued decrease in number of submitted GAS isolates may probably reflect an indirect consequence of COVID-19 related restrictions on upper respiratory tract colonization and airborne transmission of this species.

All isolates were fully susceptible to penicillin. Comparing 2021 to 2020, erythromycin resistance declined somewhat for GCS but was otherwise unchanged, as was the proportion of clindamycin-resistant isolates. The percentage of strains with inducible clindamycin resistance was virtually unchanged for all serogroups: GAS, 2.4%; GBS, 4.6%, GCS 4.3% and GGS, 11%. The percentage of fully susceptible isolates was unchanged compared to 2020 for all four serogroups.

The GAS isolates belonged to 15 different *emm* types. The majority of the received isolates (33; 79%) belonged to six *emm* types, each of which were represented by from 2 to 13 isolates (Table 8.15). The remaining nine isolates (21%) belonged to nine different *emm* types. Resistance against erythromycin and clindamycin was found only in isolates of three *emm* types: 11.0, 49.0, and 77.0.

Figure 8.19 Beta-haemolytic streptococci: Antimicrobial resistance testing results, 2013-2021. Numbers of isolates and percent resistance

DANMAP 2021

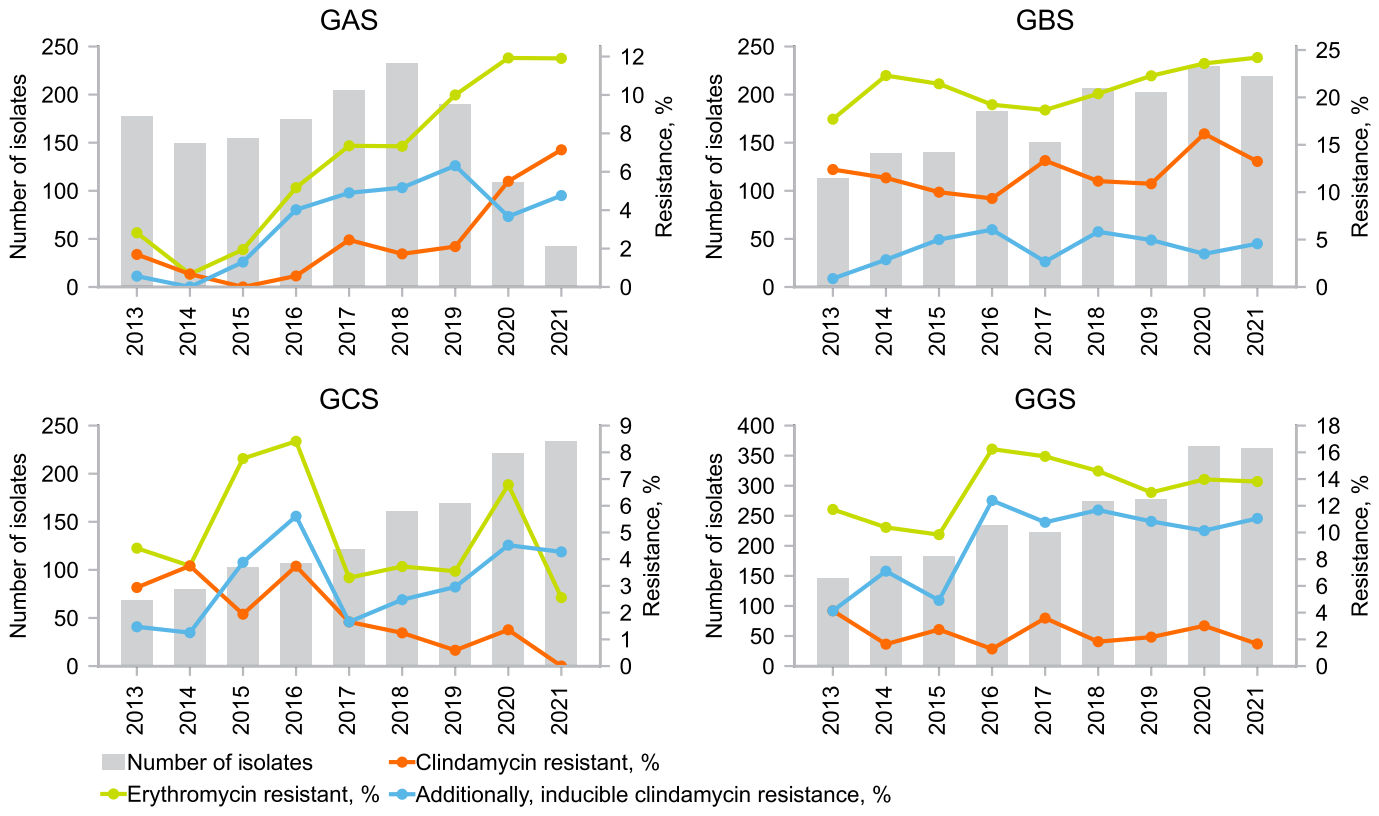

Table 8.15 Group A streptococci 2021: *emm* types, clindamycin resistance and erythromycin resistance. Number of isolates

DANMAP 2021

emm type	ERY-R	CLI-R	CLI-IR	ERY-S and CLI-S	Total
1.0				1	1
4.0				1	1
11.0	3	2		1	4
12.0				1	1
22.0				1	1
27.0				1	1
28.0				13	13
49.0	1	1		0	1
66.0				5	5
73.0				1	1
77.0	1		2	2	3
81.0				2	2
89.0				6	6
104.0				1	1
119.2				1	1
Total	5	3		37	42

CLI = clindamycin, ERY = erythromycin, IR = inducible resistance; R = resistant, S = sensitive

Conclusions

The number of submitted isolates of group A streptococci was considerably lower in 2021 than in both 2020 and in particular 2019, while the numbers for the three other serogroups were nearly identical to the numbers in 2020. All isolates were fully susceptible to penicillin. The erythromycin resistance rate remained virtually unchanged compared to 2020, except for GCS in which a decrease was found. The clindamycin resistance rate remained fairly constant in all serogroups, except for GAS in which an increase since 2018 continued.

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8.3.7 *Staphylococcus aureus*

Background

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

In 2021, 2,512 *S. aureus* bacteraemia cases corresponding to 43.0 cases per 100,000 inhabitants were reported from the departments of clinical microbiology (DCMs) in Denmark. This is a 7.3% increase compared to 2020 (2,342). Forty (1.6%) of the bacteraemia cases were caused by MRSA. During the last decade, this proportion has been between 1.3% (2012) and 2.9% (2014) and remains below most other European countries participating in EARS-Net [EARS-Net 2020]. Livestock-associated (LA-) MRSA CC398 caused six of the 40 MRSA bacteraemia cases. Within 30 days from the *S. aureus* bacteraemia onset, 558 (22%) patients died (all-cause mortality). The mortality for the MRSA bacteraemia cases was 20%.

Results from antimicrobial susceptibility testing in *S. aureus* bacteraemia isolates from 2012-2021 are presented in Table 8.16. Resistance to penicillin in 2021 was 69%, 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 (13%), erythromycin (7%), clindamycin (7%) and moxifloxacin (4%). For most antimicrobial agents, the susceptibility remained at the same level as in previous years.

Typing revealed 714 different *spa* types distributed in 27 different clonal complexes (CCs). The ten most prevalent *spa* types, representing 31% of the total number, are presented in Table 8.17 together with spa types from the most prominent types of MRSA. The distribution of the most prevalent *spa* types has been very stable over the last decade with only minor changes in the relative order. The PVL toxin was present in 24 (1%) cases of which three were MRSA. The 24 PVL presenting isolates were distributed among 17 different *spa* types and 9 different CCs.

Table 8.16 Resistance (%) in isolates from *Staphylococcus aureus* bacteraemia cases 2012-2021, Denmark DANMAP 2021

	2012	2013	2014	2015	2016	2017	2018	2019	2020*	2021
	%	%	%	%	%	%	%	%	%	%
Methicillin	1.2	1.7	2.9	1.5	2.1	2.2	1.6	2.1	1.6	1.6
Penicillin	74	76	77	71	71	72	72	72	72	69
Erythromycin	6	7	8	7	7	6	5	9	7	7
Clindamycin	6	6	8	7	6	5	4	8	7	7
Tetracycline	2	3	5	4	3	3	3	2	3	2
Fusidic acid	14	15	15	16	12	14	17	14	14	13
Rifampicin	<1	0	<1	<1	<1	<1	<1	<1	<1	<1
Moxifloxacin#	4	5	6	6	4	4	4	5	6	4
Kanamycin	1	2	2	3	1	1	2	<1	nt	nt
Linezolid	0	0	0	0	0	0	0	0	0	0
Mupirocin	<1	<1	<1	<1	0	<1	0	<1	<1	<1
Trimethoprim-sulfamethoxazole	1	1	1	<1	<1	<1	0	<1	<1	<1

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 8.17 The ten most prevalent *spa* types demonstrated in SAB and in non-LA-CC398 MRSA cases, Denmark 2021 DANMAP 2021

SAB			MRSA			
spa type	CC group	No. of cases	*spa* type	CC group	No. of cases	No. causing infections (%)
t002	CC5	112	t304	CC8	168	80 (48)
t091	CC7	108	t127	CC1	164	78 (48)
t127	CC1	103	t223	CC22	119	53 (45)
t084	CC15	96	t002	CC5	106	60 (57)
t230	CC45	92	t008	CC8	76	50 (66)
t012	CC30	63	t4549	CC8	62	52 (84)
t008	CC8	58	t005	CC22	47	30 (64)
t021	CC30	55	t1476	CC8	32	16 (50)
t701	CC8	54	t843	CC130	31	22 (71)
t015	CC45	40	t021	CC30	30	18 (60)

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

Surveillance of methicillin-resistant *S. aureus*

The COVID-19 restrictions continued to have an impact on new MRSA cases in Denmark. In 2021, 2,715 new MRSA cases were detected (46.5 per 100,000 inhabitants), a decrease of 6% compared to 2020 (2,883; Figure 8.20a) and a significant decline from the 3,657 cases registered in 2019. 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). In 2021, infections constituted 45% of the cases. The percentage of infections in the years 2010 to 2021 varied between 58% in 2010 to 38% in 2014 and 2016 (Figure 8.20b).

Figure 8.20a Number of new MRSA cases 1994-2021, Denmark, with a three years moving average DANMAP 2021

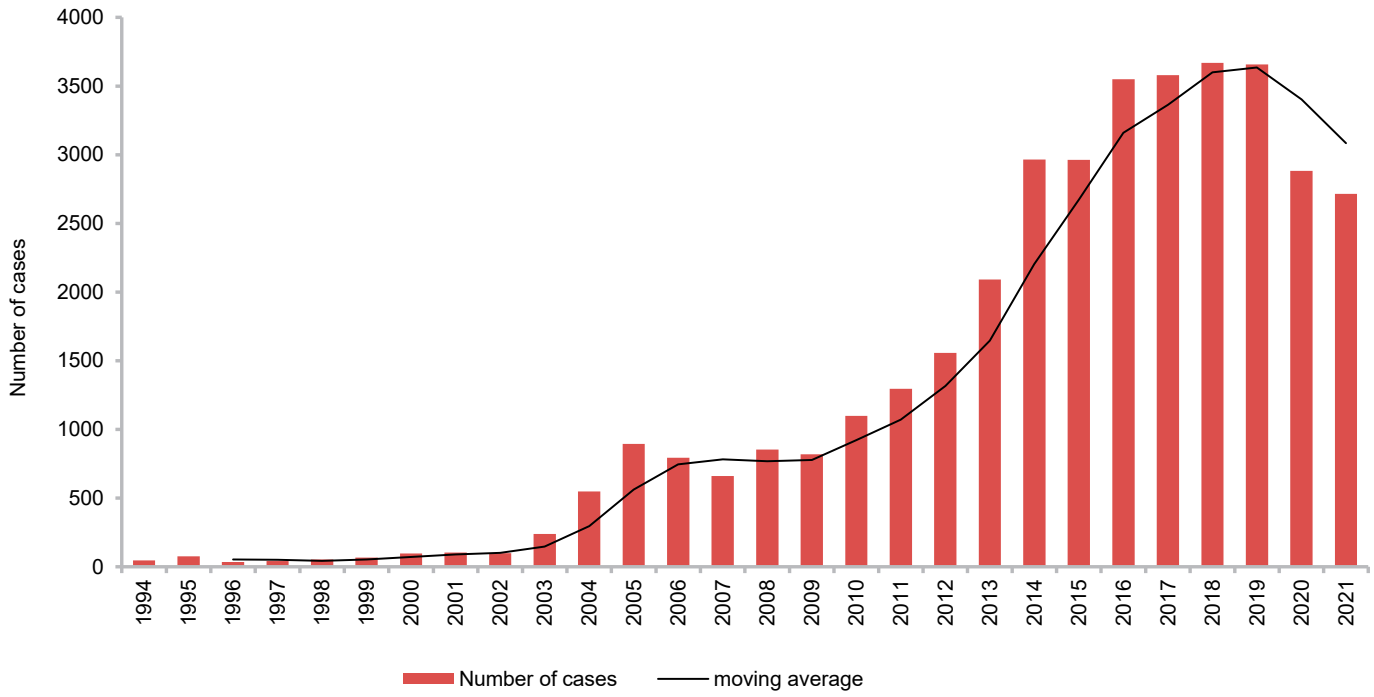
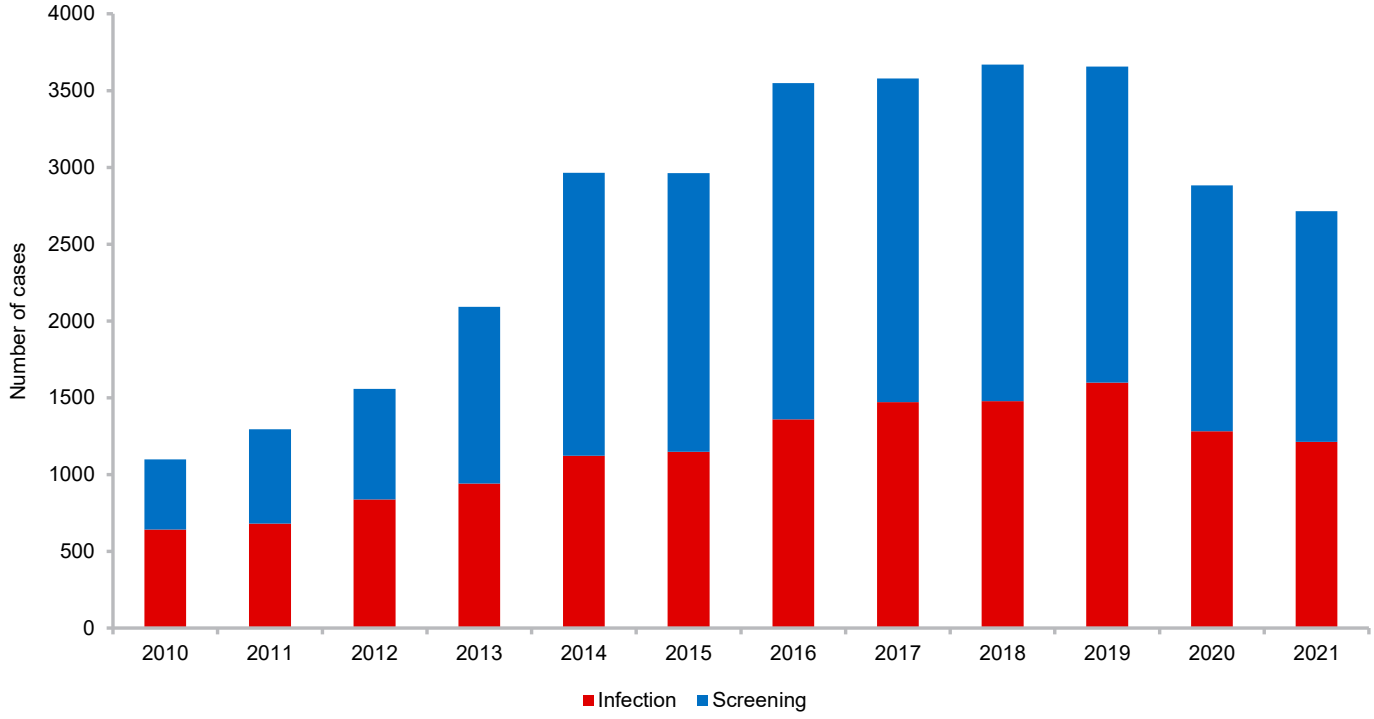


Figure 8.20b Number of new MRSA cases 2010-2021, Denmark, divided in infection and screening samples DANMAP 2021



CC398 cases constituted 36% (n = 977) of new MRSA cases, of which 959 belonged to the livestock-associated clone (LA-MRSA CC398) and the remaining 18 to a human adapted variant harbouring the PVL encoding genes. More LA-MRSA CC398 isolates (79%) were found in healthy carriers compared to MRSA of other types (41%), which likely reflects the active screening of patients with contact to livestock at admission to healthcare.

MRSA isolates carrying *mecC* were detected in 67 cases (2.5%). Forty-six of the cases (69%) had infections at the time of diagnosis. Two patients reported contact to hedgehogs, which recently has been shown to be a major environmental reservoir for *mecC* MRSA [Larsen J, et al., 2022, Nature, 602(7895)]. Three patients reported contact to livestock while the remaining 62 patients reported no known contact to any livestock. *Spa* typing revealed 317 different strain types, not including isolates belonging to LA-CC398. Among the infections, 226 *spa* types were identified. The 10 dominating non-LA-CC398 *spa* types isolated in 2021 are listed in Table 8.17. They constituted 48% of the total number of non-LA-CC398 MRSA isolates. *spa* types t304 and t223 have been among the five most prevalent *spa* types since 2016, when it was linked to the refugee crisis following the civil war in Syria. Table 8.17 does not list *spa* types in CC398, which for many years has been the dominating CC.

The PVL encoding gene was detected in 21% of the infections and in 9% of the asymptomatic carriers and most often in relation to isolates with *spa* types t008 (n = 51), t005 (n = 37), t044 (n = 23), t021 (n = 22) and t002 (n = 14).

In the course of 2021, 30 MRSA outbreaks were registered at hospitals, nursing homes and other institutions. These outbreaks comprised a total of 109 cases of which 48 had an infection. Five of the outbreaks occurred in neonatal departments, comprising a total of 42 cases. Additionally, seven outbreaks were registered in other hospital department, comprising 16 patients and ten outbreaks were observed in nursing homes (comprising a total of 21 residents).

The total number of cases and the number of cases presenting with infection according to epidemiological classification are shown in Table 8.18. Most of the cases (91%) were acquired in Denmark.

The trend of MRSA infections for 2012-2021 based on their epidemiological classification is shown in Figure 8.21. The number of infections in imported cases decreased in 2021 when compared to 2020 (- 29%), which is the lowest number in a decade and may be a consequence of less travel due to SARS-CoV2 restrictions. In addition, the number of hospital-acquired MRSA infections was lower in 2021 compared to 2020 (37 cases vs. 49, respectively).

Resistance among MRSA isolates

Resistance data for non-LA-CC398 isolates are presented in Table 8.19. The proportion of resistant isolates was similar compared to previous years, with relatively high resistance to erythromycin (29%), fusidic acid (21%), clindamycin (19%), tetracycline (21%) and moxifloxacin (19%), and low resistance (<1%) to trimethroprim-sulfamethoxazole, linezolid, mupirocin and rifampicin.

Table 8.18 Epidemiological classification of new MRSA cases, Denmark 2021 DANMAP 2021

Epidemiologic classification	Exposure	No. of cases (% of total)	No. (%) of cases with infections (% of cases in same epi. class)
Imported (IMP)		**254 (9)**	151 (59)
Hospital-acquired (HA)		**54 (2)**	25 (46)
Health-care associated, community onset (HACO)		**229 (8)**	
	with known exposure	16	11 (69)
	without known	213	170 (80)
Health care worker		**25 (1)**	13 (52)
Community-acquired (CA)		**1193 (44)**	
	with known exposure	570	74 (13)
	without known	623	497 (80)
LA-MRSA CC398		**959 (35)**	
	with known exposure	810	170 (21)
	without known	149	101 (68)
Total		**2715**	**1213 (45)**

Numbers shown in bold are totals

Figure 8.21 Number of MRSA infections according to epidemiological classification, 2012-2021 Denmark DANMAP 2021

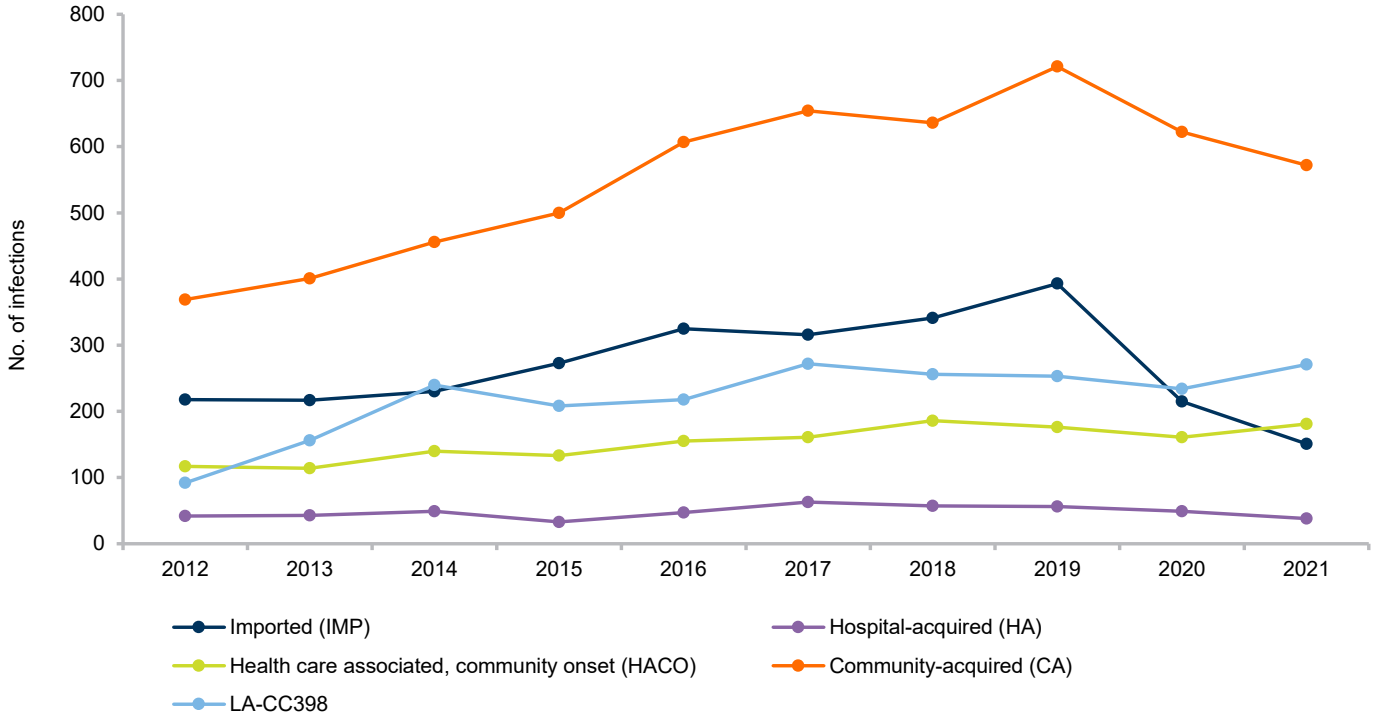


Table 8.19 Resistance (%) in non LA-CC398 MRSA isolates, 2012-2021, Denmark DANMAP 2021

	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021
Erythromycin	38	32	33	37	34	34	33	33	30	29
Clindamycin	29	24	23	29	25	27	28	23	22	19
Tetracycline	18	20	21	24	26	24	26	22	22	21
Fusidic acid	17	17	17	19	18	16	18	23	22	21
Rifampicin	1	1	<1	<1	1	1	1	<1	<1	<1
Moxifloxacin#	25	23	27	21	19	20	21	21	17	19
Linezolid	0	<1	<1	0	<1	0	<1	0	<1	<1
Mupirocin	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Trimethoprim-sulfamethoxazole	2	3	3	4	2	3	3	4	2	<1
Number of tested isolates	1324	1451	1616	1242	1184	1193	1233	1025	1920*	1520*

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

Infection prevention and control guidelines for MRSA

The Guidance on Preventing the Spread of MRSA issued by the Danish Health Authority represents the national statutory recommendations and the strategic framework for preventing the spread of MRSA. The applicable 3rd edition of the guideline is from 2016. The first edition was issued in 2006 – the year MRSA became notifiable – and the guideline was later revised in 2012. The main purpose of the guideline is to maintain a low prevalence of disease caused by MRSA associated with certain high-risk situations as previously diagnosed with MRSA, household-like contact with person tested positive for MRSA, travelling outside the Nordic countries with admission to a hospital or clinic, and frequent contact with live pigs. Individual risk factors for acquiring colonization or infection with MRSA are wounds, chronic skin diseases and invasive devices etc. As strategy the guideline emphasizes the importance of all health care staff complying with the national guidelines for infection prevention and control, and treatment of MRSA carriage. It is noteworthy, that persons who are MRSA-carriers can be treated (in contrast to persons carrying resistant bacteria in the gut, e.g. CPE) and are declared MRSA-free when tested negative minimum 6 months after completion of treatment.

Acting in compliance with the national MRSA guideline and the national guidelines for infection prevention and control (published by National Center for Infection Control at SSI) is key in preventing the spread of multidrug-resistant microorganisms with correct hand hygiene and use of personal protective equipment (PPE) among the most important control measures. 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 [https://hygiejne.ssi.dk/retningslinjer/infektionshygiejniske-retningslinjer-for-mrsa].

Conclusion

The national surveillance of MRSA has shown a marked decrease of new cases in 2020 and 2021 during the COVID-19 pandemic. This can be explained by the implemented restrictions on especially travel and changes in social behaviour affecting distancing, hand hygiene as well as changes in the health care sector. The current state of MRSA in Denmark therefore seems interconnected with the development of the COVID-19 pandemic.

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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 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 newborns after transmission from an infected mother during labour and rarely in adults following direct or indirect inoculation.

Surveillance of resistance in gonococci

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-existent 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 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 2021 were from urethra or cervix, while clinicians only rarely obtain specimens from rectum and pharynx. Occasionally, the NSR laboratory receives strains isolated from other anatomical sites, such as conjunctivae, joint fluid, blood, Bartholin's abscess, etc.

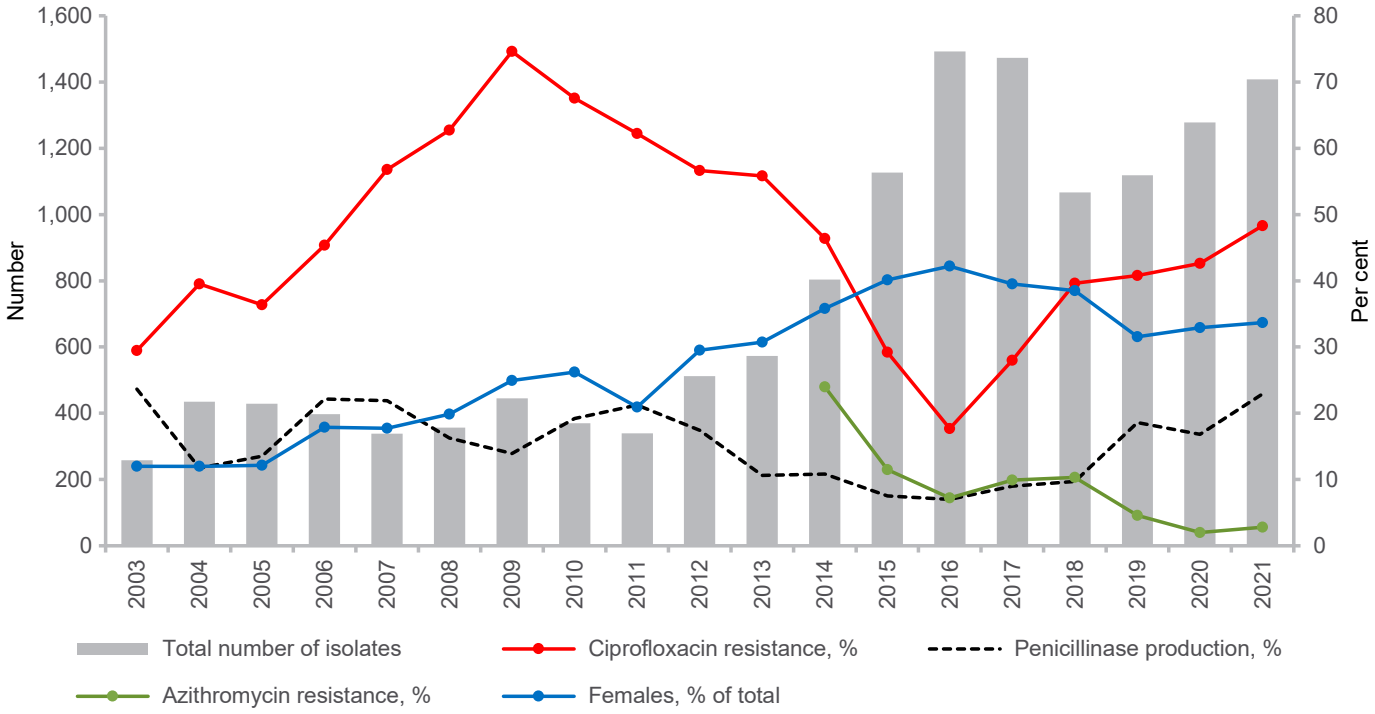
Repetitive detections of gonococci in a patient are considered to represent unique cases of gonorrhoea if separated by more than 21 days. The NSR laboratory received 1,666 isolates from 1,408 unique cases of gonorrhoea diagnosed in 2021. Only one isolate from each unique case is counted in this report.

The annual number of received isolates increased considerably from 2011 through to 2016 (Figure 8.22). This is 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 2016, but it is still substantially higher than in the beginning of the observation period (2003-2021). A decrease in the annual number of isolates from unique cases was seen in 2018 followed by an increase in 2019-2021. It should be recalled, however, that many cases diagnosed by NAATs are not followed-up by culture.

The ciprofloxacin resistance rate was 48% in 2021 (41% in 2020 and in 2019), thus still considerably lower than the peak of 75% in 2009 (Figure 8.22). Only 0.8% were classified as intermediate (MIC 0.047-0.064 mg/L).

The percentage of strains producing penicillinase was 23% (17% in 2020 and 19% in 2019). Previously, it has fluctuated between 22% in 2005 and 7% in 2016. In 2021, azithromycin resistance (MIC above the present ECOFF >1 mg/L) was found in 2.8% of the tested isolates. 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. In 2019, 4.6% of isolates had MIC >1 mg/L, and in 2020, it was 2%.

Figure 8.22 Number of submitted gonocccus isolates from males and females, and the percentage of isolates with ciprofloxacin resistance, azithromycin resistance, and penicillinase production, Denmark, 2003-2021 DANMAP 2021



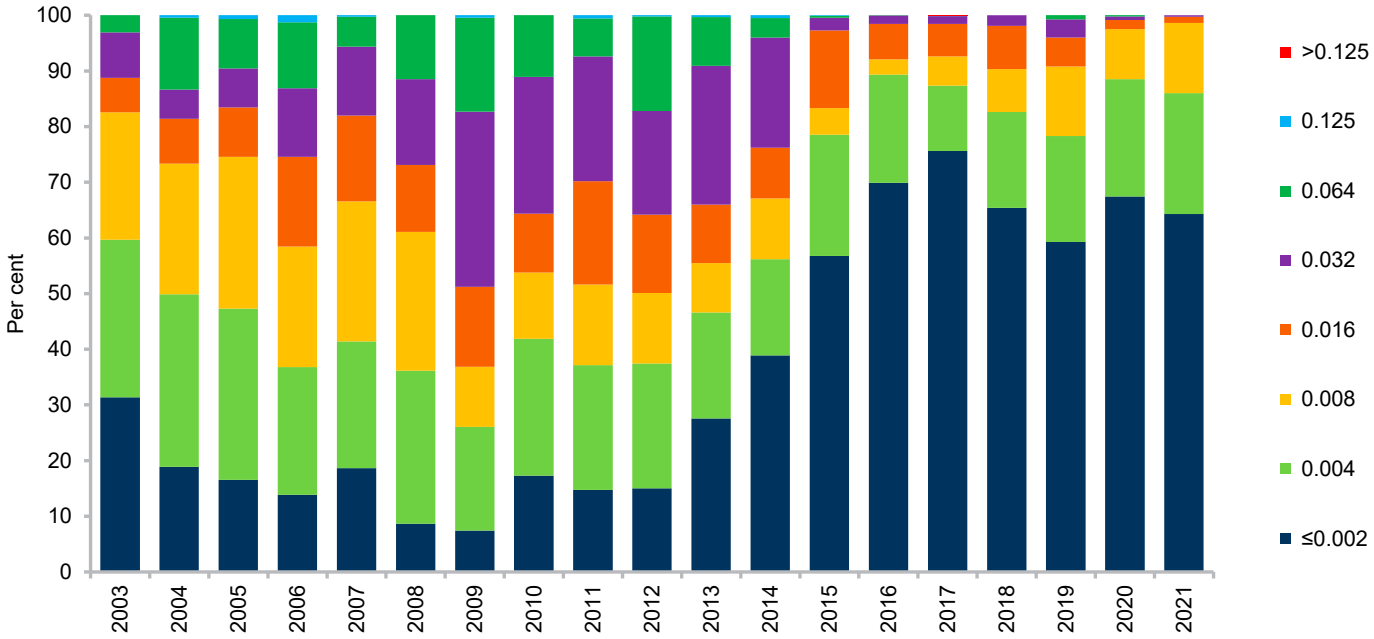
Ceftriaxone resistant gonococci (MIC >0.125 mg/L) as well as ceftriaxone treatment failure in patients with gonorrhoea have occurred in several countries during recent years. As shown in Figure 8.23, the ceftriaxone MIC distribution changed from 2003 through 2009 with the highly susceptible isolates becoming gradually less frequent. However, since then, this change has completely reversed.

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 treatment of gonorrhoea. Ciprofloxacin (500 mg p.o. as a single dose) may still be used for treatment if the strain is fully susceptible and if appropriate investigation has excluded pharyngeal gonorrhoea. 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 use of the combination therapeutic regimen has gradually been abandoned during 2019 and onwards by many clinicians.

In a subset of 117 isolates tested as part of the Danish participation in Euro-GASP, resistance against cefixime (MIC >0.125 mg/L) was 0% in 2021, like in 2020. Cefixime is an oral cephalosporin that has never been used in Denmark. Susceptibility testing for spectinomycin and gentamicin was not performed in 2021.

Figure 8.23 Distribution of ceftriaxone MIC (mg/L) values in gonococci, Denmark, 2003–2021 DANMAP 2021



Conclusions

The ciprofloxacin resistance rate was somewhat higher in 2021 than in 2020 and the ceftriaxone MIC distribution was virtually unchanged. Although resistance problems among gonococci are still not present in Denmark, the frequent emergence globally of gonococcal resistance to antibiotics underlines the importance of maintaining the centralised national surveillance of antimicrobial resistance in gonococci.

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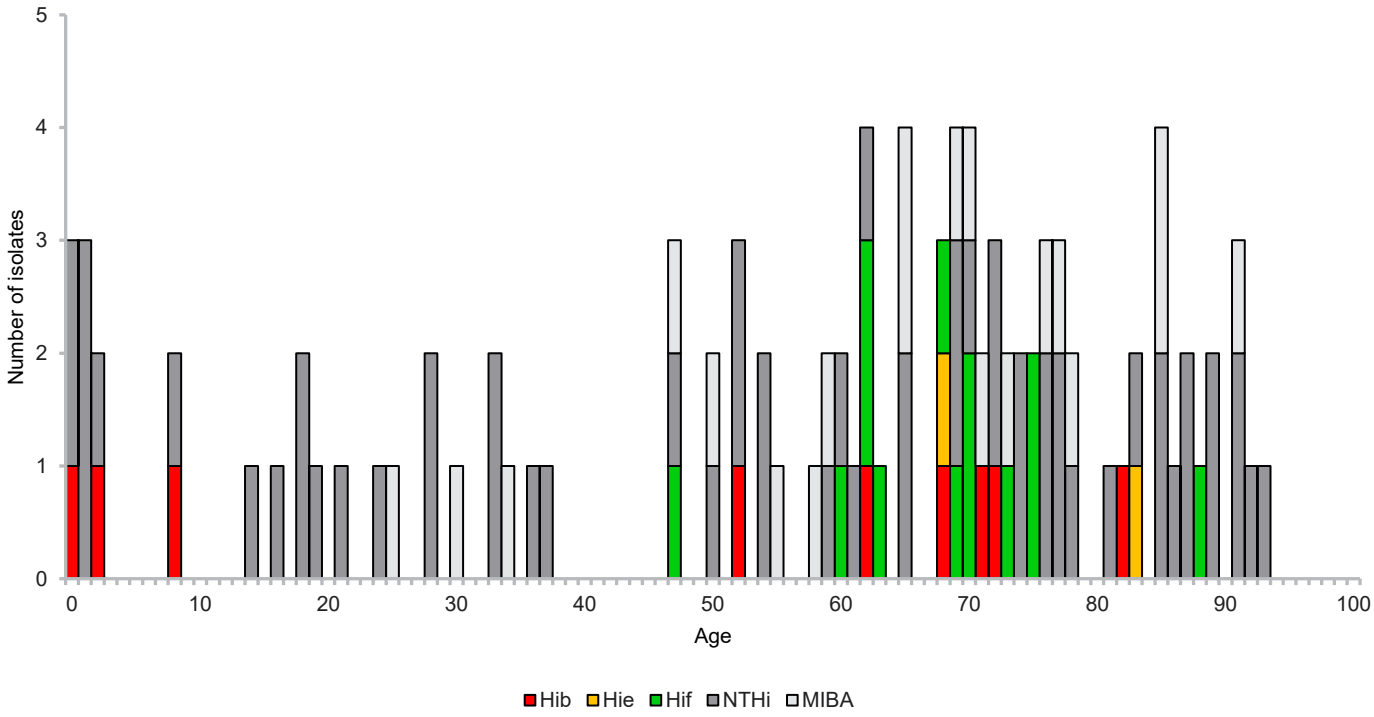
8.3.9 *Haemophilus influenzae*

Haemophilus influenzae is part of the normal upper respiratory tract flora, where colonisation varies with age. *H. influenzae* can also cause infections, with otitis media and bacterial sinusitis being the most common clinical manifestations. Invasive infections with *H. influenzae* happen relatively rarely and occur predominantly in the very young or elderly patients, but may also afflict individuals with underlying conditions, such as chronic obstructive pulmonary disease or cancer. Of the invasive infections, sepsis dominates with 80-90%, and approx. 10% are meningitis. *H. influenzae* is classified into six capsular serotypes (a, b, c, d, e and f, also known as Hia, Hib, Hic, Hid, Hie and Hif), as well as noncapsular (non-typeable, NTHi). Introduction of the polysaccharide type b vaccine in 1993 significantly reduced the number of cases with systemic infection caused by *H. influenzae* type b (Hib) isolates. Hib is now included in the combination-vaccine for infants, given at 3, 5 and 12 months of age (currently *Pentavac*), the vaccine does not protect against the other serotypes. Before the introduction of the Hib vaccine, around 80 cases of Hib meningitis were found annually among infants in Denmark, and this has now been reduced to 0-2 cases per year. The total number of *H. influenzae* meningitis cases among small children below two years of age is currently between one and three per year including all serotypes. For all cases of invasive infections with *H. influenzae* in Denmark, there are usually around one hundred per year. Of these, 10-15% are Hib, 10-15% are Hif and only a very few are either Hia or Hie. NTHi is the predominant type found among all invasive *H. influenzae* infections with 70-80% of all cases.

Invasive *Haemophilus influenzae*

Surveillance of invasive Hib is mandatory by submission of the isolate to Statens Serum Institut (SSI). By tradition, most departments of clinical microbiology are voluntarily submitting all isolates of invasive *H. influenzae* which allows monitoring of all serotypes. Isolates are submitted for the majority of cases, and the remaining cases can be identified through the Danish Microbiological Database (MiBa). All invasive infections with *H. influenzae* are registered in SSI's surveillance database, and serotypes are available for the majority of cases. In 2021, all received isolates were analysed at SSI by whole genome sequencing, and the data were examined for determination of serotype and biotype, for the presence of plasmid-borne beta-lactamase genes *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). For all cases, phenotypic antimicrobial susceptibilities were retrieved through MiBa, when available.

Figure 8.24 Different serotypes in invasive *H. influenzae* cases according to age, 2021, Denmark DANMAP 2021



The present report includes all episodes of invasive *H. influenzae* as identified through MiBa, where the date of sampling was in 2021. A total of 101 cases were identified, of which isolates from 81 (80%) were received at the reference laboratory. *H. influenzae* were isolated from cerebrospinal fluid in ten of the 101 cases, from blood in 89, and from pleural fluid in two cases. The serotypes of the 81 received isolates were: no Hia, nine Hib (11%), two Hie (3%) 13 Hif (16%) and 57 NTHi (70%). The age-distribution and serotypes of the cases is presented in Figure 8.24.

Ten of the received isolates harboured the *TEM-1* gene (two Hib, one Hif, seven NTHi), and these isolates all had corresponding phenotypical beta-lactamase activities as shown by being coded as resistant in MiBa for both penicillin and ampicillin. None of the isolates harboured the *ROB-1* gene. Eleven isolates harboured mutations in the *ftsI* gene, and all of these were also found coded as resistant in MiBa for both penicillin and ampicillin (two isolates did not have available data for ampicillin). Of the eleven isolates with *ftsI* mutations, two also had a *TEM-1* beta-lactamase and were therefore designated as β-lactamase-positive amoxicillin-clavulanate-resistant (BLPACR). The remaining nine are designated as β-lactamase-negative ampicillin-resistant (BLNAR). Although some of these isolates were noted in MiBa as sensitive to ampicillin, MIC values were not available, and it is therefore not known whether these have elevated MIC values compared to the other isolates.

Table 8.20 Distribution of antimicrobial susceptibilities in invasive *H. influenzae* according to serotypes, 2021, Denmark DANMAP 2021

	Hia	Hib	Hie	Hif	NTHi	Unknown	All (2021)	All (2020)	All (2019)	All (2018)
Penicillin: no result registered	-	1	-	-	1	6	8	1	1	1
Penicillin: I and S	-	5 (63%)	2 (100%)	9 (69%)	39 (70%)	9 (64%)	64 (69%)	41 (71%)	84 (74%)	89 (74%)
Penicillin: R	-	3 (38%)	-	4 (31%)	17 (30%)	5 (36%)	29 (31%)	17 (29%)	29 (26%)	31 (26%)
Ampicillin: no result registered	-	-	-	-	2	6	8	2	5	4
Ampicillin: I and S	-	6 (67%)	2 (100%)	11 (85%)	42 (76%)	9 (64%)	70 (75%)	45 (79%)	85 (78%)	94 (80%)
Ampicillin: R	-	3 (33%)	-	2 (15%)	13 (24%)	5 (36%)	23 (25%)	12 (21%)	24 (22%)	23 (20%)
Cefuroxime: no result registered	-	1	-	3	21	6	31	13	1	18
Cefuroxime: I and S	-	6 (75%)	2 (100%)	6 (60%)	29 (81%)	13 (93%)	56 (80%)	34 (74%)	99 (88%)	87 (84%)
Cefuroxime: R	-	2 (25%)	-	4 (40%)	7 (19%)	1 (7%)	14 (20%)	12 (26%)	14 (12%)	16 (16%)
Amoxi/Clav: no result registered	-	2	-	5	20	8	35	18	3	29
Amoxi/Clav: I and S	-	6 (86%)	2 (100%)	8 (100%)	33 (89%)	11 (92%)	60 (91%)	34 (83%)	100 (90%)	83 (90%)
Amoxi/Clav: R	-	1 (14%)	-	-	4 (11%)	1 (8%)	6 (9%)	7 (17%)	11 (10%)	9 (10%)

* The group “unknown” represent the 20 cases that were registered in MiBa, but where an isolate was not received at the reference laboratory at SSI, and therefore not serotyped

Phenotypic susceptibility results for penicillin, ampicillin, amoxicillin/clavulanic acid and cefuroxime, are presented by serotype in Table 8.20. Due to differences across the departments of clinical microbiology in the reporting of non-resistant isolates, the "S" and "I" interpretations are combined in the table. MIC values were not available.

The results from antimicrobial susceptibility testing showed, that in total there was 31% resistance to penicillin, 25% to ampicillin, 20% to cefuroxime and 9% to amoxicillin/clavulanic acid. Some variation across serotypes was observed. These figures are very similar to what was observed in 2020, 2019 and 2018.

Conclusions

The majority of *H. influenzae* isolates from invasive infections are of the non-capsular type. This is similar to previous years and also similar to what has been observed in other European countries [R. Whittaker, Emerg Infect Dis, 2017, Mar;23(3):396-404]. The majority of the invasive *H. influenzae* isolates in Denmark in 2021 were found to be non-resistant to the antimicrobials tested, and the presence of resistance was similar to what was seen in previous years. The number of cases in 2021 was 101 and therefore similar to what was found in the years prior to 2020 (between 101 and 127 in the years 2012 to 2019). In 2020 there were just 59 cases, which was probably an effect of the COVID-19 restrictions.

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Textbox 8.1

Increasing terbinafine resistance in Danish *Trichophyton* isolates 2019-2020

Dermatophytoses (tinea) are fungal infections of hair, nail and skin. The diagnosis relies on microscopy, culture and increasingly on PCR. Most cases are caused by *Trichophyton* species (mainly *T. rubrum* and species in the *T. mentagrophytes/interdigitale* species complex (TMISC)). In 2020, *Trichophyton indotineae* (prev. published as *T. mentagrophytes* ITS genotype VIII or *T. interdigitale*) was proposed as a new species after having emerged as a cause of recalcitrant tinea corporis and cruris in India. Correct identification of species within the TMISC requires DNA sequencing.

Terbinafine (topical or systemic) is a first-line agent for *Trichophyton* infections. Terbinafine resistance is mainly coupled to mutations in the target gene squalene epoxidase (SQLE). It has increasingly been reported (despite susceptibility testing not being routinely performed) in isolates of *T. rubrum* and *T. interdigitale*, in Europe, and in *T. indotineae* in Asia and Europe. Denmark has no surveillance scheme for dermatophytes. In 2019, a Danish retrospective laboratory study demonstrated 14 cases of terbinafine-resistant *T. rubrum* and *T. interdigitale* isolates (2013-2018), all harbouring SQLE alterations (1).

In 2020, EUCAST established a protocol for testing microconidia-forming dermatophytes and tentative ECOFFs for terbinafine/amorolfine/itraconazole/voriconazole for *T. rubrum* and *T. indotineae*. This enables separation between isolates with and without acquired resistance.

Here, we report EUCAST susceptibility data and SQLE profiles for isolates from 2019-2020 and compare with data from 2013-2018 (1). All TMISC isolates were ITS sequenced to ensure correct species identification.

Sixty-three isolates from 59 patients were evaluated. *T. rubrum* accounted for 81% and other *Trichopyton* species for 19%. Approximately 60% of *T. rubrum* and TMISC isolates were terbinafine non-wild-type and/or had known/novel SQLE alterations with possible implications for terbinafine susceptibility (Fig. 1A). All infections with terbinafine-resistant TMISC isolates 2019-2020 were caused by *T. indotineae*. One terbinafine-resistant *T. interdigitale* isolate from 2018 was *T. indotineae* upon ITS sequencing. Triazole resistance was low (4.5%) in *T. rubrum*.

Compared to 2013-2018, the number of patients with terbinafine-resistant *Trichophyton* isolates increased (figure 1B). For *T. rubrum* this is partly explained by an increased number of requests for susceptibility testing. Although terbinafine-resistant *T. indotineae* was first detected in 2018, it accounted for 19% of resistance (4 of 21 patients) in 2020 and appears to be establishing itself in Denmark.

Figure 1 Squalene Epoxidase Gene Mutations (SQLE) alterations found in resistant *Trichophyton rubrum/indotineae* isolates (first isolate/patient), Denmark, 2019-2020 DANMAP 2021

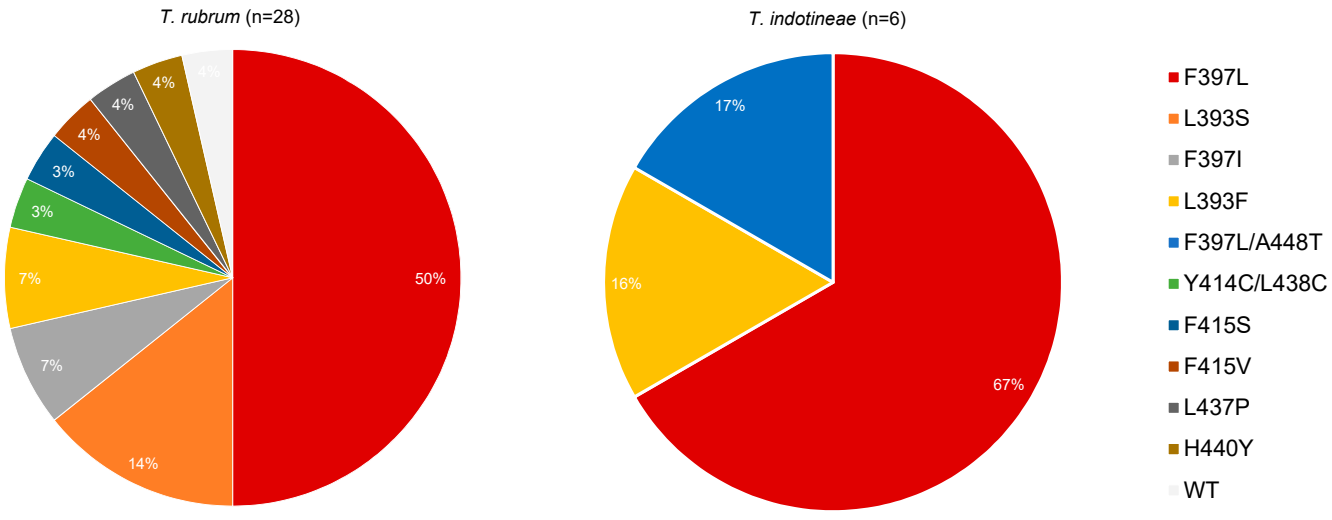
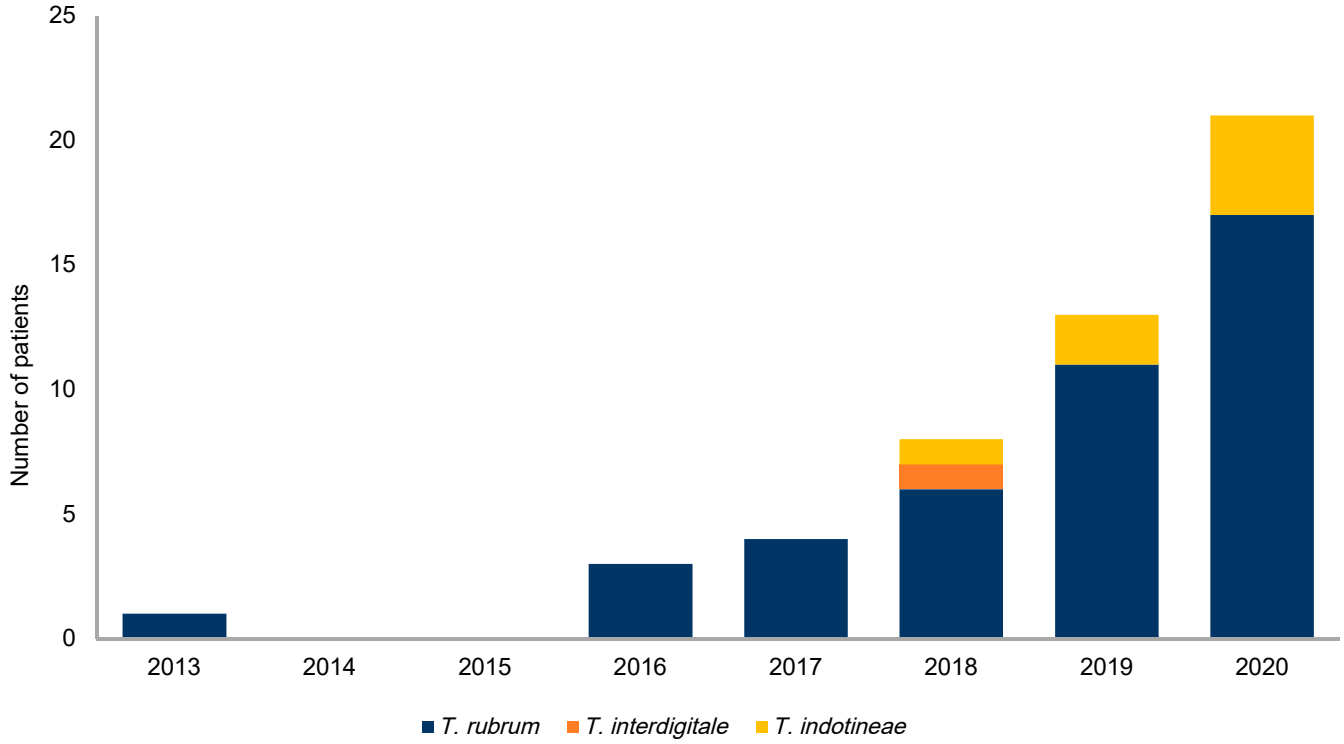


Figure 2 Number of patients with terbinafine-resistant *Trichophyton* isolates, Denmark, 2013-2020 DANMAP 2021



Annual number of Danish patient with *Trichophyton* isolates with terbinafine resistance and/or SQLE alterations. Terbinafine resistant isolates primarily based on a previous study (2013-2018) included for comparison (1). Two patients diagnosed with resistant *Trichophyton* in 2019 also harboured resistant isolates in 2017 and 2018, respectively

In conclusion, terbinafine resistance is an emerging problem in Denmark. Further studies are warranted. Clinicians should be aware that terbinafine resistance is an increasing problem in *Trichophyton* species. The infection may progess and become wide-spread in the individual patient before the clinician realises that the fungus is terbinafine resistant. Meanwhile there is a risk of transmitting the infection to other individuals (epidemic spread). Treatment is complicated in case of terbinafine resistance. Itraconazole (or griseofulvin for tinea capitis) are second line systemic treatment options. Griseofulvin is only available after specific license application to the Danish Medical Agency. Itraconazole has many drug-drug interactions and may lead to resistance development in the resident *Candida* flora. Protracted or high-dose treatment may be needed in recalcitrant cases. Susceptibility testing is highly recommended in non-responding cases.

Figures and text adapted from (2).

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[2] Astvad KMT, Hare RK, Jørgensen KM, Saunte DML, Thomsen PK, Arendrup MC. 2022. Increasing Terbinafine Resistance in Danish Trichophyton Isolates 2019-2020. J Fungi 8:1-13.

9

MATERIALS AND METHODS

9. Materials and methods

9.1 General information

For the DANMAP 2021 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.

9.2 Data on antimicrobial consumption in animals

9.2.1 Data

In Denmark, all antimicrobial agents used for treatment are available on prescription only. Until 2007, antimicrobial agents were exclusively sold by pharmacies or as medicated feed from the feed mills. 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 holder on presenta tion of a prescription.

In 2021, 97% of all antimicrobial agents were purchased through pharmacies and the drug trading companies, while 2% were purchased from feed mills. These numbers did not include prescribed zinc oxide from feeding mills for pigs. For cattle, 83% of antimicrobial agents used in 2020 were purchased from pharmacies, compared to only 7% in 2004. In aquaculture, approximately 80% were purchased through the feed mills.

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. In 2021, a new and updated platform for VetStat was launched, see Textbox 4.1, Chapter 4.

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 of 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 2021 were extracted from VetStat on 24 August 2022.

9.2.2 Methods

In DANMAP, we report use of antimicrobials in different animal populations. As a first step, the amount of antimicro bial agents used in animals is measured in kg active compound. This enables an overall crude comparison of consumption in dif ferent animal species and in 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, sows or 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 principle 3 and 4 are conflicting, principle 5 is applied.

Denominator - live biomass

The number of animals in a population (in epidemiological terms: the population at risk) is represented by their live biomass. The biomass of a species is calculated, taking into account average live bodyweight and the average lifespan in each age group. The estimation of live biomass and thus the number of standard animals at risk per day depends on the available data sources for each species. For DANMAP 2021, 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 size of the breeding animals has probably increased over the last decade, but this was not accounted for.

Cattle production: The live biomass of the cattle population is estimated from census data [Statistics Denmark] and the average live weight of the different age groups. The Danish cattle population is mainly dairy, particularly Holstein Friesian, but also other breeds such as Jersey and a small population of beef cattle. Most of the cattle slaughtered are dairy cows and bull calves of dairy origin. The average live weight was estimated for 10 different age and gender categories.

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 9.8.2.

In 2021, DAPD calculations were carried out for pigs and cattle.

For examplse , the antimicrobial use per pig produced is calculated as:

$$DAPD = \frac{DADD_{sows} + DADD_{weaners} + DADD_{finishers}}{\Sigma biomassdays}$$

Where DADDs, DADDw, DADDf are the amounts of antimicrobial agents used in sows, weaners and finishers, respectively and Σbiomassdays_all is the sum of estimated biomass-days for each age group of pigs.

9.3 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 new legislation requires monitoring of indicator *E. coli* and ESBL-, AmpC- or 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 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).

9.3.1 Animals

In 2021, most of the sampling for DANMAP was allocated to the mandatory sampling of caeca from pigs and cattle, and additional samples of caeca from pigs were collected for monitoring of Enterococci. Additional sampling of caeca from broilers was also carried out.

Caecal samples from healthy cattle (<1 year), pigs and broilers were collected by meat inspection staff at the slaughterhouses. Samples were collected throughout the year, in major Danish slaughterhouses slaughtering conventionally produced chicken (two slaughterhouses), pigs (five slaughterhouses) and cattle (five slaughterhouses).

These slaughterhouses handled at least 90% of the total number of broilers, cattle and pigs slaughtered in Denmark in 2021.

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, four 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* and *Campylobacter jejuni*.

Pig and cattle samples were also examined for *Campylobacter coli*. Furthermore, pig and cattle samples were also examined for *Salmonella* and ESBL/AmpC/carbapenemase-producing *E. coli*, and a selection of pig samples were examined for the presence of *Enterococcus faecium* and *Enterococcus faecalis* (Table 9.1).

Pathogenic bacteria from pigs comprised Actinobacillus pleuropneumoniae, haemolytic *Escherichia coli* and *Streptococcus suis* isolates identified in clinical samples submitted by veterinarians to the Veterinary Laboratory, The Danish Agriculture and Food Council.

9.3.2 Meat

In 2021, ESBL/AmpC/carbapenemase-producing *E. coli* were isolated from packages of fresh, chilled pig- and cattle meat collected in Danish wholesale and retail outlets. These samples were collected throughout the year by DVFA officers (Table 9.1). Products with added saltwater or other types of marinade as well as minced meat were not included. Packages of meat were selected at retail without pre-selecting 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 originated from the national control programme at the slaughterhouses (Table 9.1). Pig carcases were swabbed in four designated areas (the jaw, breast, back and ham) after min. 12 hours of chilling (covering 10x10 cm). All samples were processed at DVFA-approved Industry laboratories and isolates were sent to the DVFA laboratory. *Salmonella* isolates from imported pork originated from samples collected at wholesale and retail outlets and processed at the DVFA laboratory.

9.4 Microbiological methods – isolates from animals and meat

9.4.1 *Salmonella*

Salmonella from pigs and pork not originating from the national *Salmonella* surveillance program was 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. Only one isolate per serotype was selected for antimicrobial resistance testing from each herd, flock or slaughter batch.

Salmonella from carcasses originating from the national *Salmonella* surveillance program was isolated and serotyped according to the White-Kauffmann-Le Minor scheme at DVFA-approved Industry laboratories.

9.4.2 *Campylobacter*

Campylobacter from broiler, pig 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, pig herd, or cattle herd was selected for antimicrobial resistance testing.

Table 9.1 Legislative and voluntary sampling plans under national control programmes and EU harmonised monitoring that contributed isolates to DANMAP 2021 DANMAP 2021

Bacteria	Origin of isolates	Legislative reporting frequency (2020/1729/EU)	Number of tested and positive samples in 2021
Campylobacter spp.	Caecal samples from broilers [a] Caecal samples from fattening pigs [a] Caecal samples from cattle <1 year [a]	Even years Odd years Odd years	163 flocks (44 positive) 272 animals (127 positive) 233 animals (187 positive
Salmonella spp.	Caecal samples from fattening pigs Caecal samples from cattle <1 year Fresh pork at retail (domestic and imported) Fresh beef at retail (domestic and imported) Carcase swabs from fattening pigs [c] Carcase swabs from cattle <1 year [c]	Odd years Odd years Odd years Odd years	755 animals (94 positive) 293 animals (2 positive) 169 units (13 positive) 336 units (0 positive) 19029 animals (85 positive) 8594 animals (3 positive)
Enterococcus spp.	Caecal samples from fattening pigs [b]		484 animals (171 positive)
Indicator *E. coli*	Caecal samples from broilers Caecal samples from fattening pigs Caecal samples from cattle <1 year	Even years Odd years Odd years	141 flocks (132 positive) 182 animals (172 positive) 168 animals (165 positive)
Specific monitoring of ESBL/AmpC and carbapenemase-producing *E. coli*	Caecal samples from fattening pigs Caecal samples from cattle <1 year Fresh pork at retail (Danish) Fresh pork at retail (Imported) Fresh beef at retail (Danish) Fresh beef at retail (Imported) Fresh pork at border control posts (imported) Fresh beef at border control posts (imported) WGS data for collected ESBL/AmpC isolates	Odd years Odd years Odd years Odd years Odd years Odd years Odd years Odd years Odd years	272 animals (58 positive) 293 animals (18 positive) 27 units (9 positive) 310 units (7 positive) 62 units (7 positive) 218 units (8 positive) 3 units (0 positive) 3 units (0 positive) 92 isolates(d)

a) Broilers: *C. jejuni* (n=32), 12 unspecified isolates. Cattle: *C. jejuni* (n=178), *C. coli* (n=11), *C. lari* (n=1), 13 unspecified isolates. Pigs: *C. coli* (n=126), *C. coli* (n=11), *C. jejuni* (n=4), 24 unspecified isolates
b) Pigs: *E. faecalis* (n=81, of which n=8 were also positive for *E. faecium*), *E. faecium* (n=98)
c) 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 refers to individual animals and the total number of positives refers to individual and pooled samples.
d) One isolate from each of the 107 positive samples was sequenced, however bioinformatics results were only obtained for 92 isolates (14 from fresh beef at retail, 13 from fresh pork at retail, 17 from cattle <1 yr and 48 from pigs)

9.4.3 *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 meat and caecal samples 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 cycles), followed by bioinformatics analysis using the WGS Portal v1.15.1. for EU harmonised monitoring. Only one ESBL/AmpC-producing *E. coli* isolate per cattle herd, pig herd, and meat sample was selected for antimicrobial resistance testing.

9.4.4 Enterococci

Indicator enterococci were isolated from pig caeca by dipping a cotton swab in the caecal material, transferring it to 2 ml buffered peptone water and whirl mixing, after which 100 µl were inoculated onto Slanetz agar and incubated at 41,5 °C for 48 hours. 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, while *E. faecium* was not tested.

9.5 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.

9.6 Whole genome sequencing – isolates from animals and meat

In addition to *Salmonella* serotyping performed by sequencing (section 9.4.1), whole genome sequencing (WGS) and in silico 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 WGS portal for EU harmonized AMR monitoring (https://wgsportal.efsa.europa.eu). This pipeline follows the protocol provided by EURL-AR (https://www.eurl-ar.eu/protocols.aspx), to extract AMR genes in a standardized format from raw sequencing reads using ResFinder 4.1, which includes chromosomal mutations leading to resistance to beta-lactams, quinolones and colistin as well as acquired resistance genes [Zankari et al. 2012. J Antimicrob Chemother. 67(11):2640; Zankari et al. 2017. J Antimicrob Chemother. 72(10):2764]. The service also detects ST types based on MLST.

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.

9.7 Data handling - isolates from animals and meat

For the samples processed at the DVFA laboratory, sampling details and laboratory results were stored in the DVFA Laboratory system. Following validation by DVFA, data were 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 9.2 Interpretation criteria for MIC-testing according to EUCAST- and EFSA-provided epidemiological cut-off values (ECOFFs)
DANMAP 2021

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
Ampicillin	>8 [a]	>8	>4	Not tested	Not tested
Azithromycin	>16	>16	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/Clavulan 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/Clavulan acid	>2 [a]	>0.5	Not tested	Not tested	Not tested
Chloramphenicol	>16	>16	>32	>16 [d]	>16
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 [d]	>0.5 [a]
Erythromycin	Not tested	Not tested	>4	>4	>8
Gentamicin	>2	>2	>64	>2	>2
Imipenem	>1	>0.5	Not tested	Not tested	Not tested
Linezolid	Not tested	Not tested	>4 [a]	Not tested	Not tested
Meropenem	>0.125 [a]	>0.125 [a]	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
Sulfonamide	>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 2021:EN-6652]
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 included in the test panel for *Campylobacter* spp.

Table 9.3 Definitions of antimicrobial classes for calculation of multidrug-resistance in *Salmonella* and indicator *E. coli*

DANMAP 2021

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

9.7.1 Interpretation of MIC values

MIC values were retained as continuous variables, from which binary variables (resistant/sensitive) were created using the relevant cut-off from 2021 for all years. Since 2007, data have been interpreted using EUCAST epidemiological cut-off (ECOFF) values with a few exceptions described in Table 9.2. All MIC-distributions are presented in the web annex at www.danmap.org. An isolate is considered multidrug-resistant if resistant to three or more of the antimicrobial classes defined in Table 9.3 and fully sensitive if susceptible to all antimicrobial agents included in the test panel.

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) (Table 9.4).

Table 9.4 Epidemiological cut-offs and clinical breakpoints for isolates of pathogenic bacteria from pigs DANMAP 2021

Antimicrobial agent	*A. pleuropneumoniae*		*E. coli*		*S. suis*	
	ECOFF, non-wild-type > (µg/ml)	Clinical breakpoint, R > (µg/ml)	ECOFF, non-wild-type > (µg/ml)	Clinical breakpoint, R > (µg/ml)	ECOFF, non-wild-type > (µg/ml)	Clinical breakpoint, R > (µg/ml)
Ampicillin	0.5 [a]	1	8	16 [b]	Not tested	Not tested
Amoxicillin-clavulanic acid	Not tested	Not tested	Not available	16/8 [b]	Not tested	Not tested
Apramycin	Not tested	Not tested	Not available	Not available	Not tested	Not tested
Cefotaxime	Not tested	Not tested	0.25	Not available	Not tested	Not tested
Cefoxitin	Not tested	Not tested	Not tested	Not tested	Not available	Not available
Ceftiofur	Not available	4	1	4	Not tested	Not tested
Chloramphenicol	Not tested	Not tested	16	16 [b]	Not available	8 [b]
Ciprofloxacin	Not available	Not available	0.064	Not available	Not available	Not available
Colistin	Not tested	Not tested	2	Not available	Not tested	Not tested
Erythromycin	Not available	Not available	Not tested	Not tested	0.25 [a]	Not available
Florfenicol	Not available	4	16	8	Not available	4
Gentamicin	Not tested	Not tested	2	8 [b]	Not available	Not available
Nalidixic acid	Not tested	Not tested	8	Not available	Not tested	Not tested
Neomycin	Not tested	Not tested	8	Not available	Not tested	Not tested
Penicillin	Not available	Not available	Not tested	Not tested	Not available	0.5
Spectinomycin	Not available	Not available	64	Not available	Not available	Not available
Streptomycin	Not tested	Not tested	16	Not available	Not available	Not available
Sulfamethoxazole	Not tested	Not tested	Not available	Not available	Not available	Not available
Tetracycline	Not available	1	8	8 [b]	Not available	1
Tiamulin	Not available	16	Not tested	Not tested	Not available	Not available
Tilmicosin	Not available	16	Not tested	Not tested	Not tested	Not tested
Trimethoprim	Not tested	Not tested	2	Not available	Not available	Not available
Trimethoprim-sulfamethoxazole	0.125	Not available	Not tested	Not tested	0.25 [a]	Not available
Tulathromycin	Not available	Not available	Not tested	Not tested	Not tested	Not tested

MIC values were interpreted with ECOFFs (1st choice) or tentative ECOFFs (2nd choice) set by EUCAST, or with CLSI-approved animal-specific clinical breakpoints (3rd choice) or human clinical breakpoints (4th choice) when ECOFFs were unavailable
a) Tentative ECOFF
b) Human breakpoint
Abbreviations: ECOFF, epidemiological cut-off; R, resistant

9.7.2 ESBL/AmpC phenotypes

Classification of CP-, ESBL- or AmpC-producing phenotypes was done according to the scheme provided by EFSA .[EFSA 2018. EFSA Journal 16(2):5182]:

1. CP phenotype if meropenem MIC >0.12 µg/ml;
2. 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);
3. ESBL-AmpC phenotype if cefotaxime and/or ceftazidime MIC >1 µg/ml and meropenem MIC <=0.12 µg/ml and cefoxitin MIC
4. >8 µg/ml and synergy (clavulanic acid and cefotaxime/ceftazidime);
5. 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);
6. Other phenotype if not in 1-4.

Synergy is defined as ≥3 twofold concentration decrease in MIC for clavulanic acid combined with cefotaxime/ceftazidime vs the MIC of cefotaxime/ceftazidime alone.

9.7.3 Statistical tests

Significance tests of differences between proportions of resistant isolates were calculated using Chi-square, or Fisher's Exact Tests as appropriate 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 3.6.1 [R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org].

9.8 Data on antimicrobial consumption in humans

9.8.1 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 2020, indication codes were available for 95% of prescriptions, but specific indication codes still only accounted for 78%.

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 2020, 178.848 DDD (0.6%) 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.

9.8.2 Method

Primarily somatic hospitals were included in the DANMAP reporting. Data from private hospitals and clinics, psychiatric hospitals, specialised non-acute care clinics, rehabilitation centres and hospices were excluded from DANMAP in most calculations since their activity and functions are not comparable to public, somatic hospitals and therefor may skew the data. Their consumption accounts for approximately 4% of the antimicrobial consumption at hospitals in Denmark.

The present report includes data on the consumption of "antibacterials for systemic use", or group J01, of the 2021 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.

9.8.3 DDD

Defined daily dose is the assumed average maintenance dose per day for a drug used for its main indication in adults. DDDs provide a fixed unit of measurement independent of price and formulation, enabling the assessment of trends in drug consumption and to perform comparisons between population groups. The DDDs are defined and revised yearly by the WHO Collaborating Centre for Drug Statistics and Methodology [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 9.4). From DANMAP 2018, the new DDD values were applied and all tables and figures were updated ten years back.

9.8.4 DBD

DDD/100 occupied bed-days. The number of occupied bed-days are calculated as the 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.

Table 9.5 New DDDs assigned by WHO Collaborating Centre per January 2019 DANMAP 2021

ATC5 code	ATC level name	Previous DDD			New DDD		
		Weight	Unit	Route of administration	Weight	Unit	Route of administration
J01CA01	Ampicillin	2.0	g	Parenteral	6.0	g	Parenteral
J01CA04	Amoxicillin	1.0	g	Oral	1.5	g	Oral
J01CA04	Amoxicillin	1.0	g	Parenteral	3.0	g	Parenteral
J01CA17	Temocillin	2.0	g	Parenteral	4.0	g	Parenteral
J01CR02	Amoxicillin and beta-lactamase inhibitor	1.0	g	Oral	1.5	g	Oral
J01DE01	Cefepime	2.0	g	Parenteral	4.0	g	Parenteral
J01DH02	Meropenem	2.0	g	Parenteral	3.0	g	Parenteral
J01MA02	Ciprofloxacin	0.5	g	Parenteral	0.8	g	Parenteral
J01XB01	Colistin	3.0	MU	Parenteral	9.0	MU	Parenteral

9.8.5 DAD

DDD/100 admissions. One admission is registered whenever a patient is admitted to a specific 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.

9.8.6 DaDDD

Danish adjusted daily dose. This unit was developed for DANMAP 2018 as an attempt to better picture the actual dosages used for antimicrobial treatment in Denmark. DaDDD units were made by combining recommended dosages in Danish treatment guidelines with data from the prescription register, thus defining a Danish maintenance dose for each given drug. The work with DaDDD was initiated by an expert group under the Danish Regional Learning and Quality teams (LKT) developing measurable units for consumption at Danish hospitals. The DANMAP group further developed these units to also apply to drugs given orally. DaDDD, their counterparts DDD and the conversion factors are presented in Table 9.5 and Table 9.6 for the primary and hospital sector, respectively.

For further information regarding the LKT initiative in 2017-2019, please go to www.kvalitetsteams.dk > Lærings- og kvalitetsteams > Rationelt antibiotikaforbrug på hospitaler (only available in Danish). The LKT report with results from the project can be found at www.kvalitetsteams.dk.

Table 9.6 Danish adjusted DDD for penicillins in the primary sector, 2019 DANMAP 2021

ATC5 code	Antimicrobial agent	WHO DDDs in grams	Danish adjused DDDs in grams	Conversion factor	Primary indication
J01CA02	Pivampicillin	1.05	2.10	0.50	Urinary tract infection
J01CA04	Amoxicillin	1.50	1.50	1.00	Otitis media
J01CA08	Pivmecillinam	0.60	1.20	0.50	Urinary tract infection
J01CE02	Phenoxymethylpenicillin	2.00	1.90	1.05	Upper respiratory tract infection
J01CF01	Dicloxacillin	2.00	3.00	0.67	Skin- and soft tissue infection
J01CF05	Flucloxacillin	2.00	3.00	0.67	Skin- and soft tissue infection
J01CR02	Amoxicillin and beta-lactamase inhibitors	1.50	1.50	1.00	Upper and lower respiratory tract infection

Solely per oral administration routes

Table 9.7 Danish adjusted DDD for main antimicrobials in the hospital sector, 2019 DANMAP 2021

ATC5 code	Antimicrobial agent	WHO DDDs in gram	Danish adjusted DDDs	Conversion factor	Route of administration
J01CA01	Ampicillin	6.00	8.00	0.75	Parenteral
J01CA02	Pivampicillin	1.05	2.10	0.50	Oral
J01CA04	Amoxicillin	1.50	1.50	1.00	Oral
J01CA08	Pivmecillinam	0.60	1.20	0.50	Oral
J01CE01	Benzylpenicillin	3.60	4.80	0.75	Parenteral
J01CE02	Phenoxymethylpenicillin	2.00	2.67	0.75	Oral
J01CF01	Dicloxacillin	2.00	4.00	0.50	Oral
J01CF05	Flucloxacillin	2.0	4.0	0.5	Oral
J01CR02	Amoxicillin and beta-lactamase inhibitor	1.50	1.50	1.00	Oral
J01CR05	Piperacillin and beta-lactamase inhibitor	14.00	11.97	1.17	Parenteral
J01DC02	Cefuroxim	3.00	4.48	0.67	Parenteral
J01DH02	Meropenem	3.00	3.00	1.00	Parenteral
J01FA09	Clarithromycin	0.50	1.00	0.50	Oral
J01FA10	Azithromycin	0.30	1.00	0.30	Oral
J01GB03	Gentamicin	0.24	0.35	0.69	Parenteral
J01MA02	Ciprofloxacin	0.80	0.80	1.00	Parenteral

Figures based on DaDDD can be found in chapter 5, antimicrobial consumption in humans, Figure 5.5b and Figure 5.14b, presenting data from primary sector and hospital sector, respectively.

9.8.7 Contacts in primary health care

Activity in primary health care is based on reimbursement data from "Sygesikringsregisteret". A contact is defined as a reimbursed patient consultation with a health care professional in primary health care. In general practice, consultations can be physical contacts, phone contacts and e-mail contacts. In 2020, video contacts were added as an additional option.

9.9 *Salmonella* and *Campylobacter* isolates from humans

9.9.1 Data source

Antimicrobial susceptibility was performed on human clinical isolates submitted to Statens Serum Institut (SSI). *Salmonella* isolates were submitted from all clinical microbiology laboratories in Denmark and *Campylobacter* isolates were submitted from clinical microbiology laboratories representing the island of Zealand excluding the Capital Region, Funen, and Northern Jutland. As in previous years, SSI collected information on travel history from the patients. Cases were categorised as "domestically acquired" if the patients had not travelled abroad within the week prior to the onset of disease.

9.9.2 Microbiological methods

Salmonella isolates were analysed by whole genome sequencing and the serotypes were derived from the DNA sequences. In a few cases, the DNA information was supplemented with slide agglutination according to the Kauffman-White Scheme. *Campylobacter* species identification was performed by the use of MALDI-TOFF.

9.9.3 Susceptibility testing

Antimicrobial susceptibility testing of *Salmonella* and *Campylobacter* was performed as Minimum Inhibitory Concentration (MIC) determination using the Sensititre broth microdilution system (Trek Diagnostic Systems Ltd.). Inoculation and incubation procedures were in accordance with the CLSI guidelines [Clinical and Laboratory Standards Institute, USA] and the European standard ISO 20776-1:2006.

9.9.4 Data handling

Data on *Salmonella* and *Campylobacter* infections are stored in the Danish Registry of Enteric Pathogens (SQL database) that is maintained by SSI. This register includes only one isolate per patient within a window of six months and includes data on susceptibility testing of gastrointestinal pathogens.

9.10 *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter* species, *Enterococcus faecium* and *Enterococcus faecalis* isolates from humans

9.10.1 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].

9.10.2 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.

9.10.3 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.

9.11 ESBL-producing bacterial isolates from humans

9.11.1 Data source

Since 2014, the Danish DCMs have on a voluntary basis submitted 3rd generation cephalosporin resistant *Escherichia coli* isolates from bloodstream infections for verification and genotyping at the Antimicrobial Resistance Reference Laboratory, Statens Serum Institut.

9.11.2 Microbiological methods of isolates from patients

Since 2014, whole genome sequencing (WGS) and in silico bioinformatics analysis have been applied for isolates predicted to carry ESBL and/or AmpC genes based on initial phenotypic tests, to characterize the genetic background of the ESBL and/or AmpC phenotypes. Only one isolate from each patient was included if less than 12 months were between isolation of the two isolates.

9.11.3 Data handling

The quality of the raw sequencing and assembly data was ensured using the analytical tool BIFROST [https://github.com/ssi-dk/bifrost/]. An in-house bacterial analysis pipeline, building on a weekly update of the ResFinder database [https://bitbucket.org/genomicepidemiology/resfinder/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.cbs.dtu.dk]. Possible clonal clusters were detected using the SeqSphere+ (Ridom) software to call cgMLST types (*E. coli* scheme).

9.12 CPO isolates from humans

9.12.1 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.

9.12.2 Microbiological methods

All submitted isolates (originating both from screening and clinical samples) predicted to carry a carbapenemase based on initial phenotypic tests were 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.

9.12.3 Data handling

The quality of the raw sequencing and assembly data was ensured using the analytical tool BIFROST [https://github.com/ssi-dk/bifrost/]. An in-house bacterial analysis pipeline, building on a weekly update of the ResFinder database [https://bitbucket.org/genomicepidemiology/resfinder], was used for the in silico detection of acquired CPO genes and MLST from assembled WGS data. Possible clonal clusters were detected using the SeqSphere+ (Ridom) software to call cgMLST types where such schemes are available (*E. coli*, *Klebsiella pneumoniae* and *Acinetobacter baumannii*). For outbreak 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.

9.13 VRE isolates from humans

9.13.1 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.

9.13.2 Microbiological methods

All clinical VRE isolates have been whole-genome sequenced. Only one isolate from each patient was included if less than 12 months were between isolation of the two isolates.

9.13.3 Data handling

The quality of the raw sequencing and assembly data was ensured using the analytical tool BIFROST [https://github.com/ssi-dk/bifrost/]. An in-house bacterial analysis pipeline, building on a weekly update of the ResFinder database [https://bitbucket.org/genomicepidemiology/resfinder], was used for the in silico detection of genes related to vancomycin resistance in enterococci and MLST from assembled WGS data. Possible clonal clusters were detected using the SeqSphere+ (Ridom) software to call cgMLST types.

9.14 Invasive *Streptococcus pneumoniae* isolates from humans

9.14.1 Data source

Invasive pneumococcal disease is a notifiable disease in Denmark and it is mandatory to submit all invasive isolates of *S. pneumoniae* 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).

9.14.2 Microbiological methods

Identification or confirmation of the species *S. pneumoniae* was based on: visual evaluation of colonies, positive optochin test and test with either latex omni test (ImmuLex™ S. pneumoniae Omni, SSI Diagnostica, Denmark) or Neufeld based Omni serum (SSI Diagnostica, Denmark). If challenging results occurred, MALDI-TOF, bile solubility test, or whole genome sequencing were performed to further confirm the correct species identification. For non-viable isolates, species identification was based on the detection of the lytA 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.

9.14.3 Susceptibility testing

Screening for penicillin- and erythromycin-resistant *S. pneumoniae* was performed with 1 µg oxacillin discs and 15 µg erythromycin discs (Oxoid, Roskilde, Denmark), respectively, on Mueller-Hinton agar (Mueller-Hinton plate, 5% blood, 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.

9.14.4 Data handling

Only cases with isolates from blood or spinal fluid were included in the DANMAP report. Repeated samples within a 30 days window were classified as duplicates and were omitted from the analysis.

9.15 Isolates of beta-haemolytic streptococci of groups A, B, C, and G from invasive infections in humans

9.15.1 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.

9.15.2 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 [http://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.

9.15.3 Susceptibility testing

Screening for penicillin, erythromycin and clindamycin re-sistance 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 Clinical Breakpoint Tables v. 11.0).

Isolates that were either resistant or susceptible to increased exposure were categorised together as resistant.

9.15.4 Data handling

A case of invasive BHS disease was defined as the isolation of BHS from a normally sterile site (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.

9.16 Invasive *Haemophilus influenzae* isolates from humans

9.16.1 Data source

Invasive infections with *Haemophilus influenzae* type b (Hib) is a notifiable disease in Denmark and all invasive isolates nationwide are sent to the reference laboratory at SSI. By tradition, invasive isolates of other serotypes have also been submitted on a voluntary basis, and thus a broader picture of invasive H. influenzae in Denmark can be obtained. All cases were identified through MiBa and registered in the surveillance database at SSI. Cases, where isolates were not submitted to the reference laboratory were registered as "unknown serotype".

9.16.2 Microbiological methods

At SSI, the received isolates were analysed by whole-genome sequencing, from which serotype and biotype were extracted.

9.16.3 Susceptibility testing

Susceptibility data for the 2021 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).

9.16.4 Data handling

A case was defined as isolation of H. influenzae from normally sterile sites (e.g. blood, spinal fluid, pleura, joint). Repeated samples within a 30 days window were classified as duplicates and were omitted from the analysis.

9.17 *Staphylococcus aureus* including MRSA isolates from humans

9.17.1 Data source

Blood isolates were referred on a voluntary basis by all DCMs to the National reference laboratory for antimicrobial resistance at SSI. Detection of methicillin-resistant *Staphylococcus aureus* (MRSA) is a notifiable condition in Denmark and therefore all MRSA isolates from all sample types were sent to the reference laboratory.

9.17.2 Microbiological methods

At SSI, all isolates were initially tested using a multiplex PCR detecting the *spa, mecA, hsd, scn* and *pvl* (LukF-PV) genes [Larsen et al. 2008. Clin Microbiol Infect. 14: 611-614; Stegger et al. 2012. Clin Microbiol Infect. 18: 395-400]. *spa* was used as *S. aureus* specific marker and for subsequent typing by Sanger sequencing [Harmsen et al. 2003. J Clin Microbiol. 41: 5442-5448], mecA to determine MRSA status, and scn and *hsd* as markers for human adaptation and relation to CC398, respectively. All bacteremia cases and *mecA* negative presumed MRSA were tested for presence of the *mecC* gene. *spa*-negative isolates were confirmed as *S. aureus* by MALDI-TOF. Based on the spa type and known association with MLST typing, each isolate was assigned to a clonal complex (CC).

9.17.3 Susceptibility testing

Data on antimicrobial susceptibility was extracted from MiBa.

9.17.4 Data handling

For blood isolates, a case was defined as a patient with a positive blood culture. Subsequent isolates from the same patient was only included if the positive blood cultures were obtained at least one month apart (new episode).

For MRSA, data on the characteristics of the isolates and the clinical/epidemiological information were obtained from the Danish MRSA register at SSI (mandatory reportable). Patients were registered, regardless of colonisation or clinical infec-

tion, the first time they were diagnosed with MRSA or when a new subtype was demonstrated. Based on the reported information, MRSA cases were classified as colonisation/active screening (i.e. surveillance samples to detect nasal, throat, gut or skin colonisation), imported infection (i.e. acquired outside Denmark), infection acquired in a Danish hospital, defined as diagnosed >48 hours after hospitalisation with no sign of infection at admittance (HA-MRSA) or infection diagnosed outside hospitals (community onset). MRSA cases with community onset were further classified according to risk factors during the previous 6 months as either health-care associated with community onset (HACO) or community-acquired (CA). Health-care associated risk factors included prior hospitalisations, stay in long-term care facilities and being a health-care worker. Community risk factors included known MRSA-positive household members or other close contacts. Due to the increasing numbers of cases belonging to CC398, this type was treated separately as both epidemiology and relevant exposure are different from other CA cases.

9.18 Gonococci isolates

9.18.1 Data source

Isolates of gonococci (*Neisseria gonorrhoeae*) were submitted from the departments of clinical microbiology to SSI on a voluntary basis. The isolates were obtained by culture of specimens from a variety of anatomical locations, most often urethra, cervix, rectum, throat, and rarely from other sites, e.g. eyes, joint fluid, and blood.

9.18.2 Microbiological methods

The bacteriological identification of the received isolates was performed by MALDI-TOF.

9.18.3 Susceptibility testing

For all isolates the MICs of azithromycin, ceftriaxon and ciprofloxacin were determined with ETEST (Biomérieux) on chocolate agar incubated at 35 °C in 5% CO_2. The breakpoints used were those defined by EUCAST (EUCAST version 11.0).

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 Clinical Breakpoint Tables v. 11.0). A cefinase disc technique was used to examine the isolates for beta-lactamase production.

9.18.4 Data handling

Only one isolate from each unique case of gonorrhoea were included in the DANMAP report. Laboratory demonstration of gonococci in repetitive specimens was considered to represent a new case of gonorrhoea if the specimens were obtained with an interval of more than 21 days.

10

TERMINOLOGY

List of abbreviations

AGP	Antimicrobial growth promoter
AMU	Antimicrobial use
AMR	Antimicrobial resistance
ATC	Anatomical Therapeutic Chemical Classification System
ATCvet	Anatomical Therapeutic Chemical Classification System for veterinary medicines
ATU	Area of Technical Uncertainty
CA	Community-acquired
CC	Clonal complex
CDI	Clostridium difficile infections
CHR	Central Husbandry Register
CPE	Carbapenemase producing Enterobacterales
CPO	Carbapenemase producing organisms
CPR	Danish Civil Registry, register for social security numbers
DAD	Defined Daily Doses per 100 admissions
DADD	Defined Animal Daily Dose
DaDDD	Danish adjusted Defined Daily Doses
DAPD	Defined Animal Daily Dose per 1,000 animals per day
DBD	Defined Daily Doses per 100 occupied bed-days
DCM	Department of clinical microbiology
DDD	Defined Daily Dose
DID	Defined Daily Doses per 1,000 inhabitants per day (DDD/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*
NAAT	Nucleic acid amplification test
OIE	World Organisation for Animal Health
PCR	Polymerase chain reaction
PHC	Primary health care
RFCA	Regional Veterinary and Food Control Authorities
SEGES	Knowledge Centre for Agriculture
SSI	Statens Serum Institut
ST	Serotype/Sequence type
VASC	Veterinary advisory service contracts
VMP	Veterinary medicinal products
VetStat	Danish Register of Veterinary Medicines
VRE	Vancomycin-resistant enterococci
VVE	Vancomycin-variable enterococci
WGS	Whole-genome sequencing
WHO	World Health Organization

Glossary

Anatomical Therapeutic Chemical (ATC) classification: An international classification system for drug consumption studies. The ATC code identifies the therapeutic ingredient(s) of each drug for human use according to the organ or system on which it acts and its chemical, pharmacological and therapeutic properties. Antibacterials for systemic use are known as ATC group J01. The ATC classification is maintained by the WHO Collaborating Centre for Drug Statistics and Methodology (Oslo, Norway) [www.whocc.no/atcddd/indexdatabase/]. The ATC classification for veterinary medicinal products, ATCvet, is based on the same main principles as the ATC classification system for medicines for human use and is also maintained by the WHO Collaborating Centre for Drug Statistics and Methodology [www.whocc.no/atcvet/database/].

Antibacterial agents: Synthetic (chemotherapeutics) or natural (antibiotics) substances that destroy bacteria or suppress bacterial growth or reproduction [Source: Dorland's Illustrated Medical Dictionary]. In the section on human consumption, 'antibacterial agents' are referred to as 'antimicrobial agents' (see below).

Antimicrobial agents: The term 'antimicrobial agents' covers antibacterial, antiviral, coccidiostatic and antimycotic agents. In the section on veterinary consumption, the broad term 'antimicrobial agents' is generally used because coccidiostats are included. Antiviral substances are not used in veterinary medicine, and antimycotics are only registered for topical veterinary use and used mainly in companion animals. Antimycobacterial agents are not included. The term 'antibacterial agents' is only used in the veterinary section for precision, to distinguish from use of coccidiostats as feed additives (poultry only). In the chapter on human consumption, the term 'antimicrobial agents' refers to all antibacterial agents for systemic use (J01 in the ATC system) including metronidazole and vancomycin, which are used for systemic treatment but registered under the ATC code P01AB01 and A07AA09, respectively.

Broiler: A type of chicken raised specifically for meat production. In Denmark, the average weight after slaughter is 1.51 kg.

CentralHusbandryRegister (CHR): This is a register of all Danish farms defined as geographical sites housing production animals. It contains information concerning ownership, farm size, animal species, age groups, number of animals and production type. Each farm has a unique farm identity number (CHR-number).

Defined Daily Dose (DDD): This is the assumed average maintenance dose per day for a drug used for its main indication in adults. It should be emphasised that the Defined Daily Dose is a unit of measurement and does not necessarily reflect the recommended or prescribed daily dose. DDDs provide a fixed unit of measurement independent of price and formulation, enabling the assessment of trends in drug consumption and to perform comparisons between population groups. The DDDs are defined and revised yearly by the WHO Collaborating Centre for Drug Statistics and Methodology [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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