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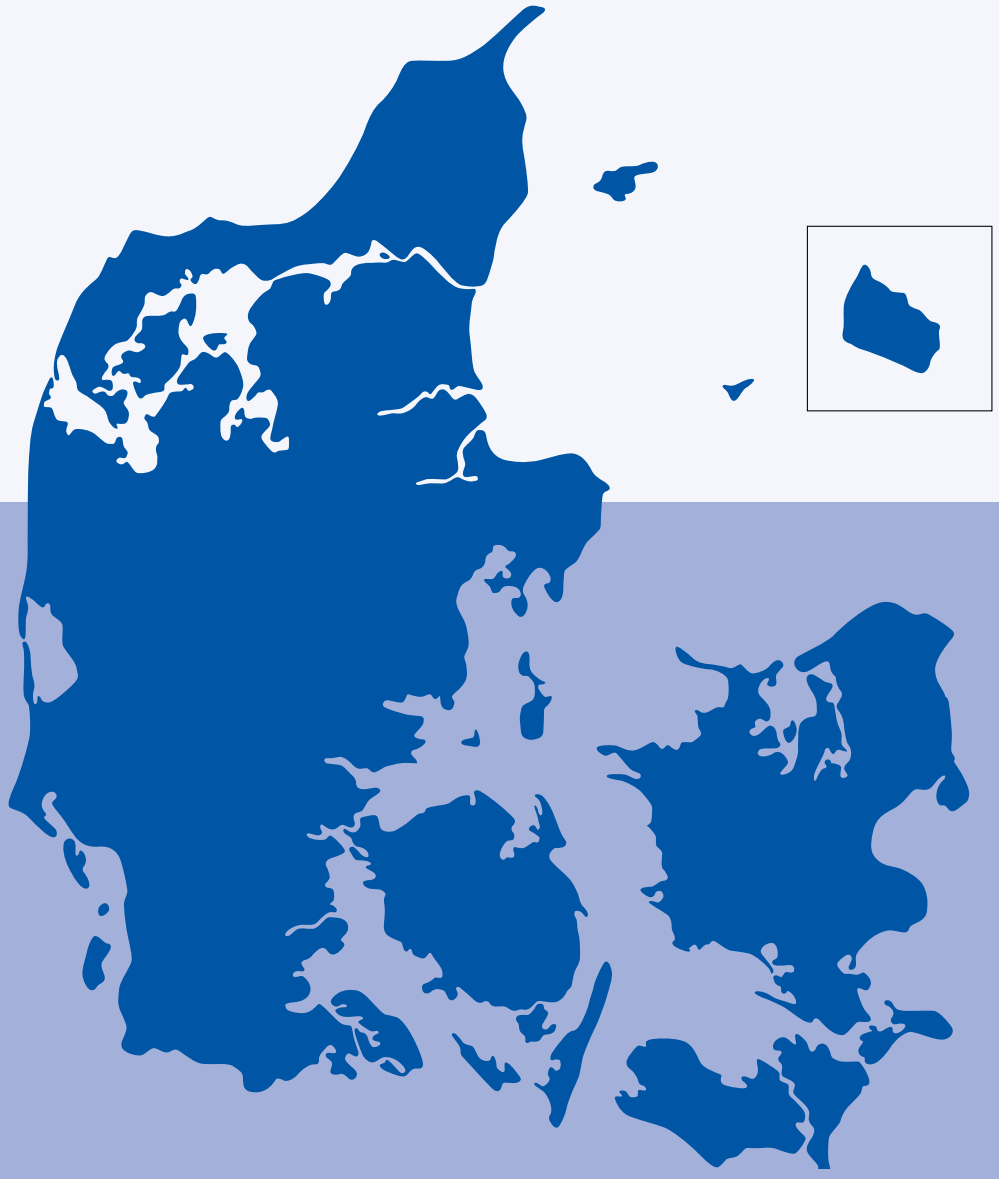
DANMAP 2020

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 2020

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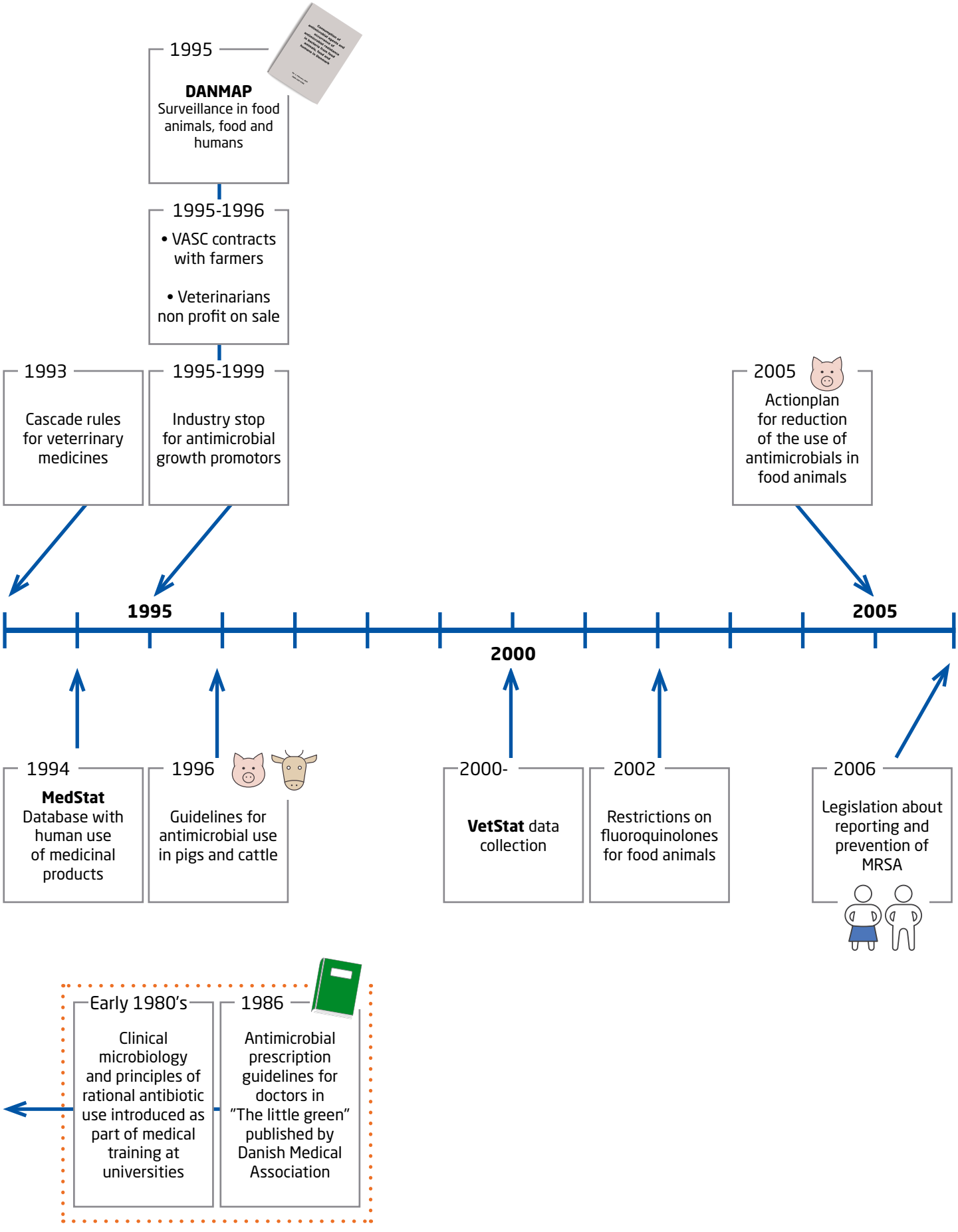
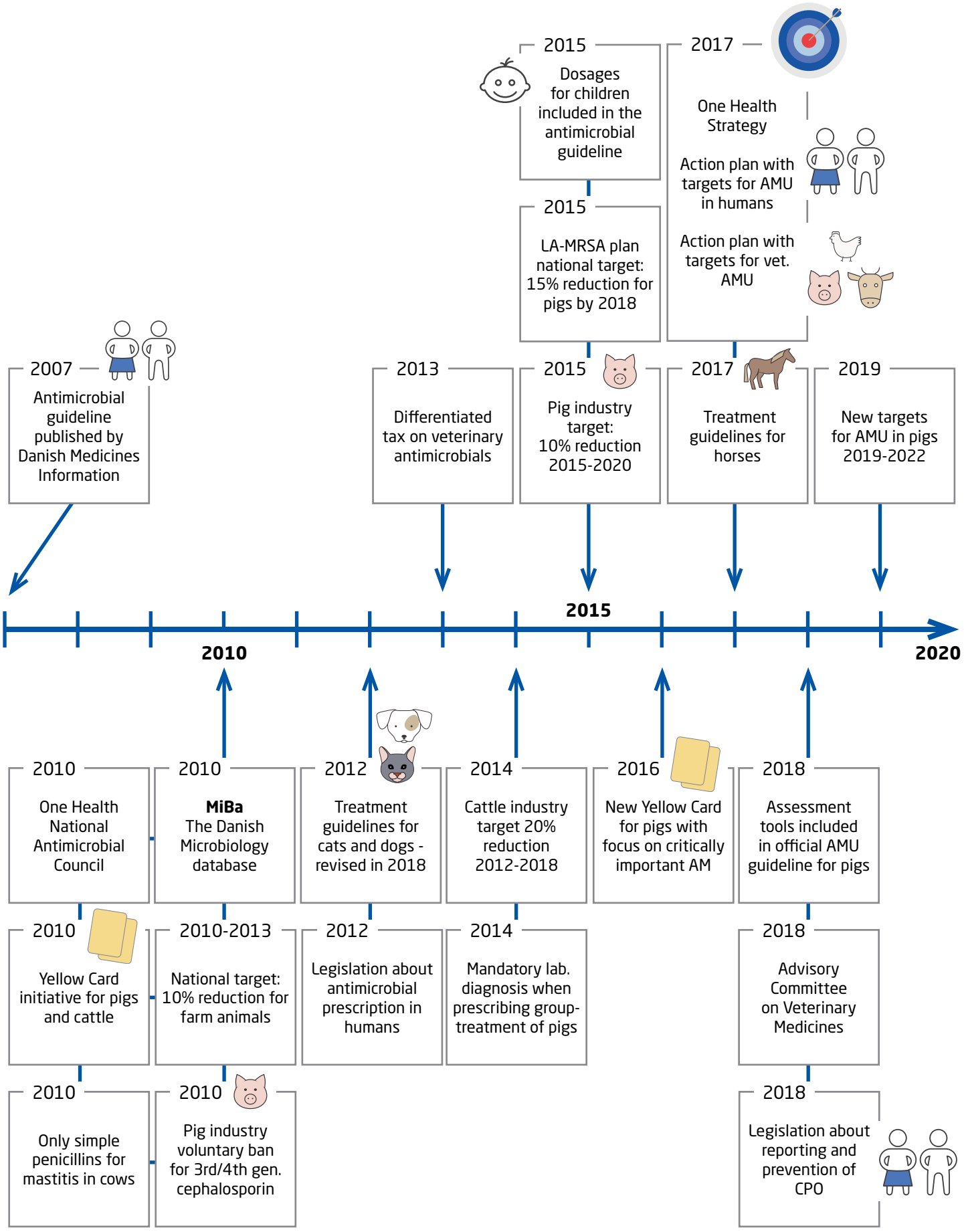
Additional information and supporting data on antimicrobial consumption and antimicrobial resistance is presented in the web annex at www.DANMAP.org.

Timeline with initiatives for the prevention and control of AMR and prudent antimicrobial use in animal and public health in Denmark




2007
Antimicrobial guideline published by Danish Medicines Information
2010
One Health National Antimicrobial Council
2010
Yellow Card initiative for pigs and cattle
2010
Only simple penicillins for mastitis in cows
2010
MiBa The Danish Microbiology database
2010-2013
National target: 10% reduction for farm animals
2010
Pig industry voluntary ban for 3rd/4th gen. cephalosporin
2012
Treatment guidelines for cats and dogs - revised in 2018
2012
Legislation about antimicrobial prescription in humans
2013
Differentiated tax on veterinary antimicrobials
2014
Cattle industry target 20% reduction 2012-2018
2014
Mandatory lab. diagnosis when prescribing group-treatment of pigs
2015
Dosages for children included in the antimicrobial guideline
2015
LA-MRSA plan national target: 15% reduction for pigs by 2018
2015
Pig industry target: 10% reduction 2015-2020
2016
New Yellow Card for pigs with focus on critically important AM
2017
One Health Strategy
Action plan with targets for AMU in humans
Action plan with targets for vet. AMU
2017
Treatment guidelines for horses
2018
Assessment tools included in official AMU guideline for pigs
2018
Advisory Committee on Veterinary Medicines
2018
Legislation about reporting and prevention of CPO
2019
New targets for AMU in pigs 2019-2022
2010
2015
2020

1. Editorial

DANMAP – the beginning

In the seventies and eighties antimicrobial resistance in hospital infections was very low in Denmark and antimicrobial usage in humans in society and in hospitals was likewise low by international comparison, and mainly based on narrow spectrum antimicrobials. Strong educational efforts and implementation of effective policies for prudent antimicrobial usage had controlled a high incidence of MRSA in hospitals in Denmark in the late sixties.

I still recall Danish clinical microbiologists complaining that their Southern European colleagues, got all the fame and glory in the world of antimicrobial resistance, because they were often the first to identify the new multi-resistant bacteria and discover the new resistance mechanisms. Being the first to report was ironically often associated with having indiscriminate antimicrobial usage in the country.

The nineties became the decade of food safety and zoonoses in Denmark, and the topic was at the top of the political, public and media's agenda. In just a few years, Denmark rose to become an international role model in surveillance and control of foodborne zoonoses and pre-harvest food safety.

It is impossible to explain the origin of DANMAP, without describing the origin of the Danish Zoonosis Centre and the Danish Integrated Zoonosis Monitoring and Control Program (which never got a catchy abbreviation like DANMAP).

In the late eighties *Salmonella* from broilers (slaughter chicken) had gotten some attention, and efforts by the poultry producers to control *Salmonella* had been implemented with moderate success. In 1992-93, I had recently finished my PhD on *Staphylococcus hyicus* infections in pigs. I carried out the project at the National Veterinary Laboratory (NVL), with Vibeke Thamdrup-Rosdahl (SSI) as a very active mentor and co-supervisor. After a short postdoc-period, I became researcher at NVL with the task to develop and implement lab-methods to investigate the epidemiology of zoonotic and foodborne infectious agents in a formal collaboration between NVL and SSI. A collaboration agreement dated 15 April 1993 on collection, storage and exchange of bacterial isolates was the basis for the work.

One of my first projects in 1993 was a retrospective comparison of human and animal *Salmonella* Typhimurium using phage typing. It showed, to our surprise, that pigs had overtaken poultry as the main reservoir for human salmonellosis in Denmark, even if the levels of *Salmonella* in pork were at least ten times lower than in chicken meat.

Inspired by the good results, SSI (Vibeke Thamdrup Rosdahl) and NVL (Knud Børge Pedersen) put forward a proposal to the Ministry of Agriculture to establish a "Zoonosis Centre" between NVL and SSI as a new national center without walls.

This coincided in time with the implementation of the recently adopted EU Zoonosis Directive (92/117/EØF) in DK, requiring member states to actively control certain zoonoses and submit an annual report on the overall zoonosis-situation in the country.

A large outbreak of human salmonellosis associated with a pig slaughterhouse in Funen in May 1993 got large media and political attention. The politicians were now actively calling for solutions to the growing problems with *Salmonella* in pigs and pork.

Based on these events, Government now called for a plan to control *Salmonella* in pigs and pork in 1993. We developed the action plan in collaboration between the NVL, the pork producers' organization and the authorities. The approach was radically new because it proposed to control *Salmonella* at the farm-level (pre-harvest), and not in meat processing, and it intended to identify *Salmonella* infected swine herds by serological testing (ELISA); something never done before.

Preparing a paper for a small Danish journal in 1993, I realized that it was possible to estimate the primary sources of all human *Salmonella* infections in Denmark based on sero- and phagetype distributions of *Salmonella* – I called it "smittekilderegnskabet" (the deterministic source attribution model).

The source attribution concept immediately caught the attention of authorities and media, because it showed quantitative associations between primary bacterial reservoirs and human infections hitherto not visible, and documented the value of systematic comparative typing of human and animal *Salmonella* isolates.

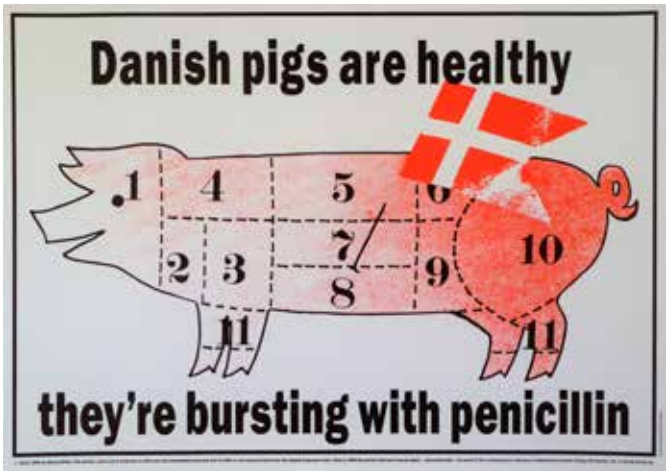
NVL, SSI and the veterinary, food and health authorities, now proposed to establish a formal body, *The Danish Zoonosis Centre*, to routinely monitor the zoonosis situation, coordinate national activities among private and public stakeholders and evaluate the effect of control program based on research. Furthermore, the center would prepare the annual report on zoonosis to the EU. The center, initially with a staff of two, was inaugurated early in January 1994, based on a grant from the Ministry of Agriculture.

Thanks to the *Salmonella* action plans and the activities of the zoonosis center, a program for systematic collection, characterization and storage of zoonotic pathogens from feed, animals, food and humans was established during 1993/94 – the Danish Integrated Zoonosis Monitoring Program.

In the past, most of the bacterial isolates from human illness, food and animals were discarded after the laboratory analysis, making it virtually impossible to retrospectively investigate trends and patterns, or to find sources of foodborne outbreaks. The center established standards for identification and epidemiological characterization between the many different national, regional and municipal laboratories involved (more than 25 laboratories at that time), recording of relevant attendant epidemiological data, and central collection and storage of relevant isolates at -80°C at NVL and SSI.

In addition to the integrated farm-to-patient surveillance approach, the most innovative elements in the program was the central collection of all relevant data (computers were quite new, and the Internet even newer), systematic collection of fecal material from farms and/or slaughterhouses (caecal sampling), new sensitive isolation methods for zoonotic pathogens and routine sero- and phage typing of *Salmonella* isolates. Furthermore, a brilliant cohort of young researchers were trained in a unique combination of field- and big-data zoonosis epidemiology. DANMAP was, to a large extent, built on this surveillance infrastructure.

In 1994, data on veterinarians' growing use of tetracycline (the "yellow powder") in pig production emerged in Denmark. Niels Frimodt-Møller and I decided to initiate a comparative study on antimicrobial resistance in *Salmonella* Typhimurium from humans and animals. The finding of a case of human infection with a multi-resistant *Salmonella* in Aarhus added to the media's attention to the link between veterinary antimicrobial usage and human health. A debate, which was not new in Denmark, thanks to Witte's well known poster from 1978. Now we had the scientific tools to investigate the links thoroughly.



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Just before Christmas 1994 Vibeke Thamdrup Rosdahl called and asked if we had any knowledge of occurrence of vancomycinresistant enterococci (VRE) in Danish farm animals. Wolfgang Witte and Ingo Klare from the Robert Koch Institute in Berlin had inquired, because they had detected VRE in German food animals, and speculated of an association to the use of an antibiotic named avoparcin in farm animals. Frank Aarestrup, who was doing parts of his PhD work in my lab, volunteered to do a quick search for VRE in Danish poultry.

Finding VRE in abundance in conventional, but not in organically raised poultry, indicated an association with use of avoparcin, a vancomycin-like glycopeptide, used in most conventional poultry flocks as a feed additive for growth promotion, so the explanation seemed straightforward (at least to a microbiologist).

A journalist from DR who was in my lab preparing a documentary on the "yellow powder" and resistant *Salmonella* quickly decided to change the topic, and prepared a documentary on antimicrobial growth promoters and the potential human health threats instead.

We obviously discussed the results with the Ministry, authorities and food animal producers. There was a strong determination to act; initially voluntarily by the animal producers, but soon after (and maybe inspired by the strong reactions to the aforementioned DR documentary which aired on 16 May), the Minister of Agriculture Henrik Dam Kristensen decided to ban Avoparcin in Denmark on 20 May 1995.

Feed additives for food animals are EU jurisdiction, so a member state cannot unilaterally ban the sale, unless new information documents an imminent threat to human or animal health. Thus, the EU Commission requested Denmark to prove its case within six weeks. An intense work period ensued, where literally "all hands were on deck" 24-7. The result was the "Avoparcin Report", delivered to the Commission on the 14 July 1995, which documented step-by-step, that Avoparcin did not live up to the microbial safety requirements for its approval as a feed additive, and that it constituted a potential threat to human health. The SCAN Committee of the EU, after a long process and some intense meetings in which Niels Frimodt-Møller and I had the "pleasure" of defending the Danish case, reluctantly had to agree to all our findings, however, they would not recommend an EU ban, because the human health risk wasn't quantified!

Only in 1997, did the EU Commission ban Avoparcin in all EU member states, against the recommendation of SCAN, but with a majority of member states backing "*the precautionary principle*", which obligates lawmakers to prioritize human health over other factors in the case of scientific uncertainty.

The Avoparcin case taught us a lot about the quality of the scientific evidence needed to qualify political decision-making in the realm of EU legislation, and it served as a "blueprint" for the other cases made by Denmark against the remaining antimicrobial growth promoters.

The growth promoter cases were the main political justification for establishing a systematic and integrated monitoring

program for antimicrobial resistance and antimicrobial usage in Denmark. There was a need scientifically document the health risks, the consequences on agricultural productivity and the long-term effects on antimicrobial resistance in animals, food and humans.

Early in May of 1995 Vibeke Thamdrup-Rosdahl, Niels Frimodt-Møller, Knud Børge Pedersen and myself met to discuss the possibilities of expanding the zoonosis monitoring program with antimicrobial susceptibility testing, and also to include s.c. indicator bacteria (enterococci and *E. coli*) in light of the recent findings. There were many obstacles to overcome, such as agreeing on harmonized susceptibility testing methods, finding the necessary funding, and getting the support of the industry for sampling. A draft plan was prepared in the beginning of May 1995.

Consequently, MoA and MOH jointly requested NVL and SSI to establish a coordinated program to monitor and conduct research on antimicrobial resistance (including antimicrobial growth promoters) in animal and humans pathogens as well as in s.c. indicator bacteria. This was the starting point of DANMAP.

Late in May, we submitted the proposal for an integrated antimicrobial resistance- and antimicrobial usage-monitoring program to the Ministry of Agriculture and Fisheries and the Ministry of Health. Niels Frimodt-Møller came up with the excellent name DANMAP. The proposed budget was roughly one mill. €, and the two ministries agreed to fund it jointly, with MoA accepting the majority of the costs.

We published the first report from DANMAP in July 1996. It was a report on occurrence of resistance towards antimicrobial growth promoters in animal pathogens and indicator bacteria. February 1997 came the first comprehensive DANMAP report, bringing together, for the first time, data on isolates from animals, food and humans, from the entire country. Furthermore, it provided estimates of antimicrobial usage in humans and animals.

On January 7, 1998, NVL handed in the report on the assessment of the growth promoter Virginamycin to MoA, and on 16 January 1998, Henrik Dam Kristensen banned it. A cliché could be that; "and the rest is history". In 2000, after a string of cases brought forward by DK, EU banned all antimicrobial growth promoters, effective from 2006. Pfizer and Alpharma brought the EU decision before the Court of Justice of the European Union, claiming that it was unlawful. Niels Frimodt-Møller and I had the pleasure to defend the strong Danish scientific evidence before the judges. The court turned down the claims of the drug industry and confirmed that the EU bans were legally justified based the available scientific evidence.

With time, harmonization and improvement of laboratory methods for susceptibility testing and epidemiological characterization have taken place, and sampling methods and strategies have been improved and expanded to make the picture even more complete. Data management and analysis is improved, and most importantly, the data on antimicrobial usage have improved greatly, in animals, with the establishment of VETSTAT in 1999, which monitors animal antimicrobial usage at farm- and prescriber-level.

With DANMAP, we got many things right from the beginning. The strongest parts of DANMAP are consistency and continuity. Sampling strategies and methods are innovative and epidemiologically sound. Harmonized laboratory methods, not least MIC testing, make surveillance data comparable over time and across borders. Furthermore, the policy of storing isolates continues to be a source of valuable studies, as DANMAP matures.

The impact of DANMAP cannot be overestimated. The initial "spark", was the need to generate solid data to support Denmark's bans of the antimicrobial growth promoters in EU, however, DANMAP quickly became a global role model, copied in EU, US, Australia, and many other countries and regions of the world. In the US, where NARMS is the counterpart to DANMAP, they described Denmark "monitoring heaven". In the EU, we were essential contributors to the decision to terminate the non-therapeutic usage of antimicrobials in food animals, cutting total consumption by more than 50% in the EU territory. Despite many attempts, we were not equally successful in supporting our American colleagues in changing the US policies, however the process is ongoing and small improvements happen regularly.

I left DANMAP more than a decade ago, taking on new challenges in the field of university leadership. However, looking back over the last 25 years, I am pleased to see that our ideas and visions have stood the test of time. DANMAP continues to generate timely surveillance data for action, and serves as a unique Danish infrastructure, with global outreach, for detailed and holistic research on antimicrobial resistance. More than 3,300 hits for the term "DANMAP" in Google Scholar is a good indicator of that.

The early papers and reports show the many names of the dedicated and hard-working people who contributed to DANMAP in the early days (too many to list here). DANMAP is a unique multidisciplinary and multi-sector collaboration built on the open and timely sharing information, isolates and data, to maximize scientific value and societal impact.

Finally, resistance to antimicrobials continue to emerge and grow. It constitutes one of the major global threats to human and animal health. We must not let our guard down – not now and not ever.

Professor Henrik C. Wegener
Rector, University of Copenhagen

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.

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- The meat inspection staff and the company personnel at the participating slaughterhouses for collecting samples from animals at slaughter. Without their careful recording of the animals' farm of origin, the results would be less useful
- DTU Centre for Diagnostics and the Laboratory of Swine Diseases, the Danish Agriculture and Food Council, Kjellerup, for their contributions on AMR in animal pathogens
- The staff of the Regional Veterinary and Food Control Authorities for collecting food samples and isolating bacteria
- The Department of Medication Statistics and Research Support at the Danish Health Data Authority (formerly the Danish Medicines Agency and SSI) for collecting and transmitting data on veterinary consumption of antimicrobial agents from the pharmacies
- The Danish Veterinary and Food Administration for collecting and transmitting data on veterinary consumption of antimicrobial agents from VetStat, statistics on consumption measured in tonnage, financing data collection and providing data for interpretation
- The Danish Agriculture and Food Council for cooperation regarding the estimation of live biomass of production animals

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- 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
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- Colleagues at the Data Integration and Analysis Secretariat at SSI for discussing hospital activity data
- Members of the Praktiserende Lægers Organisation (PLO) for discussing healthcare activity data in primary health care
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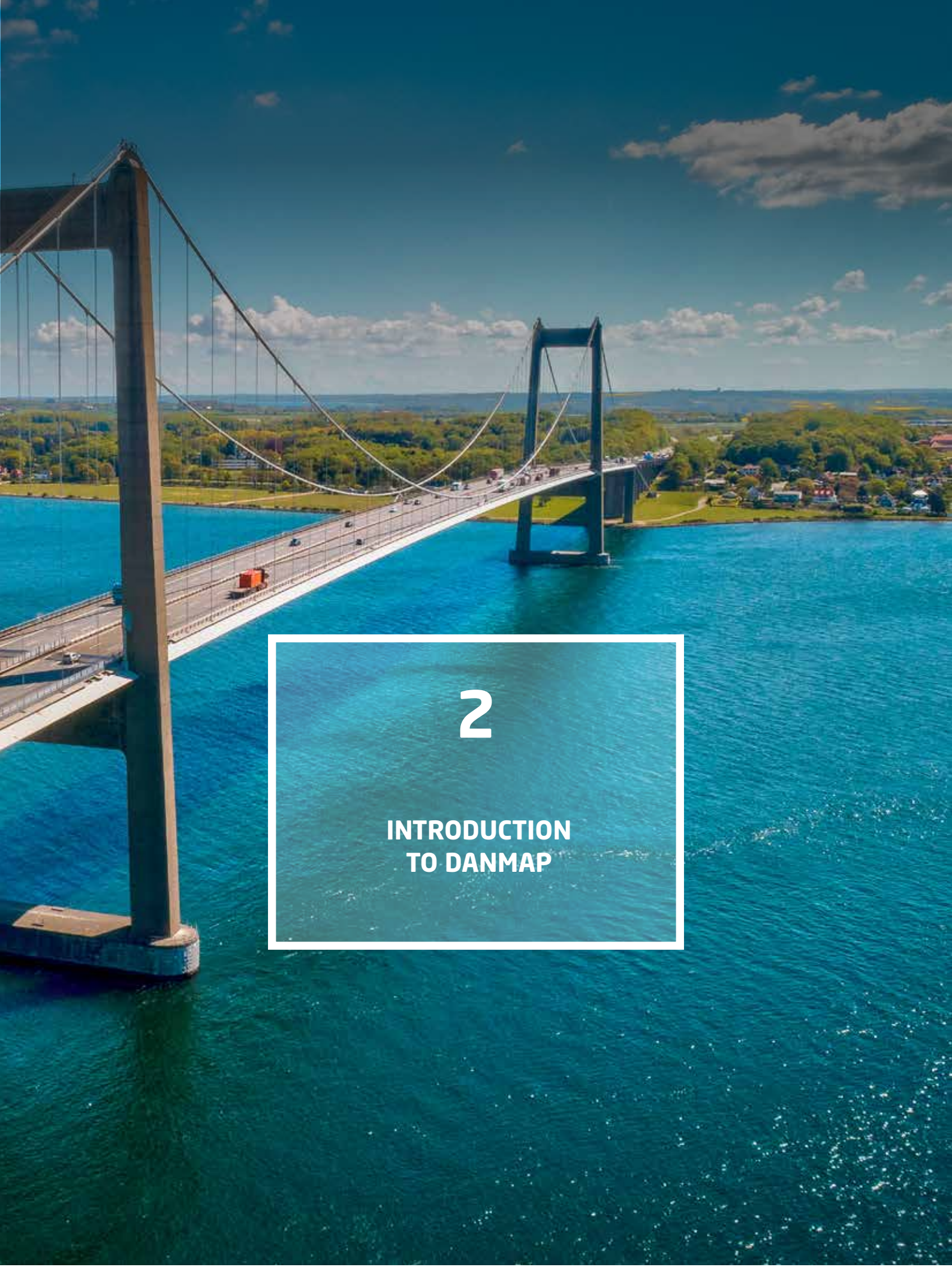
- The Danish Health Data Authority and the Register of Medicinal Products Statistics for providing data on antimicrobial consumption on healthcare activity
- All Danish hospital pharmacies for providing data on antimicrobials consumed at hospitals through special deliverances



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

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

The monitoring programme was initially developed in 1995 by researchers, based on frequent discussions and exchange of knowledge and results from research. Since then, DANMAP has evolved into a governmentally supported organisation.

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

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

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

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

Three categories of bacteria are always included in DANMAP:

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

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

The DANMAP programme is funded jointly by the Ministry of Health and the Ministry of 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.

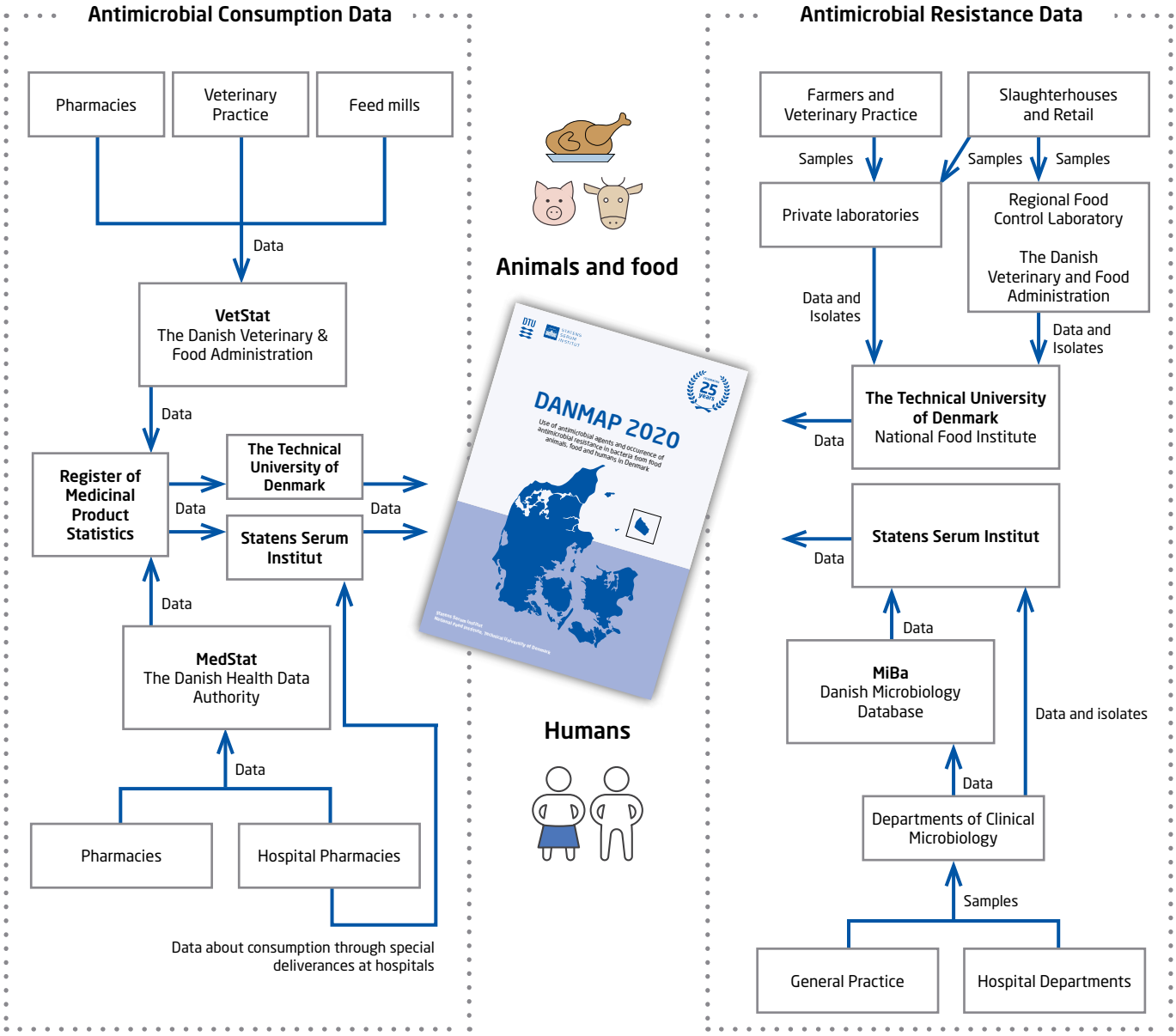
For further information on the development and history of DANMAP, please read chapter 2, "DANMAP – A 20 year perspective" in DANMAP 2015.

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

Figure 2.1 Organisation of the DANMAP collaboration regarding data and data flow DANMAP 2020



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 2020, 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 2020

North Denmark Region

No. of inhabitants	589,936
No. of inhabitants/km^2	75
No. of inhabitants/GP	2,070

Central Denmark Region

No. of inhabitants	1,326,340
No. of inhabitants/km^2	102
No. of inhabitants/GP	1,662

Capital Region of Denmark

No. of inhabitants	1,846,023
No. of inhabitants/km^2	720
No. of inhabitants/GP	1,787

Region Zealand

No. of inhabitants	837,359
No. of inhabitants/km^2	116
No. of inhabitants/GP	1,924

Region of Southern Denmark

No. of inhabitants	1,223,105
No. of inhabitants/km^2	100
No. of inhabitants/GP	1,578

GP = general practitioner
DCM = department of clinical microbiology
NRL = National Reference Laboratories

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 25 years, the human population in Denmark has increased from approximately 5.2 million inhabitants in 1995 to 5.8 million in 2020 [www.dst.dk]. Simultaneously, the average age has increased gradually. In 2020, the national average age was 41.8 years. The population and the respective regional distribution, in 2020, 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 differed from 2019. Figure 2.4 shows the number of consultations in general per thousand inhabitants from 2011 to 2020. The number of per 1,000 inhabitants consultations was 8.5% higher in 2020 compared to 2019.

Data on regional and national health care activity at hospitals in 2011 and 2020 are presented in Table 2.1. Denmark has a very high bed occupancy rate at hospitals and can reach capacity during winter time for example due to high influenza activity. In 2020, the number of admissions at Danish somatic hospitals was registered to be 706,562 and the number of bed-days was registered to be 3,046,919. From 2011-2020, the number of bed-days decreased by 22%, the number of admissions decreased by 10% whereas the Danish population grew by 5.0%.

Chapter 3 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, 2011-2020 DANMAP 2020

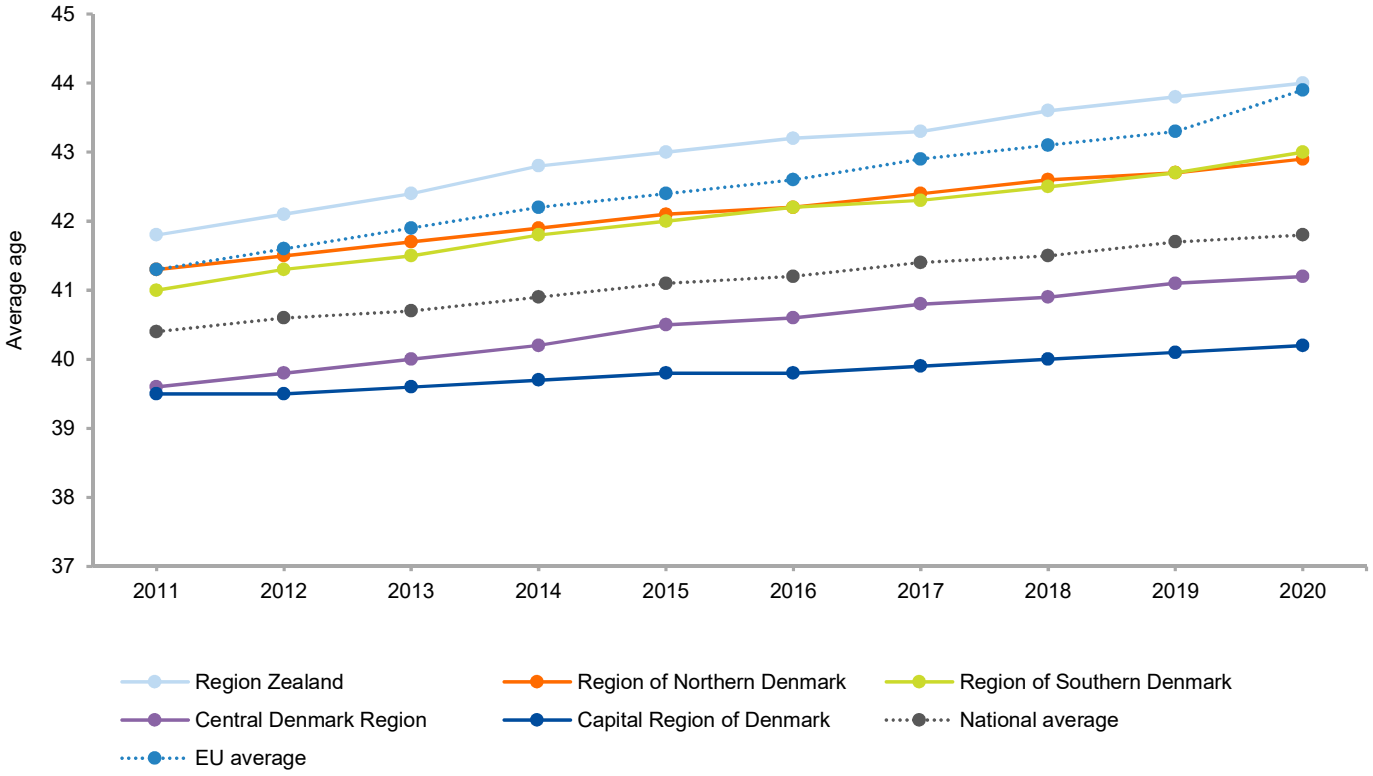
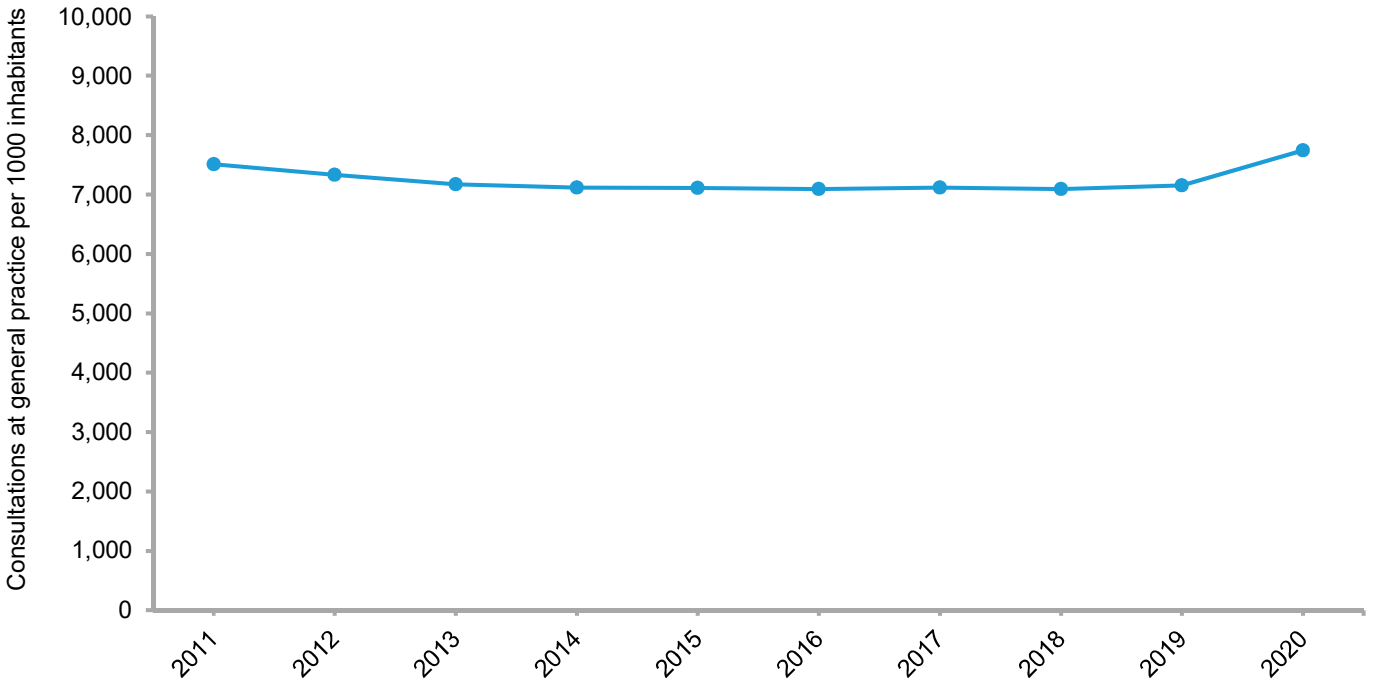


Figure 2.4 Number of consultations per 1,000 inhabitants in general practice, Denmark, 2011-2020 DANMAP 2020



Data were extracted from The National Health Insurance Service Registry and Register of Health Insurance Service Providers

Table 2.1 Activity at Danish hospitals, 2011 and 2020 DANMAP 2020

Region	Number of bed-days at somatic hospitals		Number of admission to somatic hospitals		Population	
	2011	2020	2011	2020	2011	2020
Capital Region of Denmark	1,350,091	1,028,383	259,339	240,051	1,699,387	1,846,023
Region Zealand	552,847	447,788	105,625	103,104	819,763	837,359
Region of Southern Denmark	803,531	621,465	168,510	146,092	1,200,656	1,223,105
Central Denmark Region	796,032	630,075	176,191	147,458	1,260,993	1,326,340
North Denmark Region	412,798	319,209	79,654	69,857	579,829	589,936
Denmark	3,915,299	3,046,919	789,319	706,562	5,560,628	5,822,763

Data 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

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, chicken and mink. 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 and mink, Denmark DANMAP 2020

Year	Pigs		Cattle		Poultry		Fur animals - mink	
	Total	Exported[a]	Slaughter cattle	Dairy cows	Broilers	Turkeys[b]	Females	Kits
2011	29399	7632	551	575	115454	960	2776	15325
2012	29047	8794	539	580	111080	1103	2936	16147
2013	28996	9318	551	574	117315	692	3143	17634
2014	30002	10517	556	563	115497	595	3296	17963
2015	30874	11563	513	561	114238	598	3387	18798
2016	31660	12771	540	571	120685	834	3161	17260
2017	31662	13679	509	570	117602	601	3410	18378
2018	32571	14028	533	575	122268	642	3385	17634
2019	31694	14542	518	567	123976	661	2495	13224
2020	32025	14399	500	567	120508	684	2492	13459

Source: Statistics Denmark [www.dst.dk] and Kopenhagen Fur. Export data for poultry from Statistics Denmark (personal communication) and export of 15-50 kg live pigs from the Danish Agriculture and Food Council
a) Export of 15-50 kg live pigs. These are included in total number of heads, but antimicrobial use after export until slaughter is not registered as it takes place outside of Denmark
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 2020

Year	Pork	Beef	Broiler meat[a]	Turkey meat	Milk	Farmed fish[b]	
						Land based	Marine net ponds
2011	2008	145	175	9	4801	40	11
2012	1902	138	168	12	4928	44	14
2013	1896	140	177	8	5025	48	15
2014	1924	143	174	9	5113	47	14
2015	1954	135	172	9	5278	52	16
2016	1943	142	182	10	5376	49	12
2017	1896	135	178	7	5478	51	14
2018	1967	141	185	10	5615	53	14
2019	1870	137	187	8	5615	53	14
2020	1955	132	195	8	5666	56	14

Source: Statistics Denmark [www.dst.dk]. Export data for poultry and average weight after slaughter from Statistics Denmark (personal communication)
a) In 2020, a final slaughtered weight of 1.62 kg per broiler produced and 12.0 kg per turkey produced were assumed
b) The numbers for 2020 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 2020

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
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, tedizolide, fosfomycin
QJ01XQ	Pleuromutilins	Tiamulin, valnemulin	
QP51AG04	Antiprotozoals, sulfonamides	Sulfaclozine	
Not in ATCvet [b]	Oligosaccharides	(Avilamycin)	
Not in ATCvet [b]	Flavofosfolipols	(Flavomycin)	

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

3

IMPACT OF THE COVID-19 PANDEMIC

3. Impact of the COVID-19 pandemic

Highlights

The total number of consultations in **primary health care** was 7% higher in 2020 compared to 2019. Consultation types changed during the pandemic with a shift to phone/video/email appointments - only 36% of consultations were held face-to-face in April 2020 compared to 57% in April 2019. The proportion of phone/video/email appointments decreased after easing of restrictions in May 2020 but continued to stay higher for the rest of the year compared to 2019.

The total number of prescriptions issued in primary care per 1,000 inhabitants dropped sharply following the national lockdown introduced in Denmark in March 2020. The reduction was driven by fewer prescriptions of beta-lactamase sensitive penicillins and macrolides for respiratory infections. This probably reflected changes in patients' healthcare seeking behaviour and the impact of national measures aimed at reducing transmission of SARS-CoV-2 on the incidence of bacterial respiratory infections. Numbers of prescriptions increased again in May/June 2020 but stayed lower for the rest of 2020 compared to 2018 and 2019.

All doctor types in primary care (e.g. general practitioners, ear-nose-throat specialists, dermato venereology specialists, dentists, hospital doctors prescribing for patients in the community) issued fewer prescriptions during the first pandemic wave. Following the easing of restrictions in May/June 2020 general practitioners continued to prescribe fewer antimicrobials but hospital doctors issued more prescriptions for patients in the community than in previous years, possibly due to earlier discharge/fewer admissions of patients to hospital.

The number of **hospital** admissions decreased sharply during the first pandemic wave in March 2020 and was 27% lower in April 2020 compared to April 2019. Admissions returned to levels seen in previous years over the summer but dropped slightly again in November 2020 with increasing incidence of SARS-CoV-2 cases.

Total consumption of antimicrobials in hospitals was markedly lower during the first wave compared to the same time period in 2018 and 2019 when measured in Daily Defined Doses (DDD). This reflects the decreased number of patients in hospitals following cancellations of non-urgent procedures and rapid discharge of patients to free up beds for critical care. However, consumption was higher during the first and second wave than in previous years when measured in DAD (DDD per 100 admissions). The higher use **per patient admitted** was most likely due to the different case mix in hospitals, i.e. more seriously ill patients.

This trend was mirrored in the consumption of the main antimicrobial groups used for treatment of seriously ill patients (carbapenems, combinations of penicillins, incl. beta-lactamase inhibitors). Usage of these critical antimicrobials was markedly higher than in previous years when measured in DAD, i.e. consumption **per patient admitted** to hospital, especially during the first pandemic wave.

3.1 Introduction

In March 2020, the World Health Organisation (WHO) declared a rapidly spreading outbreak caused by a novel coronavirus (SARS-CoV-2) a pandemic. The first confirmed SARS-CoV-2 case in Denmark had already been reported on 27 February 2020. Due to the high transmissibility of the virus and the clinical severity of the associated coronavirus disease (COVID-19) a lockdown of society was introduced by the Danish Government in March 2020 and gradually lifted from mid-April 2020. The lockdown was reintroduced during late autumn/winter 2020 due to a second wave of the pandemic. These restrictions as well as the actual COVID-19 cases affected healthcare delivery and public life in Denmark throughout 2020 and beyond. The DANMAP report 2020 reflects this by including a new chapter, which explores aspects of healthcare delivery and antimicrobial consumption in the context of the COVID-19 pandemic.

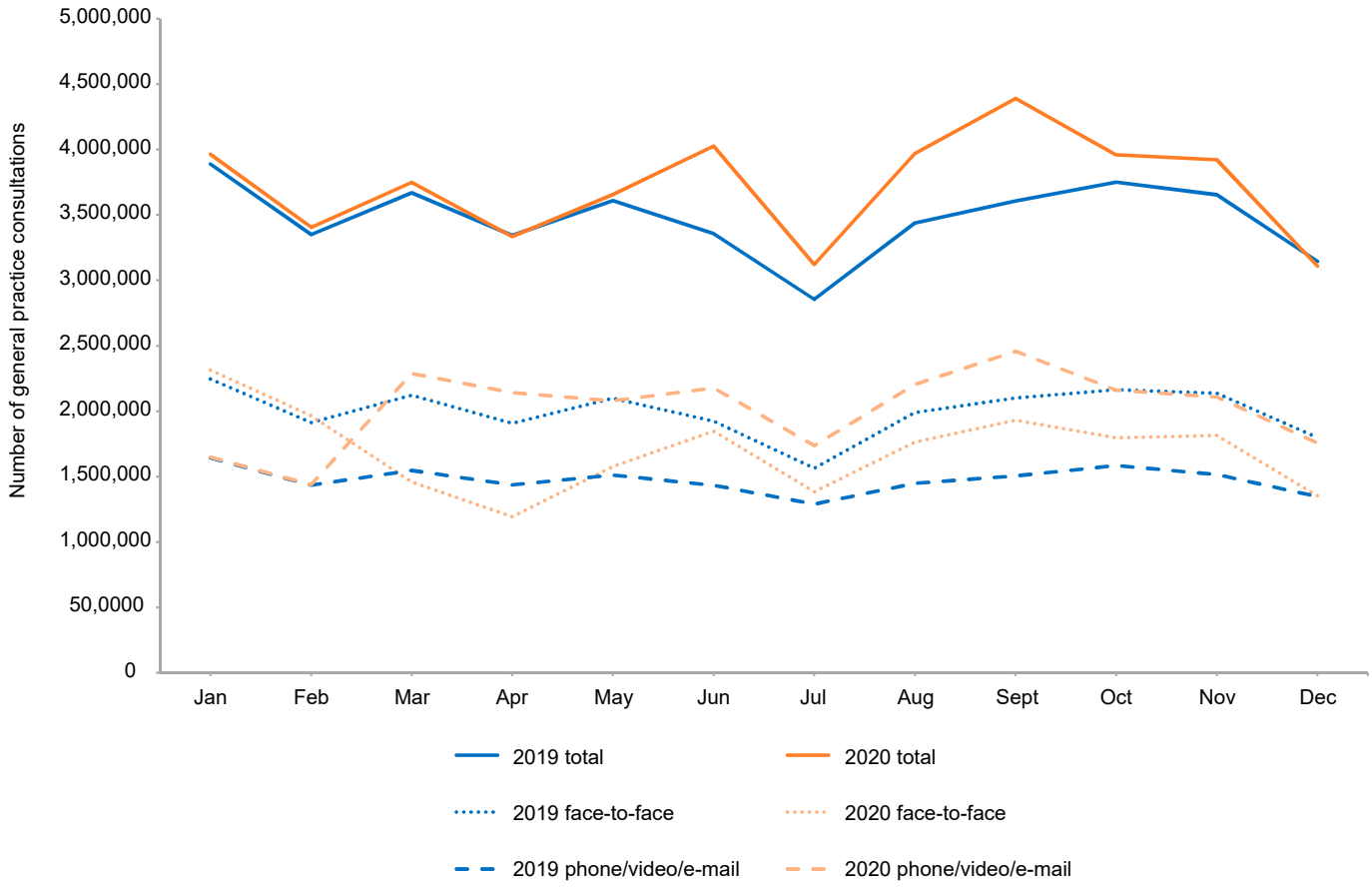
Antimicrobial consumption data are shown for the first time by month in a DANMAP report to allow a more granular analysis of the impact of the COVID-19 pandemic on prescribing trends in 2020 compared to previous years. The 'first wave' of the pandemic was defined as the time period from 27 February 2020 (first SARS-CoV-2 case in Denmark) to the beginning of May 2020 (low incidence of SARS-CoV-2 cases, most restrictions lifted) and the 'second wave' from the beginning of December 2020 (start of exponential growth of SARS-CoV-2 incidence and second series of restrictions/lockdown [large shops, high schools are closing]).

3.2 Impact of COVID-19 on healthcare delivery

3.2.1 Consultations in primary health care, 2019-2020

The total number of consultations was 7% higher in 2020 compared to 2019 (Figure 3.1). When comparing types of consultations (face-to-face/phone/video/email) a clear shift to phone/video/email consultations can be seen during the pandemic's first wave – only 36% of consultations were held face-to-face in April 2020 compared to 57% in April 2019. The proportion of phone/video/email consultations decreased again after easing of restrictions in May 2020 but continued to stay higher for the rest of the year compared to the same time period in 2019.

Figure 3.1 Total number of general practice consultations and types of consultations by month, Denmark, 2019-2020 DANMAP 2020

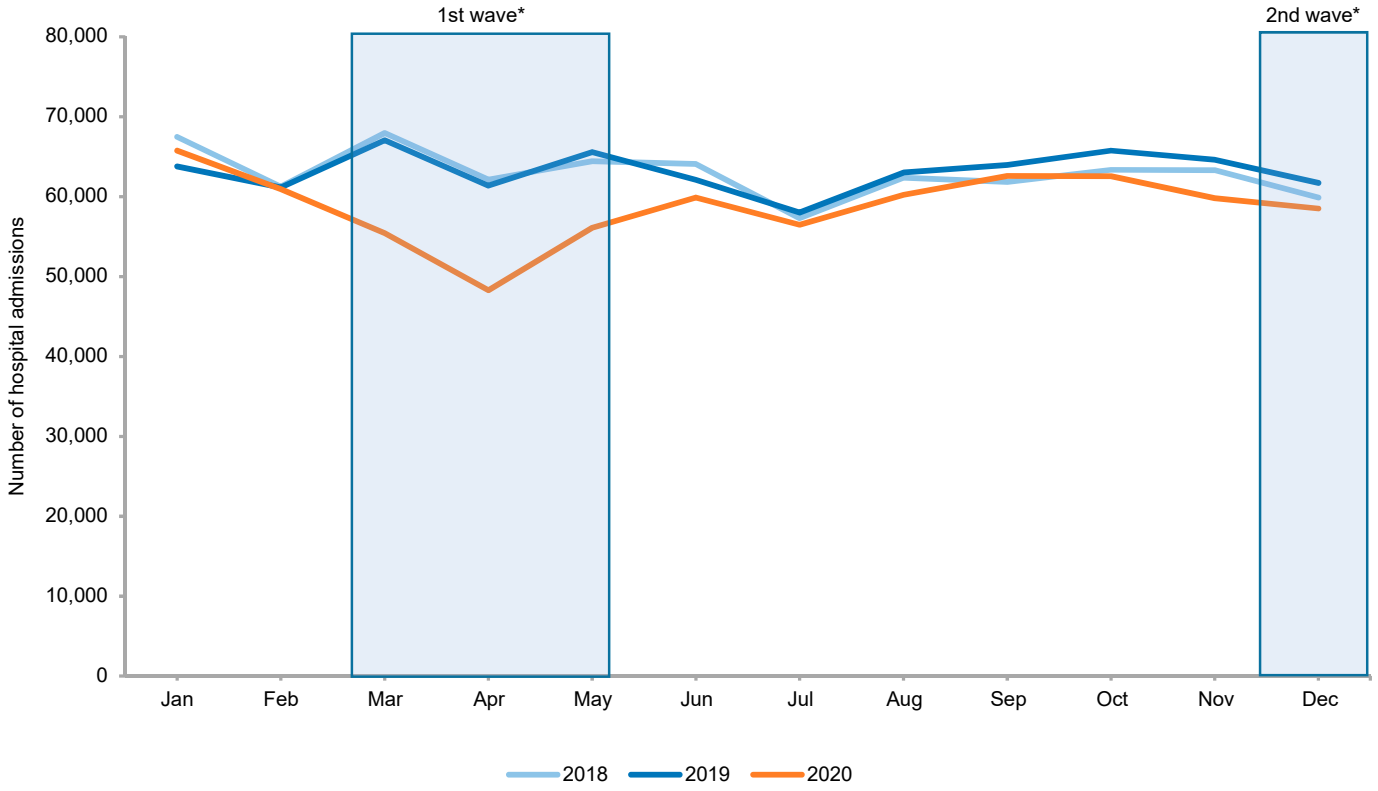


Data were extracted by The Danish Health Data Authority from The National Health Insurance Service Registry and Register of Health Insurance Service Providers

3.2.2 Hospital admissions, 2018-2020

The number of hospital admissions was similar to previous years at the beginning of 2020. However, admission numbers decreased sharply during the first pandemic wave in March 2020 and were 21% lower in April 2020 compared to April 2019 (Figure 3.2). Numbers returned to levels seen in previous years over the summer and autumn but dropped slightly again in November 2020 with increasing incidence of SARS-CoV-2 cases. When using bed-days to monitor hospital activity, similar trends were observed (data not shown).

Figure 3.2 Number of admissions to somatic hospitals by month, Denmark, 2018-2020 DANMAP 2020



Data are based on new definitions implemented in The National Patient Register in 2019
* The 'first wave' of the pandemic is defined as the period from 27 February 2020 (first SARS-CoV-2 cases in Denmark) to beginning of May 2020 (low incidence of SARS-CoV-2 cases, most restrictions lifted) and the 'second wave' as week beginning of December 2020 (start of exponential growth of incidence and second series of restrictions/lockdown [large shops, high schools are closing])

3.3 Impact of COVID-19 on antimicrobial consumption in primary health care

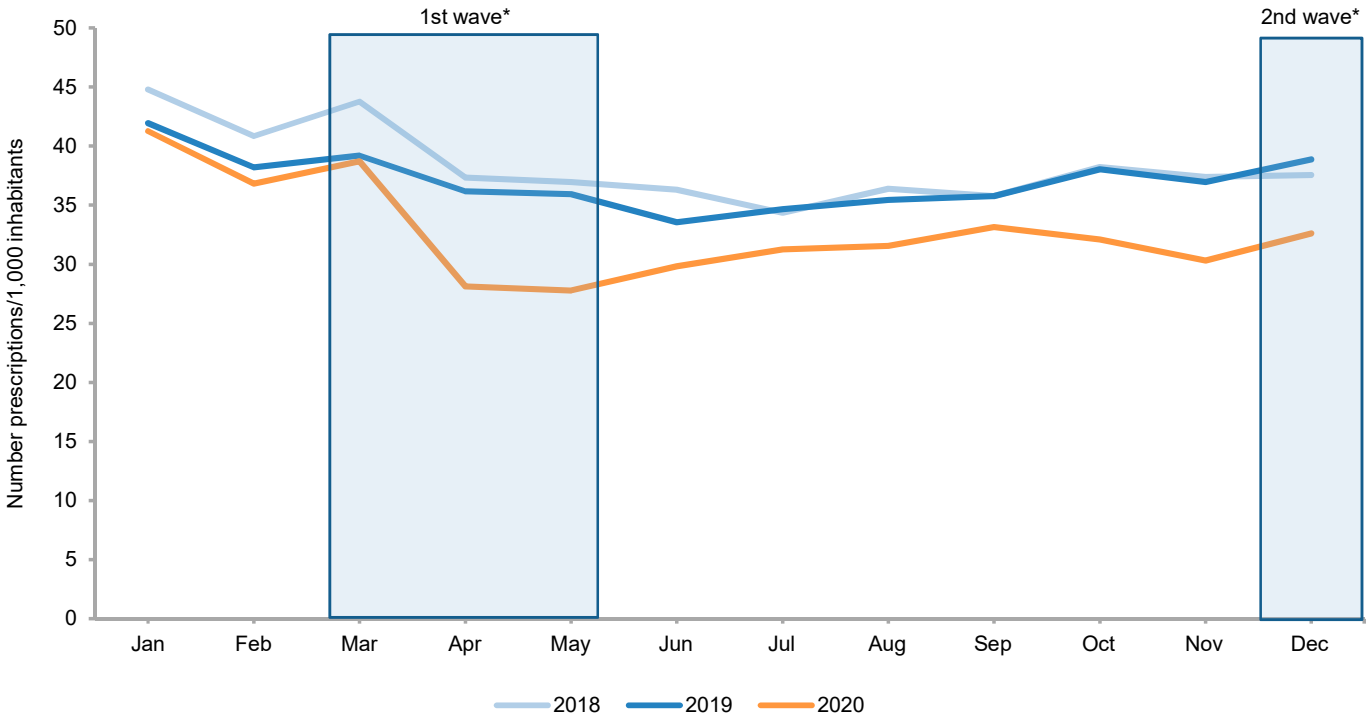
3.3.1 Total consumption

The number of total prescriptions per 1,000 inhabitants issued in primary health care was slightly lower at the beginning of 2020 compared to 2018 and 2019 (Figure 3.3). This continued a trend of decreased prescribing by GPs following the Government's introduction of the National Action Plan in 2017. However, consumption of antimicrobials dropped sharply during the first pandemic wave and continued to stay lower for the rest of the year compared to 2018 and 2019.

These changes in prescribing levels could be due to changes in healthcare access and healthcare seeking behaviour by patients as discussed in the above section 3.2 'Impact of COVID-19 on healthcare delivery'. In addition, restrictions aimed at reducing transmission of SARS-CoV-2 also reduced the incidence of respiratory infections caused by other pathogens, for example *Streptococcus pneumoniae* and group A streptococci. The numbers of invasive *S. pneumoniae* isolates, i.e. the most serious outcome of *S. pneumoniae* infections, reported to the Danish Microbiology database (MiBa) were noticeably lower during the COVID-19 pandemic than in 2018 and 2019 (see Figure 3.4 and section 8.3.5 *'Streptococcus pneumoniae'* for more information). The same trends for the pandemic year are shown for group A streptococci in section 8.3.6 'Beta-haemolytic streptococci'. Given that streptococcal community-acquired pneumonia, pharyngitis or tonsillitis warrant antimicrobial treatment by GPs, a significant drop in the incidence of these bacteria will also result in fewer prescriptions.

Figure 3.3 Total consumption of antimicrobial agents for systemic use in primary health care by month, prescriptions per 1,000 inhabitants, Denmark, 2018-2020

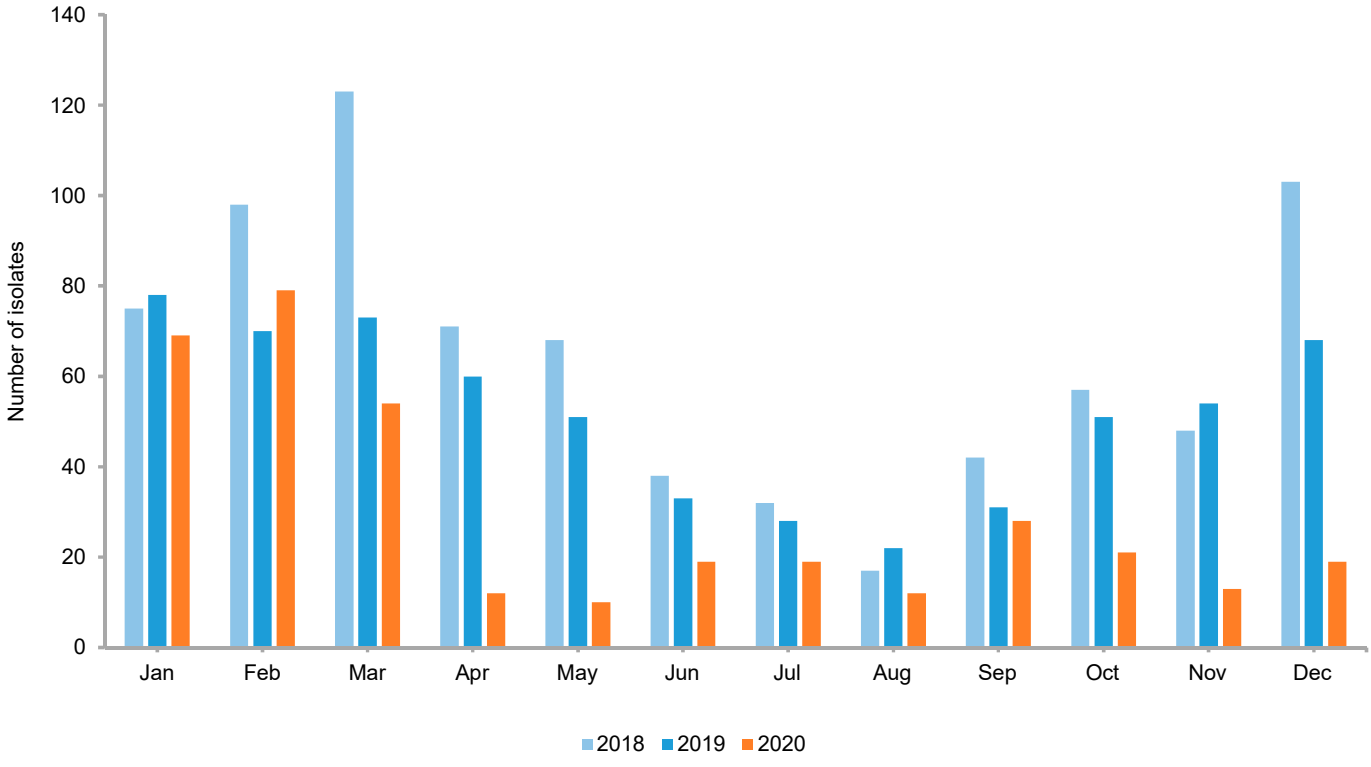
DANMAP 2020



Data used in this figure is based on registered sales to individuals
Data are based on the 2021 edition of the Anatomical Therapeutic Chemical (ATC) classification system
* The 'first wave' of the pandemic is defined as the period from 27 February 2020 (first SARS-CoV-2 cases in Denmark) to beginning of May 2020 (low incidence of SARS-CoV-2 cases, most restrictions lifted) and the 'second wave' as week beginning of December 2020 (start of exponential growth of incidence and second series of restrictions/lockdown [large shops, high schools are closing])

Figure 3.4 Number of invasive isolates of *Streptococcus pneumoniae*, Denmark, 2018-2020

DANMAP 2020



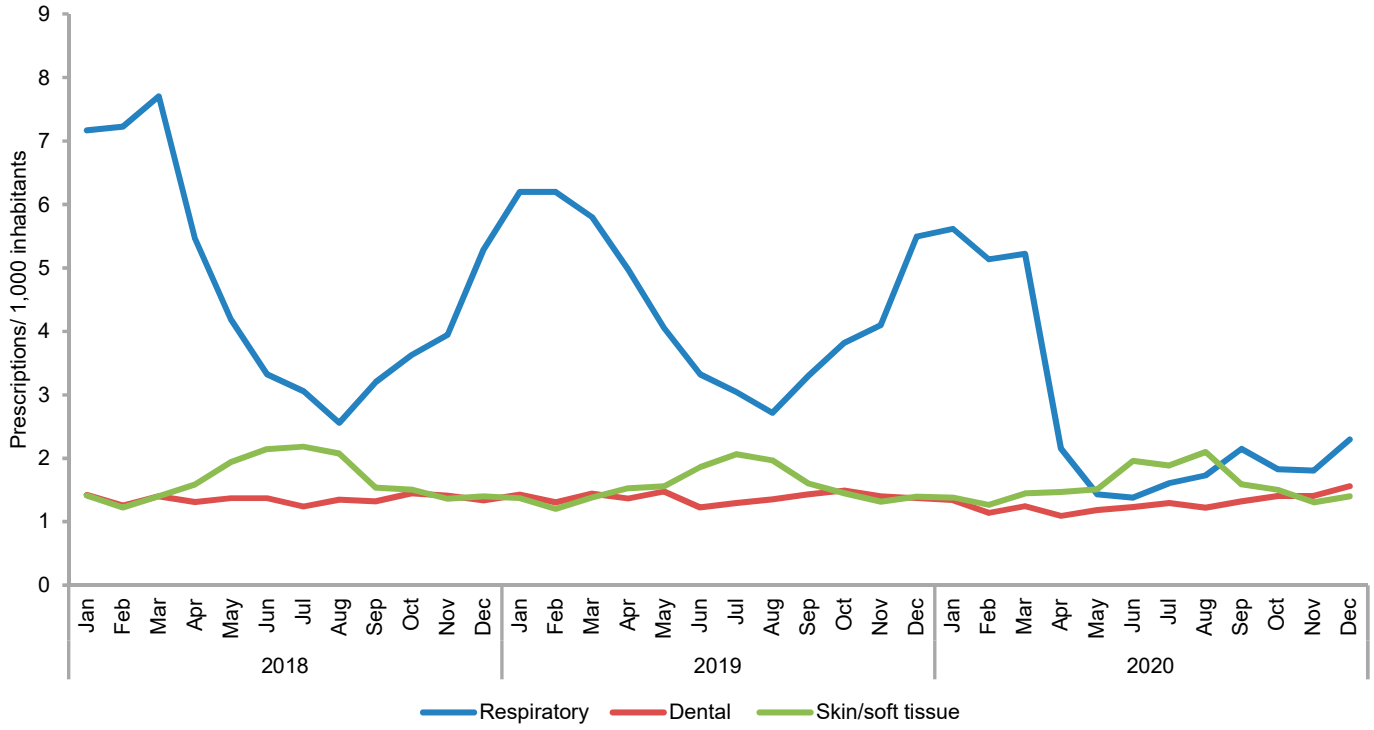
3.3.2 Consumption of selected key antimicrobials including prescribing indications

The main indications provided by prescribers in primary health care for treatment with beta-lactamase sensitive penicillins are upper/lower respiratory infections, skin & soft tissue infections and oral infections.

In 2020, consumption of beta-lactamase sensitive penicillins prescribed for treatment of respiratory infections showed slightly lower levels compared to previous years until March followed by a sharp drop and lower levels from April onwards (Figure 3.5). Beta-lactamase sensitive penicillins were also less prescribed for oral 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. Consumption of beta-lactamase sensitive penicillins prescribed for skin & soft tissue infections was on a similar level in 2020 as in the two previous years.

Figure 3.5 Consumption of beta-lactamase sensitive penicillins in primary health care by indication, prescriptions per 1,000 inhabitants, Denmark, 2018-2020

DANMAP 2020



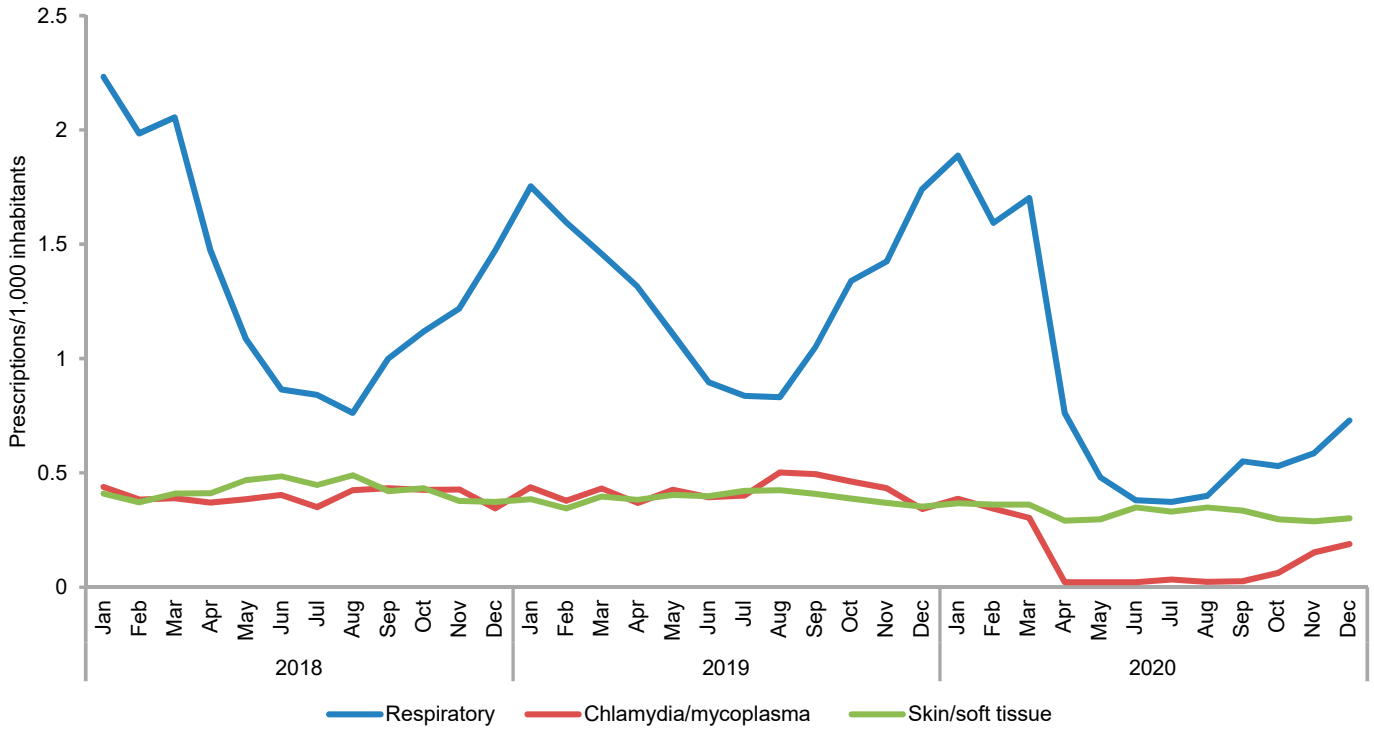
Data used in this figure is based on registered sales to individuals
Data are based on the 2021 edition of the Anatomical Therapeutic Chemical (ATC) classification system

The number of macrolide prescriptions for respiratory infections followed the same pattern as beta-lactamase sensitive penicillin prescriptions with a significant drop and lower levels from April 2020 onwards most likely reflecting the impact of restriction measures on the incidence of respiratory infections (Figure 3.6).

Macrolide prescriptions for skin & soft tissue infections showed same seasonality but slightly lower levels from April 2020 onwards compared to previous years.

Prescriptions for chlamydia/mycoplasma infections were slightly lower at the beginning of 2020 compared to 2018 and 2019. This could be due to a change in guidance for chlamydia infections from first-line treatment with azithromycin (macrolide) to doxycycline (tetracycline) published in 2019. A further, very pronounced reduction in prescriptions was reported in March 2020. The number of Chlamydia infections captured by SSI's surveillance (Overvågning i tal, grafer og kort https://statistik.ssi.dk/) show that numbers were low during the national lockdown between March and May 2020. This could be due to more limited healthcare access and sampling of specimen but behavioural changes and limited social contact may have played a role as well and led to a true reduction in Clamydia transmission during lockdown. Macrolide prescribing for chlamydia/mycoplasma infections stayed low for the rest of 2020 - despite an increase in Chlamydia incidence from June 2020 onwards - which could be due to adherence to the new guideline.

Figure 3.6 Consumption of macrolides in primary health care by indication, prescriptions per 1,000 inhabitants, Denmark, 2018-2020
DANMAP 2020



Data used in this figure is based on registered sales to individuals
Data are based on the 2021 edition of the Anatomical Therapeutic Chemical (ATC) classification system

3.3.3 Prescribing by doctor type in primary health care

The number of prescriptions per 1,000 inhabitants decreased markedly during the first pandemic wave for all doctor types (general practitioners, ear-nose-throat specialists, dermato- venereology specialists, dentists and hospital doctors prescribing for patients in the community) in primary health care. This is likely due to the lower incidence of infections described in the above sections as well as changes in access to healthcare.

Following the easing of restrictions in Denmark in May/June 2020 the level of prescribing by general practitioners increased but stayed below the levels recorded for 2018 and 2019 until the end of 2020. Dentists issued similar numbers of prescriptions compared to previous years following lifting of lockdown. However, prescriptions issued by hospital doctors for patients in the community rose above the levels seen in previous years after lockdown was lifted (Figure 3.7). These differences in prescribing behaviour highlight that the impact of the pandemic affected healthcare providers differently and the importance of targeted antimicrobial stewardship in primary health care.

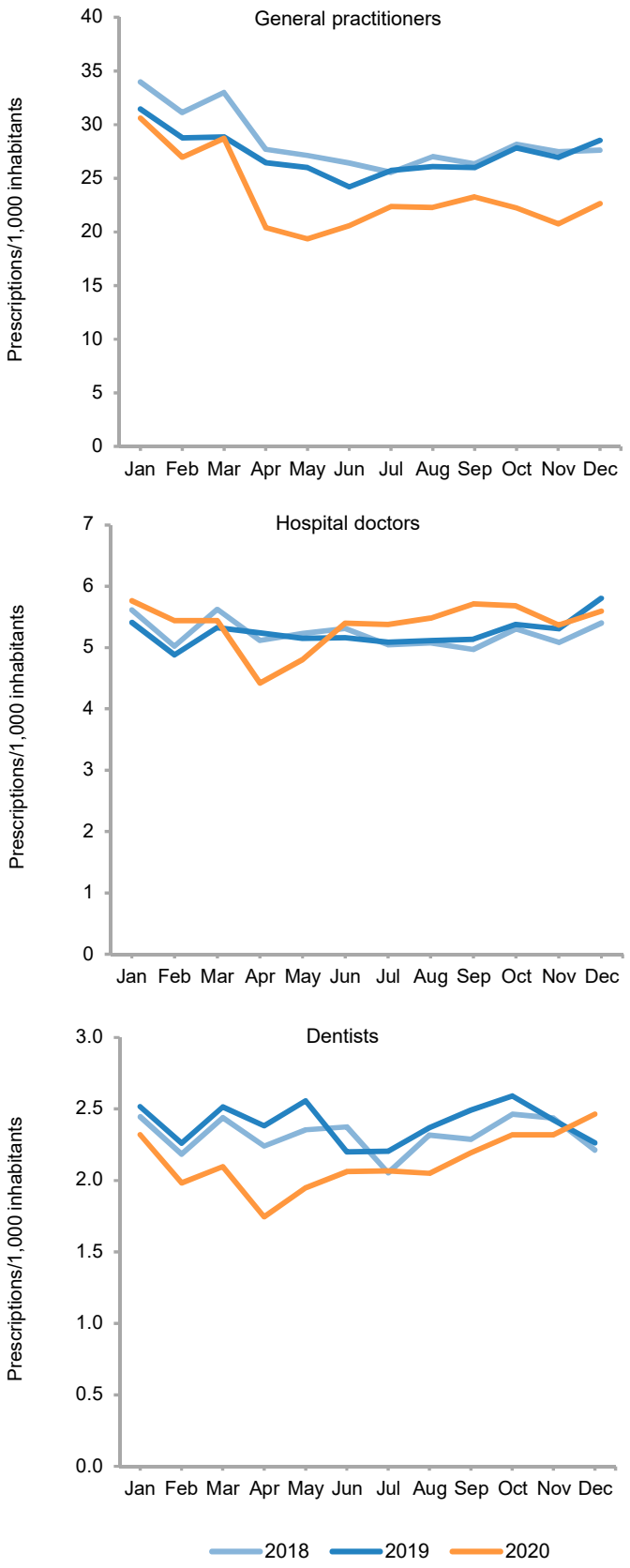
3.4 Impact of COVID-19 on antimicrobial consumption in hospital care

3.4.1 Total consumption

Total hospital consumption of systemic antimicrobial agents measured in Daily Defined Doses (DDD) was lower most months in 2020 compared to the same months in 2018 and 2019. This trend was most marked during the first pandemic wave (Figure 3.8) and probably reflects the impact of the pandemic on hospital care, i.e. cancellation of non-urgent procedures leading to fewer hospital admissions and consequently fewer patients requiring antimicrobial treatment such as surgical prophylaxis. Consumption measured in DDD returned to similar levels seen in 2018 and 2019 during summer and autumn 2020 ("return to normal") but increased above previous years' levels during the second wave.

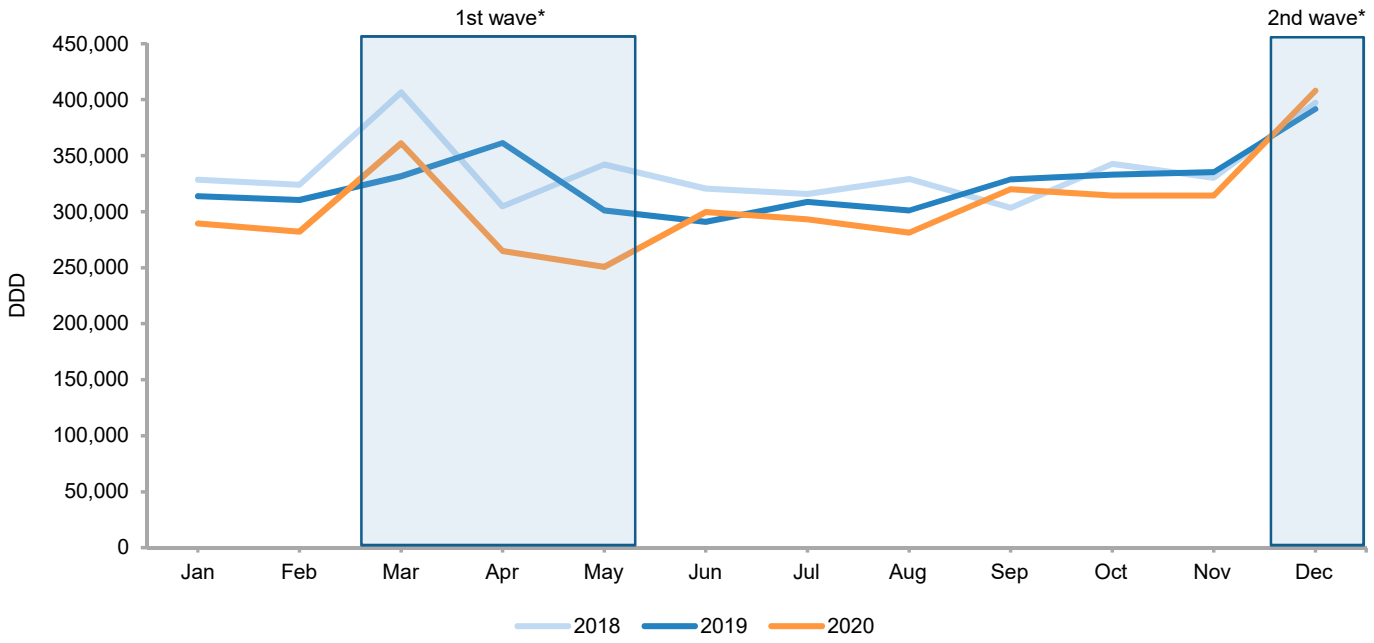
However, when measuring antimicrobial consumption in relation to hospital activity using Daily Defined Doses per 100 admissions (DAD), a steep increase in consumption per patient admitted was observed in March and April 2020 (Figure 3.9). It probably reflects the change in patient-mix during the first pandemic wave with fewer but mostly seriously ill patients. It may also reflect changes in behaviour, i.e a more risk-averse approach by prescribers, due high occupancy rates on many intensive care units and the uncertainty around treatment of COVID-19 patients in the first few weeks of the pandemic.

Figure 3.7 Number of prescriptions in primary care issued by doctor type, prescriptions per 1,000 inhabitants, Denmark, 2018-2020 DANMAP 2020



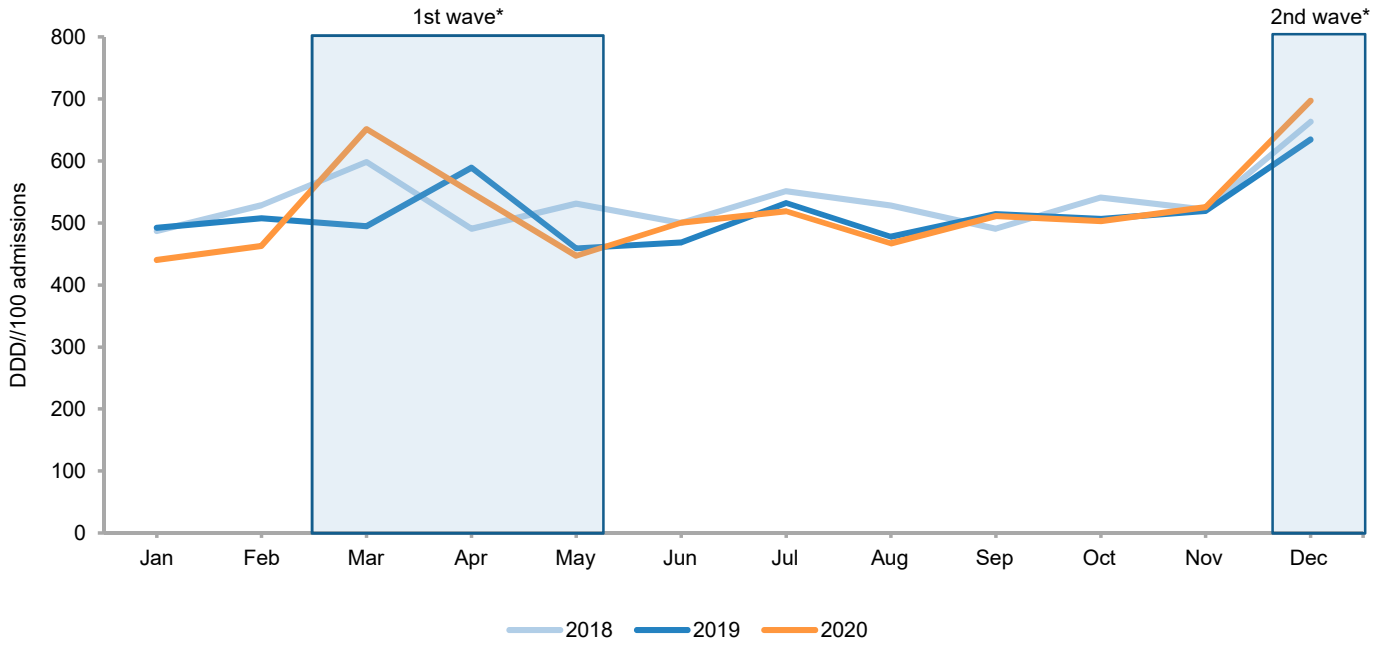
Data used in this figure is based on registered sales to individuals
Data are based on the 2021 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 3.8 Total consumption of antimicrobial agents for systemic use in somatic hospitals, Daily Defined Doses (DDD), Denmark, 2018-2020
DANMAP 2020



Data are based on the 2021 edition of the Anatomical Therapeutic Chemical (ATC) classification system
* The 'first wave' of the pandemic is defined as the period from 27 February 2020 (first SARS-CoV-2 cases in Denmark) to beginning of May 2020 (low incidence of SARS-CoV-2 cases, most restrictions lifted) and the 'second wave' as week beginning of December 2020 (start of exponential growth of incidence and second series of restrictions/lockdown [large shops, high schools are closing])

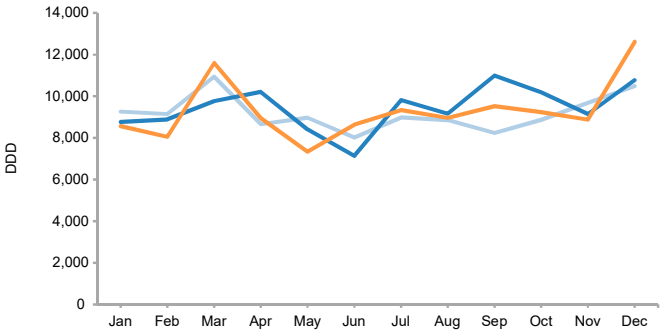
Figure 3.9 Total consumption of antimicrobial agents for systemic use in somatic hospitals, DDD per 100 admissions (DAD), Denmark, 2018-2020
DANMAP 2020



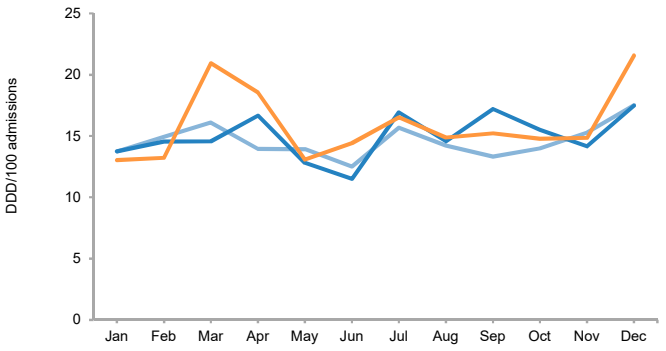
Data are based on the 2021 edition of the Anatomical Therapeutic Chemical (ATC) classification system
Data on admissions are based on new definitions implemented in The National Patient Register in 2019
* The 'first wave' of the pandemic is defined as the period from 27 February 2020 (first SARS-CoV-2 cases in Denmark) to beginning of May 2020 (low incidence of SARS-CoV-2 cases, most restrictions lifted) and the 'second wave' as week beginning of December 2020 (start of exponential growth of incidence and second series of restrictions/lockdown [large shops, high schools are closing])

Figure 3.10 Consumption of key antimicrobials used for treatment of seriously ill patients in hospital, measured in DDD and DAD, Denmark, 2018-2020 DANMAP 2020

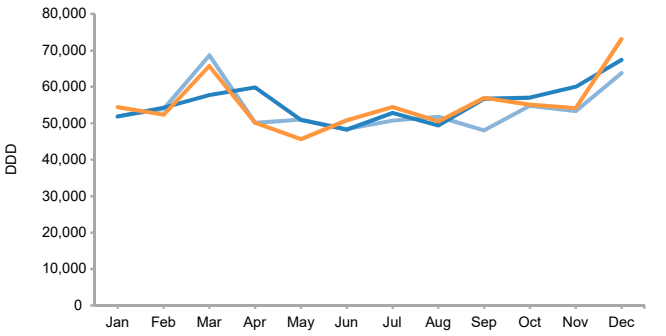
a) carbapenems (measured in DDD)



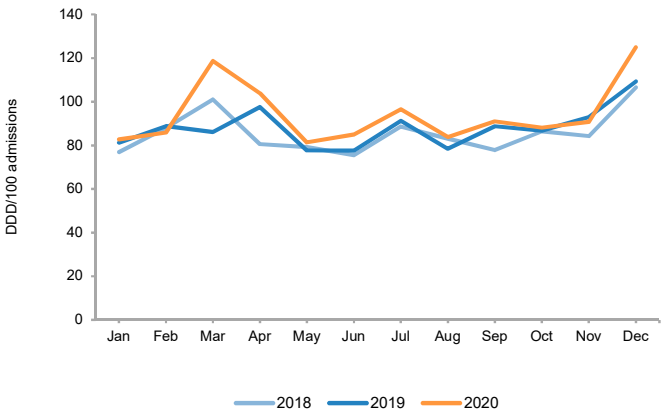
b) carbapenems (measured in DAD)



c) combinations of penicillins (incl. inhibitors) (measured in DDD)



d) combinations of penicillins (incl. inhibitors) (measured in DAD)



Data are based on the 2021 edition of the Anatomical Therapeutic Chemical (ATC) classification system
Data on admissions are based on new definitions implemented in The National Patient Register in 2019

3.4.2 Consumption of key antimicrobial groups in hospitals, DDD and DAD, 2018-2020

Consumption of the main antimicrobial groups for treatment of critically ill patients at hospitals is shown in Figure 3.10. Usage levels measured in DDD of carbapenems (ertapenem, imipenem, meropenem) and penicillin/beta-lactamase inhibitor combinations (amoxicillin/clavulanic acid, piperacillin/tazobactam) were similar in 2020 compared to 2018 and 2019 despite fewer patients admitted to hospital (section 3.2.2 'Hospitals admissions, 2018-2020'). However, when measured in DAD, i.e. in relation to the number of hospital admissions, it shows high levels of consumption per patient admitted during both pandemic waves in 2020. The value of preserving these antimicrobials, also highlighted by the National Action Plan's goal to reduce the use of antimicrobials of special critical interest (carbapenems, cephalosporins, fluoroquinolones), stresses the importance of monitoring consumption levels and of the continuation of antimicrobial stewardship activities at local and national level.

We would like to acknowledge Maja Laursen, Frederik Løgstrup Magnusson, Mads Nørgaard-Madsen, Rikke Thoft Nielsen, Steen Hoffmann and Ute Wolff Sönksen.

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4

ANTIMICROBIAL CONSUMPTION IN ANIMALS

4. Antimicrobial consumption in animals

Highlights: The **total use** of antimicrobials in animals amounted to 99.5 tonnes of active compound in 2020. This breaks with the consistently decreasing trend observed from 2013 to 2019. Nonetheless, approximately 28 tonnes (-22%) less were used than in 2010.

The **pig sector** used approximately 76% of all veterinary-prescribed antimicrobials, equal to 75.9 tonnes active compound. Adjusting for changes in production and export of pigs in 2020, an estimated 2.3% of all pigs received antimicrobial treatment per day (23 DAPD), similar to the level in 2019. In weaner pigs, the use decreased from 91 in 2019 to 89 DAPD in 2020, while the use in finishers, sows and piglets remained at levels similar to 2019, 17 DAPD and 19 DAPD, respectively.

Over time the types of antimicrobials used in pigs have changed notably. The use of tetracyclines in pigs has decreased significantly since 2009, as a response to the differentiated Yellow Card initiative. Similarly, critically important antimicrobials have been phased out. During the same period, discernible, but smaller, increases in the use of macrolides and aminoglycosides occurred.

Following a 13% decrease in the use of zinc oxide from 2015 to 2019, the use of medical zinc oxide amounted to 494 tonnes in 2020, representing a 4% increase compared to 2019. This increase may partly be explained by the increased number of pigs produced in 2020. With an annual use of almost 500 tonnes medical zinc, the industry is quite far from the target of zero use, which must be reached by June 2022.

In 2020, antimicrobials prescribed for **cattle** amounted to 12.7 tonnes. Approximately two thirds were used to treat older cattle (>1 year). Over the past decade, the antimicrobial use for older cattle (>1 year) has decreased from 3.9 to 3.4 DAPD and increased from 5.5 to 8.7 DAPD in young cattle (<1 year). The Industry has banned the use of 3rd and 4th generation cephalosporins for cattle and no use was recorded for cattle in 2020.

The use of antimicrobials for **fur animals** (mink) decreased markedly in 2020. The overall use amounted to 2,454 kg in 2020, which was 38% less than in 2019 and equivalent to a treatment proportion of approximately 2% (19 DAPD). In the last months of 2020, the majority of Danish mink were culled due to the COVID-19 situation, and little or no antimicrobial use was registered for mink from October to December.

Since 2011, the use of antimicrobials in **dogs and cats** has decreased, with a marked reduction in the use of cephalosporins. In 2020, more than half of all veterinary-prescribed cephalosporins were prescribed for dogs and cats. In addition, all flouroquinolones and all 3rd and 4th generation cephalosporins were prescribed for pets.

4.1 Introduction

The DANMAP programme began monitoring the national use of antimicrobial agents in humans and animals in 1995. Since the early 1990s, there has been increased political and public focus on the consumption of antimicrobial agents in the Danish animal production. This has resulted in discontinued use 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.

Figure 4.1 presents the total use of antimicrobials in animals and humans since 1990 and 1997, respectively. Over time, the patterns of antimicrobial use in animals have been affected by risk management measures, established to reduce consumption, as well as by changes in the animal production, especially increases in pig production.

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

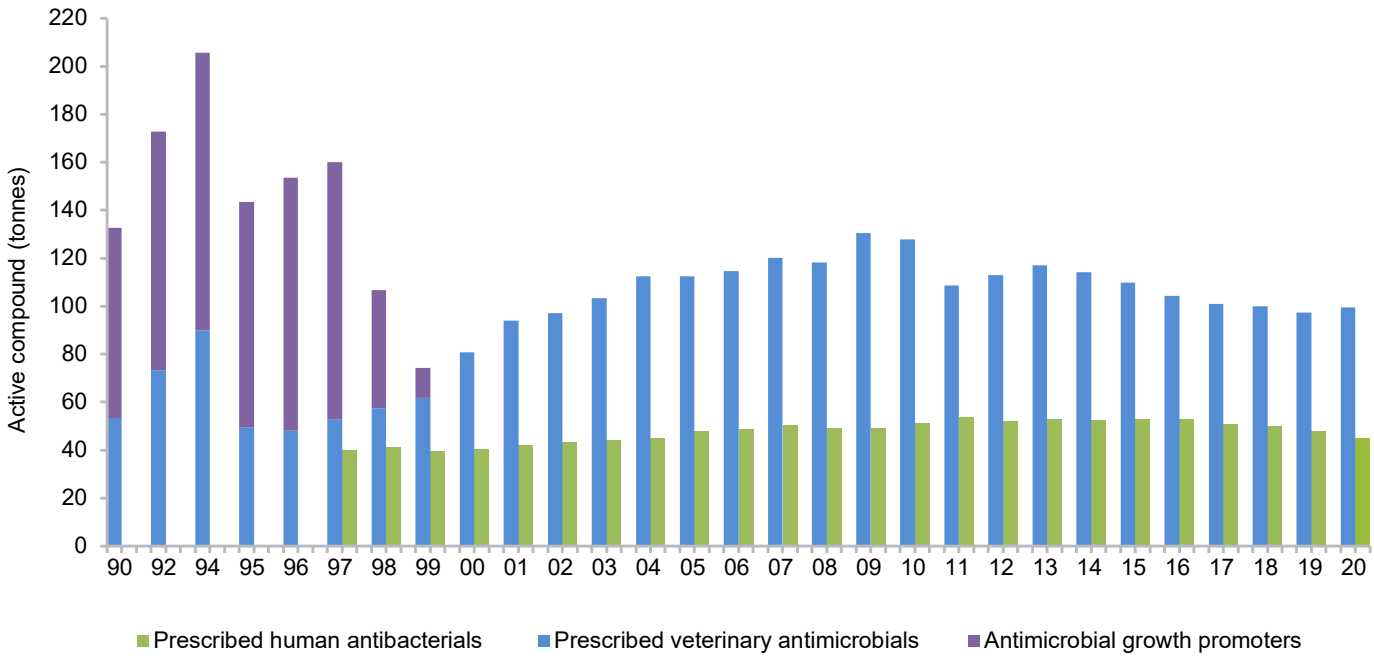
Other important interventions were the restriction on the use of fluoroquinolones in production animals through legislation implemented in 2002 and 2003 and the voluntary ban on the use of cephalosporins in the pig industry in 2010, followed by a similar initiative in the dairy cattle industry in 2014.

Furthermore, the cattle industry implemented a ban on use of 3rd and 4th generation cephalosporins for cattle from 1 September 2019.

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

To achieve the action plan goals, the Yellow Card initiative was established in 2010, introducing surveillance at herd level and specifying threshold values for antimicrobial use in individual herds to enable taking legal action against pig farmers with high antimicrobial use per pig [DANMAP 2010]. As a result, a distinct decrease in consumption was observed from 2010 to 2011. In 2019, a new national target was determined for an 8% reduction in the use of antimicrobials in the pig sector by 2022.

Figure 4.1 Prescribed antimicrobial agents for humans and all animal species, tonnes of active compound, Denmark DANMAP 2020



Sources: Human therapeutics: The Danish Health Data Authority. 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 2020, consumption data were extracted from VetStat on 3 March 2021 and include all antimicrobial agents registered for use in animals

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

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

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

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

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

In 2012, to promote prudent use of antimicrobials in dogs and cats the Danish Veterinary Association (DVA) published treatment guidelines developed by clinical specialists and experts from the Faculty of Health and Medical Sciences at the University of Copenhagen and the National Food Institute, Technical University of Denmark. Revised treatment guidelines for dogs and cats were published in 2018. Similarly, DVA published treatment guidelines for use of antimicrobials in horses in 2017.

4.1.1 Data sources

In Denmark, antimicrobials are available for treatment by prescription only. Sales data on pescribed antimicrobials have been collected in Denmark since 1990.

Since 2001, data on all medicines prescribed for use in animals, including vaccines and coccidiostatic agents (non-prescription) have been recorded in the national database VetStat. Since 2010, the VetStat database is hosted and maintained by DVFA. The 2020 data presented in this report were extracted from VetStat on 3 March 2021 and have been analysed and interpreted for DANMAP by the National Food Institute, Technical University of Denmark.

4.1.2 Methods

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

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

Since 2012, "defined animal daily dose" (DADD) and "proportion of population in treatment per day" (DAPD) have been presented 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].

DADD Defined animal daily dose

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

DAPD Proportion of population in treatment per day

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

DAPD, is a statistical measure that provides a rough estimate of the proportion of animals treated daily with a particular antimicrobial agent. For example, 10 DAPD means that an estimated 1% of the population, on average, receives a certain treatment on a given day (see section 9.2). In principle, DAPD as a metric is analogous to DID (defined daily dose per 1,000 inhabitants per day, see section 9.8), the metric used to measure antimicrobial consumption in the human sector.

In DANMAP 2020, treatment proportions are calculated for pigs, cattle, and fur animals.

Export

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

Approximately 44% of the pigs produced in 2020 were exported as live pigs with an average weight of 30 kg (Table 2.2). In comparison, this percentage was approximately 24% in 2010. When estimating DAPD for the total pig production, we include changes in export of weaners by calculating an adjusted treatment proportion, referred to as DAPDadj, see section 9.2.

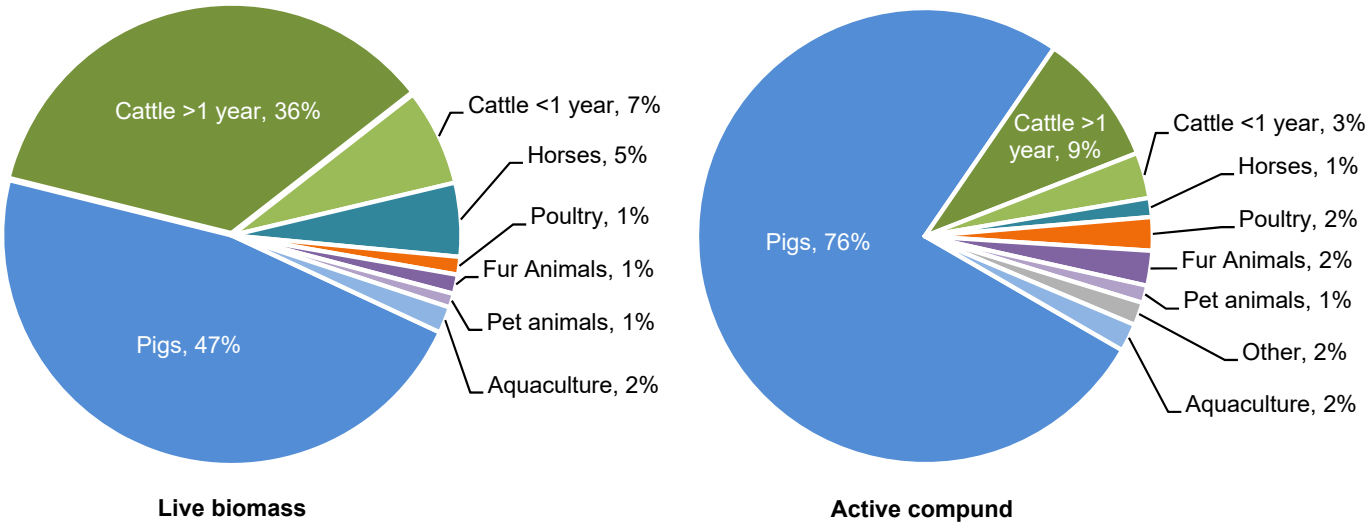
4.2 Total antimicrobial consumption in animals

The total use of antimicrobial agents in all animals amounted to 99.5 tonnes active compound, representing a 2% (+2,250 kg) increase compared to 2019 (Figure 4.1). Similar to previous years, the 2020 antimicrobial use in pigs, cattle, fur animals, and poultry comprised approximately 76%, 12%, 2% and 2%, respectively, of the total antimicrobial use in animals (Figure 4.2). The pig industry is the main driver of antimicrobial usage in animals in Denmark, due to the magnitude of the production. Cattle and pigs comprise almost equal proportions of the total live biomass. However, the vast proportion of cattle biomass consists of dairy cows, which have very low usage of antimicrobials compared with growing animals.

Historically, the overall use of kg active compound was 52% lower in 2020 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 with an average weight of 30 kg. Since then, the proportion of exported live pigs has continued to increase, while there has been an overall gradual decrease in the use of antimicrobials in animals.

Figure 4.2 Distribution of live biomass and antimicrobial consumption in main animal species, tonnes, Denmark DANMAP 2020



The live biomass is estimated from census data (pigs, cattle and pet animals) and production data (poultry, fur animals, and aquaculture). The live biomass estimates for poultry (turkeys and broilers), aquaculture, horses and pet animals are based on 2012 data and may well be underestimated. The estimation procedures are described in section 9.2

Table 4.1 Antimicrobial use by animal species and age group, kg active compound, Denmark DANMAP 2020

Therapeutic group	Aminoglycosides	Amphenicols	Cephalosporins[a]	Fluoroquinolones	Lincosamides	Macrolides	Other AB	Other quinolones	Penicillins, b-lactamase sensitive	Penicillins, others[b]	Pleuromutilins	Sulfonamides and trimethoprim	Tetracyclines	Total 2020	Total 2019
Pigs	*8666*	*561*	*<1*	*0*	*2059*	*11862*	*<1*	*0*	*18837*	*10107*	*7094*	*5325*	*11397*	*75908*	*72632*
Sows and piglets	2102	380	0	0	495	572	<1	0	9622	3565	684	4216	1063	22698	21560
Finishers	169	14	<1	0	695	3176	<1	0	6797	996	3910	96	2957	18811	17790
Weaners	6395	167	<1	0	869	8114	0	0	2418	5545	2500	1013	7376	34399	33282
Cattle	*893*	*936*	*66*	*<1*	*13*	*215*	*<1*	*0*	*7840*	*695*	*0*	*715*	*1287*	*12660*	*12350*
Intramammaries	26	0	66	0	11	0	<1	0	256	186	0	<1	0	544	523
Cows and bulls	227	14	<1	<1	<1	76	<1	0	6596	395	0	615	678	8602	8607
Calves <12 months	590	910	<1	<1	1	136	<1	0	841	108	0	90	560	3236	2985
Heifers and steers	50	13	<1	0	<1	3	<1	0	146	7	0	10	48	278	236
Poultry	*58*	*0*	*0*	*<1*	*29*	*130*	*0*	*0*	*301*	*218*	*<1*	*55*	*1571*	*2362*	*1612*
All poultry incl turkey	58	0	0	<1	29	130	0	0	301	218	<1	55	1571	2362	1612
Other production animal	*76*	*359*	*<1*	*<1*	*36*	*350*	*<1*	*565*	*10*	*1708*	*0*	*1120*	*227*	*4451*	*6510*
Aquaculture	0	341	0	<1	0	0	0	565	0	24	0	1030	1	1961	2522
Fur animals	73	17	0	0	36	347	<1	0	<1	1682	0	86	214	2454	3955
Other[c]	3	<1	<1	<1	<1	4	<1	0	9	3	0	3	12	36	33
Companion animals	*7*	*1*	*88*	*16*	*69*	*4*	*52*	*0*	*32*	*667*	*<1*	*1601*	*38*	*2575*	*2423*
Pets[d]	5	1	88	16	69	4	52	0	24	667	<1	271	34	1232	1183
Horses	2	<1	<1	<1	0	<1	<1	0	8	<1	0	1330	3	1343	1240
Unspecified[e]	*311*	*14*	*2*	*1*	*11*	*17*	*1*	*-1*	*921*	*135*	*6*	*28*	*141*	*1588*	*1766*
Total	10012	1872	155	17	2217	12579	53	564	27941	13531	7101	8842	14660	99543	97293

Data for 2020 were extracted from VetStat on 3 March 2021. Combination products are split into active compounds
a) In 2020, the use of 3rd and 4th generation cephalosporins in cattle, pets, pigs, and horses was 7.5 kg, 1.1 kg, 0.2 kg, and 0.1 kg, respectively
b) Penicillins with extended spectrum and combination penicillins, incl. beta-lactamase inhibitors
c) Mainly sheep and goats
d) Where no animal species was given, antimicrobial agents were allocated to pets based on relevant type of preparation (e.g. tablets, capsules, eye and eardrops, etc.) or registration (3rd generation cephalosporins and fluoroquinolones only). Approximately 220 kg of the sulfonamides and trimethoprim registered for pets are products (oral paste) typically used for horses. Other AB comprise mainly metronidazole
e) Includes data where the animal species were not registered or where the age group applies to the designated animal species

4.3 Antimicrobial consumption by animal species

4.3.1 Antimicrobial consumption in pigs

The majority of antimicrobials in animals are used in the pig production. The total antimicrobial consumption in pigs was 75.9 tonnes of active compound in 2020, which was 3,276 kg more than in 2019 (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 achieved in 2019, where the use had been reduced by 16%. In 2019, a new target was determined for an 8% reduction; antimicrobial use in the pig production should decrease by 2% each year from 2019-2022 compared to the 2018 level. With the increase in antimicrobial use in 2020, this goal was not achieved.

Treatment proportion

The treatment proportion (DAPD) of the total population reflects the trends in selection pressure within the population. DAPD is much higher in weaners than in finishers and sows. DAPDs in the pig population overall and by age group are presented in Figures 4.3 and 4.4, and DADDs are shown in the web annex (Table A4.1 and in the DADD description).

Historically, DAPD increased from 2004 to 2009, followed by a clear decrease in 2010 and 2011 with introduction of the Yellow Card initiative. Since 2013, the overall treatment proportion, for all age groups, decreased gradually until 2018. When adjusted for export, an estimated 2.3% of all pigs received antimicrobial treatment per day (23 DAPD), similar to levels in 2018 and 2019, but a 30% decrease measured in DAPDadj compared to 2010 (Figure 4.3).

While there was an increase in the antimicrobial use in pigs when inspecting crude consumption data (Table 4.1), the changes to the overall treatment proportion are more subtle and vary between age groups and antimicrobial classes. In 2020, DAPD decreased slightly in weaners and finishers, and increased slightly in sows compared to 2019 (Figure 4.3, Table A4.1 in the web annex). Thus, on a given day in 2020, 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 2020 the treatment proportion was at the lowest level registered in the last 16 years, with the most marked changes following the most 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% (42 DAPD) in 2010 to less than 2% (18 DAPD) in 2020. In contrast, the use of other antimicrobial agents has increased, particularly the use of aminoglycosides (mainly neomycin), macrolides, and extended-spectrum penicillins (Figures 4.3 and 4.4).

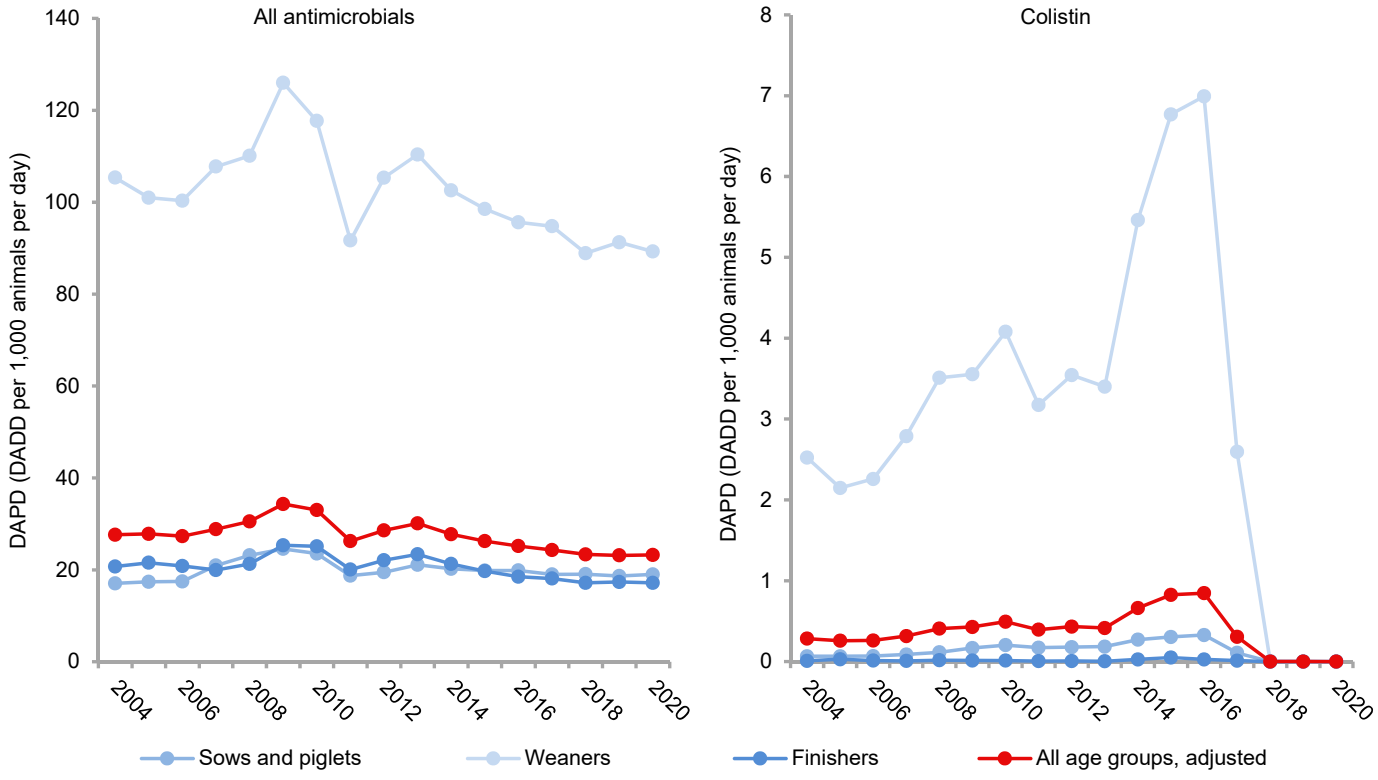
The critically important antimicrobial agents, fluoroquinolones and 3rd and 4th generation cephalosporins, were not used in pigs in 2020 (Figure 4.5 and Table 4.1).

Use of medical zinc in pigs

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 4.6).

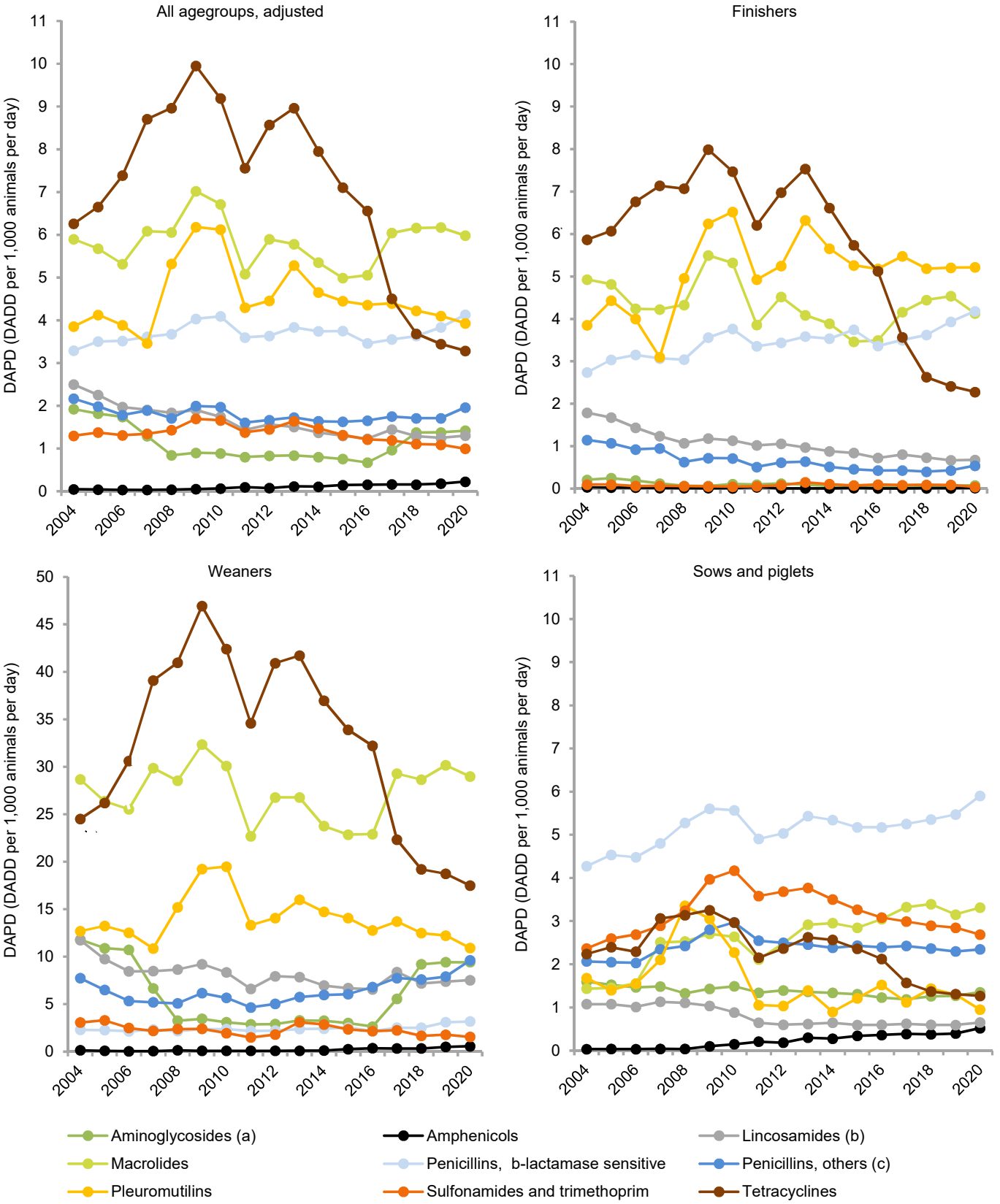
In 2017, the European Commission announced an EU-wide withdrawal of medical zinc for pigs effective from June 2022. Already in 2016, the Danish pig industry launched an action plan to help pig producers reduce the use of medical zinc. This was followed up by an updated action plan in 2018 and a number of research projects are on-going and web tools to help producers reduce the use, have been developed. From 2015 to 2019 the use of zinc oxide was reduced. However, in 2020, the use of medical zinc increased by 4% compared to 2019. Some of this increase may reflect of the increased number of pigs produced in 2020. Nonetheless, an annual use of more than 494 tonnes of medical zinc oxide remains far from the target of zero use by June 2022.

Figure 4.3 Total antimicrobial use and use of colistin in the pig production, DAPD, Denmark DANMAP 2020



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

Figure 4.4 Antimicrobial use in the total pig production and in each age group, DAPD, Denmark DANMAP 2020



DAPDs are calculated as the number of standard doses for one kg animal divided by the estimated live biomass in the age group or the total population (in tonnes). DAPDs for all age groups" are adjusted for export of pigs at 30 kg (see text)
The age group “sows and piglets” includes treatment in boars, where boars constitute 4-5% of the estimated live biomass for the age group.
Intramammaries, gynaecologicals, and topical drugs are not included (approximately 90 kg in 2020).
a) Aminoglycosides/benzylpenicillinprocain combinations comprise 51% of this group in 2020
b) Lincosamides/spectinomycin combinations comprise 76% of this group in 2020
c) Penicillins with extended spectrum and combination penicillins, incl. beta-lactamase inhibitors, mainly amoxicillin and amoxicillin/clavulanic acid combinations

Figure 4.5 Use of 3rd and 4th generation cephalosporins in pigs and cattle, kg active compound, Denmark DANMAP 2020

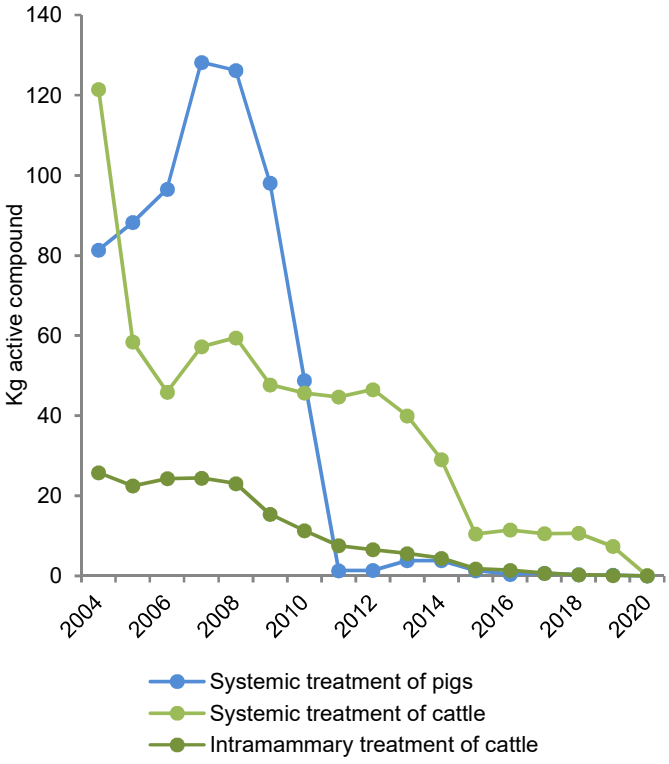
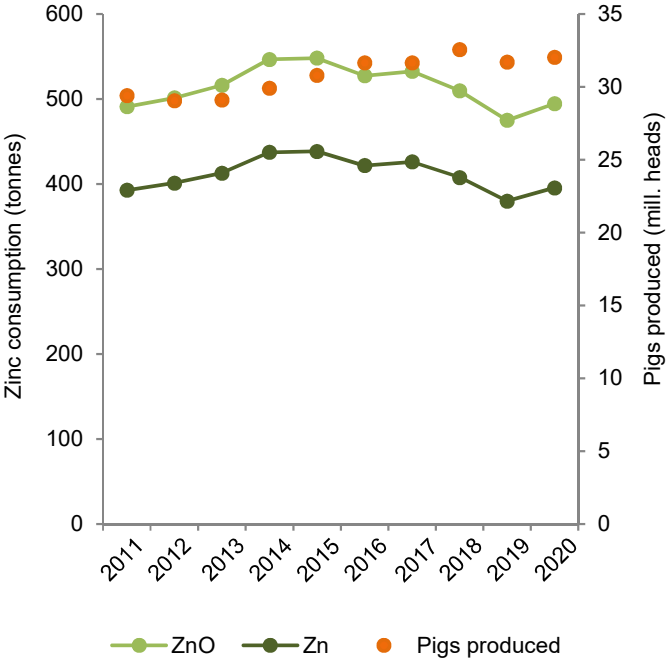


Figure 4.6 Usage (in tonnes) of medical zinc - zinc oxide (ZnO) and zinc (Zn) - in the pig production, Denmark DANMAP 2020



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

4.3.2 Antimicrobial consumption in cattle

Legislation-supported thresholds for antimicrobial use in cattle have been in place since 2011. The overall consumption of antimicrobials in cattle has fluctuated between 12 and 13 tonnes for the past 5 years. In 2020, approximately 12.6 tonnes were recorded for use in cattle, of which 544 kg were used for intramammary therapeutic or dry-cow treatment.

Around 70% of the antimicrobials (kg active compound) used for cattle were used to treat adult cattle (>12 months) (Table 4.1).The production of veal and beef has remained relatively stable over the past 5-10 years, while the production of milk has increased (Table 2.3).

Measured in kg active compound, there has been a gradual reduction in the overall use of antimicrobials for systemic treatment in adult cattle over the past decade. The consumption was 10% lower in 2020 compared to 2016 and 20% lower than in 2011. However, measure in treatment proportion, the use for adult cattle has been between 3 and 4 DAPD since 2011 and in 2020 it was 3.6 DAPD, compared to 3.2 DAPD in 2019 and 3.9 DAPD in 2011.

The main indication for systemic treatment in adult cattle was mastitis, and beta-lactamase sensitive penicillin accounted 69% of the antimicrobials used for this age group followed by tetracycline (14%). The use of macrolides constituted 2% in 2020 (Figures 4.7 and 4.8, Table A4.2 in the web annex).

In contrast, the antimicrobial use in calves and young cattle has increased over the past decade from 2,250 kg or 5.3 DAPD in 2011 to 3,236 kg or 8.7 DAPD in 2020, equivalent to an increase of 44% or 63%, respectively. The main indication for systemic treatment in calves is respiratory disease followed by joint/limb and gastrointestinal infections . An increasing number of calves are are reared in large herds, and generally, there is a correlation between the herd size and frequency of antimicobial treatment.

In calves and young cattle, treatment (DAPD) with amphenicols (florfenicol) has increased steadily over the past decade, and amphenicols have become the most frequently prescribed antimicrobial class (30%), followed by tetracyclines and macrolides (21% and 18%, respectively).

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

In 2014, the cattle industry began to phase out the use of 3rd and 4th generation cephalosporins used for systemic treatment (oral and injectable), resulting in a significant drop in 2015, and the annual usage stabilised at approximately 10 kg. On 1 September 2019, the cattle industry implemented a voluntary ban on use of 3rd and 4th generation cephalosporins in all cattle, and no use was registered in 2020 (Figure 4.5).

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

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

The number of treatments per cow has remained stable for the past decade. However, the usage pattern has changed and the relative proportion of dry-cow treatment has shifted from 22% in 2011 to 55% in 2020. Dry-cow treatment is only permitted following diagnostic testing, where the presence of bacteria causing mastitis has been confirmed.

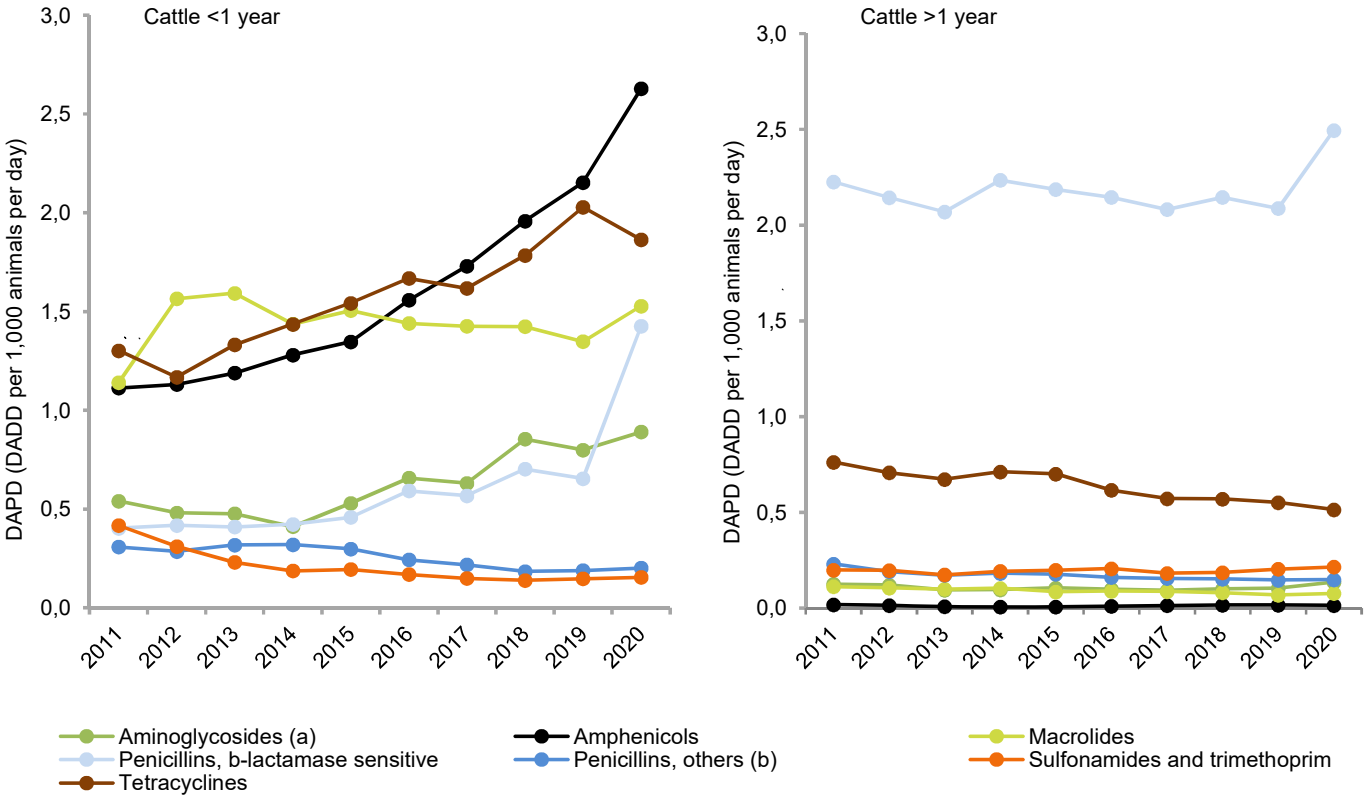
In line with the strategy, the use of beta-lactamase sensitive penicillins increased for dry-cow treatments, whereas use of 1st generation cefalosporins and extended spectrum penicillins decreased from 2012 to 2017.

In 2019, there was a remarkable shift in the drycow treatments and the use of the beta-lactamase sensitive benzylpenicillin for this purpose almost ceased, while the use of the extended spectrum penicillins, especially cloxacillin, increased. This 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, the use shifted again towards beta-lactamase sensitive penicillins, but extended spectrum penicillins were still used more than the beta-lactamase sensitive penicillins (Figure 4.8).

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

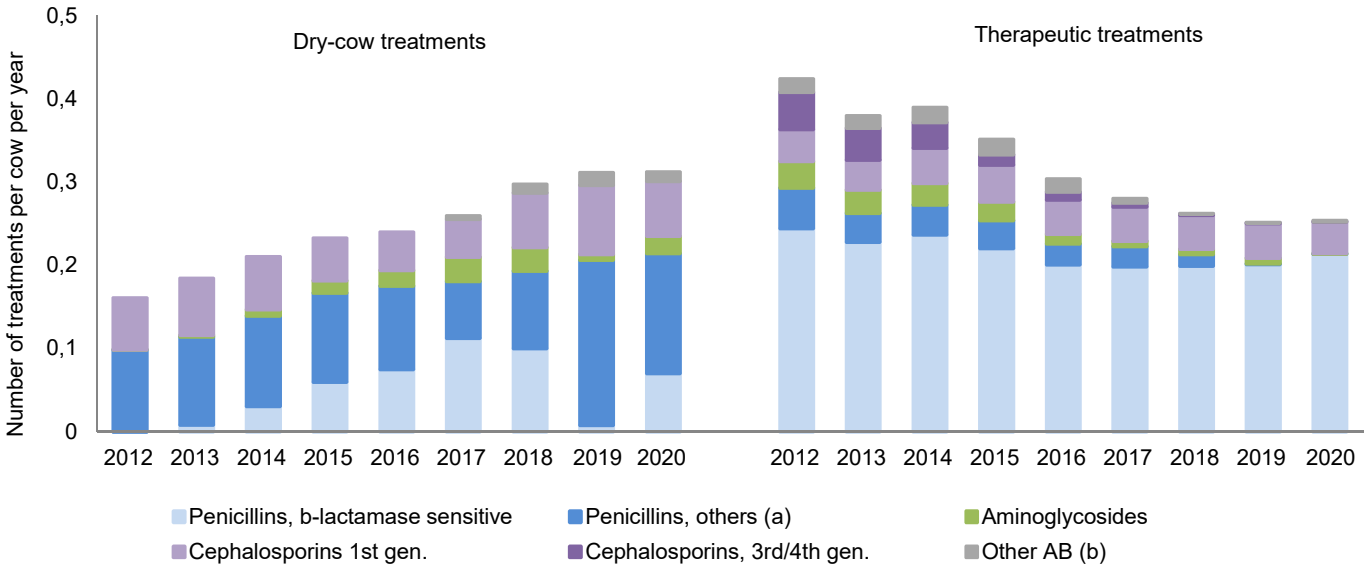
Figure 4.7 Use of antimicrobial agents in cattle, DAPD, Denmark

DANMAP 2020



Intramammaries, gynecologicals and topical drugs not included. DAPDs are calculated as the number of standard doses for one kg animal divided by the estimated live biomass in the age group (in tonnes)
a) Aminoglycosides/benzylpenicillinprocain combinations comprise 30% of this group in 2020
b) Penicillins with extended spectrum and combination penicillins, incl. b-lactamase inhibitors, mainly amoxicillin and ampicilin. Amoxicillin/clavulanic acid combinations comprise 62% of this group in 2020

Figure 4.8 Use of antimicrobial agents for intramammary application in cattle, treatments per cow per year, Denmark DANMAP 2020



For intramammary treatment, the ADDs listed in VetStat has been used for estimating the number of doses. For products used for dry cow treatment, the ADD was primarily 4 tubes per dose (95% in 2020), whereas for products used for therapeutic treatments the ADD varied from 1 to 5 tubes per dose, primarily 2 or 3 tubes (100% in 2020). Number of cows per year from Statistics Denmark (Table HDYR07)
a) Penicillins with extended spectrum and combination penicillins, incl. Beta-lactamase inhibitors, mainly cloxacillin
b) Includes lincomycin for dry-cow treatments

4.3.3 Antimicrobial consumption in poultry

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

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

In 2020, the usage increased by 47% to 2,362 kg (Table 4.2). Approximately 43% were used for broilers and 44% for turkeys. The increased antimicrobial use in 2020 was caused by more treatment requiring infections, including several *E. coli* outbreaks, compared to 2019. Several of these infections occurred late in the production period, requiring quite large amounts of antimicrobials due to the size of the birds, and this had a marked effect on the annual use [personal communication, Mie Nielsen Blom, Danish Agriculture and Food Council]. Increases were seen mainly for tetracyclines prescribed for respiratory disease and macrolides prescribed for enteritis. For the past decade, cephalosporins have not been used in the poultry industry, and the use of fluoroquinolones has been close to zero. Colistin has not been used since 2016.

4.3.4 Antimicrobial consumption in aquaculture, fur animals and companion animals

Aquaculture

Antimicrobial consumption in aquaculture varies considerably with water temperatures, because bacterial diseases are more likely to occur when temperatures are high. The summer of 2020 was not as warm as the previous summers and this was reflected in a 22% decrease in antimicrobial use from 2,522 kg in 2019 to 1,961kg in 2020 (Table 4.3).

Mainly three compounds are used to treat bacterial infections in aquaculture: sulfonamide/trimethoprim (53%), 1st generation quinolones (29%), and amphenicols (17%) (Table 4.3).

The aquaculture industry has continued focus on developing improved vaccination strategies to reduce the risk of diseases that may require antibiotic treatment.

Table 4.2 Use of antimicrobial agents in poultry, kg active compound, Denmark DANMAP 2020

	Aminoglycosides	Amphenicols	Fluoroquinolones	Lincosamides	Macrolides	Other AB	Other quinolones	Penicillins, b-lactamase sensitive	Penicillins, others [a]	Pleuromutilins	Sulfonamides and trimethoprim	Tetracyclines	Total
2011	1	1	<1	<1	157	<1	0	50	286	5	42	227	771
2012	28	5	<1	14	304	2	1	29	157	6	23	163	732
2013	36	9	<1	18	301	1	0	155	192	3	62	519	1296
2014	21	9	<1	10	359	2	0	120	326	<1	83	617	1548
2015	258	4	1	129	114	7	0	184	500	<1	446	796	2441
2016	60	5	<1	24	153	6	0	239	225	<1	111	749	1571
2017	65	5	<1	32	206	0	1	321	293	<1	85	483	1491
2018	51	0	<1	26	162	0	0	323	212	<1	37	516	1326
2019	55	<1	<1	28	228	0	0	333	215	<1	64	689	1612
2020	58	0	<1	29	130	0	0	301	218	<1	55	1571	2362

Data for 2020 were extracted from VetStat on 3 March 2021. VetStat does not differentiate between use in the different sectors of poultry production. Combination drugs are divided into active compounds
a) Penicillins with extended spectrum and combination penicillins, incl. beta-lactamase inhibitors, mainly amoxicillin

Fur animals (mink)

The Danish production of mink has increased over the last decade from 13 million animals in 2004 to 18 million in 2017 (Table 2.2). In 2020, the number of produced mink decreased to approximately 15.3 million.

In June 2020, COVID-19 was found in mink on three farms in Northern Jutland. These mink were culled. From August onwards the disease spread to more farms across Jutland. From 1 October 2020 the Danish government decided to resume culling of mink on infected farms and on farms within a radius of 7.8 km from infected farms. The decision was based on the recommendations from the Danish veterinary and health authorities. This did not stop the infection from spreading further and by 4 November a total of 207 farms were infected. Eventually a decision to cull all Danish mink due to the risk of COVID-19 was taken by the Government. The task was completed by 5 February 2021. A temporary ban on mink farming in Denmark is in place until 31. December 2021.

The use of antimicrobials in mink was lower than in 2019 (Figure 4.9). This was not only due to the decreased use from September 2020, but the antimicrobial use was also lower every month from May-September, compared to 2019. Overall, the use fell by 38% measured in kg active compound (from 3,955 kg to 2,454 kg) or by 20%, when measured in DAPD (from 24 to 19 DAPD). The reduction in use prior to September, may have been due to fewer disease outbreaks, but may also have been an effect of the implemented action plan described in Textbox 4.4, DANMAP 2018.

The use of tetracyclines, penicillins with extended spectrum, combination penicillins, and macrolides are shown in Figure 4.9. The use of fluoroquinolones and cephalosporins in the fur animal production has been close to zero for more than a decade (Table A4.3 in the web annex).

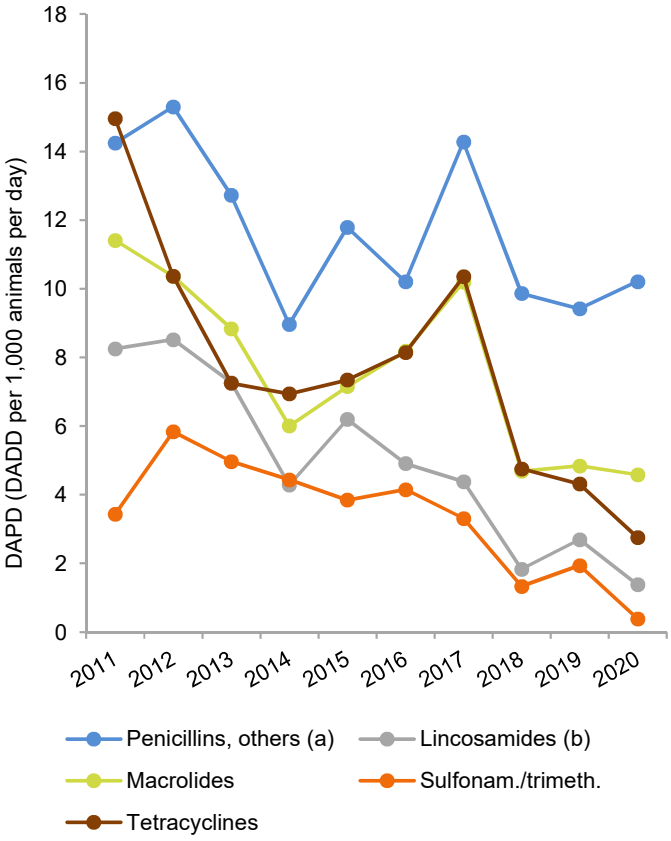
The use of tetracyclines, penicillins with extended spectrum, combination penicillins, and macrolides are shown in Figure 4.9. The use of fluoroquinolones and cephalosporins in the fur animal production has been close to zero for more than a decade (Table A4.3 in the web annex).

Table 4.3 Use of antimicrobial agents for aquaculture, kg active compound, Denmark DANMAP 2020

	Amphenicols	Other quinolones [a]	Sulfonamides and trimethoprim	Other AB [b]	Total
2011	187	357	2160	7	2711
2012	162	539	2192	25	2918
2013	180	946	2279	11	3416
2014	297	1678	3132	9	5116
2015	311	1005	1650	4	2970
2016	315	893	1086	13	2307
2017	350	637	679	31	1697
2018	323	896	2293	45	3557
2019	293	447	1721	61	2522
2020	341	565	1030	25	1961

Data for 2020 were extracted from VetStat on 3 March 2021
a) Oxolonic acid
b) Other antibiotics include mainly amoxicillin (96%)

Figure 4.9 Use of antimicrobial agents in fur animals, DAPD, Denmark DANMAP 2020



The DAPD are calculated as the number of standard doses for one kg animal divided by the estimated live biomass in the total population (in tonnes)
a) Penicillins with extended spectrum and combination penicillins, incl. b-lactamase inhibitors (mainly amoxicillin/clavulanic acid)
b) Lincosamides/spectinomycin combinations only

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. The methods used for estimating the consumption for companion animals are described in DANMAP 2016.

The total amount of antimicrobials estimated for use in horses was 1,343 kg and 1,232 kg in pets (Tables 4.4 and 4.5). As in previous years, a substantial amount of sulfonamide/trimethoprim registered as used for dogs and cats appears as oral paste, which is normally used for horses. Thus, a substantial amount of sulfonamide/trimethoprim included in Table 4.5 has most likely been used for horses (255 kg in 2020 and 220 kg in 2019).

A large proportion of antimicrobials used for dogs and cats are prescribed for the treatment of chronic or recurrent disease, mainly dermatitis. Due to the close contact between owners and their pets, the repeated use of critically important antimicrobials may pose a risk to the owners, and the use of these antimicrobials is therefore monitored carefully. Since the treatment guidelines by DVA were published in November 2012 (revised in 2018), the use of cephalosporins has been reduced by 68% (from 272 kg in 2012).

The use of fluoroquinolones in pets, mainly dogs and cats, was 16 kg and represented the majority (91%) of fluoroquinolones used in all animals in 2020. Similarly, the pets accounted for half (88 kg, 56%) of all the cephalosporins used in animals (Table 4.5). In 2020, 3rd and 4th generation cephalosporins were only used in in pets (1.2 kg active compound), which is approximately half the amount used in 2011, before the treatment guidelines were published.

Table 4.4 Estimated use of antimicrobial agents for horses measured in kg active compound, Denmark DANMAP 2020

	Sulfonamides and trimethoprim[a]	Penicillins, b-lactamase sensitive	Tetracyclines	Aminoglycosides	Other AB	Total
2011	1043	14	1	1	1	1060
2012	1000	14	3	<1	1	1018
2013	893	13	5	1	2	914
2014	1024	15	6	1	1	1047
2015	1049	10	4	3	1	1067
2016	1117	8	5	<1	1	1131
2017	1172	9	3	<1	0	1184
2018	1179	10	4	<1	1	1194
2019	1227	8	4	<1	1	1240
2020	1330	8	3	2	0	1343

Data for were extracted from VetStat 3rd March 2021. The estimates include all antimicrobial agents registered, by the either the pharmacy or veterinarians for use in horses. Antimicrobial agents, where no animal species was given, were allocated to horses based on relevant type of preparation (e.g. oral paste) or registration. Antimicrobials administered parenterally - with no information on animal species - are not included
Oral paste with sulfonamides and trimethoprim are typically used for horses, however these products may mistakenly be registered for pets (2020 = 255 kg; 2019=220 kg, included in Table 4.7)

Table 4.5 Estimated use of antimicrobial agents for dogs and cats measured in kg active compound, Denmark DANMAP 2020

	Aminoglycosides	Amphenicols	Cephalosporins[a]	Fluoroquinolones[b]	Lincosamides	Macrolides	Other AB[c]	Other quinolones	Penicillins, b-lactamase sensitive	Penicillins, others[d]	Pleuromutilins	Sulfonamides and trimethoprim[e]	Tetracyclines	Total
2011	8	<1	313	15	67	13	45	0	47	634	<1	296	50	1491
2012	7	<1	272	15	67	7	50	0	42	651	<1	306	51	1471
2013	7	<1	231	15	63	5	43	0	31	642	<1	292	45	1374
2014	8	<1	213	14	69	6	35	1	31	653	<1	300	35	1366
2015	7	<1	157	14	68	5	33	0	25	655	1	235	39	1240
2016	6	<1	137	15	69	3	31	<1	20	718	<1	275	40	1317
2017	6	1	111	14	67	2	31	0	19	718	<1	280	38	1287
2018	6	<1	97	15	62	2	41	1	20	681	<1	261	37	1224
2019	5	<1	93	14	63	9	38	0	20	662	<1	246	32	1183
2020	5	1	88	16	69	4	52	0	24	667	<1	271	34	1232

Data were extracted from VetStat on 3rd March 2021. Data include all antimicrobial agents registered, by the either the pharmacy or veterinarians for use in pets. Furthermore, antimicrobial agents, where no animal species is given, were allocated to pets based on relevant type of preparation (e.g. tablets, capsules, eye- and eardrops etc.) or registration (3rd generation cephalosporins and fluoroquinolones only)
a) Include the use of 3rd generation cephalosporin product Convenia (1.1 kg in 2019 and 2020) where no animal species is given
b) Include the use of low concentration fluoroquinolones (maximum of 50 mg/g) dispensed parenterally or orally (5.1 kg in 2020) where no animal species is given
c) Other antibiotics include mainly metronidazol
d) Penicillins with extended spectrum and combination penicillins, incl. b-lactamase inhibitors. Include use of tablets where no animal species is given
e) Oral paste with sulfonamides and trimethoprim are typically used for horses, however these products may mistakenly be registered for pets (2020 = 255 kg; 2019=220 kg, included in Table 4.7)

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Textbox 4.1

The "joint effort" information initiative – encouragement to pig farmers to reduce the use of antimicrobials

The large Danish pig production accounts for the vast majority (approximately 75%) of antimicrobials used in animals in Denmark. Therefore, many national initiatives to reduce the use of antimicrobials are aimed at the pig sector. This also include the 'yellow card' initiative, which specifically addresses pig farmers with the highest usage. A 'yellow card' is issued to a farmer if the consumption of antimicrobials, in a certain age group in a pig herd, exceeds a fixed, national threshold. The pig farmer is then obliged to make an action plan to reduce the use of antimicrobials below the threshold level.

The "joint effort" information initiative, launched in 2020, aimed at raising awareness among pig farmers regarding their use of antimicrobials and promoting a change of habits. The Veterinary and Food Administration sent out letters to the pig farmers, accrediting farmers with a low level of antimicrobial consumption, and encouraged to reduce consumption further, if possible.

All pig farmers with a Veterinary Advisory Service Contract (VASC) received two letters. The first letter, sent in June 2020, provided information on the consumption of antimicrobials at the farm, in the third quarter of 2019. Furthermore, it encouraged the farmers to revise and possibly reduce the use of antimicrobials in the next quarter (the third quarter of 2020).

A second letter was sent in November 2020 as the conclusion of the "joint effort" initiative. The second letter summed up changes in antimicrobial consumption in the third quarter of 2020, compared to the third quarter of 2019. It also provided information on the average consumption in ADD per 100 animals per day. The key messages of each letter are listed in Box 1. In addition, veterinarians with VASCs with the affected pig farmers also received letters.

If antimicrobial consumption was below the national average, the letter would say:

"We would like to compliment you for the fact that you are currently below the national average in the third quarter of 2020, because it makes a big difference. We hope you will continue the good habits in the future. Stick to the good habits. When farmers like you use less antimicrobials, it is often because you have acquired good habits that support less consumption."

If antimicrobial consumption was above the national average, the letter read as follows:

"Unfortunately, you are above the national average in the third quarter of 2020. There can be many reasons for this. We hope you will take this opportunity to consider whether you have the opportunity to reduce your antimicrobial use in the future."

When comparing antimicrobial consumption in third quarter 2019 with third quarter 2020, 56% of the farms had reduced or unchanged antimicrobial consumption, while antimicrobial consumption increased in the remaining 44%. The change in consumption was measured in Animal Daily Dose (ADD) per 100 animals per day, thereby taking the number of pigs in the herd into account.

The "joint effort" initiative was a way to directly address pig farmers. They were not obliged to open the letters, but the expectation was that most of them did. The Danish Veterinary and Food Administration designed the campaign with help from a consulting firm. Aarhus University carried out qualitative interviews with a randomly selected group of the farmers, to gain further knowledge on how such an initiative is perceived, and how similar initiatives could be carried out in the future. The results are available in 2021.

Denmark aims at a prudent use of antimicrobials, and has obtained good results from setting national targets for the reduction in the use of antimicrobials. In 2019, new national targets were determined, aiming at 8% reduction in the use of antimicrobials in the pig sector by 2022, compared to 2018.

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5

ANTIMICROBIAL CONSUMPTION IN HUMANS

5. Antimicrobial consumption in humans

Highlights

Total consumption. In 2020, total human consumption of systemic antimicrobials was 14.71 Defined Daily Doses per 1,000 inhabitants per day (DID) in Denmark. This is 22.3% lower than the consumption in 2011 (18.95 DID) and 6.7% lower than the consumption in 2019 (15.77 DID). These reductions were mainly driven by decreased consumption in the primary health care sector.

Consumption in primary health care. In 2020, total antimicrobial consumption in primary health care was 12.83 DID, a 25% reduction since 2011 (17.05 DID) and a 6.8% reduction since 2019 (13.77 DID). However, Defined Daily Doses (DDD) per prescription increased over the past decade. Decreased consumption was observed across all age groups between 2019 and 2020 except for adolescents (20-24 years of age). All doctor types except for hospital doctors prescribed fewer antimicrobials in primary care in 2020 compared to 2019. Antimicrobials prescribed by hospital doctors for patients in the community increased from 63.0 prescriptions per 1,000 inhabitants in 2019 to 64.5 in 2020.

Consumption in hospital care. In 2020, the antimicrobial consumption at Danish hospitals was 1.82 DID. This corresponds to 123.25 DDD per 100 occupied bed-days (DBD), a 33% increase since 2011 (92.5 DBD) and a 3.8% increase since 2019 (118.74 DBD). Measured in DDD per 100 admissions (DAD), the consumption in 2020 (531.51 DAD) was 15.8% higher than in 2011 (458.82 DAD) and 0.98% higher than in 2019 (526.34 DAD).

Consumption of penicillins. In 2020, penicillins accounted for 63% of the consumption in primary health care and 55% of the consumption in hospital care. For decades, beta-lactamase sensitive penicillins were the most used antimicrobials in primary health care in Denmark. This changed in 2020; penicillins with extended spectrum were the most used antimicrobials (25%). In hospitals, the consumption of all penicillins increased over the last decade. Most markedly for combination of penicillins incl. beta-lactamase inhibitors (+197%).

Consumption of tetracyclines and macrolides. In 2020, tetracycline consumption in primary health care increased by 14.5% and macrolide consumption decreased by 18.8% compared to 2019. This reflects compliance with new guidance for chlamydia treatment issued in 2019.

National Action Plan on the reduction of antibiotics in humans. The number of redeemed prescriptions per 1,000 inhabitants in primary health care decreased from 462 in 2016 to 329 in 2020 (Goal 1: 350 prescriptions per 1,000 inhabitants in 2020). The proportion of prescriptions for beta-lactamase sensitive penicillins in primary health care decreased from 31% in 2016 to 27% in 2020 (Goal 2: 36% by 2020). The consumption of antimicrobials of special critical interest at hospitals decreased by 7.4% between 2016 and 2020 with an increase of 3.8% between 2019 and 2020 (Goal 3: 10% reduction by 2020). In the light of the COVID-19 pandemic, the Ministry of Health has extended the National Action Plan until 2021 acknowledging the significant impact the pandemic had on healthcare provision (Chapter 3 `Impact of the COVID-19 pandemic') and to allow more time for achieving the goals.

5.1 Introduction

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

In Denmark, only medical doctors, veterinarians and dentists are allowed to prescribe systemic antimicrobials and sale is through publicly registered and licensed pharmacies. Recording of the consumption in the primary care sector is based on sales from pharmacies to individuals or 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, all available from the Medicinal Product Statistics. Since 2004, the Register of Medicinal Product Statistics also includes the indication for prescribing the medication. No over-the-counter sale takes place. This enables an almost complete surveillance of all systemic antimicrobials used in Denmark in the primary 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 no reliable denominator for measuring antimicrobial consumption in these facilities is available. However, in Figure 5.1 and 5.2 the total antimicrobial consumption in Denmark is presented, shown as Defined Daily Doses per 1,000 inhabitants per day, including data from all healthcare providers, both public and private sectors. The recent upgrade of The National Patient Register, implemented during 2019, has had a major impact on the calculations of the consumption of antimicrobials at hospitals due to new definitions used for bed-days and other hospital activity measures; this is described in paragraph 9.8 in Chapter 9 'Materials and methods'.

Ongoing restructuring measures of the Danish health system since 2018 have led to functions being transferred from hospital ambulatory care to smaller health units, rehabilitation centres and GPs in the municipalities. 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 of consumption levels 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 topically applied antimicrobials, tuberculostica and antiviral is not included in this chapter, whereas consumption of antifungal drugs is described in Textbox 8.4.

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

Danish adjusted DDDs (DaDDD) were developed in 2017 for monitoring hospital consumption and in 2018 for primary health care monitoring. For more information regarding DaDDD, please refer to paragraphs 9.5 and 9.6 in Chapter 9 'Materials and methods'.

Changes in consumption within the last decade have often followed initiatives calling for a more prudent use of antibiotics due to concerns about increases seen in the past. In 2012, the National Antibiotic Council was established following decisions on a national AMR strategy from 2010 that captured both better monitoring of and research on AMR and antibiotic use in the human and the animal sector. In the following years, the `happy audit´ study on better diagnostics guiding antibiotic prescribing were undertaken and published by general practitioners, updated antibiotic guidelines were issued by different medical associations and since 2013, annual - except for in the pandemic year 2020 - antibiotic awareness campaigns aimed at the public were launched by the Ministry of Health.

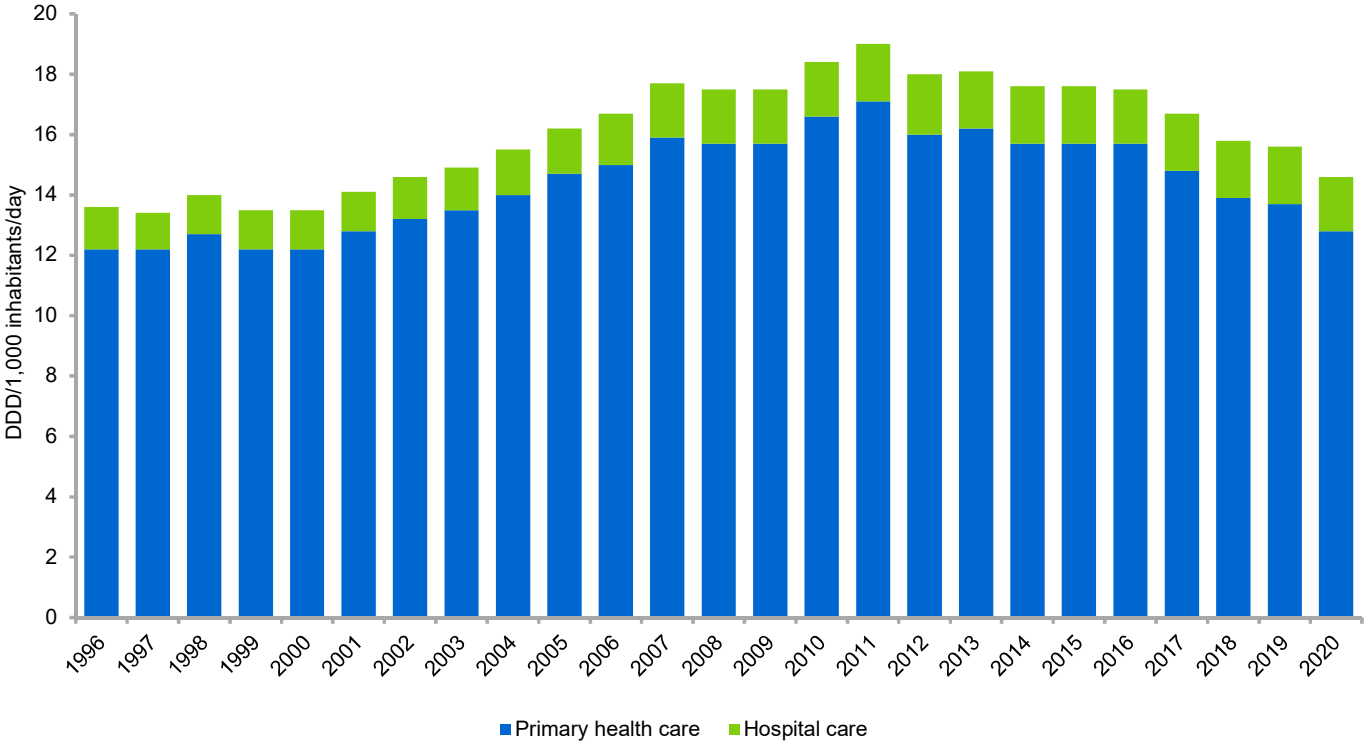
The COVID-19 pandemic had a significant impact on the Danish healthcare system. For more detailed analyses regarding the pandemic's impact on antimicrobial prescribing trends in 2020, please see Chapter 2 'Impact of the COVID-19 pandemic'.

5.2 Total consumption at all healthcare systems in Denmark

Historically, the consumption of systemic antimicrobials in Denmark showed no significant trends during the first five years of systematic registration from 1996 to 2000, where consumption was estimated to be between 13 and 14 DDD per 1,000 inhabitants per day (DID; based on new WHO DDD values and therefore not comparable to data presented in reports preceding the DANMAP 2018 report). However, between 2001 and 2011, consumption of antimicrobials increased steadily peaking at a total of 18.95 DID in 2011. Since 2011, consumption has decreased markedly (Figure 5.1).

Figure 5.1 Total consumption of systemic antimicrobial agents in humans since the beginning of DANMAP, DDD per 1,000 inhabitants per day, Denmark, 1996-2020

DANMAP 2020



Data for this figure are based on the total sales in Denmark
Data are based on the 2021 edition of the Anatomical Therapeutic Chemical (ATC) classification system

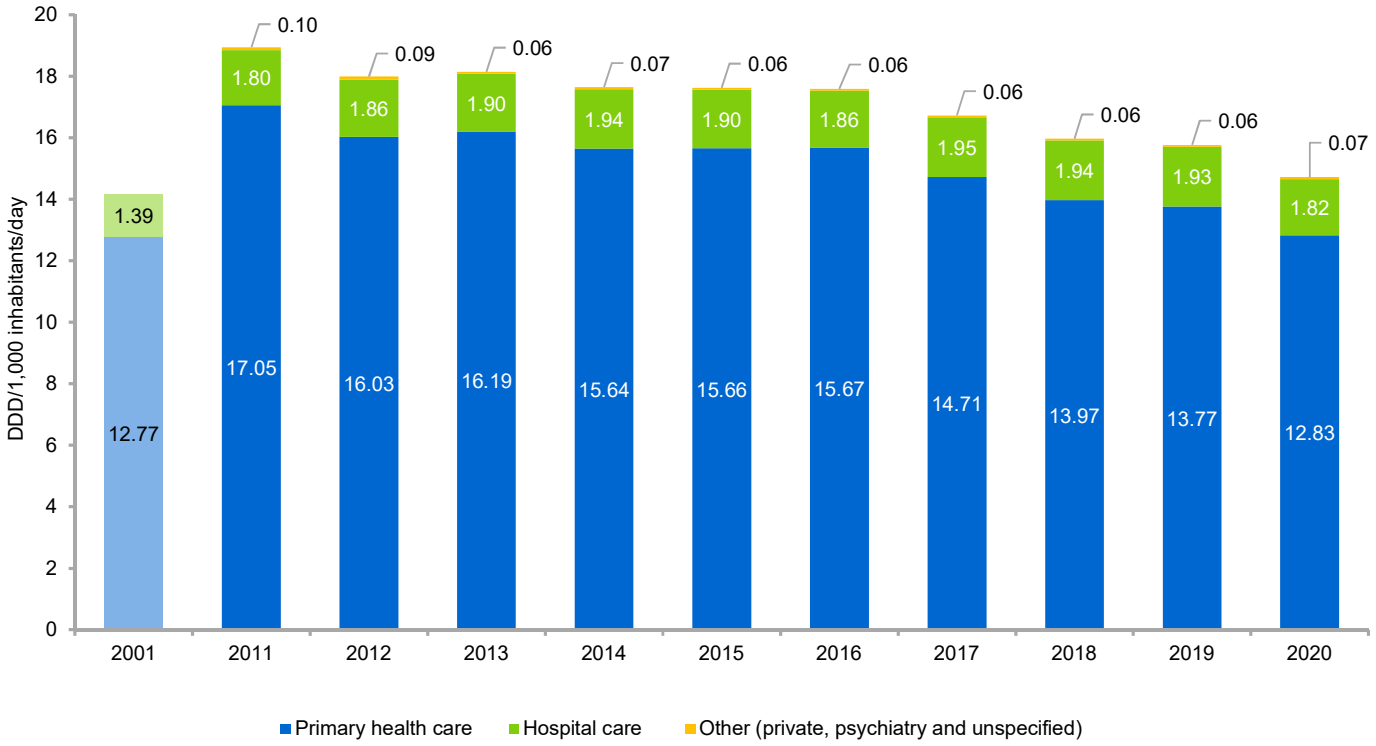
In 2020, total consumption of antimicrobials was 14.71 DID (including all public and private healthcare facilities), which is 6.7% lower than the consumption in 2019 (15.77 DID) and 22.3% lower than the consumption in 2011 (18.95 DID) (Figure 5.2). The primary sector accounted for 12.83 DID, the somatic hospital sector for 1.82 DID and psychiatry, private hospital or unspecified use ("other") for 0.07 DID in 2020. The total consumption in 2020 corresponded to 45,080 kg active compound consumed (Table A5.1 in web annex).

The decrease in total antimicrobial consumption seen since 2011 in Denmark has mainly been driven by reduced prescribing in primary health care, which accounted for 90% of total consumption in 2011 and for 87% in 2020. At hospitals, antimicrobial consumption increased from 2011 to 2019 and decreased in 2020, probably due the COVID-19 pandemic (Figure 5.2). Consumption at hospitals increased from accounting 9.5% of total consumption in 2011 to 12.4% in 2020. Changes of total consumption for the hospital and primary sector over the decade can be found in Figure A5.5 in web annex.

Consumption of the main antimicrobial drug classes in primary health care and somatic hospitals is presented in Figure 5.3. 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.

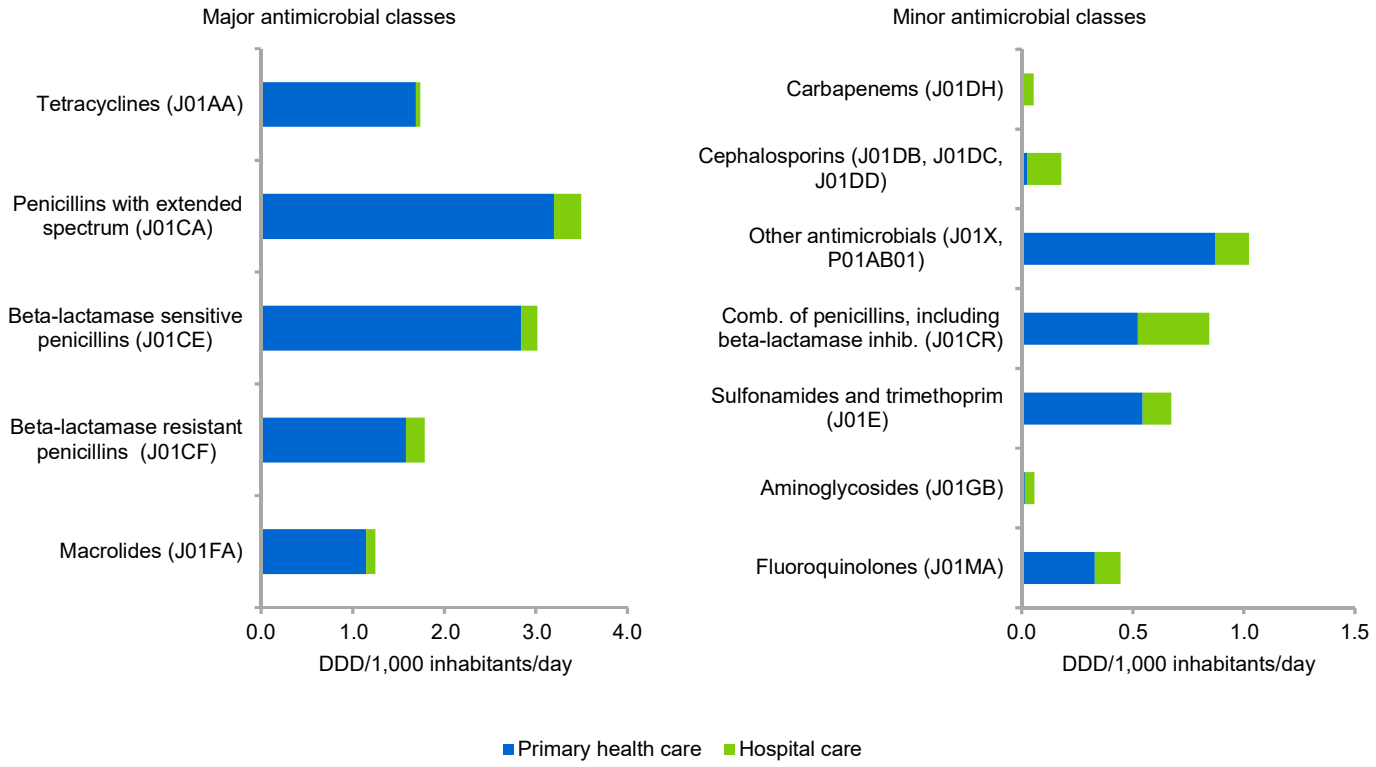
Consumption of antimicrobials in primary health care and somatic hospitals in the five Danish health regions is presented in Figure 5.4. Although consumption per inhabitant differs between the regions, for all five regions marked decreases were observed in the primary sector since 2016, more pronounced in the first years and in 2020. The Capital Region and the Region Zealand, two neighbouring regions, showed highest total consumptions of 14.65 DID and 15.28 DID, respectively. When compared to the other regions, the Capital Region showed a relatively high consumption in hospitals, and the Region Zealand a relatively high consumption in primary health care. The Central Region had the lowest total consumption with 13.31 DID.

Figure 5.2 Total consumption of systemic antimicrobial agents in humans, DDD per 1,000 inhabitants per day, Denmark, 2001 and 2011-2020
DANMAP 2020



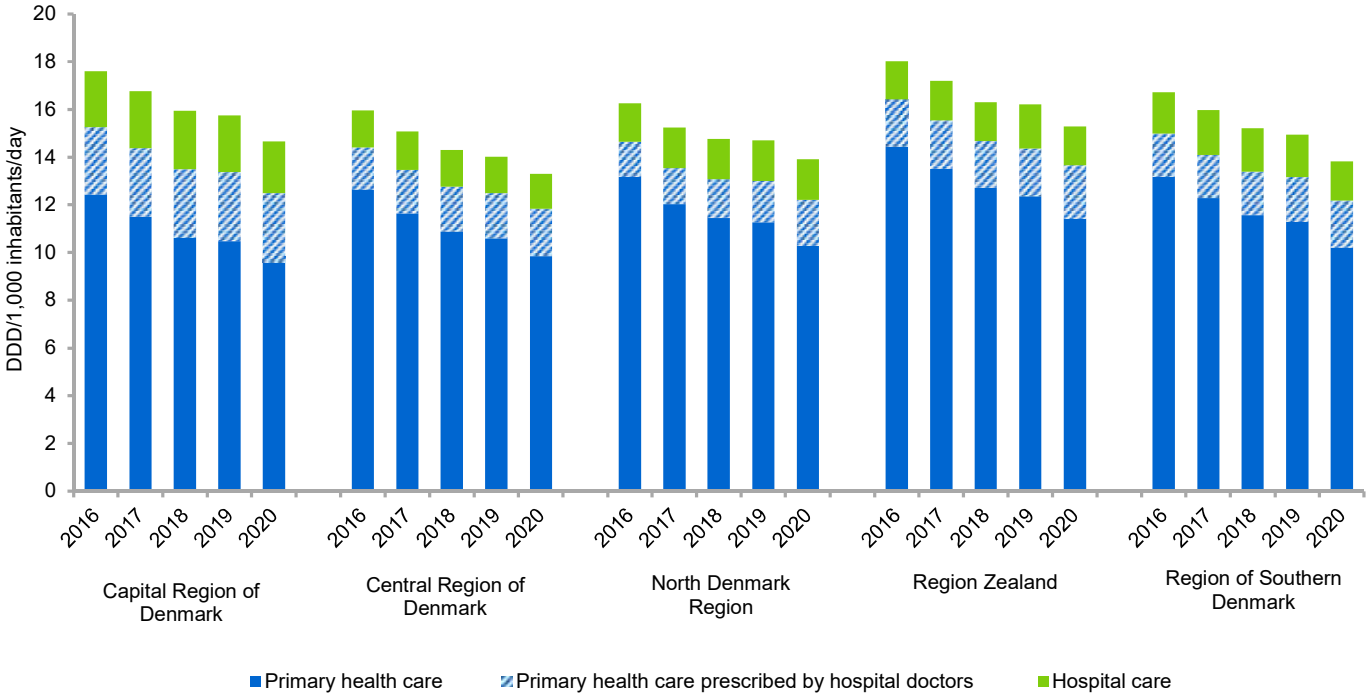
Data for this figure are based on the total sales in Denmark
Data are based on the 2021 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 5.3 Distribution of main antimicrobial classes used for humans in primary and hospital care, DDD per 1,000 inhabitants per day, Denmark, 2020
DANMAP 2020



Data used in this figure are based on registered sales to individuals and consumption at somatic hospitals
Data are based on the 2021 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 5.4 Consumption of systemic antimicrobial agents in primary health care and at somatic hospitals, DDD per 1,000 inhabitants per day, by Danish region, 2016-2020
DANMAP 2020



Data used in this figure is based on registered sales to individuals and consumption at somatic hospitals
Data are based on the 2021 edition of the Anatomical Therapeutic Chemical (ATC) classification system

For more information on population size and hospital activity in the five health regions, please see Figure 2.2 and Table 2.1 in Chapter 2 'Introduction'.

To allow comparison of the human and the animal sector, Figure 4.1 and Table A5.1 in web annex, show total antimicrobial consumption measured in kg active substance.

5.3 Primary health care

5.3.1 Total consumption in primary health care measured in DDD per 1,000 inhabitants per day

In 2020, the consumption of antimicrobials in primary health care based on total sales from pharmacies was 12.83 DID, a decline of 6.8% compared to 2019 (13.77 DID) (Table 5.1). This is a higher decrease than observed between 2018 and 2019 (1.4%). Over the past decade the overall decrease was 25% (17.05 DID in 2011).

For decades, beta-lactamase sensitive penicillins were the most used antimicrobials in primary health care in Denmark. However, penicillins with extended spectrum were the most used antimicrobials in 2020 (Figure 5.5). Beta-lactamase sensitive penicillins accounted for 2.84 DID (22%), penicillins with extended spectrum for 3.19 DID (25%), beta-lactamase resistant penicillins for 1.58 DID (12.4%), tetracyclines for 1.69 DID (13%) and macrolides for 1.15 DID (9%). A decade ago, in 2011, beta-lactamase sensitive penicillins accounted for 31%, penicillins with extended spectrum for 20%, beta-lactamase resistant penicillins for only 7%, while macrolides accounted for 15% (DANMAP 2011). For most other antimicrobial groups, the proportion of total consumption did not change notably.

5.3.2 Trends in consumption of the main antimicrobial groups in primary health care measured in DDD per 1,000 inhabitants per day

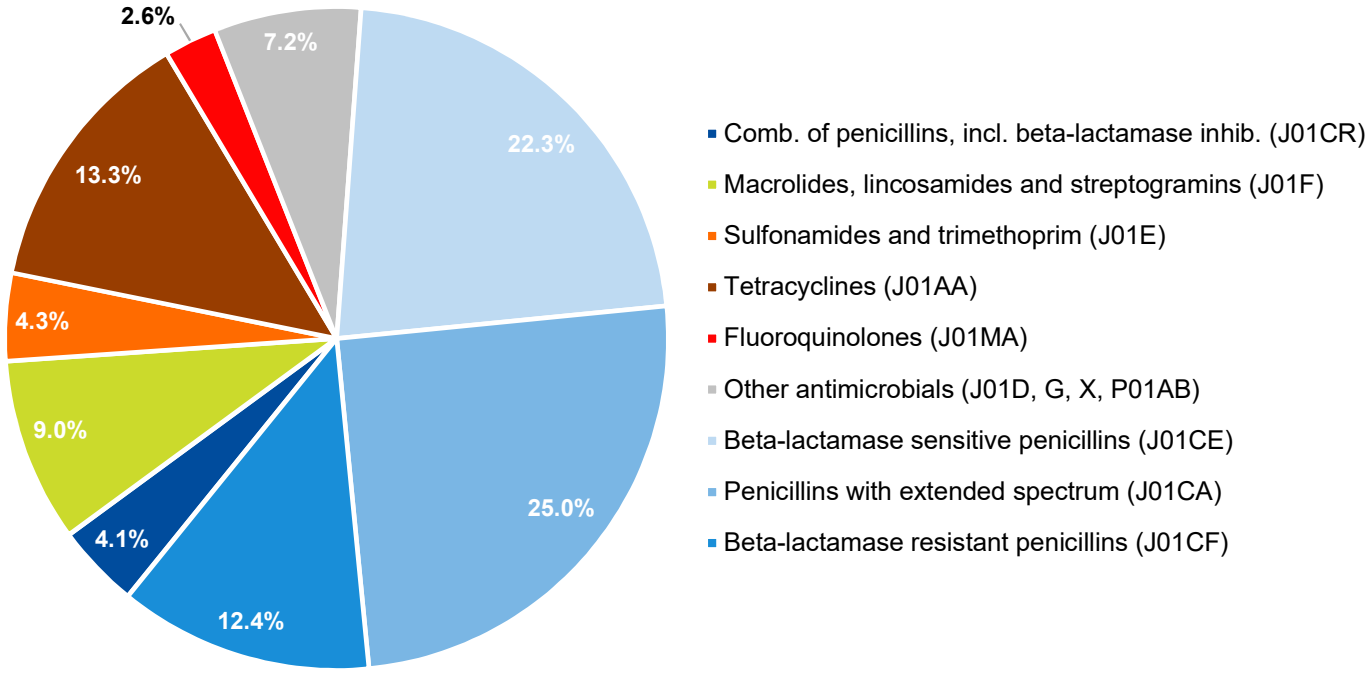
Following the continuous increases in antimicrobial consumption in primary health care in Denmark from the mid 90ties until 2011, the subsequent observed decreases were primarily driven by reduced consumption of beta-lactamase sensitive penicillins, the dominating antimicrobial class in Denmark until 2019, macrolides and to a lesser degree by fluoroquinolones (Figure 5.6). Consumption trends for penicillins, tetracyclines, macrolides and fluoroquinolones will be discussed in more detail in the sections below.

Penicillins

In Denmark, 8.14 DID (63%) of antimicrobials consumed in primary health care in 2020 belonged to the four penicillin groups (penicillins with extended spectrum; beta-lactamase sensitive penicillins; beta-lactamase resistant penicillins; combinations of penicillins, including beta-lactamase inhibitors). Other beta-lactams such as cephalosporins, monobactams and carbapenems were either used at extremely low level or are restricted to hospital use only.

In comparison, the four groups of penicillins accounted for 10.22 DID, 60% of all antimicrobials consumed, a decade ago in 2011. Over the same time period, a shift within the groups could be observed, consumption decreased for beta-lactamase sensitive penicillins from 5.29 DID in 2011 to 2.84 in 2020 and increased for penicillins with extended spectrum and the beta-lactamase resistant penicillins. In 2020 beta-lactamase sensitive penicillins constituted 35% of all penicillins used, while ten years back, in 2011, the share was 52% (Figure A5.6 in web annex).

Figure 5.5 Consumption of antimicrobial agents in primary health care by antimicrobial group (%) based on DDD, Denmark, 2020
DANMAP 2020



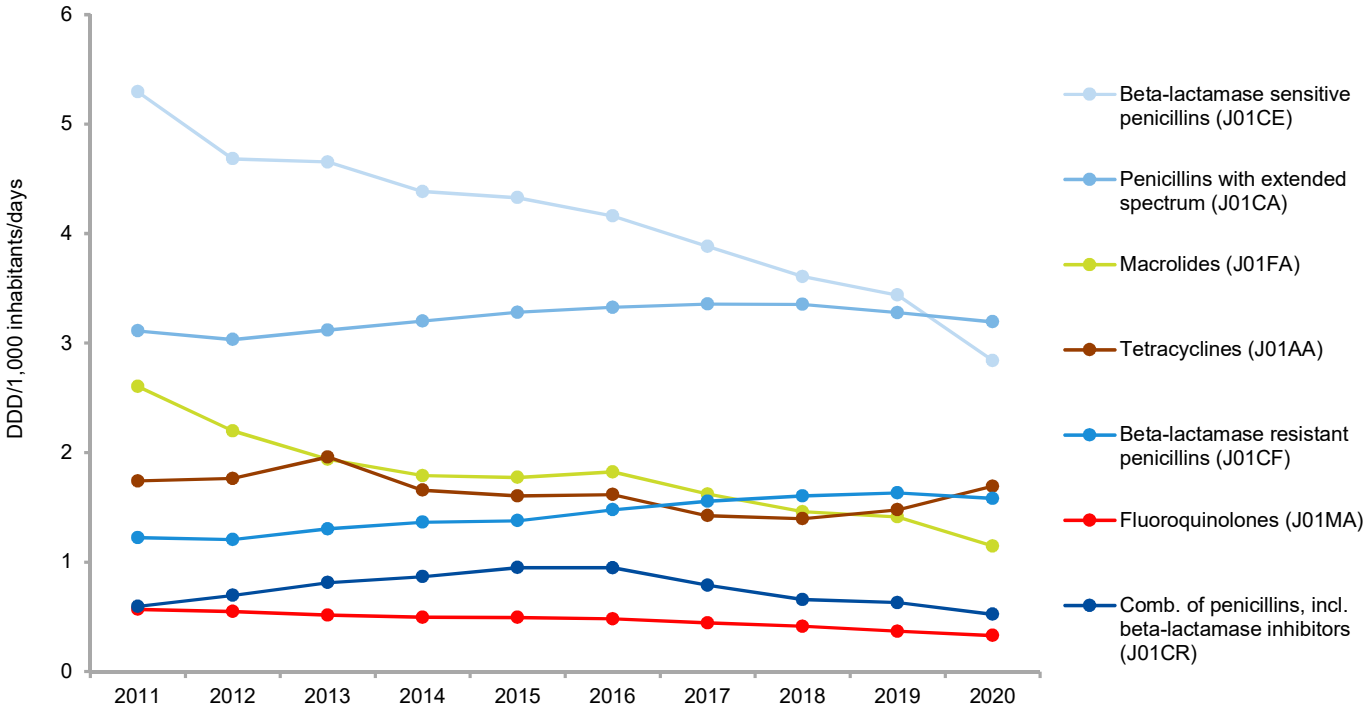
Data used for this figure are based on total sales in Denmark (individuals and clinics)
Data are based on the 2021 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Table 5.1 Consumption of antimicrobial agents for systemic use in primary health care, DDD per 1,000 inhabitants per day, Denmark, 2001 and 2011-2020
DANMAP 2020

ATC group	Therapeutic group	Year										
		2001	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
J01AA	Tetracyclines	1.00	1.74	1.76	1.96	1.66	1.60	1.62	1.42	1.40	1.48	1.69
J01CA	Penicillins with extended spectrum	2.12	3.11	3.03	3.12	3.20	3.28	3.33	3.36	3.35	3.28	3.19
J01CE	Beta-lactamase sensitive penicillins	4.97	5.29	4.68	4.65	4.38	4.33	4.16	3.88	3.61	3.44	2.84
J01CF	Beta-lactamase resistant penicillins	0.66	1.22	1.21	1.30	1.36	1.38	1.48	1.56	1.60	1.63	1.58
J01CR	Combinations of penicillins, including beta-lactamase inhibitors	0.02	0.60	0.70	0.81	0.87	0.95	0.95	0.79	0.66	0.63	0.52
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	0.35	0.50	0.52	0.53	0.55	0.56	0.56	0.56	0.53	0.45	0.43
J01EB	Short-acting sulfonamides	0.36	0.24	0.22	0.22	0.21	0.18	0.16	0.15	0.14	0.13	0.11
J01EE	Combination of sulfonamides and trimethoprim, including derivates	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
J01FA	Macrolides	2.12	2.60	2.20	1.94	1.79	1.77	1.82	1.62	1.46	1.41	1.15
J01FF	Lincosamides	0.01	0.04	0.04	0.05	0.05	0.05	0.06	0.06	0.06	0.06	0.07
J01GB	Aminoglycosides	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
J01MA	Fluroquinolones	0.17	0.57	0.55	0.52	0.50	0.49	0.48	0.44	0.41	0.37	0.33
J01XC	Steroid antibacterials (combination fusidic acid)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00
J01XE	Nitrofuran derivates (nitrofurantoin)	0.39	0.50	0.50	0.49	0.48	0.45	0.43	0.26	0.15	0.27	0.27
J01XX	Other antibacerials (metheamine >99%)	0.33	0.26	0.25	0.24	0.24	0.25	0.27	0.28	0.29	0.32	0.34
J01XD and P01AB01	Nitroimidazole derivates (metronidazole)	0.17	0.28	0.28	0.28	0.28	0.28	0.28	0.25	0.24	0.24	0.23
J01 and P01AB01	Antibacterial agents for systemic use (total)	12.77	17.05	16.03	16.19	15.64	15.66	15.67	14.71	13.97	13.77	12.83

Data used for this table are based on total sales in Denmark (individuals and clinics)
Data are based on the 2021 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 5.6 Consumption of leading antimicrobial groups for systemic use in primary health care, DDD per 1,000 inhabitants per day, Denmark, 2011-2020
DANMAP 2020

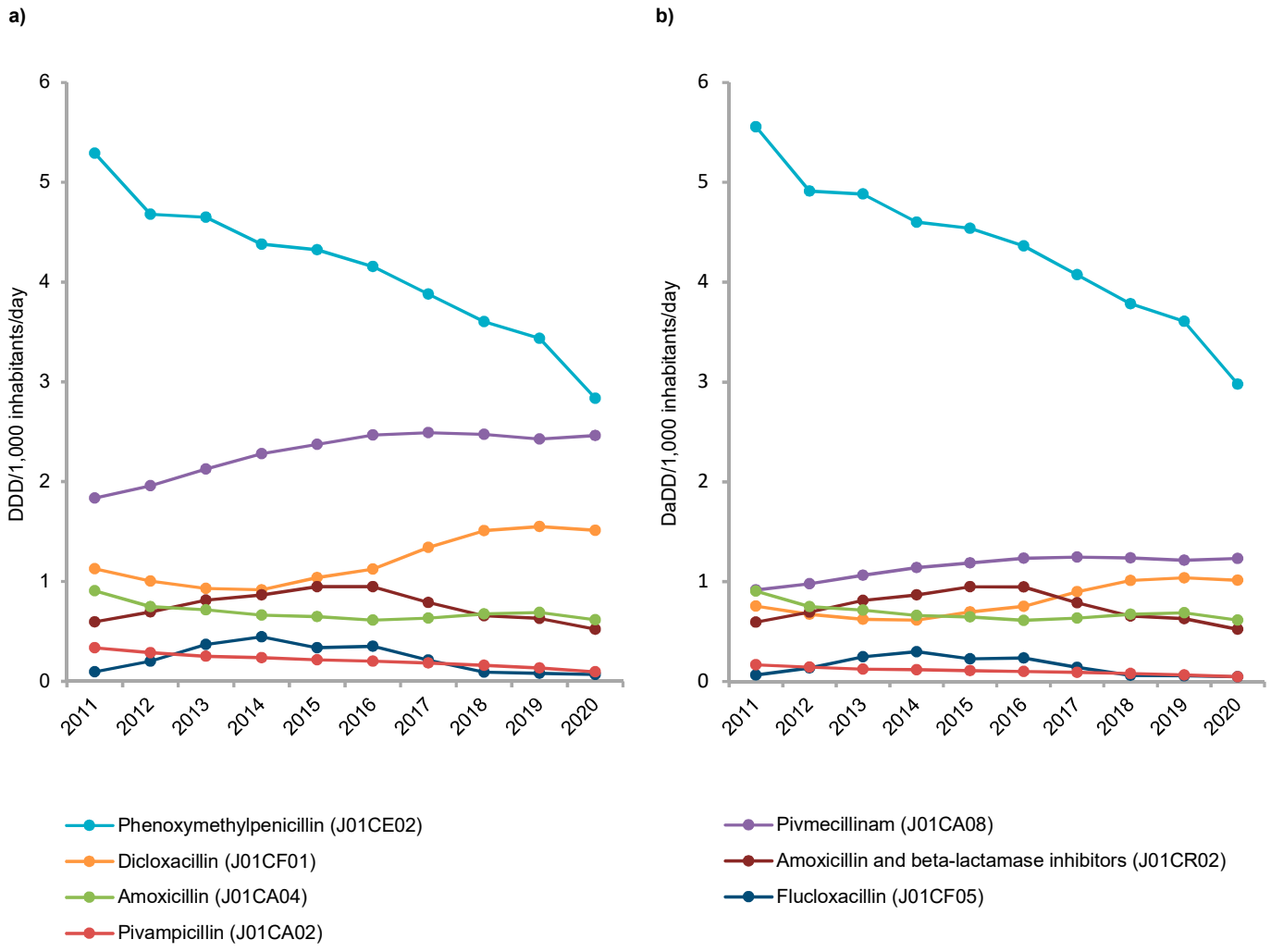


Data used for this figure are based on total sales in Denmark (individuals and clinics)
Data are based on the 2021 edition of the Anatomical Therapeutic Chemical (ATC) classification system

From 2011 to 2020, consumption of beta-lactamase sensitive penicillins had decreased by 46% (from 5.29 DID in 2011), while beta-lactamase resistant penicillins increased by 29% (1.22 DID in 2011). For the penicillins with extended spectrum consumption increased during the first years of the decade, from 3.11 DID in 2011 to 3.36 DID in 2017 (7.9%), but since then levelled off. The consumption decreased by 2.6% between 2019 and 2020.

Combination penicillins increased continuously from their introduction to the Danish market in 2009 until 2015 (0.95 DID), showed no changes for 2016 and since declined, accounting for 0.52 DID in 2020 (a reduction of 17% compared to 2019). 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 2020 (Figure 5.7a). Over the decade pivmecillinam increased by 34% from 1.84 DID in 2011 to 2.46 DID in 2020. Over the same time period pivampicillin decreased by 71% from 0.34 DID to 0.10 DID and amoxicillin decreased by 32% from 0.91 DID to 0.62 DID, respectively. Consumption of amoxicillin fluctuated within the decade, decreasing from 2011 to 2016 (0.61 DID), increasing from 2016-2019 by 12% and decreasing from 2019-2020 by 11%. Increases in the use of pivmecillinam were related to changed recommendations for the treatment of urinary tract infections (see section 5.3.7), 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 regarding more prudent use of antimicrobials in young children. Figure 5.7b shows consumption levels for the main penicillins by Danish adjusted DDD (DaDDD) to reflect dosage recommendations from Danish treatment guidelines.

Figure 5.7 Consumption of main penicillins in the primary health care: a) DDD per 1,000 inhabitants per day and b) DaDDD per 1,000 inhabitants per day, Denmark, 2011-2020
DANMAP 2020



Data used for this figure are based on total sales in Denmark (individuals and clinics)
Data are based on the 2021 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Tetracyclines and macrolides

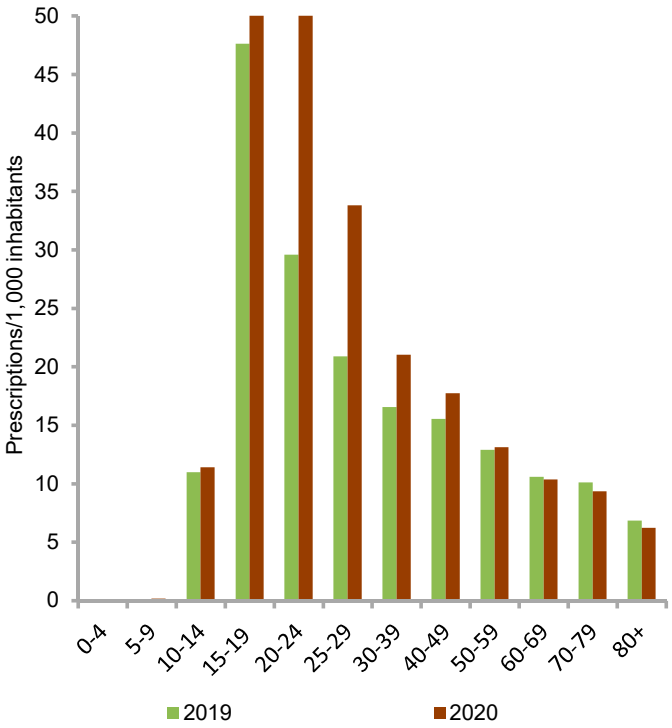
In 2020, tetracycline consumption in primary health care was 1.69 DID, corresponding to 13% of the total consumption in this sector and macrolides accounted for 9% (1.15 DID) (Figure 5.6).

During the last decade, the consumption of tetracyclines decreased by 2.8% from 1.74 DID in 2011. However, in 2020, the consumption increased by 14.5% compared to 2019. Macrolides decreased by 56% over the last decade (from 2.60 DID in 2011) and showed an additional marked reduction of 18.8% between 2019 and 2020.

These changes in tetracycline and macrolide consumption reflect compliance with the new guideline for chlamydia treatment issued by the Danish Dermatological Society in 2019 (Table 5.2). 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.

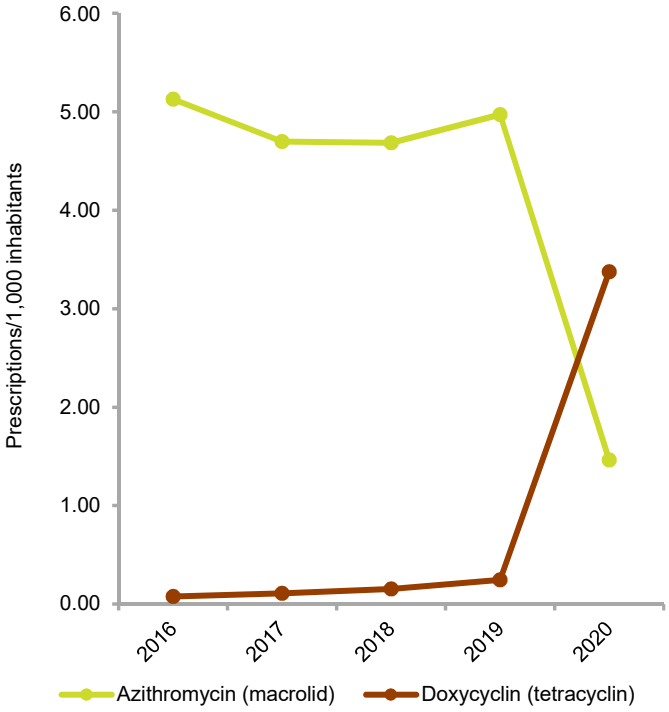
As Table 5.2 shows, acne is, as in previous years, the most common indication for tetracycline prescriptions in primary health care. The age distribution of patients with a tetracycline prescription, mainly young adults, also reflects the two main indications, acne and sexually transmitted chlamydia/mycoplasma infections (Figure 5.9).

Figure 5.9 Consumption of tetracyclines by age group, prescriptions per 1,000 inhabitants, Denmark, 2019-2020 DANMAP 2020



Data used for this figure are based on registered sales to individuals
Data are based on the 2021 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 5.8 Consumption of tetracyclines and macrolides for chlamydia treatment, Denmark, 2016-2020 DANMAP 2020



Data used in this table is based on registered sales to individuals
Data are based on the 2021 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Table 5.2 Percentage distribution of tetracyclines consumption by clinical indication code, Denmark, 2018-2020 DANMAP 2020

Indication written on the prescription	Year		
	2018	2019	2020
Against acne	52.1	52.5	48.9
Against Chlamydia and mycoplasma infection	0.8	1.2	11.1
Against Borrelia infection	3.9	4.2	2.7
Against pelvic inflammatory disease	1.8	1.8	2.0
Prevention of malaria	6.5	5.7	1.5
Against skin and soft tissue infection	1.5	1.5	1.5
Other and unspecified indications	33.3	33.2	32.3

Data used for this table is based on registered sales to individuals
Data are based on the 2021 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Fluoroquinolones

Fluoroquinolones are defined as antimicrobials of critical interest in Denmark due to their resistance potential and their reserved use for treatment of severe infections. In primary health care, the main specific indications for prescriptions of fluoroquinolones are complicated urinary tract infections. Over the past decade, the consumption of these antimicrobials in primary health care decreased continuously. In 2020, fluoroquinolones accounted for 2.6% of antimicrobial consumption

in primary care and decreased from 0.37 DID in 2019 to 0.33 DID in 2020 (Figure 5.5 and Figure 5.6). However, more than a quarter of the consumption was prescribed without a specific indication (Table 5.3). This share did not change after 2018 despite the mandatory requirement of providing a specific indication on prescriptions.

Table 5.3 Percentage of distribution of fluoroquinolones consumption by clinical indication code, Denmark, 2018-2020
DANMAP 2020

Indication written on the prescription	Year		
	2018	2019	2020
Unspecific indication, e.g. 'against infection'	27.7	27.8	27.8
Against urinary tract infection	23.9	21.9	22.6
Unknown indication	10.3	10.2	9.8
Against diarrhea	12.7	12.0	8.9
Against epididymis infection	5.5	6.9	7.8
Against skin and soft tissue infection	5.0	5.9	6.6
Against pneumonia	5.6	5.3	4.5
Other indications	9.4	9.9	12.0

Data used for this table is based on registered sales to individuals
Data are based on the 2021 edition of the Anatomical Therapeutic Chemical (ATC) classification system

5.3.3 Measures at user level

In this and the following sections, the consumption of antimicrobials is described at user level by using either the number of prescriptions per 1,000 inhabitants or the number of treated patients per 1,000 inhabitants. The measures are thus based on all information available through the sales to individuals and do not include the approximately 4% of antimicrobials, mainly penicillins, sold to clinics, dentists and doctors on call.

A comparison of trends over time for different indicators of consumption is presented in Figure 5.10. In 2020, the average DDD/prescription was 11.5, an increase of 6.0% compared to 10.9 DDD per prescription in 2019, and an increase of 22.4% compared to 9.4 DDD per prescription in 2011.

However, all three other indicators showed a decrease in consumption in primary health care over the last decade and pronounced reduction between 2019 and 2020.

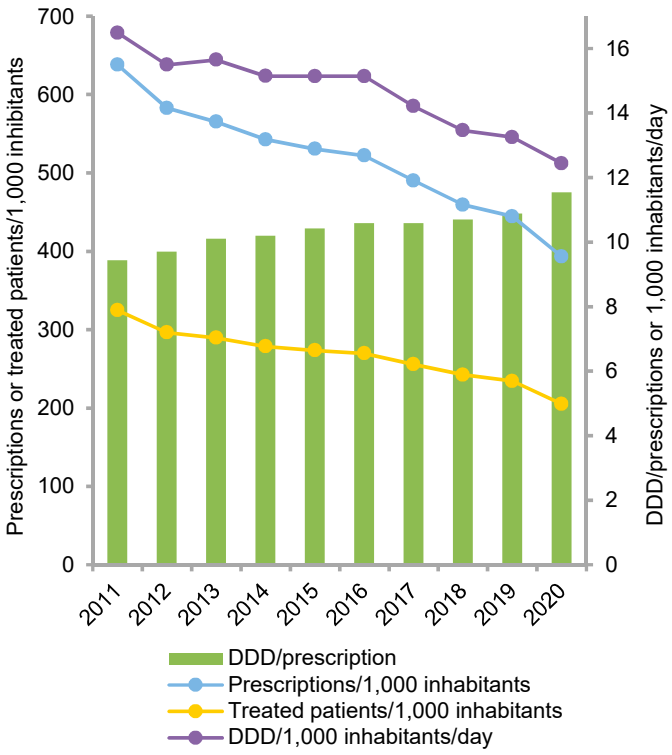
In 2020, the total number of prescriptions was 393 per 1,000 inhabitants, an 11.5% reduction from the 445 prescriptions per 1,000 inhabitants in 2019 and a 38% reduction compared to the 638 prescriptions per 1,000 inhabitants in 2011. Decreases were observed for all antimicrobial drug classes apart from tetracyclines and "Other antibacterials", which increased from 2019 to 2020 (Table 5.4).

In 2020, the average number of prescriptions redeemed per patient was 1.92 (not shown) and the total number of patients treated was 205 per 1,000 inhabitants (Table 5.5). In comparison, the number was 1.96 prescriptions per patient and 325 treated patients per 1,000 inhabitants in 2011.

Trends for the leading antimicrobials between 2019 and 2020 followed mainly the trends already described for consumption by DIDs. The most pronounced increase in the number of prescriptions per 1,000 inhabitants was observed for tetracyclines (34%). Correspondingly, prescriptions of macrolides per 1,000 inhabitants decreased by 34%. Other pronounced decreases in the number of prescriptions per 1,000 inhabitants were observed for beta-lactamase sensitive penicillins (19%), combination penicillins, including beta-lactamase inhibitors (17%), and fluoroquinolones (14%), (Table 5.4).

When measured in the number of patients treated per 1,000 inhabitants tetracyclines increased by 43% whereas numbers for macrolides decreased by 35%, for beta-lactamase sensitive penicillins by 19%, for combination penicillins, including beta-lactamase inhibitors by 17%, and for fluoroquinolones by 16% (Table 5.5).

Figure 5.10 Trends in consumption of systemic antimicrobial agents in primary health care using four different measurements, Denmark, 2011-2020
DANMAP 2020



Data used for this figure are based on registered sales to individuals
Data are based on the 2021 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Table 5.4 Number of prescriptions per 1,000 inhabitants for leading antimicrobial agents in primary health care, Denmark, 2001 and 2011-2020

DANMAP 2020

ATC group	Therapeutic group	Year										
		2001	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
J01AA	Tetracyclines	18.99	22.70	22.56	22.89	20.00	17.90	17.18	15.89	14.63	15.11	20.19
J01CA	Penicillins with extended spectrum	99.89	125.17	115.91	114.30	113.83	113.53	113.16	114.37	114.31	112.19	105.93
J01CE	Beta-lactamase sensitive penicillins	228.41	213.32	186.91	180.54	170.70	163.09	157.13	148.52	136.81	128.77	104.07
J01CF	Beta-lactamase resistant penicillins	27.40	42.75	40.42	41.25	41.04	40.81	41.87	41.87	43.35	43.16	42.87
J01CR	Combinations of penicillins, including betalctamase inhibitors	1.01	21.11	24.71	28.01	29.02	30.73	31.13	27.09	23.71	23.07	19.14
J01E	Sulfonamides and trimethoprim	53.03	45.05	43.86	43.53	41.51	38.39	36.41	34.29	31.74	28.14	25.59
J01FA	Macrolides	91.63	104.22	85.89	74.51	68.01	68.00	68.85	60.00	52.64	50.71	33.66
J01MA	Fluoroquinolones	9.58	23.15	22.14	20.65	19.67	19.50	18.74	17.37	15.97	13.99	12.07
J01X	Other antibacterials (methenamine >99%)	12.66	18.24	18.03	17.41	16.73	16.28	15.82	10.18	6.76	10.29	10.62
P01AB01	Nitroimidazole derivatives (metronidazole)	13.03	19.69	19.68	19.26	19.06	19.15	18.63	17.26	16.31	15.78	15.62
J01 and P01AB01	Antibacterial agents for systemic use	556.68	638.08	582.80	565.26	542.53	530.56	522.19	490.08	459.39	444.53	393.34

Data used in this table is based on registered sales to individual
Data are based on the 2021 edition of the Anatomical Therapeutic Chemical (ATC) classification system

5.3.4 Prescribers in primary health care

Although Denmark has a very homogenous population with relatively small geographic and socioeconomic variations compared to other countries, considerable differences in the prescribing habits among medical doctors are frequently observed. In 2020, the Central Denmark Region had the lowest prescribing activity when compared to all other four regions with 11.84 DID and 374 prescriptions per 1,000 inhabitants per year (Table 5.6). The Region Zealand had the highest prescribing activity with 13.67 DID and 436 prescriptions per 1,000 inhabitants per year. For all regions, DIDs and number of prescriptions redeemed decreased clearly between 2016 and 2020.

There may be several reasons to explain the differences in antimicrobial consumption trends at regional level, e.g. variations in the density of the population and number of general practitioners as well as the proportion of elderly or chronically ill in a given geographic area. Due to differing organisation of general practitioners and clinical practices across the country, comparison of prescribing habits at individual clinical praxis level is difficult. A clinical praxis can be based on a single physician but can also be a collaboration of up to seven physicians sharing facilities and staff. In addition, due to the lack of general practitioners in some areas, several new models of *sundhedshuse* "health houses" served by physicians and other health staff have been established. General practitioners can monitor their own prescription habits through ordiprax+, an online dashboard with restricted access which visualises prescribing data and enables comparisons with other praxis' on a regional level (Textbox 5.2).

Regional medicine consultants also have access to ordiprax+ allowing them to monitor consumption and provide advice to individual practices. Since 2018, general practitioners in defined geographical areas have formed "quality clusters" for mutual support.

In Figure 5.11a and b, the number of prescriptions at municipality level are shown for years 2016 and 2020, respectively. For 2020, the numbers span from 329 to 548 prescriptions per 1,000 inhabitants. Four years earlier, in 2016, the corresponding interval was 434-727 prescriptions per 1,000 inhabitants. From the 98 municipalities in Denmark, four were excluded from the figure due to very small populations (typically islands).

Prescribing levels 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 5.7. It shows a decrease in the numbers of prescriptions by all doctor types except for hospital doctors between 2019 and 2020. The most marked reduction can be seen for general practitioners who issued 14% fewer antimicrobials during this 1-year period. However, prescriptions by hospital doctors continued to increase due to ongoing changes in the healthcare system. In 2020, hospital doctors accounted for 65 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 5.5 Number of treated patients per 1,000 inhabitants for leading antimicrobial agents in primary health care, Denmark, 2001 and 2011-2020

DANMAP 2020

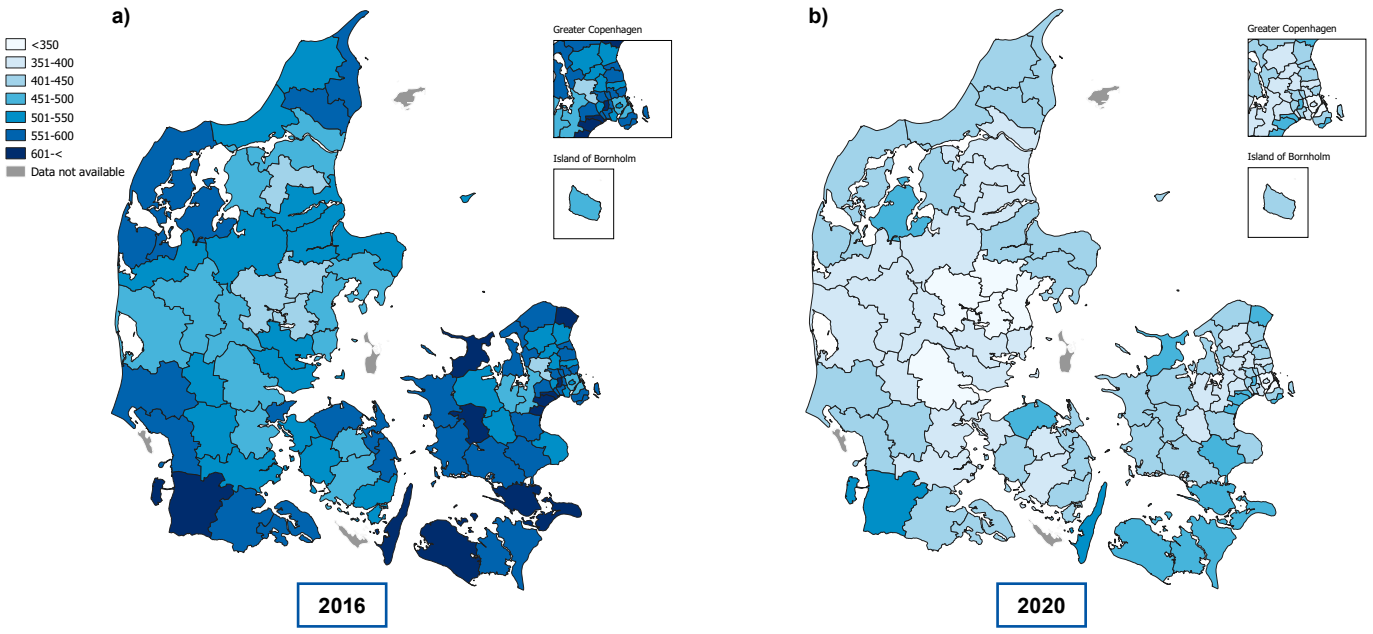
ATC group	Therapeutic group	Year										
		2001	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
J01AA	Tetracyclines	11.79	13.66	13.53	13.86	12.20	11.32	11.04	10.35	9.69	10.10	14.43
J01CA	Penicillins with extended spectrum	69.59	84.19	77.31	76.10	75.32	74.87	74.05	74.04	73.56	71.97	67.14
J01CE	Beta-lactamase sensitive penicillins	173.65	164.34	145.53	142.19	134.79	130.06	125.69	119.32	110.90	104.70	84.93
J01CF	Beta-lactamase resistant penicillins	19.22	30.34	28.51	29.07	29.24	28.85	29.70	29.96	31.10	31.06	30.52
J01CR	Combinations of penicillins, including betalctamase inhibitors	0.69	14.95	17.32	19.71	20.52	22.03	22.17	19.89	17.73	17.33	14.43
J01E	Sulfonamides and trimethoprim	36.36	27.63	26.48	26.16	24.65	22.45	21.17	19.87	18.42	16.63	15.04
J01FA	Macrolides	67.72	78.75	64.73	56.16	51.38	51.75	53.21	46.01	40.11	38.45	25.13
J01MA	Fluoroquinolones	7.47	18.10	17.25	16.04	15.30	15.04	14.37	13.36	12.26	10.74	9.01
J01X	Other antibacterials (methenamine >99%)	6.66	7.74	7.54	7.48	7.16	7.35	7.47	5.01	3.62	5.66	5.80
P01AB01	Nitroimidazole derivatives (metronidazole)	11.29	16.90	16.86	16.51	16.31	16.47	16.03	14.84	14.05	13.57	13.36
J01 and P01AB01	Antibacterial agents for systemic use	304.30	324.91	296.40	289.54	278.62	273.49	269.72	255.72	242.55	234.34	205.28

Data used in this table are based on registered sales to individuals
Data are based on the 2021 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 5.12 shows the main antimicrobial groups prescribed by medical specialty in primary health care in 2020, including hospital doctors issuing prescriptions for patients in ambulatory care. In 2020, 67% of antimicrobial prescriptions from specialists in dermato-venerology were for tetracyclines, which are indicated for treatment of severe acne and sexually transmitted chlamydia/mycoplasma infections. More than half (57%) of all prescriptions by dentists were for the narrow-spectrum beta-lactamase sensitive penicillins reflecting adherence to the recommended first-line treatment for common dental infections in the primary health care.

Figure 5.11 Number of prescriptions from primary health care per 1,000 inhabitants in Danish municipalities in a) 2016 and b) 2020

DANMAP 2020



Data used in this figure are based on registered sales to individuals
Data are based on the 2021 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Table 5.6 Consumption of antimicrobial agents for systemic use in primary health care at regional level, Denmark, 2016-2020 DANMAP 2020

Region	Indicator	Year				
		2016	2017	2018	2019	2020
Capital Region	DDD/1,000 inhabitants/day	15.26	14.36	13.49	13.37	12.49
	Prescriptions/1,000 inhabitants	519	489	453	441	382
Region Zealand	DDD/1,000 inhabitants/day	16.43	15.53	14.68	14.36	13.67
	Prescriptions/1,000 inhabitants	575	539	501	482	436
Region of Southern Denmark	DDD/1,000 inhabitants/day	14.98	14.09	13.39	13.16	12.18
	Prescriptions/1,000 inhabitants	530	497	470	455	401
Central Denmark Region	DDD/1,000 inhabitants/day	14.41	13.46	12.76	12.49	11.84
	Prescriptions/1,000 inhabitants	487	458	431	417	374
North Denmark Region	DDD/1,000 inhabitants/day	14.64	13.54	13.07	13.00	12.21
	Prescriptions/1,000 inhabitants	509	472	452	436	390
Denmark (total)	DDD/1,000 inhabitants/day	15.14	14.21	13.46	13.25	12.43
	Prescriptions/1,000 inhabitants	522	490	459	445	393

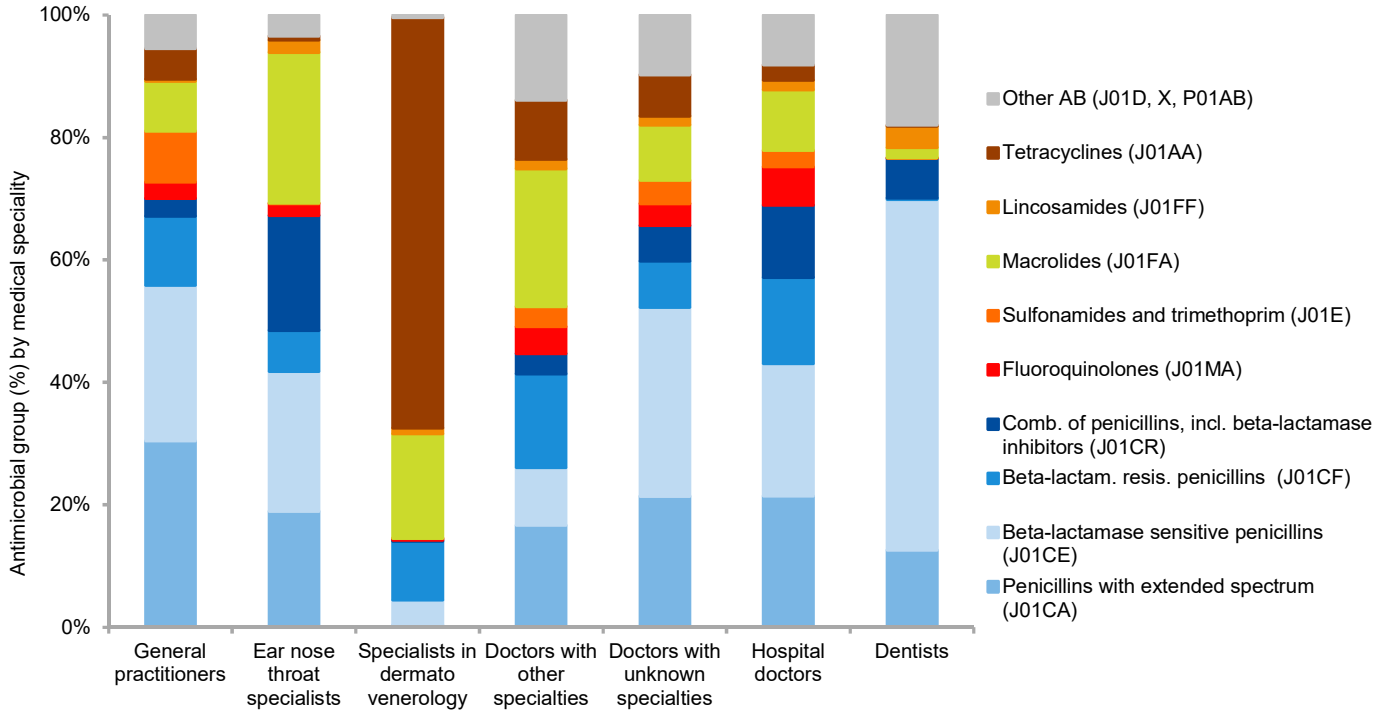
Data used in this table are based on registered sales to individuals
Data are based on the 2021 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Table 5.7 Number of prescriptions per 1,000 inhabitants for different doctor types, Denmark, 2018-2020 DANMAP 2020

Doctor type	Year		
	2018	2019	2020
General practitioners	341.5	326.8	280.2
Ear nose throat specialists	8.4	7.8	6.1
Specialists in dermato venerology	5.2	5.4	5.3
Doctors with other specialties	4.2	4.2	4.1
Doctors with unknown specialties	9.7	8.7	7.8
Hospital doctors	62.8	63.0	64.5
Dentists	27.8	28.8	25.6

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

Figure 5.12 Antimicrobial groups prescribed by main medical specialities, primary health care, Denmark, 2020 DANMAP 2020

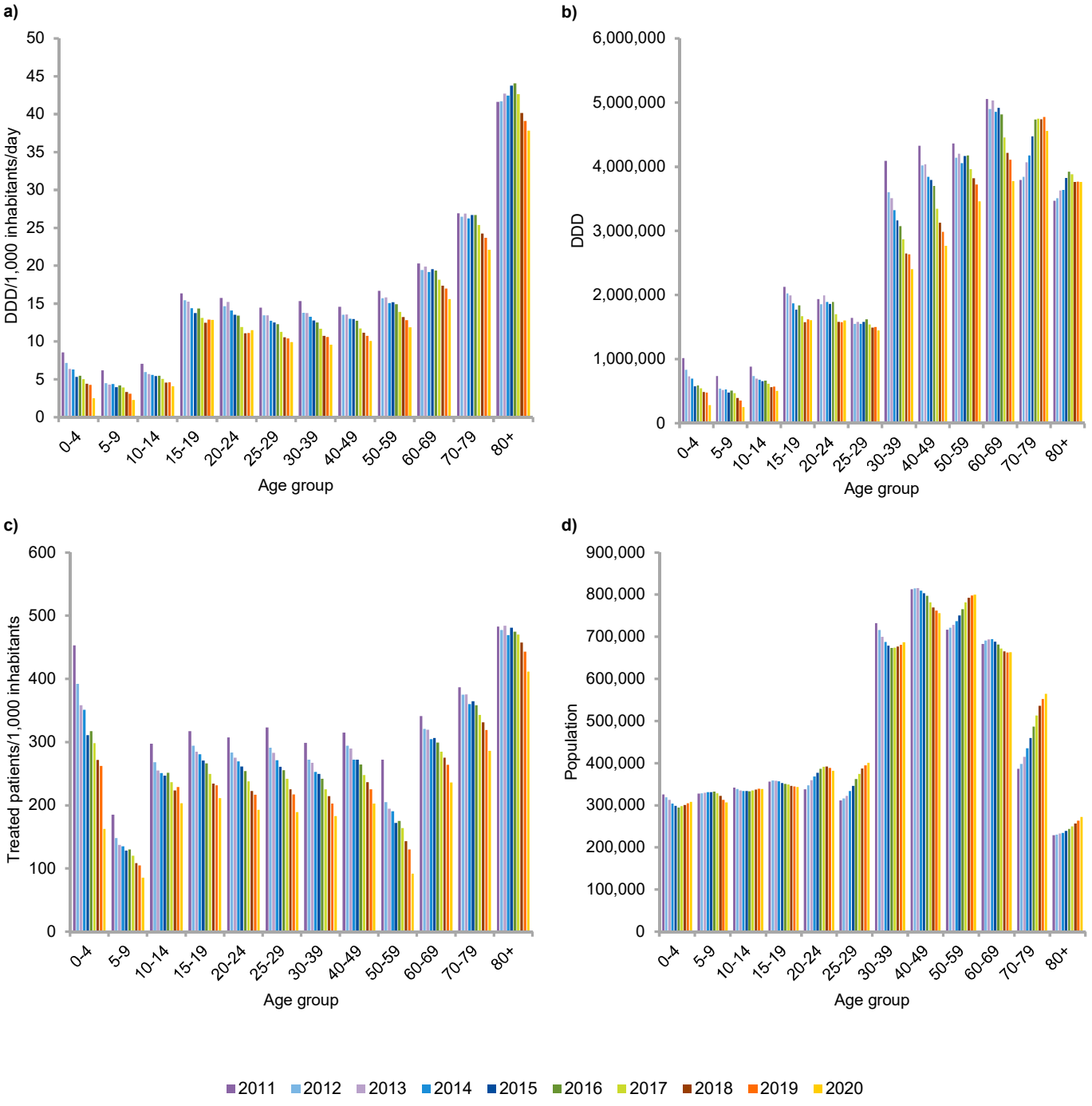


Data used in this figure are based on registered sales to individuals
Data are based on the 2021 edition of the Anatomical Therapeutic Chemical (ATC) classification system

5.3.5 Consumption by age group

Figure 5.13a-c presents consumption by age group based on different denominators: Figure 5.13a presents consumption in DDD per 1,000 inhabitants per day, Figure 5.13b in crude DDD, i.e. not corrected for population size. Figure 5.13c presents the number of patients treated per 1,000 inhabitants. Figure 5.13d presents the actual population size by age group. All figures show data from 2011 to 2020. Children and adolescents are presented in five-year age groups, while adults are shown in 10-year age groups.

Figure 5.13a to d Consumption of systemic antimicrobial agents in primary health care by age group, measured in DDD per 1,000 inhabitants per day, DDD, treated patients per 1,000 inhabitants and population size, Denmark, 2011-2020 DANMAP 2020



Data used in this figure are based on registered sales to individuals
Data are based on the 2021 edition of the Anatomical Therapeutic Chemical (ATC) classification system
Population size in Figure 5.13 is based on data from Statistics Denmark at www.dst.dk

5.3.6 Consumption of antimicrobials in children

Measures 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 since doses are calculated 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. However, assuming that dosage regimens did not change considerably in Denmark within the last decade, it is possible to compare the consumption measured in DDD within each age group over time.

In 2020, the overall consumption in children of 0-19 years continued to decrease compared to previous years. In total, there were 136 treated patients per 1,000 inhabitants, receiving 212 prescriptions per 1,000 inhabitants. In 2019, the corresponding numbers were 181 treated patients and 287 prescriptions per 1,000 inhabitants (reduction by 25% and 26% between 2019 and 2020, respectively). Since 2011, the consumption decreased from 300 treated patients per 1,000 inhabitants and 523 prescriptions per 1,000 inhabitants (reduction by 55% and 59% between 2011 and 2020, respectively).

Consumption in the 0-4 year olds. Consumption of antimicrobial agents in the youngest age group decreased by 64% between 2011 (453 treated patients per 1,000 inhabitants) and 2020 (162 treated patients per 1,000 inhabitants). Since 2019, the consumption decreased by 38% (262 treated patients per 1,000 inhabitants) (Figure 5.14a). On average, each treated patient received 2.0 prescriptions in 2011, which decreased to 1.5 prescriptions in 2020 (not shown). In 2020, the total consumption in this age group corresponded to 2.50 DID, 41% less than in 2019 (4.27 DID) (Figure 5.13a). The antimicrobials used also changed during the last decade. In 2011, penicillins with extended spectrum were the main antimicrobial agents used to treat children between 0-4 years (267 patients per 1,000 inhabitants, 59% of total consumption). In 2020, beta-lactamase sensitive penicillins were the most prescribed (78 patients per 1,000 inhabitants, 48% of total consumption), (Figure 5.14a).

Consumption in the 5-9 year olds. In 2020, 92 patients per 1,000 inhabitants of 5-9 years were treated with antimicrobial agents (Figure 5.13c). This is 66% lower than 2011 (272 patients per 1,000 inhabitants) and 30% lower than 2019 (130 patients per 1,000 inhabitants). On average, each treated patient received 1.5 prescriptions in 2011, which decreased to 1.4 prescriptions in 2020 (not shown). In 2020, the total consumption corresponded to 2.3 DID, 63% less than in 2011 (6.2 DID) and 26% less than in 2019 (3.1 DID), (Figure 5.13a). The distribution of the antimicrobials used to treat 5-9 year olds did not change markedly over the last decade (Figure 5.14b), and beta-lactamase sensitive penicillins remained the main antimicrobial agent used (54 patients per 1,000 inhabitants, 59% in 2020).

Consumption in the 10-14 year olds. In 2020, the total consumption of antimicrobial agents (85 patients per 1,000 inhabitants) was 54% lower than a decade ago (185 patients per 1,000 inhabitants) and 19% lower than 2019 (105 patients per 1,000 inhabitants) (Figure 5.13c). On average, each treated patient received 1.5 prescriptions in 2020, which increased from 1.4 prescriptions in 2019 (not shown). In 2020, the total consumption corresponded to 4.1 DID, 42% less than in 2011 (7.1 DID) (Figure 5.13a). Beta-lactamase sensitive penicillins remained the main antimicrobial agent even with a reduction of 25% from 2019-2020, (Figure 5.14c).

Consumption in the 15-19 year olds. Consumption of antimicrobial agents in older teenagers decreased in 2020; thus the increase observed in 2019 did not continue (Figure 5.13a-c). In 2020, 203 patients per 1,000 inhabitants were treated with antimicrobial agents which was 11% lower than in 2019 (229 patients per 1,000 inhabitants). This reduction was driven by a 50% decrease in macrolides and a 21% decrease in beta-lactamase sensitive penicillins (Figure 5.14d). In 2020, the consumption corresponded to 339 prescriptions per 1,000 inhabitants and 12.9 DID which is 11% and 0.4% lower than 2019, respectively.

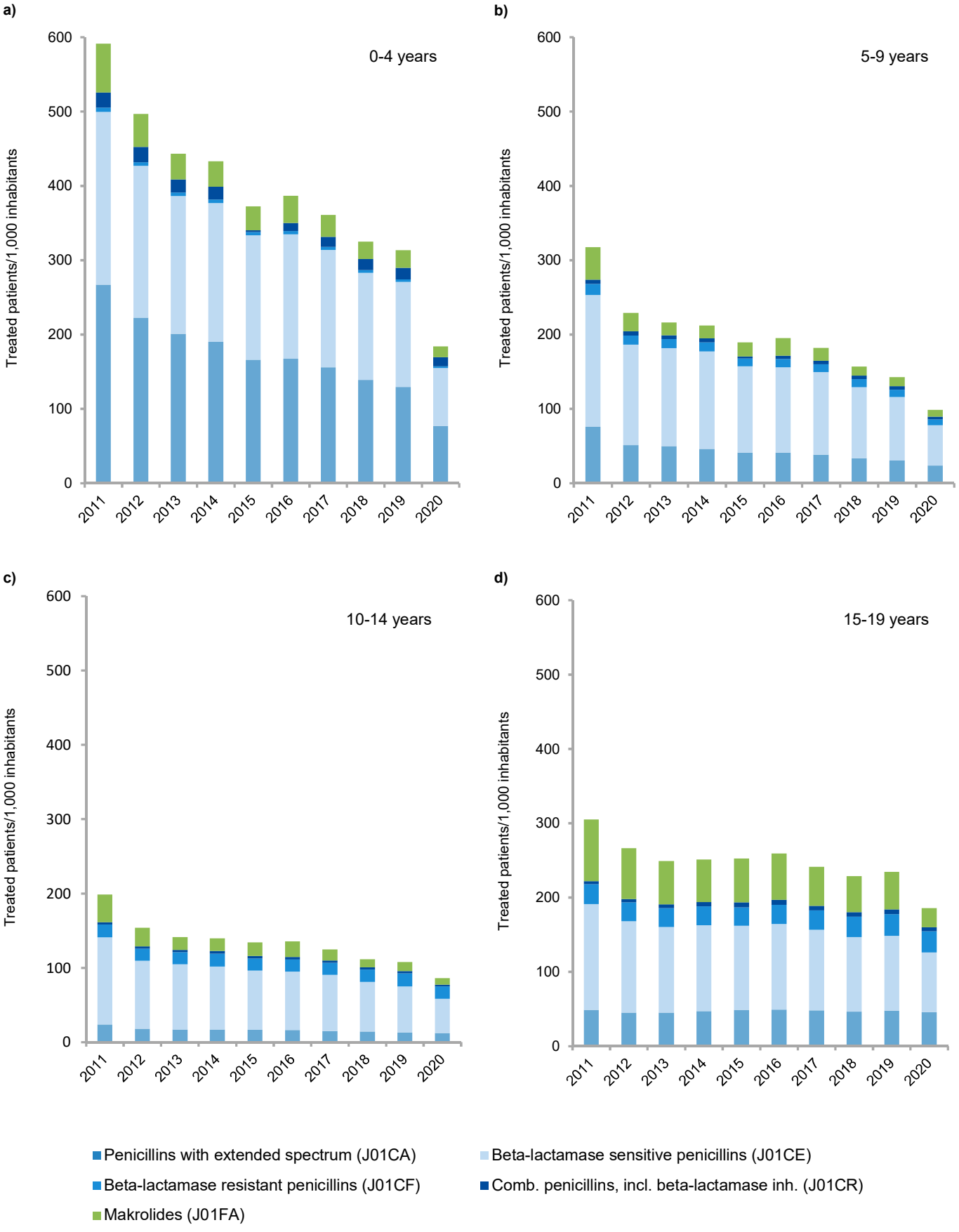
Macrolides play an important role in the treatment of bacterial respiratory infections in children and adolescents. Macrolides were also used as first-line treatment for chlamydia infections until the recent change in guidance already described in section 5.3.2.

The latter indication may be the reason for the relatively high consumption of macrolides in the 15-19 year olds, which accounted for 26 patients per 1,000 inhabitants in 2020. For the younger age groups, 0-4 year olds, 5-9 year olds and 10-14 year olds the corresponding numbers were 15, 9 and 9 patients respectively per 1,000 inhabitants per year (Figure 5.14). However, penicillins are the most used antimicrobial agents for children and adolescents, constituting between 60% and 87% of all antimicrobials prescribed depending on age group (Figure 5.14).

Comparison of consumption among girls and boys showed different tendencies in different age groups (not shown). The youngest boys (0-4 year olds) received 10% more prescriptions per 1,000 inhabitants than the girls (258 versus 235). The opposite was observed among older children: girls aged 5-9 years received 32% more prescriptions per 1,000 inhabitants than boys (149 versus 113), girls aged 10-14 years received 34% more prescriptions per 1,000 inhabitants than boys (145 versus 108) and girls aged 15-19 years received 107% more prescriptions per 1,000 inhabitants than boys (462 versus 223).

Figure 5.14 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, 2011-2020

DANMAP 2020



Data used in this figure are based on registered sales to individuals
Data are based on the 2021 edition of the Anatomical Therapeutic Chemical (ATC) classification system

5.3.7 Consumption of antimicrobials according to gender

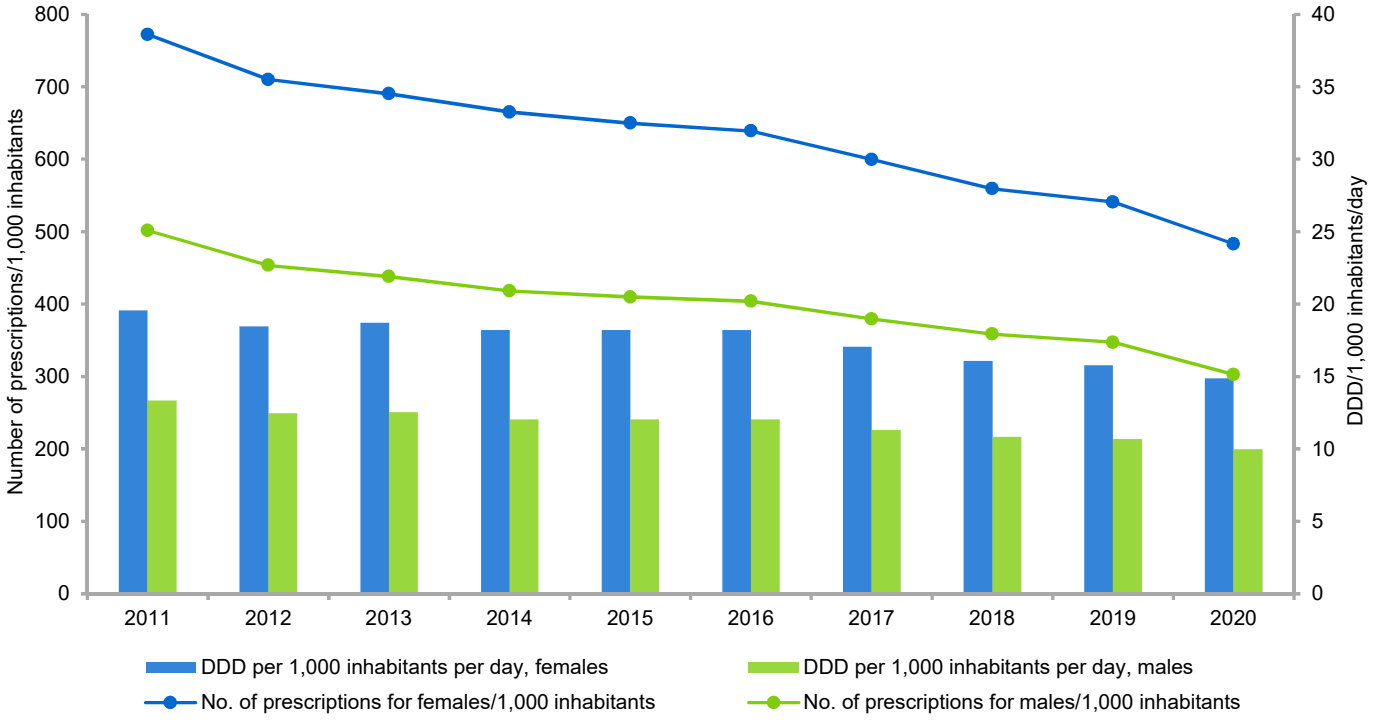
Differences between genders regarding consumption of antimicrobials are well known. In general, females receive more treatment – a trend driven by higher incidence of urinary tract infections and potentially different healthcare-seeking behaviours. Thus, the consumption of pivmecillinam, sulfonamides, trimethoprim and nitrofurantoin, all indicated for treatment of urinary tract infections, is approximately three times higher for females than for males. Also for beta-lactamase sensitive penicillins and macrolides the differences in consumption, especially when measured in DID, are substantial (Figure 5.15 and b). From 2011 to 2020, the number of treated females (all age group) decreased by 34% from 376 to 246 per 1,000 inhabitants and the number of treated males by 40% from 273 to 164 per 1,000 inhabitants.

During the same period, the number of DDD per prescription increased for females from 9.21 to 11.2 (22%), and for males from 9.7 to 12.0 (24%). Altogether from 2011-2020, the consumption in females decreased by 24% from 19.6 DID to 14.9 DID and in males by 25% from 13.4 DID to 10.0 DID.

Drugs for treatment of respiratory tract infections. For both females and males a decrease in the consumption of beta-lactamase sensitive penicillins and macrolides was observed over the last decade. A noted decrease was observed between 2019 and 2020. For females, beta-lactamase sensitive penicillins decreased from 5.6 DID to 3.0 DID and macrolides from 3.0 DID to 1.3 DID from 2019 to 2020. For males, the corresponding changes were from 4.5 DID to 2.5 DID and from 2.1 DID to 0.9 DID respectively (Figure 5.16a).

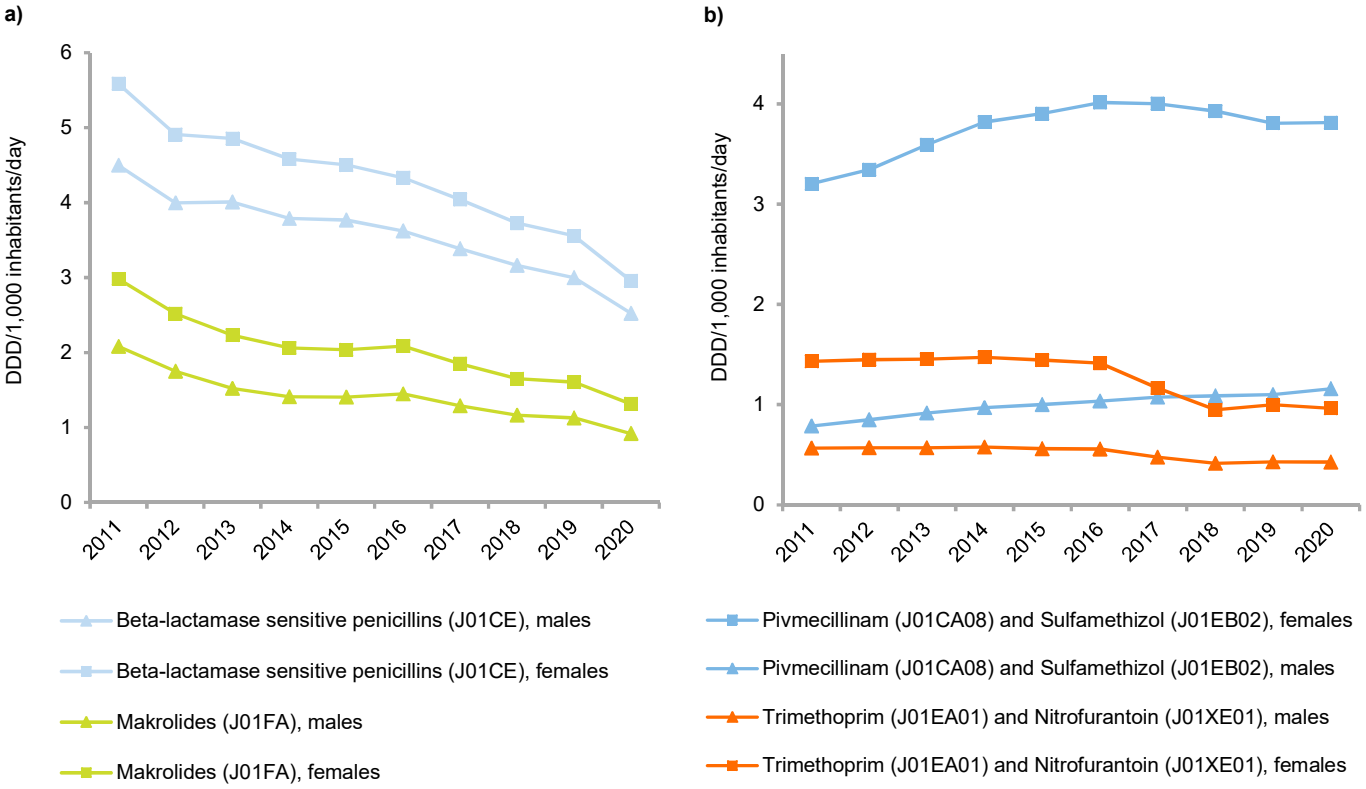
Drugs for treatment of urinary tract infections. Figure 5.16b presents the consumption of antimicrobials for urinary tract infections grouped into pivmecillinam and sulfamethizol (against acute infections) in addition to trimethoprim and nitrofurantoin (more often used in the prevention of UTI in elderly or in recurring infections) for 2011 to 2020. The data reflect again the higher incidence of urinary tract infections and corresponding higher use of antimicrobials in females as well as the impact of the national antimicrobial awareness campaign in 2016 which focussed on prevention of and prudent prescribing for urinary tract infections in older women.

Figure 5.15 Consumption of systemic antimicrobials in primary health care by gender, measured in prescriptions per 1,000 inhabitants and DDD per 1,000 inhabitants per day, Denmark, 2011-2020
DANMAP 2020



Data used in this figure are based on registered sales to individuals
Data are based on the 2021 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 5.16 Consumption of key antimicrobials used for treatment of a) respiratory tract infections and b) urinary tract infections, DDD per 1,000 inhabitants per day, Denmark, 2011-2020 DANMAP 2020



Data used in this figure are based on registered sales to individuals
Data based on the 2021 edition of the Anatomical Therapeutic Chemical (ATC) classification system

5.4 Hospital care

5.4.1 Introduction

Data on antimicrobial consumption at Danish hospitals are provided to DANMAP by the Danish Health Data Authority. The data are based on the Register of Medicinal Product Statistic which covers deliverances from hospital pharmacies to clinical departments and includes all generic products supplied through general trade agreements between the regions and the private sector company Amgros. For more information, see section 9.8, Chapter 9 'Materials and methods'.

DANMAP 2020 covers the total sales of systemic antimicrobials (ATC code J01 as well as ATC code P01AB01 and A07AA09) of all Danish hospital pharmacies. However, only Figure 5.1 and 5.2 include consumption at private hospitals and psychiatric departments (accounting for approximately 2-3% of the total hospital consumption in 2020). In all other figures and calculations, only consumption at somatic hospitals with acute care functions is presented.

In DANMAP, hospital consumption data are presented at national and regional level. Information on patient-level consumption is still not available for the hospital sector. This information will be available through the national "Hospital Medicine Register", which is currently under development.

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

Table 5.8 Consumption of antimicrobial agents for systemic use in somatic hospitals, DDD per 100 bed-days, Denmark, 2011-2020

DANMAP 2020

ATC group	Therapeutic group	Year									
		2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
J01AA	Tetracyclines	1.24	1.79	1.70	1.78	1.99	2.42	2.17	2.76	3.63	3.09
J01CA	Penicillins with extended spectrum	12.93	14.38	14.92	14.65	15.55	16.67	16.80	17.90	18.56	20.04
J01CE	Beta-lactamase sensitive penicillins	10.29	10.74	10.90	10.02	10.00	10.56	10.83	12.11	11.32	11.35
J01CF	Beta-lactamase resistant penicillins	9.29	9.46	10.17	10.00	10.21	10.76	10.64	12.18	12.96	13.88
J01CR	Comb. of penicillins. incl. beta-lactamase inhibitors	7.39	10.72	12.65	13.74	16.13	17.33	14.83	19.17	19.96	21.91
J01DB	First-generation cephalosporins	0.13	0.14	0.13	0.07	0.05	0.05	0.04	0.04	0.03	0.04
J01DC	Second-generation cephalosporins	16.33	15.40	14.23	12.23	11.15	10.63	11.73	10.48	9.38	9.18
J01DD	Third-generation cephalosporins	1.22	1.19	1.25	1.08	1.14	1.19	1.41	1.40	1.38	1.36
J01DF	Monobactams	0.16	0.17	0.16	0.07	0.03	0.01	0.01	0.01	0.01	0.01
J01DH	Carbapenems	2.71	2.97	3.23	3.55	3.20	3.11	3.05	3.26	3.42	3.71
J01EA	Trimethoprim and derivatives	0.35	0.42	0.44	0.50	0.44	0.43	0.44	0.51	0.46	0.51
J01EB	Short-acting sulfonamides	0.24	0.20	0.19	0.15	0.13	0.12	0.11	0.12	0.10	0.07
J01EE	Comb. of sulfonamides and trimethoprim. incl. derivatives	4.00	4.34	5.10	5.20	5.74	6.17	5.95	6.97	7.70	8.30
J01FA	Macrolides	3.71	3.90	3.79	3.92	4.79	5.41	6.06	7.29	7.77	6.97
J01FF	Lincosamides	0.54	0.70	0.74	0.70	0.63	0.72	0.69	0.89	0.86	0.82
J01GB	Aminoglycosides	2.30	2.43	2.49	2.20	2.37	2.24	2.37	2.50	2.82	2.90
J01MA	Fluoroquinolones	9.59	9.66	9.99	9.28	9.13	8.62	7.66	8.15	7.83	8.01
J01XA	Glycopeptides	1.38	1.45	1.52	1.24	1.27	1.25	1.39	1.48	1.54	1.71
J01XB	Polymyxins	0.26	0.26	0.31	0.24	0.21	0.22	0.20	0.26	0.25	0.27
J01XC	Steroid antibacterials (fusidic acid)	0.29	0.26	0.26	0.25	0.18	0.13	0.07	0.07	0.07	0.06
J01XD	Imidazole derivatives	4.22	4.52	4.74	4.76	4.64	5.19	4.94	5.04	4.75	4.86
J01XE	Nitrofuran derivatives (nitrofurantoin)	0.33	0.38	0.39	0.34	0.30	0.27	0.27	0.31	0.33	0.40
J01XX05	Methenamine	0.10	0.09	0.08	0.06	0.10	0.09	0.08	0.12	0.09	0.10
J01XX08	Linezolid	0.33	0.36	0.41	0.36	0.48	0.42	0.39	0.61	0.61	0.56
J01XX09	Daptomycin	0.02	0.02	0.03	0.06	0.04	0.06	0.09	0.17	0.07	0.11
P01AB01	Nitroimidazole derivatives (metronidazole)	2.67	2.64	2.60	2.13	2.21	2.51	2.16	2.26	2.21	2.27
A07AA09	Intestinal antiinfectives (vancomycin)	0.46	0.54	0.57	0.56	0.52	0.56	0.55	0.58	0.63	0.76
J01, P01AB01, A07AA09	Antibacterial agents for systemic use, including metronidazole and vancomycin	92.50	99.13	103.02	99.14	102.63	107.14	104.96	116.62	118.74	123.25

Data used in this table are based consumption at somatic hospitals
Data are based on the 2021 edition of the Anatomical Therapeutic Chemical (ATC) classification system
Data on bed-days are based on new definitions implemented in The National Patient Register in 2019

During the past decade, hospitalisation patterns in Denmark changed notably. Earlier discharge of patients from hospitals, i.e. decreased numbers of bed-days, increasing ambulatory care functions, including surgical procedures, are causing increased pressure on the health care system at municipality level and warrant restructuring of healthcare provision such as rehabilitation beds for patients after discharge from hospital (Figure A5.3 and A5.4 in web annex).

This in turn has led to increasing numbers of more serious infections being treated outside of the hospital setting and subsequent selection pressure potentially leading to the emergence of antimicrobial resistant infections in settings like residential homes.

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

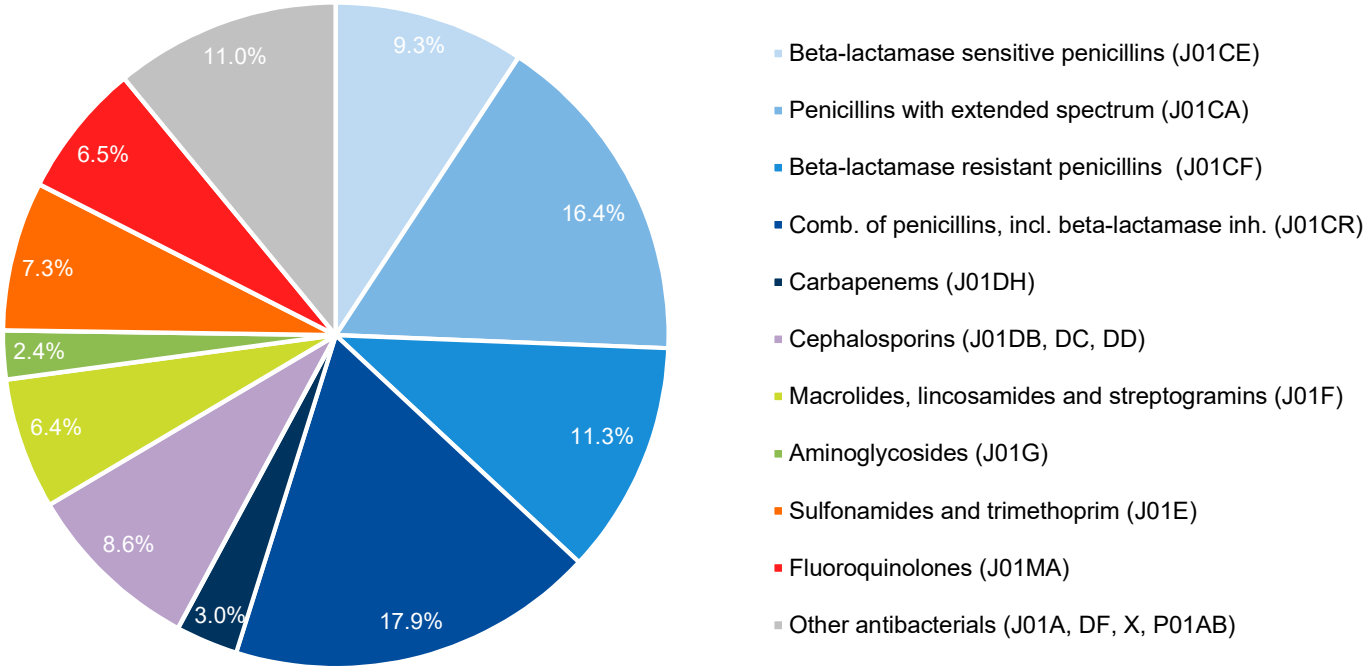
In 2020, the consumption of antimicrobial agents at somatic hospitals was 123.25 DBD. Compared to previous years, this is 3.8% higher than the observed 118.74 DBD in 2019 and 33% higher than the 92.5 DBD measured a decade ago in 2011. The level of consumption in 2020 was the highest measured this decade (Table 5.8).

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 67.19 DBD, corresponding to 55% of the total consumption of antimicrobials at somatic hospitals in Denmark in 2020 (Figure 5.17, Table 5.8).

Combinations of penicillins, including beta-lactamase inhibitors, accounted for 21.91 DBD, making it the largest group consumed in 2020 (18%) (Figure 5.17). Penicillins with extended spectrum were the second largest group consumed at Danish hospitals and accounted for 20.04 DBD (16%), an 8.0% increase compared to 2019 (18.56 DBD). Beta-lactamase sensitive penicillins accounted for 11.35 DBD (9.2%) and beta-lactamase resistant penicillins for 13.88 DBD (11.3%), a 7.1% increase from 2019 (12.96 DBD).

Over the past decade, consumption of all penicillin groups increased. Combinations of penicillins, including beta-lactamase inhibitors, increased steeply by 197% between 2011 and 2020 and beta-lactamase resistant penicillins and penicillins with extended spectrum increased by 49% and 55%, respectively (see Figure 5.19 for respective changes in DBD).

Figure 5.17 Consumption of antimicrobial agents in somatic hospitals by antimicrobial group (%) based on DDD, Denmark, 2020
DANMAP 2020



Data used in this figure are based on consumption at somatic hospitals
Data are based on the 2021 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Notable trends were also observed for tetracyclines and for combinations of sulfonamides and trimethoprim over the time period 2011 to 2020 (Table 5.8). Tetracycline consumption has increased continuously during the past decade; in 2011, these antimicrobials accounted for 1.24 DBD and for 3.09 DBD in 2020, an increase of 149%. However the consumption decreased by 15% from 2019 to 2020. Consumption of combinations of sulfonamides and trimethoprim increased from 4.00 DBD in 2011 to 8.30 DBD in 2020, a total increase of 107% over the decade (Table 5.8, Figure 5.18a and 5.19).

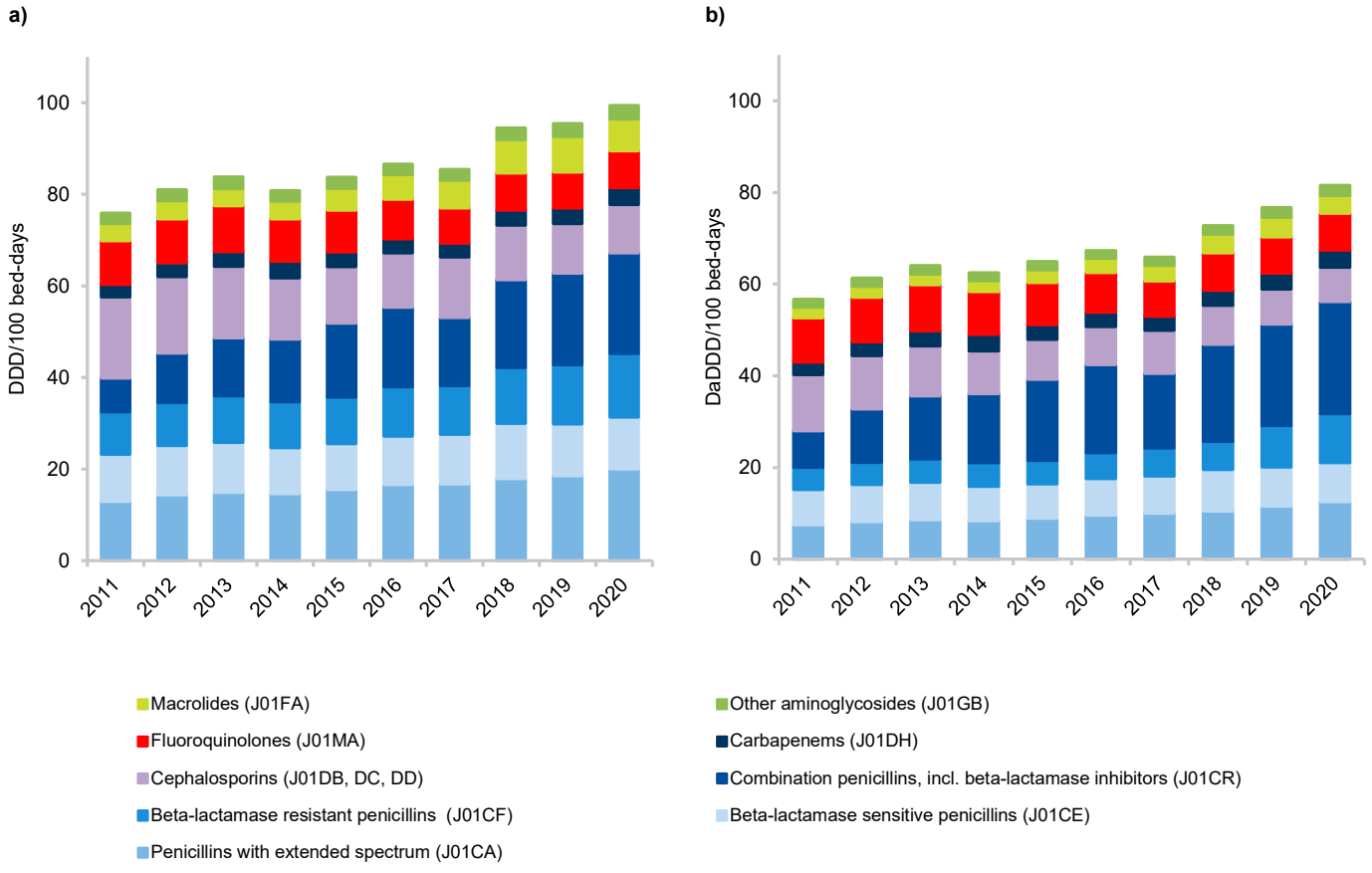
Linezolid consumption decreased to 0.56 DBD in 2020 after it peaked in 2019 (0.61 DBD). Over the past decade, the consumption of linezolid increased by 67.9% (0.33 DBD). Consumption of daptomycin peaked in 2018 (0.17 DBD), decreased in 2019 (0.07 DBD) and increased again in 2020 (0.11 DBD) (Table 5.8). Although the overall consumption of both antimicrobials is low, these changes are noteworthy since both should be reserved for treatment of serious infections caused by vancomycin-resistant enterococci (VRE) or methicillin-resistant *Staphylococcus aureus*. The continuous increase in vancomycin consumption is of concern given the increasing numbers of cases of VRE and vancomycin-variable enterococci (VVE) reported in Denmark over recent years (section 8.3.3 and 8.3.4, Chapter 8 'Resistance in human pathogens').

Trends for consumption of antimicrobials of critical interest are being discussed in section 5.4.4.

Trends in hospital consumption at regional level measured in DDD per 100 bed-days and DDD per 1,000 inhabitants per day are presented in Figure 5.20a and 5.20b. The Capital Region of Denmark shows the highest level of consumption for both measures when compared to the other regions in 2020. It is also notable that consumption increased for each region between 2019 and 2020 when measured in DBD but decreased over the same period when measured in DID. This reflects that hospital activity changed during the COVID-19 pandemic and more antimicrobials were used in relation to hospital patients' bed-days whereas population figures were less affected. Please see Chapter 2 'Impact of the COVID-19 pandemic' for more detailed information.

Figure 5.18 Consumption at somatic hospitals by leading groups of antimicrobial agents (J01), a) DDD per 100 bed-days and b) DaDDD per 100 bed-days for comparison, Denmark, 2011-2020

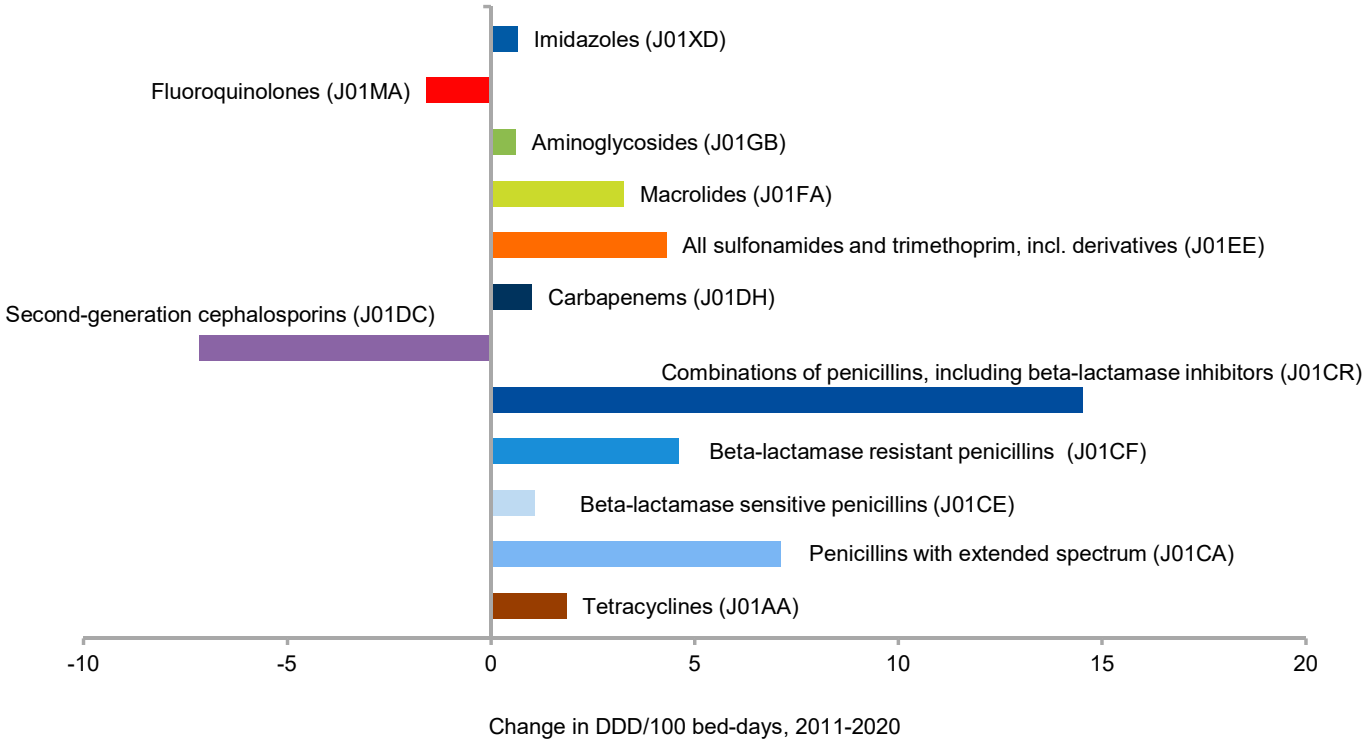
DANMAP 2020



Data used in this table are based consumption at somatic hospitals
Data are based on the 2021 edition of the Anatomical Therapeutic Chemical (ATC) classification system
Data on bed-days are based on new definitions implemented in The National Patient Register in 2019

Figure 5.19 Changes in the consumption of leading groups of antimicrobial agents at somatic hospitals, DDD per 100 bed-days, Denmark, 2011-2020

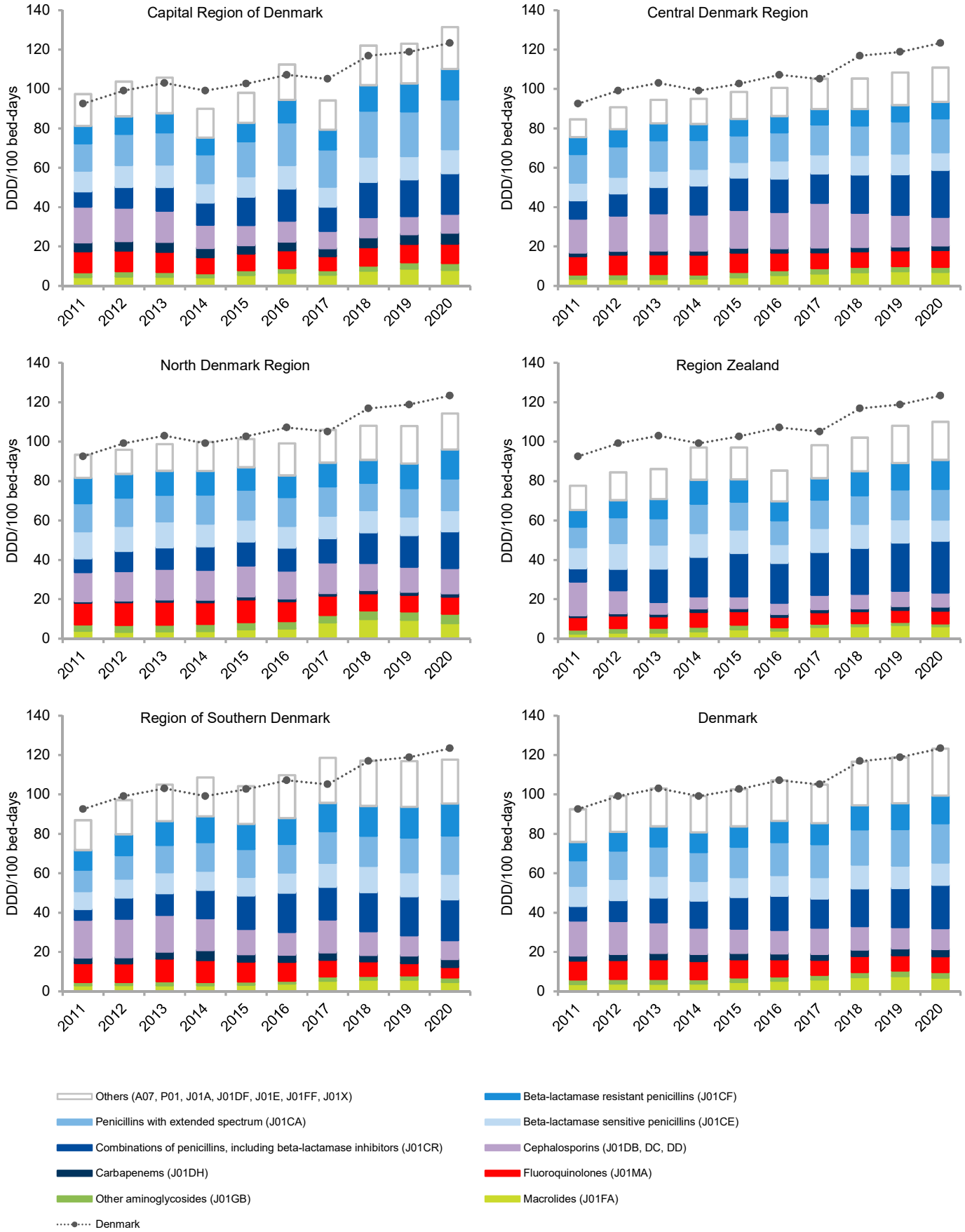
DANMAP 2020



Data used in this figure is based on consumption at somatic hospitals
Data are based on the 2021 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 5.20a Consumption of antimicrobial agents for systemic use in the five health regions, DDD per 100 bed-days, Denmark, 2011-2020

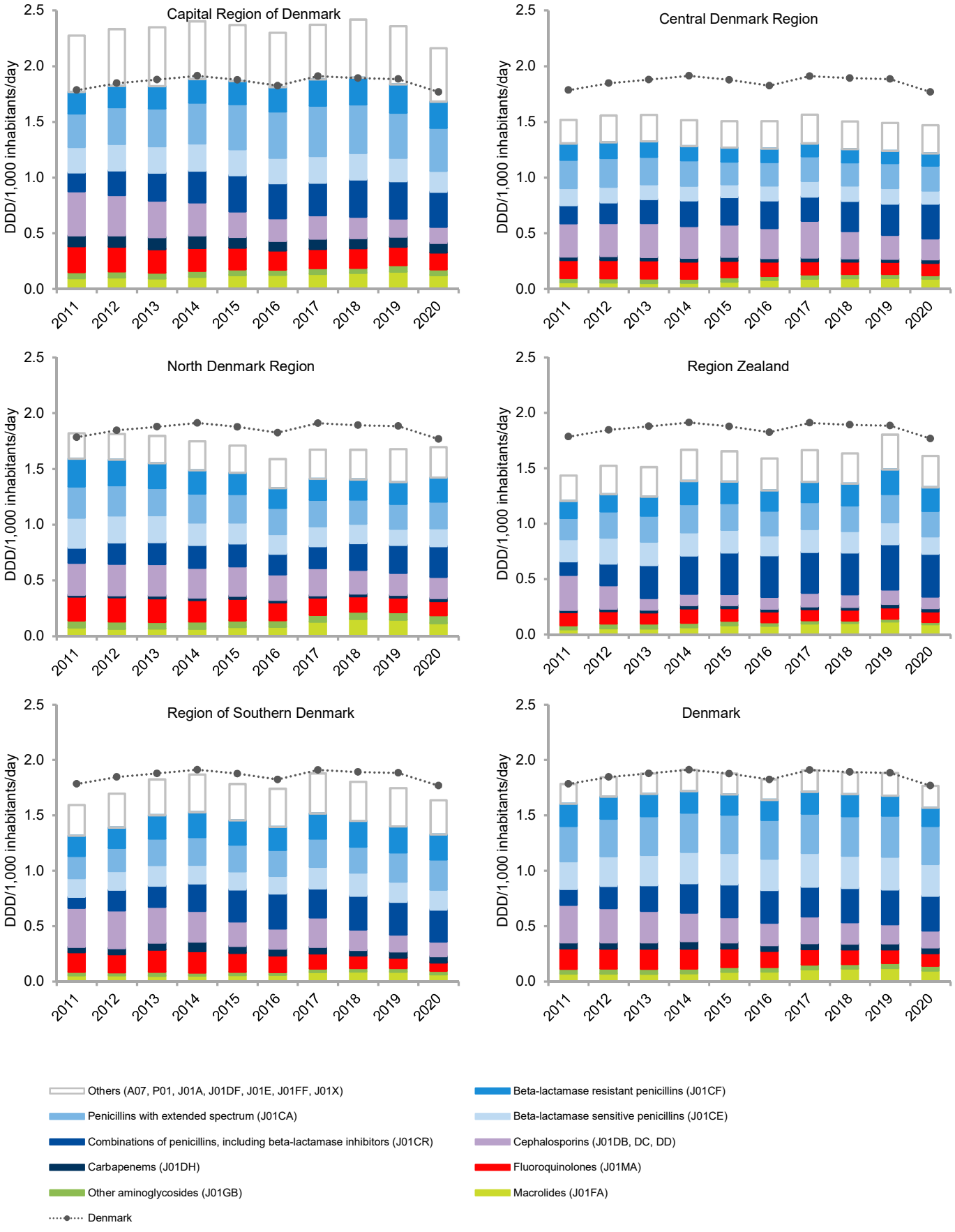
DANMAP 2020



Data used in this table are based consumption at somatic hospitals
Data are based on the 2021 edition of the Anatomical Therapeutic Chemical (ATC) classification system
Data on bed-days are based on new definitions implemented in The National Patient Register in 2019

Figure 5.20b Consumption of antimicrobial agents for systemic use in the five health regions, DDD per 1,000 inhabitants per day, Denmark, 2011-2020

DANMAP 2020



Data used in this table are based consumption at somatic hospitals
Data are based on the 2021 edition of the Anatomical Therapeutic Chemical (ATC) classification system

5.4.3 Public somatic hospitals - DDD per 100 admissions (DAD)

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), rather than by bed-occupancy.

In 2020, the total consumption was 531.51 DAD, a 0.98% increase compared to the 526.34 DAD in 2019 and a 15.84% increase compared to the 458.82 DAD in 2011. In 2020, the consumption reached the highest level measured the last decade, (Table 5.9). The trends in DDD per 100 admissions reflect for most antimicrobials the trends observed in DDD per 100 bed-days. However, the observed rates of increases were more marked, when measured in DDD per 100 bed-days than in DDD per 100 admissions for all antimicrobial classes, (Tables 5.8 and 5.9). This could be due to the change in hospital activity, as presented in Figure A5.3 in web annex. Trends in consumption measured in DDD per 1,000 inhabitants per day and DDD per 100 bed-days are presented in Figure 5.20 a and b.

Table 5.9 Consumption of antimicrobial agents for systemic use in somatic hospitals, DDD per 100 admissions, Denmark, 2011-2020

DANMAP 2020

ATC group	Therapeutic group	Year									
		2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
J01AA	Tetracyclines	6.15	8.76	8.25	8.97	9.83	11.32	10.92	12.49	16.11	13.31
J01CA	Penicillins with extended spectrum	64.16	70.34	72.25	74.01	76.63	78.08	84.42	80.93	82.25	86.42
J01CE	Beta-lactamase sensitive penicillins	51.03	52.55	52.78	50.64	49.26	49.49	54.46	54.77	50.17	48.94
J01CF	Beta-lactamase resistant penicillins	46.08	46.27	49.26	50.55	50.30	50.41	53.48	55.06	57.46	59.88
J01CR	Comb. of penicillins. incl. beta-lactamase inhibitors	36.65	52.45	61.26	69.45	79.48	81.20	74.53	86.70	88.49	94.50
J01DB	First-generation cephalosporins	0.66	0.68	0.63	0.34	0.24	0.23	0.22	0.20	0.14	0.16
J01DC	Second-generation cephalosporins	81.02	75.32	68.88	61.81	54.97	49.81	58.97	47.39	41.57	39.57
J01DD	Third-generation cephalosporins	6.07	5.82	6.07	5.43	5.63	5.55	7.11	6.32	6.10	5.87
J01DF	Monobactams	0.81	0.82	0.80	0.34	0.15	0.06	0.04	0.03	0.05	0.04
J01DH	Carbapenems	13.44	14.52	15.64	17.96	15.79	14.55	15.35	14.73	15.18	15.98
J01EA	Trimethoprim and derivatives	1.75	2.03	2.12	2.54	2.15	2.02	2.21	2.30	2.04	2.21
J01EB	Short-acting sulfonamides	1.20	1.00	0.91	0.77	0.65	0.54	0.54	0.52	0.45	0.31
J01EE	Comb. of sulfonamides and trimethoprim. incl. derivatives	19.86	21.24	24.67	26.29	28.31	28.91	29.90	31.53	34.13	35.79
J01FA	Macrolides	18.40	19.08	18.35	19.80	23.60	25.34	30.48	32.99	34.44	30.06
J01FF	Lincosamides	2.70	3.41	3.59	3.52	3.09	3.37	3.45	4.01	3.80	3.54
J01GB	Aminoglycosides	11.42	11.87	12.08	11.11	11.70	10.51	11.91	11.29	12.50	12.52
J01MA	Fluoroquinolones	47.56	47.27	48.36	46.91	45.01	40.38	38.51	36.84	34.69	34.54
J01XA	Glycopeptides	6.87	7.07	7.37	6.27	6.27	5.85	7.00	6.67	6.84	7.36
J01XB	Polymyxins	1.27	1.27	1.51	1.22	1.04	1.04	1.03	1.20	1.13	1.17
J01XC	Steroid antibacterials (fusidic acid)	1.42	1.28	1.25	1.25	0.89	0.61	0.36	0.33	0.29	0.26
J01XD	Imidazole derivatives	20.95	22.11	22.95	24.05	22.87	24.34	24.83	22.79	21.04	20.97
J01XE	Nitrofuran derivatives (nitrofurantoin)	1.62	1.83	1.90	1.71	1.46	1.28	1.35	1.42	1.44	1.72
J01XX05	Methenamine	0.52	0.46	0.41	0.30	0.48	0.43	0.38	0.55	0.40	0.45
J01XX08	Linezolid	1.65	1.74	1.99	1.84	2.37	1.96	1.98	2.75	2.72	2.41
J01XX09	Daptomycin	0.08	0.10	0.13	0.30	0.21	0.27	0.44	0.75	0.33	0.48
P01AB01	Nitroimidazole derivatives (metronidazole)	13.23	12.92	12.60	10.74	10.88	11.76	10.88	10.24	9.81	9.79
A07AA09	Intestinal antiinfectives (vancomycin)	2.27	2.63	2.77	2.83	2.54	2.62	2.78	2.61	2.79	3.28
J01, P01AB01, A07AA09	Antibacterial agents for systemic use, including metronidazole and vancomycin	458.82	484.84	498.76	500.97	505.81	501.93	527.54	527.40	526.34	531.51

Data used in this table are based consumption at somatic hospitals
Data are based on the 2021 edition of the Anatomical Therapeutic Chemical (ATC) classification system
Data on admissions are based on new definitions implemented in The National Patient Register in 2019

5.4.4 Changes in the consumption of antimicrobials of critical interest

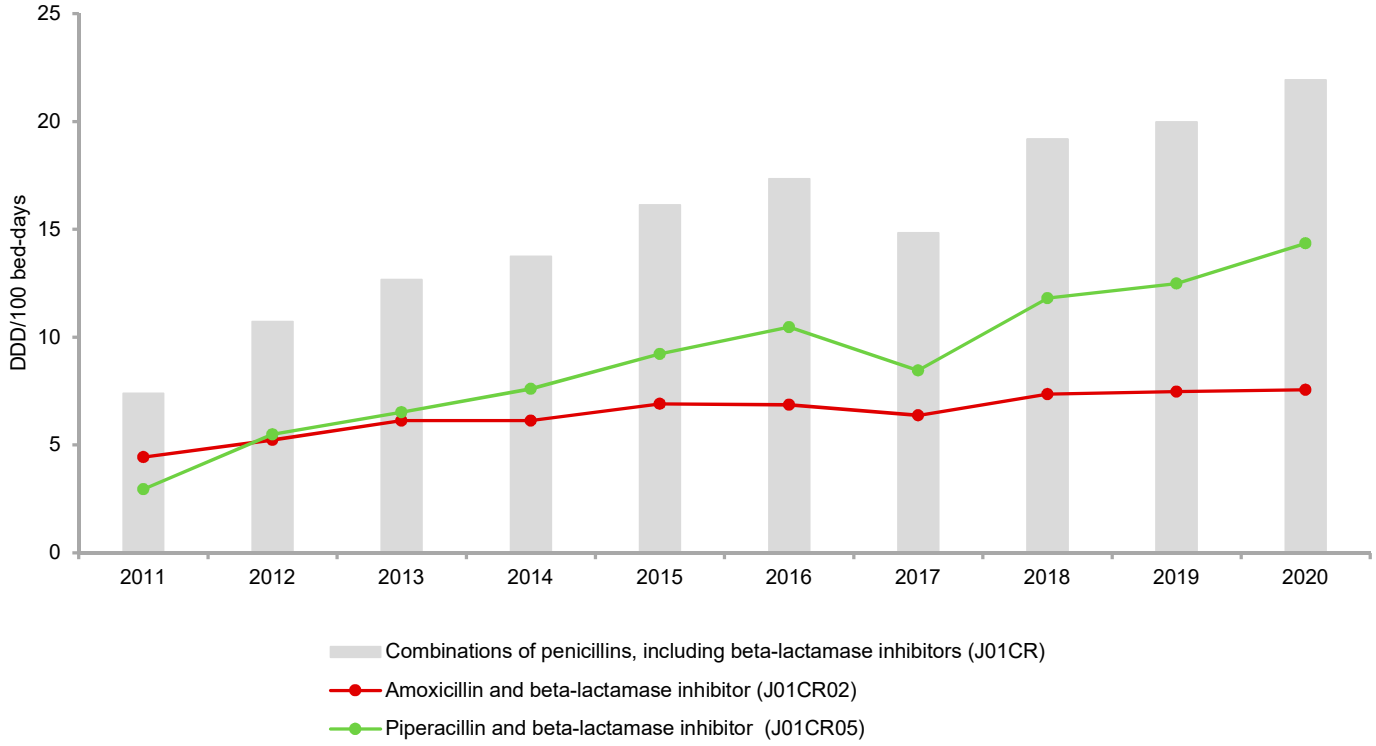
In Denmark, cephalosporins, fluoroquinolones and carbapenems have been defined as antimicrobials of special critical interest due to their resistance potential and their reserved use for treatment of severe infections.

In 2020, the antimicrobials of special critical interest constituted 18% of the total consumption at somatic hospitals compared to 32% in 2011 (Table 5.8).

For many years, 2nd generation cephalosporins were first-line treatment of patients with sepsis in Denmark. Due to links between the selection pressure caused by cephalosporins and associated risk of *Clostridium difficile*, VRE and MRSA infections, guidelines switched to recommending piperacillin with tazobactam and more recently to either beta-lactamase sensitive penicillins or penicillins with extended spectrum (in combination with gentamicin). Following global shortage of piperacillin/tazobactam in 2017 (Figure 5.21) an increase in the use of 2nd and 3rd generation cephalosporin use could be seen but levels slightly decreased again since 2018 (Table 5.9).

In 2020, cephalosporins accounted for 8.6%, fluoroquinolones for 6.5% and carbapenems for 3.0% of the total antimicrobial consumption in somatic hospitals in Denmark (Figure 5.17).

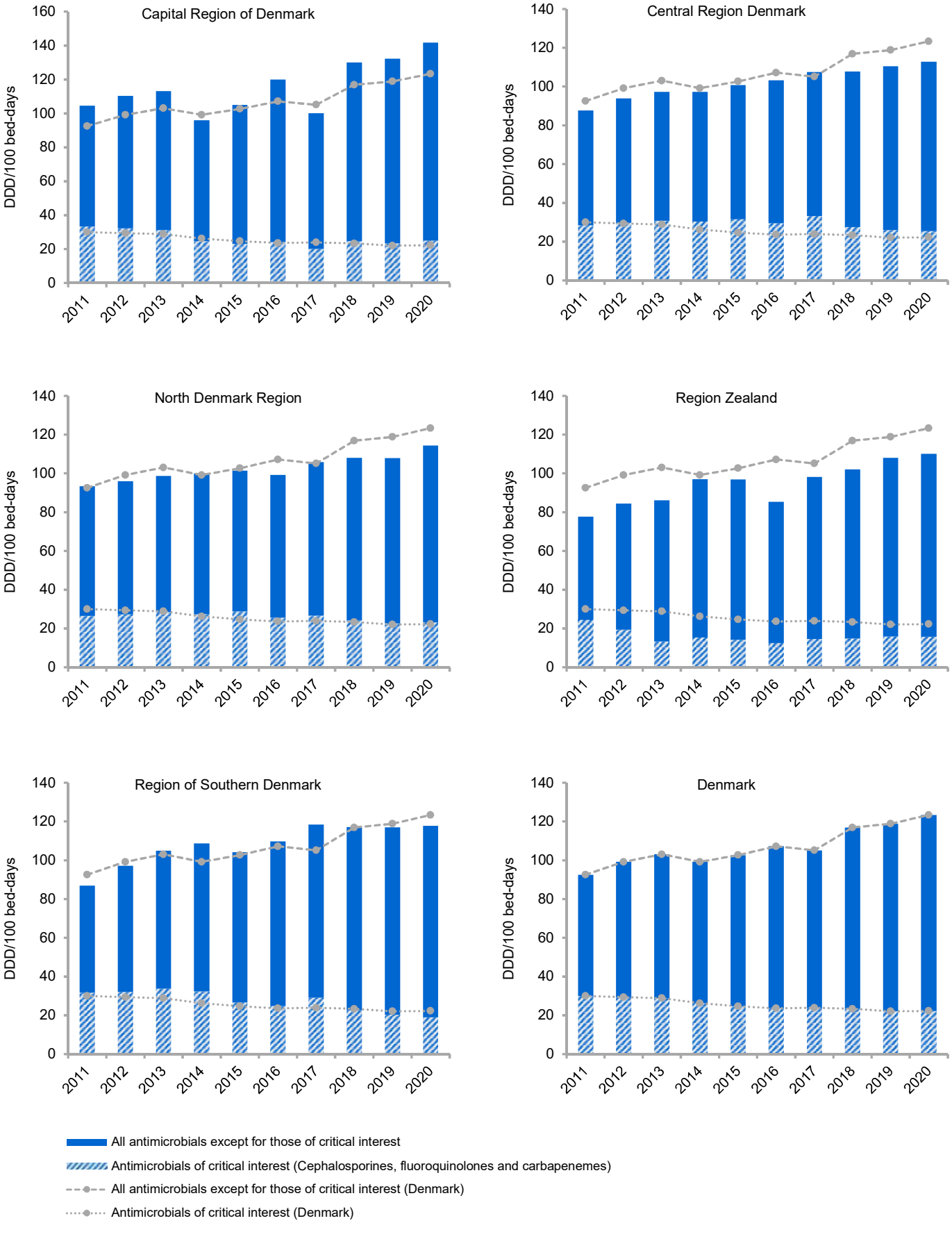
Figure 5.21 Consumption of combination penicillins at somatic hospitals, DDD per 100 bed-days, Denmark, 2011-2020 DANMAP 2020



Data used in this figure are based consumption at somatic hospitals
Data are based on the 2021 edition of the Anatomical Therapeutic Chemical (ATC) classification system
Data on bed-days are based on new definitions implemented in The National Patient Register in 2019

Figure 5.22 Consumption of antimicrobials of special critical interest in the five health regions, DDD per 100 bed-days, Denmark, 2011-2020

DANMAP 2020



Data used in this figure are based on consumption at somatic hospitals
Data are based on the 2021 edition of the Anatomical Therapeutic Chemical (ATC) classification system
Data on bed-days are based on new definitions implemented in The National Patient Register in 2019

The trends in the consumption antimicrobial of special critical interest at regional and national level between 2011 and 2020 are presented in Figure 5.22.

Monitoring of the consumption of antimicrobials of special critical interest will continue supported by local, regional and national quality improvement initiatives such as "National Quality and Learning Teams [www.kvalitetsteams.dk] (only available in Danish) and the government's National Action Plan goal to reduce the use of these antimicrobials in hospitals.

We would like to acknowledge Maja Laursen and Frederik Løgstrup Magnusson 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.

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Textbox 5.1

National Action Plan on the reduction of antibiotics in humans, 2016-2020/21

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

- The first goal targets an overall reduction in antimicrobial consumption in primary health care, from 462 redeemed prescriptions per 1,000 inhabitants in 2016 to 350 redeemed prescriptions per 1,000 inhabitants in 2020 (prescriptions issued by general practitioners, medical specialists and dentists)
- 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 third goal aims to achieve a 10% reduction of the consumption of the three antimicrobials of special critical interest (cephalosporins, fluoroquinolones and carbapenems) at hospitals between 2016 and 2020, measured in DBD

The following results have been achieved between 2016 and 2020:

Goal 1: The number of prescriptions in primary healthcare (general practitioners, medical specialists and dentists) were reduced to 329 prescriptions per 1,000 inhabitants in 2020. Figure 1 shows the number of prescriptions redeemed at pharmacies in Denmark from 2016-2020. Already in 2016, some of the general practitioners prescribed less than 350 prescriptions per 1,000 inhabitants (DANMAP 2017).

Figure 1 Prescribing trends in the primary sector, prescriptions per 1,000 inhabitants, Denmark DANMAP 2020

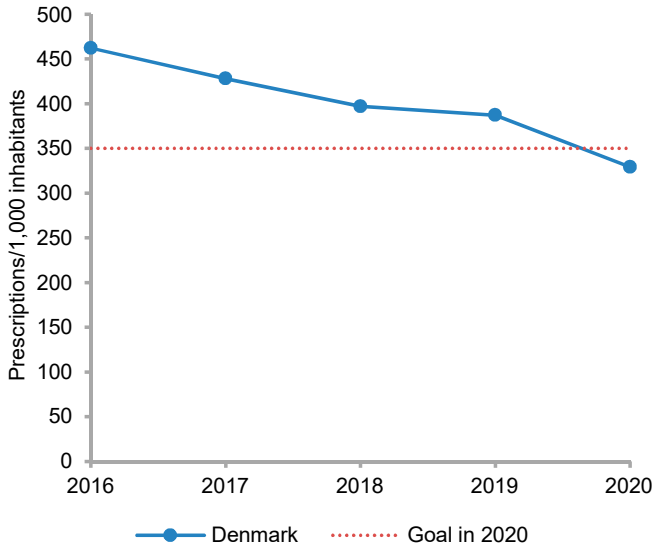
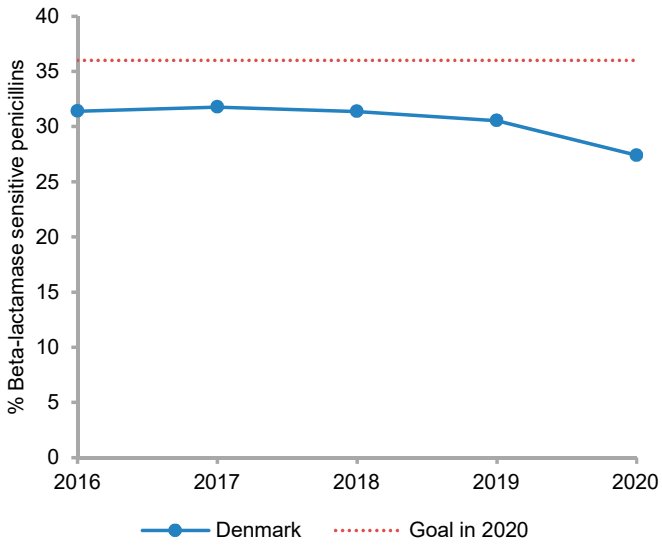


Figure 2 Share of beta-lactamase sensitive penicillins out of total consumption of antimicrobials, primary sector, Denmark DANMAP 2020

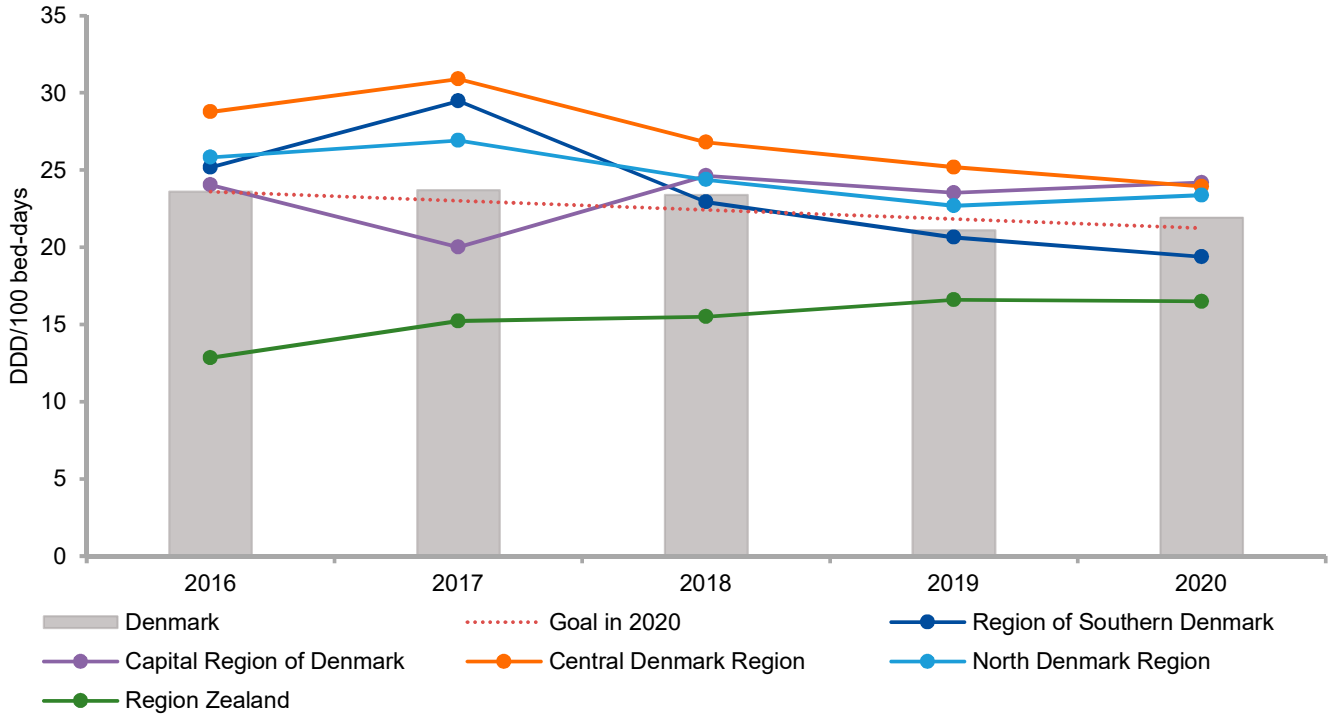


Data used in these figures are is based on registered sales to individuals
Data are based on the 2021 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Goal 2: The proportion of beta-lactamase sensitive penicillin (based on number of prescriptions issued by general practitioners, medical specialists and dentists) remained unchanged (approximately 31%) at national level between 2016 and 2019 (Figure 2). However, in 2020 the proportion decreased to 27%.

Goal 3: The consumption of antimicrobials of special critical interest at hospitals decreased by 7.2% at national level from 23.6 DBD in 2016 to 21.9 DBD in 2020 (Figure 3). It should be noted that between 2019 and 2020 the consumption increased by 3.8%. At regional level, the consumption decreased in hospitals in the Central Denmark Region (-16.8%) as well as in hospitals in the North Denmark Region (-9.5%) and Region of Southern Denmark (-23.0%). The level of consumption of antimicrobials of special critical interest increased in hospitals in the Capital Region of Denmark (+0.6%) and Region Zealand (+28.4%) between 2016 and 2020; however, the consumption in Region Zealand remained lower than the consumption levels in the other regions.

Figure 3 Consumption of critically important antimicrobials on regional level incl. 10% reduction goal, DDD per 100 bed-days, Denmark
DANMAP 2020



Data used in this figure is based on consumption at somatic hospitals
Data are based on the 2021 edition of the Anatomical Therapeutic Chemical (ATC) classification system
Data on bed-days are based on new definitions implemented in The National Patient Register in 2019

In the light of the COVID-19 pandemic, the Ministry of Health has 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.

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Textbox 5.2

ordiprax+ - Visualisation of General Practitioners' prescribing patterns

Background: In Denmark, knowledge of general practitioners' (GP) prescription pattern is part of the accreditation of GPs. Accreditation aims at ensuring quality improvement among GPs. The new online platform, ordiprax+ [www.esundhed.dk/Emner/Laegemidler/ordiprax], is a tool that enables GPs to monitor their own prescription patterns and can be used in accreditation. ordiprax+ is developed by the Danish Health Data Authority in collaboration with the Danish GP organization and other stakeholders.

Objectives: The purpose of ordiprax+ is to enhance rational use of pharmacotherapy among GPs by visualising prescribing data. ordiprax+ makes it possible for GPs to track their own prescription patterns over time and compare them with those of other GPs in the same cluster or with national levels. Regional pharmaceutical advisers can also access data for GPs in their own region. Therefore, the tool can assist GPs in evaluating treatments in cooperation with pharmaceutical advisers and when working within GP clusters on quality development initiatives.

Methods: In ordiprax+, data from the Danish National Health Service Register are being linked to the GP Register thereby linking Danish inhabitants who are registered at a GP with information on the GP. Information from the National Prescription Registry on all prescribed drugs in primary care is also being added to the dataset. The registries are linked by using personal social security numbers and GP registration numbers.

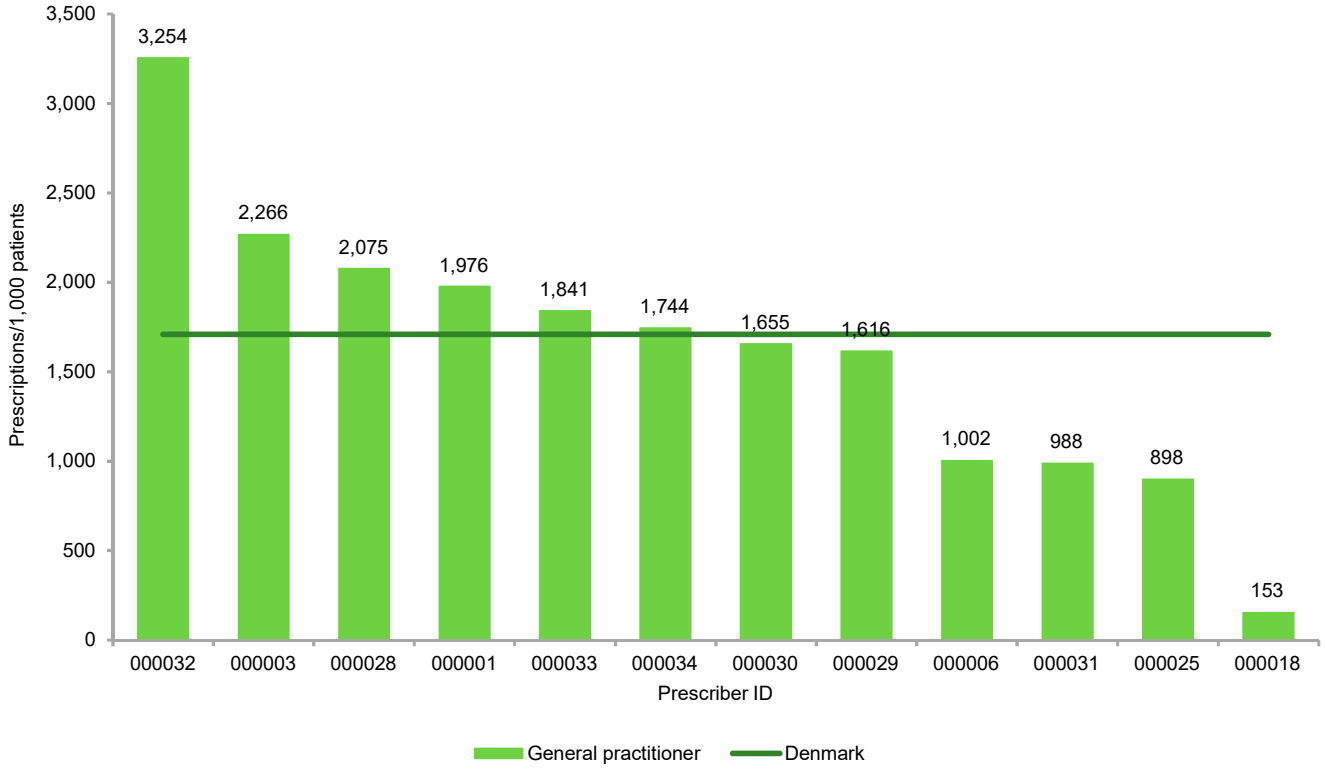
With regards to antibiotics, ordiprax+ contains data on all redeemed prescriptions of antibiotics for systemic use from Danish inhabitants' own GP during the last three years. Antibiotics are grouped according to the fourth and fifth level in the ATC classification system and as narrow-/broad-spectrum antibiotics respectively.

ordiprax+ is accessible as an online platform with regulated access, which ensures data confidentiality. Data are updated once a month and presented with the visualization software Qlik®.

Results: GPs can use ordiprax+ in their evaluation and quality improvement of own prescription patterns both in their everyday practice and when meeting with other GPs within their cluster. GPs can use, for instance, ordiprax+ to monitor the number of antibiotic prescriptions they prescribe per 1,000 patients and the percentage of penicillin V over the total amount of antibiotics prescribed by them and compare these to the level in Denmark, their own GP cluster and individual GPs in the cluster.

Visualisation of GPs' own prescribing patterns for antibiotics can therefore support rational prescribing and working towards the national goals for reducing antibiotics in primary care (Danish National Action Plan 2017 Goal 1: 350 redeemed prescriptions per 1,000 inhabitants in 2020. Goal 2: 36 percent of total number of prescriptions issued by GPs, medical specialists or dentists should be for beta-lactamase sensitive penicillins in 2020. For more information on the National Action Plan goals please see Textbox 5.1 'National Action Plan on the reduction of antibiotics in humans, 2016-2020/21').

Figure 1 Example of a graph in ordiprax+ showing the distribution of the number of redeemed prescriptions of antibiotics per 1,000 patients issued by GPs in one cluster over a one year period as well as national level (data are fictive) DANMAP 2020



Conclusions: ordiprax+ can assist GPs with quality improvement, thereby enhancing:

- Reduction of prescriptions of certain inappropriate drugs
- A more rational use of pharmacotherapy.

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6

RESISTANCE IN ZOONOTIC BACTERIA AND ANIMAL PATHOGENS

6. Resistance in zoonotic bacteria and animal pathogens

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

In 2020, fluoroquinolone (ciprofloxacin) resistance remained common in *Campylobacter jejuni* isolates obtained from human cases (51%), broilers (38%) and cattle (27%). Among *S.* Typhimurium isolates from human cases acquired in Denmark, resistance to fluoroquinolone (ciprofloxacin) remained low (1%). Macrolide (erythromycin) resistance was not observed in human isolates from domestically acquired infections, cattle and broilers (<1%).

The level of azithromycin resistance in *Salmonella* Typhimurium isolates was 1% in human isolates, while no azithromycin was resistance detected in isolates from Danish pork. 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 was not observed in *S.* Typhimurium isolates from pigs. One percent of *S.* Typhimurium isolates from human cases were resistant to 3rd generation cephalosporins. Carbapenem resistance was not observed in *S.* Typhimurium from humans. Meropenem (carbapenem) resistance has never been observed in animal or food isolates of *S.* Typhimurium.

6.1 Introduction

Zoonoses are infectious diseases that can be transmitted between animals and humans, either through direct contact with animals or indirectly by contaminated food, water, 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 2020 [www.food.dtu.dk].

Campylobacter and *Salmonella* surveillance has been part of the DANMAP programme since 1995. It monitors antimicrobial resistance in isolates from broilers, cattle, pigs, fresh meat and from human clinical cases.

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

Macrolides are used to treat infections in animals in Denmark, whereas fluoroquinolones and cephalosporins are not used in food-producing animals. In 2020, 12,579 kg of macrolides were prescribed for animals. The majority (94% equivalent to 11,862 kg) of these were used in pigs, while 215 kg and 130 kg were used for cattle and poultry, respectively (Table 4.1).

In humans, monitoring of antimicrobial resistance is performed on clinical isolates. *Salmonella* isolates, and a geographically stratified selection of *C. jejuni* isolates are susceptibility tested in accordance with the ECDC recommendations. Travel histories of the patients are collected, when possible.

Since 2014, isolation and antimicrobial susceptibility testing of *Campylobacter* has been performed in accordance with the EU harmonised monitoring of antimicrobial resistance [Decision 2013/652/EU]. In 2020, *Campylobacter* isolates were obtained from healthy animals at slaughter (caecal samples) and *Salmonella* isolates were obtained from boot swabs collected at farms and carcases at slaughter. Further details on sampling, analysis, susceptibility testing and interpretations are presented in Chapter 9.

6.2 *Campylobacter*

A total of 323 human *C. jejuni* isolates were susceptibility tested. The isolates represented 220 domestically acquired infections, 34 travel-associated infections and 69 infections of unknown origin, and included 38 outbreak related isolates from three different outbreaks. A total of 163 *C. jejuni* isolates from broilers and 93 isolates from cattle were also susceptibility tested. The MIC distributions for *C. jejuni* from broilers, cattle and humans are presented in the web annex (Tables A6.1- A6.2). For further details on methodology, see Chapter 9.

6.2.1 Resistance in *Campylobacter jejuni*

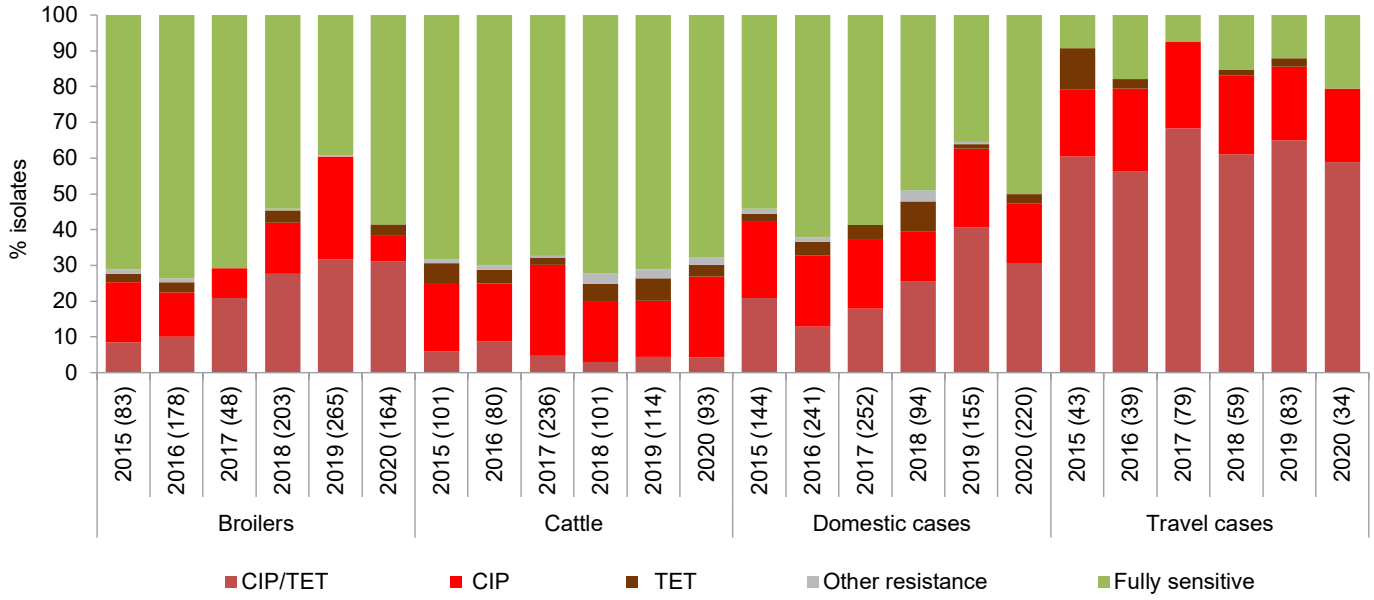
For the first time since 2016, the percentage of fully sensitive *C. jejuni* isolates from broilers and domestic human cases increased, (Figure 6.1). In 2020, 55% of *C. jejuni* from broilers and 50% from domestic human cases were sensitive to all antimicrobials tested. Compared to 2019, the percentage of isolates with resistance to only ciprofloxacin was lower in broilers and humans, as was the percentage of isolates with combined resistance to ciprofloxacin and tetracycline, among isolates from human cases acquired in Denmark. In cattle, the level of fully sensitive isolates remained the same as in previous years (67-72%). The distribution of AMR profiles are presented in the web annex (Table A6.3).

Macrolide resistance in *Campylobacter* is monitored by erythromycin. No erythromycin resistance was observed in *C. jejuni* from cattle, broilers or domestically acquired human cases in 2020. Only one isolate from a travel-related case was erythromycin resistant (Table 6.1). During the last ten years, macrolide resistance never exceeded 7% in human isolates in any year and the levels of erythromycin resistance in *C. jejuni* isolates from Danish broilers and cattle have also remained low with between zero and two resistant isolates per year (Figure 6.2). This suggests that the actual prevalence of macrolide resistance in animal and food isolates remains very close to the limit of detection by the current sampling scheme and is only captured sporadically.

Fluoroquinolone resistance in *Campylobacter* is monitored using ciprofloxacin. Over the last 20 years, ciprofloxacin resistance has slowly but continually increase in *C. jejuni* from Danish broilers. Even though the increased discontinued in 2020, the increasing trend remains statistically significant when assessed over the last 10 years (Figure 6.2). Fluoroquinolones are not used in food production animals in Denmark, suggesting that the increase in ciprofloxacin resistance in broilers is driven by something other than direct usage of fluoroquinolones. The general increase in fluoroquinolone resistance has coincided with an increase in the level of tetracycline resistance, but as for ciprofloxacin, the level of tetracycline resistance has also decreased in isolates from broilers from 2019 to 2020.

In general, the use of antimicrobials in the Danish poultry sector is low and limited to only a few antimicrobials. Tetracycline is the most commonly used antimicrobial (Figure 6.2). The high level of *C. jejuni* isolates with both ciprofloxacin and tetracycline resistance suggests the potential for co-selection of ciprofloxacin resistance by the use of tetracycline in poultry. However, this possible link warrants further investigation.

Figure 6.1 Distribution (%) of AMR profiles in *Campylobacter jejuni* from broilers, cattle and human cases, Denmark DANMAP 2020



The number of isolates included each year is shown in parentheses. Broilers include isolates from Danish broiler meat when available. 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. Fully sensitive: all isolates with neither ciprofloxacin nor tetracycline resistance. CIP/TET, CIP and TET isolates may be resistant to erythromycin, nalidixic acid or streptomycin

Table 6.1 Resistance (%) in *Campylobacter jejuni* isolates from broilers, cattle and human cases, Denmark DANMAP 2020

	Broilers	Cattle	Human			
Antimicrobial agent	Danish %	Danish %	Domestically acquired %	Travel abroad reported %	Unknown origin %	Total %
Ciprofloxacin	38	27	47	79	48	51
Erythromycin	0	0	0	3	0	0
Gentamicin	0	0	0	3	0	1
Nalidixic acid	38	27	47	79	46	50
Streptomycin	18	4	6	9	16	9
Tetracycline	34	8	34	59	35	37
Fully sensitive (%)	55	71	50	21	51	47
Number of isolates	163	93	220	34	69	323

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

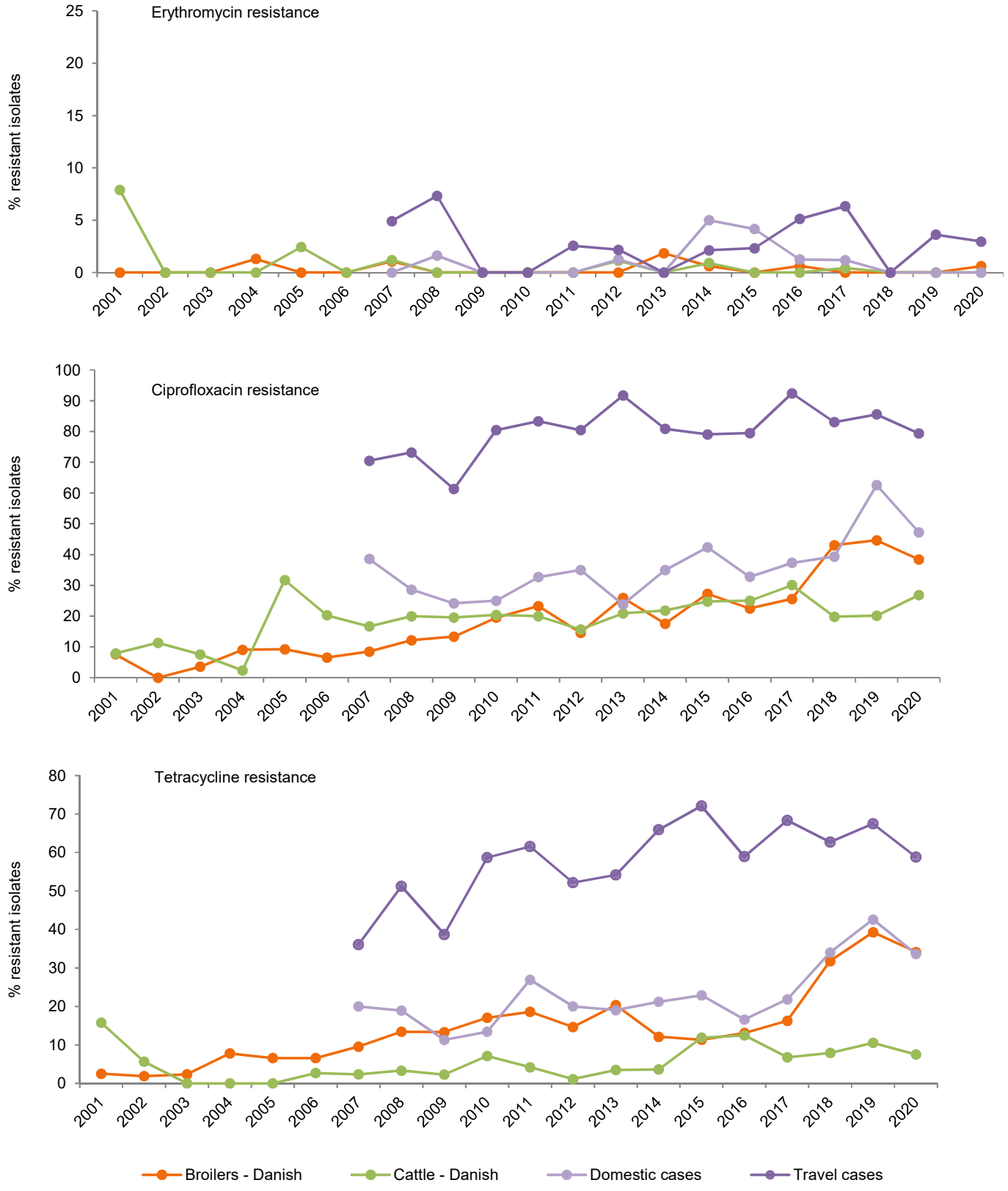
The occurrence of resistance to ciprofloxacin and tetracycline was higher in travel-associated isolates (79% and 59%, respectively) than in isolates from domestically acquired infections (47% and 34%, respectively). For both ciprofloxacin and tetracycline, the level of resistance decreased from 2019 to 2020. The levels of tetracycline resistance in isolates from humans and Danish broilers have been almost similar throughout the period from 2016 to 2020.

Gentamicin resistant isolates were not observed in 2020 among cattle and broiler isolates but one isolate from a travel-associated case were resistant. During the last 20 years of monitoring, gentamicin resistance has been low or absent among *C. jejuni* from human cases and gentamicin resistant *C. jejuni* from broilers and cattle have not been observed during this period.

Among human *C. jejuni* isolates, the level of streptomycin resistance was similar to previous years with 9% resistant isolates. In broilers, a marked increase in streptomycin resistant *C. jejuni* was observed, from 2% (N=56) in 2019 to 18% (N=164) in 2020.

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

DANMAP 2020



6.3 *Salmonella*

DANMAP focuses on resistance in *Salmonella* Typhimurium, as this serotype is present in clinical human isolates and in isolates from production animals. Clonal dissemination seems to play an important role for the occurrence of antimicrobial resistance among *S.* Typhimurium. The global dissemination of genomic islands conferring resistance to ampicillin, streptomycin, sulfonamide and tetracycline (the ASSuT profile) among *S.* Typhimurium and its monophasic variants has in increased the occurrence of multi-resistant *Salmonella* in Europe in the recent years [EFSA journal 19(4):6490]. This is also the case for Danish *S.* Typhimurium isolates from humans and production animals where resistance towards ampicillin, sulfonamide and tetracycline are common. However, these antimicrobials are not used for treatment of salmonellosis, and thus the public health impact of ASuT resistance is of less direct importance than resistance to critically important antibiotics such as macrolides, fluoroquinolones, and cephalosporins that are used for treatment of humans.

6.3.1 Resistance in *S.* Typhimurium

In DANMAP, *S.* Typhimurium includes the monophasic variants with antigenic formulas S. 4, [5],12:i:-, unless otherwise stated. In 2020, a total of 147 isolates of *S.* Typhimurium from human cases and 47 isolates from pork were collated for AMR testing. The human isolates included 61 diphasic and 86 monophasic variants and the pork isolates included 7 diphasic and 40 monophasic variants. The human monophasic isolates were dominated by ST34 (80 isolates) and the diphasic variants were dominated by ST19 (42) and ST36 (14). Thirty-five isolates were associated with five outbreaks that encompassed between four and seven isolates. Fourteen isolates were from travel-associated cases. This number was low compared to the previous years due to lack of travel activity in 2020, and therefore the data for travel-associated isolates are not presented separately in 2020.

Fluoroquinolone resistance in *Salmonella* is monitored using ciprofloxacin. During the last ten years, ciprofloxacin resistance in *S.* Typhimurium from Danish pigs and pork has been rare. In 2020, no ciprofloxacin resistance was found in 47 pork isolates. The level of ciprofloxacin resistance in isolates from domestically acquired human cases was 1% and ciprofloxacin resistance was observed in four of 14 isolates from travel-associated cases. These figures are similar to observations in previous years (Figure 6.3).

Since 2014, macrolide resistance in *Salmonella* has been monitored using azithromycin, and only a few azithromycin-resistant *S.* Typhimurium isolates have been found each year. From 2014- 2020, resistance levels never exceeded 6% annually (Figure 6.3). In 2020, azithromycin resistance was found in 1% of *S.* Typhimurium isolates from domestically acquired human cases, but was not observed in *S.* Typhimurium isolates from Danish pork (Table 6.2 and Figure 6.3).

Table 6.2 Resistance (%) in *Salmonella* Typhimurium isolates from pork and humans, Denmark DANMAP 2020

	Pork	Human	
	Danish	Domestically acquired	Total
Antimicrobial agent	%	%	%
Ampicillin	79	54	54
Azithromycin	0	1	1
Cefotaxime	0	0	1
Ceftazidime	0	0	1
Chloramphenicol	9	5	7
Ciprofloxacin	0	1	3
Colistin	0	1	1
Gentamicin	2	1	2
Meropenem	0	0	0
Nalidixic acid	0	1	1
Sulfonamide	87	54	54
Tetracycline	77	55	54
Tigecycline	0	1	1
Trimethoprim	13	10	12
Fully sensitive (%)	4	38	39
Number of isolates	47	96	147

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

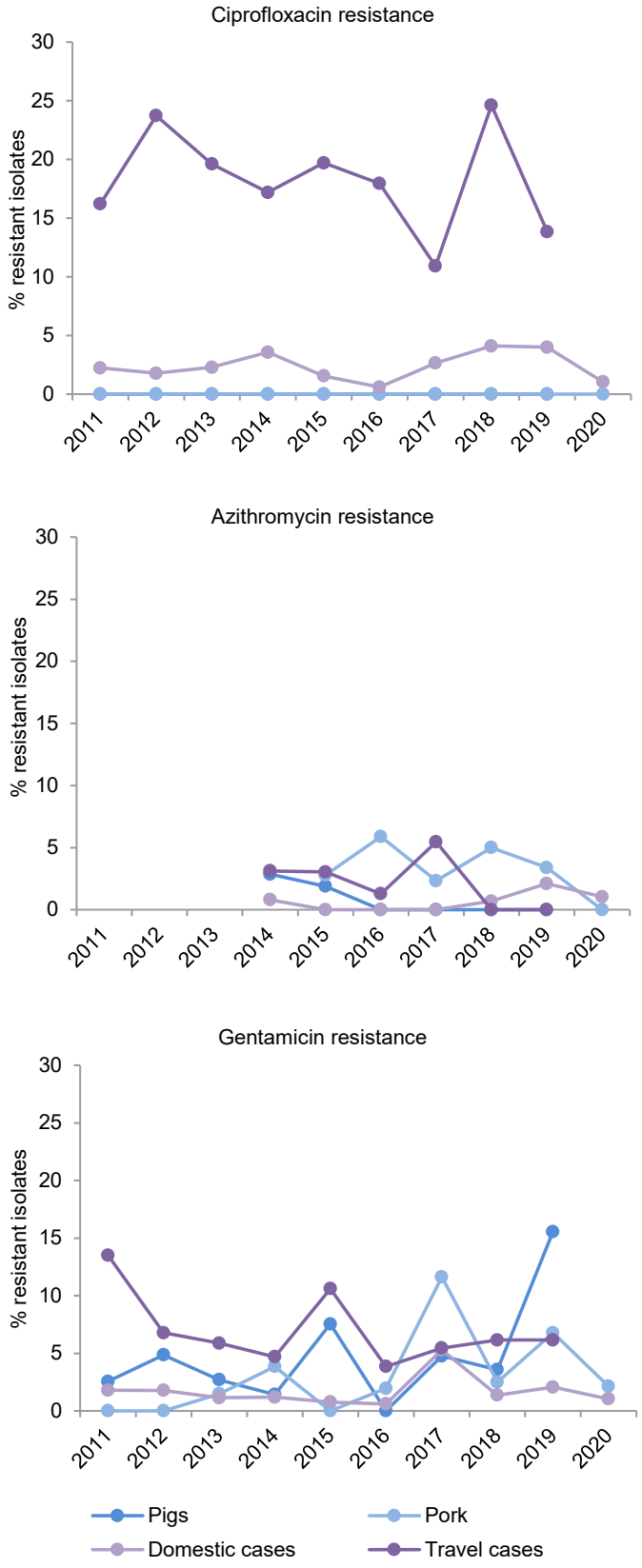
The rare detection of resistance to ciprofloxacin and azithromycin indicate that, the actual prevalence in pigs and pork is close to the detection limit. Sampling in pigs has therefore been changed after 2019, i.e. sampling of pigs at slaughter has increased, but are collected only every second year (odd years).

The peak in resistance to gentamicin in isolates from pigs and pork observed in 2019, was not observed in the *S.* Typhimurium collected from pork during 2020. The levels of gentamicin resistance in domestically acquired human isolates have been low and stable over the last years, and, in 2020, only three *S.* Typhimurium isolates were found resistant towards gentamicin.

Among human isolates, the level of resistance towards third generation cephalosporins was low, and the combination of cefotaxime and ceftazidime resistance was only found in two isolates. As in the previous years, none of the isolates from pork were resistant to third generation cephalosporins. Meropenem (carbapenem) resistance has never been observed in animal or food isolates of *S.* Typhimurium.

Resistance to tigecycline and colistin in *S.* Typhimurium is rare in Denmark and was not found in pork isolates in 2020.

Figure 6.3 Ciprofloxacin, azithromycin and gentamicin resistance (%) among *S.* Typhimurium from pigs, domestic pork and human cases, Denmark DANMAP 2020



Includes isolates verified as monophasic variants of *S.* Typhimurium with antigeneic 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. The data for travel cases are not shown for 2020, due to the low number of isolates (14)

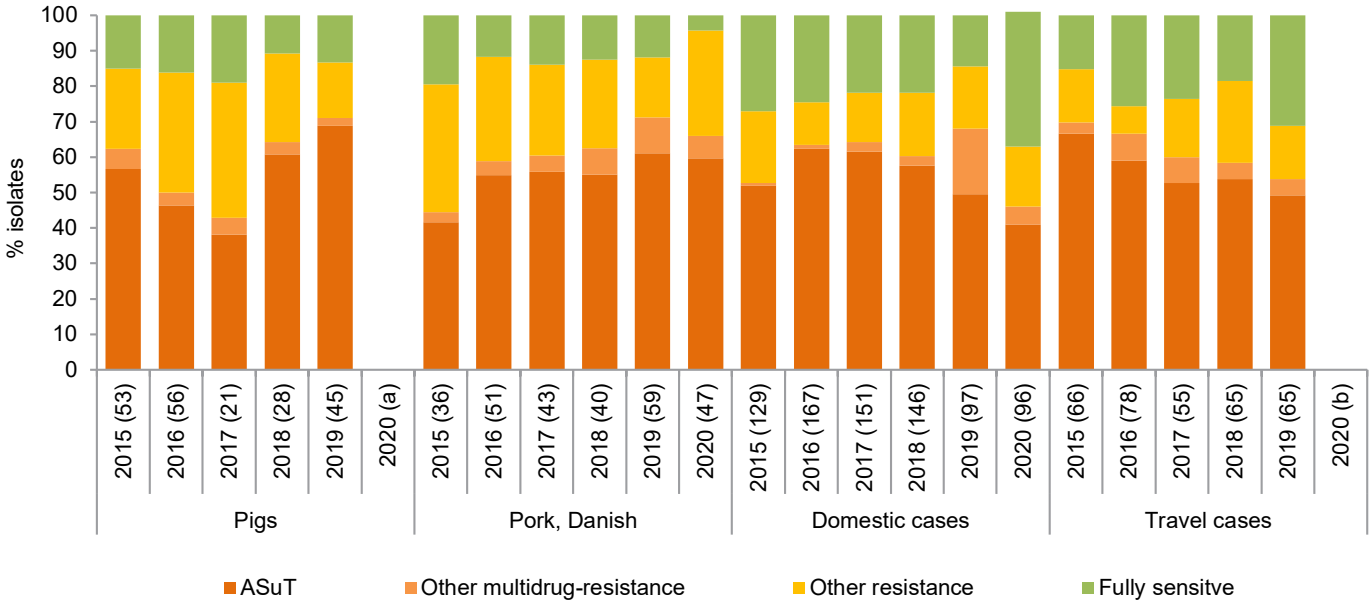
The MIC distributions and occurrence of resistance among *S.* Typhimurium isolates from humans and pork are presented in the web annex (Tables A6.3-A6.6).

Most of the *S.* Typhimurium isolates were resistant to several antimicrobials. Only 4% of the isolates from pork and 39% of the isolates from human cases were fully sensitive to all tested antimicrobials in 2020 compared to 12% and 14% in 2019, respectively (Figure 6.4).

The increasing multidrug-resistance among *S.* Typhimurium from Danish pork observed from 2015 to 2019 levelled out (Figure 6.4). As in previous years, the ASuT phenotype was the most frequent resistance profile among the included *S.* Typhimurium isolates, similar to most European countries and especially high among the monophasic isolates. See the AMR profile distribution in the web annex (Table A6.7).

The reduction in usage of tetracycline during weaning of pigs continued during 2020 (Chapter 4), but as previously reported, this was not reflected in the levels of resistance in *S.* Typhimurium. In isolates from pork, tetracycline resistance continued to increase from 44% in 2003 to 65% in 2011 and 77% in 2020. The increased use since 2017 of macrolides for weaner and finisher pigs did not result in a measurable increase in resistance to azithromycin. It is likely that the observed changes in resistance patterns are a reflection of spread of specific clones of *S.* Typhimurium, rather than changes in antimicrobial usage.

Figure 6.4 Distribution (%) of multidrug-resistant, resistant and fully sensitive *S.* Typhimurium from pigs, domestic pork and human cases, Denmark
DANMAP 2020



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 the 12 antimicrobial classes included in the test panel (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 (14)

6.3.2 Resistance in other *Salmonella* serotypes

S. Derby is common in pigs. The resistance levels in *S.* Derby are generally lower than in *S.* Typhimurium, and in 2020, 23 of the 29 *S.* Derby isolates from pork (79%) were sensitive to all tested antimicrobials. Resistance to tetracycline, sulfonamide, and trimethoprim and ampicillin was most common, either alone or in combination (Figure 6.5). Gentamicin resistance has been rare and resistance to ciprofloxacin, azithromycin or other antimicrobials of critical importance has not been observed during the last ten years of monitoring AMR in *S.* Derby from Danish pork.

S. Dublin: A total of 32 human isolates of *S.* Dublin were susceptibility tested. *S.* Dublin is intrinsically (naturally) resistant to colistin. Most of the isolates, 26, were fully sensitive, and resistance to third generation cephalosporins was not observed. Three strains exhibited multi-resistance and two of these were resistant to azithromycin.

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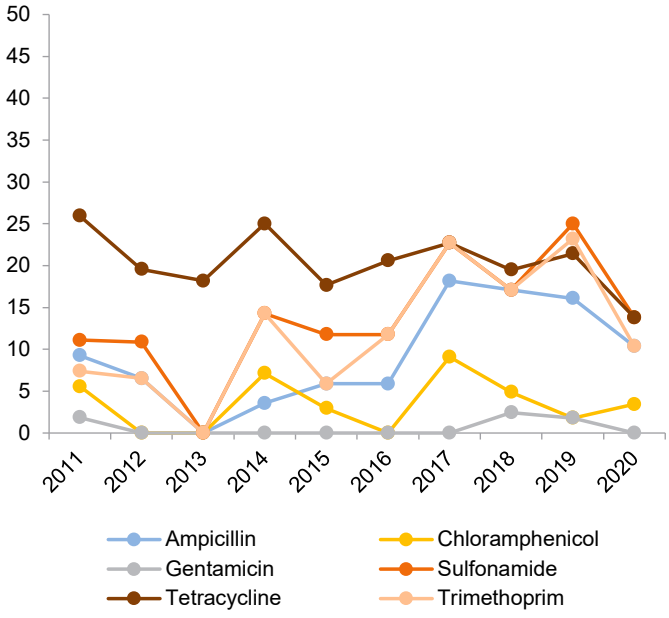
Figure 6.5 Resistance (%) in *Salmonella* Derby isolates from Danish pork, Denmark
DANMAP 2020



Textbox 6.1

Resistance in bacteria from diagnostic submissions from pigs

Background and data source: In Denmark, the veterinary pathogens from pigs most commonly susceptibility tested, comprise haemolytic *Escherichia coli*, *Streptococcus suis*, and *Actinobacillus pleuropneumoniae*. Data were obtained from the routine diagnostic laboratory analyses of isolates from dead and diseased pigs, submitted to SEGES Pig Research Centre's Laboratory in Kjellerup.

The antimicrobial susceptibility testing was carried out, using the broth microdilution method according to the CLSI guidelines, using SensiTitre panels. Since internationally approved clinical breakpoints were not available for all drug-bacterium combinations, the occurrence of resistance is presented using the interpretation criteria currently used at both DTU Centre for Diagnostics and SEGES Laboratory. These are mainly CLSI breakpoints, preferably porcine. For *E. coli*, the breakpoints are mainly human. If no CLSI breakpoints were found, ECOFFs or tentative ECOFFs were applied. When the applied breakpoints are adjusted according to newly established breakpoints, these are used retrospectively in this text box.

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

Table 1 Resistance (%) among bacteria from diagnostic submissions from pigs, Denmark DANMAP 2020

Antimicrobial agent	*Actinobacillus pleuropneumoniae*	*Haemolytic Escherichia coli*	*Streptococcus suis*
	%	%	%
Amoxicillin/clavulanic acid	-	6	-
Ampicillin	0	61	-
Apramycin	-	13	-
Cefotaxime	-	4	-
Cefoxithin			17
Ceftiofur	0	0.4	-
Chloramphenicol	-	19	0
Ciprofloxacin	0	0	1
Colistin	-	1	-
Erythromycin	100	-	56
Florfenicol	0	16	0
Gentamicin	-	14	1
Nalidixic acid	-	6	-
Neomycin	-	25	-
Penicillin	0	-	4
Spectinomycin	0	54	28
Streptomycin	-	75	44
Sulfametoxazol			81
Sulfonamide	-	68	-
Sulfonam./trimeth.	0	-	4
Tetracycline	1	63	72
Tiamulin	1	-	24
Tilmicosin	0	-	-
Trimethoprim	-	53	7
Tulathromycin	0	-	-
Number of isolates	116	255	145

Isolates from the routine diagnostic laboratory investigation of isolates from dead and diseased pigs submitted to SEGES Pig Research Centre's Laboratory for Pig Diseases in Kjellerup. Occurrences of resistant isolates are presented according to the clinical breakpoints that are currently used at DTU Centre for Diagnostics and Laboratory for Pig Diseases. Clinical breakpoint and MIC distributions are presented in the web annex (Table A6.10 - A6.12)

continued ... Textbox 6.1

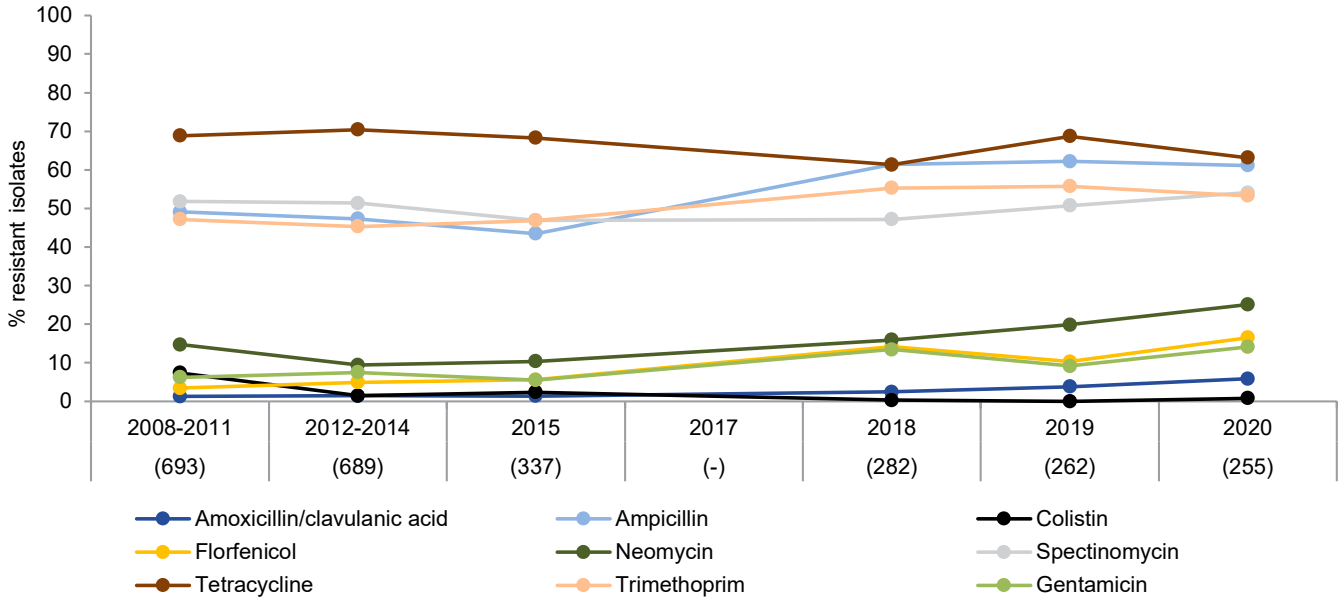
E. coli - haemolytic pathogenic strains

Enterotoxigenic *E. coli* (ETEC), often in combination with *Brachyspira pilosicoli* and *Lawsonia intracellularis*, are the most prevalent causes of bacterial diarrhoea in Danish pigs. In Denmark, identification of diarrhoeal pathogens is mandatory prior to antimicrobial flock treatment of pigs, and laboratory confirmation of the herd diagnosis must be performed at least annually. Since 2014, PCR screening of faecal samples has therefore been widely used for identifying diarrheal pathogens, including *E. coli* F4 and F8. Diagnostic samples were mostly submitted for culturing and PCR typing of haemolytic *E. coli*, and subsequent susceptibility testing.

The haemolytic *E. coli* reported here, originate both from the mandatory testing in relation to flock treatment of diarrhoea, and from diagnostic porcine samples, such as organs or faecal samples, submitted to investigate a disease problem, typically sudden death or diarrhoea. Therefore, the haemolytic *E. coli* included in the analyses originate almost exclusively from porcine enteritis or oedema disease.

Before 2018, *E. coli* isolates were identified by serotyping at the SEGES laboratory, with the most virulent haemolytic *E. coli* (ETEC and VTEC) strains belonging to serovars O138, O139, O141, and O149. The ETEC strains were also mostly positive for F4 or F18 fimbrial adhesins, which are used for attachment to the intestinal mucosa, while the VTEC strains almost always possess F18. The data for 2018-2020 represent mostly F4 or F18 positive *E. coli*, while data for 2008-2017 represent the *E. coli* serovars O138, O139, O141, and O149. Typically, but not always, F4 positive strains are serovar O149, while F18 positive strains belong to the serovars O138, O139 or O141.

Figure 1 Resistance (%) among haemolytic *Escherichia coli* from pigs, Denmark DANMAP 2020



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

With few exceptions, the resistance levels in the pathogenic *E. coli* have generally been quite stable throughout the last decade. High resistance levels were recorded for ampicillin, streptomycin, sulfonamides, tetracyclines, trimethoprim, and spectinomycin (Figure 1 and Table 1). Ampicillin resistance was significantly higher in 2018-2020 compared to previous years, even though the use of aminopenicillins in weaners has only increased slightly (see section 4.3.1). The use of tetracyclines in pigs has decreased dramatically since 2014 particularly after 2016, most likely due to the Yellow Card initiative [Antunes and Jensen, 2019; Jensen, 2018;]. However, the decline has not resulted in significant reductions in tetracycline resistance.

Most cases of porcine diarrhoea that require treatment occur during the weaning period. According to the official guidelines, neomycin, aminopenicillins, sulfonamide/trimethoprim and spectinomycin are the "compounds of choice" for oral treatment of *E. coli* diarrhoea. However, tetracyclines remains one of the most frequently used compounds in weaners. In pigs, aminoglycosides are only used for gastrointestinal infections, and particularly the use of neomycin has increased in recent years, after a reintroduction in 2017. In 2020, resistance to neomycin reached 25%, but remained lower than the level of resistance (37%) before it was taken off the market in 2007. Resistance levels towards the other aminoglycosides used for local gastrointestinal treatment, apramycin and gentamicin, were higher in 2018-2020 than in previous years, but appeared to be steady. The vast majority of apramycin resistant isolates were also resistant to gentamicin (Table 1). Resistance to florfenicol was also significantly higher in 2018-2020 compared to previous years, and in 2020 the occurrence reached 17%, which it the highest level recorded to date (Figure 1).

Resistance to ciprofloxacin, ceftiofur and colistin in the pathogenic *E. coli* has been almost absent for years. This is most likely a consequence of the very restricted use of critically important antimicrobials in Danish pigs (see section 4.3.1). The use of colistin has been regulated through the Yellow Card legislation since 2017 and has not been used in Danish pigs since 2018. Colistin resistance was very low already in 2017, and in 2020 only two resistant isolates (1%) were found.

Amoxicillin-clavulanic acid in combination (amoxiclav) for oral treatment of pigs has been available only since 2015. While the use of this combination decreased from 2015-2019, there was a marked increase from 2019 to 2020, especially for weaners. Resistance to amoxiclav has increased continuously from less than 1.5% during 2008-2015, reaching 5.7% in 2020. Since this type of combination drug is important both in humans and animals in cases of multi resistance, and active against some ESBL types, it is important to closely monitor the resistance to amoxiclav.

These data document high or increasing levels of resistance to many of the antimicrobial compounds on the panels, except for those groups rendered critically important to humans. At present, florfenicol, apramycin, and gentamicin are exceptions to this pattern, with relatively stable resistance levels for the last three years. However, the resistance has almost tripled compared to 2015 and previous years. These findings stress the importance of performing susceptibility testing for pathogenic *E. coli* in pigs, especially in case of herd diagnoses associated with recurrent use of antimicrobials.

Actinobacillus pleuropneumoniae

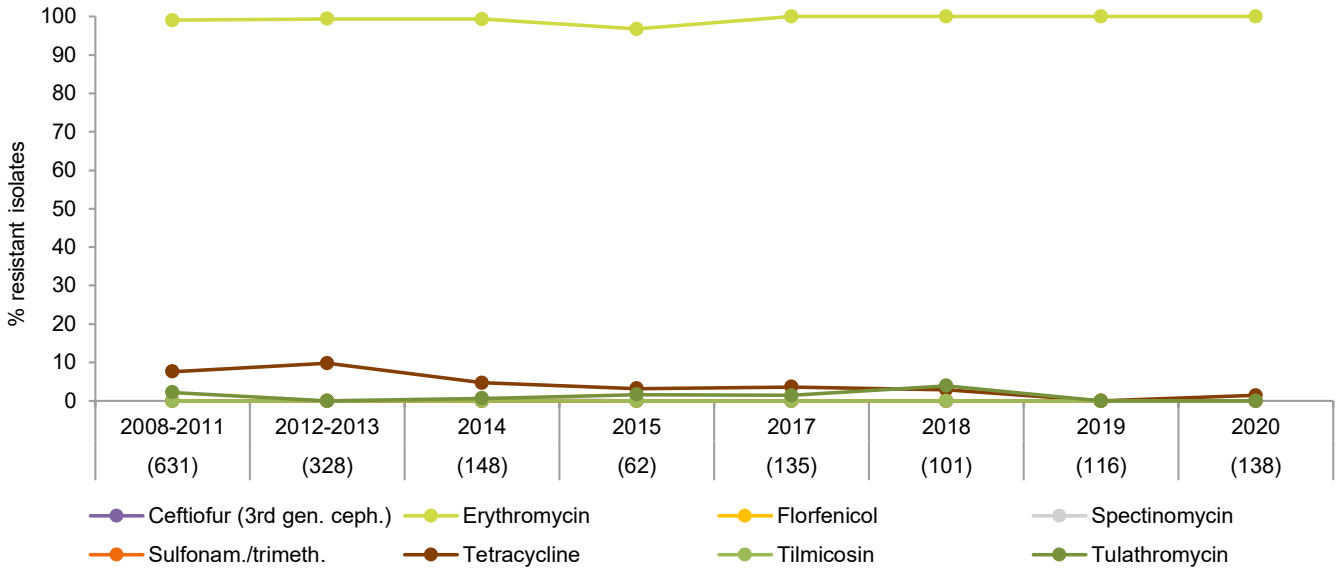
Actinobacillus pleuropneumoniae causes severe pleuropneumonia in pigs, although severity varies between serotypes. In 2020, 125 of the 138 susceptibility tested isolates were serotyped, and 85 were identified as serotype 02, 32 were 06, 3 were 012, and 4 were 01, 05, 07, and 018, respectively. In Outbreaks usually require rapid onset of treatment, before susceptibility testing can be performed, to minimise disease burden. Fortunately, *A. pleuropneumoniae* has very low occurrence of resistance to most of the available antimicrobials. No resistance to florfenicol, sulfonamide-trimethoprim and tilmicosin has been observed for the last decade, and the occurrence of resistance remains absent or very low to penicillins, spectinomycin and tiamulin (Table 1). It is also worth noting that no resistance to ciprofloxacin and ceftiofur has been observed for more than a decade.

In this report, the breakpoints were adjusted for ampicillin and tetracycline according to CLSI breakpoints. For tetracycline, this adjustment caused a few more isolates to be deemed resistant (a few percent more annually), while the vast majority (78%) are intermediate susceptible (MIC=1). In 2019-2020, the occurrence of tetracycline resistance was close to none, after a continuous decrease from the 9% resistance recorded in 2012-2013. This may be related to the steep decline in use of tetracycline in this period.

Almost all isolates were resistant to erythromycin, but this compound is not available for veterinary use. Macrolides are frequently used for treatment of pneumonia, and the resistance to the other macrolides is low (Figure 2). Tulathromycin is frequently used, and in previous years some resistant isolates were observed using a t-ECOFF at 8 (R≥16), reaching 4% in 2018, but these were not resistant according to the CLSI breakpoint (R≥128). In 2020, only one isolate was deemed resistant using R≥16, but susceptible according to the CLSI breakpoint. For tilmicosin, no resistance has been observed for more than a decade. Tildipirosin and gamithromycin are also macrolides with extended spectrum and have been available in recent years, however up till now, susceptibility testing to those antimicrobials have not been available for Danish veterinarians. Preliminary results from a recent research project (Jensen, Salomonsen et al., unpublished) suggest that resistance to these macrolides is also very low or absent in Danish *A. pleuropneumoniae*.

continued ... Textbox 6.1

Figure 2 Resistance (%) among *Actinobacillus pleuropneumoniae* from pigs, Denmark DANMAP 2020



Isolates from the routine diagnostic laboratory investigation of isolates from dead and diseased pigs submitted to SEGES Pig Research Centre's Laboratory for Pig Diseases in Kjellerup. Occurrences of resistant isolates are presented according to the clinical breakpoints (mainly porcine CLSI breakpoints) that are currently in use at both DTU Centre for Diagnostics and Laboratory for Pig Diseases. Clinical breakpoint and MIC distributions are presented in the web annex (Table A6.11)

Streptococcus suis

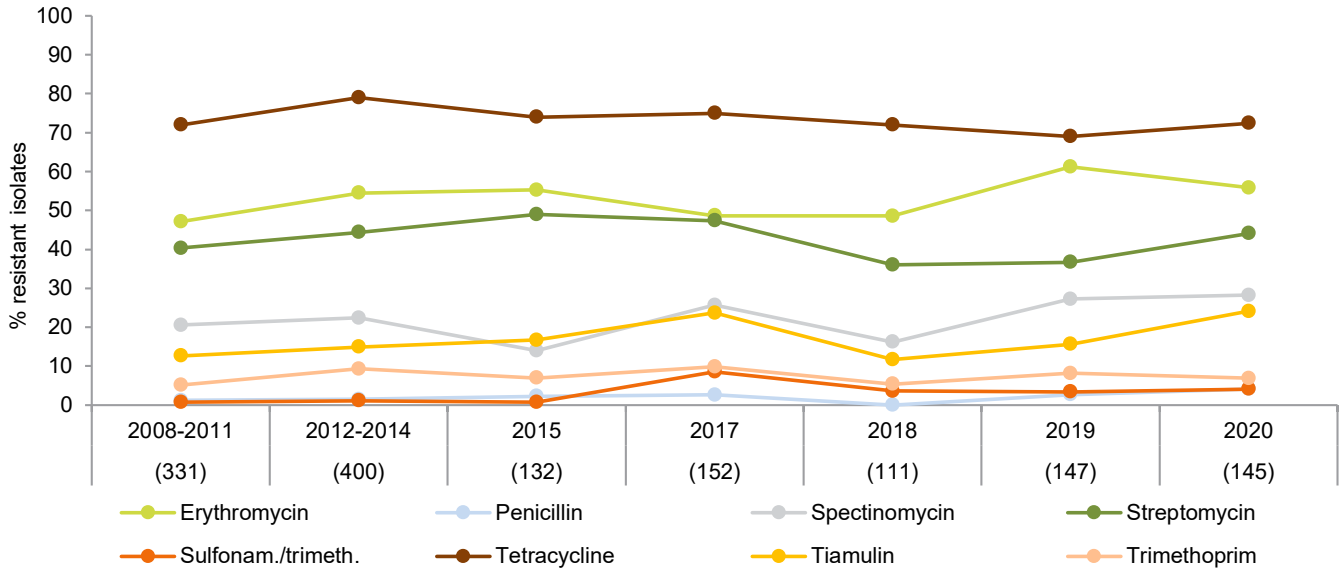
Streptococcus suis may cause a wide variety of serious infections in pigs, such as meningitis, otitis media, arthritis, pneumonia, and septicaemia, often leading to both low welfare and increased mortality.

In 2020, a single *S. suis* isolate was deemed resistant to ciprofloxacin. This is the first ciprofloxacin resistant isolate observed in Danish pigs for more than a decade. This strain was isolated from a case of meningitis, as was six other isolates with intermediate susceptibility to ciprofloxacin. The 7 isolates were all resistant to sulfamethoxazole. Four of these were also resistant to cefoxitim (FOX) and resistant or intermediate susceptible to penicillin (PEN). Three of the six isolates were resistant to streptomycin, sulfamethoxazole, tetracycline, tiamulin, and trimethoprim (two of these also resistant to FOX and PEN). Thus, even though the resistance levels in *S. suis* are generally low, multi-resistant isolates do occur, causing severe infections.

As in previous years, the highest levels of resistance were seen for sulfamethoxazole, tetracycline, erythromycin, and streptomycin, in descending order (Figure 3). Sulfonamides are only available in combination with trimethoprim. Resistance to sulfonamide-trimethoprim remained at a low level, with 3.4% resistant isolates in 2020, as most isolates (93%) are susceptible to trimethoprim. The occurrence of resistance to tetracycline has remained stable around 70% for more than a decade, despite the decreasing use of tetracyclines in pigs since 2014.

For *S. suis*, there are still several good treatment options using compounds for which there is very low levels of resistance (Table 1): Almost all isolates are susceptible to penicillin, which should be the drug of choice. Furthermore, all the *S. suis* isolates were also susceptible to sulfonamide-trimethoprim and florfenicol, which are also recommended 1st choice antimicrobials in the official guidelines. However, the occurrence of multi-resistant isolates point to the importance of a resistance testing of clinical isolates of *S. suis*.

Figure 3 Resistance (%) among *Streptococcus suis* from pigs, Denmark DANMAP 2020



Isolates from the routine diagnostic laboratory investigation of isolates from dead and diseased pigs submitted to SEGES Pig Research Centre's Laboratory for Pig Diseases in Kjellerup. Occurrences of resistant isolates are presented according to the clinical breakpoints (mainly porcine CLSI breakpoints) that are currently in use at both DTU Centre for Diagnostics and Laboratory for Pig Diseases. Clinical breakpoint and MIC distributions are presented in the web annex (Table A6.12)

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Textbox 6.2

Implementing whole-genome sequencing as a supplement to antimicrobial resistance surveillance of food animal pathogens

Background: In Denmark, there is a great focus on low usage of antimicrobials and prevention of antimicrobial resistance (AMR) in the food animal production sector. In this context, the Danish Veterinary and Food Administration has asked the Danish Veterinary Consortium (DK-VET) to set up a surveillance system to monitor AMR in pathogens isolated from livestock, as a basis to identify and evaluate risk factors for emergence and spread of AMR and to assure effective but low levels of antimicrobial usage. The DK-VET task group consists of experts from Statens Serum Institut and University of Copenhagen. The task was divided into a survey phase and a planning phase, both conducted in 2020, and an implementation phase, which started in January 2021.

Survey phase: Veterinary diagnostic laboratories were contacted to obtain information about the availability of clinical isolates from diagnostic specimens. In Denmark, clinical specimens from pigs, mink and rainbow trout are sent to centralised diagnostic laboratories, which perform bacterial culture, species identification and antimicrobial susceptibility testing (AST). The diagnostic laboratories receiving clinical specimens from the Danish pig and rainbow trout production systems expressed interest in participating in the surveillance program. Surveillance of antimicrobial resistance in clinical isolates from mink is currently not relevant due to the decision to cull the Danish mink population at the beginning of 2021 following a series of SARS-CoV-2 outbreaks. In contrast, clinical isolates from cattle and poultry are not systematically collected in Denmark, and it is therefore currently not possible to perform AMR surveillance in these production systems.

Planning phase: Each year, the AMR surveillance program will include up to 700 clinical isolates representing the most important pathogens in the different food animal production systems across Denmark. The clinical isolates will undergo the following investigations:

- Whole-genome sequencing (WGS) on Illumina platforms
- WGS-based detection of AMR genes and AMR mutations
- WGS-based strain typing (e.g., multilocus sequence typing, serotyping, fimbriae typing, detection of toxins, etc.)
- Phylogenetic analysis of WGS data to infer the population structure and distribution of AMR genes in individual bacterial species
- WGS of selected clinical isolates on Nanopore platform to determine whether the detected AMR genes are located on mobile genetic elements (MGEs)
- The result will be published in the annual DANMAP reports

Implementation phase: WGS-based AMR surveillance in pathogens from pigs commenced in January 2021, inclusion of pathogens from rainbow trout is awaiting final approval.

Expected outcomes: WGS as a supplement to AST is expected to lead to the following outcomes:

- Detection of genotypic resistance towards many antimicrobials relevant for veterinary as well as human medicine
- Detection of genotypic resistance when AST is not performed
- Increased knowledge about how AMR and pathogens spread in the different food animal production systems (clonal spread of bacteria or horizontal transfer of MGEs between bacterial populations)

The knowledge gained through this new AMR surveillance scheme will facilitate the identification of possible interventions against emergence and spread of AMR and pathogens within the different food animal production systems (e.g., antimicrobial usage, vaccine usage and animal movements between herds).

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7

RESISTANCE IN INDICATOR BACTERIA

7. Resistance in indicator bacteria

Highlights: In 2020, the levels of antimicrobial resistance in **indicator *E. coli*** from broilers, pigs and cattle were overall very similar to previous years. More than half (58%) of the broiler isolates and the majority of cattle isolates (91%) were susceptible to all antimicrobials in the test panels. From pigs, slightly less than half (46%) of the isolates were fully sensitive. During the 6-year period with EU harmonised monitoring, there has been no statistically significant increasing or decreasing trends in the relative distribution of fully sensitive isolates from broilers, cattle or pigs.

As in 2019, no colistin, meropenem or tigecycline resistance was detected. Resistance to ciprofloxacin continues to be low in *E. coli* from cattle and pigs. However, the slow but steady increase in resistance to ciprofloxacin/nalidixic acid in *E. coli* from broilers, observed over the last ten years, continued in 2020. Also among isolates from broilers, there has been a decrease in resistance to ampicillin, chloramphenicol, tetracycline and trimethoprim over the last 5-year period. Furthermore, the relative occurrence of multidrug-resistance in isolates from broilers has decreased significantly over the past 6 years, but levelled out in 2020.

In 2020, selective isolation methods showed a continued decreasing trend in occurrence of ***E. coli* producing ESBL/AmpC in Danish broilers and broiler**. Importantly, again all samples examined for carbapenemase-producing *E. coli* (including OXA-48) were found negative.

In 2020, 48% of *E. faecium* and 38% of *E. faecalis* isolated from broilers were fully susceptible. No resistance to ampicillin, gentamicin, linezolid or tigecycline was detected in any of the isolates. Resistance towards teicoplanin and vancomycin was only detected in a single *E. faecium* isolate (<1%), and the 11 daptomycin resistant *E. faecium* all had a MIC value very close to the cutoff.

E. faecalis isolates were either resistant to tetracycline alone (24%) or in combination with erythromycin (33%), and a single isolate was resistant to tetracycline and chloramphenicol. ***E. faecium*** isolates presented various resistance profiles. Resistance to only quinupristin/dalfopristin was the most common profile, and 8% of the resistant isolates were resistant to three or more antimicrobials.

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

7.1 Introduction

Escherichia coli and *Enterococcus* are included in the DANMAP programme to monitor occurrence of antimicrobial resistance in different reservoirs 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 horisontal transfer of antimicrobial resistance genes, and iii) they have the potential to cause infections in both animals and humans, and to transfer antimicrobial resistance to pathogenic bacteria of the same or other species.

E. coli exhibiting resistance to 3rd generation cephalosporins via production of extended-spectrum beta-lactamases (ESBLs) and AmpC beta-lactamases (AmpCs) is one of the fastest spreading antimicrobial resistances in both humans and production animals worldwide. Several studies report similar ESBL/AmpC genes, plasmids and/or clones of *E. coli* isolates in animals, meat and human infections, which suggests a zoonotic link [Roer et al 2019. J Antimicrob Chemother 74(3):557; Valcek et al 2019. J Antimicrob Chemother 74(8):2171]. This is supported by an attribution study estimating that although most community-acquired ESBL/AmpC carriage could be attributed to human-to-human transmission, food and direct contact with companion or farm animals also contributed to maintaining the spread between humans and non-human sources [Mughini-Gras et al 2019.Lancet Planet Health 2019 Aug;3(8):e357-e369].

Carbapenemase-producing *Enterobactericeae* (CPE) are a great threat to human health, because carbapenems are last-line antimicrobial drugs for treatment of infections caused by multidrug-resistant Gram-negative bacteria. Currently, CPE have been detected sporadically in production animals in EU but never in Denmark [EFSA/ECDC 2021. EFSA journal 19(4):6490].

Since 2014, isolation and antimicrobial susceptibility testing of indicator *E. coli*, extended-spectrum cephalosporinase- and carbapenemase-producing *E. coli* (ESC and CPE) and enterococci are performed in accordance with the EU harmonised monitoring of antimicrobial resistance [Decision 2013/652/EU]. In 2020, isolates were obtained from randomly selected caecal samples collected at slaughter, and fresh chilled meat collected at retail. Details on sampling, analysis, susceptibility testing and interpretations are presented in Chapter 9.

7.2 Indicator *Escherichia coli*

E. coli isolates were obtained from the majority of samples from broilers (172/182), pigs (185/189) and cattle (154/158). MIC distributions and occurrence of resistance among indicator *E. coli* are presented in the web annex (Table A7.1). These results were obtained using the non-selective isolation procedure. Results obtained by selective procedures for detection of cefotaxime-resistant *E. coli* are presented in section 7.3.

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

More than half (58%) of the broiler isolates and most of cattle isolates (91%) were susceptible to all antimicrobials in the test panel. From pigs, slightly less than half (46%) of the isolates were fully sensitive (Table 7.1).

A clear negative association between the probability of full sensitivity and the overall consumption of antimicrobials in food-producing animals has been observed (EFSA/ECDC/EMA 2021, JIACRA III, DOI 10.2900/056892). In Denmark, however, there has been no statistically significant increasing or decreasing trend in the relative distribution of fully sensitive isolates from broilers, cattle or pigs during the 6-year period with EU harmonised monitoring (Figure 7.1), despite a marked decrease in the consumption of antimicrobials (Figure 4.1).

Table 7.1 Resistance (%) in *Escherichia coli* isolates from broilers, calttle and pigs, Denmark DANMAP 2020

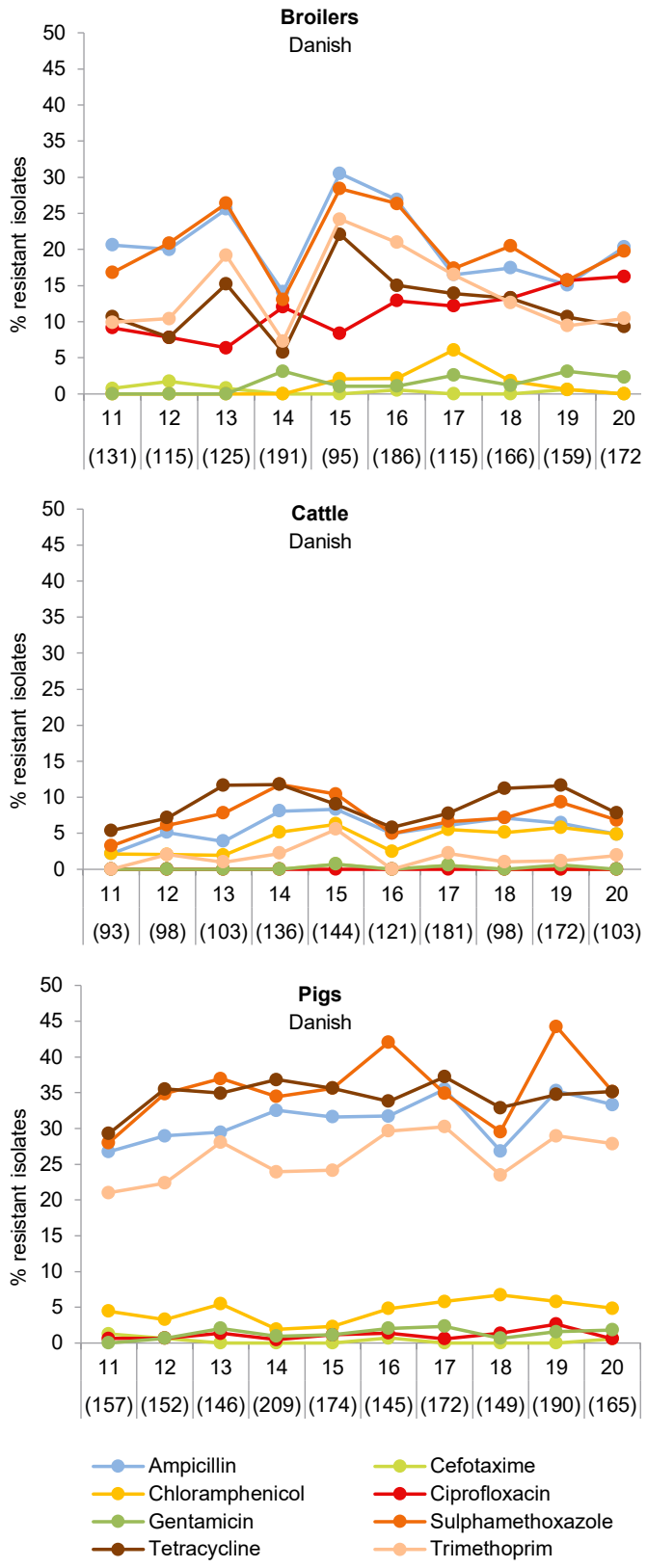
	Broilers	Cattle	Pigs
Antimicrobial agent	Danish %	Danish %	Danish %
Ampicillin	20	5	33
Azithromycin	<1	0	2
Cefotaxime	0	0	<1
Ceftazidime	0	0	<1
Chloramphenicol	0	5	5
Ciprofloxacin	16	0	<1
Colistin	0	0	0
Gentamicin	2	0	2
Meropenem	0	0	0
Nalidixic acid	15	0	<1
Sulphamethoxazole	20	7	35
Tetracycline	9	8	35
Tigecycline	0	0	0
Trimethoprim	10	2	28
Fully sensitive (%)	58	91	46
Number of isolates	172	103	165

An isolate is considered fully sensitive if susceptible to all antimicrobial agents included in the test panel

Compared to 2019, only minor fluctuations in occurrence of resistance were observed. Resistance to ampicillin and tetracycline was significantly higher in isolates from pigs compared to broilers and cattle. Nalidixic acid resistance was higher in isolates from broilers compared to pigs; and ampicillin, sulfonamide and trimethoprim resistance was lower in isolates from cattle than from broilers (Table 7.1).

As in previous years, no colistin, meropenem or tigecycline resistance was detected among the isolates. Azithromycin resistance was detected in a few isolates from pigs and broilers and one isolate from pig was found resistant to 3rd generation cephalosporins using non-selective methods (Table 7.1).

Figure 7.1 Resistance (%) among *Escherichia coli* isolates from broilers, cattle and pigs, Denmark DANMAP 2020



The number of isolates included each year is shown in parentheses

Resistance to ciprofloxacin continues to be low in *E. coli* from cattle and pigs. However, in broilers, there has been an increasing trend in resistance to ciprofloxacin and nalidixic acid over the last decade. Resistance to ciprofloxacin, but not to nalidixic acid, was observed only in a few isolates from broilers. Also among isolates from broilers, there has been a decrease in resistance to ampicillin, chloramphenicol, tetracycline and trimethoprim over the last 5-year period (Figure 7.1).

The proportions of multidrug-resistant isolates from broilers, cattle and pigs were at the same levels as in 2019. Among the multidrug-resistant isolates, combined resistance to ampicillin, sulfonamide and tetracycline (ASuT) was commonly observed (Figure 7.2). Over the 6-year period with EU harmonised monitoring, the relative occurrence of multidrug-resistance in isolates from broilers has decreased significantly, but levelled out in 2020.

A single ESBL-producing isolate, with resistance to ampicillin, azithromycin, ceftazidime, cefotaxime, cefepime, cefoxatin, sulfonamide, tetracycline and trimethoprim, was found in 2020. The distributions of all AMR profiles are presented in web annex Table A7.2.

7.2.2 Perspectives

From a European perspective, several countries report decreasing trends in antimicrobial resistance in indicator *E. coli*, especially in broilers and turkeys (data from 2018 and 2019). Indicator *E. coli* from Danish broilers and calves <1 year show a noticeably lower occurrence of resistance to any antimicrobial compared to the indicator *E. coli* from other countries apart from the Nordic countries [EFSA/ ECDC 2021,EFSA Journal 2021;19(4):6490, 179 pp]. The positive trends in several countries may to some extent, be due to the overall decline in sales of antimicrobials for use in animals since 2011, as noted in the recent ESVAC report (EMA, 2020).

Denmark remains among the countries reporting the lowest occurrence of chloramphenicol and, more importantly, ciprofloxacin resistance in indicator *E. coli* from pigs, whereas the reported occurrence of ampicillin, azithromycin, sulfonamide, trimethoprim and tetracycline resistance was comparable to the average reported in the EU Member States.

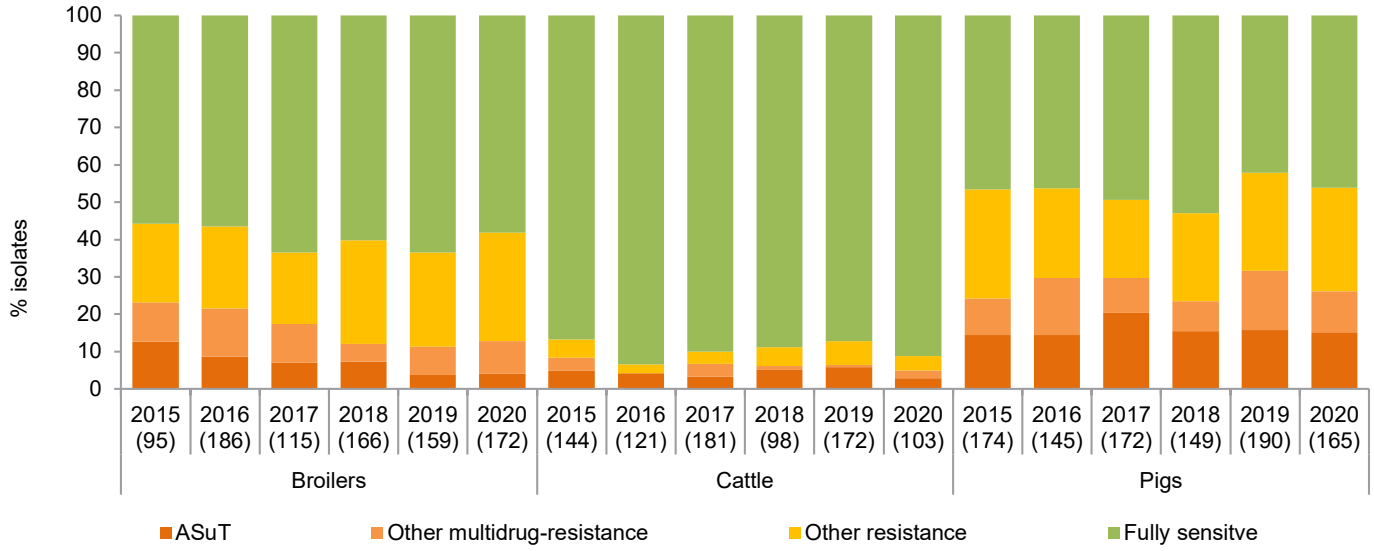
The antimicrobial resistance phenotypes mostly relevant to human health are ciprofloxacin resistance in *E. coli* from broilers and azithromycin resistance in *E. coli* from pigs.

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

In 2020, ESBL/AmpC/carbapenemase-producing *E. coli* were isolated from packages of fresh, chilled broiler meat collected from Danish wholesale and retail outlets. In accordance with the harmonised EU monitoring, packages of broiler meat were selected at retail without pre-selecting by country of origin. Only 8% of the randomly collected samples of broiler meat was from imported products.

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

DANMAP 2020



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

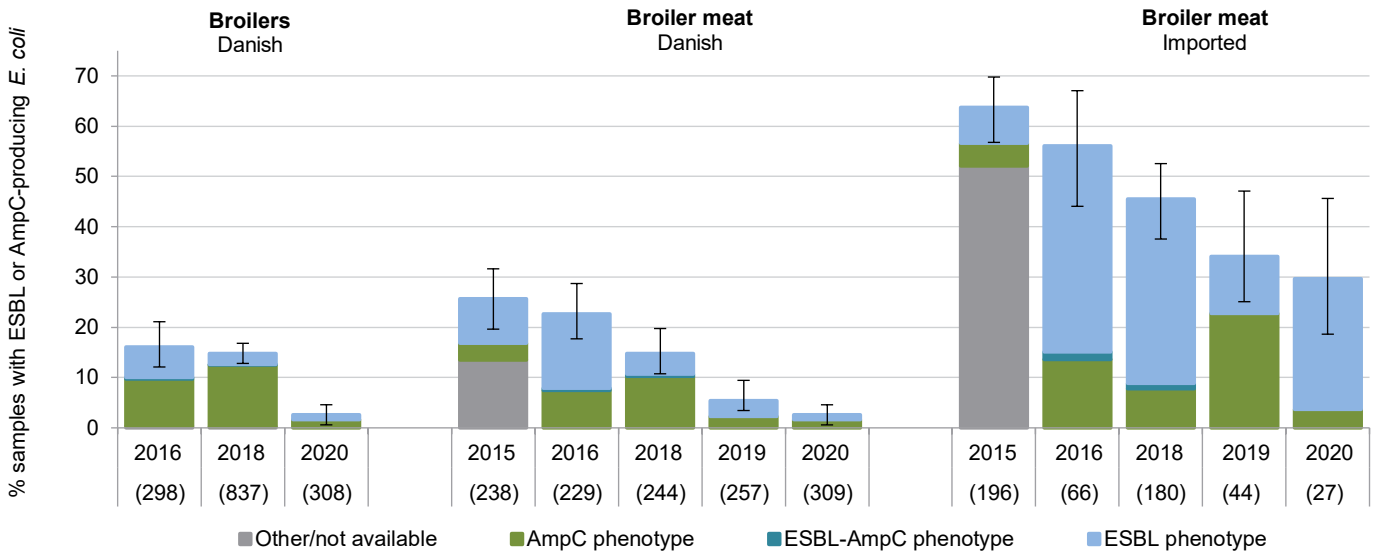
Using selective procedures, extended-spectrum cephalosporinase-producing *E. coli* (ESBL/AmpC isolates) were obtained from broilers (8/308 samples) and broiler meat (Danish: 8/309 samples, imported: 8/27 samples). The selective procedures for detection of carbapenemase-producing *E. coli* (including oxacillinase-producing OXA-48-like enzymes), recovered no CPE/Oxa48 isolates from broiler or broiler meat samples. MIC distributions and occurrence of resistance among ESBL/AmpC-producing *E. coli* isolates are presented in the web annex (Tables A7.3 and A7.4).

7.3.1 ESBL/AmpC-producing *E. coli* from broilers and broiler meat

Following selective enrichment, cefotaxime resistant *E. coli* were obtained from 3% (CI 95%: 1-5%) of samples from Danish broilers and broiler meat, and from 30% (CI 95%: 14-41%) of imported broiler meat samples. Over the 6-year period with EU harmonised monitoring, the prevalence of ESBL/AmpC has decreased significantly in Danish broilers and broiler meat, as well as in the imported broiler meat (Figure 7.3).

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

DANMAP 2020



The number of samples tested per year is presented in the parentheses. Confidence intervals for total proportion of samples positive for phenotypic ESBL and/or AmpC producing *E. coli* - calculated as 95% binomial proportions presenting Wilson intervals. CPE, ESB and AmpC phenotypes are classified according to the scheme provided by EFSA (2015 data based on WGS). In 2020, WGS revealed ESBL genes among AmpC phenotypes from broilers (n=2) and broiler meat (Danish n=1, Import n=1). Broilers are sampled in even years. Broiler meat was not sampled in 2017

All the ESBL/AmpC producing isolates were resistant to 3rd generation cephalosporins (cefotaxime and ceftazidime) as well as ampicillin. Resistance to 4th generation cephalosporins (cefepime) was found in all isolates from imported broiler meat and half of the isolates from Danish broilers and meat (Tabel 7.2). Resistance to quinolones was far more frequent in the ESBL/AmpC producing *E coli*, compared to the general indicator *E. coli* population, and has increased from 40% and 15% in 2016 to 88% and 63% in 2020, in isolates from broilers and broiler meat, respectively. No resistance to colistin, carbapenems or tigecycline was observed. One isolate was resistant to azithomycin (Table 7.2).

As previously, different combinations of additional multidrug resistance, including penta-resistance, was observed in the ESBL- and AmpC-producing *E. coli* isolates, primarily resistance to ampicillin, ciprofloxacin/nalidixic acid, tetracycline, sulfonamides and trimethoprim (see web annex Table A7.5).

The genetic basis for ESBL- and AmpC enzymes was detected in all isolates recovered by selective enrichment and corresponded to the phenotypes derived from the susceptibility testing for 20 of the 24 isolates. In four isolates, WGS revealed both ESBL and AmpC genes (Table 7.3), even though the susceptibility testing did not show a synergetic effect of combining clavulanic acid with cefotaxime or ceftazidime (resulting in AmpC phenotypes).

Among the AmpC-producing isolates from Danish broilers recovered in 2020, resistance was mainly conferred by upregulated AmpC promotor C-42T mutations (2 out of 3 isolates), whereas CMY-2 was the most common encoding gene in isolates from Danish broiler meat (2 out of 3 isolates). The gene DHA-1 was detected in a single isolate recovered from Danish broilers. Among all ESBL-producing isolates, 5 different genes were detected as the only encoding gene (CTX-M-1, CTX-M-14, CTX-M-32, SHV-12 and TEM-52) (Table 7.3). Two ESBL-producing isolates contained two ESBL genes, TEM-1 in combination with either a CTX-M-55 or a SHV-12 gene. Three isolates harboured both ESBL and AmpC encoding genes. TEM-1 in combination with upregulated AmpC promotor C-42T mutation was detected in one isolate from Danish broilers and another from Danish broiler meat, whereas TEM-1 in combination with CMY-2 was detected in an isolate from imported broiler meat.

Table 7.2 Resistance (%) and beta-lactam resistance phenotype distributions (%) in ESBL/AmpC-producing *E. coli* recovered by selective enrichment from broilers and broiler meat, Denmark
DANMAP 2020

	Broilers	Broilers meat	
Antimicrobial agent	Danish %	Danish %	Import %
Ampicillin	100	100	100
Azithromycin	13	0	0
Cefepime	50	50	100
Cefotaxime	100	100	100
Cefotaxime/clavulansyre	63	63	13
Cefoxitin	63	63	13
Ceftazidime	100	100	100
Ceftazidime/clavulansyre	63	63	13
Chloramphenicol	13	13	50
Ciprofloxacin	88	63	50
Colistin	0	0	0
Ertapenem	0	0	0
Gentamicin	13	13	0
Imipenem	0	0	0
Meropenem	0	0	0
Nalidixic acid	75	63	38
Sulphamethoxazole	25	25	88
Temocillin	0	0	0
Tetracycline	25	25	88
Tigecycline	0	0	0
Trimethoprim	25	25	63
CPE phenotypes	0	0	0
AmpC phenotypes	63	63	13
ESBL phenotypes	38	38	88
Number of isolates	8	8	8
Number of samples	308	309	27

Classification of CPE, ESBL and AmpC phenotypes is based on the MIC results according to the scheme provided by EFSA (see Materials and methods, section 9.7)

Table 7.3 Number of ESBL and AmpC enzymes detected in *E. coli* isolates from animals and meat recovered by selective enrichment, Denmark
DANMAP 2020

	Broilers	Broiler meat	
Enzymes	Danish	Danish	Import
CTX-M-1	1		3
CTX-M-14	1		
CTX-M-32			1
CTX-M-55			1
DHA-1	1		
SHV-12	1	1	1
TEM-1	2	1	3
TEM-52		2	1
CMY-2		3	1
Chromosomal AmpC	4	2	
Number of AmpC genotypes	3	4	0
Number of ESBL genotypes	3	3	7
Number of AmpC & ESBL genotypes	2	1	1
Number of positive samples	8	8	8
Number of tested samples	308	309	27

Isolates recovered by the selective enrichment methods described in the EURL-AR laboratory protocol. ESBL/AmpC enzymes are determined by WGS. For 2020 data, all MLST and ESBL/AmpC Enzymes combinations are listed in Web annex table A7.3

In total, 17 MLSTs were observed and only ST350 and ST4663 were found in more than one isolate, and found in isolates obtained from both Danish broilers and Danish broiler meat.

The MLST and ESBL and AmpC enzymes combinations are listed in web annex Table A7.6. The isolates which harboured the CMY-2 genotype were attributed to ST155, ST101 and ST442.

7.3.2 Perspectives

A significant reduction in the ESBL/AmpC producing *E. coli* in Danish broiler and broiler meat, has occured since 2018 (Figure 7.3). This reduction is likely the result of the requirements that imported breeding and production animals must be tested and found negative for ESBL/AmpC before they are allowed into the country.

The enzymes of the ESBL-, AmpC-producing *E. coli* seem to be fairly consistent with the same enzymes being observed each year, but attributed to different MLSTs, indicating the occurrence of horizontal gene transfer.

When comparing the ESBL/AmpC-producing isolates from broiler meat to the isolates from human bloodstream infections collected during 2020, no coincidence of combination of ST and ESBL-/pAmpC-genes was identified.

Still no carbapenemase-producing *E. coli* were detected in the 644 samples tested in 2020.

This is opposed to the increase reported in other countries in Europe, where carbapenemase-producing *E. coli* is emerging or is persistent in the primary production [Bonardi et al. 2019. Ital J Food Saf 8:7956].

7.4 Enterococci

Enterococci were obtained from 97% of the 289 broiler samples. MIC distributions and occurrence of resistance among *E. faecalis* and *E. faecium* isolates from broilers are presented in the web annex (Table A7.7).

7.4.1 Enterococci from broilers

E. faecium was selected for susceptibility testing when present (N=258). If only *E. faecalis* was detected, these isolates were tested (N=21, Table 7.4). Overall, 48% of the *E. faecium* isolates and 38% of the *E. faecalis* isolates were susceptible to all antimicrobials in the test panel (excluding resistance to quinupristin/dalfopristin for *E. faecalis*).

In 2020, no ampicillin, gentamicin, linezolid or tigecycline resistance was detected in any *Enterococcus* isolates from broilers. One *E. faecium* isolate harboured a VanHAX enzyme conferring resistance to vancomycin and teicoplanin. Resistance to quinupristin/dalfopristin (44%) and tetracycline (12%) were the most common among *E. faecium*, whereas moderate to high proportion of tetracycline (62%) and erythromycin (38%) resistance was observed among *E. faecalis* isolates. Resistance to ciprofloxacin and daptomycin was low in *E. faecium* and absent in *E. faecalis* isolates (Table 7.4). The 11 daptomycin resistant *E. faecium* all had a MIC value of 8 mg/L, a level very close to the cutoff at >4 mg/L. The genetic background for the phenotypic daptomycin resistance was not determined.

Despite the 4-year and 7-year data gap for *E. faecalis* and *E. faecium* isolates, respectively, tetracycline resistance appears to be on an increasing trend (Figure 7.4).

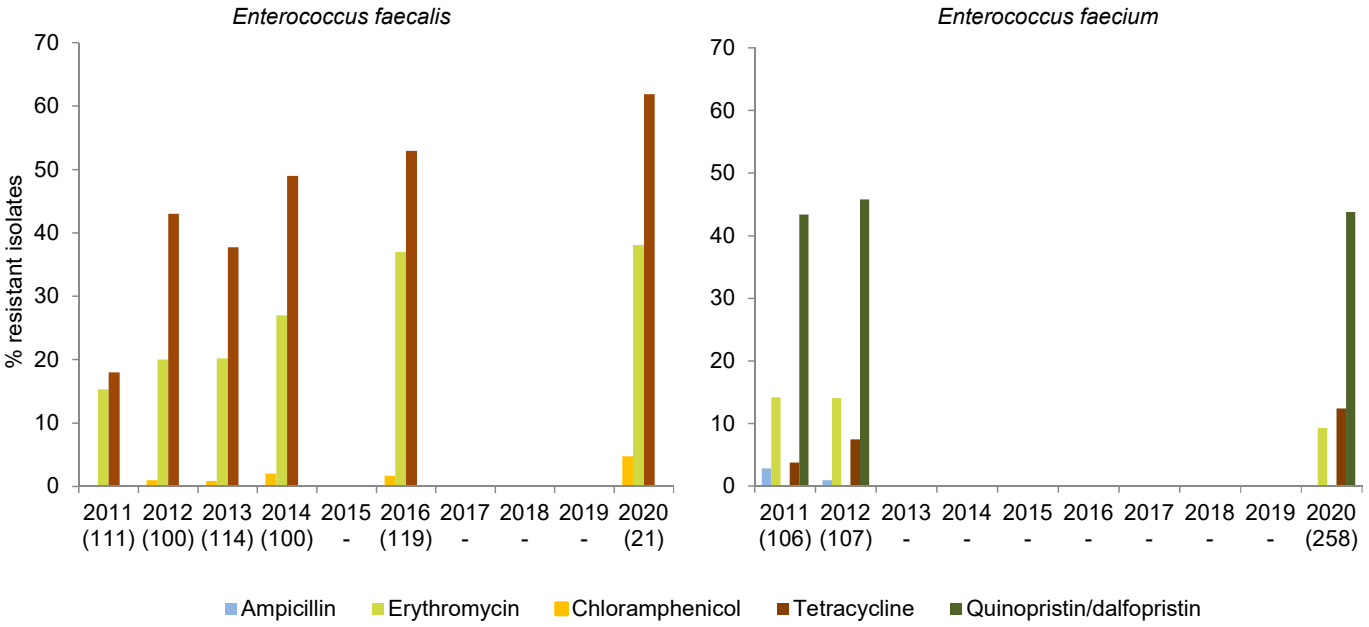
Among the resistant *E. faecium*, 9 different resistance profiles were observed. Resistance to only quinupristin/dalfopristin was most common (74 of 135 resistant isolates), however multidrug resistance was also observed, with 8% of the resistant isolates presenting resistance to three antimicrobials or more. The most common profiles among multidrug resistance included resistance to quinupristin/dalfopristin in combinaton with tetracyclin (10 isolates), erythromycin (8 isolates) or in combination with both antimicrobials (7 isolates). The 13 resistant *E. faecalis* isolates were either resistant to tetracycline alone (24%) or in combination with erythromycin (33%), and a single isolate was resistant to tetracycline and chloramphenicol (see web annex Table A7.8).

Table 7.4 Resistance (%) in *Enterococcus* isolates from broilers, Denmark DANMAP 2020

Antimicrobial agent	*Enterococcus faecalis* %	*Enterococcus faecium* %
Ampicillin	0	0
Chloramphenicol	5	0
Ciprofloxacin	0	3
Daptomycin	0	4
Erythromycin	38	9
Gentamicin	0	0
Linezolid	0	0
Quinopristin/dalfopristin		44
Teicoplanin	0	<1
Tetracycline	62	12
Tigecycline	0	0
Vancomycin	0	<1
Number of isolates	21	258

E. faecalis are assumed intrinsically resistant to streptogramins (quinopristin/ dalfopristin)

Figure 7.4 Resistance (%) among *Enterococcus* isolates from broilers, Denmark DANMAP 2020



E. faecalis are assumed intrinsically resistant to streptogramins (quinopristin/dalfopristin)
According to regulation 2013/652/EU, sampling of *Enterococcus* is voluntary. Since 2014, data on *E. faecalis* and *E. faecium* in broilers have been sampled for DANMAP in 2016 (*E. Faecalis*, only) and in 2020

7.4.2 Perspectives

Enterococci are commensal bacteria in the intestine in both animals and humans, however, both *E. faecalis* and *E. faecium* can cause human disease. DANMAP 2020 showed that *E. faecium* and *E. faecalis* isolates from broilers exhibited no resistance to linezolid and tigecycline, and <1% of the tested *E. faecium* isolates exhibited resistance towards teicoplanin and vancomycin (Table 7.4). These antimicrobials are critically important to human medicine and are considered last resort compounds to treat severe infections caused by Gram positive bacteria.

In humans, an increase in infections caused by invasive enterococci, mainly *E. faecium*, has been observed since 2002, but did not increase further in 2020. The proportion of invasive vancomycin-resistant *E. faecium* isolates reported in Danish Microbiology Database (MiBa) is relatively high in Denmark (9.4% in 2020), (Figure 8.11).

When comparing the resistance profiles of the human *E. faecium* isolates to the resistance profiles of the *E. faecium* broiler isolates from 2020, some differences can be observed. In contrast to the human isolates, the broiler isolates showed no linezolid, teicoplanin resistance and very little resistance towards vancomycin and ampicillin (<1%).

Comparing resistance profiles of the *E. faecalis* isolates, a few more similarities can be found. Human isolates exhibited a low proportion of linezolid resistance vs. no linezolid resistance among broiler isolates. None of the isolates showed teicoplanin, tigecycline or vancomycin resistance.

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Textbox 7.1

Potential of metagenomics for monitoring of resistance and resistance association to antimicrobial use compared to indicator *Escherichia coli*

Background: Recently, at the pig farm level, metagenomic analysis has shown promises for adequate antimicrobial resistance (AMR) surveillance, and at the country level genotypic AMR correlated with antimicrobial use (AMU). Resistome similarities have also been found between pigs and pig farm workers, suggesting the occurrence of workers' occupational exposure to AMR genes. Metagenomics is considered a promising, although challenging, future approach to AMR integrated surveillance. This study was conducted to further evaluate the usefulness of metagenomics for monitoring of AMR and compare it to the value of the presently used indicator *Escherichia coli*.

Methods: Data was gathered on antimicrobial use in Danish pig farms, and on phenotypic resistance of indicator *E. coli* isolated from faeces of fattening pigs slaughtered in Denmark, as reported in DANMAP.

Additionally, 62 pooled faecal samples from fattening pigs collected at slaughter for DANMAP throughout two time periods - 1999-2004 and 2015-2018 - were shotgun-sequenced, with each pool corresponding to 25 individual samples. Across all samples, the resistomes included a total of 272 individual antimicrobial resistance genes (ARGs). ARG counts were normalised by gene-length and transformed to relative abundance for further analyses. Relative abundance was calculated with data aggregated at individual gene level and at antimicrobial class level, according to annotation in the database ResFinder 4.0. Cluster analysis of the resistomes was performed at both aggregation levels.

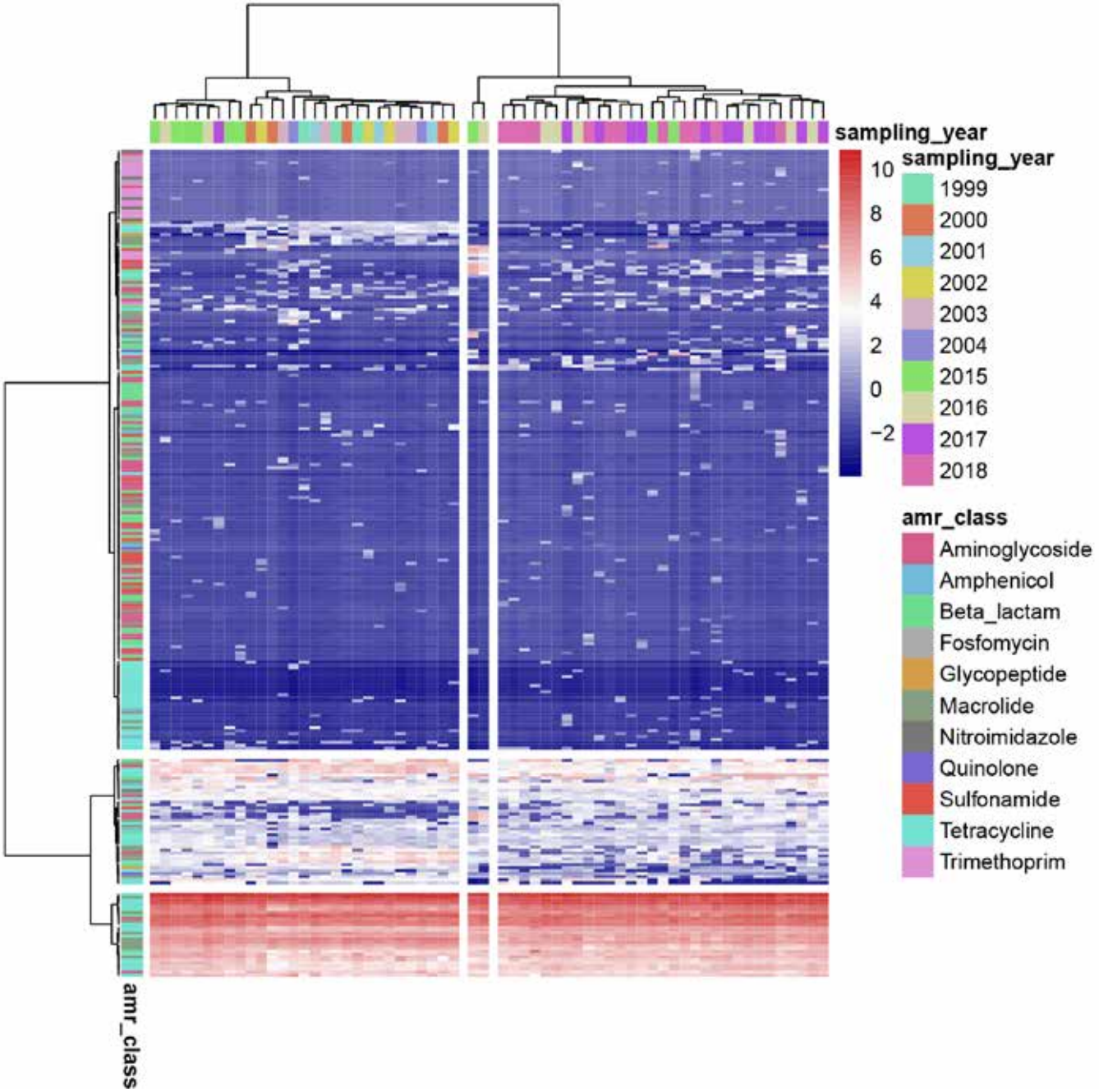
Using the multivariate approach of Procrustes rotation analysis, the correlation between AMU and phenotypic resistance in indicator *E. coli* was compared to the correlation between AMU and genotypic resistance determined by metagenomics.

Results and discussion: The cluster analysis of resistomes at individual gene level (Figure 1) showed most samples from latest years (2016-2018) agglomerating separately from those of earlier years (1999-2004), and samples from year 2015 spread among both clusters. The continuous colour scale shows the relative abundance of an ARG. Individual resistance genes also formed two main clusters, one gathering ARGs with overall low relative abundance over time and the other gathering genes with intermediate and high relative abundance. There was no evident clustering based on antimicrobial class.

Contrarily, in the cluster analysis of the resistomes at antimicrobial class level (Figure 2), antimicrobial classes clustered based on their general relative abundance across all sampling years. The two main clusters separated the four most relatively abundant classes (macrolide, tetracycline, beta-lactam and aminoglycoside) from least abundant ones. Each main cluster further sub-divided based on general relative abundance gradients across all samples. The three classes with lowest relative abundance over time were fosfomycin, quinolone and trimethoprim. Sulfonamide, glycopeptide, phenicol and nitroimidazole presented intermediate relative abundance. Among the four classes with highest abundance over time, macrolide and tetracycline were the most abundant. In Figure 2, the colour trend from left to right represents the change in relative abundance over time for a class. Glycopeptide showed obvious lower relative abundance in the latest sampling period (2015-2018), whereas phenicol, sulfonamide and trimethoprim showed obvious higher relative abundance.

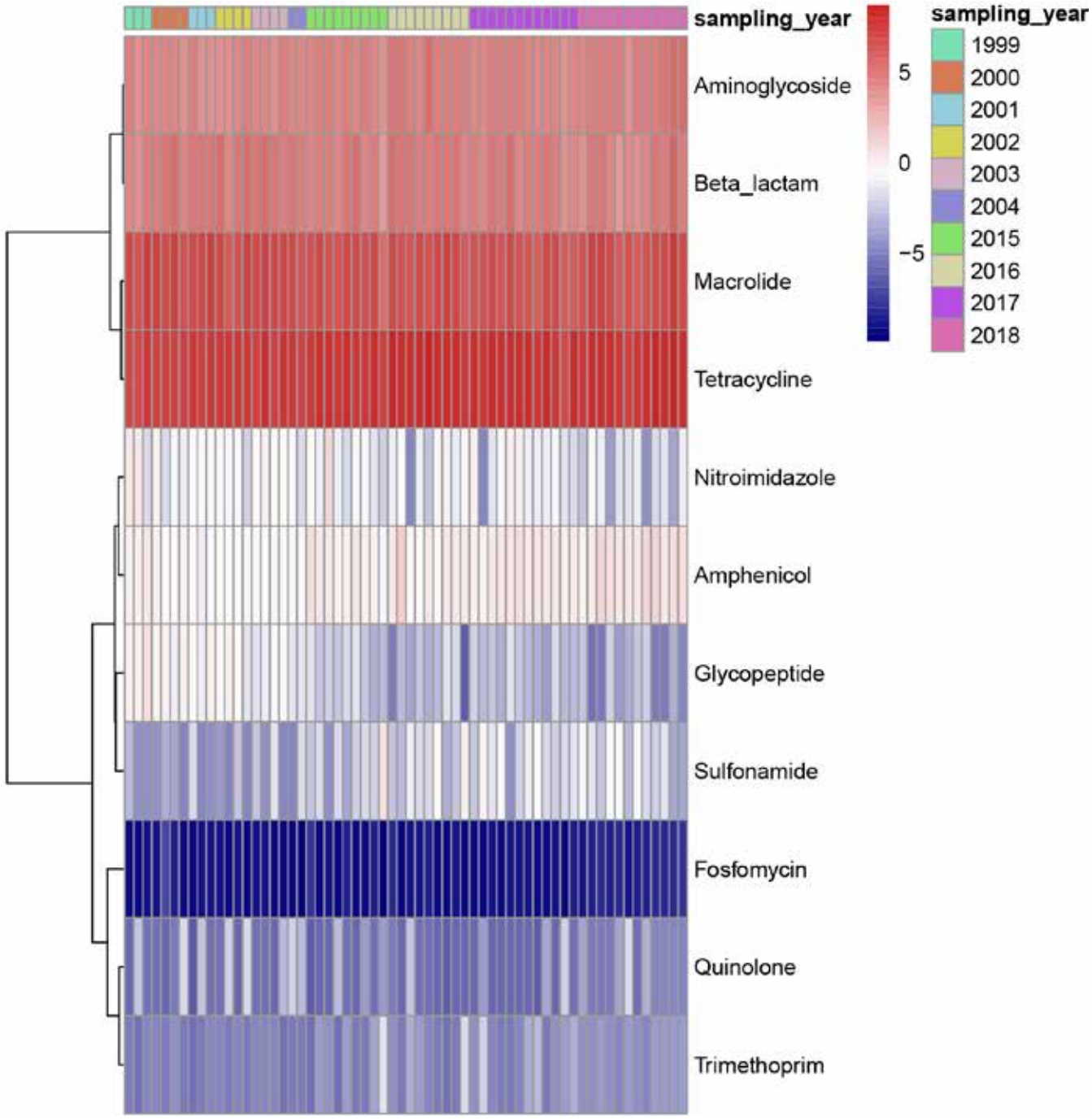
continued ... Textbox 7.1

Figure 1 Heatmap of relative abundance of individual antimicrobial resistance genes DANMAP 2020



Relative abundances expressed as centered log ratios (clr) were used to produce a heatmap at antimicrobial resistance gene level. The value for each ARG in a sample represents that gene's ratio to the average abundance across all genes. Samples were clustered by row (antimicrobial class) and by column (year) based on Euclidean distances and by Ward agglomeration method (dissimilarities not squared). Clr values are coloured on a continuous scale from red (high relative abundance) to blue (low relative abundance)

Figure 2 Heatmap of relative abundance of antimicrobial resistance classes DANMAP 2020



Relative abundances expressed as centered log ratios (clr) were used to produce a heatmap at antimicrobial class level. The value for each class in a sample represents that class' ratio to the average abundance across all classes in the same sample. Samples were clustered by row (antimicrobial class) based on Euclidean distances and by Ward agglomeration method (dissimilarities not squared), and were annotated by year. clr values are coloured on a continuous scale from red (high relative abundance) to blue (low relative abundance)

continued ... Textbox 7.1

The Procrustes analysis showed that in the period 2015-2018 AMU correlated equally well to *E. coli* phenotypic resistance (*r* values of 0.80 for sows/piglets, 0.68 for weaners and 0.70 for fatteners) and to genotypic resistance measured by metagenomics (*r* values of 0.82 for sows/piglets, 0.75 for weaners and 0.79 for fatteners). However, correlation between genotypic resistance and resistant *E. coli* was of low significance. These results indicate that at least a similar level of AMR monitoring seems possible to achieve with metagenomics, in relation to the phenotypic testing of indicator *E. coli*, and that the outcomes of both methods should be complementary, not directly compared. Furthermore, this multivariate analysis of the association between AMU and resistant *E. coli* delivered higher correlation values than a univariate, where each antimicrobial class is analysed individually (Spearman rank correlation test – results not shown). This suggests that multivariate approaches to investigate the evolution of AMR in association with AMU should be considered with monitoring data spanning several antimicrobial classes.

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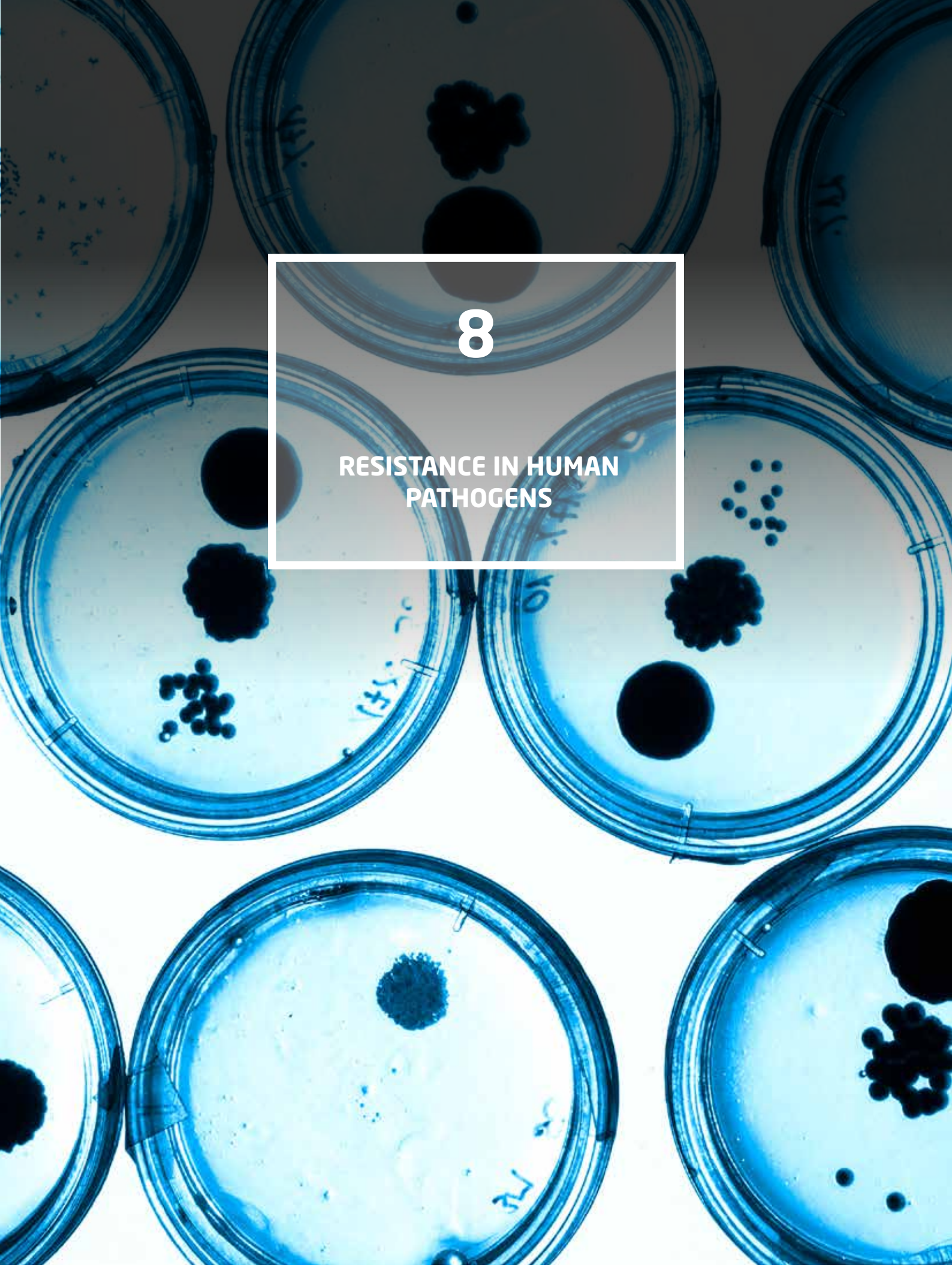


8

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 key bacteria increased by 41% in Denmark between 2011 and 2020. *Escherichia coli* was the most frequent causative organism. *Streptococcus pneumoniae* was the only species with an overall decrease over this time.

Escherichia coli. Invasive and urinary tract *E. coli* infections increased continuously between 2011 and 2020. Resistance to carbapenems has increased since 2011 but is still very low (0.1%). The number of ESBL- and/or AmpC positive invasive *E. coli* isolates decreased by 6% in 2020 (352 isolates) compared to 2019. CTX-M-15 remained by far the most prevalent ESBL enzyme. Seven carbapenemase-producers were identified among the 193 whole genome sequenced ESBL- and/or pAmpC *E. coli* blood isolates.

Klebsiella pneumonia. Antimicrobial resistance in invasive and urine *K. pneumoniae* isolates decreased for most antimicrobials over the last ten years. However, piperacillin-tazobactam and carbapenem resistance increased to 9.1% and 0.8% respectively in 2020.

Carbapenemase-producing Organisms/Enterobacterales (CPO/CPE). The increase in CPO/CPE infections continued with 171 new CPE patients in 2020. In addition to 11 ongoing hospital outbreaks, 9 new CPE hospital clusters were identified by WGS. In contrast, travel-associated CPO-infections decreased (17% in 2020, 43% in 2019).

Enterococci. The number of invasive enterococcal infections, mainly caused by *E. faecium*, increased by 21% between 2011 and 2020. Resistance to ampicillin and vancomycin stayed high (92.4% and 9.4% respectively) but the number of vancomycin-resistant (VRE) and vancomycin-variable enterococci (VVE) seemed to stabilise. WGS showed a large shift in clones (cluster types; CTs) with *vanA E. faecium* ST203-CT859 clone dominant in 2015-2018, followed by a shift to *vanA E. faecium* ST1421-CT1134. A third clone, *vanB E. faecium* ST80-CT2406, emerged in 2020 .The numbers of linezolid resistant enterococci (LRE) and linezolid vancomycin resistant enterococci (LVRE) were small in 2020 (7 and 8 isolates respectively) but those findings are concerning, as treatment options are limited.

Streptococcus pneumonia. The number of cases of invasive pneumococcal disease (IPD) in 2020 was markedly lower than in 2019, likely due to COVID-19 restrictions.

Beta-haemolytic streptococci. The number of invasive isolates of beta-haemolytic streptococci group A was 43% lower in 2020 than in 2019. In contrast, the number of submitted isolates of serogroups B, C, and G increased by 13%, 31% and 32% respectively. All isolates were fully susceptible to penicillin.

Staphylococcus aureus. The number of *Staphylococcus aureus* bacteraemia cases continued to increase (2,342 cases in 2020 compared to 2233 in 2019). Thirty-eight (1.6%) of the bacteraemia cases were methicillin-resistant (MRSA) out of which almost a third of cases (11) were Livestock-Associated MRSA (LA-MRSA). Surveillance of all MRSA cases (infection and colonization) showed a decrease of 21% of reported cases in 2020 compared to 2019, most likely due to COVID-19 restrictions. The number of MRSA outbreaks was higher but involved fewer patients (31 outbreaks in 2020, 23 in 2019; 130 patients in 2020, 157 in 2019).

8.1 Introduction

In Denmark, all microbiological tests from hospitals and general practitioners are analysed by ten Departments of Clinical Microbiology (DCMs). All DCMs are located at hospitals in the five regions of Denmark. The national surveillance of resistance in human clinical bacteria is based on data from routine diagnostics performed at the ten DCMs and on resistance and typing results from isolates analysed at the reference laboratories at SSI. Isolates are received at SSI either based on a mutual agreement of voluntary submission of specific species and/or types of resistances or as part of a mandatory surveillance programme of diseases made notifiable by the Danish Health Authority (Table 8.1).

Table 8.1 Summary of species/types, sampling and sources of isolates for national resistance surveillance in humans, 2020

DANMAP 2020

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 all sample sites
3rd generation cephalosporin resistant *Escherichia coli*	First isolate per patient within 12 months from blood
Vancomycin-resistant enterococci	First isolate per patient within 12 months irrespective of sample site (excluding screening samples)
Enterococci with exceptional phenotype (e.g. linezolid, daptomycin and tigecycline R)	First isolate per patient within 12 months irrespective of sample site (clinical and screening samples)
All bacterial species with other exceptional phenotypes (e.g. acquired colistin resistance)	One isolate per patient per episode irrespective of sample site
Mandatory submissions of isolates to the reference laboratories at SSI	
Species or type	**Sampling**
Carbapenemase-producing organisms	First isolate per patient within 12 months irrespective of sample site (clinical and screening samples)
Methicillin-resistant *Staphylococcus aureus*	First isolate from all new cases of MRSA postive patients irrespective of sample site (clinical and screening samples)
Streptococcus pneumoniae	One isolate per patient per episode from blood or cerebrospinal fluid
Haemophilus influenzae serotype b, Hib	All invasive isolates

Regarding submissions of isolates to the reference laboratories normally more isolates are received, but for the statistics one patient only counts once

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

Voluntary national surveillance of resistance in Denmark is based on data from routine diagnostics from the DCMs. The surveillance includes invasive isolates of *Escherichia coli, Klebsiella pneumoniae, Enterococcus faecalis, Enterococcus faecium, Pseudomonas aeruginosa* and *Acinetobacter* species. For resistance in urine isolates *E. coli* and *K. pneumoniae* are included. The surveillance has been performed since 1995 – in the very beginning based on reporting from two DCMs, but quickly joined and supported by most DCMs in Denmark. From 2010 to 2014, DANMAP received data from all except one DCM resulting in a coverage of approximately 95% of the population. Since 2015, all DCMs have participated in the programme resulting in a 100% population coverage. Since 2018, all resistance data for DANMAP were extracted directly from the Danish Microbiology Database (MiBa), a nationwide, automatically updated database of microbiological test results [https://miba.ssi.dk/Service/English.aspx]. A description of MiBa and the usage and validation of MiBa-data is given in DANMAP 2018, Textbox 8.1. Materials and methods are described in section 9.10 in Chapter 9.

8.1.2 Surveillance based on data from the reference laboratories

In addition to the MiBa-based surveillance, resistance is also being monitored in specified isolates submitted to the reference laboratories at SSI. Isolate-based surveillance gives the opportunity to characterise specific resistance mechanisms in microorganisms of interest. Since 2015/2016, this has been

mainly performed by the use of whole genome sequencing (WGS). Voluntary submission of specified isolates has been in place since 1957; beginning with the submission of all isolates of *Staphylococcus aureus* from bloodstream infections. The submission of invasive beta-haemolytic streptococci is also voluntary, while invasive *Streptococcus pneumoniae* and *Haemophilus influenzae* serotype b (Hib) are mandatory to submit. The detection of methicillin-resistant *S. aureus* (MRSA) and *Neisseria gonorrhoeae* from all clinical sites is notifiable. Submission of MRSA isolates to SSI is mandatory, while submission of *N. gonorrhoeae* isolates is voluntary. In addition, the DCMs voluntarily submit isolates of ESBL-producing *E. coli* from blood and vancomycin-resistant enterococci (VRE) from all clinical sites, based on a mutual agreement to survey the development and spread of these often multidrug-resistant bacteria at Danish hospitals. The Danish Health Authority made carbapenemase-producing organisms (CPO) notifiable, and submission of all clinical and screening isolates regardless of sample site has been mandatory as of 5th September 2018. Previously, CPO were submitted on a voluntary basis.

8.1.3 Surveillance of invasive cases

A key function of DANMAP's monitoring of antimicrobial resistance is to survey the proportion of resistant bacteria in the total number of invasive cases (blood and cerebrospinal fluid isolates). This is harmonised with the monitoring performed by the European Antimicrobial Resistance Surveillance Network (EARS-Net). Figure 8.1 presents the total numbers of invasive cases in Denmark from 2011 to 2020 for the bacterial species included in the surveillance programmes for both DANMAP and EARS-Net. Invasive cases caused by *Acinetobacter* species are not shown in Figure 8.1, due to the small numbers of isolates (ranging from 55 to 84 cases/year between 2011 and 2020) but are being included in the DANMAP and EARS-Net surveillance. For all species included in the two surveillance schemes, the following case definition applies: The first sample, by date of sample collection, of each given bacterial species per unique patient per year of observation are included. Duplicates, within the year of observation, from the same patient are excluded.

Between 2011 and 2020, the total number of registered invasive cases increased by 41% from 8,504 to 11,983 cases in Denmark. *E. coli*: 3,642 to 5,870 cases (61% increase), *S. aureus*: 1,452 to 2,391 (65%) and *K. pneumoniae* 910 to 1,413 (55%).

The only species with an overall decreasing number of cases during the past 10 years was *S. pneumoniae*. A significant drop of 43% in total *S. pneumoniae* cases was observed between 2019 and 2020 (from 639 cases in 2019 to 363 in 2020).

Figure 8.1 Number of invasive cases for bacterial species under surveillance, Denmark, 2011-2020 DANMAP 2020

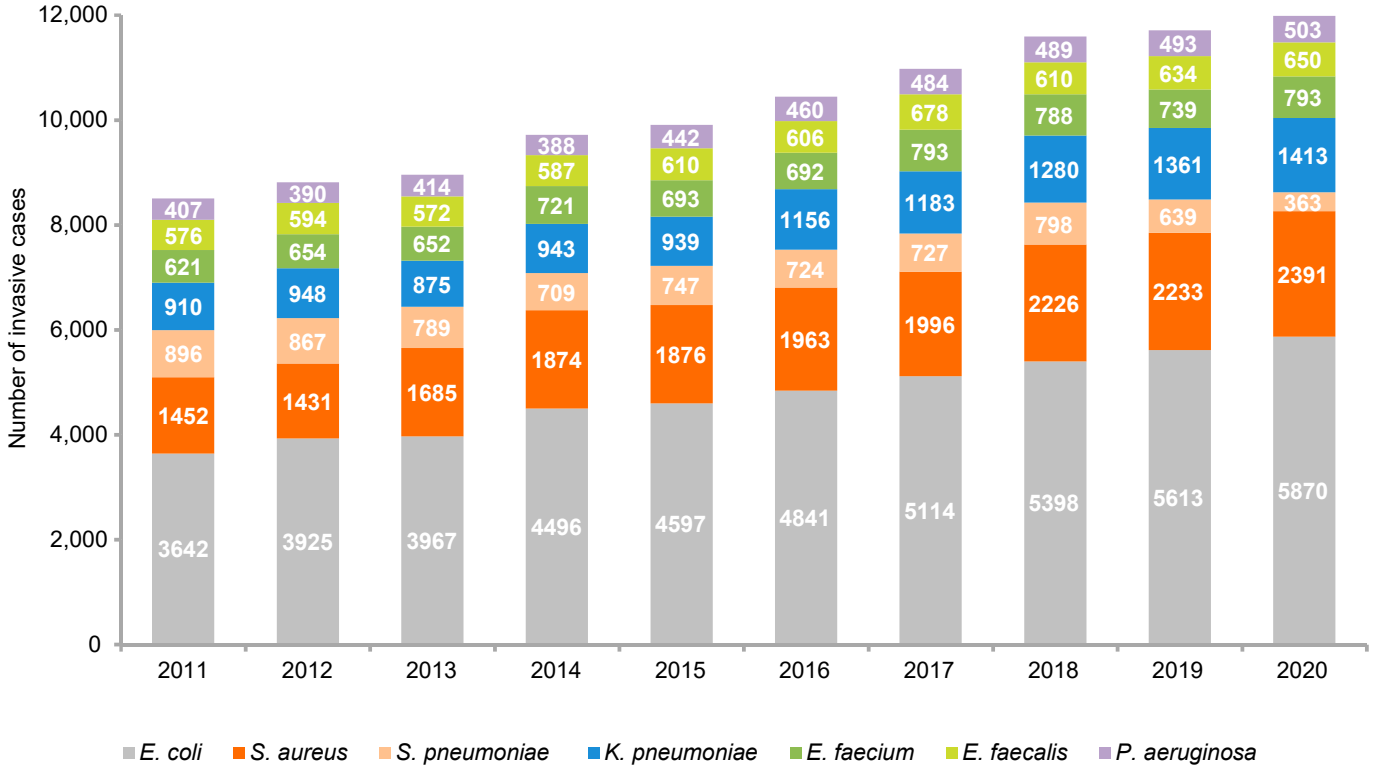
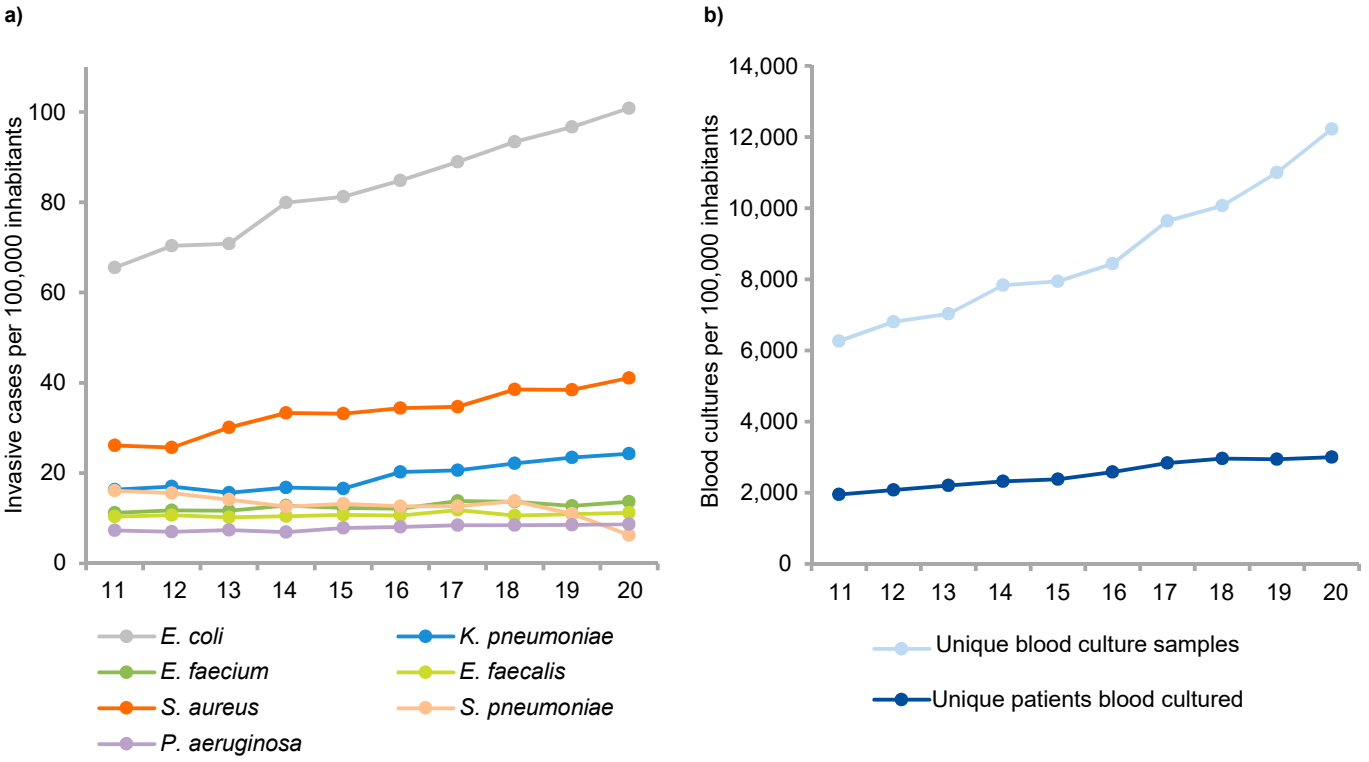


Figure 8.2a shows the number of invasive cases per 100,000 inhabitants in Denmark per year from 2011 to 2020. During this period, the Danish population increased by 4.7% (from 5,560,628 inhabitants in 2011 to 5,822,763 inhabitants in 2020). Figure 8.2b shows the total number of blood cultures taken per 100,000 inhabitants per year in the same period. Additionally, the number of unique patients with a blood culture taken per 100,000 inhabitants per year is shown. In the ten-year period the number of unique patients with at least one blood culture taken per year increased from 1,950 patients per 100,000 inhabitants in 2011 to 3,001 patients per 100,000 inhabitants in 2020 (an increase of 54%). The total number of blood samples (unique samples in MiBa) taken per 100,000 inhabitants increased even more (95%). Thus, on average more patients have more blood cultures taken each year.

Changes in hospital workflow (reduced bed-days), health-care-related infections, improved diagnostics and increased numbers of elderly at risk of infections may also affect the increased number of total infections.

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

Figure 8.2 Invasive cases and blood cultures taken per 100,000 inhabitants, Denmark, 2011-2020 DANMAP 2020



8.2 Surveillance based on MiBa data

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.

Invasive cases from hospital patients

In 2020, a total of 5,870 unique patients with invasive *E. coli* isolates were identified in MiBa. Antimicrobial susceptibility interpretations for key antimicrobials were also extracted for these isolates from MiBa (Chapter 9 'Material and methods').

The percentages of resistance in invasive *E. coli* isolates for each key antimicrobial are presented in Table 8.2, as well as urine samples from hospitals and primary health care (see details in later paragraphs). Figures 8.3a and 8.3b show the total annual numbers of invasive isolates and percentages of resistant isolates, respectively, between 2011 and 2020. Time trends including significance levels, based on the percentage of resistant *E. coli* isolates five and ten years back, respectively, are presented in Figure 8.3c.

Table 8.2 *Escherichia coli*. Resistance (%) in isolates from humans, Denmark, 2020 DANMAP 2020

	Invasive isolates, hospitals %	Urine isolates, hospitals %	Urine isolates, primary health care %
Ampicillin	46	41	36
Mecillinam	14	7.2	4.9
Piperacillin/tazobactam	5.4	4.3	5.1*
Amoxicillin/clavulanic acid	26	12	6.8*
Sulfonamide		30*	27
Trimethoprim		22	21
Nitrofuratoin		1*	0.8
Gentamicin	5.4	4.7	3.8*
Ciprofloxacin	11	9.6	7.5
Cefuroxime	9	7.2	5.5*
3rd generation cephalosporins	6.2	6.3	5
Carbapenem	0.1	0.0	0.0*
Max number of isolates tested for resistance to the presented antibiotics	5866	48962	88462

Included are results from all DCMs where >75% of the isolates in each antibiotic/sample group were susceptibility tested
* Less than 6 (out of 10 DCMs) tested >75% of the isolates. For carbapenems, piperacillin/tazobactam and gentamicin in urines from primary health care, only one DCM tested >75% of their isolates

As mentioned in the introduction, an increase in the number of invasive *E. coli* cases was observed over the past decade corresponding to 65.5 cases and 108.8 cases per 100,000 inhabitants respectively (a 54% increase, data not shown) over this time period.

In 2020, 9.2% 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 6.2% with a decreasing tendency the past decade.

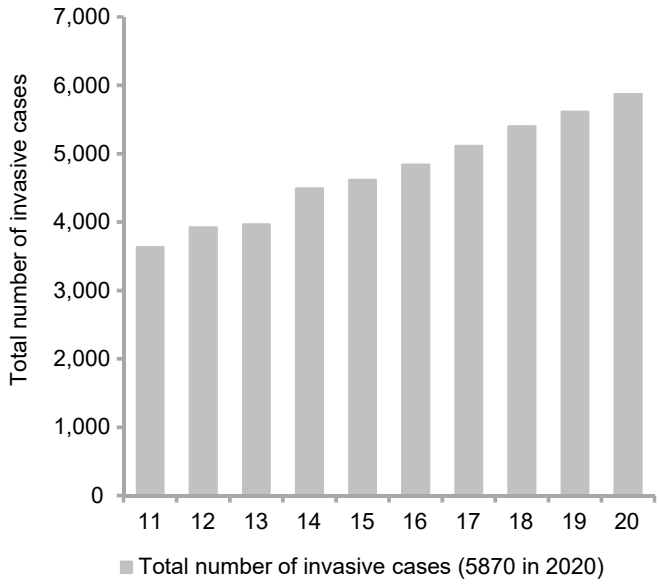
The ciprofloxacin resistance for invasive *E. coli* was 11.1%, ranging from 14.1 to 11.1% the last decade. The increased ciprofloxacin resistance rate in 2017 compared to 2016 mainly reflected a relatively large EUCAST breakpoint change in 2017. Ciprofloxacin breakpoints were changed (lowered) once again 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 3.9% of invasive *E. coli* isolates being reported as resistant in 2011 to 5.4% in 2020. Gentamicin resistance was 5.4% in 2020 and resistance rates have declined over the last decade. For more details, see Figure 8.3b.

The number of carbapenem resistant invasive *E. coli* isolates remained very low in 2020 with seven isolates categorised carbapenem-resistant and four 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 1.5%, Table 8.3.

Figure 8.3 Invasive *Escherichia coli* isolates from humans: a) annual numbers isolates, b) proportion of resistant isolates and c) trends and significance levels, Denmark, 2011-2020

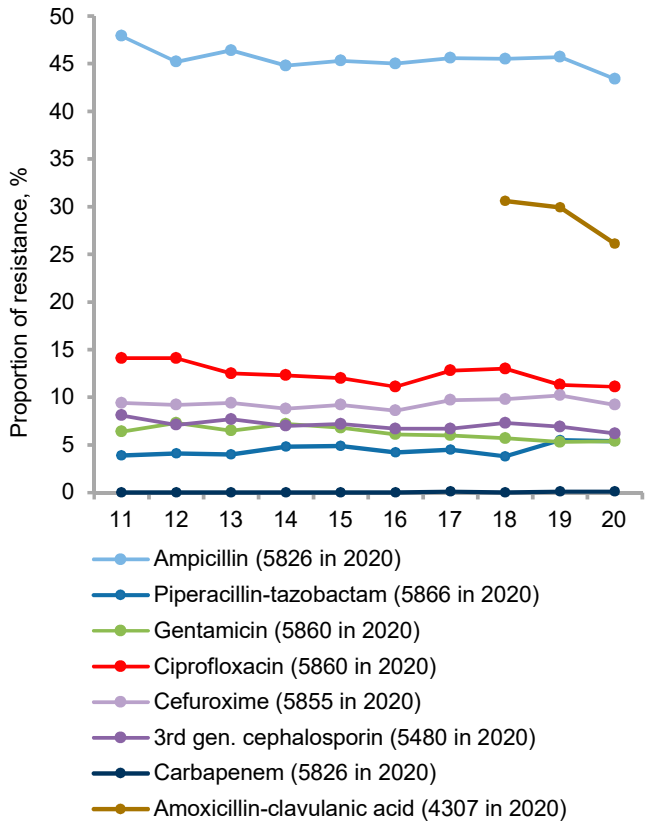
DANMAP 2020

a)



The number (n) in parentheses represents the numbers of isolates tested in 2019.

b)



c)

Substance	Percent resistant invasive *E. coli* isolates										Time trends (Cochran-Armitage test)	
	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	10 years (2011-2020)	5 years (2016-2020)
Ampicillin	47.9	45.2	46.4	44.8	45.3	45	45.6	45.5	45.7	43.4	↓ p = 0.002#	↑ p = 0.277
Piperacillin-tazobactam	3.9	4.1	4	4.8	4.9	4.2	4.5	3.8	5.5	5.4	↑ p = 5.375e-05#	↑ p = 1.957e-05#
Gentamicin	6.4	7.3	6.5	7.2	6.8	6.1	6	5.7	5.3	5.4	↓ p = 1.657e-07#	↓ p = 0.027#
Ciprofloxacin	14.1	14.1	12.5	12.3	12	11.1	12.8	13	11.3	11.1	↓ p = 4.351e-06#	↑ p = 0.3516
Cefuroxime	9.4	9.2	9.4	8.8	9.2	8.6	9.7	9.8	10.2	9.2	↑ p = 0.102	↑ p = 0.149
3rd gen.cephalosporins	8.1	7.1	7.7	7	7.2	6.7	6.7	7.3	6.9	6.2	↓ p = 0.001#	↓ p = 0.248
Carbapenem	0	0	0	0	0	0	0.1	0	0.1	0.1	↑ p = 0.002#	↑ p = 0.023#
Total number of isolates	3636	3922	3967	4492	4618	4841	5114	5398	5613	5870		

Significant trend in the overall data

Table 8.3 *Escherichia coli*. Combined resistance to 3rd generation cephalosporins, ciprofloxacin, and gentamicin (multiresistance) in invasive isolates from humans, Denmark, 2014-2020

DANMAP 2020

	2014 % (N)	2015 % (N)	2016 % (N)	2017 % (N)	2018 % (N)	2019 % (N)	2020 % (N)
Resistance	1.8 (72)	2.3 (93)	1.8 (87)	1.8 (88)	2.0 (100)	1.8 (93)	1.5 (82)
Percentage (no.) of isolates tested for combined resistance (multiresistance)	90 (4039)	88 (4071)	98 (4763)	95 (4883)	93 (4997)	94 (5259)	93 (5470)
Total number of invasive isolates	4495	4614	4841	5114	5398	5613	5870

Urinary tract infections in hospitals

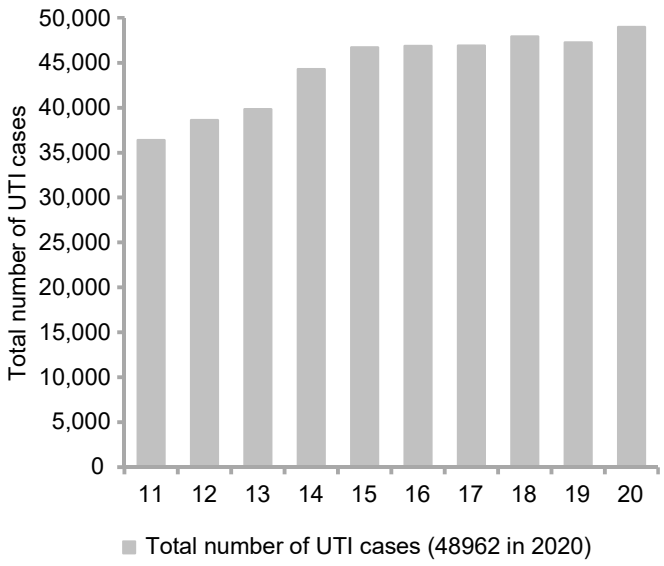
In 2020, 48,962 unique hospital patients had *E. coli* isolated from urine samples, a 35% and 3.5% increase compared to 2011 and 2019 respectively.

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

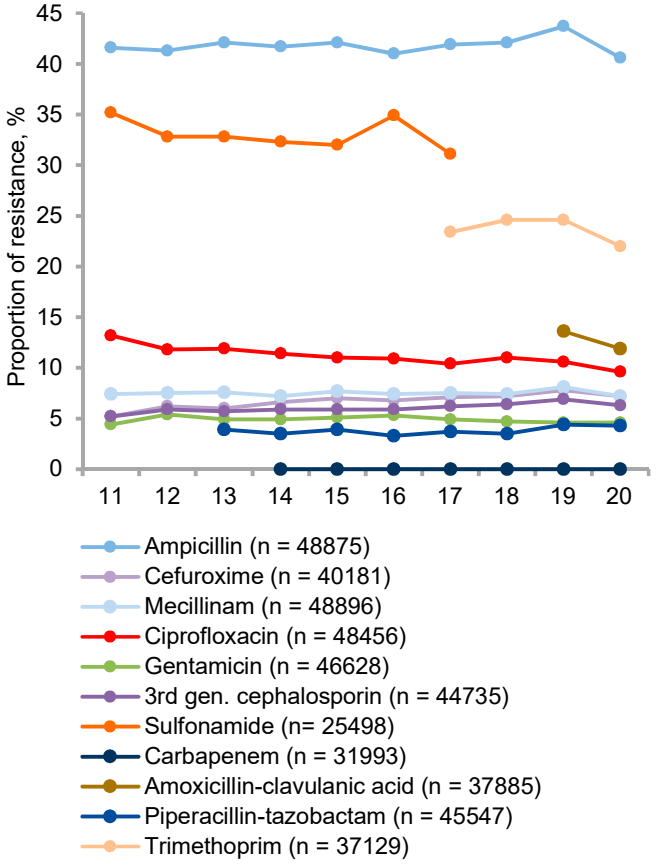
Summarized antimicrobial susceptibility results for *E. coli* isolates (invasive and urine specimen) are shown in Table 8.2. In Figure 8.4, resistance for *E. coli* urine isolates from hospitalised patients are shown for 2011-2020.

Figure 8.4 Urine *Escherichia coli* isolates from humans (hospitals): a) annual numbers isolates, b) proportion of resistant isolates and c) trends and significance levels, Denmark, 2011-2020 DANMAP 2020

a)



b)



The number (n) in parentheses represents the numbers of isolates tested in 2020.

* UTI = Urinary tract infection

c)

Substance	Percent resistant *E. coli* isolates from hospital urine										Time trends (Cochran-Armitage test)	
	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	10 years (2011-2020)	5 years (2016-2020)
Ampicillin	41.6	41.3	42.1	41.7	42.1	41	41.9	42.1	43.7	40.6	↑ p = 0.06	↑ p = 0.186
Mecillinam	7.4	7.5	7.6	7.2	7.7	7.4	7.5	7.4	8.1	7.2	↑ p = 0.0003#	↑ p = 0.326
Piperacillin-tazobactam			3.9	3.5	3.9	3.3	3.7	3.5	4.4	4.3	NA	↑ p <2.2e-16#
Sulfonamide	35.2	32.8	32.8*	32.3*	32	34.9	31.1	31*	31.4*	29.4*	NA	NA
Gentamicin	4.4	5.4	4.9	4.9	5.1	5.3	4.9	4.7	4.6	4.7	↓ p = 0.106	↓ p = 0.0002#
Ciprofloxacin	13.2	11.8	11.9	11.4	11	10.9	10.4	11	10.6	9.6	↓ p <2.2e-16#	↓ p = 5.345e-07#
Cefuroxime	5.2	6.2	6	6.6	7	6.8	7.1	7.2	7.8	7.2	↓ p <2.2e-17#	↑ p <2.2e-16#
3rd gen. cephalosporins	5.2	5.9	5.7	5.9	5.9	5.9	6.2	6.4	6.9	6.3	↓ p <2.2e-18#	↑ p = 7.876e-10#
Carbapenem				0	0	0	0	0	0	0	↓ p <2.2e-19#	NA
Total number of isolates	36389	38610	39820	44288	46723	46865	46884	47914	47235	48962		

* Less than 6 DCMs reported routine testing
\# Significant trend in the overall data

As can be seen in Figure 8.4c, a significant decrease in ciprofloxacin and gentamicin resistance was observed for the past five and ten years. However, 3rd generation cephalosporins and cefuroxime resistance increased significantly over the past five years.

In 2020, 22 carbapenem-resistant and 17 carbapenem-susceptible, increased exposure *E. coli* urine isolates from hospitalised patients were reported, compared to 19 and 16 isolates in 2019, respectively.

Urinary tract infections in primary health care

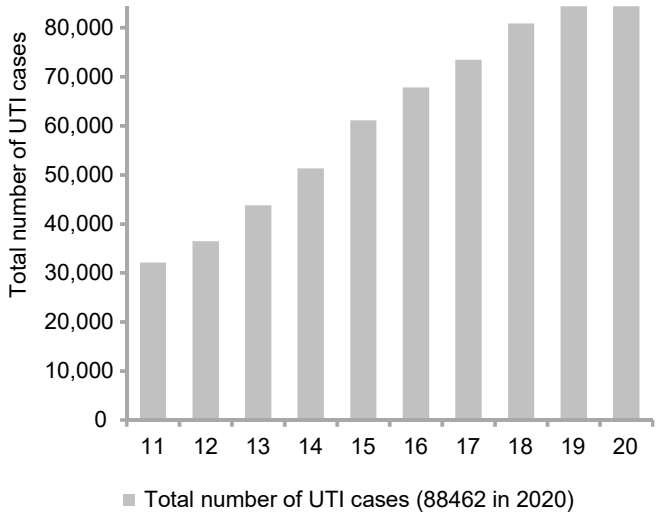
In 2020, 88,462 unique patients in primary health care had *E. coli* isolated from urine samples, a 64% and 2.2% increase compared to 2011 and 2019, respectively.

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.

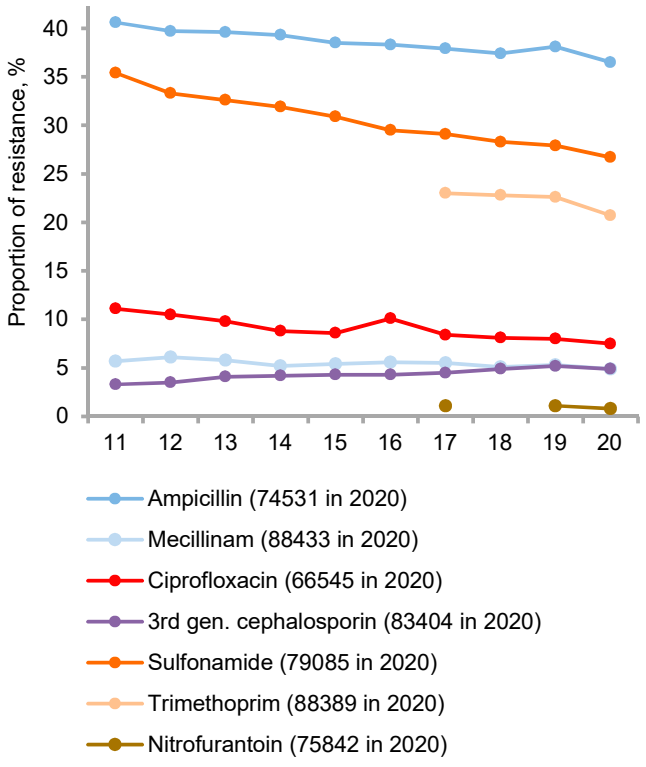
A continuous slow increase in resistance to 3rd generation cephalosporins was observed between 2011 and 2019. However, from 2019 to 2020 it decreased (0.6%). In contrast, a slow decline in mecillinam resistance and a more pronounced decline in sulfonamide resistance were observed over the same time period. A significant decrease in ciprofloxacin and ampicillin resistance was observed during both the five and ten year periods. For more details, see Figure 8.5.

Figure 8.5 Urine *Escherichia coli* isolates from humans (primary health care): a) annual numbers isolates, b) proportion of resistant isolates and c) trends and significance levels, Denmark, 2011-2020 DANMAP 2020

a)



b)



The number (n) in parentheses represents the numbers of isolates tested in 2020

* UTI = Urinary tract infection

c)

Substance	Resistance in *E. coli* urine isolates from primary health care										Time trends (Cochran-Armitage test)	
	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	10 years (2011-2020)	5 years (2016-2020)
Ampicillin	40.6	39.7	39.6	39.3	38.5	38.3	37.9	37.4	38.1	36.5	↓ p <2.2e-16#	↓ p = 0.023#
Mecillinam	5.7	6.1	5.8	5.2	5.4	5.6	5.5	5.1	5.3	4.9	↓ p = 7.078e-10#	↓ p = 6.142e-11#
Sulfonamide	35.4	33.3	32.6	31.9	30.9	29.5	29.1	28.3	27.9	26.8	↓ p <2.2e-16#	↓ p <2.2e-16#
Ciprofloxacin	11.1	10.5	9.8	8.8	8.6	10.1	8.4	8.1	8	7.6	↓ p <2.2e-16#	↓ p <2.2e-16#
3rd gen. cephalosporins	3.3	3.5	4.1	4.2	4.3	4.3	4.5	4.9	5.2	5	↑ p <2.2e-16#	↑ p <2.2e-16#
Total number of isolates	32117	36475	43770	51272	61083	67798	73497	80851	86508	88462		

Significant trend in the overall data

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 2011. Around 37-40% 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* has been stable and decreased slightly from 10.2% to 9.2% and 6.9% to 6.2% from 2019 to 2020, respectively. Resistance to carbapenems in invasive *E. coli* has increased since 2011, but is still very low.

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8.2.2 *Klebsiella pneumoniae*

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.

Invasive cases from hospitals

In 2020, a total of 1,361 unique patients were registered in MiBa with invasive *K. pneumoniae* isolates.

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 2011 and 2020. Time trends including statistical significance levels, based on the percentage of resistant *K. pneumoniae* isolates five and ten years back, respectively, are presented in Figure 8.6c.

As for invasive *E. coli* cases, a continuous increase in the number of invasive *K. pneumoniae* cases was observed over the last decade, from 910 cases in 2011 to 1,413 cases in 2020. 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).

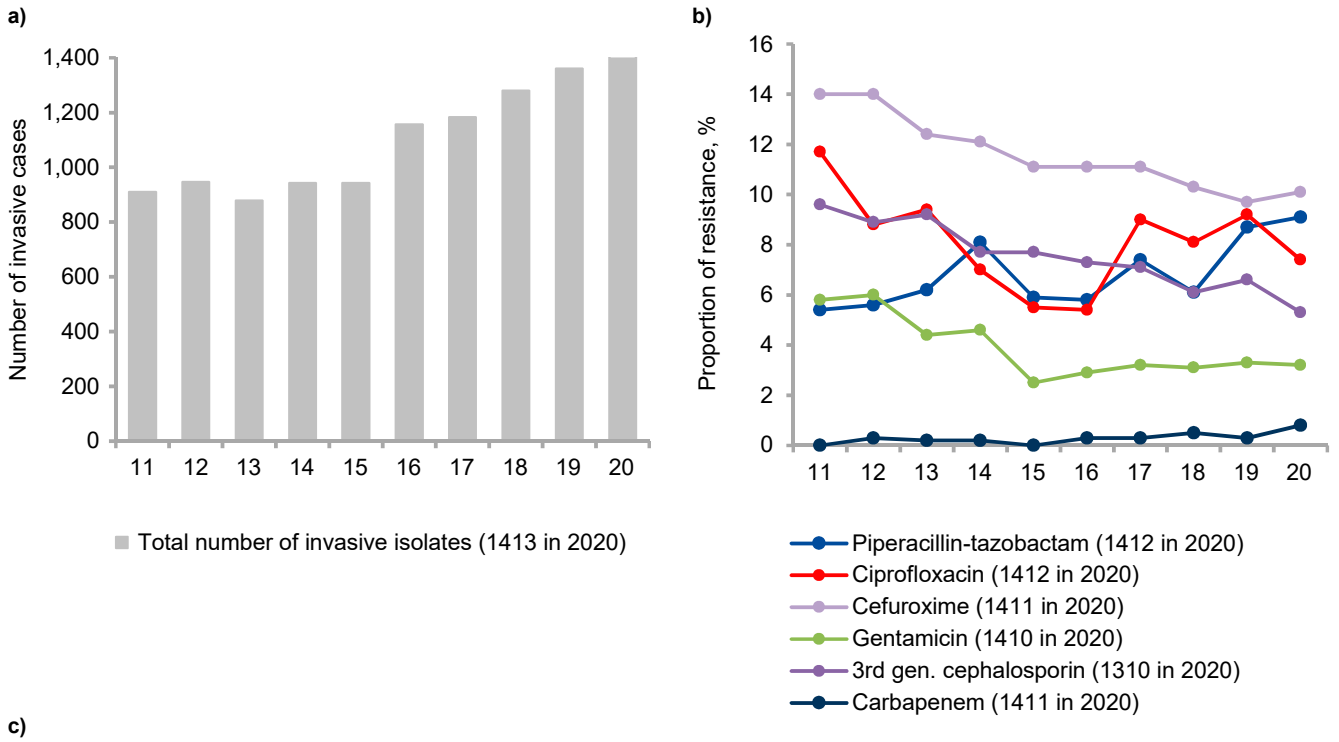
Resistance in invasive *K. pneumoniae* isolates has decreased over the past 10 years for gentamicin, ciprofloxacin, cefuroxime and 3rd generation cephalosporins respectively. For more details, see Figure 8.6b and 8.6c. The increased proportion of ciprofloxacin-resistant isolates in 2017 when compared 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 and significance levels were not calculated. 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.

Table 8.4 *Klebsiella pneumoniae*. Resistance (%) in isolates from humans, Denmark, 2020 DANMAP 2020

Substance	Invasive isolates, hospitals %	Urine isolates, hospitals %	Urine isolates, primary health care %
Mecillinam	14	12	9.4
Piperacillin/tazobactam	9.1	8	9.3*
Amoxicillin/clavulanic acid	16.9	9.3	5.9
Sulfonamide		17.1*	15.5
Trimethoprim		17.4	16.6
Nitrofuratoin		31.1*	41.6*
Gentamicin	3.2	2.9	1.5*
Ciprofloxacin	7.4	7.2	5.1
Cefuroxime	10.1	8.5	4.7*
3rd generation cephalosporins	5.3	5.5	4.4
Carbapenem	0.8	0.1	0.1*
Max. number of isolates tested for resistance to the presented antibiotics	1412	7814	9387

Included are results from all DCMs where >75% of the isolates in each antibiotic/sample group were susceptibility tested
* Less than 6 (out of 10 DCMs) tested >75% of the isolates. For carbapenems, piperacillin/tazobactam and gentamicin in urines from primary health care, only one DCM tested >75% of their isolates

Figure 8.6 Invasive *Klebsiella pneumoniae* isolates from humans: a) annual numbers isolates, b) proportion of resistant isolates and c) trends and significance levels, Denmark, 2011-2020 DANMAP 2020



c)

Substance	Percent resistant invasive *K. pneumoniae* isolates										Time trends (Cochran-Armitage test)	
	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	10 years (2011-2020)	5 years (2016-2020)
Piperacillin/tazobactam	5.4	5.6	6.2	8.1	5.9	5.8	7.4	6.1	8.7	9.1	↑ p = 4.554e-05#	↑ p = 0.0001#
Gentamicin	5.8	6.0	4.4	4.6	2.5	2.9	3.2	3.1	3.3	3.2	↓ p = 1.338e-06#	↑ p = 0.315
Ciprofloxacin	11.7	8.8	9.4	7	5.5	5.4	9.0	8.1	9.2	7.4	↓ p = 0.037#	↑ p = 0.041#
Cefuroxime	14.0	14.0	12.4	12.1	11.1	11.1	11.1	10.3	9.7	10.1	↓ p = 5.855e-06#	↓ p = 0.111
3rd gen. cephalosporins	9.6	8.9	9.2	7.7	7.7	7.3	7.1	6.1	6.6	5.3	↓ p = 8.291e-07#	↓ p = 0.025#
Carbapenem	0.0	0.3	0.2	0.2	0	0.3	0.3	0.5	0.3	0.8	↑ p = 0.002#	↑ p = 0.027#
Total number of isolates	910	947	879	943	943	1156	1183	1280	1361	1413		

Significant trend in the overall data

Carbapenem resistance in invasive *K. pneumoniae* is very low (11 resistant isolates out of 1,411 tested [0.8%] in 2020). The increase shown in Figure 8.6b and 8.6c is mainly due to hospital outbreaks (section 8.3.2 Carbapenemase-producing bacteria in Denmark, 2020). The level of multidrug-resistant (combined resistance to 3rd generation cephalosporins, ciprofloxacin and gentamicin) invasive *K. pneumoniae* was below 2% in 2020, Table 8.5. None of the invasive *K. pneumoniae* isolates were registered resistant to colistin, susceptibility to colistin is not routinely tested though.

Table 8.5 *K. pneumoniae*. Combined resistance to 3rd generation cephalosporins, ciprofloxacin, and gentamicin (multidrug-resistance) in invasive isolates from humans, Denmark, 2014-2020 DANMAP 2020

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

Urinary tract infections in hospitals

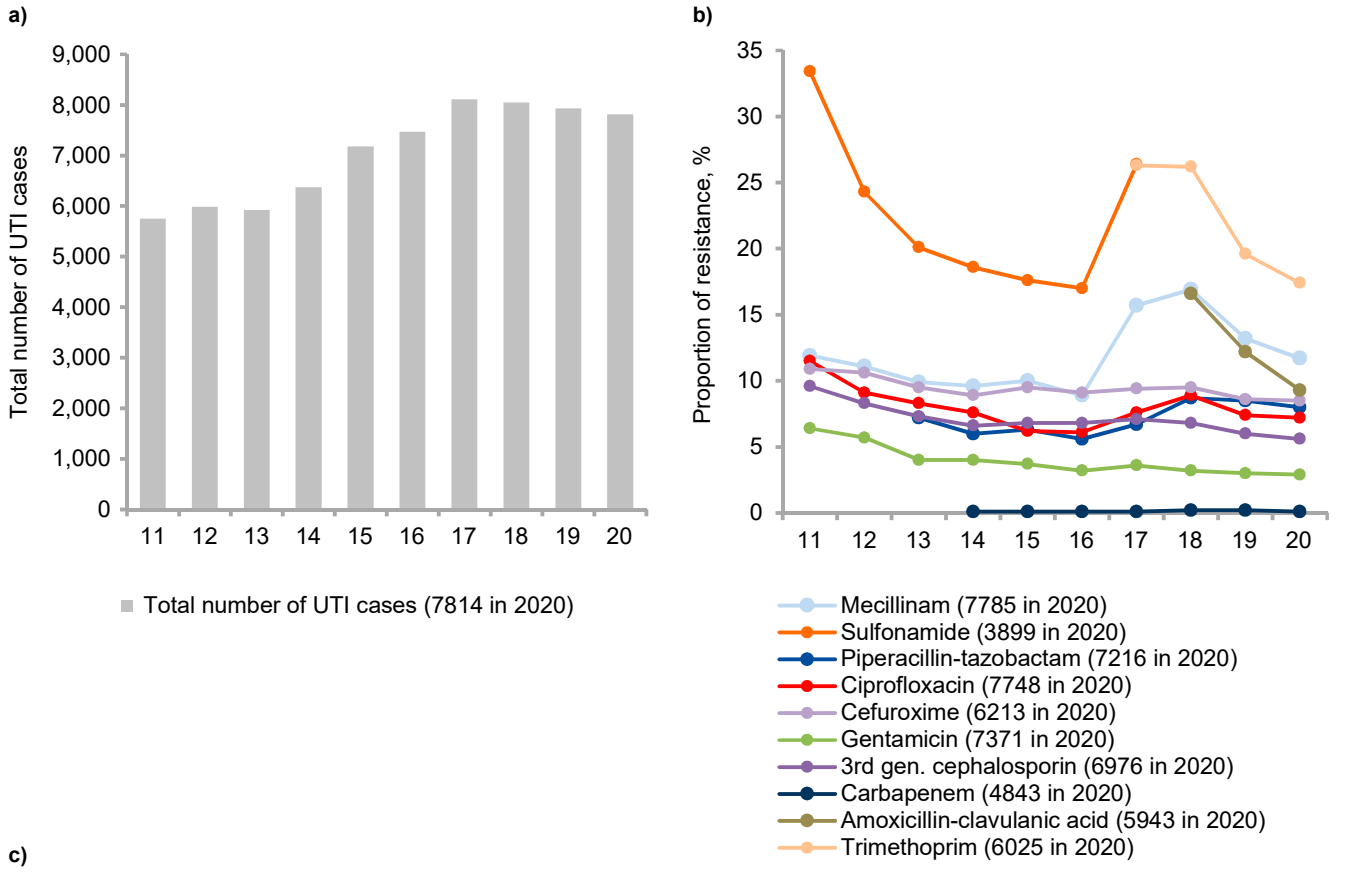
In 2020, 7,814 unique hospital patients had *K. pneumoniae* isolated from urine samples in Denmark. This represents a 26% increase and 1.4% decrease compared to 2011 and 2019, respectively.

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 2011-2020.

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 seems to be decreasing in 2019 and 2020. For more details, see Figure 8.7. Amoxicillin/clavulanic acid resistance has only been reported since 2017 in urinary isolates from hospitals, due to less than six DCMs testing isolates previously. The proportion of resistance decreased since then.

In 2020, there were 15 carbapenem-resistant and seven carbapenem-susceptible, increased exposure *K. pneumoniae* urine isolates from hospital patients reported in MiBa, respectively, compared to 15 and 11 isolates in 2019, respectively.

Figure 8.7 Urine *Klebsiella pneumoniae* isolates from humans (hospitals): a) annual numbers isolates, b) proportion of resistant isolates and c) trends and significance levels, Denmark, 2011-2020 DANMAP 2020



c)

Substance	Resistance in *K. pneumoniae* urine isolates from hospitals										Time trends (Cochran-Armitage test)	
	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	10 years (2011-2020)	5 years (2016-2020)
Mecillinam	11.9	11.1	9.9	9.6	10	8.9	15.7	16.9	13.2	11.6	↑ p <2.2e-16#	↑ p = 0.04#
Piperacillin/tazobactam			7.2	6	6.3	5.6	6.7	8.7	8.5	8	NA	↑ p = 6.757e-13#
Gentamicin	6.4	5.7	4	4	3.7	3.2	3.6	3.2	3	2.9	↓ p <2.2e-16	↓ p = 0.029#
Ciprofloxacin	11.5	9.1	8.3	7.6	6.2	6.1	7.6	8.9	7.4	7.2	↓ p = 1.287e-09#	↑ p = 0.015#
Cefuroxime	10.9	10.6	9.5	8.9	9.5	9.1	9.4	9.5	8.6	8.5	↓ p = 0.001#	↑ p = 0.323
3rd gen. cephalosporins	9.6	8.3	7.3	6.6	6.8	6.8	7.1	6.8	6	5.5	↓ p = 1.003e-13#	↓ p = 0.003#
Total number of isolates	5746	5982	5919	6372	7175	7467	8106	8047	7926	7814		

Significant trend in the overall data
UTI: Urinary tract infection

Urinary tract infections in primary health care

In 2020, 9,387 unique patients in primary health care had *K. pneumoniae* isolated from urine samples, a 63% increase and 3% decrease compared to 2011 and 2019 respectively.

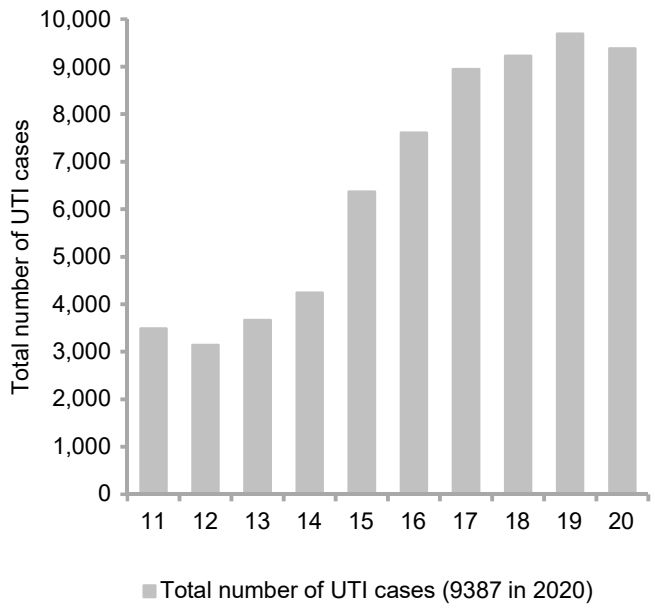
As for the results from invasive isolates and isolates from hospital urine samples susceptibility results for all tested antimicrobials are shown in Table 8.4. In Figure 8.8, resistance for *K. pneumoniae* urine isolates from primary health care is shown for the past decade.

Following a sharp increase in 2017, resistance to mecillinam and sulfonamides/trimethoprim decreased in 2019 and 2020. For more details, see Figure 8.8.

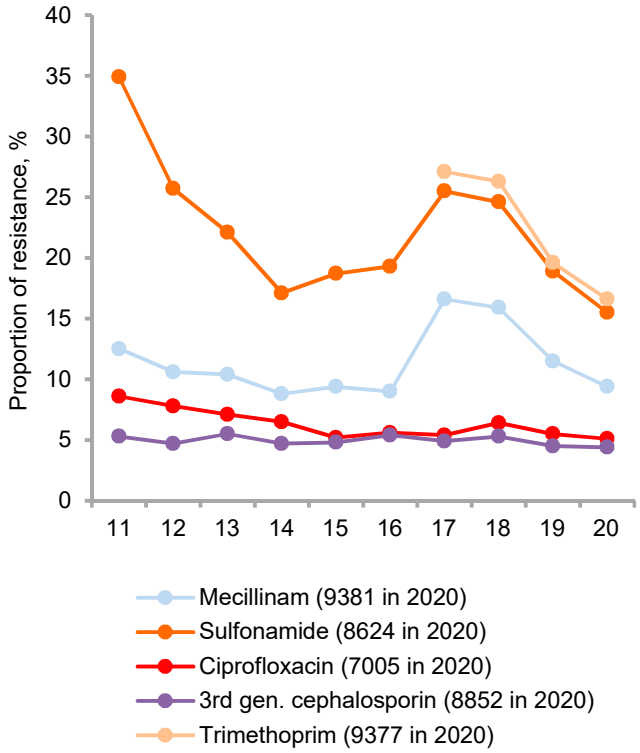
Four carbapenem-resistant isolates and one carbapenem-susceptible, increased exposure isolate were registered in 2020 compared to three and two in 2019 respectively. However, susceptibility to carbapenems is only routinely reported to MiBa by one DCM.

Figure 8.8 Urine *Klebsiella pneumoniae* isolates from humans (primary health care): a) annual numbers isolates, b) proportion of resistant isolates and c) trends and significance levels, Denmark, 2011-2020 DANMAP 2020

a)



b)



c)

Substance	Resistance in *K. pneumoniae* urine isolates from primary health care										Time trends (Cochran-Armitage test)	
	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	10 years (2011-2020)	5 years (2016-2020)
Mecillinam	12.5	10.6	10.4	8.8	9.4	9	16.6	15.9	11.5	9.4	↑ p = 0.0001#	↓ p = 2.485e-09#
Sulfonamide	34.9	25.7	22.1	17.1	18.7	19.3	25.5	24.6	18.9	15.5	↓ p <2.2e-16#	↓ p <2.2e-16#
Ciprofloxacin	8.6	7.8	7.1	6.5	5.2	5.6	5.4	6.4	5.5	5.1	↓ p = 2.229e-09#	↑ p = 0.261
3rd gen. cephalosporins	5.3	4.7	5.5	4.7	4.8	5.4	4.9	5.3	4.5	4.4	↓ p = 0.003#	↓ p = 0.004#
Total number of isolates	3489	3142	3669	4246	6372	7615	8948	9227	9696	9387		

Significant trend in the overall data
UTI: Urinary tract infection

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 been decreasing in 2019 and 2020. Even though precautions should be taken when interpreting reported susceptibility results for piperacillin-tazobactam, there has been an increased number of isolates with decreased susceptibility by disk diffusion (zone diameters <17mm). The carbapenem resistance in *K. pneumoniae* is very low (<1%).

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8.2.3 *Pseudomonas aeruginosa*

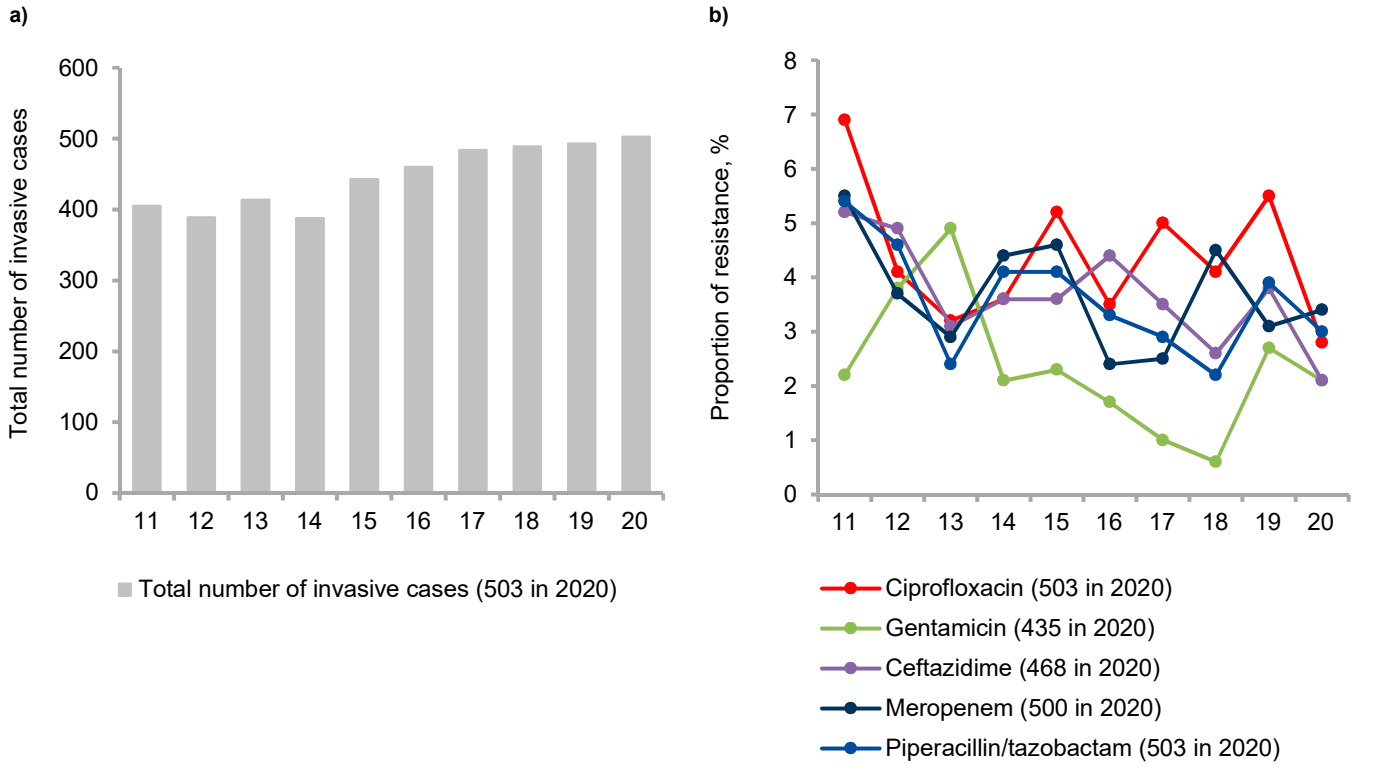
Pseudomonas aeruginosa is an opportunistic pathogen which can colonise the lung, urinary tract, burn wounds, superficial wounds and can cause invasive infections. The pathogen has properties of biofilm formation and ability to colonise medical devices (e.g. indwelling catheters and implants) and in most 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, chromosomal gene mutations and has the ability to horizontally acquire resistance mechanism of β-lactamases (extended-spectrum β-lactamases (ESBLs) and carbapenemases (especially class B carbapenemases or metallo-β-lactamases (MBLs)). 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 antibiotics, such as ceftazidime-avibactam and ceftolozane-tazobactam, may be used against some multidrug resistant Gram-negative bacteria and *Pseudomonas aeruginosa* strains.

Invasive cases from hospital patients

In 2020, a total of 503 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 – 6.9% the past 10 years. In 2020, meropenem resistance was reported in 3.5% of the cases, and only 1.4% of the cases were resistant to three or more of the five antimicrobials under surveillance. None of the invasive *P. aeruginosa* isolates were reported as colistin-resistant to MiBa.

Figure 8.9 Invasive *Pseudomonas aeruginosa* isolates from humans: a) annual numbers isolates and b) proportion of resistant isolates, Denmark, 2011-2020 DANMAP 2020



Conclusion

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

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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, UTI 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, which are recommended for treatment, include fluoroquinolones, aminoglycosides, carbapenems and colistin.

Invasive cases from hospitals

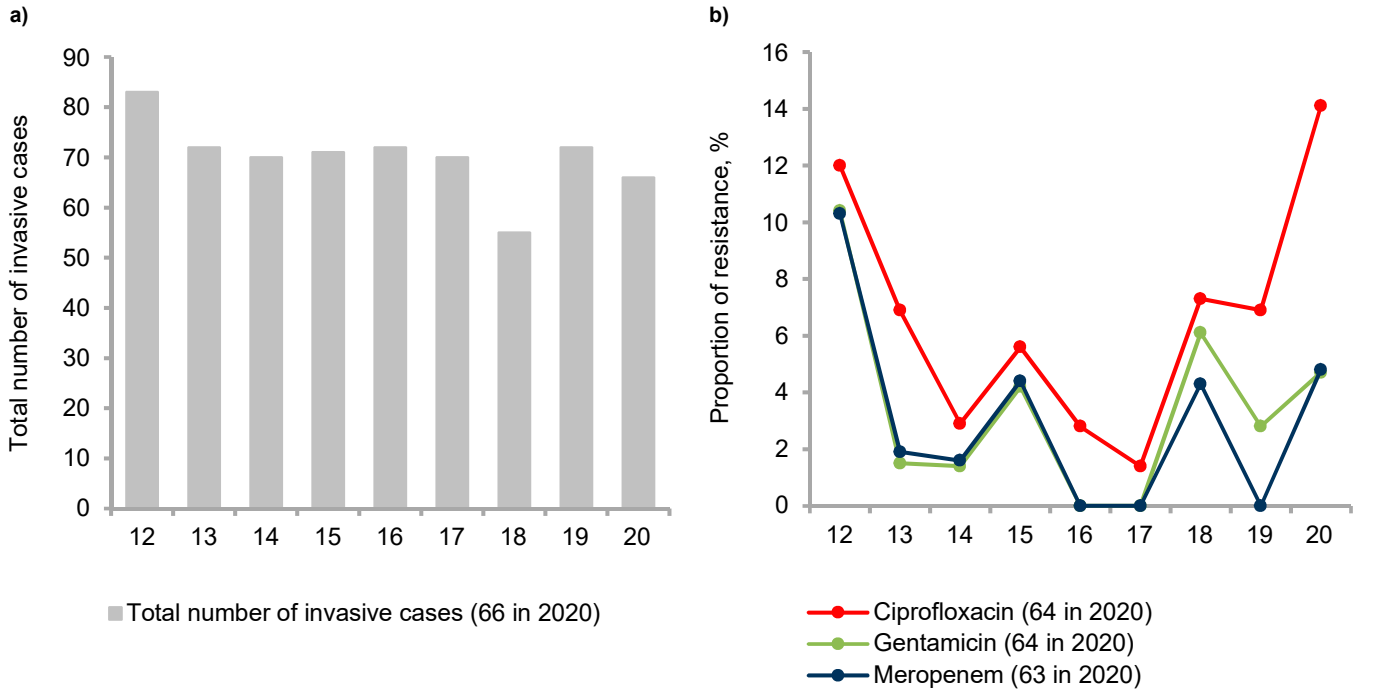
In 2020, a total of 66 unique patients with invasive *Acinetobacter* species were reported. Data are presented in Table 8.6 and in Figure 8.10.

Table 8.6 *Acinetobacter* spp. tested and resistant invasive isolates, Denmark, 2012-2020 DANMAP 2020

	2012		2013		2014		2015		2016		2017		2018		2019		2020	
	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
Gentamicin	8	77	1	65	1	70	3	71	0	70	0	70	3	49	2	72	3	64
Meropenem	6	58	1	52	1	62	3	68	0	69	0	67	2	47	0	72	3	63
Total number of invasive isolates	84		72		72		71		72		70		55		72		66	

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

Figure 8.10 Invasive *Acinetobacter* spp. isolates from humans: a) annual numbers isolates and b) proportion of resistant isolates, Denmark, 2012-2020 DANMAP 2020



The number of invasive *Acinetobacter* spp. cases in 2020 were slightly lower than the numbers in 2013 - 2017 and 2019. Three of the 66 isolates identified in 2020 were resistant to meropenem, nine were resistant to ciprofloxacin and three were resistant to gentamicin. Three isolates had combined resistance to ciprofloxacin and gentamicin and none was reported resistant to colistin, however, susceptibility to colistin, is not routinely tested.

Conclusion

In general, the number of invasive *Acinetobacter* spp. cases as well as the proportion of invasive isolates resistant to key antimicrobials is low in Denmark.

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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 2020, 650 unique patients with invasive *E. faecalis* isolates and 793 unique patients with invasive *E. faecium* isolates were reported in MiBa.

The proportion of resistant invasive *E. faecalis/faecium* isolates in 2020 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 2011 to 2020.

The total number of invasive cases caused by *E. faecalis* and *E. faecium* increased by 21% from 2011 to 2020.

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 vancomycin resistant *E. faecium* seems to have stabilised around 9% of all invasive isolates for the past three years.

The proportion of high-level gentamicin resistance is based on reporting from one DCM in 2010-2017, four DMCs in 2018 and from three DCMs in 2020. These limited data show a decreasing trend in high-level gentamicin resistance in invasive *E. faecalis* from 36% in 2010 to 7.4% in 2020. High-level gentamicin in *E. faecium* was 30% in 2020 (Table 8.7).

During 2020, six invasive isolates of *E. faecalis* and three invasive isolate of *E. faecium* from nine unique patients were reported linezolid resistant (Table 8.7). In 2019, the numbers were eight *E. faecalis* and one *E. faecium*. All linezolid resistant invasive isolates identified in MiBa in 2020 were reported susceptible to vancomycin.

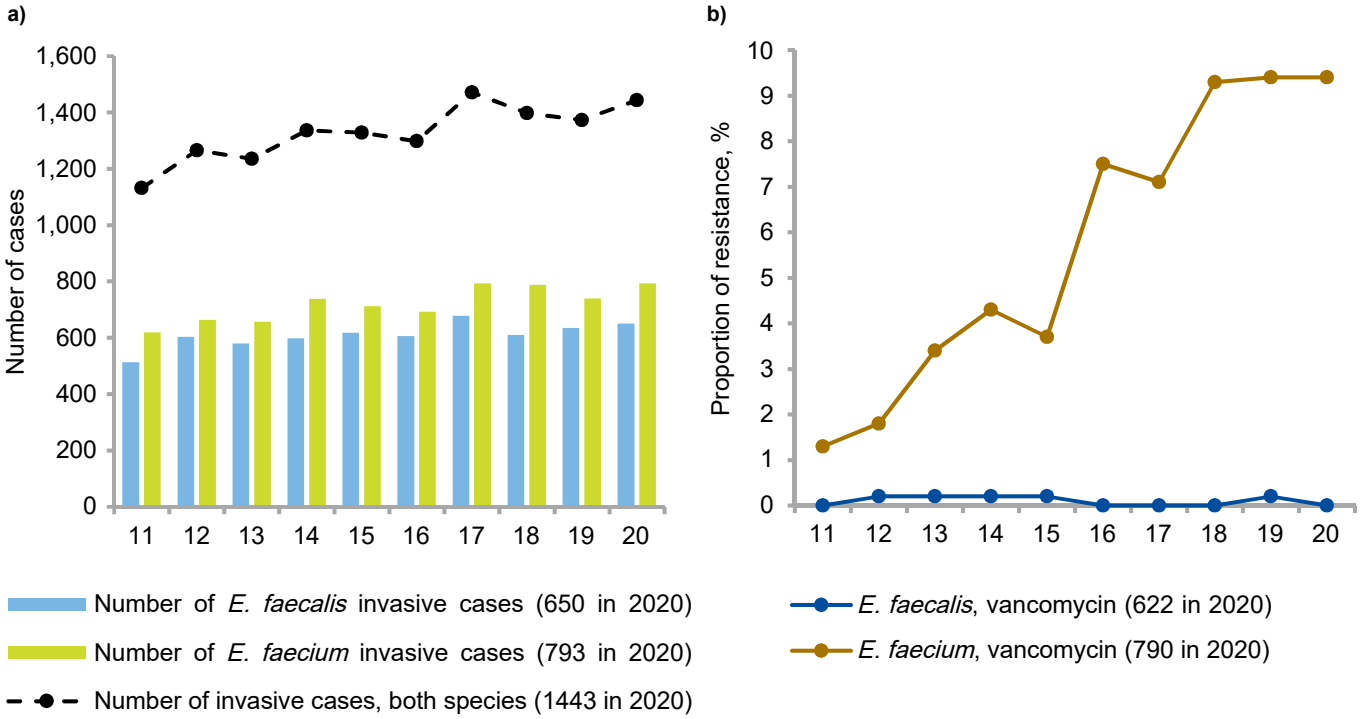
Table 8.7 Enterococci. Resistance (%) in invasive isolates from humans, Denmark, 2020 DANMAP 2020

	E. faecalis	*E. faecium*	Number of included isolates (number of DCM)	
	%	%	*E. faecalis*	*E. faecium*
Ampicillin	0	92.4	650 (10)	768 (9)
Vancomycin	0	9.4	622 (9)	790 (10)
Linezolid	1.2	0.5	507 (6)	621 (6)
High-level gentamicin	7.4	30	349 (3)	298 (3)
Teicoplanin	0	3.2	224 (2)	253 (2)
Tigecycline	0.9	2.5	112 (1)	120 (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 numbers isolates and b) proportion of vancomycin resistant isolates, Denmark, 2011-2020

DANMAP 2020



Conclusion

The number of invasive enterococcal infections, mainly caused by *E. faecium*, increased by 21% between 2011 and 2020. Resistance to ampicillin and vancomycin stayed high (92.4% and 9.4% respectively) in 2020 limiting treatment options and highlighting the importance of antimicrobial stewardship and infection prevention and control measures.

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8.3 Surveillance based on data from the reference laboratories

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

Background

Resistance to 3rd generation cephalosporins in *Escherichia coli* often occurs through production of extended-spectrum beta-lactamases (ESBLs), carbapenemases, plasmid-mediated AmpC (pAmpC) or through mutations within the promoter/attenuator region of the chromosomal *ampC* leading to AmpC 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 to the National Reference Laboratory for Antimicrobial Resistance at Statens Serum Institut (SSI). Isolates were selected by adhering to the EUCAST criteria for reduced susceptibility towards cefpodoxime, ceftriaxone, cefotaxime or ceftazidime for further characterization.

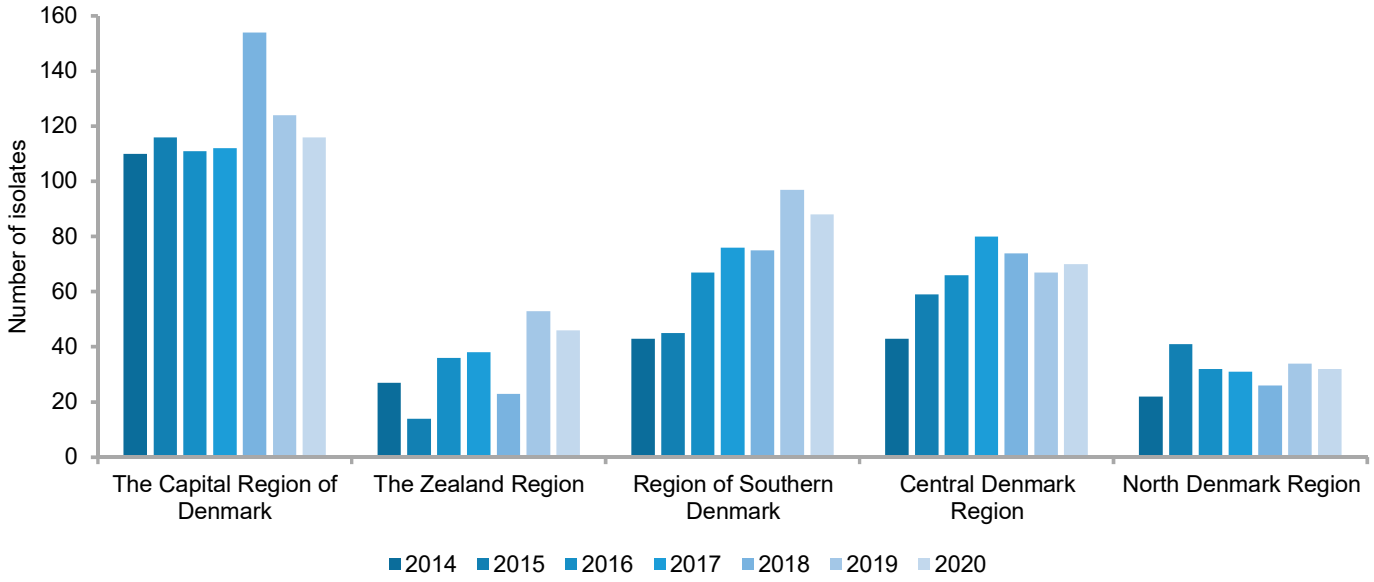
At SSI, the 3GC-R *E. coli* collected in Denmark through 2020, were phenotypically tested for ESBL-production. ESBL- and/or pAmpC-positive isolates from January, March, May, July, September, and November were subjected to whole-genome sequencing (WGS) and characterized according to Multi Locus Sequence Types (MLSTs), and the encoding ESBL-, pAmpC- and carbapenemase genes.

Results

In 2020, a total of 352 *E. coli* isolates from unique patients, were identified by phenotypic testing, as ESBL and/or AmpC positive isolates. Demographic data was available for all 352 *E. coli* isolates in 2020; 190 (54%) of the patients were men compared to 204 (55%) in 2019, and 162 (46%) were women compared to 169 (45%) in 2019. The average age at diagnosis was 71 years, ranging from below one year to 100 years.

The regional distribution of the 352 isolates with ESBL- and AmpC phenotype was compared to data from previous years (Figure 8.12).

Figure 8.12 ESBL/pAmpC producing *E. coli* from bloodstream infections by region, Denmark, 2014-2020 DANMAP 2020



From 2014 to 2019, the reported cases of ESBL/pAmpC *E. coli* in bloodstream infections have changed from 245 to 375 per year, a 53% increase. For the first time since 2014 however, the number of cases decreased 6% from 2019 to 2020, to the level of 2018 (from 373 to 352 isolates). In The Capital Region, the number of reported cases decreased from 124 cases in 2019 to 116 cases in 2020 ($p = 0.003$). In addition, a significant decrease from 97 cases in 2019 to 88 cases in 2020 were observed in the Region of Southern Denmark ($p = 0.03$). For the remaining three regions, the reported number of cases were stable in 2020 compared to 2019.

WGS data were obtained from 193 *E. coli* isolates (as only isolates from every second months were sequenced). Genes encoding ESBL and/or pAmpC were detected in all isolates (7 of which also having carbapenemase encoding genes detected).

In 2020, 20 different ESBL-, and pAmpC-beta-lactamases were detected among the 193 sequenced isolates (Table 8.8). As in previous years, CTX-M-15 was the most prevalent enzyme, remaining stable in occurrence at 53% in 2020, compared to 43% in 2019. The presence of CTX-M-14 decreased from 17% in 2019 to 8% in 2020 (p=0.004). Two new ESBL-enzymes were detected among the isolates in 2020, CTX-M-242 and CTX-M-244.

Table 8.8 Most common ESBL enzymes and carbapenemases detected in *E. coli* from bloodstream infections, Denmark, 2014-2020 DANMAP 2020

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

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

Table 8.9 Distribution of MLSTs in *E. coli* from bloodstream infections, Denmark, 2014-2020 DANMAP 2020

	DANMAP 2014		DANMAP 2015		DANMAP 2016		DANMAP 2017		DANMAP 2018		DANMAP 2019		DANMAP 2020	
MLST	Numbers	%	Numbers	%	Numbers	%	Numbers	%	Numbers	%	Numbers*	%	Numbers*	%
ST131	124	51	135	49	177	57	175	52	189	54	93	47	89	46
ST38	18	7	23	8	21	7	23	7	22	6	13	7	8	4
ST69	10	4	10	4	16	5	20	6	27	8	14	7	20	10
ST648	7	3	10	4	5	2	8	2	6	2	4	2	0	0
ST12	5	2	9	3	14	4	6	2	5	1	5	3	2	1
ST1193	2	1	5	2	10	3	7	2	8	2	6	3	9	5
ST10	0	0	6	2	2	1	4	1	7	2	5	3	1	1
ST23	1	0	0	0	2	1	3	1	1	0	11	6	3	2
ST73	3	1	2	1	4	1	2	1	6	2	4	2	8	4
Other STs[1]	75	31	75	27	61	20	89	26	81	23	42	21	53	27

[1] Found in less than 2% in 2020
* Numbers based on sequenced data from odd months

In addition, 7 carbapenemase producers were observed during 2020 among the 193 whole genome sequenced blood infection isolates (4%); three NDM- and four OXA-48-group producing isolates of which five isolates (2 NDM-5/OXA-181 producers, as well as one NDM-5-, one OXA-181- and one OXA-436 producer) belonged to known outbreaks while the remaining 2 isolates (one NDM-1- and one OXA-244 producer) occurred sporadically.

In 2020, the 193 whole genome sequenced *E. coli* isolates belonged to 41 different MLSTs, with the most common sequence type (ST) being ST131 (46%), followed by ST69 (10%) and ST1193 (5%) (Table 8.9).

The proportion of ST23 isolates decreased from 6% in 2019 to 2% in 2020 ($p=0.03$). Besides this, no significant changes in the distribution of MLSTs were observed in 2020 (Table 8.9).

Among the 89 *E. coli* isolates belonging to ST131, CTX-M-15 (61%) was the most common enzyme, followed by CTX-M-27 (28%), and CTX-M-14 (3%). The distribution of ESBL and/or pAmpC enzymes observed within ST131 remained stable in 2020.

Conclusion

In 2020, the number of ESBL- and/or AmpC positive isolates decreased from 373 to 352 isolates (6% decrease). CTX-M-15 remained by far the most prevalent ESBL enzyme in Danish *E. coli* from bloodstream infections in 2020, whereas the occurrence of CTX-M-14 decreased. In isolates belonging to ST131, the relative distribution of ESBL/pAmpC enzymes was stable in 2020 compared to 2019.

In 2020, 7 carbapenemase producers were observed among the 193 whole genome sequenced ESBL- and/or pAmpC blood infection isolates.

With the exception of a decrease in ST23, the relative distribution of sequence types for the 193 whole genome sequenced isolates were similar to the distributions in the previous years; the worldwide disseminated ST131 clone was still strongly represented in 2020 (46%). This suggests that the ESBL/pAmpC *E. coli* population in Danish patients is relatively stable over time, but does not rule out that local outbreaks of these bacteria may occur occationally.

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8.3.2 Carbapenemase-producing organisms (CPO) in Denmark, 2020

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).

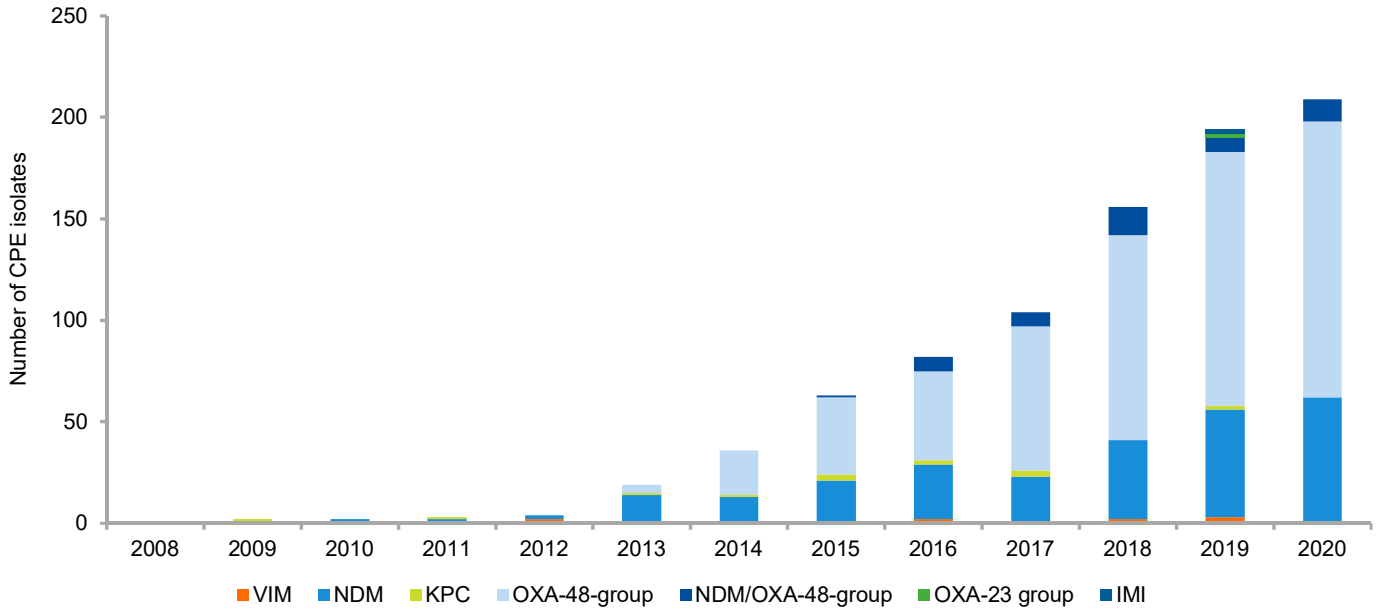
Carbapenemase-producing organisms (CPO) comprise two main groups: 1) Intestinal bacteria: Carbapenemase-producing Enterobacterales (CPE), e.g., *Escherichia coli* and *Klebsiella pneumoniae*, and 2) environmental bacteria: *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. The term CPE is used to 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 2020, 238 CPO were identified from 207 patients compared with 221 CPO isolates from 187 patients in 2019. 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 carbapenemases. Seventeen out of all CPOs were from bloodstream infections compared to ten out of all CPOs in 2019.

Collection of travel information is part of the CPO surveillance in Denmark. Thus, information of traveling abroad in the period of 6 months prior to testing positive for CPO is reported to SSI, together with the clinical sample information. In 2020, due to COVID-19, travel abroad has decreased, which in turn has affected the number of travel associated CPO-infections to a much lower proportion. In 2019, 80 of 187 CPO-positive persons (43%) reported travelling outside the Nordic countries. In 2020, only 35 of 207 CPO-positive persons (17%) reported travelling outside the Nordic countries.

Figure 8.13 Numbers of carbapenemase-producing Enterobacterales (CPE), Denmark, 2008-2020 DANMAP 2020



Carbapenemase-producing Enterobacterales
In 2020, 210 CPE isolates were reported from 183 patients compared to 194 CPE from 168 patients in 2019 resulting in a 8.2% increase compared to 2019. In 2020, 11 of the 210 CPE isolates produced both NDM and OXA-48 group enzymes, 137 produced OXA-48 group enzymes and 61 were NDM-producing. Furthermore, one VIM-producing isolate was identified. No OXA-23 group, IMI- or KPC-producing CPE isolates were identified (Figure 8.13).

In 2020, 171 new patients were identified among the 183 CPE patients, compared to previous years. Thus, 12 patients in 2020 have had an infection with CPE before 2020. Comparing the species, multilocus sequence typing (MLST) and core-genome multilocus sequence typing (cgMLST) data, resistance genes and plasmid replicons for the isolates isolated from each patient, six patients had a second case of infection with a highly similar strain as the first case reported in previous years. Four of the six cases were caused by a reinfection with *E. coli*, and two cases with *K. pneumoniae* ranging from 392–714 days between the first and second infection. In 2020, a study on recurrent bloodstream infections caused by ESBL/pAmpC *E. coli* was published. The study showed recurrent infections with more than 1,000 days between first and second episode, likely due to long-term host colonisation [Roer L. et al. Int J Antimicrob Agents. 56:106041]. It is therefore highly plausible that patients carrying a CPE may also risk reinfection with the same strain at a later stage due to long-term colonisation. Analysis of patient's long term carrier- or recolonisation ability by CPE should be carefully investigated in the future, to be able to implement preventive measures such as mandatory isolation upon (re-)hospitalization.

A case of blaNDM-1-positive *Salmonella* Kottbus
In 2020, the first case of blaNDM-1-positive *S.* Kottbus located on a 43 kb IncN2-plasmid was identified in a patient in Denmark. The plasmid was also found in *E. coli* and *C. freundii* from the same patient on the same 43 kb IncN2-plasmid, suggesting horizontal gene transfer. The patient had no known travel history outside Europe and could be the first confirmed case of blaNDM-1-positive *Salmonella* not related to travel outside Europe (Nielsen HL et al. Euro Surveill. 2021;26(26):pii=2100569).

Carbapenemase-producing *Acinetobacter* spp.
In 2020, 21 carbapenemase-producing *Acinetobacter* spp. isolates were reported from 21 patients, compared to 20 isolates in 2019. Six of the patients had been travelling abroad prior to identification of the carbapenemase-producing *Acinetobacter* spp. In 2020, 21 carbapenemase-producing *Acinetobacter baumannii* with the following enzymes were identified: OXA-23 (20), and NDM-1/OXA-23 (1).

Carbapenemase-producing *Pseudomonas* spp.
In 2020, seven carbapenemase-producing *Pseudomonas* spp. isolates were reported from seven patients, which was the same number as in 2019. Three of the patients had been travelling abroad prior to detection of the carbapenemase-producing *Pseudomonas* spp. isolates. In 2020, five carbapenemase-producing *Pseudomonas aeruginosa* with the following enzymes were identified: VIM-2 (3), NDM-1 (1), and IMP-4 (1). Furthermore, one BIC-1- and one BIC-1-like-producing *Pseudomonas fluorescens* was identified.

Outbreaks with CPO during 2020
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 colonization of the patients, cluster analysis is carried out regardless of the time of isolation. When epidemiological investigations can establish a link (e.g. the patients had been at the same hospital ward at the same time) between at least two patients in a genomic cluster, the patients are classified as belonging to a "verified outbreak". When no epidemiological link can be established, the patients related to the genomic cluster is classified as a possible outbreak (Materials and methods, section 9.12).

In 2020, a total of 20 CPO-outbreaks were registered compared to 16 CPO-outbreaks in 2019. In 11 of the outbreaks, it was possible to establish an epidemiological links between the patients. All epidemiological links were found in healthcare settings, caused by patients sharing the same ward or hospital. Eleven of the 20 outbreaks has been ongoing for more than two years and one up to nine years, meaning that new patients has been identified as belonging to the same cgMLST cluster as found in the previous years. In total, 65 new patients was affected in 2020 by these ongoing outbreaks. Of the 20 outbreaks registered in 2020, nine was new outbreak clusters, representing 29 patients. In three of these outbreaks, epidemiological investigations showed that the patients had been at the same hospital ward at the same time (Table 8.10).

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 2020 [Hammerum et al. 2016, J. Antimicrob. Agents, 71:3117-3124]. The outbreak is now present also in the Central Denmark Region. Until the end of 2020, 52 hospitalised patients have been involved in this outbreak. Among these, 13 new cases were identified in 2020 of which eight had been hospitalised in the Central Denmark Region. None of the new cases has 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 2020, 60 patients in total have been involved in this outbreak. During 2020, 19 new patients were part of this outbreak of which six were identified in the Capital Region. 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 has a prior history of travel.

In 2020, the Central Denmark Region reported an increased number of patients with *K. pneumoniae* ST231 OXA-232. The first person in Denmark with this specific type was identified in 2016 and had been travelling to India prior to infection. In 2018, unrelated to the first patient, five hospitalised patients were reported in the Central Denmark Region followed by one patient in 2019. During 2020, the outbreak strain has spread affecting 16 hospitalized patients (ID33).

In 2020, Carbapenemase-producing *A. baumannii* OXA-23 ST195 was involved in a CPO-outbreak in the Capital Region affecting 11 hospitalised patients in an intensive care (ID 1058) (see Textbox 8.1).

Infection prevention and control guidelines for CPO

The National Health Authority has published the first national guideline on prevention of the spread of CPO in 2018 [www.sst.dk/da/udgivelser/2018/vejledning-og-bekendtgoerelse-om-forebyggelse-mod-spredning-af-cpo]. The guideline presents the Danish 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 risk situations as e.g. 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

Table 8.10 Outbreaks of carbapenemase-producing Enterobacterales (CPE) and carbapenemase-producing organisms (CPO) during 2020, n=20, Denmark

DANMAP 2020

Outbreak ID	Year	Patients total	Patients 2020	Carbapenemase	Type of Outbreak	Species (clonal spread)	Regions	Status
Outbreaks of carbapenemase-producing Enterobacterales (CPE)								
41	2012-2020	52	13	NDM-1	Clonal/ plasmid	ST18 *C. freundii*	Capital Region/Central Denmark Region/North Denmark Region	Verified
48	2013-2020	23	4	OXA-436/OXA-48	Clonal/ plasmid	ST90 *E. cloacae*/ ST22 *C. freundii*	Capital Region/South Denmark Region/Zealand Region	Verified
24	2014-2020	10	1	OXA-181	Clonal	ST410 *E. coli*	Capital Region	Verified
25	2014-2020	7	1	OXA-48	Clonal	ST38 *E. coli*	Capital Region/Zealand Region	Verified
21	2015-2020	60	19	NDM-5/OXA-181	Clonal	ST410 *E. coli*	Capital Region/Zealand Region	Verified
22	2015-2020	6	1	OXA-181	Clonal	ST440 *E. coli*	Capital Region/Central Denmark Region	Possible
42	2015-2020	9	2	OXA-48	Clonal	ST65 *C. freundii*	Capital Region/North Denmark Region/Zealand Region	Verified
33	2016-2020	23	16	OXA-232	Clonal	ST231 *K. pneumoniae*	Central Denmark Region	Verified
35	2017-2020	4	2	OXA-48	Clonal	ST15 *K. pneumoniae*	Capital Region/Zealand Region	Possible
51	2018-2020	3	1	OXA-48	Clonal	ST73 *E. coli*	Central Denmark Region	Possible
7	2019-2020	7	5	NDM-5	Clonal	ST167 *E. coli*	Capital Region	Verified
1061*	2020	3		OXA-181	Clonal	ST22 *C. freundii*	Central Denmark Region	Possible
1054*	2020	2		OXA-48	Clonal	ST16 *K. pneumoniae*	Zealand Region	Possible
1057*	2020	3		OXA-244	Clonal	ST38 *E. coli*	Capital Region, South Denmark Region	Possible
1059*	2020	2		OXA-48	Clonal	OXA-48 *E. hormaechei*	Capital Region	Possible
1060*	2020	2		NDM-1	Clonal	ST78 *E. hormaechei*	Capital Region	Verified
1062*	2020	2		NDM-5	Clonal	ST79 *E. hormaechei*	Capital Region, Central Denmark Region	Possible
1068*	2020	2		OXA-48	Clonal	ST18 *C. freundii*	Capital Region	Possible
1062*	2020	2		NDM-5	Clonal	ST79 *E. hormaechei*	Capital Region, Central Denmark Region	Possible
1068*	2020	2		OXA-48	Clonal	ST18 *C. freundii*	Capital Region	Possible
Outbreaks of carbapenemase-producing organisms (CPO)								
1058*	2020	11		OXA-23	Clonal	ST195 *A. baumannii*	Capital Region	Verified
1067*	2020	2		OXA-23	Clonal	ST195, ST1816 *A. baumannii*	South Denmark Region	Verified

* Outbreak clusters identified in 2020

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 [www.hygiejne.ssi.dk/retningslinjer].

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.

Conclusion

The occurrence of carbapenemase-producing bacteria in Denmark continued to increase throughout 2020. Furthermore, the number of outbreaks in hospital settings continued to increase, a worrisome trend. 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 infections, which makes outbreak control difficult. Patients are known to be carriers of CPE for years, which highlights that infection control measures are important tools to prevent further spread of CPE. In 2020, travel outside the Nordic countries decreased due to COVID-19 probably resulting in lower numbers of travel-associated CPO infections.

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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 makes antibiotic treatment of enterococcal infections challenging. In addition, most hospital-acquired *E. faecium* are resistant to ampicillin, further limiting the treatment options. Vancomycin is an important drug for the treatment of severe *E. faecium* infections, however, an increase in the occurrence of vancomycin resistant enterococci (VRE) has been observed in Denmark and internationally during the past decade. Newer antibiotics such as linezolid and daptomycin can be used for treatment of VRE, but use of both of these antimicrobial agents may lead to potential adverse effects and development of resistance has been reported.

In recent years, *E. faecium* harboring the *vanA* gene complex, but being phenotypically susceptible to vancomycin, have been described in other countries. These enterococci are referred to as vancomycin-variable enterococci (VVE). VVE have caused nosocomial outbreaks; and development of reverting mutants becoming vancomycin resistant *in vitro* and *in vivo* has been described. Thus, VVE are clinically relevant, and their detection critical, in order to avoid treatment failure when using vancomycin. However, VVE cannot be selectively cultured on vancomycin-containing media and can only be detected by molecular methods. In 2015 and 2016, sporadic VVE isolates with different genetic background in relation to concurrent VRE-outbreaks were detected in the Capital Region of Denmark, [Hammerum et *al.* Euro Surveill. 2019; 24(34)]. In 2016, a new VVE clone belonging to ST1421- CT1134, which displays variable vancomycin susceptibility due to a deletion in the *vanX* gene, was detected. [TA Hansen et al., J. Antimicrob. Agents, 2018, 73: 2936-2940].

Surveillance of VRE/VVE

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

In 2017, testing of phenotypically vancomycin-susceptible *E. faecium* isolates from blood cultures for the presence of *vanA*/*vanB* genes by PCR was introduced in the DCMs in the Capital Region for detection of possible VVE. During 2018, PCR testing was expanded to all clinical *E. faecium* isolates. Similarly in 2018, molecular testing by PCR of *E. faecium* from all clinical samples was implemented in one of the four DCMs in the Region of Southern Denmark. Furthermore, *E. faecium* isolates from blood cultures were tested by PCR for *vanA*/*vanB* genes in a second DCM in the Region of Southern Denmark and in the DCM in the Central Denmark Region in 2018. In 2019, diagnostic algorithms to detect VVE expanded, as most of the DCMs across Denmark tested, as a minimum, all blood culture *E. faecium* isolates for the presence of *vanA* genes using PCR [Hammerum et *al.* Euro Surveill. 2020;25(18)]. In 2020, one DCM in the Capital Region changed their molecular PCR-testing protocol by lowering the concentration of vancomycin for selective enrichment, to be able to detect *vanB* positive enterococcus isolates directly from patient samples. Another DCM in the Capital Region started to perform *vanA*/*vanB* PCR directly on the samples. The *vanB* positive enterococcus isolates were purified and sequenced for identification and genotyping.

To determine any underreporting in the submissions to the reference laboratory, the number of VRE/VVE submitted to SSI since 2016 were compared to data from clinical VRE reported

by the DCMs to MiBa (the national Danish Microbiology Database). This comparison showed that the number of submitted VRE/VVE isolates to SSI was not complete (Figure 8.14), as MiBa contained reports of additional 145 isolates, for which samples had not been submitted to SSI. In 2020, 511 VRE/VVE isolates were submitted to SSI and by adding the 145 VRE/VVE isolates extracted from MiBa, this summed up to 656 VRE/VVE isolates from 656 patients in 2020 compared to 660 VRE/VVE isolates from 660 patients in 2019 (Figure 8.14).

Since 2013, a high increase in clinical VRE isolates has been observed. Until 2018, the increase was mostly seen for *vanA E. faecium*, but during 2019 an increase was detected for *vanB E. faecium* which continued in 2020 (Figure 8.14). In 2020, more than 50% of the sequenced isolates were *vanB* positive *E. faecium*.

From 2015 to 2020, the clinical VRE/VVE isolates received at SSI have been analysed by whole-genome sequencing (WGS) and in 2020, 511 VRE/VVE were analysed by WGS. From the WGS data, species identification, multilocus sequence type (MLST), core genome MLST (cgMLST) and *van*-gene variants were identified *in silico*.

Of the 511 clinical VRE/VVE isolates, 239 were *vanA E. faecium*, 255 *vanB E. faecium*, and 17 *vanA*/*vanB E. faecium*. In 2020 vancomycin resistant *E. faecalis* were not observed (Figure 8.14). WGS-based MLST and cgMLST analysis was performed on the 511 *E. faecium* isolates using SeqSphere+ (Ridom), which subdivided them into 57 cluster types (CTs). Two clones were predominant: ST1421-CT1134 *vanA E. faecium* and ST80-CT2406 *vanB E. faecium* (Table 8.11).

In 2017, 3% of the *E. faecium* isolates belonged to the VVE clone, ST1421-CT1134 *vanA E. faecium*. Initially, this type was only detected from clinical samples from the Capital Region. In 2018, 34% of the *E. faecium* isolates belonged to ST1421-CT1134 and were not only detected in the Capital Region but also in the Region Zealand and by one DCM in the Region of Southern Denmark 2019 [Hammerum et *al*. Euro Surveill. 2019;24(34)]. During 2019, ST1421-CT1134 *vanA E. faecium* was the most prevalent type observed (50%) (Table 8.11), thus replacing the previously dominating *vanA E. faecium* ST203-CT859). Furthermore, ST1421-CT1134 *vanA E. faecium* has spread to the Faroe Islands during 2018 and 2019 through patient transferal from Denmark [Hammerum et *al*. Euro Surveill. 2019;24(34)]. In 2020, ST1421-CT1134 *vanA E. faecium* was still the most prevalent type observed but it decreased to a relative prevalence of 36%.

ST80-CT2406 *vanB E. faecium* isolates were again first detected during 2019 in the Capital Region, in only 1% of the isolates. In 2020, the ST80-CT2406 increased to 28%, accounting for the majority of the *vanB* positive *E. faecium* detected. ST80-CT2406 was detected in four of the five Regions, with 84% detected in the Capital Region. Two DCMs in the Capital Region reported a changed procedure in 2020 for detecting the *vanB* positive *E. faecium*.

During 2015-2017, ST203-CT859 *vanA E. faecium* was the most prevalent type (together with its subtypes CT1051 and CT1507). Since 2018, prevalence of this type has been decreasing. In 2020, only 2% of the VRE/VVE *E. faecium* isolates belonged to ST203-CT859.

Figure 8.14 Numbers of *Enterococcus faecium* and *Enterococcus faecalis* isolates carrying *vanA* and *vanB* genes from clinical samples submitted to SSI 2010-2020 supplemented with data obtained from MiBa from 2016-2020, Denmark DANMAP 2020

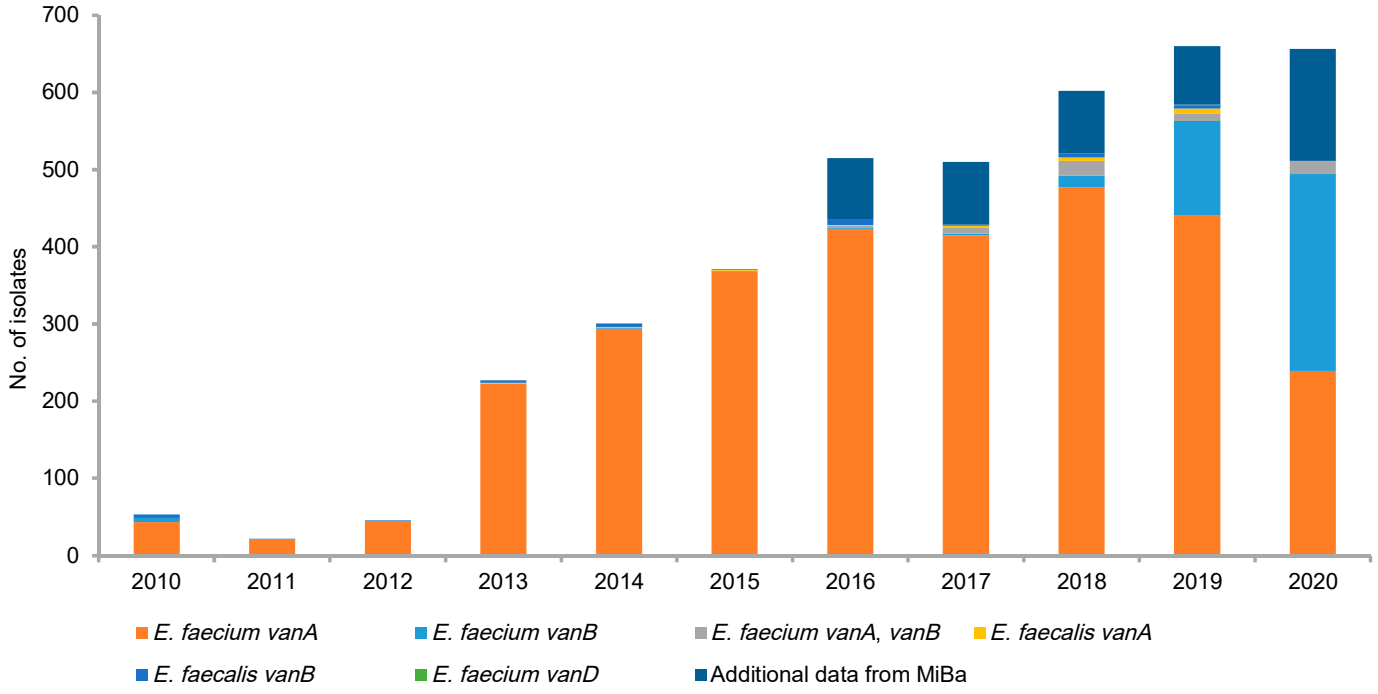

Table 8.11 Description of the most common types of *vanA* and/or *vanB Enterococcus faecium* according to MLST and cgMLST, Denmark, 2015-2020

DANMAP 2020

Types[a]	2015		2016		2017		2018		2019		2020	
	(n = 368)		(n = 427)		(n = 425)		(n = 515)		(n = 574)		(n = 511)	
ST80-CT14 *vanA*	81	22%	38	9%	15	4%	1	<1%	1	<1%	N.D.	N.D.
ST80-CT24 *vanA*	23	6%	19	5%	11	3%	2	<1%	4	<1%	N.D.	N.D.
ST80-CT866 *vanA*	14	4%	10	2%	7	2%	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
ST80-CT1064 *vanA/vanB*	N.D.	N.D.	2	<1%	8	2%	23	4%	11[b]	2%	12[c]	2%
ST80-CT1729 *vanA*	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	22	4%	2	<1%	3	<1%
ST80-CT2406/CT3024 *vanB*	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	7	1%	148[d]	29%
ST80-CT2946 *vanB*	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	17	3%
ST117-CT1180 *vanA*	N.D.	N.D.	N.D.	N.D.	9	2%	30	6%	14	2%	5[b]	<1%
ST117-CT36/CT991/CT2531 *vanB*	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	2	<1%	90	16%	39	8%
ST117-CT2531 *vanB*	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	5	1%	11	2%
ST203-CT859/CT1051/CT1507 *vanA*	188	51%	271	64%	265	63%	161	31%	54	9%	12	2%
ST1421-CT1134 *vanA*	N.D.	N.D.	2	<1%	13	3%	176	34%	285	50%	183	36%
Other types	62	17%	85	20%	97	23%	98	19%	101	18%	81	16%

a) ST, sequence type (MLST); CT, cluster type (cgMLST); N.D., not detected
b) Two isolates were *vanB* positive
c) One isolate were only *vanB* positive
d) One isolate were *vanA/vanB* positive

During 2019, the ST117-CT36 *vanB E. faecium* and its subtype ST117-CT991 *vanB E. faecium* increased in Denmark, which was both related to several introductions from hospitals abroad and spread between hospitals due to patient transfer. Only two isolates belonged to this type in 2018, whereas 90 isolates were detected during 2019. The prevalence of ST117-CT36 and its subtype ST117-CT991 decreased again in 2020, where only 28 (5%) were detected (Table 8.11).

Conclusion

Compared to the number of VRE/VVE cases in Denmark in 2019, the number of detected cases was stable in 2020. The increased focus on infection prevention and control during the COVID-19 pandemic could have been the cause of breaking the previous trend of increasing numbers. Until 2020, *vanA E. faecium* have been dominating in Denmark. During 2018-19, an increase of *vanB* vancomycin-resistant *E. faecium* was observed in the Capital Region of Denmark [Pinholt et al. 2021 J. Antimicrob. Chemother. doi:10.1093/jac/dkab198]. In 2020, *vanB E. faecium* was dominating among the clinical VRE/VVE cases. *VanB* producing isolates are harder to detect than *vanA* producing isolates, so the numbers of *vanB* producing isolates could have been larger due to missed identification of cases.

VRE can be carried in the intestines for long periods without causing any symptoms. Moreover, VRE can persist in the hospital environment, which makes infection control a difficult task. Infection control measures should as a minimum include proper cleaning, continued focus on hand hygiene, correct use of PPE, VRE/VVE screening and subsequent isolation of patients. The steady shift in VRE clones is of high concern.

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8.3.4 Linezolid-resistant/linezolid-vancomycin-resistant enterococci

Background

Linezolid can be used for treatment of infections caused by vancomycin-resistant enterococci. Resistance to linezolid in enterococci is often due to mutations in the V domain of the 23S rRNA genes. The G2576T mutation (*Escherichia coli* numbering) is the most common cause of the linezolid-resistant enterococci (LRE) phenotype. Another mutation in the 23S rRNA, G2505A, (*E. coli* numbering) has also been reported for LRE, but seems to be less frequent than the G2576T mutation. Furthermore, transferable resistance genes (*cfr, cfr(B), optrA* and *poxtA*) encoding linezolid resistance, have been described in enterococci, whereas amino acid substitutions in the ribosomal proteins L3, L4 and/or L22, suspected to cause decreased susceptibility to linezolid in staphylococci, are rare in enterococci.

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

Surveillance of linezolid-resistant enterococci (LRE)

Danish departments of clinical microbiology (DCMs) have submitted LRE on a voluntary basis for surveillance purposes to the National Reference Laboratory for Antimicrobial Resistance at Statens Serum Institut (SSI). During 2015-2019, eight linezolid-resistant *E. faecium* isolates and ten linezolid-resistant *E. faecalis* isolates were sent to SSI (one isolate per patient only).

Table 8.12 Characterisation of linezolid-resistant enterococci (LRE) and linezolid-vancomycin-resistant enterococci (LVRE) submitted to SSI, Denmark, 2020 DANMAP 2020

	No. of isolates	Species	Linezolid resistance mechanism	Vancomycin resistance gene
LRE	1	*E. faecalis*	G2576T	none
	6	*E. faecalis*	*optrA*	none
LVRE	4	*E. faecium*	G2576T	*vanA*
	4	*E. faecium*	G2576T	*vanB*

In 2020, seven linezolid-resistant *E. faecalis* isolates were 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 web-tool, LRE-Finder (https://cge.cbs.dtu.dk/services/ LRE-Finder/).

Among the LRE isolates from 2020, LRE-Finder detected one *E. faecalis* with the G2576T mutation, six *E. faecalis* isolates with *optrA* (Table 8.12).

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 by DCMs directly. During the period 2015-2019, no linezolid vancomycin-resistant *E. faecalis* isolate was detected, whereas, eleven linezolid-vancomycin-resistant *E. faecium* were identified.

In 2020, eight linezolid-vancomycin-resistant *E. faecium* were identified. All eight linezolid-resistant *E. faecium* isolates had the G2576T mutation. (Table 8.12). Four of these belonged to the VVE clone, ST1421-CT1134 *vanA E. faecium* and four belonged to ST80-CT2406/CT3024 *vanB E. faecium.* Both types were major VRE/VVE types during 2020 (TEXT VVE/VRE).

Conclusion

As in previous years, the numbers of LRE and LVRE detected in Denmark in 2020 were small. However, the findings are of concern since linezolid is used for treatment of VRE and only a limited number of antimicrobial agents are available for treatment of infections with LVRE.

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8.3.5 *Streptococcus pneumoniae*

The surveillance of pneumococci (*Streptococcus pneumoniae*) causing invasive infections 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 cases without a submitted isolate are identified through MiBa.

In Denmark, 369 cases of invasive pneumococcal disease (IPD) were registered in 2020. The cases were mainly from pneumococci found in either blood (335) or cerebrospinal fluid (29). For five cases, pneumococci had been found in other, normally sterile sites (joint, pleura), but data from these are not included in this report. In total 354 isolates were received at the reference laboratory out of the 364 cases of bacteraemia and meningitis identified in MiBa. One of the received isolates was not viable for susceptibility testing, but serotyping was nevertheless possible. Data for the ten remaining cases, from whom isolates were not provided; were retrieved from MiBa. Failure of submitting an isolate for serotyping was mainly due to non-viable isolates or diagnosis through PCR. Antimicrobial susceptibility data for those cases were retrieved, when available, through MiBa. In total, serotypes were available for 354 isolates and antimicrobial susceptibility data for both penicillin and erythromycin was available for 359 isolates.

The 364 isolates from blood or cerebrospinal fluid belonged to 36 different serotypes. For the 359 cases with available susceptibility data, 328 were susceptible to both penicillin and erythromycin (91.4%). For penicillin, 333 were susceptible (92.8%), 25 (7.0%) were classified as 'susceptible, increased exposure' and one isolate (0.3%) was classified as resistant. In total, 7.2% were non-wild type with respect to susceptibility to penicillin, Figure 8.15. For erythromycin, 346 isolates were susceptible (96.4%) and 13 isolates (3.6%) were resistant. The single isolate (serotype 17F) that was resistant to penicillin, was also resistant to erythromycin.

Antimicrobial susceptibility in pneumococci is highly correlated to serotypes (Table 8.13), 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 (IPD) in Denmark (Figure 8.16), as it is clear that IPD caused by the seven serotypes included in the vaccine (PCV7 vaccine from 2007) and later 13 serotypes (PCV13 vaccine from 2010) has decreased markedly. The current predominant serotype is serotype 8 (22.8% in 2020), of which all invasive isolates isolated in 2020, 2019 and 2018 were fully sensitive to both penicillin and erythromycin.

Figure 8.15 Non-wild type (%) in *Streptococcus pneumoniae* blood and spinal fluid isolates from humans, Denmark, 1993-2020

DANMAP 2020

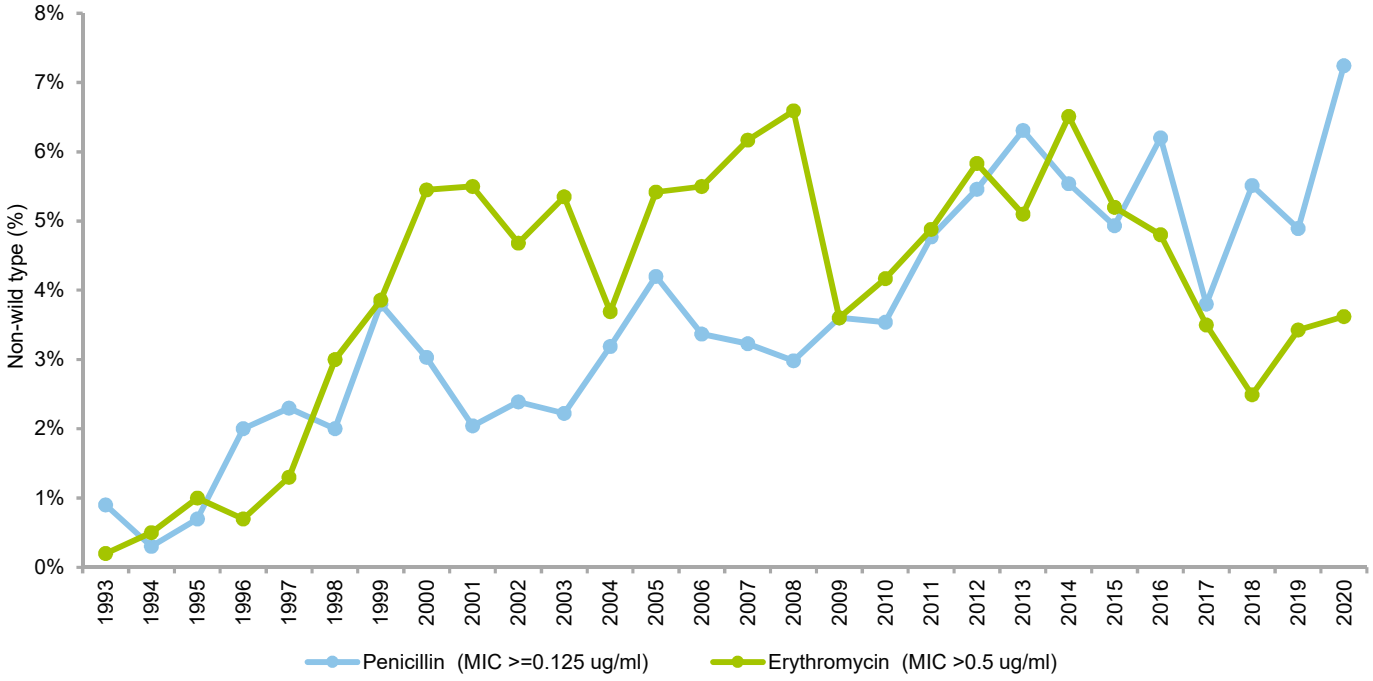


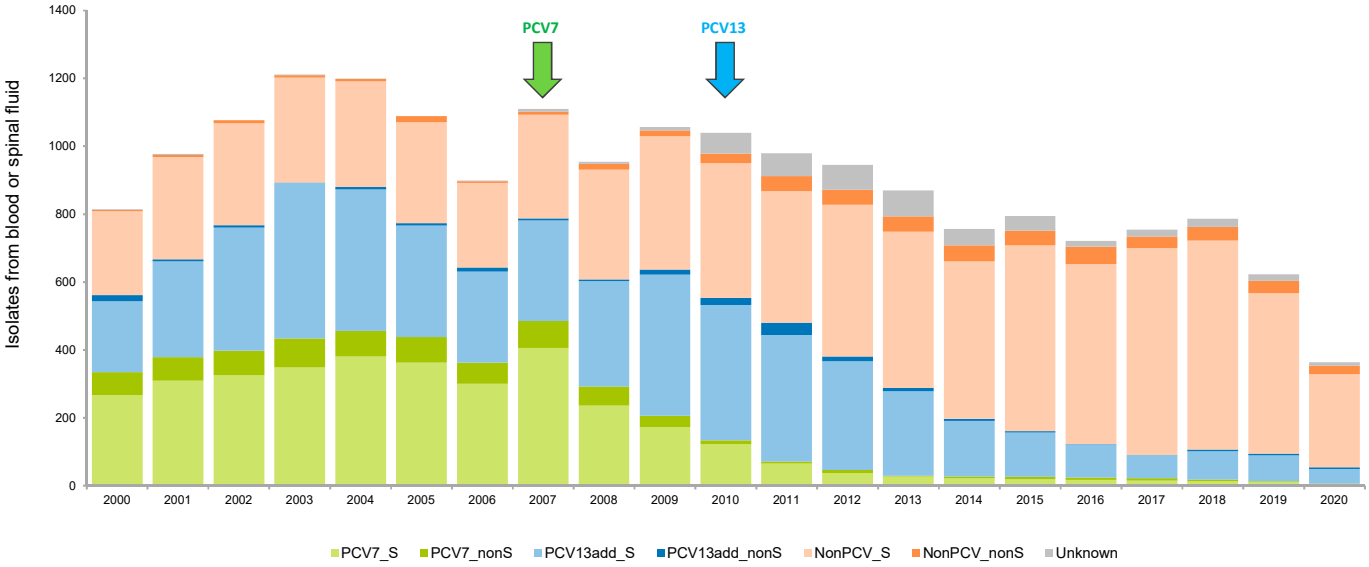
Table 8.13 Number of invasive isolates and distribution of resistance in the most common serotypes of pneumococci, Denmark, 2017-2020

DANMAP 2020

Serotype	N 2020	PEN-S_ ERY-S	PEN-S_ ERY-R	PEN-I_ ERY-S	PEN-I_ ERY-R	PEN-R_ ERY-R	Unk	% S-S	N (% S-S) 2019	N (% S-S) 2018	N (% S-S) 2017
8	83	82					1	100%	162 (100%)	194 (100%)	192 (99%)
3	37	36			1			97%	69 (100%)	70 (97%)	57 (100%)
22F	25	25						100%	49 (96%)	69 (100%)	58 (100%)
12F	22	22						100%	44 (95%)	55 (100%)	69 (99%)
9N	17	16		1				94%	28 (96%)	62 (98%)	56 (98%)
23B	12	4		8				33%	18 (50%)	14 (7%)	11 (27%)
23A	12	12						100%	14 (100%)	13 (100%)	18 (100%)
10A	12	12						100%	9 (100%)	15 (100%)	9 (100%)
24F	11	11						100%	15 (67%)	17 (76%)	19 (79%)
35B	11	9		2				82%	13 (85%)	15 (100%)	20 (90%)
11A	10	8	2					80%	17 (88%)	19 (95%)	19 (89%)
20	10	10						100%	13 (100%)	24 (100%)	26 (100%)
15A	9	6			3			67%	15 (93%)	25 (76%)	16 (63%)
35F	9	9						100%	9 (89%)	14 (100%)	13 (100%)
19A	9	7	1	1				78%	8 (50%)	11 (82%)	5 (100%)
15B	9	9						100%	8 (100%)	9 (100%)	14 (93%)
17F	8	3		3	1	1		38%	8 (63%)	12 (50%)	10 (80%)
7C	8	8						100%	8 (100%)	6 (83%)	3 (100%)
31	6	6						100%	6 (100%)	15 (100%)	8 (88%)
33F	5	4	1					80%	13 (77%)	17 (88%)	13 (92%)
16F	3	3						100%	17 (94%)	19 (84%)	21 (95%)
15C	3	3						100%	11 (82%)	4 (100%)	3 (100%)
10B	3	3						100%	9 (100%)	9 (100%)	9 (100%)
19F	3	2	1					67%	6 (100%)	7 (71%)	13 (69%)
6C	3	3						100%	5 (80%)	5 (40%)	13 (92%)
Other	24	15	0	3	2		4	75%	49 (90%)	42 (88%)	39 (87%)
Sum	364	328	5	18	7	1	5	91%	623 (93%)	762 (93%)	734 (94%)

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

Figure 8.16 *Streptococcus pneumoniae* blood and spinal fluid isolates from humans. Vaccine serotypes and non-vaccine serotypes in combination with susceptibility to penicillin and erythromycin, Denmark, 2000-2020 DANMAP 2020



PCV7_S : PCV7 serotypes, susceptible to both penicillin and erythromycin
PCV7_nonS : PCV7 serotypes, non-susceptible to either penicillin or erythromycin
PCV13add_S : PCV13 serotypes not in PCV7, susceptible to both penicillin and erythromycin
PCV13add_nonS : PCV13 serotypes not in PCV7, non-susceptible to either penicillin or erythromycin
NonPCV_S : serotypes not included in PCV7 or PCV13, susceptible to both penicillin and erythromycin
NonPCV_nonS : serotypes not included in PCV7 or PCV13, non-susceptible to either penicillin or erythromycin
Unknown : cases where either serotype or susceptibility to penicillin or erythrymycin is unknown
The two arrows indicate when PCV7 and PCV13 were introduced in the Danish childhood immunization programme

Comparing the obtained results of antimicrobial susceptibility of IPD in Denmark in 2020 to the data reported in 2019 from our neighbouring countries, the levels of penicillin non-wild type reported by EARS-Net [www.ecdc.europa.eu/en/publications-data/surveillance-antimicrobial-resistance-europe-2019] were: Sweden (6.5%), Norway (6.3%) and Germany (5.7%). The levels of erythromycin resistance were: Sweden (6.6%), Norway (5.7%) and Germany (7.7%). Thus, the results of non-wild type (I+R) for invasive pneumococci from Denmark in 2020 were slightly higher than the reported values from 2019 from neighbouring countries with respect to penicillin, and markedly lower with respect to erythromycin resistance.

Conclusion

For penicillin, the level of non-wild type in 2020 was higher than in 2019 (7.2% compared to 4.9%). For erythromycin, the level of resistance in 2020 was comparable to the level in 2019 (3.6% compared to 3.4%) Figure 8.16. The higher level of non-wild type for penicillin in 2020 compared to 2019 was due to a slightly different distribution of serotypes, and to a slightly higher level of non-wild type among some serotypes. There was no sign of a particular trend. The number of cases of IPD in 2020 was markedly lower than in 2019. This decrease is likely due to the COVID-19 restrictions in Denmark, put in place in March 2020 and sustained in various levels throughout the year. Since the initial focus of infection for IPD is often pneumonia [www.ecdc.europa.eu/en/pneumococcal-disease/facts], it makes sense that restrictions put in place to hinder the spread of one particular airway infection, SARS-CoV2, will also have an effect on the spread of other airway infections, such as pneumococcal pneumonia.

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) can be divided into serological groups according to antigenic properties of the polysaccharide capsule. In human infections, groups A, B, C and G are the most frequent species. Approximately 10% of humans are asymptomatic throat carriers of BHS of any group.

Streptococcus pyogenes (group A streptococci; GAS) cause pharyngitis, tonsillitis, otitis media, wound infections, and superficial skin infections, but also more severe infections, e.g., bacteraemia, necrotizing myofasciitis, and rarely meningitis. In Denmark, the rate of asymptomatic throat carriage of GAS is approximately 2%.

Streptococcus agalactiae (group B streptococci; GBS) may be present in the vaginal flora of 20-25% of women in the childbearing age. If these bacteria are transferred to the child during labour, meningitis and septicaemia may develop in the newborn. Such infections also occur in elderly or immuno-compromised 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 invasive isolates (i.e. from blood, cerebrospinal fluid or other normally sterile anatomical sites) submitted in 2020 to the Neisseria and Streptococcus Reference laboratory. This report includes only non-duplicate isolates, i.e. if more than one isolate of the same serogroup and type is obtained from the same patient within an interval of 30 days or less, only the first isolate is included. 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. Susceptibility testing was performed with disk diffusion methods. If resistance was observed, MIC was determined with Etest. All results were interpreted according to MIC breakpoints issued by EUCAST (http://www.eucast.org/) (version 10.0). For all isolates of GAS the *emm* type was determined by whole genome sequencing of the portion of the *emm* gene that dictates the M protein serotype.

Figure 8.17 Beta-haemolytic streptococci: Antimicrobial resistance testing results, 2013-2020. Numbers of isolates and proportion of resistant isolates, Denmark, 2013-2020

DANMAP 2020

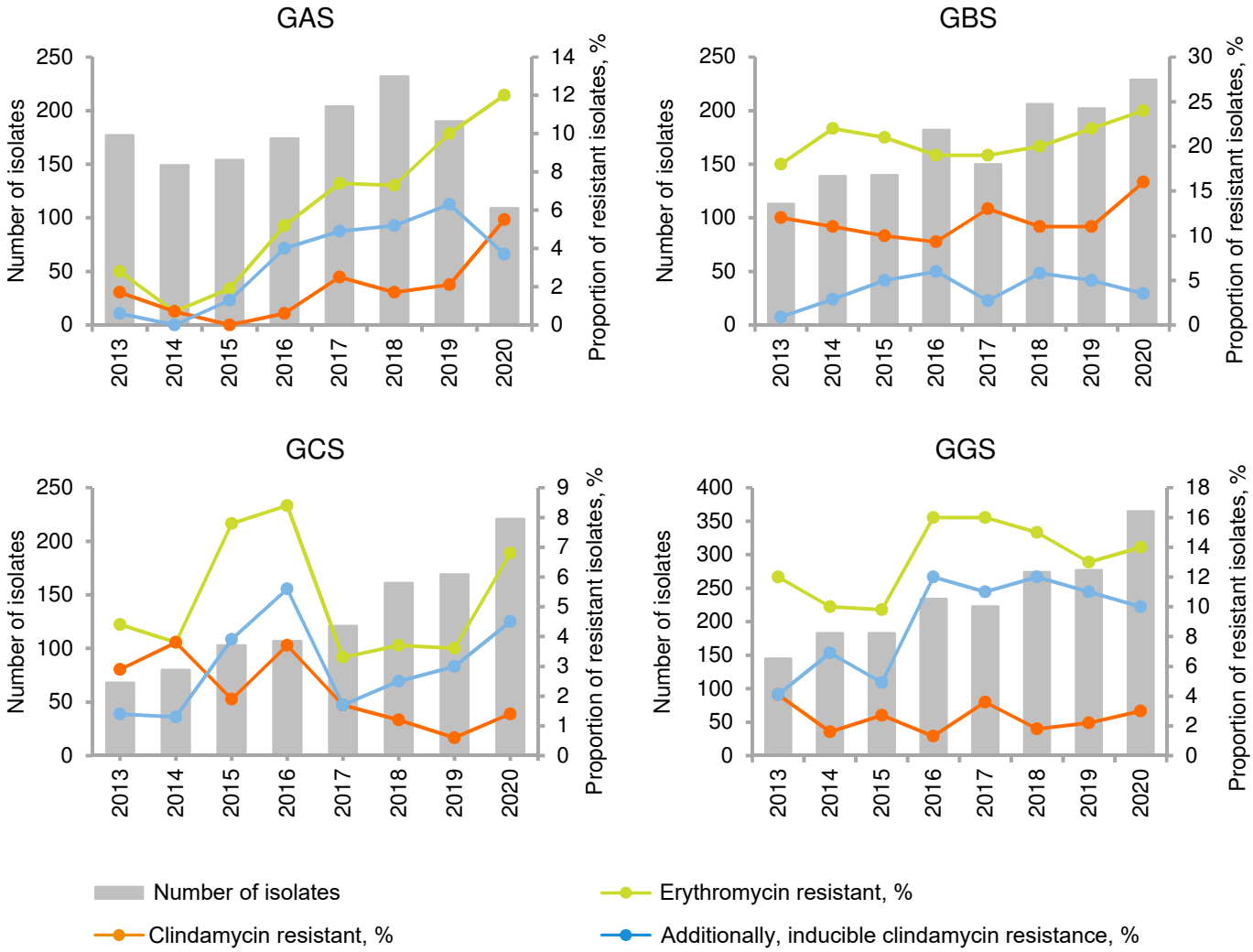

Table 8.14 Group A streptococci 2020: *emm* types, clindamycin resistance and erythromycin resistance. Number of isolates
DANMAP 2020

	CLI-R		CLI-S		
emm type	ERY-R	ERY-S	ERY-R	ERY-S	Total
89.0	0	0	0	26	26
28.0	0	0	0	20	20
1.0	0	0	0	12	12
12.0	0	0	0	7	7
75.0	0	0	0	5	5
11.0	2	0	0	1	3
4.0	0	0	0	3	3
4.19	0	0	0	3	3
44.0	0	0	0	3	3
66.0	0	0	0	3	3
Subtotal	2	0	0	83	85
Other	4	0	7	13	24
Total	6	0	7	96	109

Numbers of isolates are shown for individual *emm* types if ≥3. Otherwise, if <3, the numbers are summarized in the "Other" category.
CLI = clindamycin, ERY = erythromycin, R = resistant, S = sensitive

Figure 8.17 shows the resistance findings for the years 2013 through 2020. In 2020, the number of submitted isolates from unique cases was 924, an increase of 10% compared to 2019 (839). Corresponding changes for individual serogroups were: GAS, -43%; GBS, +13%; GCS, +31%; and GGS, +32%.

The substantial decrease in number of submitted GAS isolates may possible reflect an indirect consequence of COVID-19 related restrictions on upper respiratory tract colonization and airborne transmission of this species. There is, however, no straightforward explanation for the increase of GBS numbers and the substantial increase in numbers of GCG and GGS.

All isolates were fully susceptible to penicillin. Erythromycin resistance remained virtually unchanged compared to 2019 for all four serogroups, while the proportion of clindamycin-resistant isolates showed a slight increase. The percentage of strains with inducible clindamycin resistance was: GAS, 3.7%; GBS, 3.5 0% (both a slight decrease), GCS 4.5% (a slight increase), and GGS, 11% (virtually unchanged). The percentage of fully susceptible isolates was unchanged compared to 2019 for all four serogroups.

The GAS isolates belonged to 30 different *emm* types. The majority of the received isolates (85; 78%) belonged to ten *emm* types, each of which were represented by at least three isolates (Table 8.14). The remaining 24 isolates (22%) belonged to 20 different *emm* types.

Conclusions
The number of submitted isolates of group A *Streptococcus* was considerably lower in 2020 than in 2019, but showed a definite increase for the three other serogroups. All isolates were fully susceptible to penicillin. The erythromycin resistance rate remained virtually unchanged compared to 2019 for all four serogroups while the clindamycin resistance rate showed a slight increase.

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8.3.7 *Staphylococcus aureus*

Staphylococcus aureus is part of the normal flora of the skin and mucosa. Approximately 50% of humans will be colonized with *S. aureus* in their nose or throat at any given time, some of these carrying the strain only intermittently, others for longer periods. *S. aureus* causes infections ranging from superficial skin infections e.g. impetigo and boils to deeper and more complicated infections. These may be healthcare associated infections, 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 programme 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 bacteraemia
In 2020, altogether 2,342 *S. aureus* bacteraemia cases corresponding to 40.2 cases per 100,000 inhabitants were reported from the departments of clinical microbiology (DCMs)

in Denmark. This is a 4.9% increase compared to 2019 (2,233). Thirty-eight (1.6%) of the bacteraemia cases were caused by MRSA. During the last decade, the proportion has been between 1.3% (2010 and 2012) and 2.9% (2014) and remains below most other European countries participating in EARS-Net [https://www.ecdc.europa.eu/en/antimicrobial-resistance/surveillance-and-disease-data/data-ecdc]. Livestock-Associated Methicillin-Resistant *Staphylococcus aureus* (LA-MRSA) CC398 caused eleven of the 38 MRSA bacteraemia cases.

Within 30 days from the bacteraemia onset, 485 (21%) patients died (all cause mortality). The mortality for the MRSA bacteraemia cases was 11%.

Results from antimicrobial susceptibility testing in *S. aureus* bacteraemia isolates from 2011–2020 are presented in Table 8.15. Resistance to penicillin in 2020 was 72%. At the beginning of the 1990s resistance to penicillin was around 86% and in 2010 75%, thereby a continuing decreasing trend was observed. The highest frequency of resistance to other antimicrobials than penicillin was observed for fusidic acid (14%), erythromycin (7%), clindamycin (7%) and norfloxacin (6%). For most antimicrobial agents, the susceptibility remained at the same level as the previous years.

Table 8.15 Resistance (%) in isolates from *Staphylococcus aureus* bacteraemia cases, Denmark, 2011-2020 DANMAP 2020

Antimicrobial agent	2011 %	2012 %	2013 %	2014 %	2015 %	2016 %	2017 %	2018 %	2019 %	2020 %
Methicillin	1.4	1.2	1.7	2.9	1.5	2.1	2.2	1.6	2.1	1.6
Penicillin	77	74	76	77	71	71	72	72	72	72
Erythromycin	7	6	7	8	7	7	6	5	9	7
Clindamycin	6	6	6	8	7	6	5	4	8	7
Tetracycline	2	2	3	5	4	3	3	3	2	3
Fusidic acid	13	14	15	15	16	12	14	17	14	14
Rifampicin	<1	<1	0	<1	<1	<1	<1	<1	<1	<1
Norfloxacin	4	4	5	6	6	4	4	4	5	6
Kanamycin	<1	1	2	2	3	1	1	2	<1	nt
Linezolid	0	0	0	0	0	0	0	0	0	0
Mupirocin	<1	<1	<1	<1	<1	0	<1	0	<1	<1
Trimethoprim-sulfamethoxazole	<1	1	1	1	<1	<1	<1	0	<1	<1
Numbers tested	1515	1523	962	381	502	560	551	504	549	variable*

nt = not tested. * In 2020 resistance data was extracted from the Danish Microbiology database, MiBa

Surveillance of methicillin-resistant *S. aureus*

The COVID-19 restrictions with less international travel, social distancing and less contact to the health care system resulted in a decrease in new MRSA cases. In 2020, 2,883 MRSA cases were detected (49.5 per 100,000 inhabitants), a decrease of 21% compared to 2019 (3,657; Figure 8.18). A case was defined when a person for the first time tested positive for a specific MRSA strain regardless of clinical situation (infection or colonisation).

CC398 cases constituted 33% (n = 962) of new MRSA cases, of which 931 belonged to the LA-MRSA CC398 and the remaining 31 to a human adapted variant harbouring the PVL encoding genes. The number of LA-MRSA CC398 is lower than the previous five years.

MRSA isolates carrying *mecC* were detected in 41 cases (1.4%). Thirty of the cases (73%) had infections at the time of diagnosis. One patient reported contact to hedgehogs, which recently has been shown to be a major environmental reservoir for *mecC* MRSA [Rasmussen SL https://pubmed.ncbi.nlm.nih.gov/31490992/] [Bengtsson B. https://pubmed.ncbi.nlm.nih.gov/28757008/]. The remaining 40 patients reported no known contact to any livestock.

In the course of 2020, 31 MRSA outbreaks were registered at hospitals, nursing homes and other institutions. These outbreaks comprised a total of 130 cases. Seven of the outbreaks occurred in neonatal departments, comprising a total of 59 cases. Additionally, eight outbreaks were registered in other hospital department, comprising 20 patients and seven outbreaks were observed in nursing homes (counting a total of 17 residents).

The total number of cases and the number of cases presenting with infection according to epidemiological classification are shown in Table 8.16. Most of the cases (88%) were acquired in Denmark. At the time of diagnosis, 44% (n = 1,281) of cases had infection, which was similar to 2019 (44%).

The trend of MRSA infections for 2011-2020 based on their epidemiological classification is shown in Figure 8.19. The number of infections in all epidemiological categories decreased in 2020 when compared to 2019, most notably for imported (- 45%) and community-acquired infections (- 14%).

Figure 8.18 Number of new MRSA cases, Denmark, 1994-2020 DANMAP 2020

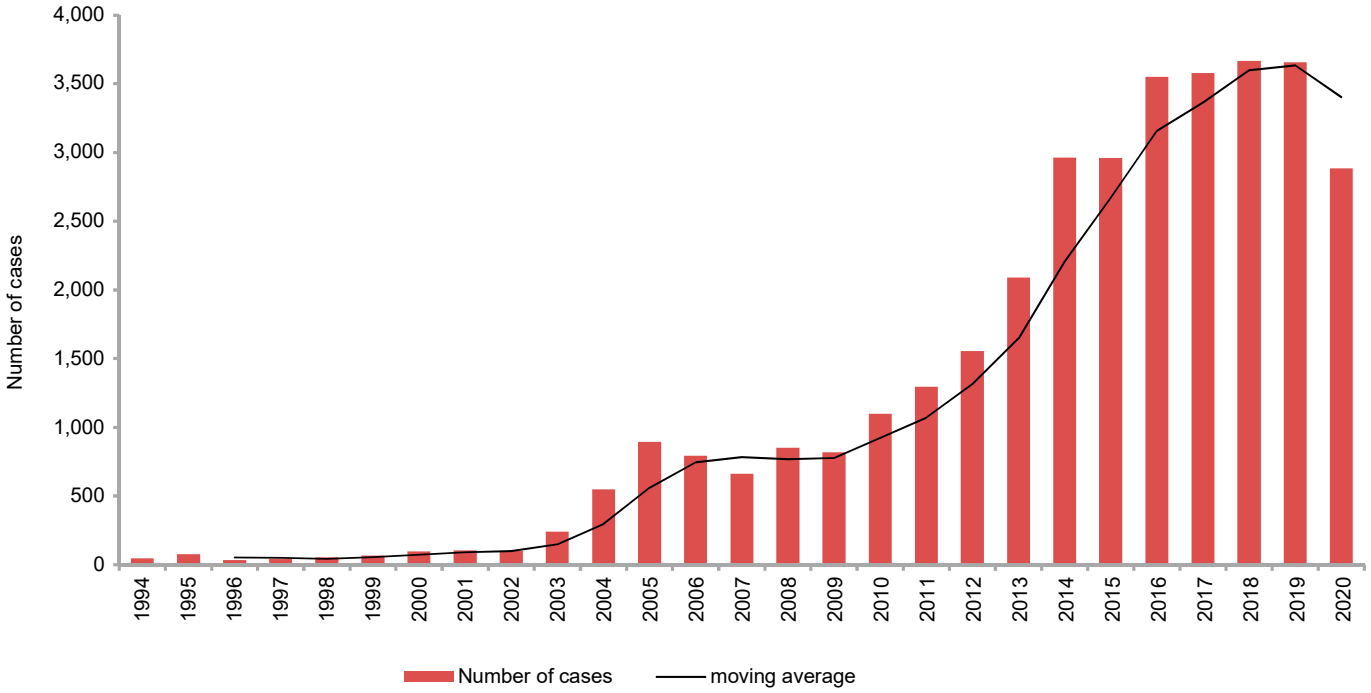


Table 8.16 Epidemiological classification of new MRSA cases, Denmark, 2020 DANMAP 2020

Epidemiologic classification	Exposure	No. of cases (% of total)	No. (%) of cases with infections
Imported (IMP)		**351 (12)**	215 (61)
Hospital-acquired (HA)		**70 (2)**	40 (57)
Healthcare associated, community onset (HACO)		**197 (7)**	
	with known exposure	16	12 (75)
	without known	181	147 (81)
Health care worker		**27 (1)**	11 (41)
Community-acquired (CA)		**1307 (45)**	
	with known exposure	659	90 (14)
	without known	648	532 (82)
LA-MRSA CC398		**931 (32)**	
	with known exposure	790	139 (18)
	without known	141	95 (67)

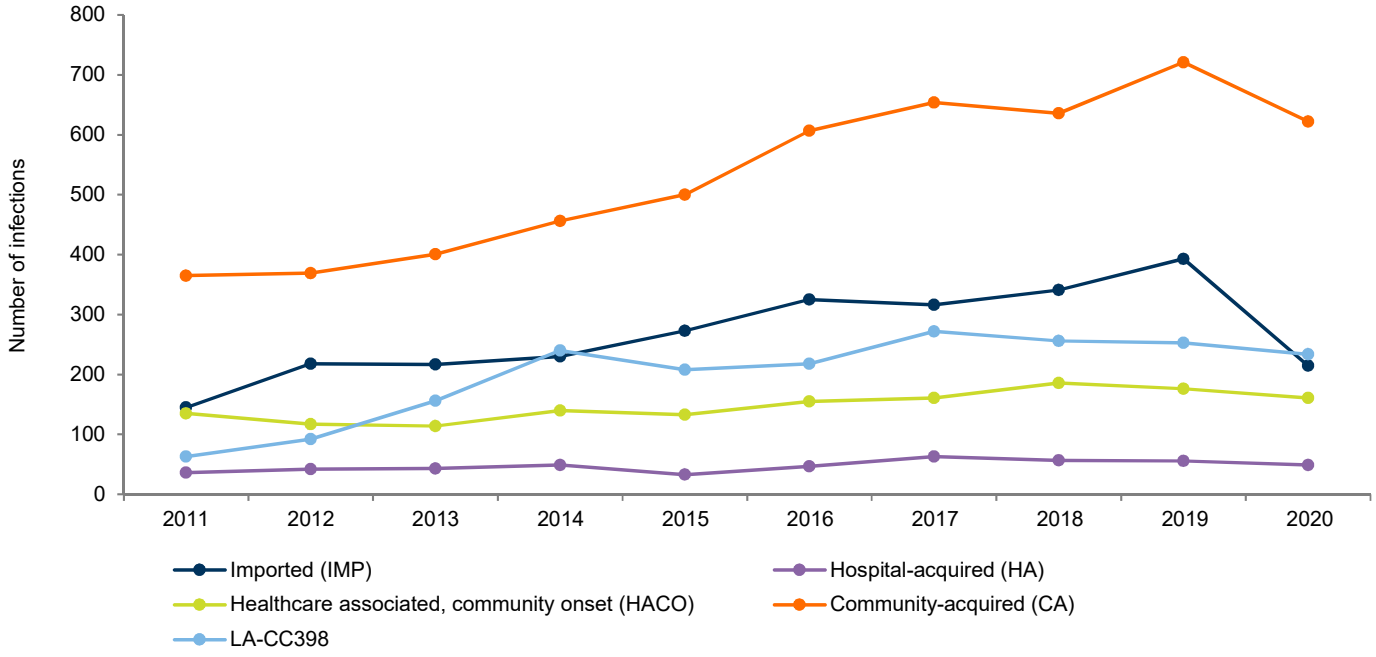
Numbers shown in bold are totals

Table 8.17 Resistance (%) in non LA-CC398 MRSA isolates, Denmark, 2011-2020 DANMAP 2020

	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
Erythromycin	37	38	32	33	37	34	34	33	33	30
Clindamycin	27	29	24	23	29	25	27	28	23	22
Tetracycline	17	18	20	21	24	26	24	26	22	22
Fusidic acid	15	17	17	17	19	18	16	18	23	22
Rifampicin	2	1	1	<1	<1	1	1	1	<1	<1
Norfloxacin	27	25	23	27	21	19	20	21	21	17
Linezolid	0	0	<1	<1	0	<1	0	<1	0	<1
Mupirocin	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Trimethoprim-sulfamethoxazole	1	2	3	3	4	2	3	3	4	2
Number of tested isolates	1125	1324	1451	1616	1242	1184	1193	1233	1025	1920*

* Not all isolates were tested for all listed antimicrobials

Figure 8.19 Number of MRSA infections according to epidemiological classification, Denmark, 2011-2020 DANMAP 2020



Resistance among MRSA isolates

Resistance data for non-LA-CC398 isolates are presented in Table 8.17. The proportion of resistant isolates were similar to previous years, with relatively high resistance to erythromycin (30%), clindamycin (22%), fusidic acid (22%), tetracycline (22%) and norfloxacin (17%), and low resistance (0-2%) to trimethoprim/sulfamethoxazole, linezolid, mupirocin and rifampicin.

Conclusions

The number of SAB continued to increase while numbers of MRSA cases decreased by 21%. The reduction in MRSA cases was expected due to the COVID-19-induced travel restrictions, limitations on social contacts and reduced number of healthcare contacts.

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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.

Gonococcal surveillance

Since 1962, the DCMs in Denmark have voluntarily submitted isolates of gonococci to the Neisseria and Streptococcus Reference (NSR) laboratory at Statens Serum Institut for national surveillance of antimicrobial resistance.

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

As part of NSR's participation in ECDC's surveillance of sexually transmitted infections since 2009, 110-120 gonococcus isolates are collected consecutively each year and investigated for susceptibility to an expanded panel of antimicrobial agents. This panel includes cefixime and in selected years also spectinomycin and sometimes gentamicin.

Results and discussion

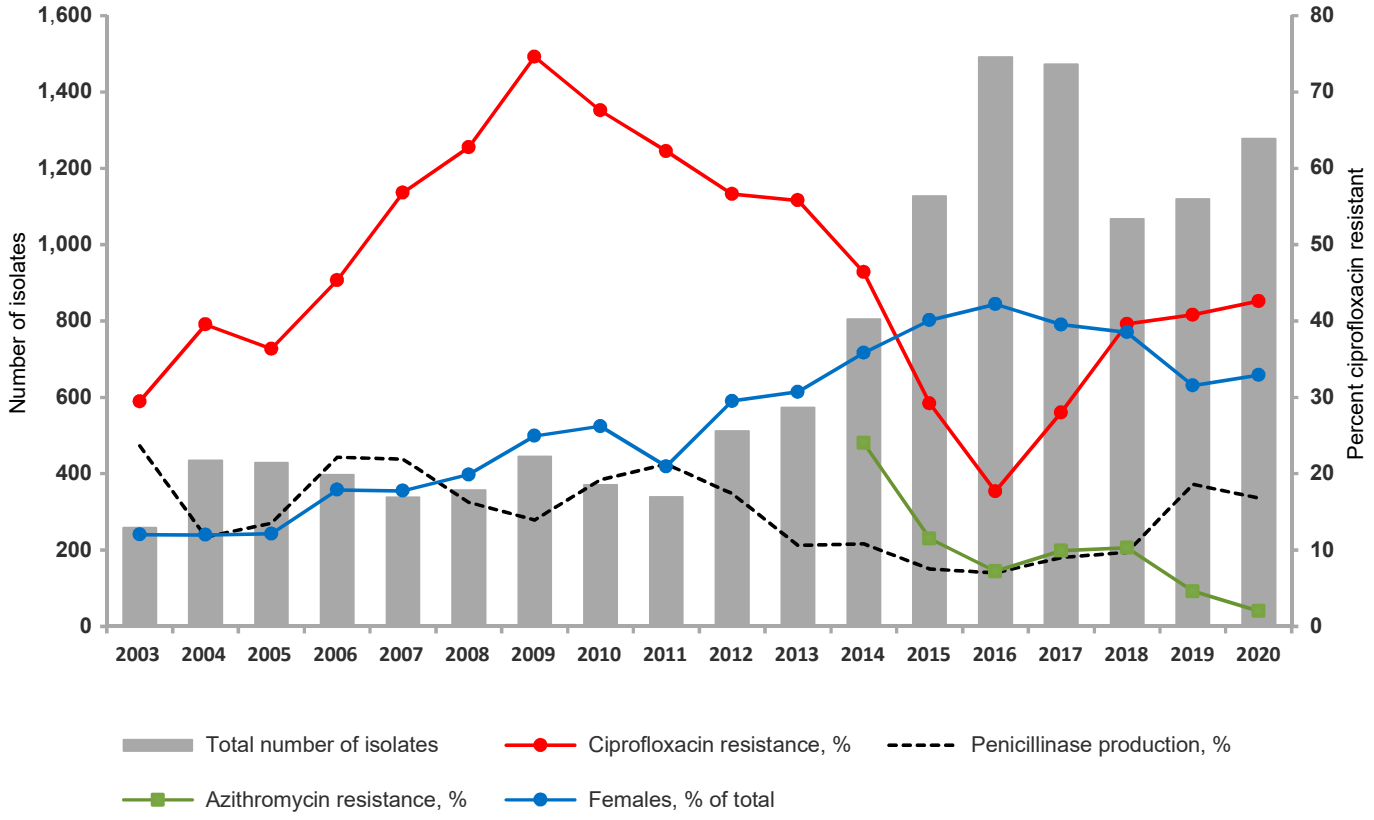
Most of the isolates received in 2020 were from urethra or cervix, while clinicians only rarely obtain specimens from rectum and pharynx. Occasionally, the NSR laboratory receives strains isolated from other anatomical sites, such as conjunctivae, joint fluid, blood, Bartholin's abscess, etc.

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 1,512 isolates from 1,278 unique cases of gonorrhoea diagnosed in 2020. Only one isolate from each unique case is counted in this report.

The annual number of isolates increased considerably between 2011 and 2016 (Figure 8.20). This was most likely due to the widespread use of combined nucleic acid amplifications tests (NAATs) for *Chlamydia trachomatis* and *N. gonorrhoeae* which identified cases of gonorrhoea previously not necessarily tested due to low-grade clinical symptoms. In addition, incidence of gonorrhoea increased, especially among young heterosexual persons including an increasing proportion of women. A slight decrease in the annual number of isolates from unique cases was seen in 2017 and a more pronounced decrease in 2018 followed by an increase in 2019-2020. However, it should be noted that many cases diagnosed by NAATs are not being followed-up by culture, i.e. without gonococci being isolated and sent to SSI for further testing.

The proportion of gonococci with ciprofloxacin resistance was 41% in 2020 and 2019, thus still considerably lower than at the peak of 75% in 2009 (Figure 8.20). There were 733 isolates (57%) with ciprofloxacin MIC ≤0.032 mg/L (S), 20 (1.6%) with MIC 0.047-0.064 mg/L (I), and 525 (41%) with MIC >0.064 mg/L (R). The proportion of strains producing penicillinase was 17% in 2020 compared to 19% in 2019 and 10% in 2018. Azithromycin resistance (MIC above the present ECOFF >1 mg/L) was found in 2% 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.

Figure 8.20 Number of submitted gonococci isolates from males and females, and the percentage of isolates with ciprofloxacin resistance, azithromycin resistance, and penicillinase production, Denmark, 2003-2020 DANMAP 2020

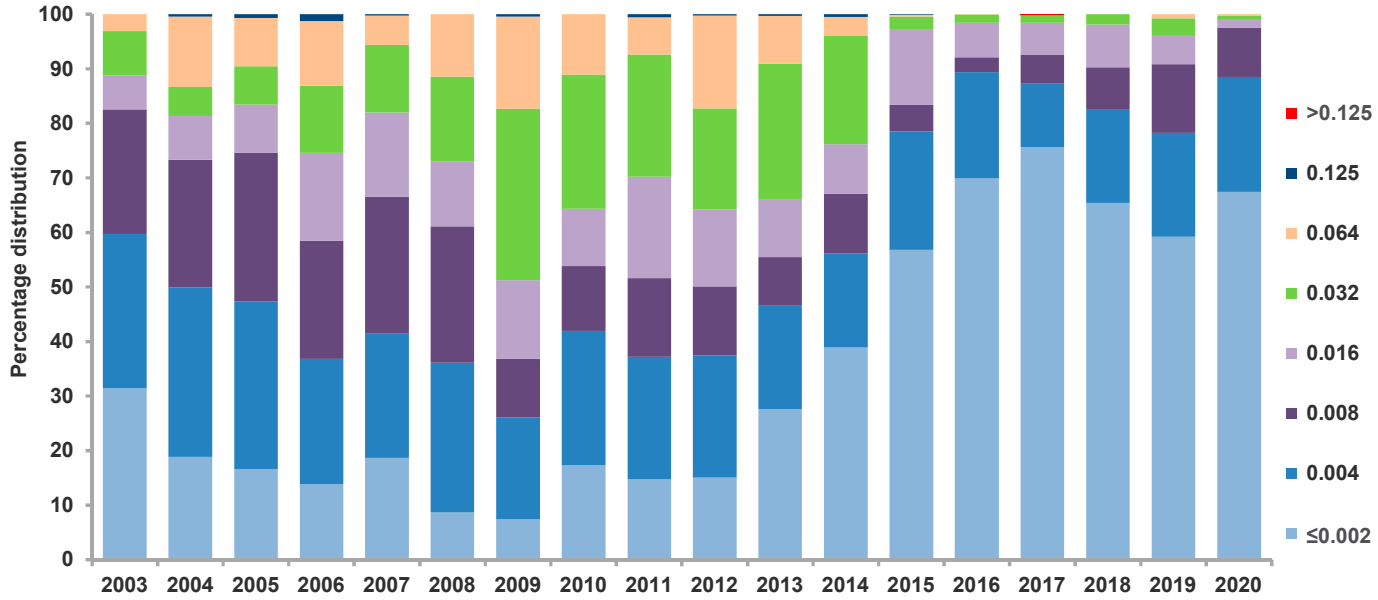


Ceftriaxone-resistant gonococci (MIC >0.125 mg/L) as well as ceftriaxone treatment failure in patients with gonorrhoea have occurred in several countries during recent years. As shown in Figure 8.21, the ceftriaxone MIC distribution changed from 2003 through 2009 with the highly susceptible isolates 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 was gradually abandoned during 2019.

In a subset of 110 isolates, resistance against cefixime (MIC >0.125 mg/L) was 0% in 2020, like in 2019. Cefixime is an oral cephalosporin that has never been used in Denmark. Resistance against spectinomycin was 0% in 2020 as well as in the years 2015 through 2019. MIC values for gentamicin were 1 to 4 mg/L, but no breakpoints are defined for this agent against gonococci.

Figure 8.21 Distribution of ceftriaxone MIC (mg/L) values in gonococci, Denmark, 2003-2020 DANMAP 2020



Conclusions

The ciprofloxacin resistance rate and the ceftriaxone MIC distribution were virtually unchanged in 2020 compared to 2019. 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. *H. influenzae* is classified into six capsular serotypes (a, b, c, d, e and f, also known as Hia, Hib, Hic, Hid, Hie and Hif), as well as noncapsular (non-typeable, NTHi). Introduction of the polysaccharide type b vaccine in 1993 significantly reduced the number of cases with systemic infection caused by *H. influenzae* type b (Hib) isolates. Before the vaccine was introduced, there were around 80 cases of Hib meningitis annually among infants in Denmark. This has been reduced to 0-2 cases per year. NTHi for which no vaccine yet exists is now the predominant type found among invasive *H. influenzae* infections.

Invasive *Haemophilus influenzae*

Surveillance of invasive Hib is mandatory by submission of the isolate to Statens Serum Institut (SSI). By tradition, most

departments of clinical microbiology are voluntarily submitting all isolates of invasive *H. influenzae*. The received isolates are then serotyped and biotyped by the reference laboratory at SSI. Thus, isolates are submitted for the majority of cases, and the remaining cases can be identified through the Danish Microbiological Database (MiBa). Thereby, all invasive infections with *H. influenzae* are registered in the surveillance database, and for the majority of cases serotypes are available. In 2020, all isolates were tested at SSI for antimicrobial susceptibility with disc diffusion assays and the betalactamase test. Whole genome sequencing was also performed on the received isolates, and the data were analysed for the presence of the plasmid-borne beta-lactamase genes TEM-1 and ROB-1. For cases where isolates were not received, antimicrobial susceptibilities were retrieved through MiBa, when available.

The present report includes all episodes of invasive *H. influenzae* as identified through MiBa, where the date of sampling was in 2020. A total of 59 cases were identified, of which isolates from 47 (80%) were received at the reference laboratory. *H. influenzae* were isolated from cerebrospinal fluid in four of the 59 cases, from blood in 50, and from pleural fluid in five cases. The serotypes of the 47 received isolates were: one Hia (2%), nine Hib (15%), five Hif (8%) and 32 NTHi (54%). The age-distribution of the cases is presented in Figure 8.22.

Figure 8.22 Different serotypes in invasive *H. influenza* cases according to age, Denmark, 2020 DANMAP 2020

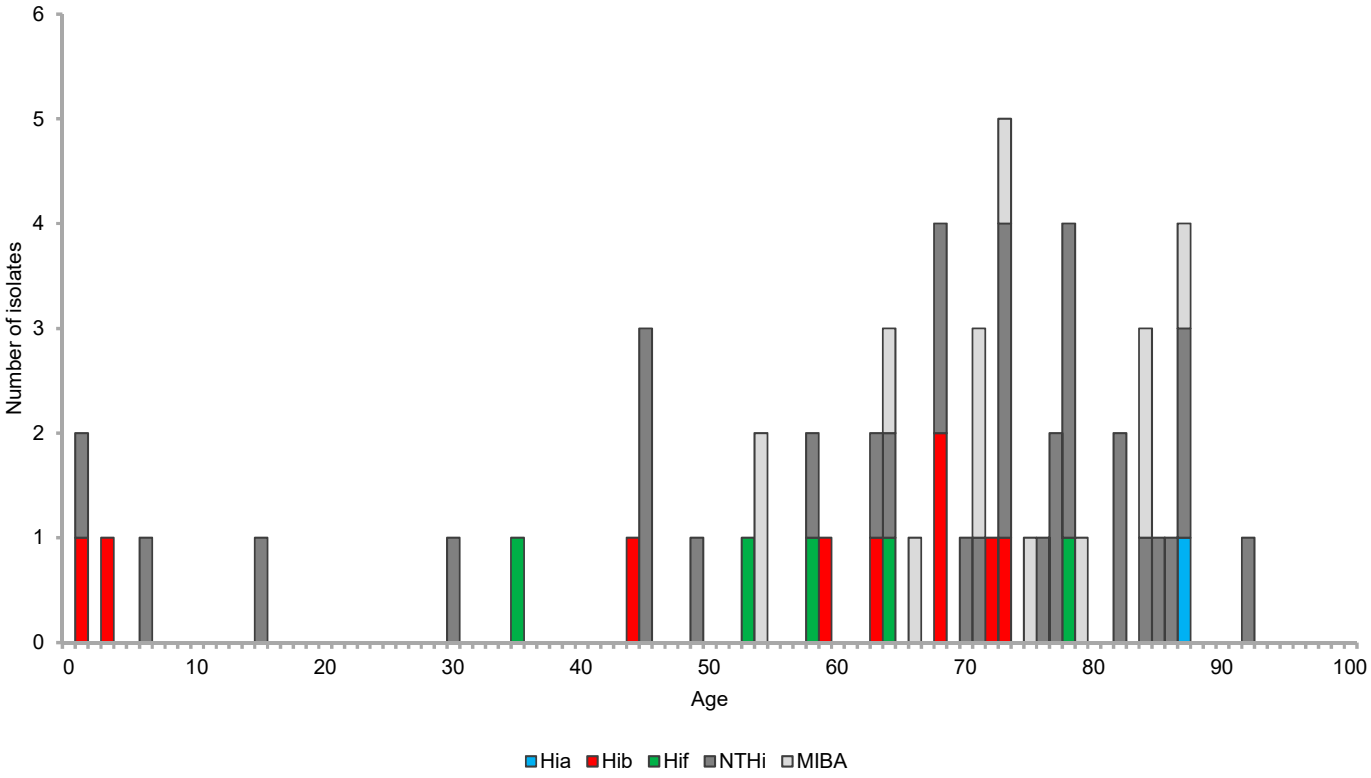

Table 8.18 Distribution of antimicrobial susceptibilities in invasive *H. influenza* according to serotypes, Denmark, 2020 DANMAP 2020

	Hia	Hib	Hie	Hif	NTHi	Unknown*	All(2020)	All(2019)	All(2018)
Penicillin: no result registered	-	-	-	-	-	1 (8%)	1 (2%)	1	1
Penicillin: I and S	1 (100%)	5 (56%)	-	5 (100%)	23 (72%)	7 (58%)	41 (69%)	84 (74%)	89 (74%)
Penicillin: R	-	4 (44%)	-	-	9 (28%)	4 (33%)	17 (29%)	29 (26%)	31 (26%)
Ampicillin: no result registered	-	-	-	-	-	2 (17%)	2 (3%)	5	4
Ampicillin: I and S	1 (100%)	7 (78%)	-	5 (100%)	25 (78%)	7 (58%)	45 (76%)	85 (78%)	94 (80%)
Ampicillin: R	-	2 (22%)	-	-	7 (22%)	3 (25%)	12 (20%)	24 (22%)	23 (20%)
Cefuroxime: no result registered	-	2 (22%)	-	1 (20%)	8 (25%)	2 (17%)	13 (22%)	1	18
Cefuroxime: I and S	1 (100%)	5 (56%)	-	3 (60%)	17 (53%)	8 (67%)	34 (58%)	99 (88%)	87 (84%)
Cefuroxime: R	-	2 (22%)	-	1 (20%)	7 (22%)	2 (17%)	12 (20%)	14 (12%)	16 (16%)
Amoxi/Clav: no result registered	-	2 (22%)	-	2 (40%)	11 (34%)	3 (25%)	18 (31%)	3	29
Amoxi/Clav: I and S	1 (100%)	5 (56%)	-	3 (60%)	17 (53%)	8 (67%)	34 (58%)	100 (90%)	83 (90%)
Amoxi/Clav: R	-	2 (22%)	-	-	4 (13%)	1 (8%)	7 (12%)	11 (10%)	9 (10%)

* The group “unknown” represent the 12 cases that were registered in MiBa, but where an isolate was not received at the reference laboratory at SSI, and therefore not serotyped

One of the received isolates harboured the TEM-1 gene (NTHi), this isolate also had corresponding phenotypical betalactamase activity. None of the isolates harboured the ROB-1 gene. Susceptibility results, divided in to serotypes, for penicillin, ampicillin, amoxicillin/clavulanic acid and cefuroxime, are presented in Table 8.18. Due to differences across the departments of clinical microbiology in the reporting of non-resistant isolates, the "S" and "I" interpretations are combined in the table.

The results from antimicrobial susceptibility testings showed that in total there was 29% resistance to penicillin, 20% to ampicillin, 20% to cefuroxime and 12% to amoxicillin/clavulanic acid. Some variation across serotypes was observed. These figures are very similar to what was observed in 2019 and 2018, except for a higher level of resistance for cefuroxime (20% vs. 12% and 16%).

In summary, the majority of isolates from invasive infections with *H. influenzae* are of the non-capsular type. This is similar to previous years and also similar to what is observed generally in Europe [R. Whittaker, Emerg Infect Dis, 2017, Mar;23(3):396-404]. The majority of the invasive *H. influenzae* isolates in Denmark in 2020 were found to be non-resistant to the antimicrobials tested, with the non-capsular isolates showing the highest degree of resistance. In 2020, signficantly fewer cases of invasive *H. influenzae* were confirmed than in previous years (114 in 2019) compared to 59 in 2020, but this is most likely due to an effect of the COVID-19 restrictions.

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Textbox 8.1

Investigation of an *Acinetobacter baumannii* OXA-23 carbapenemase hospital outbreak – no source of transmission revealed

In August 2020, a ST2/ST195 OXA-23-producing *Acinetobacter baumannii* was detected at Rigshospitalet in Copenhagen. This sequence type had not previously been detected in Denmark but had been described in previous outbreaks from other parts of the world. From August 2020 until March 2021, ST2/ST195 OXA-23-producing *Acinetobacter baumannii* isolates were detected in 13 patients at Rigshospitalet. The isolates from these patients were analysed and found to be closely related with between 4-16 SNPs differences only. The isolates carried blaOXA-23, blaOXA-66, blaADC-25 (Ambler Class C beta-lactamase), blaTEM-1D in addition to several other resistance genes.

All patients had been hospitalized at the Intensive Care Unit and the Burn Unit at some point during 2020-2021. Most patients were admitted via the Trauma Centre and had undergone treatment at one of two operating theatres. However, no direct epidemiological links between several of the patients were established.

The infection control team audited the operating theatres, the Trauma Centre and the two departments involved. Audits were cautiously performed on procedures, staff's compliance to infection control guidelines, equipment, reprocessing procedures and cleaning routines. Specific mattresses designed for disposable use had been reused and these were discarded. Finally, sinks and drains from the operating theatre and the Trauma Centre were investigated. ST2/ST195 OXA-23-producing *Acinetobacter baumannii* was detected in one drain. Routine cleaning of drains was reintroduced. We refrained from any further environmental sampling. Rectal screening of all patients at the involved department was not performed. Yet, all wounds from the involved department were sampled routinely. Two of the departments were relocated during the outbreak, including one operating theatre. Two known cases were relocated with the department and one patient had treatment in the new operation theatre. No specific source of transmission was revealed, nor did we find a probable reservoir. A thorough bundle approach was performed and discrepancies from current guidelines were corrected. No further cases have been detected at Rigshospitalet since March 2021.

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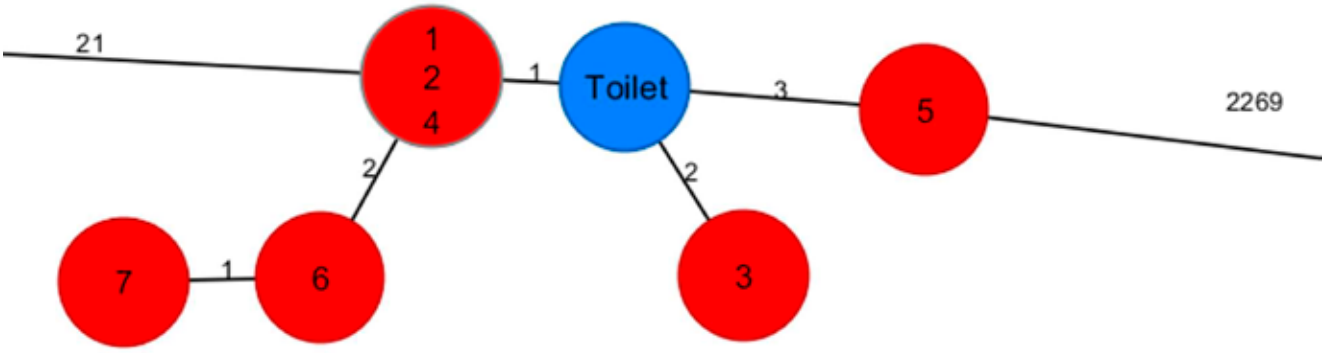
Textbox 8.2

Investigation of a local *E. coli bla*NDM-5 hospital outbreak with possible link to a toilet reservoir

Between January 2019 and November 2020, seven meropenem resistant *E. coli* producing an NDM carbapenemase were submitted from the same clinical microbial laboratory on the island of Zealand to Statens Serum Institut for whole genome sequencing (WGS). Here, further analysis found all seven *E. coli* to carry a *bla*NDM-5 gene, belong to MLST type ST167 and were closely related in clonal analysis by cgMLST (Figure 1). The seven isolates originated from seven individual patients, whom all had been visiting the same hospital on different occasions between December 2018 and September 2020, indicating a local outbreak on this location. Only two out of the seven patients had been at the same department for one day, which made it unlikely that the outbreak was solely related to direct patient-to-patient transmission. However, the five most recent patients had all been hospitalized at the same ward between February and August 2020, which instigated a thorough investigation of the hospital environment in late November 2020 focusing on contaminated toilets, sinks and shower drains. This led to the identification of an NDM-positive *E. coli* within one of the toilets of the specific ward, where the five recent patients had been hospitalized previously. WGS data analysis confirmed the presence of the *bla*NDM-5 gene and a close clonal relationship of the toilet isolate (blue circle in Figure 1) to the seven patient isolates (red circles in Figure 1).

Figure 1 cgMLST analysis of seven *Escherichia coli bla*NDM-5 patient isolates (red color) and the toilet isolate (blue color)

DANMAP 2020



Numbers in the circles indicate patient number in the outbreak
Numbers above lines indicates allelic differences between isolates in cgMLST (= number of genes differing between isolates out of 2513 genes included in the analysis)

Subsequently, an intensified cleaning procedure of the colonized toilet with chloride and peracetic acid as well as screening of patients with access to the toilet and still admitted was conducted. No additional carbapenemase producing *E. coli* were found in the toilet after termination of the cleaning procedure or in any of the recent patients with prior access to the toilet. Also, no additional patients in general have been found carrying the blaNDM-5 producing ST167 *E. coli* after the toilet was disinfected in November 2020. It is therefore plausible that the colonized toilet was involved in the local outbreak, and that eradication of the bacteria within the toilet lead to termination of the outbreak. This investigation underlines the potential of sewage systems in hospital settings as a source of prolonged nosocomial outbreaks and the importance of infection control measures.

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Textbox 8.3

Predicting β-lactam susceptibility from the genome of *Streptococcus pneumoniae* and other mitis group streptococci

Background: For *S. pneumoniae*, β-lactam susceptibility can be predicted from the amino acid sequence of the penicillin-binding proteins PBP1a, PBP2b and PBP2x. The combination of PBP-subtypes provides a PBP-profile, which correlates to a phenotypic MIC. The Mitis-group streptococci (MGS) have similar PBPs and exchange *pbp*-alleles with *S. pneumoniae*. We examined the PBP-profile and genotypic-phenotypic correlation in Danish *S. pneumoniae* isolates and in internationally collected MGS.

Methods: Isolates with available WGS and phenotypic susceptibility data were included. For each isolate, we identified best matching PBP-profile and corresponding MIC for penicillin (PEN) and ceftriaxone (CFT). We calculated category agreement (CA), essential agreement, minor-, major-, and very major discrepancy. The Blosum62 scoring matrix for amino acid substitutions was used for phylogenetic analyses. Genotypic-phenotypic correlation was examined with Deming regression.

Results: Among 88 *S. pneumoniae* isolates, a recognized PBP-profile was found in 55 isolates, with CA being 100% and 98.2% for PEN and CFT respectively. The remaining 33 *S. pneumoniae* isolates had PBP-substitutions and thus a new PBP-profile; CA was 90.9% (PEN) and 93.8% (CFT) comparing with nearest recognized PBP-profile. CA was 100% for PEN and CFT in 19 susceptible *S. pneumoniae* isolates. In 33 *S. mitis* isolates, CA was 75.8% (PEN) and 86.2 (CFT) and in 25 *S. oralis* isolates, CA was 8% (PEN) and 100% (CFT). Performing phylogeny, using the concatenated PBP-sequence, susceptible isolates of each species clustered separately while non-susceptible isolates of different species clustered together.

Conclusion: Genotypic susceptibility prediction was accurate in Danish *S. pneumoniae* isolates, particularly in isolates with recognized PBP-profiles. It seems that specific PBP-profiles are needed for correct susceptibility prediction of other MGS.

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One Day in Denmark Consortium: Valeria Bortolaia, Pimlapas Leekitcharoenphon, Dennis Schrøder Hansen, Hans Linde Nielsen, Svend Ellermann-Eriksen, Niels Nørskov-Lauritsen, Michael Kemp, Bent Løwe Røder, Niels Frimodt-Møller, Turid Snekloth Søndergaard, John Eugenio Coia, Claus Østergaard and Henrik Westh
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Textbox 8.4

Fungaemia epidemiology, resistance rates and human antifungal consumption

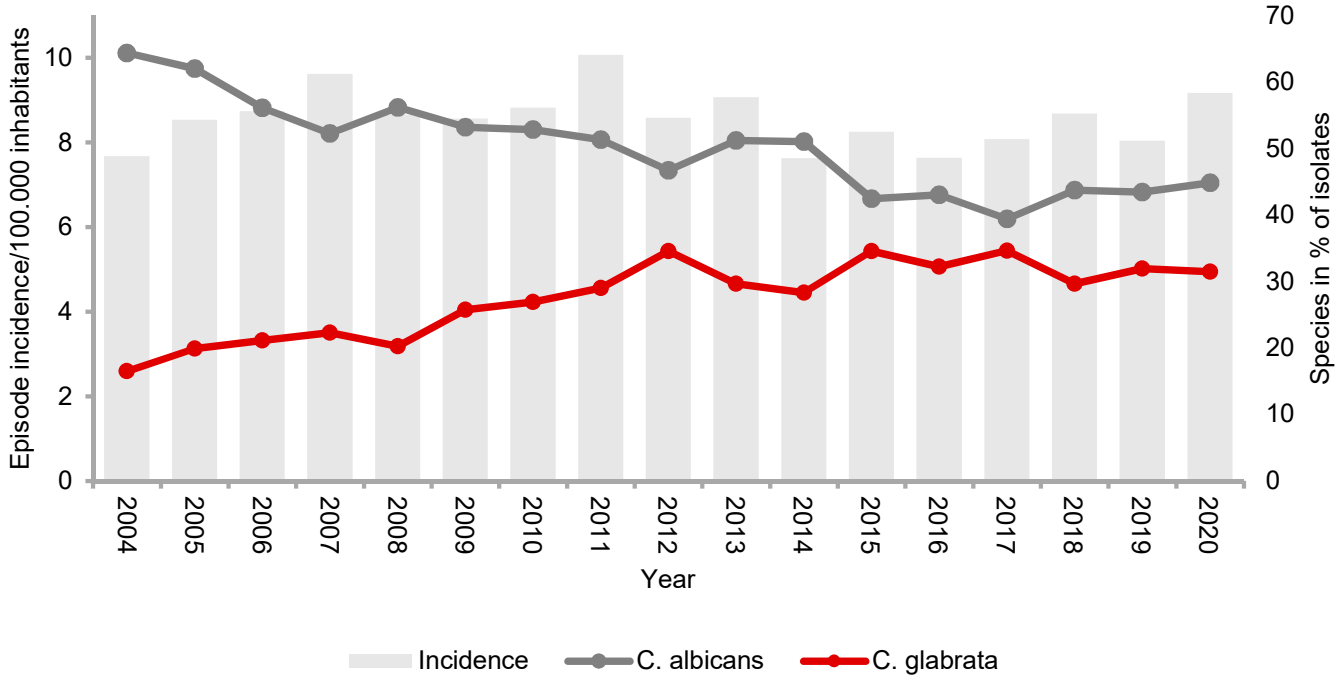
Candidaemia is the most common manifestation of fungaemia in Denmark. Candidaemia is a serious infection which occurs mainly in hospitalized patients. Risk factors include abdominal surgery, severe underlying disease requiring intensive care, the presence of central venous catheters (CVCs), immunosuppression and administration of broad-spectrum antibiotics [1,2]. The 30-day mortality rate is ~40% in Denmark and higher in ICUs [2].

A nationwide surveillance of fungaemia has existed in Denmark since 2004, involving all Danish clinical microbiology departments. All blood culture fungal isolates are referred to Statens Serum Institut for confirmatory species identification and susceptibility testing. Sequencing of *FKS* genes is performed as needed.

Denmark is a high-incidence country compared to its Nordic neighbours, with fungaemia incidence peaking at 10.1/100.000 inhabitants in 2011 [3]. The incidence has since stabilised around 8/100.000 but rose to 9.2/100.000 in 2020, (Figure 1) [4,5]. This could be related to the COVID-19 pandemic but is within the year-to-year-variation.

The first-line treatment for candidaemia is echinocandins. If antifungal susceptibility testing confirms fluconazole susceptibility and the patient's condition has stabilised there is an option for de-escalation (outpatient oral treatment possible). However, a marked shift from the susceptible species *Candida albicans* to the azole less susceptible species *Candida glabrata* has been evident since the initiation of the surveillance. This shift has been linked to the high use of azoles in Denmark (Figure 2A) [3,5].

Figure 1 Fungaemia incidence rate and species distribution over time, Denmark, 2004-2020) DANMAP 2020



Episode rate and species distribution from the Danish nationwide fungaemia surveillance 2004-20. A unique isolate is defined as: if found >21 days apart, confirmation of another species or different susceptibility pattern. Episode rate is defined as: one unique isolate or one polyfungal infection or one admission at one centre per 100.000 inhabitants. Other fungi found 2019-2020 were (% of total): Candida species (other than *C. albicans* and *C. glabrata*) (22.3%), other yeasts (including *Cryptoccocus*) (1.4%) and moulds (0.5%)

continued ... Textbox 8.4

In 2019-2020 *C. albicans* accounted for 44% and *C. glabrata* for 32% of isolates (Figure 1) [6]. The increase in *C. glabrata* since the beginning of the surveillance programme is the main cause for only ~59% of isolates being susceptible to fluconazole (at standard dosing). The azole (mainly fluconazole) use has declined from 2013 to 2020, both in the primary health care sector (-31%) and in the hospital sector (-23%), which may have contributed to the low level of acquired azole resistance (<0.5% in *C. albicans/C. dubliniensis* and <2.5% in *C. tropicalis/C. parapsilosis*, respectively).

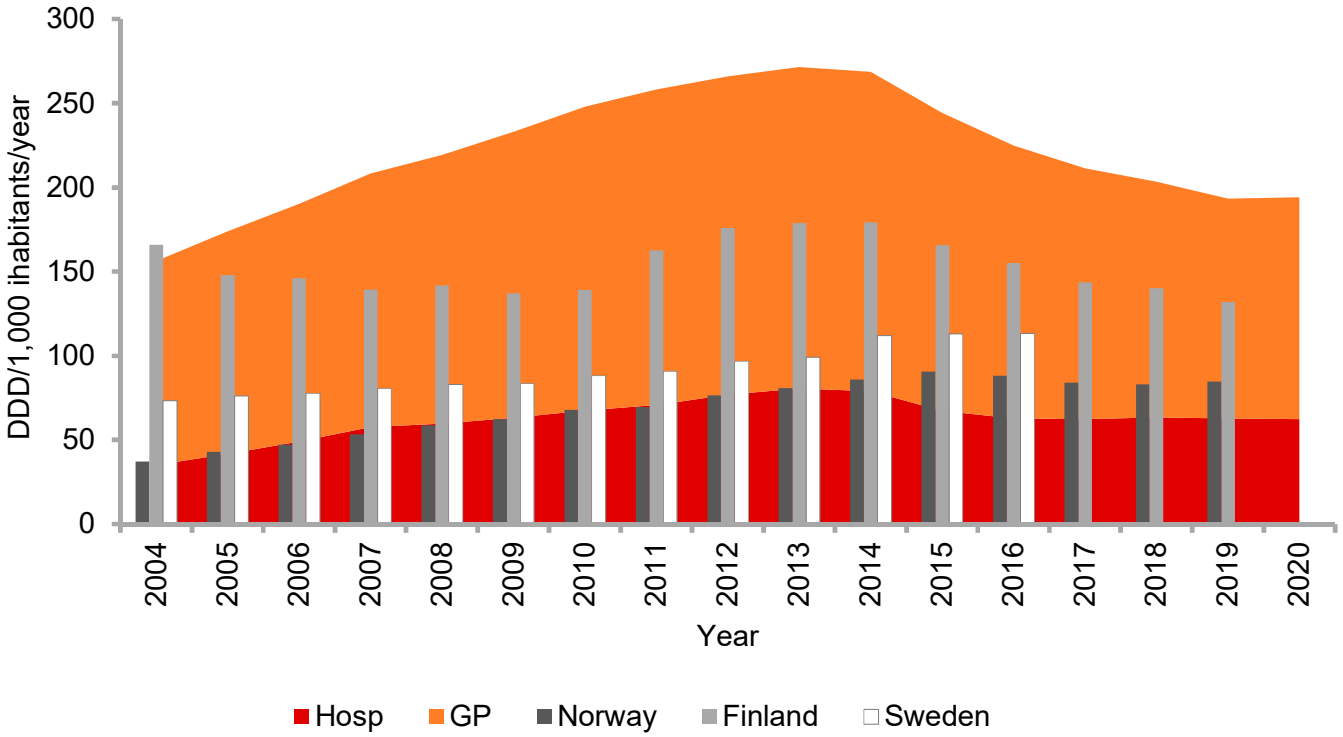
Following the introduction of echinocandins, acquired echinocandin-resistance has emerged [5]. Despite a continued increase in echinocandin usage (+26% since 2013) (Figure 2B), the acquired resistance rate 2019-2020 (1.3%) was similar to 2012-15 (1.7%) and 2016-18 (1.5%) but varied noticeably over the two years (from 0.7% to 1.8%) [6]. A 3.5% resistance rate in *C. glabrata*, specifically, was documented in 2020. This, combined with a 10% acquired fluconazole resistance rate complicates treatment of infections caused by this species.

In conclusion, the overall incidence and high proportion of *C. glabrata* remain stable despite a reduction in azole use over the last seven years. Resistance levels have not increased.

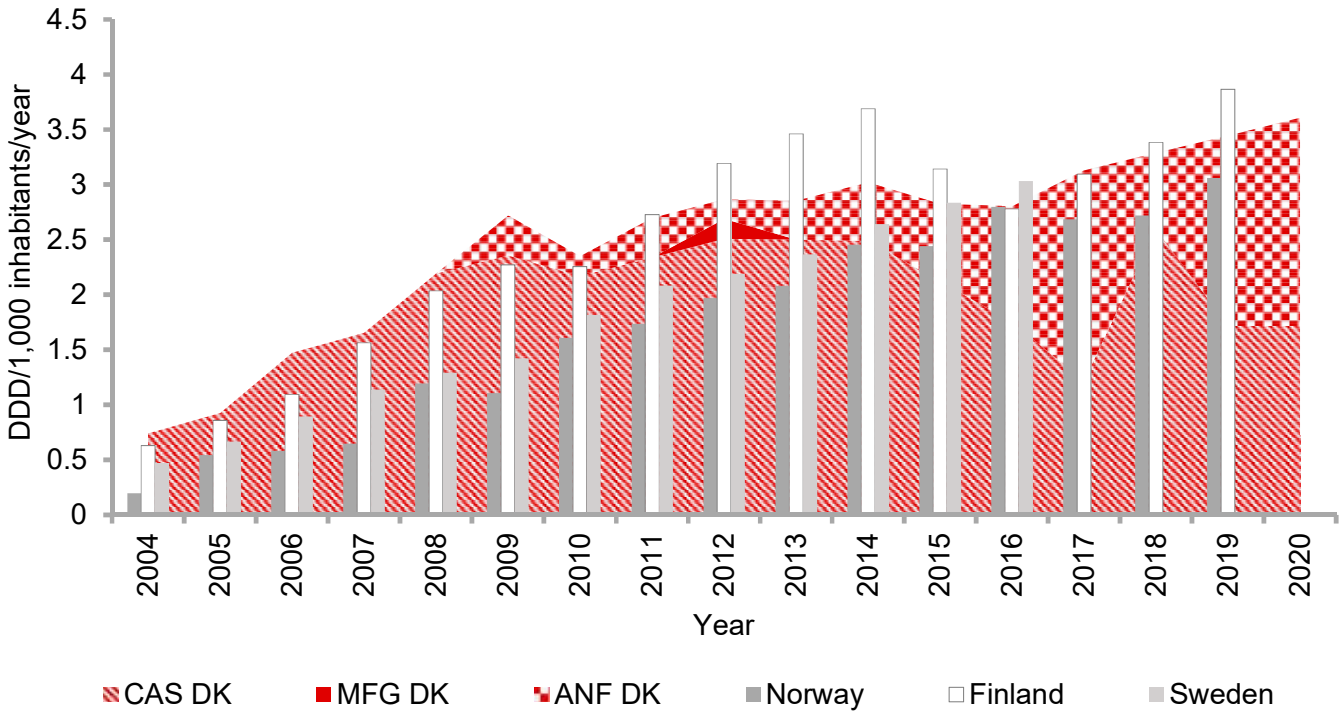
Figure 2 Annual consumption of the (A) azoles (excluding ketoconazole) and (B) echinocandins in DDD/1,000 inhabitants in 2004-2020

DANMAP 2020

a)



b)



DDD: Defined daily dosage. Hosp: Hospital sector. GP: Primary health care sector. CAS: Caspofungin. MFG: Micafungin. ANF: Anidulafungin

Data from Denmark has been found at medstat.dk 8.7.2020 (2020 data made available directly from Sundhedsdatastyrelsen). Data from Norway, Finland, and Sweden is acquired via contact to Norwegian Drug Wholesales Statistics, Norwegian Institute of Public Health (www.hfi.no), www.fimea.fi and www.socialstyrelsen.se. Data on antifungal consumption was not available from Sweden from 2017 onwards

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Textbox 8.5

Azole resistance in *Aspergillus fumigatus* - data from the first 2 years of nationwide surveillance

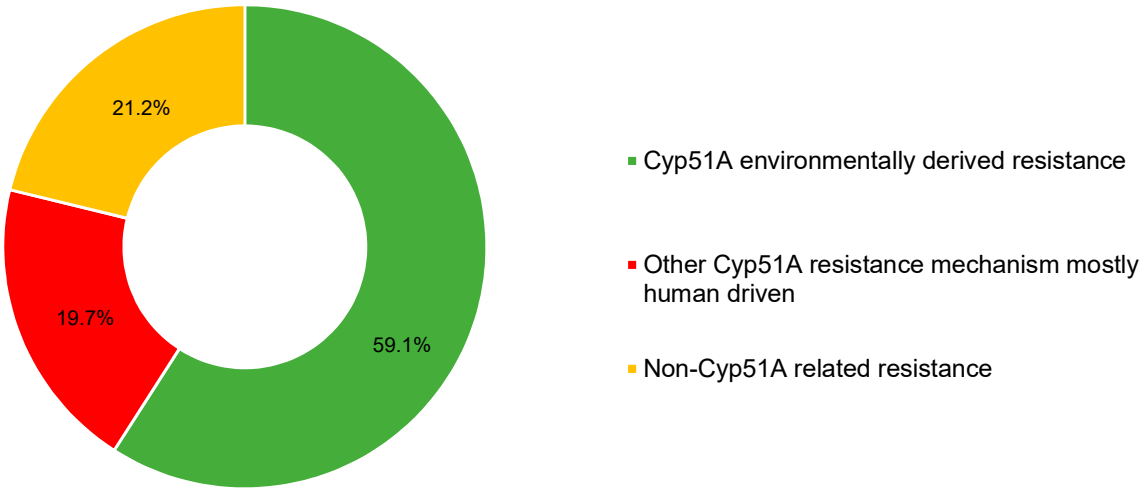
Azole resistance in *Aspergillus fumigatus* complicates treatment of patients with invasive aspergillosis and is associated with increased mortality (1). Azole resistance arises most commonly from mutations in *cyp51A* either environmentally - or in relation to azole treatment. The environmentally derived mutations TR34/L98H and TR46/Y121F/T289A have been found in samples from both the environment and patients in Denmark (2-5). Increasing azole resistance in *A. fumigatus* isolates from cystic fibrosis patients was found in Denmark over a ten year period from the years 2007 and 2009 to 2018 (5). We established a nationwide surveillance programme in 2018 and found a 5.7% azole resistance rate the first nine months and 6.1% from the first 18 months.

Nationwide surveillance of *A. fumigatus* was initiated in Denmark in October 2018. Isolates included unique *A. fumigatus* isolate defined as clinically significant and any *A. fumigatus* isolated on a defined weekday (background samples, regardless of clinical significance) were included. Isolates from the same patient were deemed unique when they were 1) found >30 days apart or 2) showed a different susceptibility pattern or a different resistance mechanism. Referred isolates underwent confirmatory species ID and susceptibility testing. Isolates categorised as azole resistant according to the EUCAST breakpoints v. 10.0 underwent *cyp51A* sequencing. During the two-year surveillance period the EUCAST breakpoints have been revised. Data presented here have been categorized according to the current EUCAST breakpoints (6).

Data from Oct-1st 2018 to September-30th 2020 consisted of 1822 susceptibility tested isolates from 1083 patients, including 98 isolates marked "Background." At the patient level 6.1% (66/1083) had at least one azole-resistant isolate. Among these, 59% (39/66) of patients harboured an isolate with TR34/L98H (3.6% of all patients), 20% (13/66) with other *cyp51A* resistance mechanisms and 21% (14/66) non-*cyp51A* related resistance (Figure 1). Among the 'Background' samples 3.2% (3/93) harboured TR34/L98H at patient level. Azole resistance was detected from samples from the primary and secondary healthcare sector. TR34/L98H was found in samples from four out of the five Regions in Denmark (Figure 2), other *cyp51A* mutations were detected in isolates referred from Zealand and Funen, but not from Jutland.

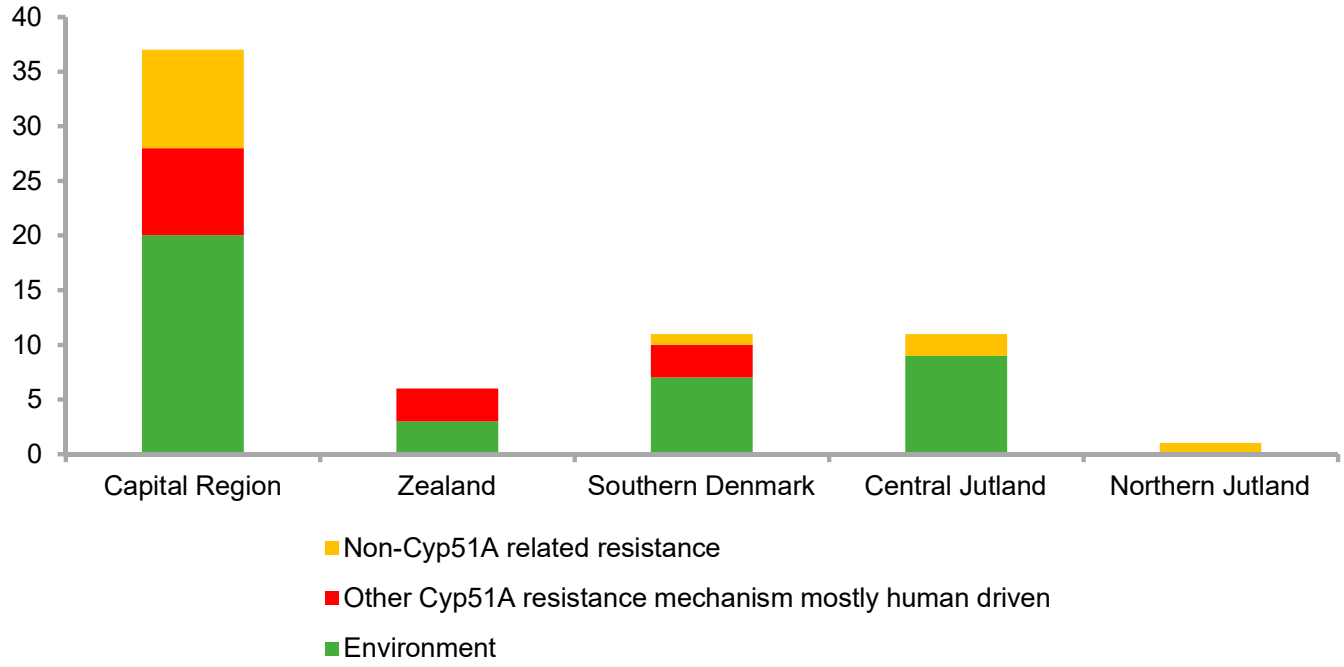
We report a nationwide two-year azole resistance rate of 6.1% in *A. fumigatus* at patient level. The majority of the resistance was due to environmentally driven mutations. This raises therapeutic challenges and highlights the need for rapid susceptibility testing.

Figure 1 Distribution of resistance mechanisms in *Aspergillus fumigatus* isolates at patient level, Denmark, Oct 2018-Sept 2020
DANMAP 2020



Some patients harboured resistant isolates in which different resistance mechanisms were detected sequentially (e.g. a *cyp51A* mutation and a *cyp51A*-wildtype). In these cases the *cyp51A* mutation is shown

Figure 2 Numbers of patients with a resistant *Aspergillus fumigatus* in the five Danish Regions, Oct 2018- Sept 2020 DANMAP 2020



The Region represented is the Region from which the isolate was referred and does not necessarily represent the patients' place of residence or the place that the resistant fungus was acquired

Malene Risum, Rasmus Krøger Hare, Maiken Cavling Arendrup and the Danish azole resistant Aspergillus study group (Jan Berg Gertsen, Lise Kristensen, Flemming Rosenvinge, Sofia Sulim, Raluca Datcu, Ea Marmolin, Karen Astvad, Jette Bangsborg, Bent Røder, Michael Pedersen, Esad Dzajic, Esad Dzajic and Steen Lomborg Andersen)
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9

MATERIALS AND METHODS

9. Materials and methods

9.1 General information

For the DANMAP 2020 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. However, in April 2007 the monopoly was suspended. Since then, private companies can obtain license to sell prescribed veterinary medicinal products for animals, but must 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 presentation of a prescription.

In 2020, 96% of all antimicrobial agents were purchased through pharmacies and the drug trading companies, while 4% were purchased from feed mills. These numbers did not include prescribed zinc oxide from feeding mills for pigs. For cattle, 85% of antimicrobial agents used in 2020 were purchased from pharmacies, compared to only 6% in 2004. In aquaculture, approximately two thirds 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.

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 writing invoices. Electronic registration of the sales at pharmacies is linked to the billing process and stock accounts at the pharmacy, which ensures a very high quality of data regarding amounts and type of medicine. Data are transferred daily from pharmacies to The Register of Medicinal Product Statistics at The Danish Health Authority and to VetStat. However, VetStat does not have any validation on data entry and typing errors from veterinarians may occur.

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 2020 were extracted from VetStat on 3 March 2021.

9.2.2 Methods

In DANMAP, we report use of antimicrobials in dispensed different animal populations. As a first step, the amount of antimicrobial agents used in animals is measured in kg active compound. This enables an overall crude comparison of consumption in different 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. This is described in the footnotes for figures and tables in chapter 4.

Numerator - DADD

Defined animal daily dose (DADD) is the average maintenance dose per day for a drug used for its main indication in the appropriate animal species. The DADD is not defined at product level but as mg active compound per kg live animal for each antimicrobial agent, administration route and animal species. DADD has been specifically defined for use in DANMAP and does not always completely match the "prescribed daily dose" or the recommended dosage in the Summaries of Product Characteristics (SPC).

The following principles are applied when setting the DADDs:

1. Minor inconsistencies are corrected (e.g. due to rounding of numbers);
2. Approved dosage for the most widely used antimicrobial products is given priority above dosage for products that are rarely used;
3. Approved dosage for older products within the group is maintained as the common DADD even if a new product is approved with a higher dosage;
4. If the dosage for a group shows large variation in approved dosages of the products, the dosages provided by "The Veterinary Formulary" [British Veterinary Association 2005, 6th edition] are applied;
5. Dosages may vary within active compound and administration route, if different dosages have been approved for different age groups, indications or formulations.

When principle 3 and 4 are conflicting, principle 5 is applied.

Denominator - live biomass

The number of animals in a population (in epidemiological terms: the population at risk) is represented by their live biomass. The biomass of a species is calculated, taking into account average live bodyweight and the average lifespan in each age group. The estimation of live biomass and thus the number of standard animals at risk per day depends on the available data sources for each species. For DANMAP 2020, only the live biomass for pigs, cattle and mink were updated.

Pig production: The estimation was based on the number of pigs produced, including exports at different ages, productivity data for each year [Statistics Denmark; Danish Agriculture and Food Council] and census data for breeding animals [Statistics Denmark]. The average weight and life span for the growing animals (piglets, weaners and finishers) was estimated from the annual productivity numbers [Danish Agriculture and Food Council]. For DANMAP 2020, productivity data from 2019 were used to estimate the biomasses for pigs, since the 2020 productivity data were not available when estimates were calculated. The estimation methods were developed in cooperation with Danish Agriculture and Food Council. There are no statistics on average weight of breeding animals available, so an estimated average weight had to be assumed. However, the size of the breeding animals has probably increased over the last decade, but this was not accounted for.

Cattle production: The live biomass of the cattle population is estimated from census data [Statistics Denmark] and the average live weight of the different age groups. The Danish cattle population is mainly dairy, particularly Holstein Friesian, but also other breeds such as Jersey and a small population of beef cattle. Most of the cattle slaughtered are dairy cows and bull calves of dairy origin. The average live weight was estimated for 10 different age and gender categories.

Fur animals: The live biomass of mink is estimated from production data [Kopenhagen Fur] and carried out as described by Jensen et al., 2016 [Prev Vet Med. 26:170].

Treatment proportion - DAPD

The treatment proportion is a statistical measure for antimicrobial use in animal populations, calculated as the annual number of DADDs administered in the population, divided by the estimated total population live biomass (in tonnes). For a single animal, the mg active compound (e.g. the number of DADDs) given in a daily treatment depends on the body weight. 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 2020, DAPD calculations were carried out for pigs, cattle and fur animals.

Due to a relative high number of pigs exported around 30 kg; an adjusted measure of the average antimicrobial use in all age groups was calculated (DAPDadj). The adjustment is based on the assumption that pigs exported at 30 kg, on average, would have received the same amount of antimicrobial agents as other pigs from farrowing to slaughter.

Antimicrobial use per pig produced (adjusted) is calculated as:

$$DAPD_{adj} = \frac{DADD_{sows} + DADD_{weaners} + (1+Q)*DADD_{finishers}}{\Sigma biomassdays_{all} + N_{export}*biomassdays_{adj}}$$

Where DADDs, DADDw, DADDf are the amounts of antimicrobial agents used in sows, weaners and finishers, respectively. Q is the proportion of pigs exported at approximately 30 kg.

Σbiomassdays_all is the sum of estimated biomass-days for each age group of pigs, N_export is the number of weaning pigs exported, and biomassdays_adj is the assumed number of lost biomass-days per exported pig.

9.3 Collection of bacterial isolates from animals and meat

9.3.1 Animals

Since 2014, most isolates available for DANMAP have been collected in accordance with the EU harmonised monitoring of antimicrobial resistance in zoonotic and commensal bacteria [Decision 2013/652/EU]. In addition to sampling for the national *Salmonella* control programmes in poultry farms, the legislation requires sampling of broilers and fattening turkeys at slaughter in even years (2014-2020), and sampling of fattening pigs and cattle <1 year at slaughter in odd years (2015-2019).

In 2020, most of the sampling for DANMAP was allocated to the mandatory sampling of caeca from broilers, but additional sampling of pigs and cattle was also carried out.

Caecal samples from healthy broilers, cattle (<1 year) and pigs were collected by meat inspection staff or abattoir personnel at the slaughterhouses. Samples were collected throughout the year, in major Danish slaughterhouses slaughtering conventionally produced chicken (three slaughterhouses), pigs (eight slaughterhouses) and cattle (five slaughterhouses). These slaughterhouses handled at least 75% of the total number of broilers, cattle and pigs slaughtered in Denmark in 2020.

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. Samples from all three animal species were examined for indicator *E. coli*. Broiler and cattle samples were also examined for *Campylobacter jejuni*. Furthermore, broiler samples were also examined for enterococci and ESBL/AmpC/carbapenemase-producing *E. coli* (Table 9.1).

All Danish flocks of layers, broilers and turkeys are tested for *Salmonella* on-farm as part of the national *Salmonella* control programme. Due to the low numbers of *Salmonella* isolates obtained from the Danish poultry production [Annual Report on Zoonoses in Denmark, 2020] these data are not included in DANMAP 2020.

9.3.2 Meat

In addition to sampling for the national *Salmonella* control programmes at slaughter, the EU harmonised monitoring requires sampling broiler meat at retail, in even years (2014-2020), and sampling of pork and beef in odd years (2015-2019) [Decision 2013/652/EU].

In 2020, ESBL/AmpC/carbapenemase-producing *E. coli* were isolated from packages of fresh, chilled broiler 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 broiler 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.

The *Salmonella* isolates from domestically produced broiler meat, beef and pork originate from the national control programme at the slaughterhouses (Table 9.1). For beef and pork, carcases are swabbed in four designated areas (the jaw, breast, back and ham) after min. 12 hours of chilling (covering 10x10 cm). For broiler meat, 300 neck-skin samples (1 g) are collected after slaughter and pooled into subsamples of 60 grams. All samples were processed at Industry laboratories.

Salmonella isolates from cattle and pig carcases were sent to the DVFA laboratory, and *Salmonella* isolates from the neck-skin sampling were sent to the laboratory at DTU. *Salmonella* from broiler meat and beef are not included in DANMAP 2020 due to low numbers of isolates available from the national surveillance programmes [Annual Report on Zoonoses in Denmark, 2020].

9.4 Microbiological methods – isolates from animals and meat

9.4.1 *Salmonella*

Salmonella was isolated in accordance with the methods issued by the NMKL [NMKL No. 187, 2007] or ISO 6579-1 [ISO6579-1:2017]. Serotyping of isolates was performed by whole genome sequencing using the Illumina MiSeq platform, paired-end sequencing 2x250 cycles. For bioinformatics, a CGE service (Centre for Genomic Epidemiology, DTU) for *Salmonella* serotyping was applied based on the genetic background for antigenic formulas given by the White-Kauffmann-Le Minor scheme. Only one isolate per serotype was selected from each herd, flock or slaughter batch.

9.4.2 *Campylobacter*

Campylobacter from broilers and cattle was isolated and identified according to the methods issued by the NMKL [NMKL No. 119, 2007] followed by species-determination by BAX® rtPCR

Table 9.1 Legislative and voluntary sampling plans under national control programmes and EU harmonised monitoring that contributed isolates to DANMAP 2020 DANMAP 2020

Bacteria	Origin of isolates	Legislative reporting frequency (2013/652/EU)	Number of tested and positive samples in 2020
Campylobacter spp.	Caecal samples from broilers [a]	Even years	691 flocks (205 positive)
	Caecal samples from cattle <1 yr [a]		158 animals (134 positive)
Salmonella spp.	On-farm samples from laying hens (production flocks)	Even years	432 flocks (8 positive)
	On-farm samples from broilers (production flocks)	Even years	3,604 flocks (13 positive)
	Neck skin samples from broilers	Even years	231 units (0 positive)
	Carcase swabs from fattening pigs	Odd years	11,202 animals (0.9% positive)
	Carcase swabs from cattle <1 year	Odd years	4,104 animals (0.3% positive)
	Fresh pork at retail (imports)		149 units (44 positive)
	Fresh beef at retail (imports)		326 units (3 positive)
	Fresh duck meat at retail (Danish and imports)		100 units (11 positive)
Enterococcus spp.	Caecal samples from broilers [b]		289 animals (281 positive)
Indicator *E. coli*	Caecal samples from broilers	Even years	182 flocks (172 positive)
	Caecal samples from fattening pigs	Odd years	189 animals (185 positive)
	Caecal samples from cattle <1 yr	Odd years	158 animals (154 positive)
Specific monitoring of ESBL/AmpC and carbapenemase-producing *E. coli* [c]	Caecal samples from broilers	Even years	308 flocks (8 positive)
	Fresh broiler meat at retail (Danish)	Even years	309 units (8 positive)
	Fresh broiler meat at retail (Imports)	Even years	27 units (8 positive)
	WGS data for collected ESBL/AmpC isolates		24 isolates

a) Broilers: *C. jejuni* (n=165), *C. coli* (n=25), *C. lari* (n=3) and 12 unspecified. Cattle: *C. jejuni* (n=121), *C. coli* (n=2), and 11 unspecified isolates
b) Broilers: *E. faecium* (n=259), *E. faecalis* (n=21) and 1 unspecified
c)Testing for carbapenemase producing *E. coli* is voluntary according to regulation 2013/652/EU. Carbapenemase producing *E. coli* was not detected in any of the analysed samples

assay. Pre-enrichment in Bolton broth was used for cattle and broiler meat samples, whereas direct spread of caecal sample on to selective agar was used for broiler samples. Only one *Campylobacter jejuni* isolate per broiler flock, cattle herd or per batch of fresh meat was selected.

9.4.3 Indicator *Escherichia coli*

Indicator *E. coli* from broilers, pigs and cattle was isolated by direct spread of caecal sample material onto violet red bile agar incubated for 24h at 44°C. Presumptive *E. coli* was identified on TBX agar incubated at 44°C o/n. Only one indicator *E. coli* isolate per flock or herd was selected. For specific isolation of ESBL/AmpC and carbapenemase-producing *E. coli* from meat and caecal samples, the present EURL-AR laboratory protocols describing the selective enrichment procedures was applied in accordance with the EU harmonised monitoring. Only one ESBL/AmpC-producing *E. coli* isolate per cattle herd, pig herd, and meat sample was selected (no carbapenemaseproducing *E. coli* isolates were detected).

9.4.4 Indicator enterococci

Indicator enterococci from broilers were isolated from chicken caeca material suspended in 10 ml buffered peptone water, and inoculated onto Slanetz agar incubated at 41,5°C for 2 days. Presumptive *E. faecium*/*E. faecalis* were identified by a real-time PCR assay. Only one isolate per flock was selected for antimicrobial resistance testing. *E. faecium* were selected where possible.

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 20776-1:2006]. The isolates were tested for antimicrobial susceptibility in accordance with the Decision 2013/652/EU about the EU harmonised monitoring of antimicrobial resistance.

The 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).

9.6 Whole genome sequencing – isolates from animals and meat

In addition to *Salmonella* serotyping performed by sequencing (section 9.4.1), whole genome sequencing (WGS) and in silico bioinformatic tools were also used to detect the genetic background of the ESBL/AmpC and carbapenemase-producing *E. coli*. At the DVFA laboratory in Ringsted, strains were sequenced using the Illumina MiSeq platform and the bioinformatics analysis was conducted at DTU National Food Institute from Centre for Genomic Epidemiology [www.genomicepidemiology. org; https://cge.cbs.dtu.dk//services/all.php]. ResFinder 4.1 was used for detection of antimicrobial resistance genes including chromosomal mutations leading to resistance to beta-lactams, quinolones and colistin as well as acquired resistance genes [Zankari et al. 2012. J Antimicrob Chemother. 67(11):2640; Zankari et al. 2017. J Antimicrob Chemother. 72(10):2764]. ST types were defined using MLST Finder 2.0.

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.

Table 9.3 Definitions of antimicrobial classes for calculation of multidrug-resistance in *Salmonella* and indicator *E. coli*
DANMAP 2020

Antimicrobial classes	*Salmonella* and *E. coli*
Beta-lactam penicillins	Ampicillin
Macrolides	Azithromycin
Cephalosporins	Cefotaxime and/or ceftazidime
Phenicols	Chloramphenicol
Quinolones	Ciprofloxacin and/or nalidixic acid
Polymycins	Colistin
Aminoglycosides	Gentamicin
Carbapenems	Meropenem
Sulfonamides	Sulfonamides
Tetracyclins	Tetracycline
Glycylcyclines	Tigecycline
Trimethoprim	Trimethoprim

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

9.7.1 Interpretation of MIC values

MIC values were retained as continuous variables in the database, from which binary variables were created using the relevant cut-off from 2019 for all years. Since 2007, data have been interpreted using EUCAST epidemiological cut-off values with a few exceptions described in Table 9.2. All MIC-distributions are presented in the web annex at www.danmap.org. Each of the tables provides information on the number of isolates, the applied interpretation of MIC-values and the estimated level of resistance and confidence intervals calculated as 95% binomial proportions presenting Wilson intervals.

An isolate is considered multidrug-resistant if resistant to three or more of the antimicrobial classes defined in Table 9.3 and fully sensitive if susceptible to all antimicrobial agents included in the test panel.

9.7.2 ESBL/AmpC phenotypes

Classification of CPE, ESBL and AmpC phenotypes was done according to the scheme provided by EFSA [EFSA 2018. EFSA Journal 16(2):5182]:

1. CPE phenotype if meropenem MIC >0.12 µg/ml;
2. ESBL phenotype if cefotaxime/ceftazidime MIC >1 µg/ml and meropenem MIC <=0.12 µg/ml and cefoxitin MIC <=8 µg/ml and synergy (clavulanic acid and cefotaxime/ceftazidime);
3. ESBL-AmpC phenotype if cefotaxime/ceftazidime MIC >1 µg/ml and meropenem MIC <=0.12 µg/ml and cefoxitin MIC >8 µg/ml and synergy (clavulanic acid and cefotaxime/ceftazidime);
4. AmpC phenotype if cefotaxime/ceftazidime MIC >1 µg/ml and meropenem MIC <=0.12 µg/ml and cefoxitin MIC >8 µg/ ml and no synergy (clavulanic acid and cefotaxime/ceftazidime);
5. Other phenotype if not in 1-4.

Synergy is defined as ≥3 twofold concentration decrease in MIC for clavulanic acid combined with cefotaxime/ceftazidime vs the MIC of cefotaxime/ceftazidime alone.

9.7.3 Statistical tests

Significance tests of differences between proportions of resistant isolates were calculated using Chi-square, or Fisher's Exact Tests as appropriate. Significance tests for trends in rates of resistance were performed by applying the Cochran-Armitage test. One-sided tests were chosen because of preliminary expected trend directions and a p-value of <0.05 is generally considered significant.

Analyses were done using SAS®Software, SAS Enterprise Guide 8.2 or Sergeant, ESG, 2018. Epitools Epidemiological Calculators. Ausvet. Available at: http://epitools.ausvet.com.au.

Table 9.2 Interpretation criteria for MIC-testing by EUCAST epidemiological cut-off values (blue fields) and the corresponding EUCAST clinical breakpoints (white fields) DANMAP 2020

Antimicrobial agent	*Salmonella*		*E. coli*		*Enterococcus*		*C. jejuni*		*S. aureus*
	ECOFF µg/ml	Clinical breakpoint µg/ml	ECOFF µg/ml	Clinical breakpoint µg/ml	ECOFF µg/ml	Clinical breakpoint µg/ml	ECOFF µg/ml	Clinical breakpoint µg/ml	Clinical breakpoint µg/ml
Ampicillin	>8	>8	>8	>8	>4	>8			
Azithromycin	>16		>16						
Cefepime	>0.125 [a]	>4	>0.125 [a]	>4					
Cefotaxime	>0.5	>2	>0.25	>2					
Cefotaxime/ clavulansyre	>0.5 [a]		>0.25						
Cefoxitin	>8		>8						>4
Ceftaroline									>1
Ceftazidime	>2	>4	>0.5	>4					
Ceftazidime/ clavulansyre	>2 [a]		>0.5 [a]						
Ceftobiprole									>2
Chloramphenicol	>16	>8	>16	>8	>32				
Ciprofloxacin	>0.064	>0.064	>0.064	>0.5	>4 [a]		>0.5	>0.5	
Clindamycin									>0.5 [b]
Colistin	>2 [a]	>2	>2	>2					
Daptomycin					>4 [a]				>1
Ertapenem	>0.064	>1	>0.064 [a]	>1					
Erythromycin					>4		>4	>4	>2
Fusidic acid									>1
Gentamicin	>2 [a]	>4	>2 [a]	>4	>32 [a]		>2 [a]		>1
Imipenem	>1	>8	>0.5	>8					
Kanamycin									>16
Linezolid					>4	>4			>4
Meropenem	>0.125 [a]		>0.125 [a]	>8					
Mupirocin									>2
Nalidixic acid	>16 [a]		>16 [a]				>16		
Norfloxacin									>4
Penicillin									>0.125
Quinopristin/ Dalfopristin					>1 [c]				
Rifampicin									>0.5
Streptomycin							>4		
Sulfamethoxazole/ trimethoprim									>4
Sulfonamide	>256 [a]		>64 [a]						
Teicoplanin					>2	>2			
Temocillin	>32 [a]		>32 [a]						
Tetracycline	>8		>8		>4	>0.5	>1	>2	>2
Tigecycline	>1 [a]	>2	>1 [a]	>2	>0.25	>0.25			
Trimethoprim	>2	>4	>2	>4					
Vancomycin					>4	>4			

EUCAST epidemiological cut-off values (ECOFFs) and EUCAST clinical breakpoints listed unless noted
a) ECOFF as provided by EFSA [EFSA Supporting publication 2021:EN-6424]
b) Inducible clindamycin resistance included
c) For Quinopristin/Dalfopristin, ECOFF only applies for *E. faecium*

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. This year, DANMAP reports monthly antimicrobial consumption data for the first time to allow analysis of the impact of the Covid-19 pandemic on antimicrobial consumption in humans in 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 2019 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 9.4 New DDDs assigned by WHO Collaborating Centre per January 2019 DANMAP 2020

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 9.5 Danish adjusted DDD for penicillins in the primary sector, 2019 DANMAP 2020

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 9.6 Danish adjusted DDD for main antimicrobials in the hospital sector, 2019 DANMAP 2020

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*, *Ps. 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 diam-

eters <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.

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.

9.10.4 Statistical test

Significance tests for trends in rates of resistance in human bacteria were performed by applying Cochran-Armitage test. One-sided tests were chosen because of preliminary expected trend directions. Cochran-Armitage test calculates probability in binomial proportions across one single, levelled variable. In this report, the test has been performed on susceptibility data from the past 10 and five years. The significance levels were calculated using the DescTools v0.99.19 package in R version 3.5.0. A p-value of ≤0.05 was considered significant. The resulting p-values are reported supplemented by an arrow indicating trend direction. Note that the significance levels serve to support the graphs and thus should be interpreted with caution.

9.11 ESBL-producing 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 investigation Data from The National Patient Register (LPR), information 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 out-

break 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, 20 mg beta-NAD, SSI Diagnostica, Denmark). Isolates, that were found non-susceptible by screening were further analysed for penicillin and erythromycin MICs by broth microdilution using the STP6F plate, Sensititre (Trek Diagnostic Systems, Thermo Scientific) as recommended by the manufacturer. All breakpoints used were as defined by EUCAST (Eucast Clinical Breakpoint Tables v.9.0). Isolates that were either resistant or susceptible increased exposure were categorised together as non-wild-type. For cases, where an isolate was not received at the reference laboratory, susceptibility data could often be found in MiBa.

9.14.4 Data handling

Only cases with isolates from blood or spinal fluid were included in the DANMAP report. Repeated samples within a 30 days window were classified as duplicates and were omitted from the analysis.

9.15 Invasive beta-haemolytic streptococci (group A, B, C and G streptococci) isolates from 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). DNA from all isolates was extracted using DNeasy Blood & Tissue kit as described by the manufacturer (Qiagen, Valencia, CA). Fragment libraries were constructed using the Nextera XT DNA Library Preparation Kit (Illumina, San Diego, CA), followed by 150-bp paired-end sequencing on a NextSeq (Illumina) according to manufacturer's instructions. The sequencing reads were assembled using SKESA [https://github.com/ncbi/SKESA]. The isolates were species typed using Kraken [https://ccb.jhu.edu], and MLST typed using https://github.com/tseemann/mlst.

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 resistance was performed with 1 unit penicillin G discs, 15 µg erythromycin discs and 2 µg clindamycin discs (Oxoid, Denmark) on Mueller-Hinton agar (Mueller-Hinton plate, 5% blood, 20 mg beta-NAD, SSI Diagnostica, Denmark). Isolates were also tested for inducible clindamycin resistance. For non-susceptible streptococci the MIC was determined with ETEST (Biomérieux), with either benzylpenicillin, erythromycin or clindamycin on Mueller-Hinton agar. The breakpoints used were as defined by the EUCAST (EUCAST Clinical Breakpoint Tables v. 10.0).

Isolates that were either resistant or susceptible increased exposure were categorised together as resistant.

9.15.4 Data handling

A case of invasive BHS disease was defined as the isolation of BHS from a normally sterile site (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 (T-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 isolates were serotyped and biotyped. Identification or confirmation of the species *H. influenzae* was based on visual evaluation of colonies, the satellitism test and biochemical reactions. Serotypes were determined by latex agglutination (ImmuLex™ *H. influenzae*, SSI Diagnostika, Denmark). Biotypes were determined by a series of biochemical reactions.

9.16.3 Susceptibility testing

Susceptibility data for the 2020 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, the presence of beta-lactamase encoding plasmids TEM-1 and ROB-1 was identified through whole-genome sequencing performed at the SSI.

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 infection, the first time they were diagnosed with MRSA or when a new subtype was demonstrated. Based on the reported information, MRSA cases were classified as colonisation/active screening (i.e. surveillance samples to detect nasal, throat, gut or skin colonisation), imported infection (i.e. acquired outside Denmark), infection acquired in a Danish hospital, defined as diagnosed >48 hours after hospitalisation with no sign of infection at admittance (HA-MRSA) or infection diagnosed outside hospitals (community onset). MRSA cases with community onset were further classified according to risk factors during the previous 6 months as either health-care associated with community onset (HACO) or community-acquired (CA). Health-care associated risk factors included prior hospitalisations, stay in long-term care facilities and being a health-care worker. Community risk factors included known MRSA-positive household members or other close contacts. Due to the increasing numbers of cases belonging to CC398, this type was treated separately as both epidemiology and relevant exposure are different from other CA cases.

9.18 Gonococci 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% CO2. The breakpoints used were those defined by EUCAST (EUCAST version 10.0).

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.

The MICs of cefixime, gentamicin, and spectinomycin were determined for 110 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. 9.0). A cefinase disc technique was used to examine the isolates for beta-lactamase production.

9.18.4 Data handling

Only one isolate from each unique case of gonorrhoea were included in the DANMAP report. Laboratory demonstration of gonococci in repetitive specimens was considered to represent a new case of gonorrhoea if the specimens were obtained with an interval of more than 21 days.

10

TERMINOLOGY

List of abbreviations

AGP	Antimicrobial growth promoter
AMU	Antimicrobial use
AMR	Antimicrobial resistance
ATC	Anatomical Therapeutic Chemical Classification System
ATCvet	Anatomical Therapeutic Chemical Classification System for veterinary medicines
ATU	Area of Technical Uncertainty
CA	Community-acquired
CC	Clonal complex
CDI	Clostridium difficile infections
CHR	Central Husbandry Register
CPE	Carbapenemase producing Enterobacterales
CPO	Carbapenemase producing organisms
CPR	Danish Civil Registry, register for social security numbers
DAD	Defined Daily Doses per 100 admissions
DADD	Defined Animal Daily Dose
DaDDD	Danish adjusted Defined Daily Doses
DAPD	Defined Animal Daily Dose per 1,000 animals per day
DBD	Defined Daily Doses per 100 occupied bed-days
DCM	Department of clinical microbiology
DDD	Defined Daily Dose
DID	Defined Daily Doses per 1,000 inhabitants per day (DDD/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 [www.whocc.no/ atcddd/ index database].

Defined Daily Dose per 100 admissions (DAD): DAD measures the amount of daily doses consumed per 100 admitted patients at hospitals during a given timeframe (one year). It is used for benchmarking consumption related to the hospital activity and will typically be compared to the consumption measured in DBD (see below). DAD and DBD will generally be applied to comparing individual hospitals and consumption of individual drug classes over time. In DANMAP DAD covers all patients attended at somatic hospitals only. Admission-days are extracted from the National Patient Registry (Landspatientregistret, LPR).

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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