

DANMAP 2016 - Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark

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DANMAP 2016

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



Statens Serum Institut
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DANMAP 2016

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This report is issued by DANMAP - The Danish Integrated Antimicrobial Resistance Monitoring and Research Programme. It presents the results of monitoring the antimicrobial use and antimicrobial resistance in food animals, food and humans in 2016. The report is produced in collaboration between the National Food Institute, Technical University of Denmark and Statens Serum Institut. The DANMAP programme is funded jointly by the Ministry of Health, the Ministry of Environment and Food and the Ministry of Higher Education and Science.

DANMAP 2016

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1. Editorial

For many years Denmark has maintained a strong focus on the transmission of antimicrobial resistance from production animals to humans. It is well documented that a high or irrational use of antimicrobials inevitably will lead to development of resistance and Denmark has introduced several interventions to reduce the antibiotic use in animals to protect public health. The control initiatives in animals include: maintaining awareness of the use of antimicrobials in especially food animals; surveillance of antimicrobial use at farm level, with penalties for farmers who have an unjustifiable high use of antimicrobials; guidelines for use of antimicrobials in pig production and in companion animals, and a farming sector led initiative to drastically reduce the use of 3rd and 4th generation cephalosporins in pigs and cattle.

The contribution from the animal reservoir to AMR in humans varies across the globe. The Danish preventive initiatives on controlling antimicrobial usage in animals continue, and so the most immediate AMR hazard to humans today does not emerge from the Danish animal reservoir. Carbapenemase Producing Organisms (CPO) are resistant to multiple antibiotics used to treat humans, and are carried and spread by healthy individuals. We suspect that the number of healthy carriers in Denmark is increasing, but we do not know for certain. While the organisms themselves often only cause disease in vulnerable patients, the genes can be transferred to other bacteria, contributing to an unknown number of combinations of bacterial species and genes that surpasses the traditionally built surveillance systems.

In DANMAP 2016 we report again that non-travel related carbapenemase resistance in humans occurs with increasing frequency in Denmark, despite a relatively low national consumption of carbapenems. While the prevalence of CPO remains very low in blood isolates from patients with bacteremias - in contrast to the situation that some countries in Southern Europe and the Middle and the Far East are experiencing - several small outbreaks at hospitals have occurred. Carriage of CPO by healthy individuals may inadvertently introduce CPO into environments with high antimicrobial consumption

and a high selective pressure. This highlights the importance of finding places of introduction and routes of transmission at Danish hospitals. A working group under the Danish Health Authority is currently working on guidelines defining risk situations, screening methods and ways of controlling spread at hospitals, it is important to remember that some farm animal environments also may constitute places of possible risks due to locally and periodically high consumptions of antimicrobials. DANMAP has to date not found CPO in animals or food of Danish origin, but the ability of AMR monitoring systems to detect rare and emerging AMR genes with a potentially very high impact needs to be reviewed.

New transmission routes present new challenges for surveillance and control. A novel approach is to look for genes rather than resistant bacterial species as it was done in a recent study, where waste from toilets of airplanes arriving in Denmark from selected destinations was analysed by metagenomics techniques. This contributed to an understanding of the diversity and amount of genes being introduced into a naïve environment. Investigations of the transmission of resistance genes from the hospital setting to the environment and numerous other studies in the use and application of metagenomics have contributed to defining the term: resistome. Looking for the resistome in the individual and in a population as part of the surveillance system is tempting, but raises the questions of what, where and when to look. A new approach like this also demands new diagnostic tools and rapid tests based on gene-analyses. Furthermore, processes for interpretation of results and data capture also need development before such data are applicable to surveillance.

The exact way this information may be used to inform surveillance programmes still needs to be determined, but we look forward to overcoming these challenges to remain at the forefront of surveillance of antimicrobial consumption and resistance with a stable yet dynamic DANMAP.

DANMAP Steering Committee

2. Acknowledgements

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3

INTRODUCTION TO DANMAP

3. Introduction to DANMAP

3.1 About DANMAP

DANMAP was established at the initiative of the Danish Ministry of Health and the Danish Ministry of Food, Agriculture and Fisheries. The programme participants are the National Food Institute and the National Veterinary Institute, both at the Technical University of Denmark (DTU), as well as Statens Serum Institut (SSI). The DANMAP programme is funded jointly by the Ministry of Health, the Ministry of Higher Education and Science, and the Ministry of Environment and Food.

The Danish Integrated Antimicrobial Resistance Monitoring and Research Programme, DANMAP, has implemented the One Health approach, comprising the entire chain from farm to fork to sickbed, since 1995. The organisation and collection of DANMAP data is presented in Figure 3.1.

The objectives of DANMAP are:

- to monitor the consumption of antimicrobial agents in food animals and humans;
- to monitor the occurrence of antimicrobial resistance in bacterial isolates from food animals, food of animal origin (e.g. meat) and humans;
- to study associations between antimicrobial consumption and antimicrobial resistance; and
- to identify routes of transmission and areas for further research studies.

The monitoring of antimicrobial resistance is based on three categories of bacteria:

Human and animal pathogens that cause infections and are thought to reflect resistance caused by the use of antimicrobial agents in the respective reservoirs;

Zoonotic bacteria that can develop resistance in the animal reservoir, which may subsequently compromise treatment outcome when causing infection in humans;

Indicator bacteria (enterococci and *E. coli*) due to their ubiquitous nature in animals, food and humans, and their ability to readily develop or transfer antimicrobial resistance in response to selective pressure in both reservoirs.

All pathogens may be considered reservoirs of resistance determinants - genes - that may be disseminated independently of the bacterial hosts.

A web annex presenting Minimum inhibitory concentration (MIC) distributions, detailed tables of antimicrobial consumption and other additional data are available for download at www.danmap.org. Current and previous DANMAP reports are also available at the website (PDF versions).

Public health risks

Bacteria become resistant either by spontaneous mutation or by transfer of resistance genes from other bacteria. Resistant strains are favoured when use of antimicrobial agents provide a selective pressure. This occurs in humans as well as in animals undergoing antimicrobial treatment. Resistant bacteria can spread between humans in the community, at healthcare centres and at hospitals. Furthermore, resistant bacteria from animals can be transmitted to humans either through direct contact with animals and their environment or through ingestion of contaminated food or other contaminated vehicles.

Antimicrobial treatment failure may occur if the ingested resistant bacteria are a direct cause of disease, or if resistance determinants are transferred to pathogenic bacteria causing the disease. Bacteria may be resistant to several - sometimes all - antimicrobial agents available for treatment, leading to life-threatening illness.

Currently, there is only a limited number of antimicrobial agents, with novel modes of actions, under development by the pharmaceutical industry. Therefore, it is vital for public health organisations to ensure the continued effectiveness of compounds considered critically important to human treatment by ensuring prudent use for both humans and animals.

Prudent use should include the restriction of critical antimicrobial agents for use in humans only, as well as the elimination of overuse, i.e. only humans and animals suffering from an infection responsive to antimicrobial treatment should be exposed to antimicrobial agents.

3.2 General information

The following sections present some general information about the human population in Denmark in 2016, as well as the production of food animals and the amount of meat available for human consumption in Denmark over the past decade. It also provides an overview of the antimicrobial agents for systemic and intramammary therapeutic use in humans and animals in 2016.

3.2.1 Populations

Over the past two decades the human population in Denmark has increased from approximately 5.2 million inhabitants in 1995 to 5.7 million in 2016 (www.dst.dk). The distribution of the population, which could potentially have received antimicrobial treatment in 2016, is shown in Figure 3.2, together with the five healthcare regions and the 10 (merged from 11 during 2016) Departments of Clinical Microbiology (DCM) in Denmark.

The production of food animals and the production of meat and milk are presented in Table 3.1. In 2016, the number of pigs produced was approximately 3% higher than the previous

year and the number of exported fattening pigs (15-50 kg) increased by approximately 9%. Since 2004, the total exports of fattening pigs have increased more than six-fold.

From 2015 to 2016, the number of cattle slaughtered increased by 5%, while the number of dairy cows increased by 2% and the amount of milk produced remained at the same level as in 2015.

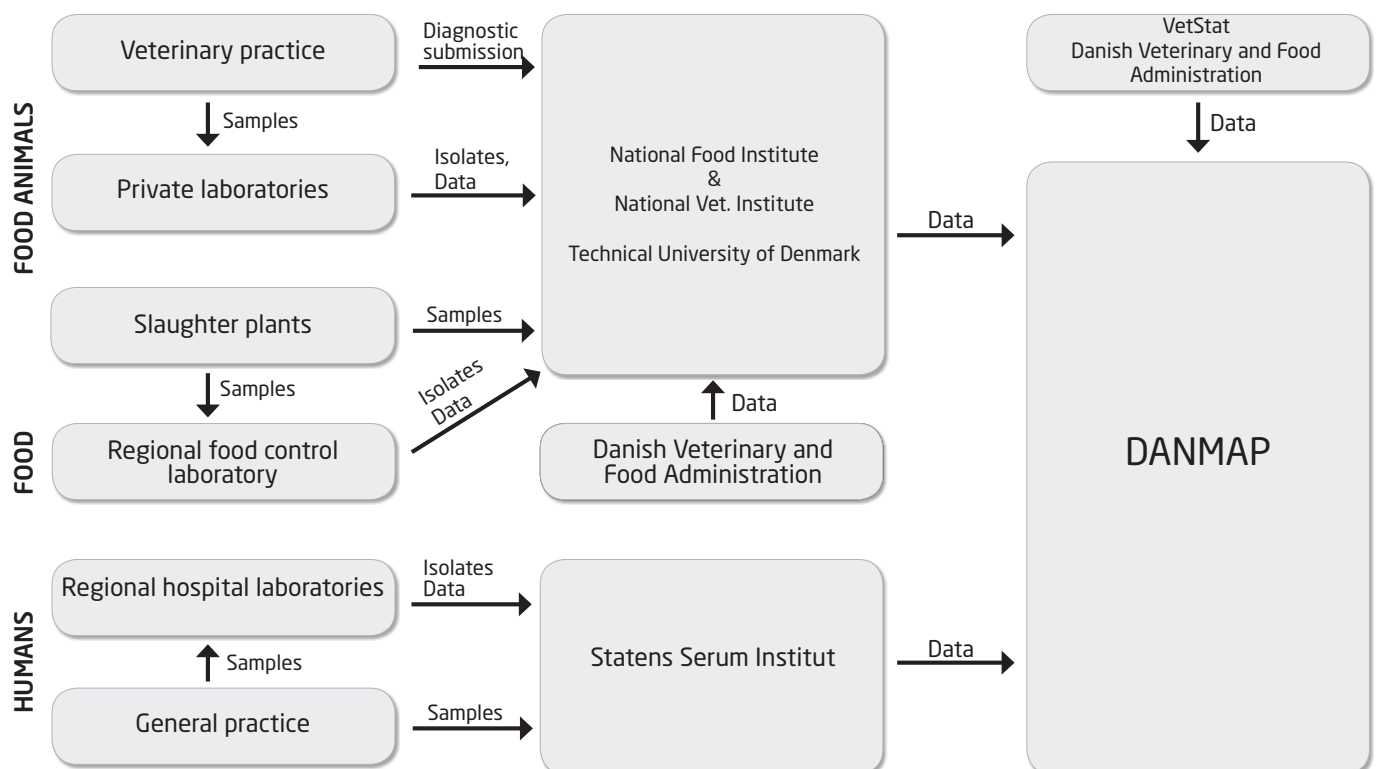
The number of broilers produced increased by approximately 6%, and approximately 19% of the broilers produced in Denmark in 2016 were exported for slaughter. The production of turkeys has fluctuated considerably over the past decade but increased for the first time since 2012. Since 2006, more than 99% of the turkeys produced have been exported for slaughter, thus the majority of turkey meat available for sale in Denmark is listed as imported.

3.2.2 Registered antimicrobial agents

Table 3.2 shows the antimicrobial agents that are registered to treat bacterial infections in humans and animals. Some of these are considered to be critically important for treat-

Figure 3.1. Organisation of DANMAP

DANMAP 2016



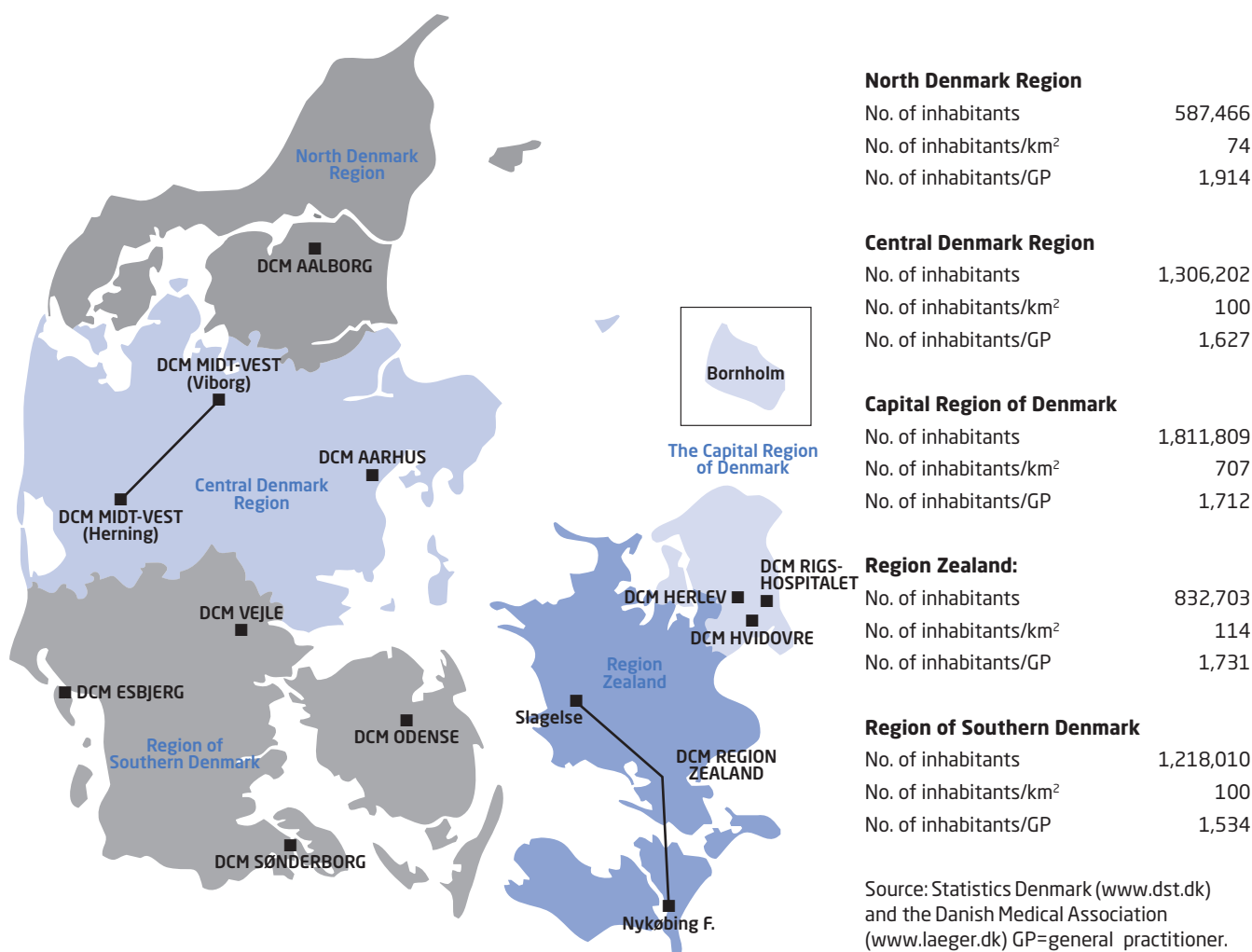
ing bacterial infections in humans. An antimicrobial agent is considered critically important if it is the only compound, or one of a limited number of compounds, available to treat serious human disease. Fluoroquinolones, 3rd and 4th generation cephalosporins, macrolides and glycopeptides are among such agents [AGISAR, 4.revision, WHO 2013]. Critically important antimicrobial agents are also used to treat diseases in food animals and pets, and bacteria that are resistant to critically important agents may be transmitted to humans. Also, bacteria that cause human disease may acquire resistance genes from bacteria of animal origin.

In Denmark, three antimicrobial classes have been appointed as critically important, namely cephalosporins, fluoroquinolones and carbapenems. Extra focus has since 2012 been on the restricted use of these in both animals and human care.

Growth promoters, which are no longer used for animals in Denmark, are shown in parentheses in Table 3.2. Most of these had effects on Gram-positive bacteria. Since 1995, the indicator enterococci from animals and meat (and in some years from healthy humans) have been used as a measure of resistance to growth promoters.

Figure 3.2 The five health care regions and Departments of Clinical Microbiology (DCM) in Denmark

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In April 2016 the DCM Midt-Vest (Herning) and the DCM Midt-Vest (Viborg) were merged with DCM Aarhus.

Table 3.1. Production of food animals and the production of meat and milk, Denmark

DANMAP 2016

Year	Broilers		Turkeys		Cattle (slaughtered)		Dairy cows		Pigs	Farmed fish ^(a)			
	1,000 heads	mill. kg ^(b)	1,000 heads	mill. kg	1,000 heads	mill. kg	1,000 heads	mill. kg milk		1,000 heads	Export 1,000 heads ^(c)	mill. kg	Fresh water
1990	94560	116	571	2,5	789	219	753	4542	16425	-	1260	-	-
1992	107188	137	761	5,4	862	236	712	4405	18442	-	1442	35	7
1994	116036	152	1091	8,6	813	210	700	4442	20651	-	1604	35	7
1996	107895	149	961	9,3	789	198	701	4494	20424	-	1592	32	8
1998	126063	168	1124	11,6	732	179	669	4468	22738	-	1770	32	7
2000	133987	181	1042	10,3	691	171	636	4520	22414	-	1748	32	7
2001	136603	192	1086	13,2	653	169	623	4418	23199	-	1836	31	8
2002	136350	190	1073	12,8	668	169	611	4455	24203	-	1892	32	8
2003	129861	197	777	11,2	625	161	596	4540	24434	-	1898	34	8
2004	130674	198	1086	19,6	632	165	569	4434	25141	1712	1967	34	9
2005	122179	183	1237	17,4	549	145	559	4449	25758	2720	1988	31	8
2006	106182	161	785	11,3	509	140	556	4492	25763	3204	1957	29	8
2007	107952	163	1009	14,4	512	141	545	4515	26311	3522	2046	31	10
2008	107595	163	1068	12,3	509	138	559	4585	27078	4943	1985	30	10
2009	108851	165	1175	11,1	507	137	569	4734	27603	6642	1898	29	11
2010	117653	178	1184	14,0	519	142	574	4830	28505	7074	1974	28	10
2011	115454	175	960	9,4	551	145	575	4801	29399	7632	2008	28	11
2012	111080	168	1103	12,4	539	138	580	4928	29047	8794	1902	28	13
2013	117315	177	692	8,3	551	140	574	5025	28996	9318	1896	28	12
2014	115497	174	595	8,8	556	143	563	5113	29926	10517	1924	30	11
2015	114238	172	598	8,8	513	135	561	5270	30874	12133	1954	32	12
2016	120685	182	834	9,9	540	142	571	5276	31660	13280	1943	32	12
Diff.(d)	6%	6%	39%	13%	5%	5%	2%	<1%	3%	9%	-1%	0	0

Source: Statistics Denmark (www.dst.dk) and The Danish AgriFish Agency. Production data for farmed fish was not available for 2016. Live animals exported prior to slaughter are included in number of animals and amount of meat produced. 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) The numbers for 2016 are not final. The production of farmed fish includes fish transferred from one production facility to another

b) Assume a final slaughtered weight of 1.51 kg per broiler produced (Danish Agriculture and Food, 2013)

c) 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

d) Difference from 2015 to 2016

Table 3.2. Antimicrobial agents registered for systemic and veterinary intramammary therapeutic use in animals and humans, Denmark

DANMAP 2016

ATC / ATCvet codes ^(a)	Therapeutic group	Antimicrobial agents within the therapeutic groups	
		Animals	Humans
J01AA / QJ01AA,QJ51AA	Tetracyclines	Chlortetracycline, doxycycline, oxytetracycline	Doxycycline, lymecycline, tetracycline, tigecycline
QJ01BA	Amphenicols	Florfenicol	
J01CA / QJ01CA	Penicillins with extended spectrum	Ampicillin, amoxicillin	Ampicillin, pivampicillin, amoxicillin, pivmecillinam, mecillinam
J01CE / QJ01CE	Beta-lactamase sensitive penicillins	Benzylpenicillin, phenoxymethylpenicillin, procaine penicillin, penethamate hydroiodide	Benzylpenicillin, phenoxymethylpenicillin
J01CF / QJ51CF	Beta-lactamase resistant penicillins	Cloxacillin, nafcillin	Dicloxacillin, flucloxacillin
J01CR / QJ01CR	Comb. of penicillins and beta-lactamase inhibitors	Amoxicillin/clavulanate	Amoxicillin/clavulanic acid, piperacillin/tazobactam
J01DB / QJ01DB,QJ51DB	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, ceftobiprol, ceftolozan/tazobactam
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, sulfamethoxazol/trimethoprim	Sulfamethoxazole/trimethoprim
J01FA / QJ01FA	Macrolides	Spiramycin, tylosin, tilmicosin, tylvalosintartrat, tulathromycin, gamithromycin, tildiprocin	Erythromycine, roxithromycine, clarithromycine, azithromycine, telithromycine
J01FF / QJ01FF	Lincosamides	Clindamycin, lincomycin	Clindamycin
QJ01XX ^(b)	Streptogramins	(Virginiamycin)	
J01GB / QJ01RA,QA07AA	Aminoglycosides	Streptomycin, dihydrostreptomycin, gentamicin, neomycin, apramycin	Tobramycin, gentamicin
J01MA / QJ01MA	Fluoroquinolones	Enrofloxacin, marbofloxacin, difloxacin, ibafloxacin, pradofloxacin	Ciprofloxacin, levofloxacin, moxifloxacin
QJ01MB	Other quinolones	Oxolinic acid	
QJ01MQ ^(b)	Quinoxalines	(Carbadox, olaquinox)	
J01XA,A07AA / Not in ATCvet ^(b,c)	Glycopeptides	(Avoparcin)	Vancomycin, teicoplanin, telavancin, dalbavancin, oritavancin
J01XB / QA07AA ^(b)	Polypeptides (incl. polymyxins)	Colistin, bacitracin	Colistin
J01XC	Steroid antibacterials		Fusidic acid
J01XD,P01AB ^(c)	Imidazole derivatives		Metronidazole
J01XE	Nitrofurane derivatives		Nitrofurantoin
J01XX / QJ01FF	Other antibacterials	Spectinomycin	Methenamine, linezolid, daptomycin, tedizolide, fosfomicin
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 anti-infectives (A07AA) and imidazole derivatives for protozoal diseases (P01AB) were, for the first time, included in DANMAP 2014, since their widespread use in the treatment of *Clostridium difficile* infections makes them belong to the most used antibiotics in human infections in Denmark



4

ANTIMICROBIAL CONSUMPTION IN ANIMALS

4. Antimicrobial consumption in animals



Highlights: The overall use of antimicrobials for animals decreased in 2016, for the third consecutive year, by approximately 5% compared with 2015. The decrease was mainly driven by the use of antimicrobials for pigs, which was 4% less than the year before. Particularly the use of tetracyclines for pigs has been reduced consistently since 2013.

Following on from two years with several serious disease outbreaks, the poultry production (excl. turkeys) sharply reduced antimicrobial use in 2016, returning to the same levels as before the disease outbreaks.

Use of antimicrobials in the aquaculture industry continued to decrease in 2016, where we recorded the lowest level of antimicrobial use in a decade.

In contrast, the fur animal industry continued to see an increase in antimicrobial use. Two studies have found an association between the quality of feed and the amount of antimicrobial agents prescribed (Textbox 4.3). The studies raise awareness of its importance in relation to fur animals' need for treatments.

The use of critically important antimicrobials in food production animals remained low. The use of colistin for pigs increased by further 40 kg in 2016. The use of colistin in production animals is of concern, since it has become increasingly important as a last resort antimicrobial in human medicine. The increase in colistin use for pigs has likely been caused by a shift from other antimicrobial agents (Textbox 4.2).

In companion animals, the use of critically important antimicrobials is relatively high compared with other species. Almost all fluoroquinolones and more than half of the cephalosporins used for animals are used for dogs and cats. Despite a small increase in use from 2015 to 2016, there has been an overall decreasing trend in the use of antimicrobials for dogs and cats since 2011. Furthermore, there has been a shift in the use of antimicrobials with a marked reduction in the relative use of cephalosporins and increase in the use of penicillins.

4.1 Introduction

The use of antimicrobial agents in humans and animals has been monitored by the DANMAP programme since 1995. Since the early 1990s, there has been both political and public focus on the use of antimicrobial agents in the Danish animal production. This resulted in discontinued use of several antimicrobial agents used for growth promotion from 1994-1999, and more recently, in a voluntary ban of use of cephalosporins in the pig and dairy cattle production, as well as in regulatory legislation regarding therapeutic use [DANMAP 2010].

Figure 4.1 shows the total use of antimicrobials for animals and humans since 1994 and 1997, respectively. Changes in the antimicrobial consumption patterns for animals can partly be explained by an increase in pig production. However,

national control initiatives to reduce consumption have clearly influenced the prescription and antimicrobial usage patterns for animals during the period. 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 preventive veterinary strategies with Veterinary Advisory Service contracts (VASCs) and regular monthly 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], which limits the use of (cheaper) extemporaneously produced medicines. The latter particularly affected the use of tetracyclines from 1994. Another important intervention was the restriction on the use of fluoroquinolones in production animals through legislation implemented in 2002 and

2003. Furthermore, in July 2010, the pig industry imposed a voluntary ban on the use of cephalosporins. This was followed by a similar initiative by dairy cattle farmers in July 2014.

From 2010 to 2011, consumption decreased following the introduction of threshold values for antimicrobial consumption adopted within the “yellow card initiative”. This enforces legal actions on pig farmers with high antimicrobial use per pig [DANMAP 2010]. Effects from other parts of the legislation may be less obvious but are also likely to have affected prescription patterns. The “yellow card” principles were revised in 2016 and are described in Textbox 4.1

Official guidelines regarding the selection of antimicrobial agents for pigs and cattle have been available since 1996. The guidelines provide specific recommendations for selection of the appropriate antimicrobial treatment of all common indications in the major production animal species. Initially, guidelines were developed by the National Veterinary Laboratory (presently, National Veterinary Institute, DTU). Since 2005, the guidelines have been updated by the Danish Veterinary and Food Administration (DVFA) in collaboration with stakeholders and university experts. The latest update was in 2010, when new dynamic evidence based treatment guidelines for pigs were launched [DANMAP 2010, www.fvst.dk]. In 2012, the Danish Veterinary Association published treatment guidelines to promote prudent use of antimicrobials in dogs and cats. The

guidelines were prepared by clinical specialists and expert scientists from the Faculty of Health and Medical Sciences at the University of Copenhagen and National Food Institute DTU. The treatment guidelines for dogs and cats will be revised in 2017.

4.1.1 Data sources

Data on antimicrobial use at the product level have been collected in Denmark since 1996, including historical data back to 1990. In Denmark, all therapeutic antibiotics are available by prescription only.

Since 2000, data on all medicine prescribed for use in animals, including vaccines, antimicrobial growth promoters (no longer permitted) and coccidiostatic agents (non-prescription) have been collected in the national database VetStat. The VetStat database is hosted and maintained by Danish Veterinary and Food Administration (DVFA). Data in VetStat are validated by the DVFA.

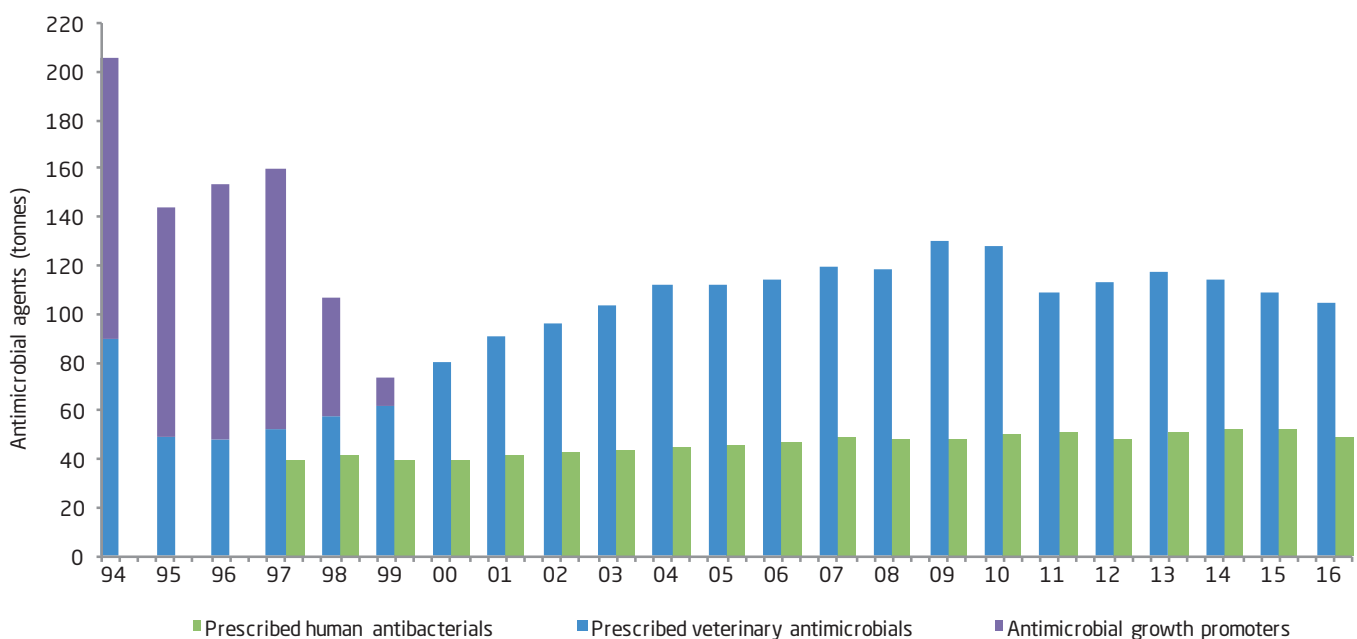
The data presented in this report were extracted from VetStat on 3rd March and 22nd August 2017. Data have been summarized for DANMAP by DTU National Food Institute.

4.1.2 Methods

Metrics of antimicrobial consumption are numerous, each with its own advantages and limitations. Therefore, the selection of

Figure 4.1. Prescribed antimicrobial agents for humans, and for all animal species, Denmark

DANMAP 2016



Sources: Human therapeutics: The Danish Health Data Authority. Antimicrobials for animals: Until 2001, data are based on reports from the pharmaceutical industry of total annual sales from the Federation of Danish pig producers and slaughterhouses (1994-1995) and Danish Medicines Agency and Danish Plant Directorate (1996-2000). Data from 2004-2016 are based on data extracted from VetStat (3rd March and 22nd August 2017) and includes all antimicrobial agents registered for use in animals.

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 and to enable international comparisons (Figure 4.1).

In DANMAP 2012, we introduced two new metrics to monitor trends in antimicrobial consumption to ensure the robustness of

the analyses over time and to facilitate comparisons between animal species, as well as comparisons between the veterinary and human sectors. The new metrics are defined below, and for additional information on methodology, please refer to Chapter 9 and the web annex [www.Danmap.org].

DADD (Defined animal daily dose)

DADD is the average maintenance dose per day for the main indication of a drug in the appropriate animal species. The DADD is not defined at product level but for each antimicrobial agent, administration route and animal species; and when

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

DANMAP 2016

ATCvet code	QJ01B	QJ01G	QJ01D	QJ01MA	QJ01FF	QJ01FA	QJ01MB	QJ01CE	QJ01CA	QJ01XQ	QJ01E	QJ01AA			
Therapeutic group	Amphenicols	Aminoglycosides	Cephalosporins	Fluoroquinolones	Lincosamides	Macrolides	Other quinolones	Penicillins, b-lactamase sensitive	Penicillins, others(a)	Pleuromutilins	Sulfonamides and trimethoprim	Tetracyclines	Other AB	Total 2015	Total 2016
Pigs	371	4163	<1	<1	1875	10113	0	15588	8286	7820	6615	22453	864	81499	78150
Sows and piglets	258	1666	<1	<1	436	528	0	8331	3512	999	4770	1698	102	23366	22300
Weaners	106	2357	<1	0	734	6665	0	1680	3961	2868	1594	13733	752	34985	34449
Finishers	7	140	<1	<1	705	2920	0	5578	813	3953	251	7022	10	23148	21401
Cattle	656	742	66	<1	3	273	0	7719	809	0	1038	1604	10	13150	12920
Intramammaries	0	31	55	0	1	0	0	257	147	0	6	0	<1	503	498
Cows and bulls excl. intramammaries	12	240	10	<1	1	114	0	6862	497	0	893	999	<1	10244	9629
Calves <12 months	635	445	1	<1	<1	155	0	467	152	0	128	567	9	2241	2561
Heifers and steers	8	26	<1	0	<1	4	0	133	13	0	10	37	<1	162	232
Poultry	5	60	0	<1	24	153	0	236	218	<1	111	748	6	2440	1560
All poultry excl. turkeys	<1	36	0	<1	18	103	0	168	97	<1	106	414	0	1931	942
Turkeys	4	24	0	0	6	50	0	67	121	0	5	334	6	509	619
Other production animal species	315	325	<1	<1	160	789	893	1	2054	0	2284	795	<1	8146	7617
Aquaculture	314	0	0	<1	0	0	893	0	11	0	1071	<1	0	2969	2289
Fur animals	2	325	0	0	160	789	0	1	2043	0	1213	794	0	5177	5327
Companion animals ^(b)	1	15	137	14	69	3	0	29	717	<1	1393	45	31	2312	2458
Pets (estimated) ^(c)	<1	14	137	14	69	3	0	20	717	<1	276	40	31	1245	1326
Horses (estimated)	<1	<1	<1	<1	0	<1	0	8	<1	0	1117	5	<1	1067	1131
Other ^(d)	5	175	3	3	7	22	<1	766	245	11	283	130	1	2194	1647
Total	1353	5480	206	17	2139	11353	892	24339	12329	7832	11724	25775	913	109741	104352

Note: Data for 2015 and 2016 were extracted from VetStat 22 August 2017. Only the ATCvet group contributing mostly to the antimicrobial group is mentioned. Combination drugs are divided into active compounds

a) Penicillins with extended spectrum and combination penicillins, incl. b-lactamase inhibitors

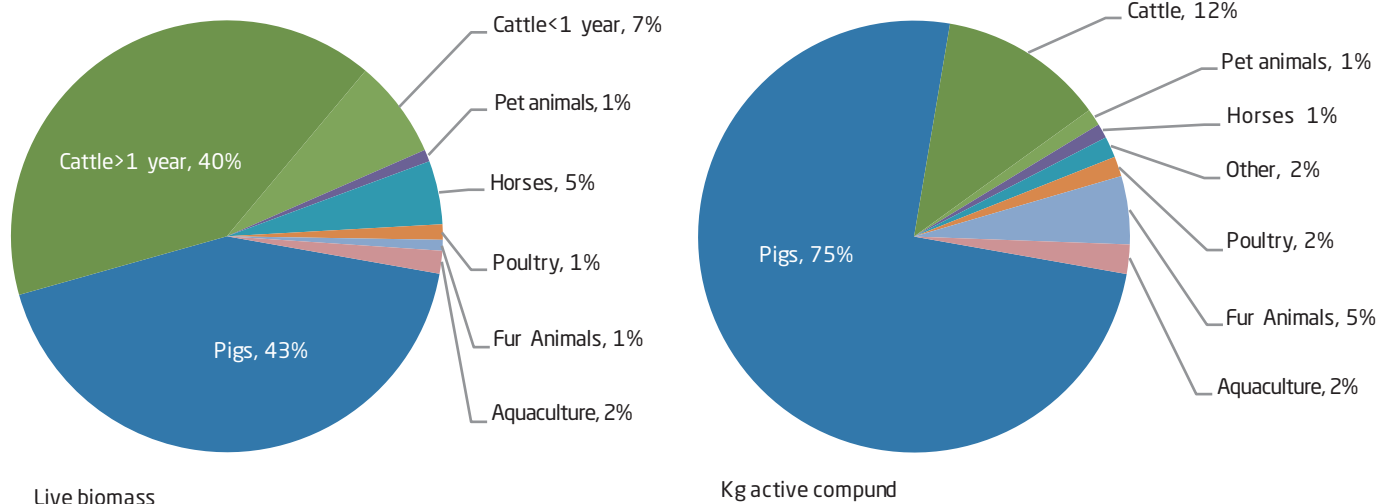
b) In DANMAP 2016, new principles have been applied to estimate the antimicrobial use for companion animals, please see section 4.3.4

c) Approximately 222 kg of the sulfonamides and trimethoprim registered for pets are products (oral paste) typically used for horses

d) This includes data on antimicrobial agents used for sheep and goats (14 kg), data where the animal species has not been defined or where the age group does not apply to the designated animal species

Figure 4.2. Live biomass (mill. kg) and antimicrobial consumption (kg) in main animal species, Denmark

DANMAP 2016



Note: The live biomass is estimated from census data (pigs, cattle and pet animals) and production data (poultry, fur animals and aquaculture). For poultry: the figures comprise only the biomass for the main production types (turkeys and broilers). The live biomass estimates for cattle, poultry, aquaculture, fur and pet animals are based on 2012 data. The estimation procedures are described in Chapter 9, Materials and Methods

appropriate, also age group. The DADDs have been specifically defined for use in DANMAP based on current knowledge and may vary from the prescribed daily dose or the recommended dosage in the Summaries of Product Characteristics (SPC) or in the VetStat database.

DAPD-proportion of population in treatment per day

Trends in antimicrobial usage for animals, both within and across species, are presented in DAPD, because this measure allows for comparison between sectors. DAPD=DADD per 1000 animals per day, where ‘animals’ are represented by their live biomass and adjusted for life-span. 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’, and allows comparison between species with large differences in body-mass and life-span.

DAPD, or estimated treatment proportion, is a statistical measure that provides a rough estimate of the proportion of animals treated daily with a particular antimicrobial agent. For example, 10 DAPDs indicate that an estimated 1% of the pig population, on average, receives a certain treatment on a given day (Section 4.3 and Chapter 9, Materials and Methods). DAPD also allows comparisons with the antimicrobial consumption in the human sector, which is measured in defined daily dose per 1,000 inhabitants per day (DID), see Chapter 10, Terminology, for a description of DID.

4.2 Total antimicrobial consumption in animals

Measured in kg active compound, the total use of antimicrobial agents used for all animals, amounted to 104.4 tonnes active compound, representing an overall 5% decrease compared with 2015, Figure 4.1 and Table 4.1.

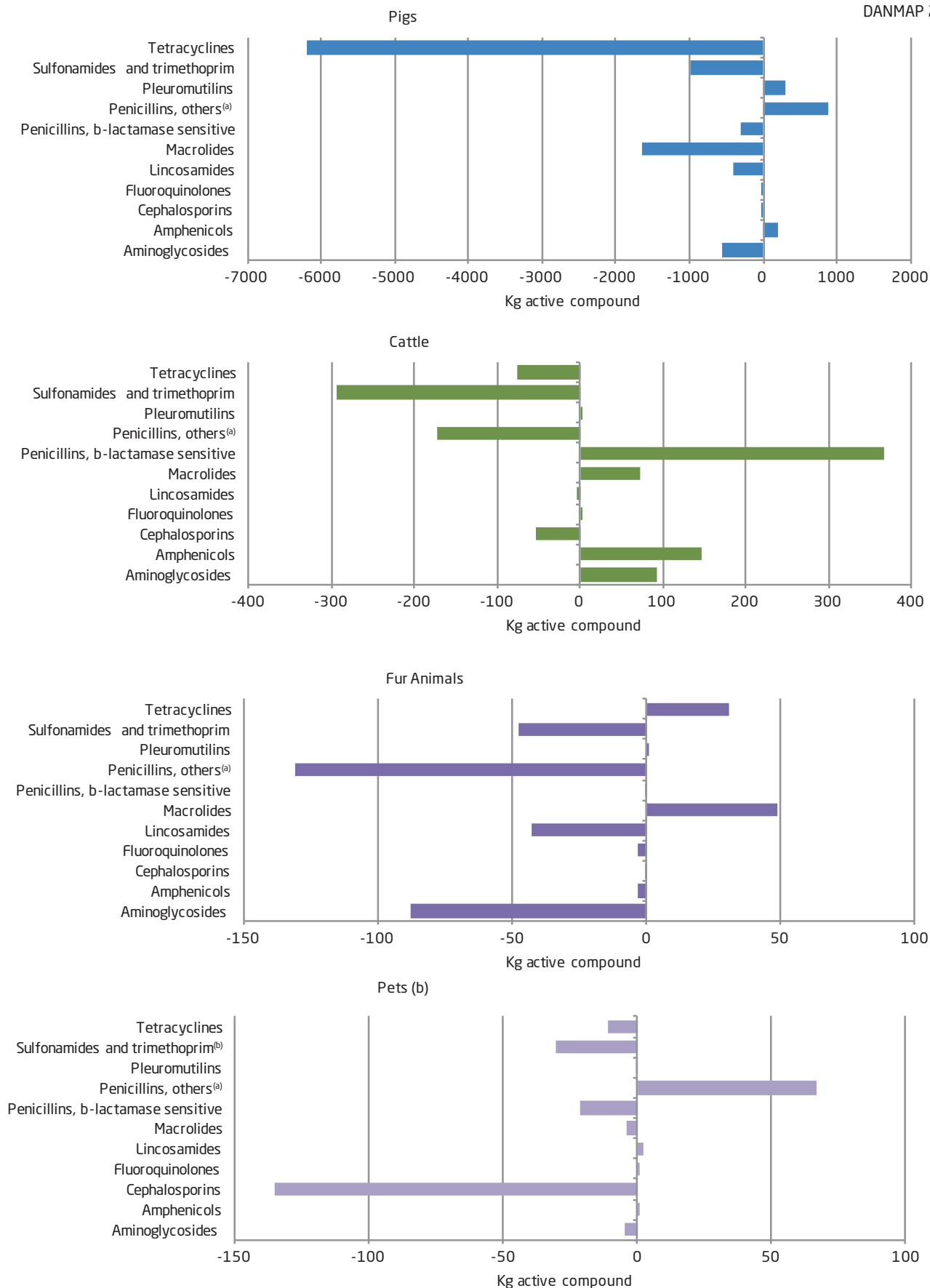
In 2016, the antimicrobial use for pigs, cattle, fur animals and poultry comprised approximately 75%, and 12%, 5% and 2% of the total antimicrobial consumption for animals, respectively (Figure 4.2). The decrease in antimicrobial use for animals was mainly attributed to a 3% decrease in the amount used in the pig industry, which is the main driver of antimicrobial consumption in animals in Denmark, due to the size of the industry. Cattle and pigs are the two major production species in Denmark and they comprise almost equal proportions of live biomass. However, the vast proportion of cattle biomass consists of dairy cows, which have very low consumption of antimicrobial agents compared with growing animals.

Historically, the overall consumption, measured as kg active compound, was 49% lower in 2016 compared with 1994 (Figure 4.1). In contrast, the total meat production increased by 15% during this period (Table 3.1). A major part of the decrease in consumption can be explained by the discontinued use of growth promoters from 1994 to 1999.

Between 2000, when antimicrobial consumption was first registered in VetStat, and 2009, when it was at its highest, the amount of kg active compound increased by 62% (Figure 4.1). This increase was driven mainly by consumption in pigs, and during this period the number of pigs produced went up by 23% (Table 3.1). At the same time, the proportion of exported live pigs (approx. 30 kg) increased and thus resulted in a decrease in the overall biomass of the pig population. Since then, the antimicrobial consumption for animals has gradually decreased and in 2016 it was 20% lower than in 2009.

Figure 4.3. Change in antimicrobial use (kg active compound) in pigs, cattle, fur animals and pets (dogs and cats) 2012 - 2016, Denmark

DANMAP 2016



Note: The figure includes the antimicrobial agents registered for use in the particular animal species. Note also the different scale on the x-axis. Poultry has not been included in the figure, since several serious disease outbreaks in 2014 and 2015 have caused considerable fluctuations in the antimicrobial consumption for poultry over the past three years, see Table 4.1 and section 4.3.3

a) Penicillins with extended spectrum and combination penicillins, incl. b-lactamase inhibitors

b) The use of antimicrobial agents for pets (dogs and cats) has been estimated as described in section 4.3.4

Figure 4.3 illustrates the changes in usage in pigs, cattle, fur animals and pets over the past five years. With the exception of fur animals, the changes in the antimicrobial use within the different animal species have been clearly affected by the different campaigns to reduce the total use and to reduce the use of particular antimicrobial classes. In particular, the use of tetracyclines for pigs has been reduced significantly, the usage patterns in cattle has shifted towards more penicillins and less sulfonamides and trimethoprim, amphenicols and cephalosporins and in dogs and cats the use has shifted away from the cephalosporins.

4.3 Antimicrobial consumption by animal species

For this issue of DANMAP, updated data from 2004-2016 were extracted from VetStat¹ and all measures for the antimicrobial usage in pigs were calculated for all years, using the updated dataset.

4.3.1 Antimicrobial consumption in pigs

In 2016, the total antimicrobial consumption in pigs (sows and piglets, weaners, finishers) was 78.2 tonnes active compound (Table 4.1), a decrease of 3.3 tonnes (4%) compared with 2015.

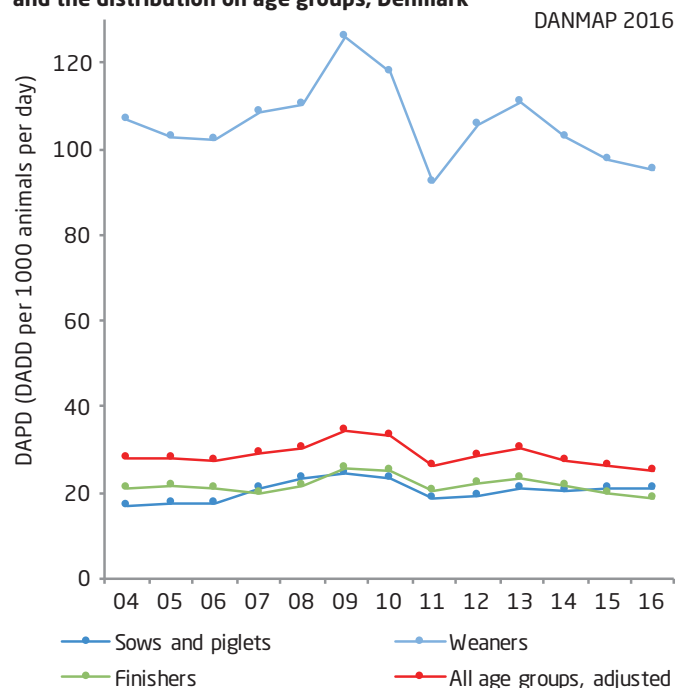
The treatment proportions (DAPD) in the pig population overall and by age group are presented in Figure 4.4 and Figure 4.5 and the DADD's are shown in the webannex (TableA4.1 and in the DADD description).

The treatment proportion (DAPD) of the total population reflects the trends in selection pressure within the population. However, the DAPD is much higher in the weaning pigs than in finishers and sows (Figure 4.4). Furthermore, the biomass of the weaning pigs is also very small (7.5- 30 kg, 4 weeks), compared with the finishers (31-107 kg, 12 weeks) and the sows. The large differences in DAPDs between age groups affects the DAPD of the total population and trends are influenced by changes in population structure. Thus, significant changes in export or productivity need to be accounted for, before interpreting the antimicrobial consumption patterns and selection pressure in the pig production. As an example, increased export of live pigs right after weaning could lead to an increase in DAPD for the remaining population, since the exported pigs were only in the country, while the treatment proportion was highest.

Historically, the treatment proportion (DAPD) increased from 2004 to 2009, followed by a decrease in 2010 and 2011, which is considered a result of the "yellow card initiative" (See DANMAP 2010).

In 2016, the antimicrobial consumption in pigs, measured in DAPD, decreased by 5% to approximately 25 DAPD (Figure 4.4) when adjusted for changes in export. Overall the number of pigs produced in 2016 increased by 3%, and the number of

Figure 4.4. Antimicrobial consumption (a) in the pig production, and the distribution on age groups, Denmark



Note: The "all age groups adjusted" is adjusted for the increasing export of pigs at 30 kg (see text). "Sows" includes treatment in boars and piglets pre-weaning

a) The DAPD is 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)

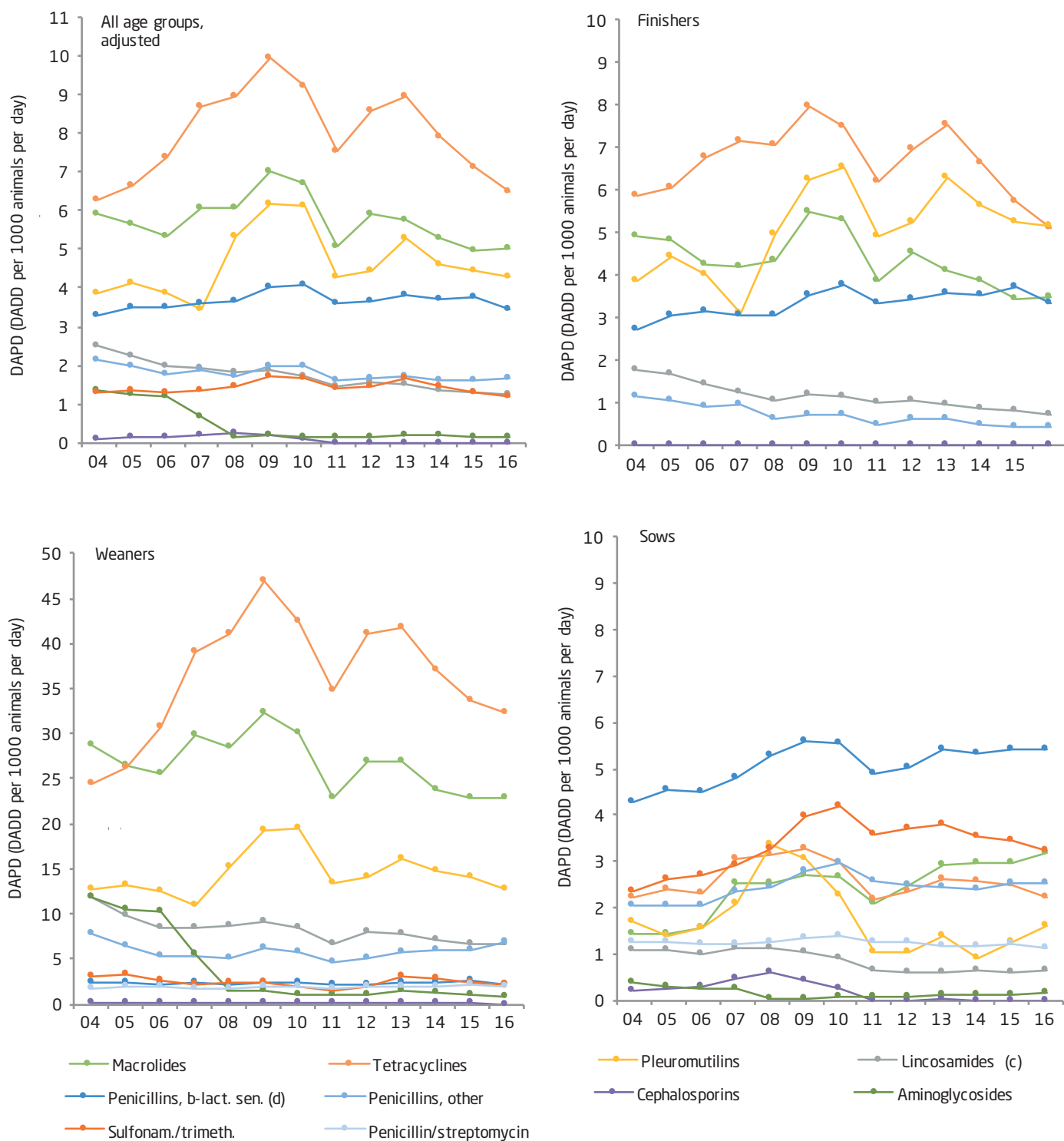
pigs exported increased by 9% (Table 3.1). Within the different age groups, the treatment proportions decreased in weaners and finishers, but remained at the same level for sows and piglets (Figure 4.4). Thus, on average, on a given day in 2016, approximately 2% of sows and piglets, 1-2% of finisher pigs and 10% of weaner pigs were under treatment with antimicrobial agents.

Also measured in DAPD, the antimicrobial use in pigs was 27% lower in 2016 than in 2009, when adjusted for changes in export (Figure 4.4). Overall, the decrease was primarily seen for tetracyclines, pleuromutilins and beta-lactamase sensitive penicillins (Figure 4.5). Tetracycline has been one of the most commonly used antimicrobials in the Danish pig production for more than a decade. It is almost exclusively administered orally, and is especially used for treatment of gastrointestinal disease in weaning pigs and finishers. The overall use of tetracyclines in the pig production fluctuated from 2009 to 2013, but has since then decreased and 2016 saw the lowest DAPD levels since 2005. Measured in DAPD, the use of tetracyclines, for all age groups was reduced by 8% from 2015 to 2016 and has decreased 35% since 2009. The proportion of weaner pigs under treatment with tetracycline, on average, on a given day has decreased from approximately 5% in 2009, to approximately 3% in 2016

¹ Data extracted from VetStat 3rd March and 22nd August 2017

Figure 4.5. Antimicrobial consumption^(a) in the total pig production^(b), and in finishers, weaners, sows and piglets, Denmark

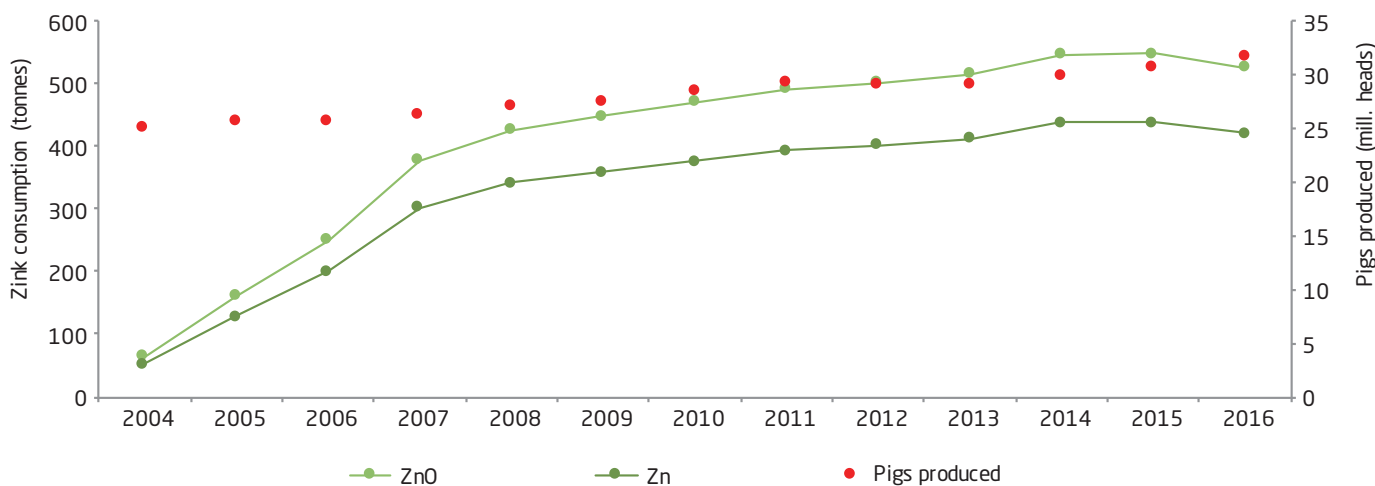
DANMAP 2016



Note: The figure includes all antimicrobial agents registered for use in pig and in specific age-groups. Amphenicols, colistin, fluoroquinolones, intramammaries, gynecologicals and topical drugs are not included in the figure

- a) The DAPP is 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)
- b) The total is adjusted for the increasing export of pigs at 30 kg (see text). "Sows" includes treatment in boars and piglets pre-weaning
- c) Lincosamide/spectinomycin combinations comprise 65% of this group
- d) Beta-lactamase sensitive penicillins

Figure 4.6 Consumption (tonnes) of zinc oxide (ZnO) and zinc (Zn) in the pig production, Denmark DANMAP 2016



While the overall consumption of antimicrobial agents for pigs has decreased almost consistently since 2009, the use of colistin for pigs has increased more than two-fold from 407 kg in 2009 to 864 kg in 2016, of which 752 kg were used for weaners. Although the use of colistin constitutes a very small fraction of the overall antimicrobial consumption in pigs (approximately 1% in 2016), the increase has potential implications on human health, since polymyxins, to which colistin belongs, have found a new role for treatment of carbapenemase resistant infections in human medicine, see also Textbox 4.2 concerning colistin.

Of the critically important antimicrobial agents, the use of fluoroquinolones and cephalosporins was close to zero in 2016.

Consumption of zinc oxide and zinc in the pig production

Zinc is relevant in the context of DANMAP, because its use may select for antimicrobial resistance in some bacteria, including MRSA. Medicinal zinc, in the form of zinc oxide, is fed to piglets after weaning to prevent or treat diarrhoea. Figure 4.6 shows the use of medicinal zinc in Danish pig production. The most commonly used product is zinc oxide (ZnO) which contains 80% zinc and which is largely insoluble in water.

For more than a decade the consumption of zinc has increased steadily, reaching a peak in 2015. However, in 2016 we observed a 4% decrease, equivalent to approximately 23 tonnes of zinc oxide or 18 tonnes of zinc, compared with 2015. Since DANMAP 2015, a number of errors were identified in VetStat data on zinc consumption. This has been adjusted and Figure 4.6 shows the updated data on consumption of medicinal zinc in pigs and reflects the situation better than figure 4.6 in DANMAP 2015.

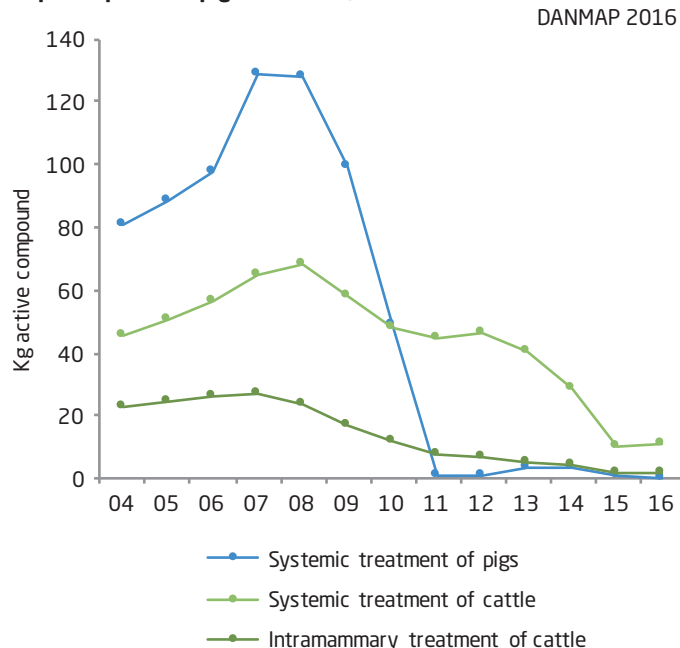
4.3.2 Antimicrobial consumption in cattle

In 2016, the overall consumption of antimicrobials in cattle decreased by approximately 3% (363 kg) compared with 2015. The production of veal and beef remained more or less at the same level from year to year, while milk production continued to increase (Table 3.1).

The use of fluoroquinolones in cattle has been low for the last decade and no use of fluoroquinolones was reported for cattle in 2016.

Approximately 11 kg of cephalosporins (all generations) were used systemically, which represents a 61% reduction since 2014, when the dairy association decided to phase out its use,

Figure 4.7. Consumption of 3rd and 4th generation cephalosporins in pigs and cattle, Denmark DANMAP 2016



and an 83% reduction compared with 2008, when cephalosporin consumption was at its peak. The use of 3rd and 4th generation cephalosporins is low in cattle and mostly used for systemic treatment (Figure 4.7).

The majority of antimicrobials administered parenterally for cattle are used for dairy cows (Table 4.1), and mainly prescribed for mastitis. From 2005 to 2013 there was a slight reduction in the overall level of intramammary treatment. Following an increase in 2014 the use of intramammary treatment has decreased again in 2015 and 2016 (Table 4.2).

Since 2014, the Danish dairy industry (the Danish Agriculture and Food Council) has had a strategy for reducing the amount of antimicrobials used for treatment of mastitis by 20% compared to the 2012 level. From 2015 to 2016, the overall use

of antimicrobials for cattle decreased by approximately 2% (corresponding to 230 kg).

Order (DK) 785/2010 provides legal regulations of use of antimicrobial agents for mastitis in cattle and the industry has emphasized that farmers should use narrow spectrum penicillins to treat mastitis caused by Gram-positive bacteria, unless sensitivity testing reveals resistance towards these antimicrobials. The overall use of intramammary treatment, measured in DADDs, decreased from 2010 to 2011, but has increased again after 2013 (Table 4.2). However, it is notable that the use of 3rd and 4th generations cephalosporins has been reduced significantly and the relative proportion of drying-off treatment versus therapeutic treatment has shifted markedly from 22% versus 78% in 2010 to 55% versus 45% in 2016 (Table 4.3).

Table 4.2. Use of antimicrobial agents for intramammary application in cattle in DADD's (1000s), Denmark

DANMAP 2016

	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
Doses per antimicrobial class	DADDs (1000's)											
Penicillins(a)	201	211	211	236	282	314	318	324	311	317	275	262
Aminoglycoside-benzylpenicillin combinations(b)	130	104	101	101	110	93	48	47	58	90	143	177
Cephalosporins, 1st generation	103	98	89	85	89	89	99	105	111	113	96	89
Cephalosporins, 3rd and 4th generation	110	124	127	112	76	51	34	30	24	21	10	9
Others(c)	21	20	16	15	14	12	9	8	0	0	11	10
Total	566	558	544	549	570	559	508	514	504	541	535	547
Total DADD per cow per year	1,0	1,0	1,0	1,0	1,0	1,0	0,9	0,9	0,9	1,0	1,0	1,0

Note: For intramammary treatment, 1 DADD is defined as the dose to treat two teats for 24 hours

a) Includes benzylpenicillin, cloxacillin, and cloxacillin-ampicillin combinations (QJ51CE, QJ51CF, QJ51RC)

b) Mainly dihydrostreptomycin-benzyl benicillin combinations; includes also combinations of penicillin/aminoglycoside with bacitracin or nafcillin (QJ51RC)

c) Lincosamides, neomycin-lincomycin combinations and trimethoprim-sulfonamide combinations

Table 4.3. Number of treatments with antimicrobial agents for intramammary application in cattle, Denmark

DANMAP 2016

	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
Total doses per indication (a)	DADDs (1000's)											
Drying off treatment (4 teats)	73	75	71	76	82	99	97	117	125	140	154	174
Therapeutic treatment (2 teats)	420	408	388	377	378	350	307	279	253	259	227	199

Note: Includes data for Intramammaries registered for use in cattle. For intramammary therapeutic treatment, 1 DADD is defined as the dose to treat two teats for 24 hours. For drying off treatment, 1 DADD is defined as the dose to treat 4 teats.

4.3.3 Antimicrobial consumption in poultry

In Denmark, poultry production comprises mainly broiler production, followed by egg layers and turkey production. In addition there is a small production of ducks, geese and game birds.

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, a few disease outbreaks in some farms can markedly affect and cause considerable fluctuations in the national statistics on antimicrobial usage. This was the case in late 2014 and throughout 2015, where the broiler industry experienced several large disease outbreaks, and the use of antimicrobials in the population increased significantly during this period. However, the problems were resolved during 2015 (personal communication: J. Dahl and M. Nielsen Blom, Danish Agriculture and Food Council) and in 2016 use of antimicrobials for poultry (excl. turkeys) decreased sharply to 1,560 active compound, a decrease of 36% compared with 2015. For broilers, amoxicillin has been the most commonly used antimicrobial agent for more than a decade. However, in 2016 tetracycline was the most commonly used antimicrobial (Table 4.1).

Currently, VetStat does not allow differentiation of the use of antimicrobials between different sectors of the poultry production. However, the consumption for turkeys was identified by combining information from the Central Husbandry Register with information provided by poultry veterinarians and the industry (personal communication: S. Astrup, PoultryVet, and M. Nielsen Blom, Danish Agriculture and Food Council) and the information in VetStat.

Over the past few years, the turkey industry has introduced a new hybrid of birds, less prone to arthritis, and improved the vaccination strategy against Turkey Rhino Tracheitis virus (personal communication: S. Astrup, PoultryVet). This may have explained the decrease in antimicrobial use in 2015. However, in 2016 the turkey production increased (Table 3.1) and the use of antimicrobial agent increased by 110 kg.

4.3.4 Antimicrobial consumption in aquaculture, fur animals and companion animals

The antimicrobial consumption in aquaculture decreased by 23% to 2,289 kg in 2016 compared with 2015 (Table 4.1). This is the lowest amount registered for more than a decade. The distribution of the different compounds was sulphonamide/trimethoprim comprised 47%, quinolones (oxolinic acid) 39% and amphenicols 14%, when measured in kg active compound.

Antimicrobial consumption in aquaculture is mostly influenced by the summer temperatures, because diseases are more likely to occur in warmer waters. In recent years, the aquaculture industry has developed new and better vaccines and improved vaccination strategies to reduce the risk of diseases that may require antibiotic treatment. A combination of favourable

weather conditions (lower temperatures during summer) and a positive effect of the revised vaccination strategies may explain the reduced consumption seen over the last few years [personal communication: N. H. Henriksen, Danish Aquaculture].

With the exception of 2013 and 2014, the use of antimicrobial agents in mink production has increased every year for more than a decade, from less than two tonnes in 2004 to more than 5 tonnes in 2016. The production of mink has also increased significantly during this period and peaked at 17.8 million in 2015. In 2016, 17.1 million mink were produced in Denmark (Source: Copenhagen Fur). In recent years, the industry has introduced interventions to reduce the antimicrobial consumption, by increasing the quality of feed and improving animal welfare (personal communication: T. Struve, Copenhagen Fur). In 2013 and 2014, this appeared to have resulted in a decrease in antimicrobial consumption of 11% and 14%, respectively. However, in 2015 the use of antimicrobial agents increased by 23% to 5,177 kg and in 2016 by further 3% to 5,327 kg active compound; mainly penicillins, sulphonamide/trimethoprim, tetracyclines and macrolides (Table 4.1). There is no apparent diagnostic explanation for this increase in antimicrobial use (personal communication: National Veterinary Institute, DTU). Recent studies have identified risk factors for antimicrobial use in mink, including feed quality, see Textbox 4.3.

Use of fluoroquinolones and cephalosporins in fur animal production has been close to zero for more than a decade.

It is noteworthy, that the use of prescribed medical zinc (zinc oxide) in mink production has increased markedly in recent years, from approximately 200 kg in 2012 to 1,045 kg in 2016. In the mink production, medical zinc is applied topically on pre-weaning mink kits in the nesting boxes to keep them dry (personal communication: M. Chriel, National Veterinary Institute, DTU).

The information available on antimicrobial consumption in companion animals is not as complete as for production animals, and a substantial amount of the antimicrobials used for companion animals are entered into VetsStat without defining animal species. For DANMAP 2016, the principles for estimating the amount of antimicrobial agents used for companion animals (pets and horses) were adjusted to improve the estimates. In the Tables 4.4 and 4.5, the following principles have been applied: 1) all antimicrobial agents registered, by the either the pharmacy or veterinarians for use in dogs, cats and horses have been included, 2) antimicrobial agents, where no animal species is given, were allocated to pets or horses based on relevant type of preparation or registration. However, the estimates may underestimate the actual use for companion animals, since the antimicrobials administered parenterally - with no information on animal species - are not included (see table 4.1)

Measured in kg active compound, the overall antimicrobial consumption for horses, specifically the use of sulphonamide/trimethoprim, has increased steadily over the past five years (Table 4.4). For pets (dogs and cats), the use decreased from 2012 to 2015, but increased in 2016 (Table 4.5). Antimicrobial use in dogs and cats from 2012 to 2016 has been described in further detail in Textbox 4.5.

A large proportion of antimicrobials used for companion animals are prescribed for 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.

In 2016, the use of fluoroquinolones for use in pets was 14 kg, compared with 13 kg in 2015. Almost all fluoroquinolones used in animals in 2016, were prescribed for pets. Similarly, the pets accounted for a significant proportion (137 kg or 66%) of the use of cephalosporins used in animals.

However, over the past three years, since the treatment guidelines by Danish Veterinary Association (November 2012) were first published, the use of cephalosporins has been reduced by 36%. Thus, there appears to be a shift away from the use of cephalosporins, sulphonamides and trimethoprim and towards penicillins (Figure 4.3) This may be an effect of treatment guidelines, recommending that use of critically important antimicrobials should be reduced as much as possible.

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

DANMAP 2016

	Aminoglycosides	Amphenicols	Cephalosporins	Fluoroquinolones	Lincosamides	Macrolides	Other AB	Other quinolones	Penicillin's, b-lactamase sensitive	Penicillin's, others ^(a)	Pleuromutilins	Sulfonamides and trimethoprim	Tetracyclines	Total
2012	<1	<1	<1	<1	0	<1	<1	0	14	<1	0	1000	3	1018
2013	1	<1	<1	<1	0	<1	<1	0	13	<1	0	893	5	914
2014	1	<1	<1	<1	0	<1	<1	0	15	<1	0	1024	6	1047
2015	3	<1	<1	<1	<1	<1	<1	0	10	<1	0	1049	4	1067
2016	<1	<1	<1	<1	0	<1	<1	0	8	<1	0	1117	5	1131

Note: Data extracted from VetStat on 3rd March and 22nd August 2017. The estimates include all antimicrobial agents registered, by the either the pharmacy or veterinarians for use in horses. Furthermore, antimicrobial agents, where no animal species is given, were allocated to horses based on relevant type of preparation (eg. oral paste) or registration. Antimicrobials administered parenterally - with no information on animal species - are not included

a) Penicillins with extended spectrum and combination penicillins, incl. b-lactamase inhibitors

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

DANMAP 2016

	Aminoglycosides	Amphenicols	Cephalosporins	Fluoroquinolones	Lincosamides	Macrolides	Other AB	Other quinolones	Penicillin's, b-lactamase sensitive	Penicillin's, others ^(a)	Pleuromutilins	Sulfonamides and trimethoprim	Tetracyclines	Total
2012	22	<1	272	13	67	7	49	0	42	651	<1	306	51	1483
2013	19	<1	231	12	63	5	42	0	31	642	<1	292	45	1383
2014	21	<1	213	12	69	6	34	1	31	653	<1	300	35	1376
2015	18	<1	157	13	68	4	32	0	25	655	1	235	39	1249
2016 ^(b)	14	<1	137	14	69	3	31	0	20	717	<1	276	40	1323

Note: Data extracted from VetStat on 3rd March and 22nd August 2017. 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 (eg. tablets, eye- or eardrops) or registration. Antimicrobials administered parenterally - with no information on animal species - are not included

a) Penicillins with extended spectrum and combination penicillins, incl. b-lactamase inhibitors

b) In 2016, approximately 222 kg of the sulfonamides and trimethoprim registered for pets are products (oral paste) typically used for horses

Textbox 4.1

New model for the yellow card initiative

The Yellow Card Initiative was introduced in 2010 to reduce the use of antimicrobials in pig production in Denmark. Use of antimicrobial agents on each holding is monitored and if it exceeds a pre-specified threshold of animal daily doses (ADD) per pig per day, a warning in form of a 'yellow card' is issued. A yellow card restricts antimicrobial usage further and requires more frequent veterinary consultations (DANMAP 2010). If the farmer does not reduce the consumption of antimicrobials to a level below the threshold, the Danish Veterinary and Food Administration (DVFA) requires a second opinion on the health issues on the farm by an independent private veterinarian. The Yellow Card Initiative works as an incentive to all pig producers to contribute to the goal of reducing the overall use of antimicrobials in the pig production (see DANMAP 2010).

On the 30th of June 2016, the DVFA further developed the Yellow Card Initiative by weighting the use of each antimicrobial agent according to the importance of the specific antimicrobial class in development of antimicrobial resistance. The weights are shown in table 1. The weight for a specific antimicrobial is multiplied by amount used to generate a "weighted ADD" to motivate the veterinarian and owner to restrain from using antimicrobial agents with higher weights. The weights are determined according to the importance of the antimicrobial agent for treating human illness and the importance in contributing to the development of antimicrobial resistance (AMR). In addition to the new weighted approach, the threshold for receiving a yellow card was lowered by 5 percent. The period for calculating the average antimicrobial consumption in a pig holding is nine months and if a yellow card is issued, the farmer is expected to reduce the antimicrobial consumption on the pig holding to a level below the threshold levels within nine months of the issuance of the injunction.

The allocation of yellow cards based on the new weighted approach, took place for the first time in May 2017. The designation was based on the antimicrobial consumption in the pig herds from July 2016 to March 2017. The weights were fully implemented by March 31st 2017, when an average of the past nine months could be calculated. The applied weights are shown in Table 1. The new approach is not appropriate for monitoring of antimicrobial consumption in the population, but is valuable in targeting specific resistance problems.

On the 31st of December 2016, the DVFA adjusted the weight for tetracyclines from 1.2 to 1.5. The adjustment was made to motivate further reduction in the consumption of tetracyclines in the pig production (Table 1). The first allocation of yellow cards including the new weight for tetracyclines will take place in November 2017 and will be based on the antimicrobial consumption in the holding from January to September 2017.

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Table 1: The weights that are used for calculating antimicrobial consumption in the pig production for the yellow card initiative in 2017

DANMAP 2016

Class of antimicrobial	Factor applied 31. March -29. September 2017	Factor applied from 30-September 2017
Fluoroquinolones, 3rd and 4th generation cephalosporins ^(a)	10.0	10.0
Tetracyclines ^(b)	1.2	1.5
Simple penicillins, sulfonamides, trimethoprim and pleuromutilins ^(c)	0.95	0.95
Other antimicrobials	1.0	1.0

a) ATC-codes: QJ01MA, QJ01DD, QJ01DE

b) ATC-codes: QJ01AA

c) ATC-codes: QJ01EQ, QJ01EW, QA07AB, QJ01CE, QJ01XQ

Textbox 4.2

Use of colistin in animals and humans - a Danish Perspective

Background: Traditionally, colistin was considered an inferior antibiotic to control and treat Gram negative infections in humans. Its toxicity profile combined with the availability of more efficient and less toxic alternatives such as beta-lactams and fluoroquinolones meant that it had low priority as a treatment option in humans. Since it played only a minor role as a human drug, it was recommended for veterinary medicine, especially to treat diarrhea in pigs. In addition to the fairly low risk profile of using colistin for animals, acquired resistance towards colistin was until recently only associated with chromosomal (non-transferrable) mechanisms, leading to no horizontal spread between resistant veterinary and human pathogenic isolates. However, plasmid-mediated (transferrable) colistin resistance conferred by the *mcr-1* gene was detected in late 2015 in *E. coli* isolates from animals, foods and patients in China [1] and was soon also found in imported poultry meat (five isolates) and in one bacterial isolate from a patient with bloodstream infection in Denmark [2] and many other countries world-wide [3]. Recently new variants of the *mcr*-gene have been described and two newly published studies from Denmark found the *mcr-3* gene in one blood isolate from a Danish patient dating back to 2014 [4] and nine *Salmonella* isolates from human infections have also been found to carry either the *mcr1* or *mcr3* gene [5]. Simultaneously, an increasing need for colistin in human medicine is emerging due to increasing prevalence of infections caused by multi-resistant bacteria. The combination of these two new situations has forced both national and international risk managers to revise the risk profile of colistin in veterinary medicine.

Use of colistin in humans

In Denmark, colistin has primarily been used for inhalation treatment of lung patients suffering from chronic infections with *Pseudomonas aeruginosa* and occasionally for treatment of patients suffering from chronic urinary tract infections or other infections with ESBL-positive and multi-resistant Gram negatives. The consumption of colistin for human treatment used to be less than one Defined Daily Dosis (DDD) per 100 admitted patients at hospitals and without any measurable consumption in the primary health sector. In 2016, the use of colistin for intravenous treatments of severely ill patients suffering from bacteremia with carbapenemase-producing *E.coli* or *K. pneumoniae* was very rare, but it is becoming one of the few treatment options in this patient group. It is therefore of utmost importance that the drug is effective and no additional resistance mechanisms are introduced into these already multi-resistant bacteria.

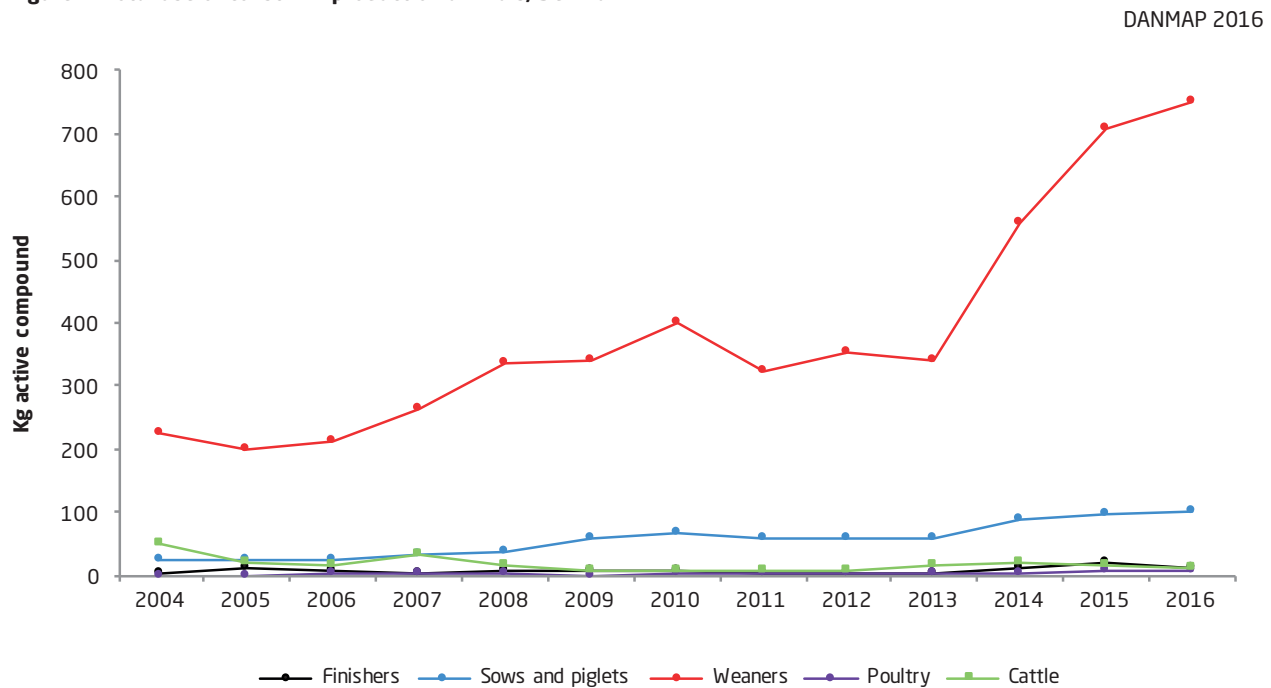
Use of colistin in production animals

In Denmark, almost all the colistin prescribed for animals (881 kg in 2016) is used in the pig production for treating gastrointestinal infections in weaners. Only smaller amounts (less than 20 kg in 2016) are used for cattle and poultry, respectively. An overview of colistin consumption in production animals 2004-2016 is shown in Figure 1. Except for 2011 and 2013, the use of colistin has increased all years since 2006, but the increase was particularly steep in 2014 and 2015. In 2010, colistin was introduced as one of the "first choice" antimicrobial agents for the treatment of gastroenteritis in the treatment guidelines for pigs. In 2014, the Danish pig producers committed themselves to reduce the consumption of tetracyclines by 50% and the increase in colistin use in 2014 and 2015 may be a response to this. However, in 2016 the increase levelled off, probably due to an increased focus on colistin use in animals and humans, because of the emergence of new colistin resistance.

One Health perspective

Immediately following the detection of the *mcr-1* gene in Denmark, the Danish Veterinary and Food Administration took initiative to convene experts from the health sector and DTU to assess the gravity of the situation. In autumn 2016, the DFVA recommended to restrict the use of colistin in pig production. In spring 2017, pharmaceutical products containing colistin were assigned a weight of 10 in the calculations of individual herd consumption of antibiotics against the farm threshold according to the differentiated yellow card scheme. This change was implemented in April 2017, and is expected to significantly reduce the use of colistin in pigs.

Figure 1. Total use of colistin in production animals, Denmark



The Danish Health Authority is currently leading a working group of experts developing guidelines on the management of carbapenemase-producing organisms, including carbapenem-resistant *Enterobacteriaceae*. The introduction of screening methods of risk patients upon admission to hospitals is expected to reduce the spread in the hospital environment and protect vulnerable patients, thus being able to keep colistin as a very last line drug for the clinical cases demanding treatment.

As part of the increased awareness of colistin resistant bacteria in humans the reference laboratory at Statens Serum Institut offers phenotypic resistance testing to clinical microbiological departments suspecting these resistance mechanisms in clinical strains, thereby broadening the surveillance of multi-resistant bacteria.

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Textbox 4.3

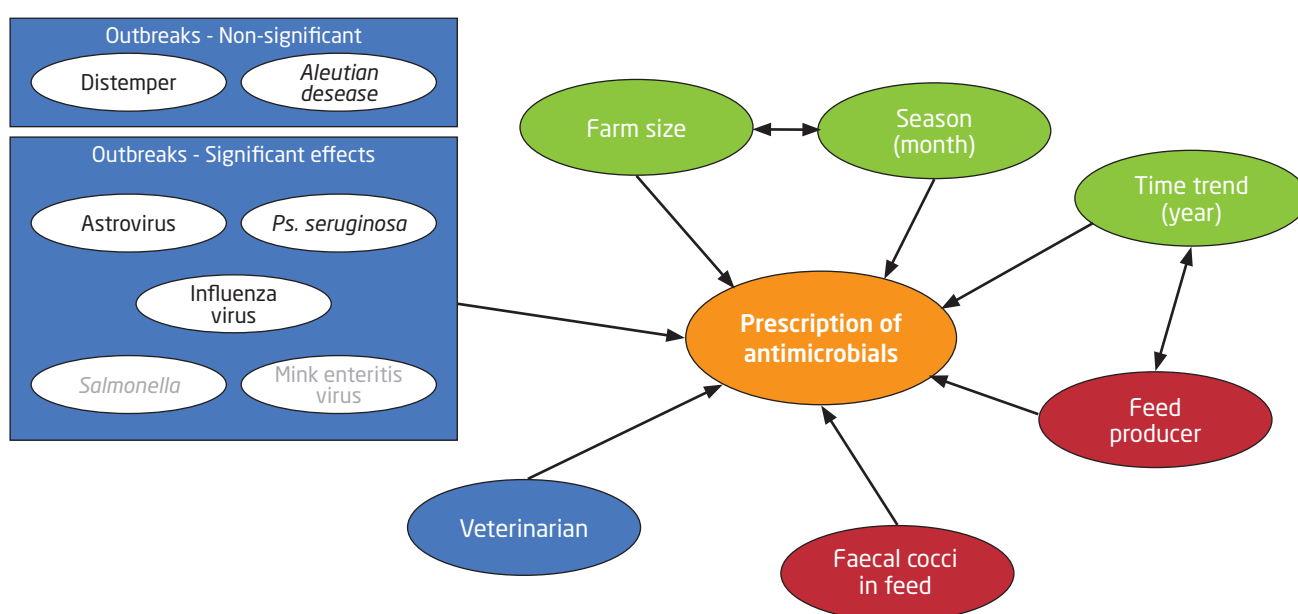
Correlation between quality of mink feed and prescription of antimicrobials for mink

Background: Due to the increasing prescription of antimicrobials in the commercial mink (*Neovison vison*) production in Denmark (from 2,420 kg in 2007 to 5,558 kg in 2012), two register based studies were carried out to identify potential causal factors for antimicrobial use. Both studies included data from all active Danish mink productions, with data on farm size and the relation between farm and feed producer obtained from the registers at Copenhagen Fur. Information on prescribed antimicrobials was obtained from the national database VetStat, October 2014 (Study 1) and October 2015 (Study 2). Data on microbiological feed quality was obtained from the Voluntary Feed Control under the Mink producers Organization.

Methods and Results: In the first study, the objective was to identify risk factors for antimicrobial use at farm level in the period 2007–2012. We found a clear significant effect of season on antimicrobial use (measured in DAPD), with a peak around the time of whelping in May and a high level in the following months. In autumn, a minor peak in the use of antimicrobials occurred throughout the study period. From 2007 to 2011, we observed a 102% increase in annual antimicrobial use. At farm level, the annual number of months with antimicrobial prescriptions was significantly ($p < 0.01$) affected by feed producer, veterinarian, disease (laboratory diagnosis of specific pathogens), farm size and year, with an interaction between feed producer and year. Furthermore, in months with antimicrobial use, the treatment proportion at farm level was significantly ($p < 0.001$) affected by year, month (season), feed producer, feed quality score, veterinarian, farm size and laboratory confirmed diagnosis of specific infections. We also found statistically significant interactions between year*feed producer and the actual farm size*month were noted. Moreover, the prescription patterns varied significantly between veterinarians. Some veterinarians prescribe not only more frequently, but also larger amounts of antimicrobials than others. Farm outbreaks of *Pseudomonas aeruginosa*, astrovirus, or influenza virus, were associated with an increase in antimicrobial use. *Salmonella* spp. and mink enteritis virus were also found to significantly influence the prescription of antimicrobial agents, but the number of diagnosed outbreaks was low (Figure 1), [1].

Figure 1. Risk factors for antimicrobial use in the Danish mink production

DANMAP 2016



continued ... Textbox 4.3

The second study focused on the potential effect of specific feed parameters on oral use of antimicrobial agents at farm level. Mink are fed moist feed, based on perishable ingredients. All feed batches controlled during 2012-2014 were included in this study; Prescription of oral antimicrobials at farm level, within time slots of 3, 5 or 7 days after receiving an included feed batch, was applied as outcome variable. A multi-variable variance analysis was carried out analysing the effect of the feed parameters (total volatile nitrogen, dry matter, crude protein and fat; total bacterial count (21°C), and counts of sulphite producing bacteria (21°C), *Clostridium* spp., faecal cocci (44°C), fungi, and mould; presence of *Salmonella* spp. and *Clostridium perfringens*). Two binomial models were applied, adjusting for significant effects (*Pseudomonas aeruginosa* outbreak, farm size, season and year). Prescription of antimicrobials was significantly associated with counts of faecal cocci in all three time slots and in both models [Jensen et al, Prev Vet Med, 2017]

Conclusion: Both studies identified important risks factors for prescription of antimicrobial agents for mink and there is a clear association between the quality of the feed and the amount of antimicrobial agents prescribed. Thus, the quality of the daily produced feed is a very important factor for the health of the mink. However, it is difficult to implement quality assurance systems for the large amounts of perishable products included in the feed because the feed has been eaten before the results are available.

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Textbox 4.4**Stronger focus on preventing illegal import of antimicrobials**

In May 2016, the Danish Veterinary and Food Administration published the report “*Strengthening the efforts regarding illegal import of antibiotics for production animals*”. As a part of the Second Veterinary Action Plan running from 2013 to 2016, a comprehensive study investigated whether illegal import of antimicrobials were used in farm animals to avoid the restrictions on antimicrobial consumption. The study also suggested ways to strengthen authority efforts to prevent illegal import of antimicrobials. The study investigated, evaluated and concluded on: Cases of illegal import of antimicrobials, the Danish enforcement and monitoring system, the temporal patterns in consumption of antimicrobials, the development of the Danish consumption of antimicrobials and the development of regulation on veterinary medicines.

The study found no evidence of systematic illegal import of antimicrobials for food producing animals, even though a few cases of illegal import were identified. The threat to the high level of food safety in Denmark was considered negligible at this point in time. It is acknowledged that a change in the risk may have a great impact on the Danish approach to control usage of antimicrobial agents and development of antimicrobial resistance. The work has led to a strengthened effort to restrict illegal import of antimicrobial agents by dissemination of information to farmers describing the risks associated with illegal import, and by raising awareness in the enforcement system.

The full report can be found here:

<https://www.foedevarestyrelsen.dk/Leksikon/Sider/Import-af-lægemedler.aspx>

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Textbox 4.5

Antimicrobial use in dogs and cats in Denmark - 2012-2016

Background: In November 2012 the first Danish National Antimicrobial Treatment Guidelines for companion animals were published by the Danish Small Animal Veterinary Association. This textbox describes the development of antimicrobial use in companion animals from 2012 to 2016.

The data on antimicrobial use in companion animals in Denmark derive from the national database VetStat. In previous Danmap reports from 2013-2015, consumption in companion animals was estimated from prescriptions for companion animals plus a proportion of veterinary antimicrobials not recorded for production animals. However, in an attempt to assess the effect of the treatment guidelines, we applied the principles described below to provide a more precise estimate of the actual antimicrobial use for dogs and cats over the past five years.

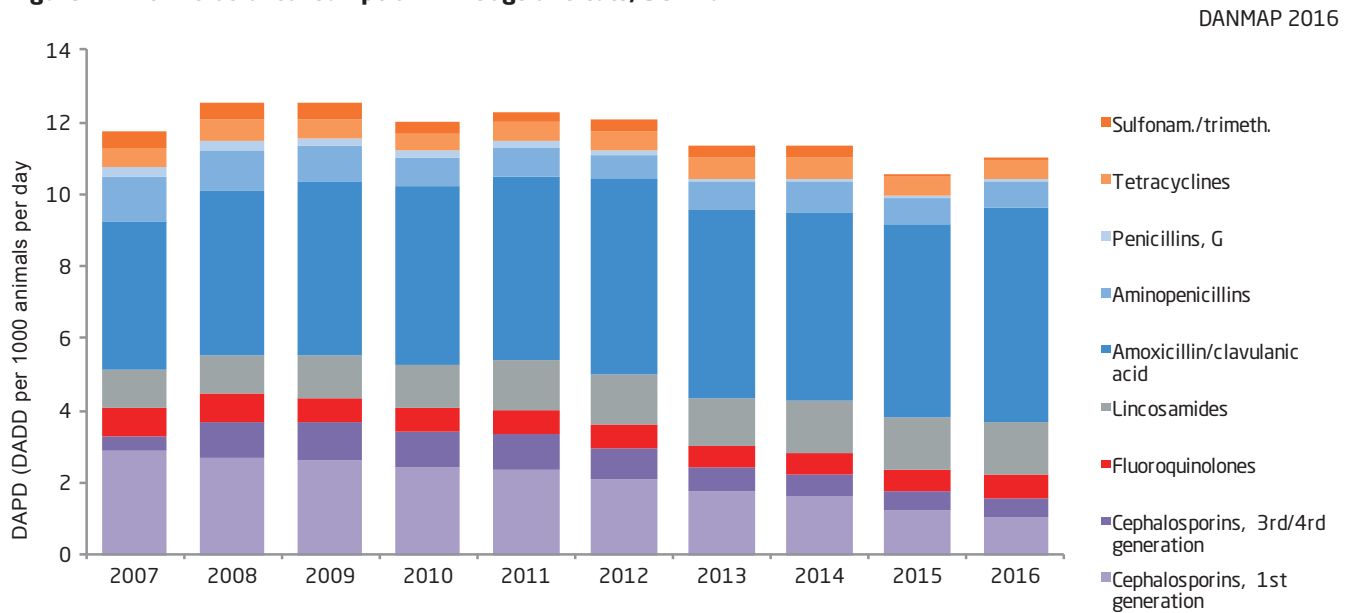
Methods: Data on prescription of antimicrobial products for companion animals were extracted from VetStat on April 18th 2017. The antimicrobial consumption was estimated according to the principles described below:

- All antimicrobials (including human products) for oral use dispensed directly to companion animal owners via pharmacies;
- Antimicrobial preparations licensed solely for use in companion animals dispensed directly via pharmacies or by the veterinarian. This group comprises exclusively products for oral use with the exception of the injectable 3rd generation cephalosporin cefovecin;
- Oral formulations registered for use only in horses or production animals, but recorded in VetStat as prescribed for companion animal use are assumed to be registration errors and are thus not included.
- For fluoroquinolones, only parental use of preparations with a low concentration of drug per ml, suitable for use in dogs and cats (50 mg/l or less) is included. Fluoroquinolone preparations with a high concentration of drug per ml are assumed to be used for horses and are not included
- Other antimicrobials for parental use formulated for use in multiple species, including companion animals and horses, are not included, as target species cannot be assumed with certainty.

The resulting estimates on antimicrobial consumption may underestimate the total consumption in companion animals by approximately 20%, as most parenteral use is not included [DANMAP 2011].

The calculations of DAPD (Defined Animal Daily Doses per 1000 animal per day) are based on estimated population numbers for dogs and cats of 546.000 and 646.000 individuals in the year 2000 [Statistics Denmark], and an average body mass of 10 kg. Thus, changes in population size and differences in treatment frequency between dogs and cats are not taken into account.

Results: The relative use of the different antimicrobial groups in 2007-2016 is illustrated in Figure 1. From 2007 to 2012, the use of antimicrobial agents for dogs and cats was estimated at levels of approximately 12-13 DAPD (Figure 1). From 2012 to 2015, the estimated antimicrobial usage in companion animals decreased by 14%, from 12.4 DAPD in 2012 to 10.7 DAPD in 2015. In 2016 the consumption increased to 11.2 DAPD, however, this still represents a 10% reduction in the consumption compared to 2012.

Figure 1. Antimicrobial consumption^(a) in dogs and cats, Denmark

Note: Includes antimicrobials (including human products, except for small amounts of metronidazole and nitrofurantoin) for oral use, dispensed directly to the companion animal owner, and antimicrobial preparations licensed solely for use in companion animals.

a) Measured in DAPD, estimating the percentage of animals treated on a given day (10 DAPD \approx 1 % under treatment on average). The DAPD is calculated as the number of standard doses for cats and dogs assuming an average weight of 10 kg. For all years, the assumed population is 1.192.000 dogs and cats [Statistics Denmark 2000]

In 2016, the most commonly used antimicrobial was amoxicillin/clavulanic acid, which accounted for 53% of the antimicrobial consumption in companion animals. Other commonly used antimicrobials were lincosamides (clindamycin), 1st generation cephalosporins, and aminopenicillins (amoxicillin). The use of 1st generation cephalosporins has markedly decreased from 16% of the total consumption in 2012 to 9% in 2016, whereas the use of lincosamides has increased in the same period from 11% to 13% of the total consumption, and thus constituted the second most common antimicrobial class used for companion animals in 2016.

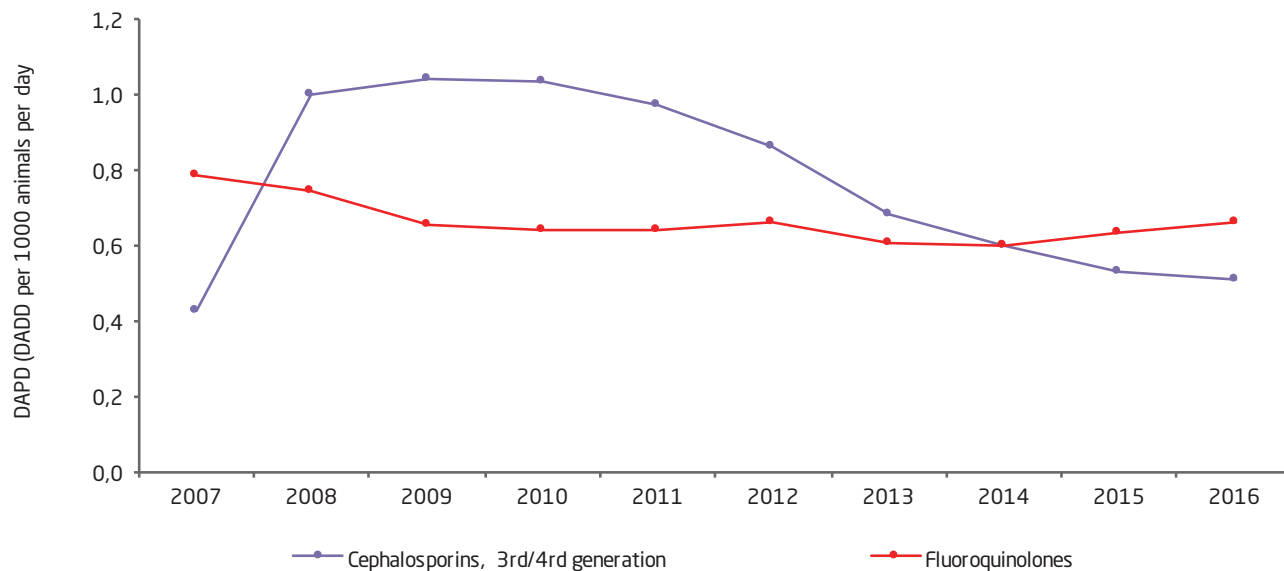
From 2014 to 2015, the use of potentiated sulfonamides decreased substantially, by 91%. It should be noted that oral formulations of potentiated sulfonamides licensed for use in dogs and cats have been unavailable on the Danish market since March 2015. The total antimicrobial consumption for companion animals in 2016 (excluding most parenteral preparations) accounted for approximately 1% of the total veterinary antimicrobial consumption.

The consumption of the third generation cephalosporin cefovecin has decreased since 2010. Between 2012 and 2016, the consumption of cefovecin decreased by 41% (Figure 2). The consumption of fluoroquinolones for oral use has remained relatively stable, at 0.66 DAPD in 2012 and 2016, with a minor transitory decrease in 2013 (Figure 2). Similarly, the estimated consumption of fluoroquinolones for parental use has been stable at 0.1 DAPD during this period. In 2016, the consumption of fluoroquinolones for companion animals accounted for 82% of the total veterinary fluoroquinolone consumption.

continued ... Textbox 4.4

Figure 2. Antimicrobial consumption^(a) of 3rd/4rd generation cephalosporins and fluoroquinolones in companion animals, Denmark

DANMAP 2016



Note: Includes only parenteral use of 3rd generation cephalosporin cefovecin and fluoroquinolones for oral use

a) The DAPD is calculated as the number of standard doses for cats and dogs assuming an average weight of 10 kg. For all years, the assumed population is 1.192.000 dogs and cats [Statistics Denmark 2000]

Concluding remarks: Using the described method for estimating antimicrobial consumption in dogs and cats, we observed a 10% decrease from 2012 to 2016. The consumption of fluoroquinolones has been stable throughout the period, and compared to other animals in Denmark, companion animals are by far more frequently treated with this antimicrobial class. In contrast, the use of the third generation cephalosporin, cefovecin, has substantially decreased since 2012.

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5

ANTIMICROBIAL CONSUMPTION IN HUMANS



5. Antimicrobial consumption in humans



Highlights: In 2016, the total consumption of antimicrobials in humans, when calculated in defined daily doses per 1000 inhabitants per day (DID), remained with 18.47 DID very close to the consumption in 2015 (18.50 DID) and to the consumption a decade ago in 2007 (18.37 DID). In general, the consumption of antimicrobials increased from registrations in the first DANMAP report in 1996 (13.60 DID) until 2011 (19.31 DID) and has since levelled off.

Penicillins remained the most frequently used antimicrobial agents in both primary health care (65%) and in hospital care (54%), but the changes in consumption observed within this drug group in the last decade continued. Thus in 2007, beta-lactamase sensitive penicillins constituted 55% of all penicillins consumed in primary health care, while in 2016 this had decreased to 39% (from 5.67 DID to 4.16 DID, a decrease of almost 30%). Simultaneously, consumption of combination penicillins increased in both sectors, from 0.19 DID to 1.42 DID in primary health care and from 2.95 defined daily doses per 100 occupied bed days (DBD) to 18.04 DBD in hospital care. In 2016, combination penicillins constituted 9% of the antimicrobial consumption in primary health care and 18% of the consumption in hospital care, making them, together with penicillins with extended spectrum, the largest antimicrobial drug group consumed at hospitals.

In 2016, Fluoroquinolones constituted 3% and 8% of the consumption in primary and hospital care, respectively. In Denmark, fluoroquinolones, cephalosporins and carbapenems are defined as critically important antimicrobials, cephalosporins and carbapenems being used solely at hospitals. In 2016, the consumption of the three drug classes constituted altogether 22% of the consumption at hospitals, a decrease from 24% observed the year before and from 32% in 2007. The consumption of cephalosporins and fluoroquinolones has shown slow but continuous decreases since 2011. The consumption of carbapenems has shown more fluctuations but decreased notably from 2015 to 2016. In 2016 fluorquinolones accounted for a consumption of 8.11 DBD, cephalosporins for 10.24 DBD and carbapenems for 3.93 DBD.

In 2016, the total antimicrobial consumption at hospitals was measured at 99.98 DBD and 310.53 defined daily doses per 100 admissions (DAD), respectively, a slight decline from 103.02 DBD and 313.38 DAD the previous year. From 2007 to 2016 the total consumption at hospitals increased with 36% and 1.2%, respectively, when measured in DBD or DAD.

Since 1999, the number of DDD's per prescription and per package have increased notably, resulting in an increase of DDD's per treated patient from 15.2 DDD in 1999 to 21.5 DDD in 2016. In contrast the number of patients treated has decreased markedly, in 2016 it was on average 270 treated patients or 522 prescriptions per 1000 inhabitants. This corresponds to reductions of 17% for both the number of treated patients and the number of prescriptions per 1000 inhabitants for the last decade. Decreases are noted for all age groups but are biggest in the youngest children (0-4 years old), where the number of treated patients decreased with 33% from 472 to 318 treated patients per 1000 inhabitants per year in 2007 and 2016, respectively.

5.1 Introduction

In Denmark, all consumption of human medicine including antimicrobials is recorded through the Register of Medicinal Product Statistics at the Danish Health Data Authority. The primary sector has reported on antimicrobial sales data since 1994, whereas the hospital sector has submitted data since 1997.

Recording of the consumption in the primary sector covers all antimicrobials on prescriptions from general practitioners, medical specialists and dentists as well as prescriptions given to hospital patients upon discharge. No over-the-counter sale takes place, all sale is through pharmacies and based on prescription only, which gives a close to total of all systemic antimicrobials used in Denmark. For the hospital sector, only data from public somatic hospitals are included - data from psychiatric hospitals, private hospitals, hospices and rehabilitation centers were omitted since they contribute with a low consumption of antimicrobials (2.3% in 2016), but in many ways differ from the patient population at public somatic hospitals.

In the primary sector, sales are reported through the pharmacies and include information on the generic and the brand name of the product, formulation, active drug, size and number of packages, as well as age, gender and regional residence of the patient. For 2016, clinical information on the indication for prescribing the drug was available for 86% of prescriptions. Yet some indication codes suffer from being unspecific, like the term "infection". Specific indications account for 70%. Better and more precise indication codes will, in the future, give the opportunity to register and evaluate on a more prudent use of antimicrobials. For the hospital sector, data are available through the hospital pharmacies and include information on

the amount and formulations of antimicrobials delivered to the different departments; these also include multi-packages and marginal products. In addition, work is going on to include details on the patient level thus improving the possibilities of monitoring actions on more prudent use.

In this chapter, the term `antimicrobial agents` covers all systemic antibacterial agents for human use listed in the Anatomical Therapeutic Chemical (ATC) Classification under the code J01. The only other antimicrobials included are metronidazole (ATC code P01AB01) and vancomycin (A07AA09), since these constitute important systemic antibacterial treatment as well. Their consumption has been included since DANMAP 2014, tables and figures were updated ten years back. Tuberculosis, antiviral and antifungal drugs are not included.

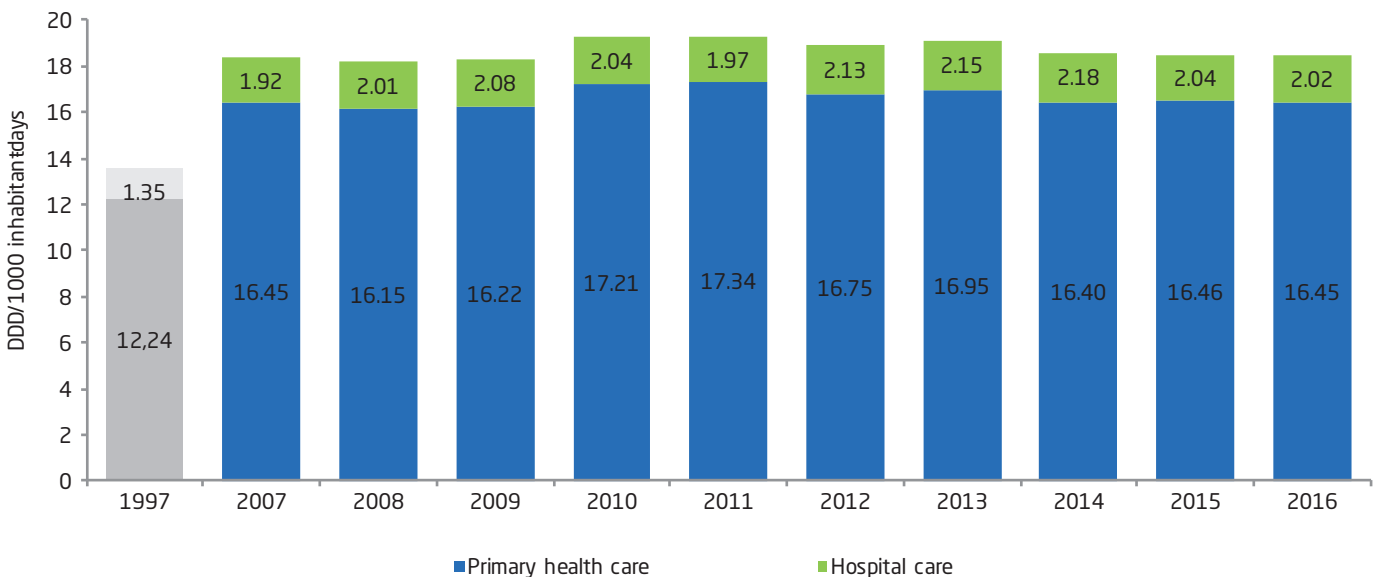
5.2 Total consumption (Primary Health Care and Hospital Care Sectors)

In 2015, the total consumption of antimicrobials in Denmark was 18.47 defined daily doses per 1000 inhabitants per day (DID), which is similar to the consumption in 2015 (18.50 DID) and to the consumption a decade ago in 2007 (18.37 DID), (Figure 5.1). The total consumption in 2016 corresponds to 49,605kg active compound consumed (Table A5.1 in web annex).

The primary sector accounts for approximately 90% of the total consumption and thus has significant impact on the overall consumption patterns. Overall, the consumption of antimicrobials showed no significant trends for the first five years of regular registration from 1996 (13.40 DID) until 2000 (13.63 DID), but increased steadily onward until 2011 (19.31 DID) and has since levelled off (Figure 5.1).

Figure 5.1 Total consumption of systemic antimicrobial agents in humans in primary health care vs hospital care. Denmark

DANMAP 2016



At the European level, Denmark reports a comparatively low consumption of antimicrobial agents and is specifically characterized by the proportionally high consumption of penicillins, which contributes to the total consumption with significant amounts (ECDC.ESAC-Net 2015). This is because penicillins in general and especially penicillins with extended spectrum contribute with high DIDs per treatment and thus account for a significant part of the measurable consumption.

Among the Scandinavian countries the proportional consumption of small-spectrum penicillins is generally high and all countries are characterized through a general awareness on the rational use of antibiotics in the population as well as among health professionals. Still, the trends in consumption over time have been quite different. While Denmark had the lowest consumption of antimicrobials in the late nineties and Sweden the highest, overall increasing trends in consumption in Denmark and simultaneously decreasing trends in Sweden, resulted in Denmark having the highest consumption of the three countries in 2015 and Sweden the lowest (<https://ecdc.europa.eu/en/antimicrobial-consumption/database/trend-country>). For Norway and Denmark consumptions in the primary sector have shown comparable trends for the last decade, both stabilizing or slightly decreasing for the last four years. The Norwegian national action plan from 2015, aiming for a 30% reduction in the total consumption (with 2012 as the reference point), did not show marked changes for 2016 but is expected to result in a lower consumption in the coming years.

5.3 Primary Health Care

5.3.1 Total consumption in Primary Health Care

In 2016, the total consumption of antimicrobials in primary health care remained with 16.45 DID close to the 16.46 DID in 2015, thus continuing the trend of a stabilized or slightly declining consumption that has been observed since the peak of 17.34 DID in 2011 (Figure 5.1, Table 5.1). Since 1997, the consumption increased with 34% from 12.24 DID.

In 2016, beta-lactamase sensitive penicillins continued to be the biggest group consumed with 4.16 DID (accounting for 25% of the total consumption in primary care), followed closely by penicillins with extended spectrum with a consumption of 3.63 DID (corresponding to 22% of the total consumption). The third biggest group consumed were the macrolides with 1.82 DID (accounting for 11% of the total consumption).

The group of penicillins accounted for altogether 10.7 DID and thus continued to constitute the majority of antimicrobials consumed in primary health care; a decade ago, in 2007, they accounted for 10.2 DID.

In Denmark, penicillins are the only beta-lactams used in primary care, other beta-lactams such as cephalosporins, monobactams and carbapenems are solely used in hospital care and primarily in somatic hospital with surgical or acute care functions. A distribution of the different antimicrobial classes between primary care and hospital care is shown in Figure A 5.1 in web annex.

Table 5.1. Consumption of antimicrobial agents for systemic use in primary health care (DDD/1000 inhabitant-days), Denmark

DANMAP 2016

ATC group ^(a)	Therapeutic group	Year										
		2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	
J01AA	Tetracyclines	1.48	1.54	1.61	1.69	1.64	1.76	1.96	1.66	1.61	1.62	
J01CA	Penicillins with extended spectrum	3.25	3.26	3.29	3.47	3.41	3.40	3.48	3.53	3.61	3.63	
J01CE	Beta-lactamase sensitive penicillins	5.67	5.30	5.12	5.25	5.31	4.68	4.65	4.38	4.33	4.16	
J01CF	Beta-lactamase resistant penicillins	1.09	1.12	1.13	1.17	1.14	1.21	1.30	1.36	1.38	1.48	
J01CR	Combinations of penicillins, including beta-lactamase inhibitors	0.19	0.27	0.45	0.68	0.89	1.05	1.22	1.30	1.42	1.42	
J01D	Cephalosporins and other -lactam antibiotics	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	
J01EA	Trimethoprim and derivatives	0.49	0.49	0.48	0.51	0.50	0.52	0.53	0.55	0.56	0.56	
J01EB	Short-acting sulfonamides	0.31	0.28	0.27	0.26	0.24	0.22	0.22	0.21	0.18	0.16	
J01EE	Combinations of sulfonamides and trimethoprim, including derivatives	0.00	0.00	0.00	0.00	0.00	0.02	0.01	0.00	0.00	0.00	
J01FA	Macrolides	2.42	2.28	2.21	2.44	2.47	2.19	1.93	1.79	1.77	1.82	
J01FF	Lincosamides	0.02	0.03	0.03	0.04	0.04	0.04	0.05	0.05	0.05	0.06	
J01GB	Aminoglycosides	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01	
J01MA	Fluoroquinolones	0.44	0.51	0.52	0.57	0.57	0.55	0.52	0.50	0.49	0.48	
J01XC	Steroid antibacterials (kombination fusidic acid)	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	
J01XE	Nitrofurantoin derivatives (nitrofurantoin)	0.47	0.47	0.49	0.51	0.50	0.49	0.49	0.48	0.45	0.43	
J01XX	Other antibacterials (methenamine >99%)	0.26	0.27	0.26	0.27	0.26	0.25	0.24	0.24	0.25	0.27	
J01XD and P01AB*	Nitroimidazole derivatives (metronidazole)	0.23	0.24	0.27	0.28	0.28	0.28	0.28	0.28	0.28	0.28	
J01+P01AB	Antibacterial agents for systemic use (total)	16.45	16.15	16.22	17.21	17.34	16.75	16.95	16.40	16.46	16.45	

a) From the 2016 edition of the Anatomical Therapeutic Chemical (ATC) classification system

*) all metronidazole preparations, formerly only listed as J01XD, 10 years retrospective data included in the DANMAP report since 2014

5.3.2 Trends in consumption of the leading antimicrobials

Even though the total consumption of penicillins has only changed slightly over the years, within the group of penicillins marked changes have been observed during the last decade. Since 2007 the consumption of beta-lactamase sensitive penicillins decreased almost continuously with 27%, paralleled by a decrease of macrolides with 25% (from 5.67 DID and 2.42 DID, respectively), (Figure 5.2a and 5.2b). For both drug classes this may be the result of a more restrictive use of antimicrobials observed in primary care in general, since an overall declining number of treated patients and of redeemed antimicrobial prescriptions parallels the decrease in DID (Table 5.2 and 5.3). Both antimicrobial classes are the main drugs in the treatment of upper airway infections, being the most common type of infections seen at general practitioners and the focus of the National antibiotic campaigns from 2013 to 2015. Recommendations on a more prudent use of antibiotics through the Danish College of General Practitioners may also have had an impact on the prescribing of beta-lactamase sensitive penicillins and macrolides. For the three other penicillin groups' continuous increases in consumption occurred in the same period - penicillins with extended spectrum increased with 12%, the beta-lactamase resistant penicillins with 36% and the combination penicillins including beta-lactamase inhibitors with more than 600% (Figure 5.2b).

Thus, while in 2007 the beta-lactamase sensitive penicillins constituted 56% within the group of penicillins consumed, in 2016 they constituted 39%. If the shifting trends in the consumption of the different penicillins are to continue, the leading antimicrobial will probably become the penicillins with extended spectrum within the next few years. Within the total

consumption of different antimicrobials the group of penicillins accounted for altogether 65% (Figure 5.3)

Tetracyclines are the fourth biggest group of antimicrobials consumed in Denmark. In 2016 they accounted for 1.62 DID, corresponding to 10% of the total consumption in primary care. During the last decade the consumption has increased with 10% from 1.48 DID in 2007. In 2013, the consumption peaked unexpectedly with 1.96 DID but has since shown continuing decreases. Tetracyclines are consumed by all age groups above 12 years and by both genders. The treatment of acne in adolescents is the main driver of consumption and contributes considerably to the total consumption with both DIDs as well as the number of redeemed prescriptions and patients treated. While the number of DIDs consumed increased in women (primarily girls from 15 to 24 years) from 1.58 DID in 2007 to 1.88 DID in 2016, it simultaneously decreased slightly in men (primarily boys from 15 to 19 years) in the same period, from 1.35 DID in 2007 to 1.33 DID in 2016 (Figure 5.7a.).

Fluoroquinolones are the smallest drug class among the leading antimicrobials, in 2016 accounting for 0.48 DID, corresponding to 3% of the total consumption in primary care. The consumption of fluoroquinolones followed the general increasing trends in the total consumption from 2007 (0.44 DID) until 2010 and 2011 (peaking with 0.57 DID) and has since been decreasing, giving an overall increase of 8.5% from 2007 to 2016. In Denmark, fluoroquinolones are designated as "antimicrobials of special, critical interest" through the National Health Authority and are mentioned in the National recommendations on the use of antibiotics issued in 2012. According to these fluoroquinolones are to be solely used for treatment of very few specific infections, where they are considered

Figure 5.2a Consumption of leading antimicrobial groups for systemic use in primary health care, Denmark

DANMAP 2016

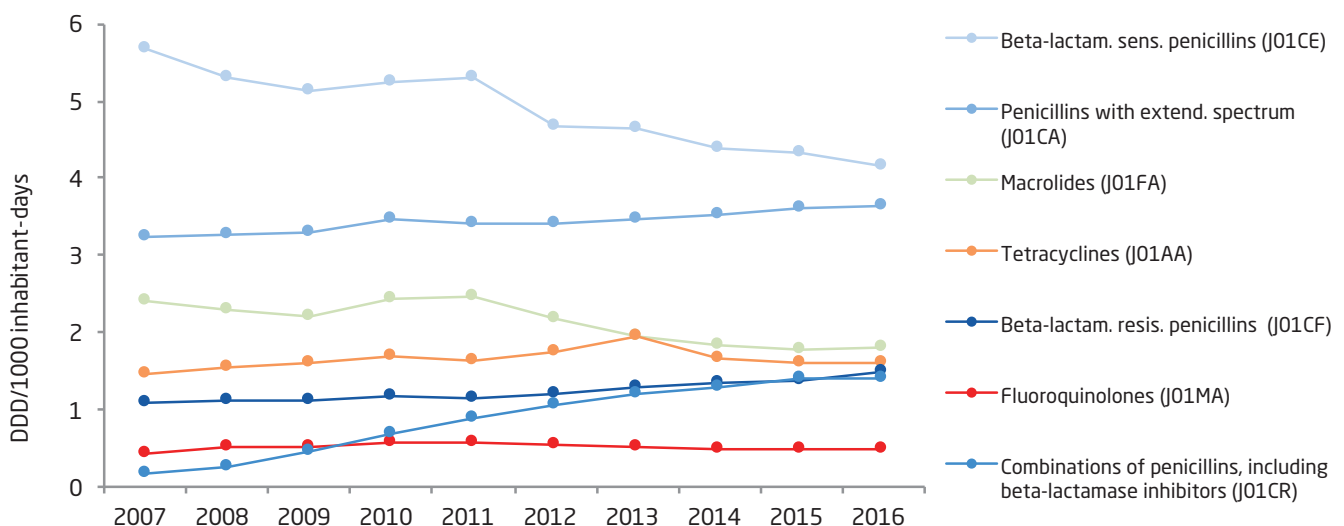
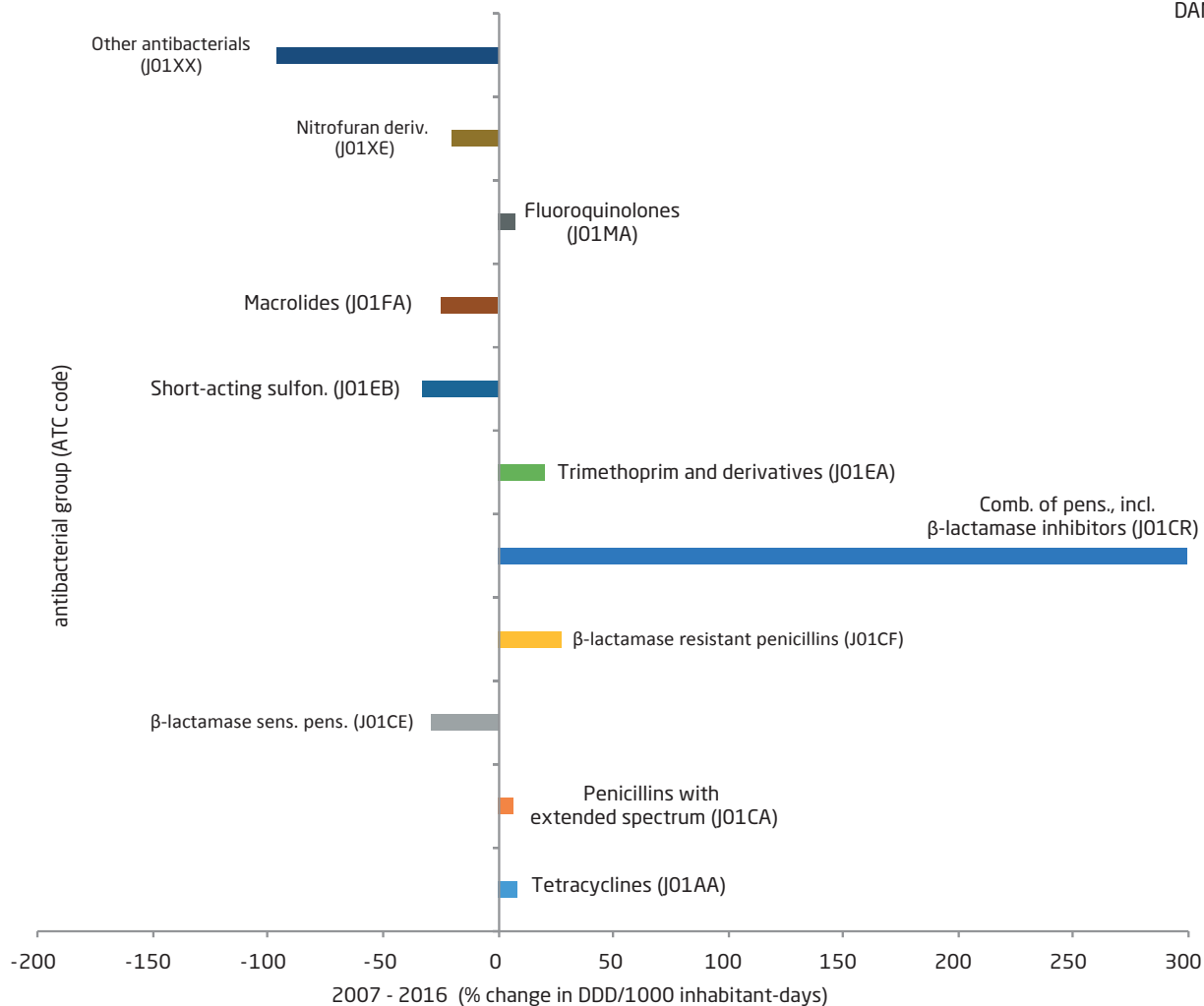


Figure 5.2b. Changes in the consumption (% DID) by leading groups of antimicrobial agents (J01) in the primary sector, Denmark

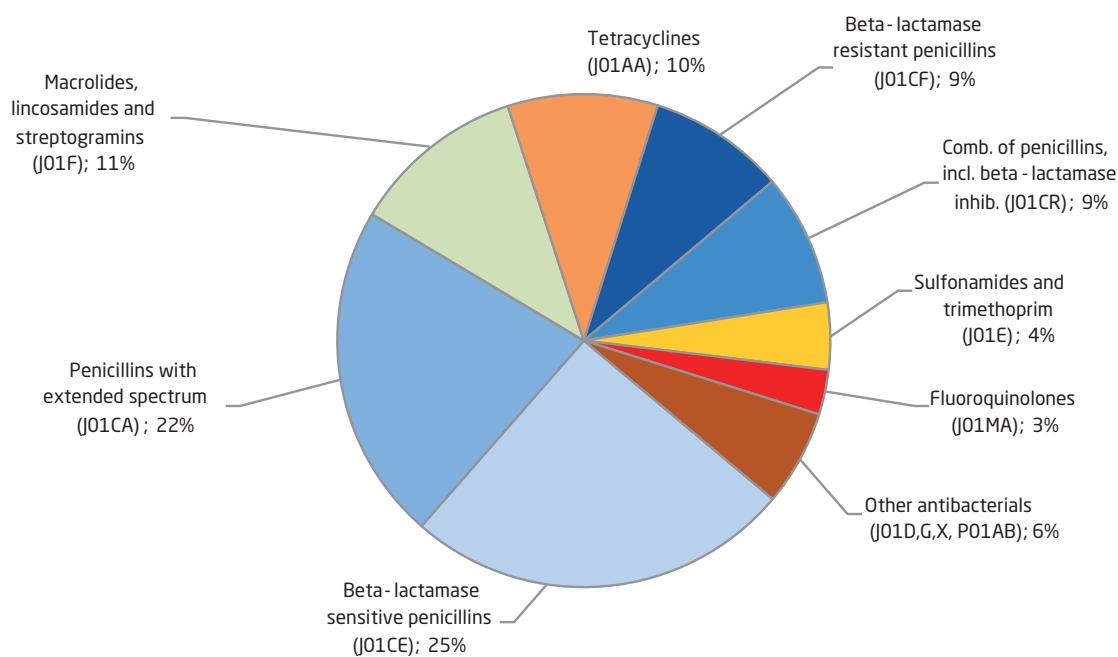
DANMAP 2016



* Combination penicillins changed with 600%.

Figure 5.3. Distribution of the total consumption of antimicrobial agents in primary health care, Denmark

DANMAP 2016



the drug of choice (fx exacerbation in a patient with chronic obstructive lung disease and penicillin allergy). They are also recommended in the case of infection with multidrug resistant bacteria, where microbiological results point towards a fluoroquinolone to be the only or best choice.

5.3.3 Penicillins

While beta-lactamase sensitive penicillins decreased almost continuously since 2007, the combination penicillins including beta-lactamase inhibitors (represented solely by amoxicillin with clavulanic acid) increased markedly in the same period from 0.19 DID in 2007 to 1.42 DID in 2016 (Figure 5.4). For the first time in this decade, no increases were observed from 2015 to 2016. The increases are probably due to a combination of changed recommendations in the treatment of exacerbation of chronic lung disease and demographic changes with a growing elderly population, but may well be a sign of the drug becoming more popular in the treatment of upper respiratory infections as well. This is to be investigated in the future when a sufficient number of the specific indication on each medical prescription will give the opportunity to look more thoroughly into the prescription habits regarding this drug class.

The increases described for the penicillins with extended spectrum from 1.09 DID in 2007 to 3.48 DID in 2016 are primarily due to increases in the consumption of pivmecillinam, accounting for about two thirds of this drug class. While pivmecillinam increased with 3.9% from 2015 to 2016 and with 88% since 2007, pivampicillin and amoxicillin decreased with 7% and 5%, respectively, from 2015 to 2016 and with 62% and 33% since 2007. In 2016, pivmecillinam accounted for 2.47 DID, amoxicillin for 0.92 DID and pivampicillin with 0.20 DID. In Denmark

pivmecillinam is the recommended first choice in the treatment of uncomplicated urinary tract infections (UTI). Its increases are paralleled by decreases in the other drugs used for the treatment of UTI, primarily sulfamethizol and nitrofurantoin (Figure 5.2b and Table 5.1 and 5.2). Amoxicillin is primarily used in the treatment of upper respiratory infections in small children (age 0 to 4 years). The decreases observed in the consumption of amoxicillin when measured in DID are mirrored in a reduced number of children treated with the drug (Figure 5.6a and 5.6b).

Increases were also observed for the beta-lactamase resistant penicillins. These comprise dicloxacillin and flucloxacillin. Flucloxacillin was introduced to the Danish market due to a shortage of dicloxacillin in 2010. Beta-lactamase resistant penicillins are used in all age groups and both genders. Their increased consumption needs yet to be investigated but is paralleled by an increased occurrence of staphylococcal skin infections observed in recent years.

5.3.4. Measures at treated patient level

In 2016, the number of prescriptions was 522 per 1000 inhabitants, a 1.6% reduction from the 531 prescriptions per 1000 inhabitants in 2015 and a 17% reduction compared to the 630 prescriptions per 1000 inhabitants in 2007 (Table 5.2). The average number of prescriptions per patient is 1.9. This has not changed significantly during the last decade, thus in 2016 the number of treated patients was 270 per 1000 inhabitants, showing a similar decrease of 17% since the 323 patients per 1000 inhabitants in 2007 (Table 5.3 and A5.2 in web annex). Trends in the number of prescriptions and treated patients for the different antimicrobial classes followed mainly the trends

Figure 5.4. Consumption of leading penicillins in primary health care, Denmark

DANMAP 2016

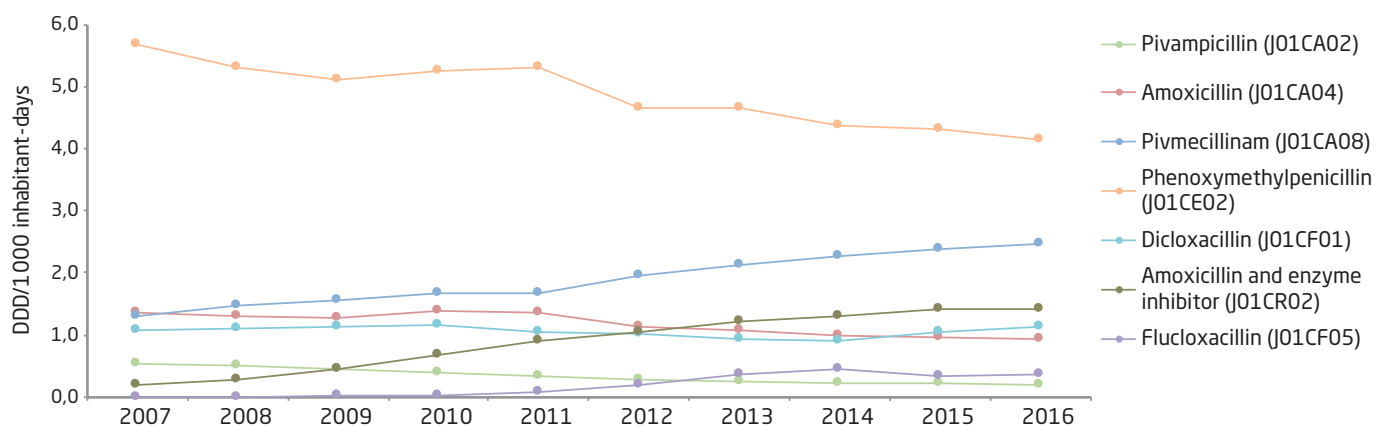


Table 5.2. Number of prescriptions per 1000 inhabitants for leading antimicrobial agents in primary health care, Denmark

DANMAP 2016

ATC group ^{a)}	Therapeutic group	Year									
		2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
J01AA	Tetracyclines	20.78	20.92	21.62	22.49	22.70	22.55	22.89	20.00	17.90	17.18
J01CA	Penicillins with extended spectrum	121.86	120.31	119.28	127.23	125.17	115.89	114.28	113.83	113.54	113.17
J01CE	Beta-lactamase sensitive penicillins	234.86	216.09	205.85	212.19	213.32	186.88	180.51	170.70	163.10	157.14
J01CF	Beta-lactamase resistant penicillins	41.83	42.22	42.10	42.32	42.75	40.41	41.24	41.04	40.82	41.87
J01CR	Combinations of penicillins, including beta-lactamase inhibitors	5.14	7.05	11.15	16.53	21.11	24.71	28.01	29.02	30.73	31.13
J01E	Sulphonamides and trimethoprim	53.03	48.89	47.17	47.35	45.05	43.85	43.53	41.51	38.39	36.42
J01FA	Macrolides	97.74	91.47	87.24	97.34	104.22	85.87	74.50	68.01	68.00	68.85
J01MA	Fluoroquinolones	19.87	22.07	21.71	23.69	23.15	22.14	20.64	19.67	19.51	18.74
J01X	Other antibacterials (methenamine >99%)	16.42	17.34	17.93	17.49	18.23	18.03	17.41	16.73	16.28	15.82
P01AB	Nitroimidazole derivatives (metronidazole)	16.91	15.32	19.02	19.67	19.69	19.67	19.26	19.06	19.16	18.63
J01 (incl. P01)	Antibacterial agents for systemic use (total)	630.08	606.26	595.28	628.78	638.08	582.69	565.16	542.53	530.62	522.23

a) From the 2016 edition of the Anatomical Therapeutic Chemical (ATC) classification system

already described for the consumed DIDs. Most pronounced for the ten year period were the decreases in the number of treated patients with beta-lactamase sensitive penicillins (- 29%), sulphonamides (- 48%) and macrolides (- 25%). Also for penicillins with extended spectrum and for tetracyclines a decrease was observed, (- 10% and - 12%, respectively). Similar decreases were noted when measured in the number of prescriptions per 1000 inhabitants for beta-lactamase sensitive penicillins (- 33%), sulphonamides (- 51%), macrolides (- 30%), tetracyclines (- 17%) and penicillins with extended spectrum (- 7%). Fluoroquinolones decreased with 5.4% and 5.7% in the number of treated patients and the number of prescriptions per 1000 inhabitants, respectively. A comparison of the dif-

ferent indicators of consumption is shown in Figure 5.5.

In 2016, each patient received an average of 21.5 DDD, a slight decline from 21.8 DDD the year before and a 9.7% increase compared to 2007 (19.6 DDD). The number of DDD/package increased similarly from 9.3 in 2007 to 10.5 in 2016 (13%). The number of packages has remained unchanged during all ten years with 2.1 packages per patient in average, (Table A5.3 in web annex).

5.3.5 National initiatives on continued reductions of the antimicrobial consumption

The National Action plan on the reduction of antibiotics

Figure 5.5. Indicators of antimicrobial consumption (J01) in primary health care, Denmark

DANMAP 2016

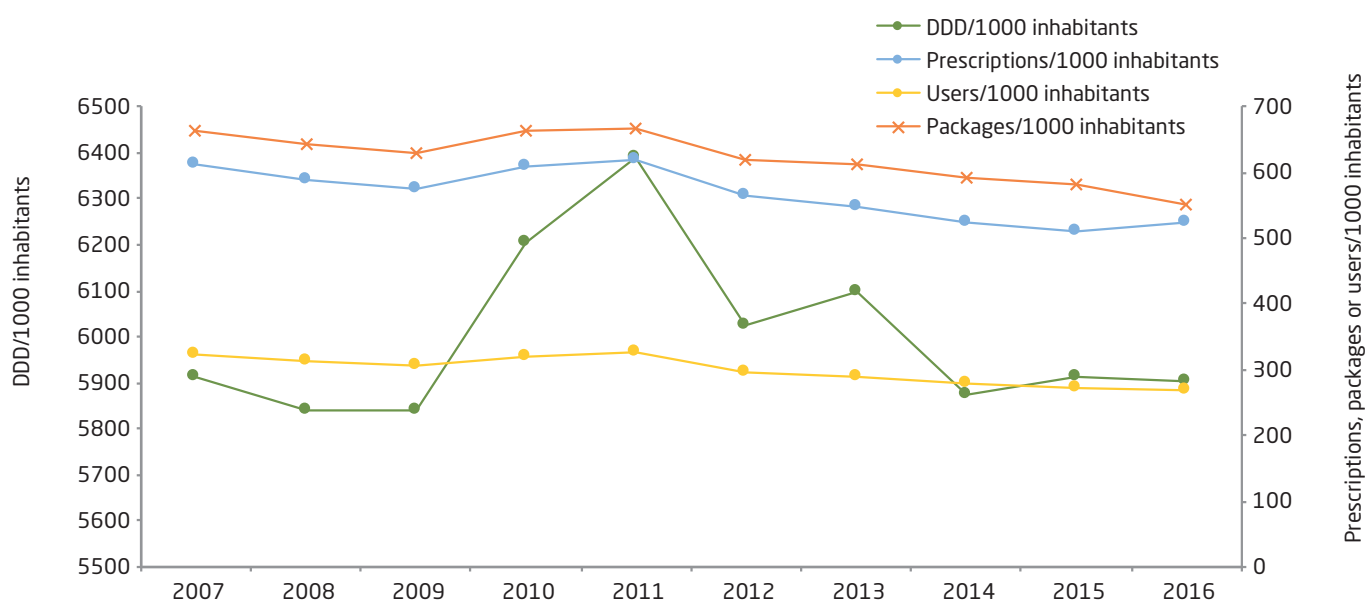


Table 5.3. Number of treated patients per 1000 inhabitants for leading antimicrobial agents in primary health care, Denmark

DANMAP 2016

ATC group ^(a)	Therapeutic group	Year									
		2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
J01AA	Tetracyclines	12.52	12.73	13.02	13.44	13.66	13.53	13.85	12.20	11.32	11.04
J01CA	Penicillins with extended spectrum	82.03	81.27	81.07	85.04	84.19	77.29	76.09	75.32	74.88	74.05
J01CE	Beta-lactamase sensitive penicillins	177.07	164.38	158.72	162.81	164.34	145.50	142.16	134.79	130.07	125.70
J01CF	Beta-lactamase resistant penicillins	29.67	29.89	29.87	30.02	30.34	28.50	29.07	29.24	28.85	29.70
J01CR	Combinations of penicillins, including beta-lactamase inhibitors	3.57	4.95	8.02	11.70	14.95	17.34	19.71	20.52	22.03	22.17
J01E	Sulphonamides and trimethoprim	33.62	30.49	29.51	29.31	27.63	26.47	26.15	24.65	22.45	21.20
J01FA	Macrolides	71.38	66.84	64.44	72.67	78.75	64.72	56.15	51.38	51.75	53.21
J01MA	Fluoroquinolones	15.19	17.05	16.87	18.45	18.10	17.24	16.04	15.30	15.04	14.37
J01XX	Other antibacterials (methenamine >99%)	7.17	7.43	7.67	7.53	7.74	7.54	7.48	7.16	7.35	7.47
J01XD and P01AB	Nitroimidazole derivatives (metronidazole)	14.44	15.32	16.28	16.73	16.90	16.86	16.51	16.31	16.47	16.04
J01 (incl. P01)	Number of treated patients in total	323.49	311.37	306.41	318.69	324.91	296.32	289.47	278.62	273.49	269.73

a) From the 2016 edition of the Anatomical Therapeutic Chemical (ATC) classification system

in humans issued in July 2017 uses two measurable goals directed at the consumption trends in the primary care. The first goal aims at a continued general reduction in the number of prescriptions issued in Denmark (from 462 prescriptions per 1000 inhabitants among general practitioners, medical specialists and dentists in 2016 to 350 prescriptions per 1000 inhabitants in 2020). The second goal focuses on the more prudent choice of antimicrobials emphasizing the importance of continued use of beta-lactamase sensitive penicillins as the drug of first choice in many common infections, especially in respiratory infections.

The National Action plan is supported through the National antibiotic campaigns aiming at a continued education of the public on general issues regarding prudent use including also hand hygiene as an important measure to reduce the spread of all kind of infections.

The National Action plan is issued from the Danish Health Ministry and supported by the National antibiotic council representing all relevant health institutions, organisations and specialties, working with the prevention, control and treatment of infections in Denmark. Together with the National Action Plan a One Health Strategy was issued, building on the National Action Plan on controlling the development of antimicrobial resistance from 2010. Both are available at the Danish Ministry of Health's homepage at www.SUM.dk.

5.3.6 Consumption of antimicrobials in children

The total consumption in children of all age groups continued the decreases observed for the last decade, regardless of the indicator used (Figures 5.6a and 5.6b). In 2016, altogether 7.9 DID were consumed in children and young from 0 to 19 years, corresponding to 218 treated patients per 1000 inhabitants (children) and 355 prescriptions issued per 1000 inhabitants.

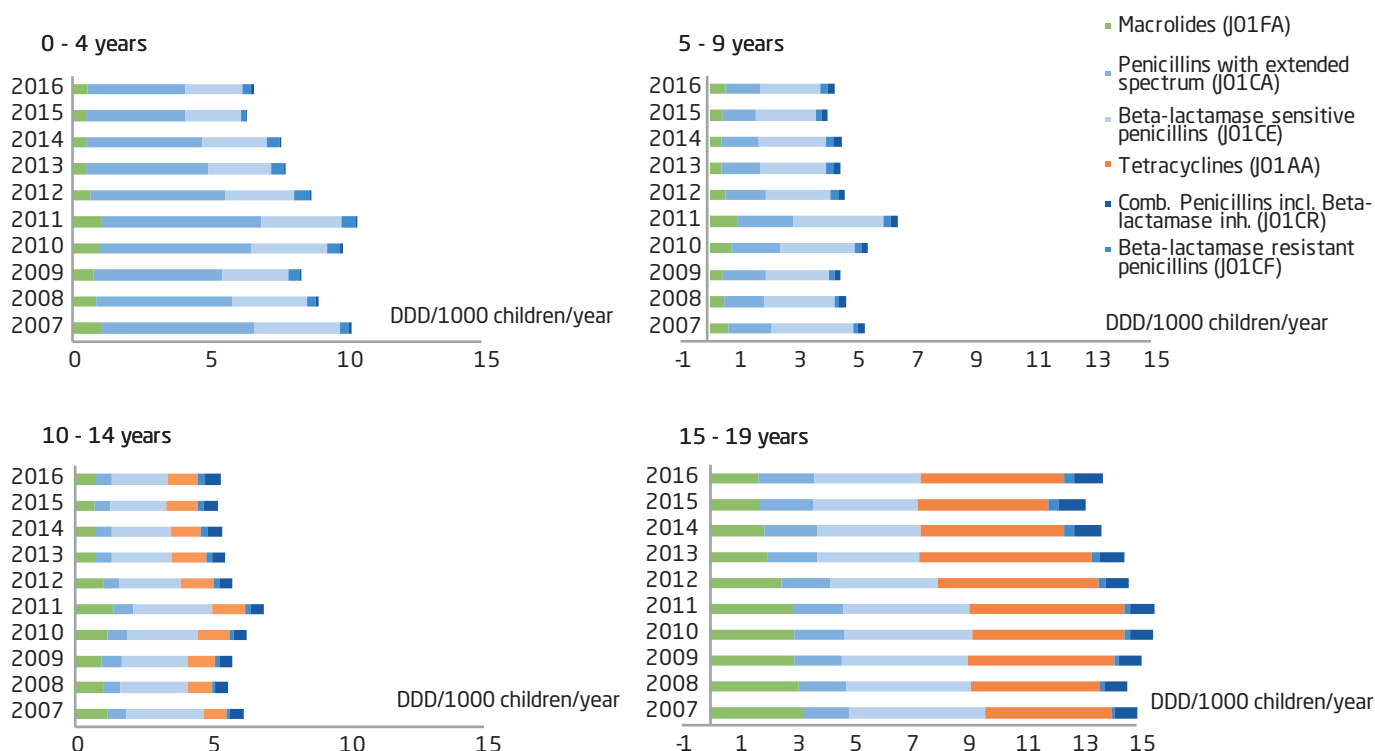
It is important to note that measuring the consumption in children in Defined Daily Doses is problematic since the system of Defined Daily Doses was developed based on the "maintenance dose per day for its main indication in adults" (https://www.whocc.no/ddd/definition_and_general_considera/). For children, different pharmacodynamics and -kinetics apply and especially dosing in the younger classes is based on doses per bodyweight in kg. Still, assuming that dosage regimens did not vary considerably over the last decade, the decreases shown for the consumption of DIDs in the different age groups show a clear tendency to reductions for especially penicillins and macrolids (Figure 5.6a).

From 2007 to 2016, the total DIDs consumed in 0 to 4 year olds decreased with 35 %, in 5 to 9 year olds with 20%, in 10 to 14 year olds with 13% and in the young adults with 8%. The corresponding number of prescriptions redeemed for all young age groups (0 to 19 year olds) decreased from 542 to 355 prescriptions per 1000 inhabitants (- 33%) and from 305 to 218 treated patients per 1000 inhabitants (- 28%) for the decade, respectively. Differences in reduction varied from a decrease of 43% in the number of prescriptions for the youngest (0 - 4 years old) to - 22% for the oldest, (15 to 19 year olds). When measured in the number of treated patients, the decreases varied from - 33% in the youngest to -19% in the adolescents.

In the youngest age group of 0 to 4 year olds, the boys receive on average 10% more prescriptions than the girls, a trend that has been quite stable. Thus in 2007, they received 488 versus 453 prescriptions per 1000 inhabitants (children) and in 2016 331 versus 304 prescriptions per 1000 inhabitants, respectively (not shown). For the 5 to 19 year olds the trends are opposite, girls receiving on average 10% more prescriptions.

Figure 5.6a. Consumption of leading antimicrobials in children/adolescents aged 0 - 19, Denmark

DANMAP 2016

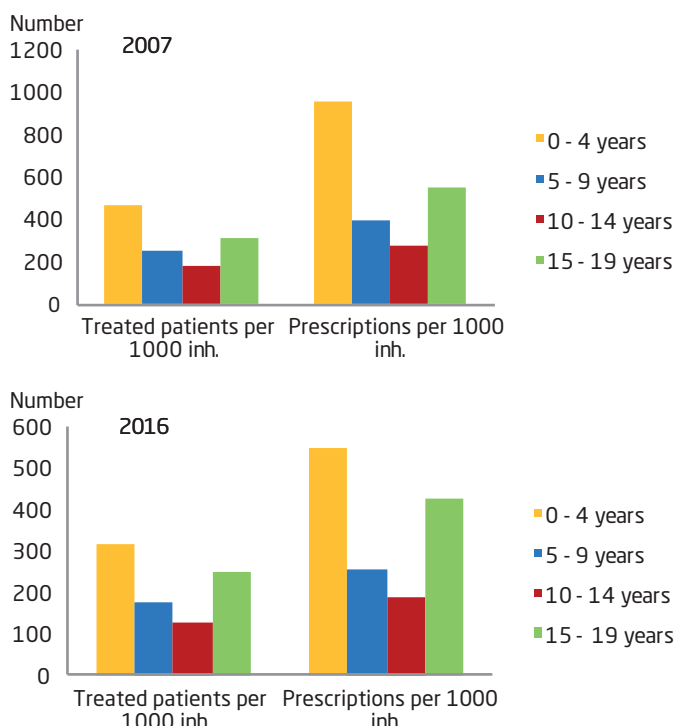


Since children contribute with a high proportion to the total antimicrobial consumption, continued focus on these age groups and their consumption habits is important for the success of the overall reduction plans for the consumption of antimicrobi-

als in humans. Thus, continued focus will be paid to this group and their parents in the National Antibiotic campaigns and in the continuing education of general practitioners and medical specialists on continued prudent use of antimicrobials. Children suffer more often than any other age group from upper airway infections caused by different virus and thus will be a core focus in the overall reduction of antimicrobials prescribed in Denmark as mentioned in the National action plan on the reduction of antibiotics from 2017.

Figure 5.6b. Number of prescriptions and treated patients per 1000 inhabitants aged 0-19 in 2007 and 2016, Denmark

DANMAP 2016



Macrolides are the drug of choice for respiratory tract infections with *Mycoplasma pneumoniae* and in patients with known or suspected allergy to penicillins. In the young age groups the 15 to 19 year olds account for the biggest consumption of macrolides (78 prescriptions per 1000 inhabitants per year compared to 34 for the 0 to 14 year olds for 2016, not shown). Since *Mycoplasma pneumoniae* primarily affects young school children and tends to occur every four to six years, the consumption of macrolides is expected to mirror these epidemics. In the winter of 2015 to 2016, a long-lasting but less fulminant epidemic with *Mycoplasma pneumoniae* occurred, spanning two seasons as had been observed with the last epidemic in 2010 to 2012 (Epi-News No 41_2016, available at www.ssi.dk). After an overall decrease of the number of prescriptions on macrolides issued from 2007 (69.3 prescriptions per 1000 inhabitants) to 2015 (40.7 prescriptions per 1000 inhabitants), the consumption increased slightly in 2016 to 44.8 prescriptions per 1000 inhabitants redeemed on average for all young age groups. Increases were noted for all, but were most obvious in the 5 to 9 year olds (from 23 to 29 pre-

Figure 5.7a. Consumption of leading antimicrobials (DDD/1000 inhabitants/day) in males and females, Denmark

DANMAP 2016

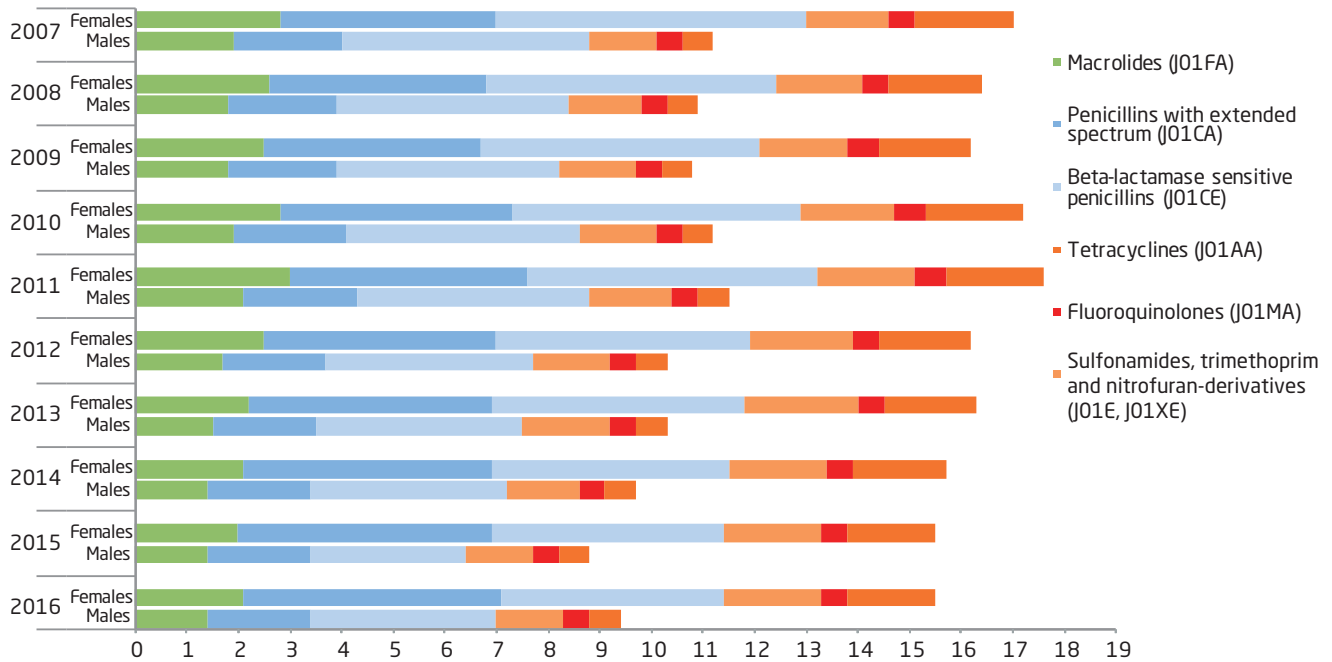


Figure 5.7b. Number of treated men/women and of prescriptions per gender per 1000 inhabitants, 2007-2016

DANMAP 2016



scriptions per 1000 inhabitants, corresponding to an increase of 30%).

Tetracyclines account for a considerable part in the consumption of antimicrobials among adolescents due to the treatment of acne. Treatments are long-lasting (up to six months) and in addition may be repeated in a situation of relapse in a patient, who may be suffering from the condition for years. Both genders are affected, but there exist clear differences in prescription habits between boys and girls. Thus, among girls the treatment periods are longer and extend into the young adults of 20 to 24 years, while boys primarily are treated in shorter periods at the age of 15 to 19 years. In 2007, 15 to 19 year old boys received, on average, 70 versus girls receiving 52 prescriptions per 1000 inhabitants per year, corresponding to 32 versus 28 patients treated per 1000 inhabitants. In 2016, the number of 15-19 year old boys receiving treatment had declined to 28 treated patients (corresponding to 49 prescriptions) per 1000 inhabitants, while the number of 15 to 19 year old girls had increased to 32 treated patients (corresponding to 54 prescriptions redeemed) per 1000 inhabitants. Still, in 2016 girls, on average, received fewer prescriptions per patient than in 2007, pointing towards shorter treatment courses and an increased awareness on a more prudent use. Danish treatment recommendations issued in October 2014 through the former "Institute for Rational Pharmacotherapy" (now the "Initiatives on Rational Pharmacotherapy" under the Danish Health Authority) underline the importance of reducing the use of tetracyclines in the young. They focus on topical (immunomodulation) treatment in mild to moderate cases and on shortening and frequently evaluating the treatment course once treatment with systemic tetracyclines is begun.

5.3.7 Consumption of antimicrobials in men and women

Differences between the genders regarding consumption of antimicrobials is well known, (Figure 5.7a and 5.7b). Women receive in general more treatments, a trend driven by a much

higher consumption of antimicrobials used for the treatment of urinary tract infections. Thus, the consumption of sulfonamides, trimethoprim and nitrofurantoin is three times higher than in the male population and the consumption of pivmecillinam in women doubles the consumption in men. For beta-lactamase sensitive penicillins and tetracyclines there are less marked differences in gender and for the consumption of fluoroquinolones no differences have been observed through the years.

From 2007 to 2016 the number of treated women per 1000 inhabitants decreased from 373 to 317 (- 15%) and the number of treated men from 273 to 222 (- 19%). In the same period, the amount of DDD/prescription increased for women from 8.9 to 10.8 and for men from 9.6 to 11.5, resulting in an overall decrease of DIDs consumed in women from 1.99 to 1.90 DID (- 5%), and a decrease in men from 1.40 to 1.26 DID (- 10%).

As urinary tract infections (UTI) are a common condition in many women and contribute significantly to the number of antimicrobial treatments in these, several Danish studies have investigated in better diagnostic tests helping to differentiate between bacterial conditions demanding antimicrobial treatment and more unspecific irritative signs from the urinary mucosa better left untreated with antibiotics. Especially in elderly women, it becomes difficult to clearly differentiate between inflammation and infection, not least due to transient asymptomatic bacteriuria. In 2016, the National antibiotic campaign thus focused on reducing the amount of antimicrobials consumed for the treatment of UTIs through two different initiatives, one using an educating movie on the social media targeted at young women, the other directed at health personnel at nursing homes dealing with often confused or dement elderly women with unspecific signs of UTI. Hopefully, the initiatives will show a measurable effect in decreased amounts of antimicrobials used for women in the future.

Table 5.4. Consumption of antimicrobial agents for systemic use in primary health care at regional level, Denmark DANMAP 2016

Region	Indicator	Year					
		2011	2012	2013	2014	2015	2016
Capital Region	DDD/1000 inhabitants/day	17.9	16.9	16.9	16.3	16.4	16.0
	Prescriptions/1000 inhabitants	657.6	599.2	576.7	549.4	533.6	519.6
Region Zealand	DDD/1000 inhabitants/day	17.8	16.7	16.9	16.5	16.9	17.2
	Prescriptions/1000 inhabitants	677.4	618.6	601.4	579.5	575.4	574.8
Region of Southern Denmark	DDD/1000 inhabitants/day	17.3	16.2	16.5	15.8	15.8	15.8
	Prescriptions/1000 inhabitants	658.0	598.4	588.9	556.5	540.3	530.7
Central Denmark Region	DDD/1000 inhabitants/day	16.0	15.3	15.5	15.1	15.2	15.1
	Prescriptions/1000 inhabitants	573.8	531.9	512.8	500.5	494.5	487.4
North Denmark Region	DDD/1000 inhabitants/day	16.4	15.2	15.4	15.1	15.2	15.4
	Prescriptions/1000 inhabitants	620.1	557.6	541.2	525.6	510.6	509.4
Denmark (total)	DDD/1000 inhabitants/day	17.3	16.8	17.0	16.4	16.5	16.2
	Prescriptions/1000 inhabitants	637.4	581.1	564.2	542.2	530.9	522.2

5.3.8 Prescribing activity in primary care

Although Denmark has a very homogenous population with relatively small geographic and socioeconomic variations, there are still considerable differences in the prescription habits among medical doctors. In 2016, the Central Region of Denmark had the lowest prescription activity with 15.1 DID and 487.4 prescriptions per 1000 inhabitants, (Table 5.4). The Region of Zealand had the highest prescription activity with 17.2 DID and 574.8 prescriptions per 1000 inhabitants. All regions have seen marked decreases in the DID and number of prescriptions issued for the five years shown, decreasing on average 6.4% in DID and 17% in the number of prescriptions per 1000 inhabitants (Table 5.4).

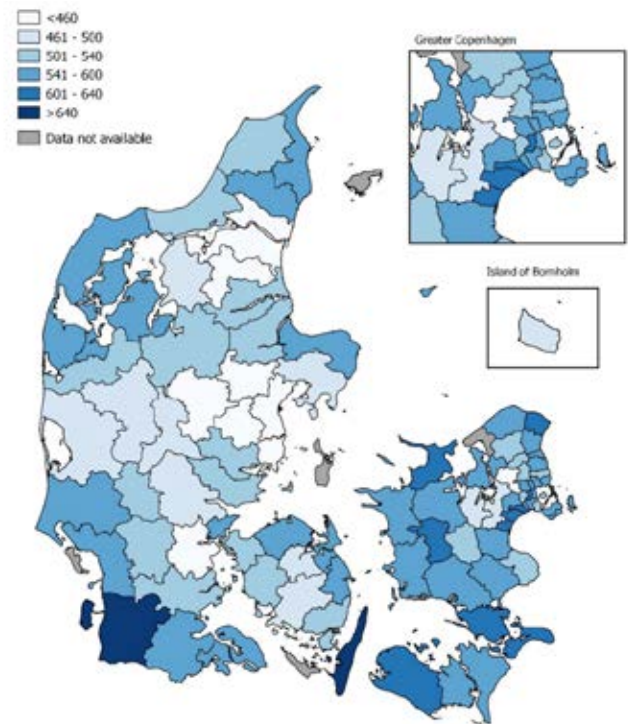
There may be several reasons for the differences in prescribing habits, e.g. the density of the population and number of general practitioners as well as the proportion of elderly or chronically ill in a given geographic area. The number of patients assigned to each clinic, driving distances to the nearest hospital or the nearest medical specialist as well as organizational differences could also play a role. Finally, the number of general practitioners in a clinical practice probably will influence the prescribing habits - in clinics with more personnel it is easier to participate in educational courses and academic meetings. A Clinic with more staff also gives the advantage to discuss individual patients with the colleagues and supervise each other on a daily basis. General practitioners can follow their own prescription habits through the website www.ordiprax.dk, a closed IT system that collects all prescribed data and enables comparison with other practices on a regional level.

Support of the general practitioners regarding their prescribing habits in general is provided through regional medicine consultants, who also have access to Ordiprax on each clinic level, thus being able to monitor consumption and give individual advice. In the Central Region of Denmark, these consultants have worked specifically with focus on a prudent use of antimicrobials.

In Figure 5.8 the number of prescriptions on municipality level is shown, spanning from 434 to 728 prescriptions per 1000 inhabitants. In 2016, most municipalities were within the range of 475 to 575 prescriptions per 1000 inhabitants. From the 98 municipalities in Denmark, four were excluded from the Figure due to very small populations (typically islands).

The part of prescriptions issued through hospital doctors has been increasing through the last decade; in 2016, they accounted for 1.3% of the antimicrobials sold at pharmacies, in 2007 it was 6% (not shown). The prescribing activity of hospital doctors has presumably increased due to the mentioned changes in hospital activity with the shortening of bed-days. These changes rely on patients completing a treatment at home and on a primary sector who takes care of and follows up on these patients. In this regard, it is important to note that

Figure 5.8 Number of primary health care prescriptions/1000 inhabitants in Danish municipalities, 2016 DANMAP 2016



(National average=522 prescriptions/1000 inhabitants)

the goals in the National Action Plan on antibiotics in Human Healthcare from 2017 directed at the consumption in primary care include prescriptions issued from General Practitioners, medical specialists and dentists but exclude the prescribing activity from hospital doctors upon discharge of the patient. This will be taken into account when defining the different initiatives on prudent use of antibiotics and most of all the monitoring of these.

5.4 Hospital Care

5.4.1 Introduction

Antimicrobial consumption at hospitals is reported to DANMAP once a year through the Register of Medicinal Product Statistics at the Danish Health Data Authority. Reporting is based on deliverances from the hospital pharmacies to the different clinical departments and includes all generic products that are supplied through general trade agreements between the hospital and different medical suppliers. In the case of production failures and shortages in deliverance of specific products, the hospitals have to apply for special deliverances through the Danish Medicines Agency. These special deliverances are reported separately to DANMAP. In general it is assumed that the amount of sold antimicrobials is comparable to the overall consumption at the different departments. Still, information is lacking on consumption at the individual patient level. Although all hospitals have been connected to the centralized "shared medicine card", collecting information on all medicine prescribed on an individual level for all Danish citizens, the sys-

tem does not yet include more detailed information regarding the hospital stay such as indication for the actual treatment or dosages given, length of treatment etc. This information is expected to be available through the future National "Hospital Medicine Register", which is currently being developed.

DANMAP 2016 covers the total sales on systemic antimicrobials (all ATC code J01 as well as ATC code P01AB01 and A07AA09) reported from all Danish hospitals. Consumption at private hospitals and psychiatric departments was excluded, in 2016 accounting for approximately 2.2% of the total hospital consumption.

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. Data are also presented as DID to enable comparison with

primary health care. During the past decade, the hospitalization pattern in Denmark changed notably: more people are admitted to somatic hospitals, while the average length of stay is shortened considerably. Since selection pressure for the emergence of antimicrobial resistance increases with increasing hospital activity, the selection pressure has increased considerably from 2007 to 2016.

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

In 2016, the consumption of antimicrobial agents in somatic hospitals was 99.98 DBD, 3% lower than the 103.06 DBD in 2015 and 4.2% lower than the highest consumption measured in 2014 (104.34 DBD). Since 2007, the consumption increased with 35% (from 74.33 DBD), (Table 5.5). This reflects the changing workflows at hospitals with an increased number of admissions and decreased number of bed-days. In 2016,

Table 5.5. Consumption of antimicrobial agents for systemic use in somatic hospitals (DDD/100 occupied bed-days), Denmark

		DANMAP 2016									
ATC group ^{a)}	Therapeutic group	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
J01AA	Tetracyclines	0.63	0.78	1.04	1.09	1.18	1.58	1.52	1.73	1.85	2.06
J01CA	Penicillins with extended spectrum	13.42	13.96	15.37	14.61	14.41	14.90	15.06	16.40	17.01	16.75
J01CE	Beta-lactamase sensitive penicillins	10.79	9.98	9.90	9.49	9.32	10.13	10.13	10.31	10.03	9.77
J01CF	Beta-lactamase resistant penicillins	6.70	6.81	7.40	7.71	7.30	8.37	9.07	9.57	9.80	8.80
J01CR	Combinations of penicillins, incl. beta-lactamase inhibitors	2.95	4.00	5.65	7.13	8.51	12.00	13.64	16.04	17.80	18.04
J01DB	First-generation cephalosporins	0.13	0.18	0.13	0.13	0.13	0.12	0.11	0.06	0.04	0.04
J01DC	Second-generation cephalosporins	12.31	13.32	15.76	16.21	16.14	14.15	12.32	11.68	10.37	9.16
J01DD	Third-generation cephalosporins	1.03	1.25	1.42	1.26	1.39	1.07	1.08	1.02	1.06	1.04
J01DF	Monobactams	0.04	0.07	0.06	0.09	0.19	0.15	0.14	0.06	0.03	0.01
J01DH	Carbapenems	2.13	2.70	3.15	4.02	4.16	3.86	4.02	4.09	4.10	3.93
J01EA	Trimethoprim and derivatives	0.44	0.44	0.44	0.36	0.36	0.38	0.41	0.52	0.46	0.38
J01EB	Short-acting sulfonamides	0.34	0.35	0.35	0.33	0.25	0.20	0.20	0.18	0.15	0.11
J01EE	Combinations of sulfonamides and trimethoprim, incl. derivatives	1.52	1.95	2.28	3.04	4.11	3.33	4.21	4.70	5.04	5.11
J01FA	Macrolides	3.08	3.06	3.42	3.52	3.69	3.56	3.39	3.88	4.56	4.78
J01FF	Lincosamides	0.35	0.41	0.50	0.47	0.53	0.62	0.64	0.65	0.57	0.62
J01GB	Aminoglycosides	1.79	1.64	1.56	1.71	1.91	2.14	2.13	1.61	1.68	1.94
J01MA	Fluoroquinolones	8.16	9.53	10.71	10.44	10.70	10.02	9.77	9.88	9.30	8.11
J01XA	Glycopeptides	0.63	0.68	0.99	1.07	1.24	1.29	1.29	1.15	1.07	1.07
J01XB	Polymyxins	0.05	0.05	0.07	0.10	0.09	0.09	0.16	0.27	0.25	0.29
J01XC	Steroid antibacterials (fusidic acid)	0.28	0.26	0.31	0.34	0.27	0.23	0.22	0.23	0.16	0.11
J01XD	Imidazole derivatives	2.62	3.27	3.84	3.93	4.19	4.16	4.08	4.48	4.25	4.51
J01XE	Nitrofurantoin derivatives (nitrofurantoin)	0.28	0.29	0.36	0.31	0.33	0.34	0.38	0.36	0.32	0.24
J01XX05	Methenamine	0.09	0.10	0.09	0.08	0.10	0.09	0.09	0.07	0.10	0.08
J01XX08	Linezolid	0.16	0.21	0.22	0.22	0.32	0.32	0.35	0.34	0.43	0.33
J01XX09	Daptomycin	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.03	0.04	0.05
P01AB01	Nitroimidazole derivatives (metronidazole)	2.20	2.43	2.91	2.99	3.12	2.89	2.70	2.48	2.05	2.17
A07AA09	Intestinal anti-infectives (vancomycin)	2.19	2.43	2.93	2.96	3.12	2.98	2.75	2.55	0.47	0.48
J01	Antibacterial agents for systemic use (total)	74.33	80.14	90.87	93.67	97.08	98.94	99.88	104.30	103.02	99.98

a) From the 2016 edition of the Anatomical Therapeutic Chemical (ATC) classification system

the number of admissions at Danish hospitals was 1,365,311, while the number of bed-days was 4,064,079 (data from the Danish National Patient Register march 2017). Since 2007, the number of bed-days decreased with altogether 19%, while the number of admissions increased with 13%. Changes in hospital activity were most pronounced from 2008 to 2009, where the number of bed-days within one year decreased with 9.8%, while the number of admissions increased with 5.7%. On average, the number of bed-days decreased with 2.2% yearly, while the number of admissions increased with 1.3% per year (Figure A5.2 in web annex).

In 2016, the four penicillin groups accounted for altogether 53.36 DBD, corresponding to 54% (Table 5.5, Figure 5.9). For the second year in a row the combination penicillins constituted with 18.04 DBD the biggest group consumed (18%), and the penicillins with extended spectrum with 16.75 DBD the second biggest (17%). Beta-lactamase sensitive penicillins accounted for 9.77 DBD (10%) and beta-lactamase resistant penicillins for 8.80 DBD (9%).

Since 2007, the combination penicillins and the penicillins with extended spectrum increased with 15.09 DBD and 3.33 DBD, respectively, reflecting the changes that have happened in the treatment of septic patients. Today, piperacillin with tazobactam or a penicillin with extended spectrum combined with an aminoglycoside are often the drug of choice in the treatment of the septic patient with a suspected Gramnegative bacte-

remia. Until 2011, it used to be cefuroxime, a second generation cephalosporin. During the last decade the consumption of beta-lactamase sensitive penicillins remained relatively stable around 9.5 to 10.5 DBD after decreasing from its top of 12.17 DBD in 2005. The decade before, from the beginning of registration in DANMAP, it had been continuously increasing from 8.02 DBD in 1997. Beta-lactamase resistant penicillins had also been increasing for all years, from 4.44 DBD in 1997 to the top of 9.80 in 2015. The 10% decrease from 2015 to 2016 was the first marked decrease observed for the whole period.

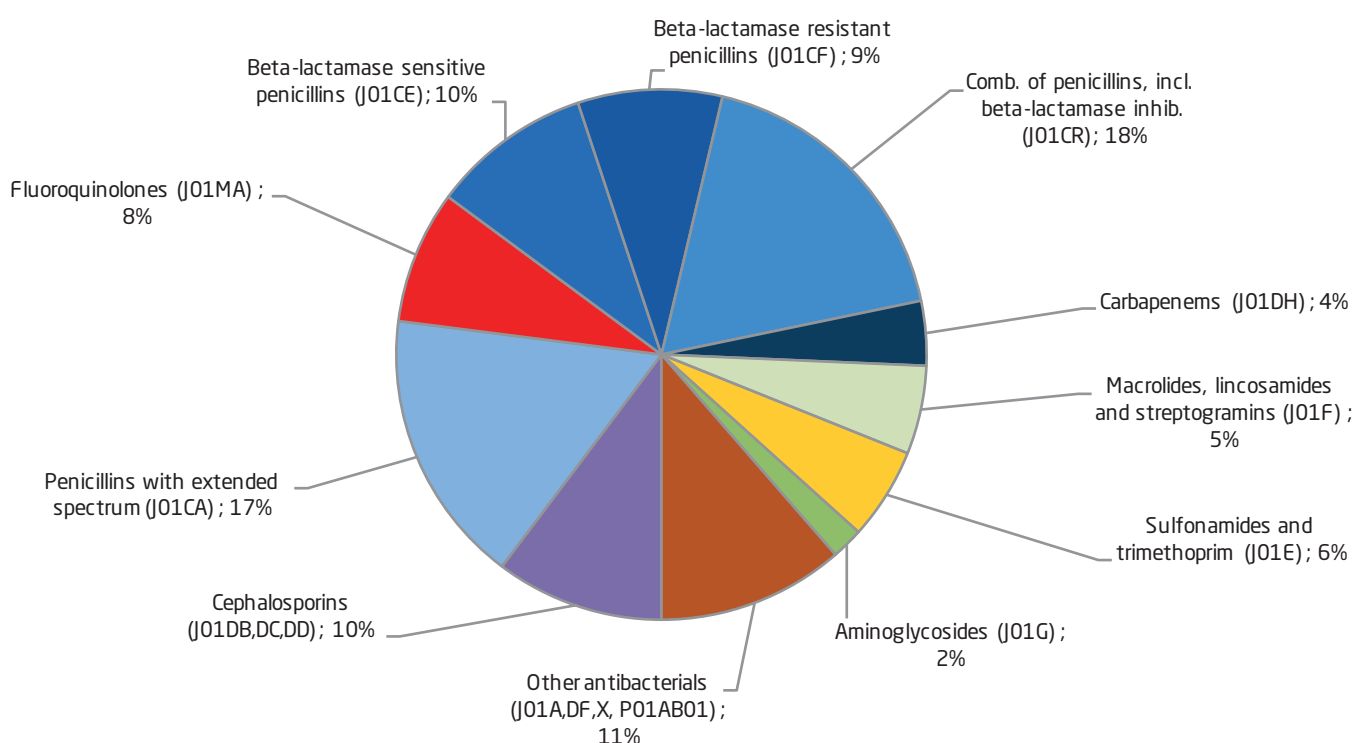
Although tetracyclines only account for a minor part of the antimicrobials consumed at hospitals it is worth noting that the drug group has seen continuous increases for the past decade; in 2007 they accounted for 0.63 DBD, while in 2016 this had increased to 2.06. From 2015 to 2016 the consumption increased with 11%. In 2016, the proportion of tigecyclin constituted 1.1% of the tetracyclines consumed.

Two other antimicrobial classes that have seen continuous increases during the past decade are the combinations of trimethoprim and sulfonamides, including derivatives, and the macrolides. From 2007 to 2016, these increased from 1.52 to 5.11 DBD (> 200%) and from 3.08 to 4.78 DBD (55%), respectively.

In 2016, cephalosporins accounted with altogether 10.24 DBD for 10%, a marked decrease from the 15% last year. Second

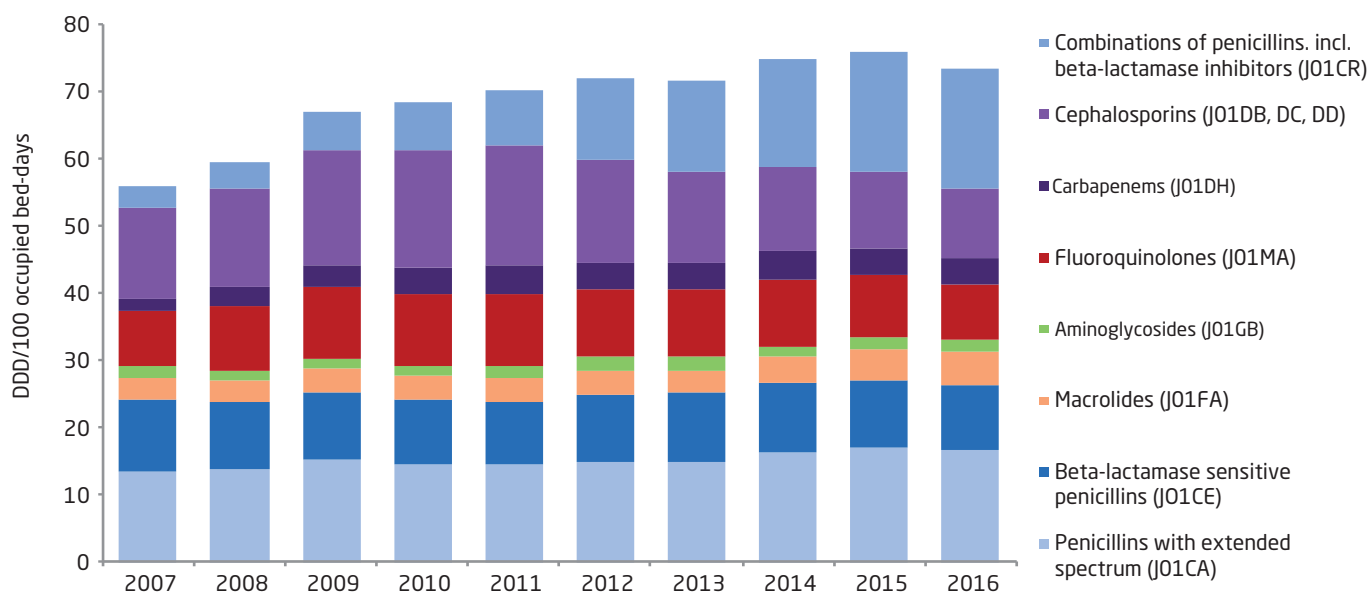
Figure 5.9. Distribution of the total consumption of antimicrobial agents in somatic hospitals, Denmark

DANMAP 2016



Total somatic hospital consumption (DBD) by leading groups of antimicrobial agents (J01), Denmark

DANMAP 2016



generation cephalosporins are the most used at hospitals and accounted for 9.16 DBD. In addition to its former important role in the treatment of septic patients, cefuroxime has been widely used for surgical prophylaxis, but in many clinical settings today, dicloxacillin (a beta-lactamase-resistant penicillin) is used instead. Fluoroquinolones accounted for 8.11 DBD, another marked (13%) reduction from 9.30 DBD in 2015. The consumption of fluoroquinolones topped in the years of 2009 to 2011 with an average of 10.60 DBD and has since shown slight declines. Carbapenems accounted in 2016 for 3.93 DBD, a 4% decline from the 4.10 DBD in 2015. This is the second time in the ten year period that a decrease in the consumption of carbapenems was observed. The last time was from 4.16 DBD in 2011 to 3.86 DBD in 2012.

Together the three groups of critically important antimicrobials in Denmark (cephalosporins, fluoroquinolones and carbapenems) constituted 22%, in 2015 it was 24% and ten years ago, in 2007, it was 32%. Trends in the consumption of cephalosporins, fluoroquinolones and carbapenems are shown in Figure 5.10 and 5.11. The consumption of these three antimicrobial groups will be monitored closely also in the future due to several local, regional and national initiatives, the most important one probably being the implementation of the third measurable goal in the National Action Plan on antibiotics from 2017 aiming at a 10% reduction in the consumption of these from 2016 to 2020. Reductions are also aimed at through the "National Quality and Learning Teams", an initiative spanning all Danish regions, working on applying principles of antibiotic stewardship in many of the acute care hospitals at emergency departments and in medical departments with a relatively

high number of acute patients. In addition, the consumption of cephalosporins will be followed with extra interest next year. Due to shortages of piperacillin with tazobactam in 2017, it may have been necessary to reintroduce cephalosporins in the treatment of acutely ill, septic patients at several hospitals. Thus for 2017 an increase of the overall consumption of cephalosporins is expected, which will be difficult to counter-balance through the different national initiatives.

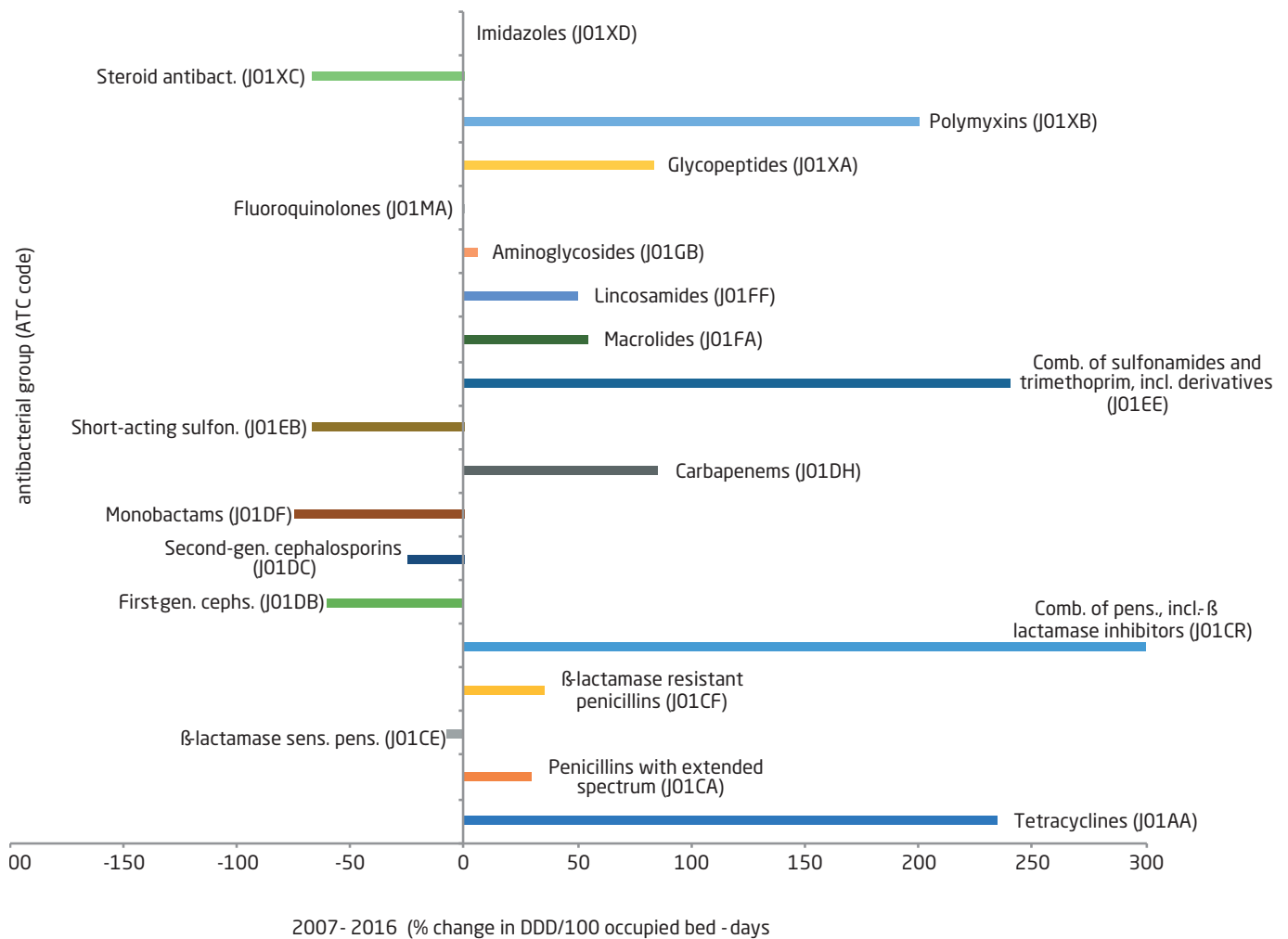
In 2016, the consumption of the leading groups of antimicrobials decreased, mainly due to the mentioned decreases observed for cephalosporins and fluoroquinolones. In 2016 the leading antimicrobials constituted 76.00 DBD of the total consumption of 99.98 DBD. In 2015 it was 73.56 DBD out of a total of 103.02 DBD. For most antimicrobial classes decreases were observed from 2015 to 2016 (Figure 5.10). During the last decade the consumption and proportions of many of the smaller antimicrobial classes increased, (Figure 5.11)

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

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

In 2016, the consumption was 310.53 DAD, a 2.8% decline from the 313.34 DAD in 2015 and 1.2% increase from 306.90 DAD in 2007. The highest top observed was in 2013 with 325.20 DAD.

Figure 5.11. Changes in the consumption (DBD) by leading groups of antimicrobial agents (J01) in the hospital sector, Denmark
 DANMAP 2016



* comb. penicillins, including beta-lactamase inhibitors increased with more than 300% (=512%)

The trends in DAD reflect for most antimicrobials the trends observed in DBD, yet differences over time may be bigger or smaller, compared to hospital activity, the number of patients treated with the specific drug class and the use of the individual antimicrobials in the treatment of acutely or chronically ill patients. The observed increases, when calculated in per cent, were thus more marked, when measured in DBD than in DAD for the following antimicrobial classes: tetracyclines, combination penicillins including beta-lactamase inhibitors, macrolides, lincosamides, glycopeptides, polymyxins, steroid antibacterials (fusidic acid), imidazole derivatives and daptomycin (Tables 5.5 and 5.6).

For three antimicrobial classes the opposite trends were observed for the past decade: penicillins with extended spectrum decreased 3.8%, when measured in DAD, but increased 25%, when measured in DBD; third generation cephalosporins decreased 25% in DAD, but increased 1.4% in DBD and aminogly-

cosides decreased with 19% in DAD, but increased with 8.3% in DBD.

At the regional level the hospital activity mirrors the density of the population. Numbers for Regional activity when calculated in bed-days and in hospital admissions are shown in Table 5.7.

For comparison with the usage of antimicrobials in animals, human consumption for the different antimicrobial classes measured in kg active substance is shown in Table A 5.1 in web annex. For comparison with the primary sector consumption at hospital level measured in DID can be found in Table A5.4 in web annex.

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Table 5.6. Consumption of antimicrobial agents for systemic use in somatic hospitals (DDD/100 admitted patients), Denmark

DANMAP 2016

ATC group(a)	Therapeutic group	Year									
		2007	2008(b)	2009	2010	2011	2012	2013	2014	2015	2016
J01AA	Tetracyclines	2.59	3.19	3.63	3.55	3.66	5.15	4.97	5.37	5.65	6.46
J01CA	Penicillins with extended spectrum	55.39	57.18	53.76	47.46	44.77	48.60	47.90	50.95	51.75	53.26
J01CE	Beta-lactamase sensitive penicillins	44.55	40.90	34.61	30.83	28.98	33.04	33.13	32.03	30.52	30.64
J01CF	Beta-lactamase resistant penicillins	27.64	27.89	25.86	25.04	22.71	27.30	29.64	29.73	29.82	27.77
J01CR	Comb. of penicillins. incl. beta-lactamase inhibitors	12.17	16.37	19.74	23.15	26.47	39.14	44.60	49.81	54.14	55.35
J01DB	First-generation cephalosporins	0.55	0.72	0.46	0.43	0.41	0.40	0.37	0.20	0.13	0.13
J01DC	Second-generation cephalosporins	50.81	54.55	55.12	52.65	50.19	46.17	40.27	36.29	31.53	28.31
J01DD	Third-generation cephalosporins	4.24	5.10	4.98	4.10	4.33	3.50	3.53	3.18	3.22	3.16
J01DF	Monobactams	0.18	0.27	0.21	0.29	0.60	0.48	0.45	0.19	0.08	0.03
J01DH	Carbapenems	8.78	11.08	11.01	13.07	12.55	12.60	13.14	12.70	12.50	11.94
J01EA	Trimethoprim and derivatives	1.81	1.80	1.56	1.17	1.11	1.23	1.33	1.62	1.40	1.29
J01EB	Short-acting sulfonamides	1.41	1.43	1.21	1.09	0.78	0.63	0.62	0.55	0.46	0.38
J01EE	Comb. of sulfonamides and trimethoprim. incl. derivatives	6.28	7.98	7.96	9.88	12.79	10.87	13.76	14.53	15.32	15.54
J01FA	Macrolides	12.70	12.53	11.97	11.45	11.47	11.61	11.08	12.04	13.90	14.83
J01FF	Lincosamides	1.46	1.69	1.74	1.52	1.63	2.01	2.09	2.03	1.75	1.88
J01GB	Aminoglycosides	7.39	6.71	5.45	5.56	5.95	6.99	6.97	5.01	5.11	5.98
J01MA	Fluoroquinolones	33.66	39.04	37.45	33.92	33.30	32.67	31.96	30.69	28.28	24.89
J01XA	Glycopeptides	2.61	2.77	3.48	3.47	3.87	4.20	4.22	3.58	3.25	3.24
J01XB	Polymyxins	0.22	0.21	0.24	0.32	0.28	0.30	0.54	0.85	0.77	0.88
J01XC	Steroid antibacterials (fusidic acid)	1.17	1.05	1.09	1.12	0.85	0.76	0.71	0.71	0.50	0.34
J01XD	Imidazole derivatives	10.83	13.39	13.43	12.76	13.03	13.55	13.33	13.93	12.93	13.68
J01XE	Nitrofurantoin derivatives (nitrofurantoin)	1.17	1.19	1.27	1.01	1.02	1.12	1.25	1.13	0.98	0.85
J01XX05	Methenamine	0.38	0.43	0.31	0.27	0.32	0.28	0.30	0.22	0.29	0.28
J01XX08	Linezolid	0.68	0.84	0.76	0.72	0.99	1.04	1.14	1.05	1.31	1.09
J01XX09	Daptomycin	0.03	0.06	0.06	0.07	0.05	0.06	0.07	0.10	0.12	0.15
P01AB01	Nitroimidazole derivatives (metronidazole)	9.10	9.99	10.20	9.72	9.70	9.44	8.83	7.72	6.24	6.73
A07AA09	Intestinal anti-infectives (vancomycin)	9.10	9.96	10.20	9.73	9.71	9.54	9.00	7.92	1.43	1.46
J01	Antibacterial agents for systemic use (total)	306.9	328.3	317.8	304.3	301.9	322.7	325.2	324.1	313.38	310.53

a) From the 2016 edition of the Anatomical Therapeutic Chemical (ATC) classification system

b) The number of admissions was affectedly low in 2008 due to a major hospital strike

Table 5.7. Activity in somatic hospitals, Denmark DANMAP 2016

Region	No. bed-days somatic hospitals ^(a)	No. admissions somatic hospitals ^(a)
The Capital Region of Denmark	1,486,698	484,004
The Sealand Region	603,831	236,853
Region of Southern Denmark	779,140	248,756
Central Denmark Region	809,151	281,664
North Denmark Region	385,259	114,034
Denmark ^(b)	4,064,079	1,365,311

Source: The Danish Health Data Authority (www.sds.dk)

a) Excluding private hospitals, psychiatric hospitals, specialized clinics, rehabilitation centres and hospices

b) Compared to 2015 no. bed-days have decreased by 2% and no. admissions remain at the same level

Textbox 5.1

Reducing antibiotic consumption among children and elderly through knowledge building - interventions joining primary and secondary health care sector

Introduction

The association between antibiotic use and antibiotic resistance is widely acknowledged. Preschool children and elderly residents of nursing homes are among the population groups with the highest antibiotic consumption. It has been estimated that a substantial amount of the antibiotics used in these two population groups are either unnecessary or inappropriate. The recognition has led to an increased awareness regarding the importance of strategies to reduce antibiotic resistance, including the promotion of appropriate use of antibiotics, especially in primary care, where the majority of antibiotics are prescribed.

The Department of Clinical Microbiology at Herlev and Gentofte Hospital has established collaborations with different players in primary health care in the Capital Region of Denmark. The department has designed interventions that apply the knowledge of microbiologists and infection control nurses towards the prevention and treatment of infections and prudent antibiotic use.

Education and acquirement of knowledge are the keywords for the interventions carried out at the Department of Clinical Microbiology. Below is described four ongoing projects targeted at children and elderly and focusing on knowledge building through collaboration with the primary care sector and Copenhagen University, Research Unit of General Practice.

Reduce antibiotic use among children

The Department of Clinical Microbiology conducted a survey among 1,170 general practitioners (GPs) in 2015 (response rate, 49%). The GPs were asked about their perceptions on parental knowledge and expectations in relation to antibiotic use. The results show that GPs perceived low parental knowledge and the GPs felt that parents, to some degree, explicitly (74 %) or implicitly (88 %) indicate that they expect antibiotics. About half the GPs (46%) were positive about the potentials of delayed antibiotic prescribing, but they rarely apply this. The results of the survey found that there could be a potential for implementing delayed prescription but also that there is a need for increasing knowledge about infections and antibiotics among parents. Based on the results of the survey and international research, two projects were designed to reduce inappropriate prescriptions.

Project 1: Is Delayed Prescription a Useful Tool to reduce Inappropriate Antibiotic Prescription among Children? Barriers and Opportunities seen by the GPs and the Parents (May 2016 to August 2017)

Delayed prescribing of antibiotics is one of most important strategies to reduce use of antibiotics, without influencing the outcome of the patients. Delayed antibiotic prescribing means that the patient is given a prescription, but it is recommended to wait and see if the symptoms will disappear spontaneously within the next few days. Patients are advised that if the symptoms get worse or are not reduced, then the prescription should be followed.

A pilot project was carried out among 48 GPs distributed in 17 clinics in the Capital Region of Denmark, primarily in Albertslund and Rudersdal municipals. The aim was to test the feasibility of delayed antibiotic prescription in preschool children with symptoms of upper respiratory tract infections over a four-month period (November 2016 to February 2017) and subsequently investigate the barriers and possibilities observed by the GPs and parents.

In total, 1031 children with symptoms on upper respiratory tract infections were seen by the GPs. Of these, 6.1 % received a delayed prescription, while 23.3 % got an immediate prescription. Large differences among the GPs were seen (Figure 1). About half of those who got a delayed prescription, redeemed it. Most of the delayed prescriptions were prescribed on Fridays.

The satisfaction rate among parents was very high, and most of them were interested in a delayed prescription again if their GP recommended it. Qualitative interviews with parents revealed that they were under strain from the labor market when their children were sick, and they hoped that antibiotics could hasten the recovery phase. Qualitative interviews with GPs

continued ... Textbox 5.1

revealed that they found that most parents were willing to wait and see if the symptoms of the child disappeared without antibiotics, but also that the parents were fragile in relation to their work-life balance.

Project 2: Knowledge building among new parents about infections and antibiotic—an intervention with child health nurses as mediators (March 2017 to December 2018)

Infants often get sick, causing parents to believe that their child is “always sick”. It is, however, normal that children get several infections during their first year of life and that the majority of these infections are respiratory infections. Although, most parents know that antibiotics are not effective in viral infections, many believe that antibiotics decrease the duration or complications. In order to increase parental knowledge, the aim of the intervention is to increase parental knowledge about infections and antibiotics, so they can be prepared for their visit to their GP.

All parents with newborn children will be visited at their home by a maternal and child health nurse (MCHN). Between 1 September 2017 to 30 August 2018, three municipalities (Egedal, Gentofte, and Hørsholm) will participate in the intervention, where a MCHN will teach the parents the basics of infections and antibiotic use at the 8-month visit. All MCHNs from the three municipalities will receive education on the topics primarily by staff from the Department of Clinical Microbiology. The parents will receive a small book authored by the Department of Clinical Microbiology, with valuable information about bacteria and virus and antibiotics.

The intervention will be evaluated qualitatively and quantitatively.

Reduce antibiotic use among elderly at nursing homes

The growth in the number of elderly and oldest old will increase the demand for long-term care services in nursing homes. Elderly at nursing homes have been considered to be at a high risk of developing bacterial resistance because of the high prevalence of infections and use of antibiotics.

Results from a European point prevalence survey found wide differences in antimicrobial use in nursing homes between Denmark, Norway, and Sweden. In Denmark, 11.3% of the residents received at least one antimicrobial treatment, whereas it was 8.3% and 2.7% in Norway and Sweden, respectively.

Antibiotics are most frequently prescribed for treatment of urinary tract infections (UTIs) among elderly at nursing homes. About half the antibiotics prescribed for urinary tract infections were for prophylactic use, which makes Denmark among the highest ranking country in Europe in the use of prophylactic antibiotics.

The elderly at nursing homes often have co-morbidities and their clinical history is often difficult to obtain, since many elderly have cognitive impairment and may also have hearing and speech difficulties. They are particularly challenged since they rarely are capable to consult their own GP and must depend on the judgment of the nursing staff.

Project 3: Less Antibiotic Use and fewer Urinary Tract Infections among Elderly at Nursing Homes through Knowledge building among Health Care Personnel (March 2017 to March 2019)

The aim of the intervention is to reduce the rate of UTIs, increase knowledge about infections and antibiotics, and reduce the rate of UTI-related admissions.

All staff at nursing homes in two municipalities (Frederikssund and Gentofte) will attend a seminar where they will be educated about infections, especially UTIs; hygiene; and antibiotics. Staff from the Department of Clinical Microbiology is primarily responsible for the seminar and the education program.

All participating nursing homes will register the number of antibiotic days, prophylaxis or acute use, and number of UTIs during a 17-month period. The nursing home staff will receive education by staff from the Department of Clinical Microbiology and survey, with baseline and follow-up measurement of changes in the staff’s knowledge about infections, UTIs, and hygiene.

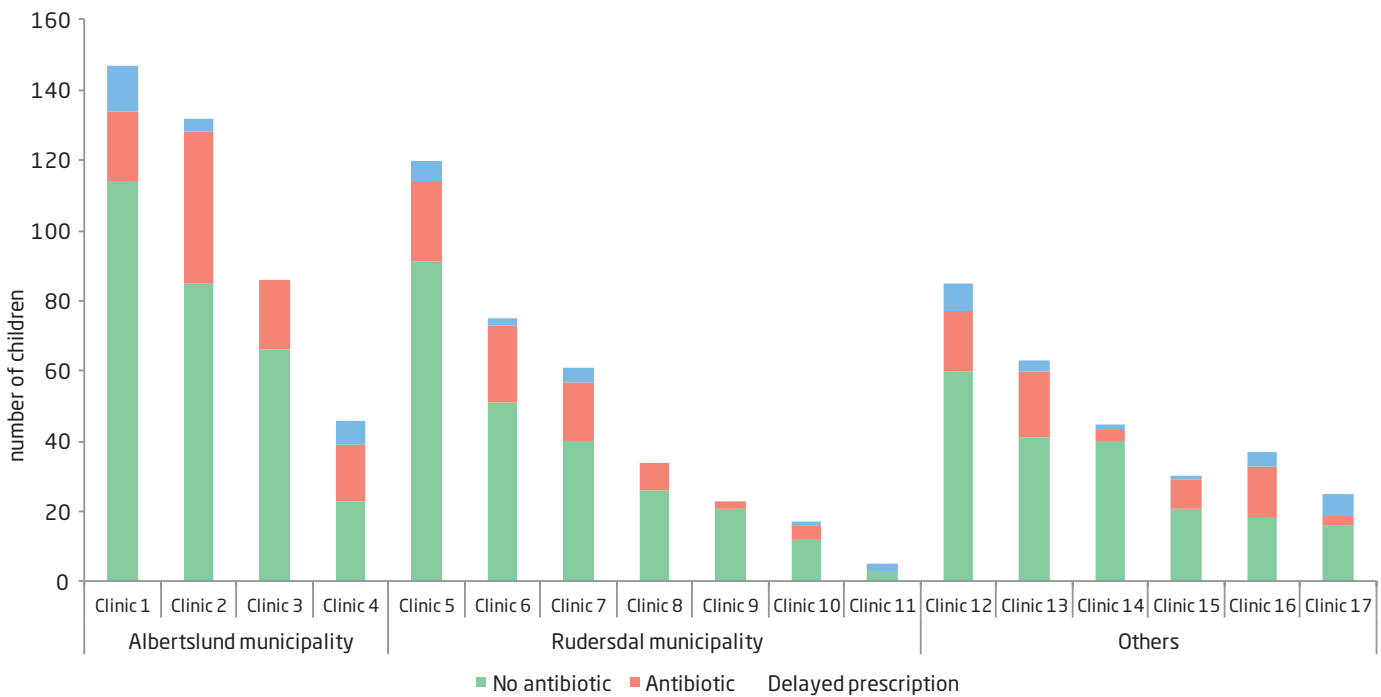
Project 4: Improving Treatment for Residents with Suspected Urinary Tract Infection in Long-Term Care Facilities-Protocol for a Cluster Randomized Trial.

The aim of this project is to reduce the rate of prescribed antibiotics through improved cooperation between nursing home staff and GPs and is a collaboration between The Department of Clinical Microbiology at Herlev and Gentofte Hospital, The Research Unit for General Practice and the Municipality of Gentofte. Nursing home staff will receive a decision aid consisting of three parts: observation of the nursing home resident, communication with the GP and a GP prescribed CRP. The effect of the decision aid on antibiotic prescription will be tested in a cluster randomized controlled trial in 20 nursing homes during a 6 month period. The decision aid will be based on international literature and national projects and further tailored to implementation through interviews, audit and focus groups with relevant stakeholders.

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Figure 1: Participating clinics (one or more GP), number of registered children, and the distribution of no antibiotics, immediate antibiotics, and delayed prescription

DANMAP 2016



Textbox 5.2

Incidence of multiresistant bacteria and consumption of antimicrobial agents in Greenland

Background: Greenland has a population of 55,847 inhabitants (January 2016) and Nuuk is the capital with around 16,000 inhabitants. Greenland has its own Ministry of Health and the country is divided into five health regions. There are five smaller hospitals, one general hospital and 11 health care centres in the five health regions. The general and largest hospital, Dronning Ingrid's Hospital, is situated in Nuuk (182 beds). Around 15-16,000 persons are admitted to hospital from once to several times a year. Patients with specific/serious diseases that cannot be treated at Dronning Ingrid's Hospital are transferred to Denmark or Iceland for further treatment (e.g. hemodialysis, cancer treatment, brain surgery etc.).

Resistant bacteria: From 2000 to 2016, 28 patients were diagnosed with methicillin-resistant *Staphylococcus aureus* (MRSA), 76 patients with ESBL-producing *Enterobacteriaceae*, two patients with vancomycin-resistant enterococci (VRE) and 136 patients with *Clostridium difficile* infection, of which 47 were diagnosed with the O27 type.

The number of patients with MRSA increased during this last year due to an outbreak in Nuuk. This outbreak consisted of six persons from the same family living in two households (a divorced family). The index person was either the mother or the father (a) of the eldest child, as both of them had been living and working in Australia some years earlier and the MRSA strain was found to be a type which is seldom seen in Denmark or Europe (t3979 CC5). Presumably they had been MRSA-carriers for years without any problems. When the mother gave birth to her second child she developed mastitis and the newborn child developed conjunctivitis in both eyes - both with the same MRSA-strain. The following examinations found both the first child and the new husband and father (b) of the second child to be MRSA-positive. As the first child commuted between both homes - the mothers' and the fathers' (a) - the father (a) and his new wife were also examined for MRSA and found to be MRSA-positive.

In families with children below the age of two years it can be difficult and cumbersome to treat a MRSA-carrier state. Since most children spontaneously "lose" their MRSA strain again before the age of two years, treatment of these families is usually not undertaken. The mother became pregnant with her third child, before the second child turned two years old; therefore an eradication-treatment of the MRSA-strain was attempted in the whole family four weeks before the expected birth. After treatment all family-members were MRSA-negative except the second child, who was still carrying MRSA on the perineum (still using diapers). No further spread of MRSA in this family was observed, neither was there found any transmission to other relatives, social contacts or to the health care system. The success was due to the family being highly compliant to hand hygiene and cleaning in their homes. This outbreak illustrates the fact, that MRSA is mainly spread through close contact in families.

In spite of outbreaks in Denmark with VRE, so far only two patients have been diagnosed with VRE in Greenland. Both patients were colonized with VRE in the rectum after being hospitalized in Denmark. No transmission was seen in the wards.

Most of the other resistant bacteria were imported from Denmark or abroad, but in some cases, especially in patients with ESBL-producing *Enterobacteriaceae*, treatment with broad-spectrum antimicrobial agents in Greenland has probably selected for these bacteria. From 2012 to 2013, there were outbreaks with *C. difficile* type O27 in the hospitals, and transmission within the country occurred. But due to a great infection control effort these outbreaks were quickly stopped. Of the 17 new patients with *C. difficile* infection diagnosed in 2016, none had the O27 type.

Consumption of antimicrobial agents: All antimicrobial agents in Greenland are purchased and disseminated from the National Pharmacy. Figure 1 a and b show the total purchase of selected antimicrobial agents in DDD per 1,000 inhabitants per day (DID) from 2007 to 2016. From 2007-2013, an increase of narrow-spectrum (18%) and broad-spectrum penicillins (12%) was observed, but from 2013 to 2014 a decrease of 23% and 4%, respectively, occurred. An increase was seen again from 2014 to 2016 of 37% and 23%, respectively. From 2015 to 2016 decreases in broad-spectrum antimicrobial agents such as macrolides (7%), fluoroquinolones (20%), and meropenem (31%) were observed. A minor increase in cephalosporins (1%) and larger increases in piperacillin-tazobactam (28%), tetracyclines (25.5%), and gentamicin (16.5%) were seen from 2015 to 2016.

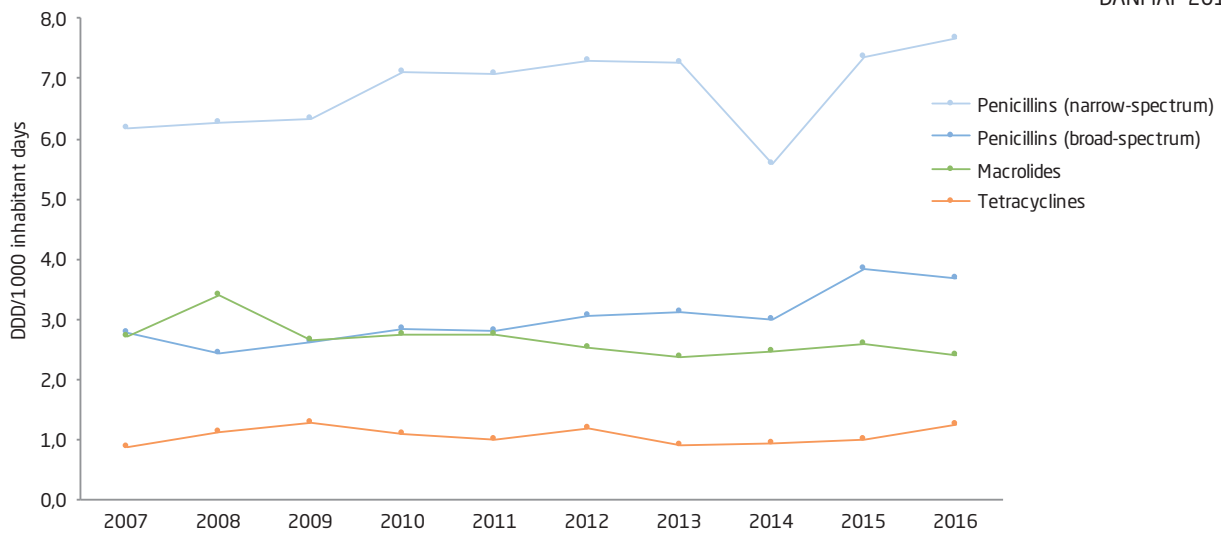
Conclusion: The consumption data for antimicrobial agents are based on purchases and fluctuations are therefore seen from year to year. However, as a result of the increased focus on prescription of antibiotics (especially at Dronning Ingrid's Hospital) a continued increase in purchases of piperacillin-tazobactam and gentamicin has been observed. Remarkable decreases were also seen for meropenem and fluoroquinolones. It is - however -worrying that the purchase of tetracyclines has increased once again.

Continued focus on the use of broad-spectrum antimicrobial agents - both in hospitals and in primary health care - and on the incidence of multiresistant bacteria in Greenland is very important also in the future.

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Figure 1a. Consumption of selected antimicrobial agents in humans in Greenland (DDD/1000 inhabitants/day) 2007-2016: consumption of narrow- and broad-spectrum penicillins, macrolides and tetracyclines

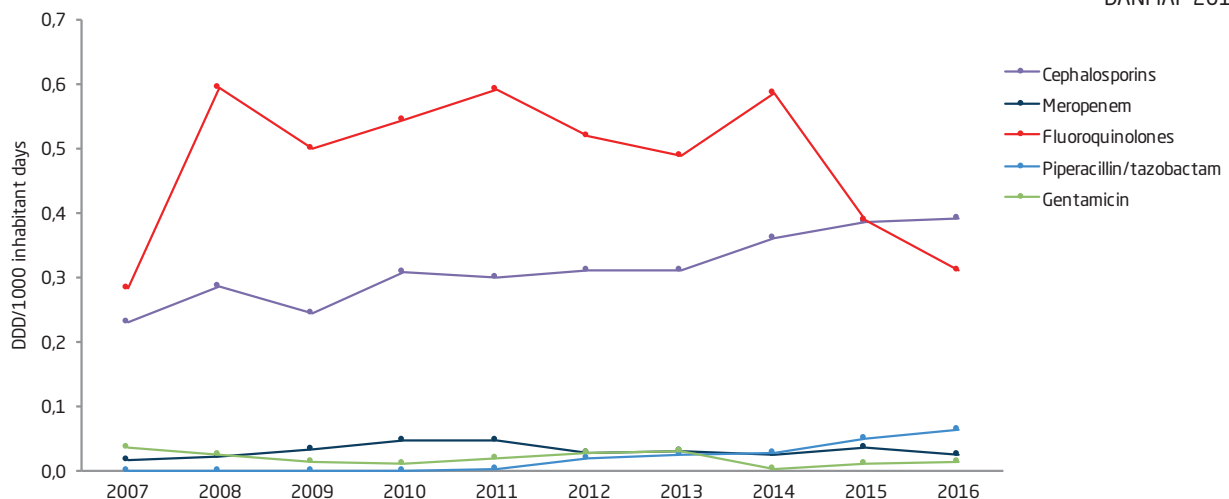
DANMAP 2016



Note: Narrow-spectrum penicillins include benzylpenicillin, phenoxymethylpenicillin and dicloxacillin, and broad-spectrum penicillins include ampicillin, pivampicillin, amoxicillin and amoxicillin with enzyme inhibitor.

Figure 1b. Consumption of selected antimicrobial agents in humans in Greenland (DDD/1000 inhabitants/day) 2007-2016: consumption of cephalosporins, meropenem, fluoroquinolones, piperacillin/tazobactam and gentamicin

DANMAP 2016



Textbox 5.3

Antimicrobial resistance and consumption of antimicrobials in the Faroe Islands

Background. The Faroe Islands (FI) consist of 18 islands, inhabited by approximately 49,000 inhabitants, 19,000 of whom live in the capital Tórshavn. The main hospital (Landsjúkrahúsið, LS, with 170 beds) is located in Tórshavn, and there are two smaller hospitals in Klaksvík (36 beds) and Súðuroy (26 beds). The healthcare system is comparable to the Danish healthcare system with general practitioners responsible for primary care and hospitals providing secondary care. LS has a local as well as a centralized function. In the case of specified diseases, patients are referred to hospitals in Denmark or other foreign hospitals.

Data and data sources. Data for antimicrobial consumption for FI and for LS were supplied by the Chief Pharmaceutical Office. Data on MRSA and other resistant bacteria were obtained from LS, as were bed-days.

Resistant microorganisms. MRSA and ESBL-producing *Enterobacteriaceae* (*Escherichia coli* and *Klebsiella pneumoniae*) are continuously surveyed and screening performed according to guidelines. Since April 2015, vancomycin-resistant enterococci (VRE) have been an increasing problem - especially at LS. A systematic periodic screening of VRE was performed in the wards throughout 2015. From 2016, screening was mainly performed after transfer of patients from hospitals abroad (including Denmark). The VRE data for 2016 are therefore based on results of screening and on clinical samples. For details, see Table 1.

Antimicrobial consumption at LS. Total antimicrobial consumption was 60.54 DDD/100 bed-days (DBD), an increase of 13 % compared to 2015. Special attention was paid to the use of three broad-spectrum antimicrobials: in 2016 cefuroxim constituted with 13.55 DBD 21 % of total antibacterial DBD, continuing steady increases since 2012, whereas both meropenem (2.37 DBD) and ciprofloxacin (4.91 DBD) increases seemed to be levelling off. The use of amoxicillin with betalactamase-inhibitor more than doubled during that period, from 1.11DBD in 2012 to 2.61 DBD in 2016. Consumption of mecillinams (pivmecillinam and mecillinam) increased from 0.45 DBD in 2012 to 3.74 DBD in 2016, with a 170 % increase from 2015 to 2016. For details see Figure 1.

Antimicrobial consumption in primary healthcare. Total antimicrobial consumption in 2016 was 13.87 DDD/1000 inhabitants/day (DID) representing a 6.9 % reduction compared to 2015 (14.90 DID). The distribution of antimicrobial consumption is shown in Figure 2. Remarkable is the decrease in the use of penicillin and ampicillins observed, from 5.44 and 1.53 DID (2012) to 5.08 and 1.35 in 2015 and with a further decrease to 4.23 and 1.06 DID in 2016, respectively. In contrast, an increase was observed in the use of penicillinase-stable penicillins and amoxicillin with betalactamase-inhibitor, from 1.14 and 0.50 DID (2012) to 1.19 and 0.77 in 2015 with a further increase to 1.35 and 0.79 DID in 2016, respectively.

Conclusion. Antimicrobial consumption is still increasing at LS, with a 123% increase alone in 2016, while the consumption observed in primary healthcare is simultaneously decreasing. Positive trends were the consumption of meropenem and ciprofloxacin, the use of which seems to be levelling off at LS, while the use of mecillinams showed increasing trends both at LS and in primary healthcare. Implementation of antibiotic stewardship and a continuous focus on adherence to general infection control precautions are the necessary steps in the effort to reduce development and spreading of antimicrobial resistance in LS and in primary healthcare.

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Table 1. Total number of cases being colonized or infected with multi-resistant bacteria

DANMAP 2016

Time span	Methicillin-resistant <i>S. aureus</i> (MRSA)	ESBL-producing <i>Enterobacteriaceae</i>	Vancomycin-resistant enterococci (VRE)
2004-2016	50		
2006-2016		55	
2015-2016			135

Figure 1. Consumption of selected antibiotics (narrow- and broad-spectrum) at LS 2012-16 (DDD/100 bed-days)

DANMAP 2016

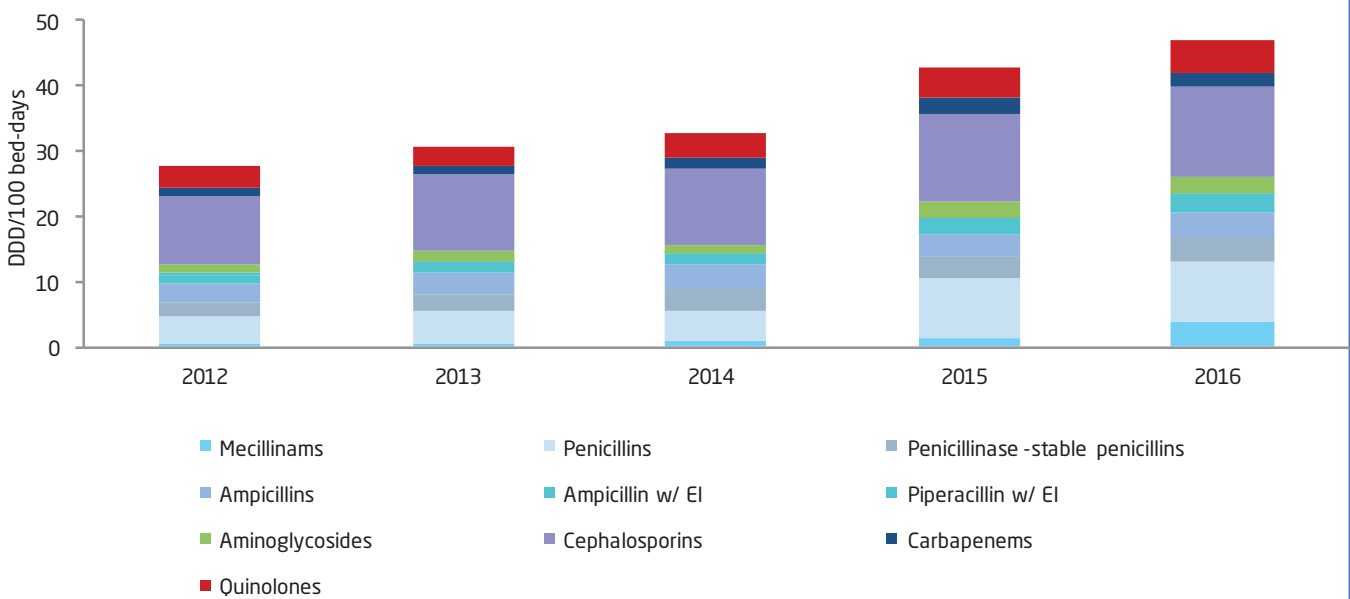
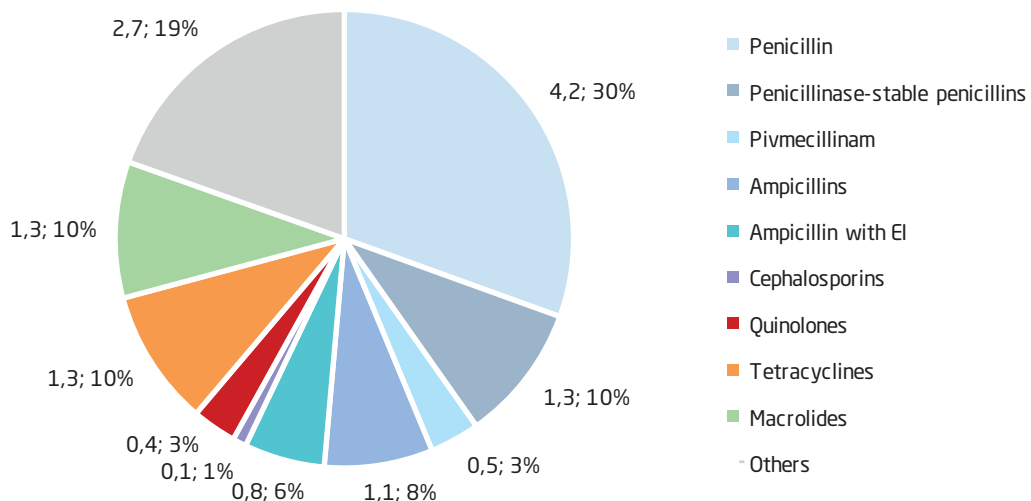


Figure 2. Consumption of antimicrobials in primary healthcare, 2016 (DDD/1000 patients/day; %)

DANMAP 2016





6

RESISTANCE IN ZOOONOTIC BACTERIA

6. Resistance in zoonotic bacteria



Highlights: In 2016, *Salmonella* Typhimurium overtook *S. Derby*, as the most prevalent serotype found in Danish pigs and domestically produced pork. The continued high occurrence of multi-resistant monophasic *S. Typhimurium* resulted in high levels of resistance to ampicillin, sulfonamide and tetracycline (61-76%) among porcine *S. Typhimurium* isolates. Similar high levels (68-71%) were observed for domestically acquired human cases. These observations highlight the importance of monophasic *S. Typhimurium* in spreading and maintaining resistance in Danish pigs and pork as well as in human cases.

Among human cases, resistance to quinolones remained higher among isolates from the travel-related cases than among cases acquired in Denmark. Due to the highly restrictive use of antibiotics critical for human treatment in the pig production, fluoroquinolone (ciprofloxacin) resistance has not been found in *S. Typhimurium* from pigs or domestically produced pork in several years. Resistance to 3rd generation cephalosporins and carbapenems has also remained very low in *S. Typhimurium* from domestically acquired human cases and were not found in the *Salmonella* isolates from Danish pigs and pork.

Since 2002, resistance to fluoroquinolones has increased to a level of approximately 20% among *Campylobacter jejuni* from Danish broilers; however most isolates remain fully sensitive. The level of fluoroquinolone resistance in *C. jejuni* continues to be a lot higher among isolates from imported broiler meat compared with isolates from Danish broilers. In *Campylobacter* isolates from cattle the resistance to fluoroquinolone also continued to increase, despite nearly zero use of these antimicrobials in cattle. The occurrence of resistance to ciprofloxacin and tetracycline in human isolates remained significantly higher in travel associated *C. jejuni* isolates compared to isolates from domestically acquired infections.

6.1 Introduction

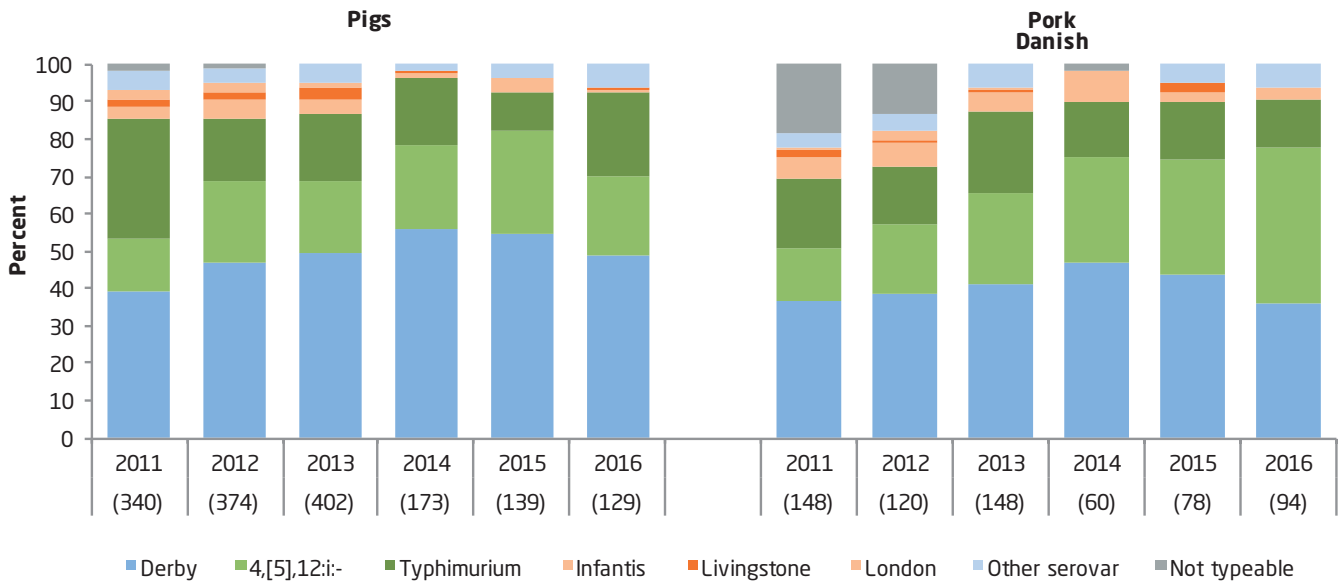
Zoonoses are infectious diseases that can be transmitted between animals and humans, either through direct contact with animals or indirectly by contaminated food. Zoonotic bacteria, such as *Salmonella* and *Campylobacter*, can develop resistance towards antimicrobial agents, which subsequently may lead to limited treatment possibilities, prolonged illness or even treatment failure of human infectious diseases. The development and spread of antimicrobial resistance is multi-factorial and can happen in many ways, including antimicrobial treatment of animals and humans, transfer of genes between bacteria or spread of very persistent and strong survivor strains carrying resistance genes.

A more detailed description of the trends and sources of zoonoses in Denmark and of national surveillance and control programmes can be found in the Annual Report on Zoonoses in Denmark 2016[www.food.dtu.dk].

S. Typhimurium, *S. Enteritidis*, *C. jejuni* and *C. coli* have been included in the DANMAP programme since 1995, where isolates were recovered for susceptibility testing in samples from broilers, cattle and pigs as well as from human cases. Sampling of fresh meat was initiated from 1997. Since 2014, sampling and testing of *Salmonella* and *Campylobacter* have been according to the EU harmonized monitoring of antimicrobial resistance [Decision 2013/652/EU].

Figure 6.1. Relative distribution (%) of *Salmonella* serotypes from pigs and Danish pork, Denmark

DANMAP 2016



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

6.2 Salmonella

Salmonella is the second most frequent zoonotic bacterial pathogen in Denmark and can have a severe impact on both animal production and human health [Annual Report on Zoonoses in Denmark 2016].

In Denmark and the rest of the Europe, *S. Enteritidis* and *S. Typhimurium* are the serotypes most frequently found to be associated with human illness. Human cases caused by *S. Enteritidis* are most commonly associated with consumption of contaminated eggs or poultry meat, whereas *S. Typhimurium* cases are mostly associated with eating contaminated meat from pigs, cattle and poultry.

Salmonella isolates for DANMAP 2016 were derived from national surveillance and control programmes. Pig isolates originate from slaughterhouses, where representative samples from healthy pigs (caecum) and pork (carcass swabs) are collected each year. Salmonellosis is a notifiable disease in humans and isolates from all reported *S. Typhimurium* cases are susceptibility tested. Only one isolate per farm, meat sample or human case was included in this report. For further details see Chapter 9, Materials and Methods.

Salmonella in domestic broilers, layers and cattle as well as some other types of Danish and imported meat are also monitored in Denmark each year. However, these are not included in DANMAP 2016, as only few isolates were found and thus, fall below the inclusion threshold for DANMAP of 15 isolates per population. The data are however reported to EFSA, and are included in the European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2016.

The DANMAP report focuses on the resistance in *S. Typhimurium*, because they are the most relevant serotype in public health. However, resistance in all *Salmonella* serotypes from pigs and pork is also presented for 2011 and onwards, which is the year Denmark started to susceptibility test all serotypes according to EU legislation.

In DANMAP, *S. Typhimurium* includes the monophasic variants with antigenic formulas *S. 4, [5],12:i:-* as recommended by the European Food Safety Authority [EFSA journal 2010. 8(10): 1826]. In the text, the term generic *S. Typhimurium* refers to results on isolates of the non-monophasic variants only.

The antimicrobials recommended by EFSA were used for susceptibility testing and MIC distributions and occurrence of resistance among isolates from pigs, pork and humans are presented in the web annex (Tables A6.1 - A6.5).

6.2.1 *Salmonella* in pigs and domestically produced pork - all serotypes

More than 700 representative pig caeca and 10,000 pig carcass swabs (pork) yielded 223 *Salmonella* isolates, which were tested for antimicrobial resistance. As in the previous years, *S. Typhimurium* (including the monophasic variants) and *S. Derby* were the most common serotypes, representing 91% of all the isolates (Figure 6.1). For the first time, *S. typhimurium* (48%) overtook *S. Derby* (43%) as the most prevalent serotype from domestically produced pigs and pork. This shift is mainly due to a large proportional increase of monophasic variants in Danish pork and a small drop in *S. Derby* isolates in pigs. Statistically significant differences in distribution of serotypes between pigs arriving at the slaughterhouses and the contamination of carcasses at the end of the slaughter line were also present.

It is the first year that this difference is observed and it is too soon to conclude on whether this is a trend or within normal variation, but this will be monitored in the coming years.

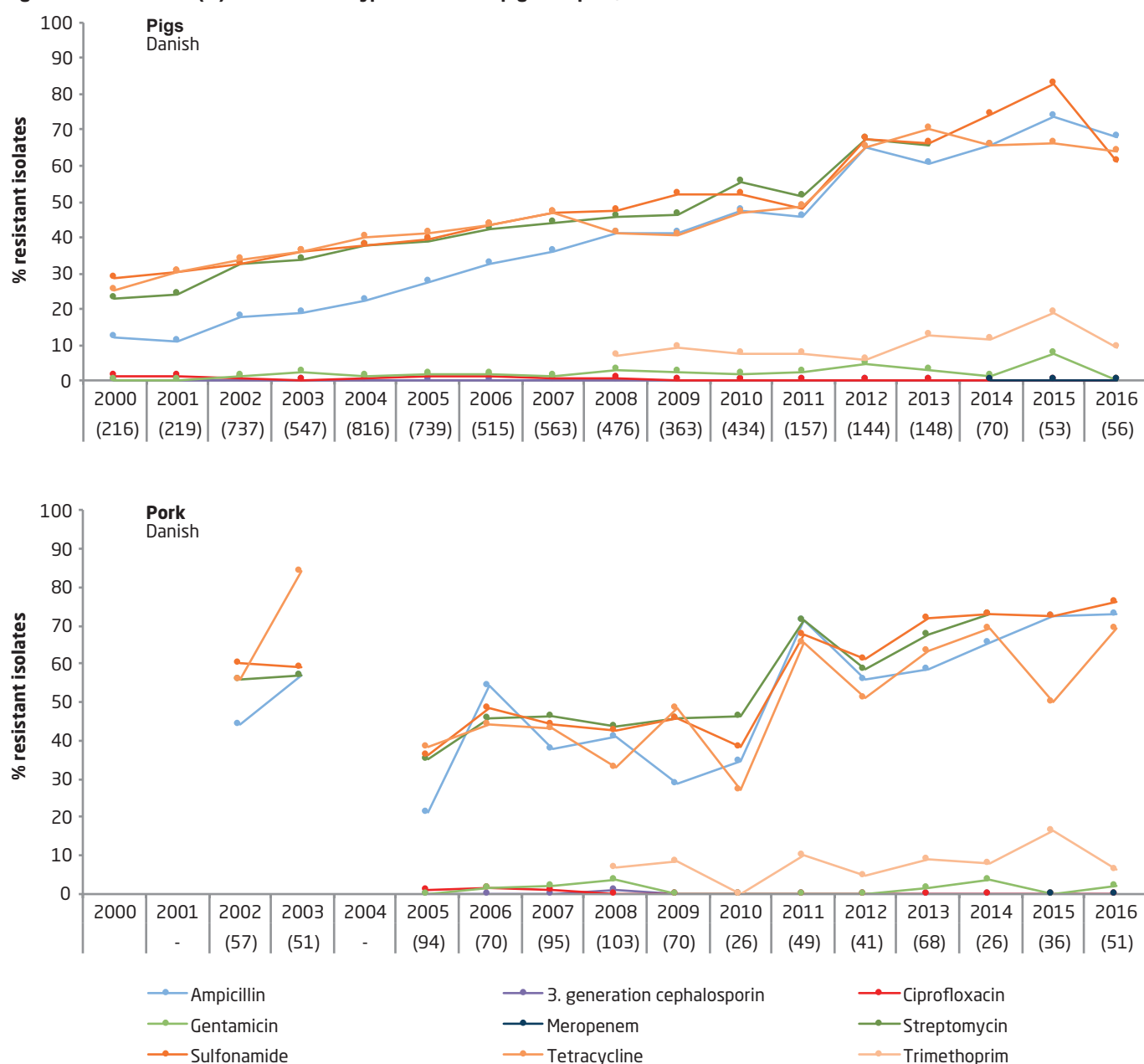
6.2.2 *S. Typhimurium* in pigs and domestically produced pork

S. Typhimurium remains the most important zoonotic serotype originating from pigs. A total of 107 *S. Typhimurium* (41 generic) and their monophasic variants (66) were isolated from Danish pigs and pork in 2016. The level of fully sensitive *S. Typhimurium* isolates in pigs remained the same as in 2015 (15% and 16%), but the level decreased in pork (19% to 12%).

Only 3% of the monophasic isolates were fully sensitive to all antibiotics in the panel versus 32% fully sensitive generic *S. Typhimurium*. This illustrates the important role of the monophasic variants in spreading and maintaining resistance and attributes some of the increase in resistance in Danish pigs and pork to the continuing spread of monophasic *Salmonella*.

As in the previous years, the highest resistance levels in *S. Typhimurium* isolates were observed to ampicillin, tetracycline and sulfonamide (Figure 6.2). Sulfonamide resistance was higher in isolates from pork than from pigs, probably reflecting the larger proportion of monophasic variants in pork (Table

Figure 6.2. Resistance (%) in *Salmonella Typhimurium*^{a)} in pigs and pork, Denmark DANMAP 2016



Note: Number of isolates included each year is presented in the parenthesis. The '-' indicate data for application of 2016 cut-offs were not available or less than 25 isolates were available

a) Includes isolates verified as monophasic variants of *S. Typhimurium* with antigenic formulas S. 4,[5],12:i:-

6.1). Resistance to sulfonamides declined sharply in live pigs in 2016. The distinct drop was independent of resistance to tetracycline and ampicillin, but seemed to be associated with the drop in trimethoprim resistance instead. The reduction was only seen in monophasic variants, not in generic *S. Typhimurium* isolates. A similar reduction was not observed in isolates from pork. Notably, the use of sulfonamide/trimethoprim, mainly used for sows, has decreased consistently over the past three years.

Tetracycline resistance in pork isolates returned to the expected trend after a sharp decline in 2015, and again reached the levels of resistance to ampicillin and sulphonamides, probably as part of the ASuT-resistance profile dominating monophasic variants. This year 65% of the monophasic isolates carried the ASuT resistance profile, occasionally in combination with resistance to one or more antibiotics. The ASuT-profile was only found in 27% of the generic Typhimurium isolates (11 of 41 isolates). Low levels of resistance to chloramphenicol and trimethoprim were observed in both pigs and pork. Very low levels of resistance to azithromycin and gentamycin were found in pork, but not in pigs.

Tetracyclines, macrolides (mainly Tylosin and Tilmicosin), pleuromutilins and beta-lactamase sensitive penicillins are the main antimicrobial agents used in pigs in Denmark (Figure 4.6).

Table 6.1. Resistance (%) among *Salmonella* Typhimurium^(a) isolates from pigs, Danish pork and human cases^(b), Denmark

DANMAP 2016

Antimicrobial agent	Pigs		Human cases		
	Danish %	Pork %	Domestically acquired %	Travel abroad reported %	Total %
Tetracycline	64	69	68	68	68
Tigecycline	0	0	<1	0	<1
Chloramphenicol	14	6	10	18	12
Ampicillin	68	73	71	65	70
Cefotaxime	0	0	3	5	4
Ceftazidime	0	0	<1	4	2
Meropenem	0	0	0	0	0
Trimethoprim	9	6	9	15	10
Sulfonamide	61	76	68	69	69
Azithromycin	0	6	0	1	<1
Gentamicin	0	2	<1	4	1
Ciprofloxacin	0	0	<1	18	6
Nalidixic acid	0	0	0	6	2
Colistin	0	0	<1	5	2
Number of isolates	56	51	167	78	297

a) Include isolates verified as monophasic variants of *S. Typhimurium* with antigenic formulas S. 4,[5],12:i:-

b) An isolate was categorised as 'domestic' if the patient did not travel outside Denmark one week prior to the onset of the disease

Overall, the use of these antimicrobials peaked in 2009, but has since then been reduced markedly. However the changes in usage of tetracycline and macrolides is not reflected in the observed levels of resistance in *S. Typhimurium* from pigs and domestically produced pork - as the trends are still dominated by the increasing occurrence of monophasic *S. 4,[5],12:i:-* clones (Figure 6.2).

None of the *S. Typhimurium* isolates from pigs or domestic pork were resistant to quinolones, cephalosporins (cefotaxime and ceftazidime) or carbapenems (meropenem), providing 95% certainty that these resistances are present in less than 5.2% of *S. Typhimurium* isolates from pigs and 5.7% *S. Typhimurium* from pork (see Textbox 6.2 and Materials and Methods section 9.7.3)

6.2.3 Resistance in other relevant *Salmonella* serotypes in pigs and domestically produced pork

S. Derby

S. Derby was isolated from 63 slaughter pigs and from 34 Danish pork samples. *S. Derby* is common among pigs, but only few human cases (n=12) were reported in Denmark in 2016 [Annual Report on Zoonoses in Denmark 2016]. The majority of the *S. Derby* isolates from pigs were fully sensitive (63%), but the proportion of fully sensitive isolates was significantly lower than last year (72%), suggesting resistance in *S. Derby* is increasing. Resistance to tetracycline, ampicillin and sulphonamide resistance were most common, either alone or in combination. Two isolates were also resistant to chloramphenicol and one to gentamycin, but no resistance to any other antimicrobials was found.

S. Infantis

Only four isolates of *S. Infantis* was isolated from Danish pigs (n=1) and pork (n=3). Three of these were fully sensitive to all antibiotics in the panel and one isolate from pork was resistant to ampicillin, sulfamethoxazole and trimethoprim.

6.2.4 *Salmonella* in humans

In 2016, *Salmonella* continued to be the second most frequent cause of bacterial intestinal infections in Denmark. A total of 1,074 human laboratory-confirmed cases of salmonellosis was reported (18.8 cases per 100,000 inhabitants). The most common serotypes were *S. Typhimurium* (including the monophasic variants) and *S. Enteritidis* with 5.6 and 4.3 cases per 100,000 inhabitants, respectively [Annual report on Zoonoses in Denmark 2016].

6.2.5 *S. Typhimurium* in humans

Salmonella Typhimurium, including the monophasic variants, was the most common serotype among the human cases (300 cases) and 297 of these isolates were susceptibility tested. The monophasic variants represented two thirds of the *S. Typhimurium* cases (192 monophasic versus 108 generic *Typhimurium*).

Information on travel history was collected through phone interviews and among the 297 human *S. Typhimurium* isolates, included in DANMAP 2016, 26% of the cases were categorised as travel associated, whereas 56% most likely were acquired in Denmark (Table 6.1). A total 57 human cases were considered 'outbreak-related' and originated from seven outbreaks, one outbreak of *S. Typhimurium* and six outbreaks of the monophasic variant of *S. Typhimurium*. Domestically acquired human cases included both sporadic and outbreak related cases (Table 6.1).

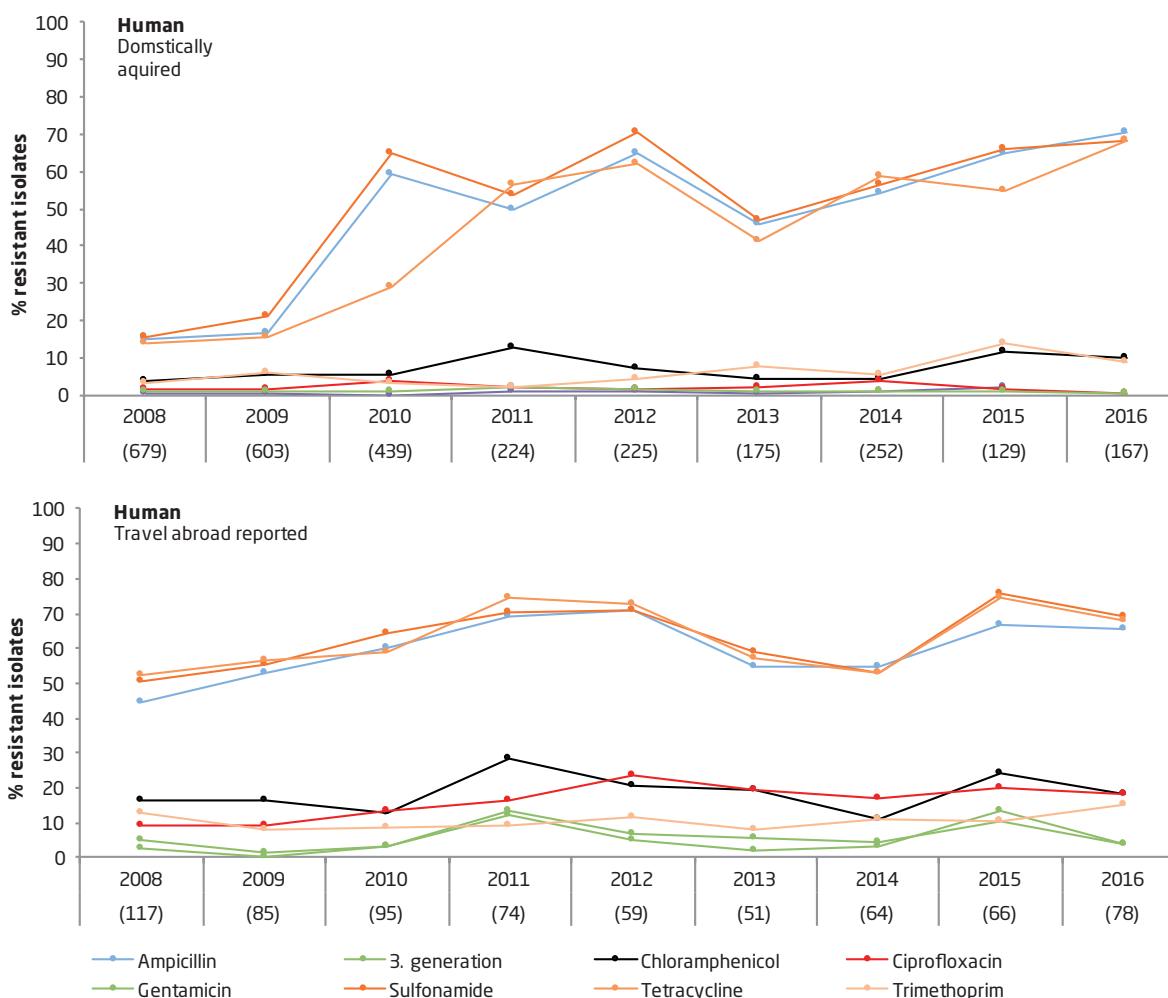
Again this year, high levels of resistance to ampicillin, sulfonamide, and tetracycline were observed. Resistance to the three different antimicrobials was observed at the same level (65-71%) among isolates from domestic and travel-associated cases (Table 6.3). For domestically acquired cases the level of tetracycline resistance increased significantly from 2015 to 2016 (from 55% to 68%).

Resistance to ampicillin, sulfonamide and tetracycline has increased since 2008, especially among the isolates from domestic acquired cases (Figure 6.3). Starting in 2008 we had one of the largest outbreaks ever seen in Denmark that ran for two years. The outbreak was caused by a fully sensitive *S. Typhimurium* clone and therefore has a huge impact on the trend graph.

There was a significantly higher level of resistance to quinolones and colistin among travel-associated isolates compared with domestic ones in 2016. Both ciprofloxacin and nalidixic acid resistance were higher in isolates from travel-related cases (18% and 6% respectively) compared with isolates from domestic cases (1% and 0% respectively). Resistance to colistin was also significantly higher in travel-associated cases (5%) when compared to domestically acquired cases (1%).

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Figure 6.3. Resistance (%) in *Salmonella Typhimurium*^(a) in human cases^(b), Denmark DANMAP 2016



a) Include isolates verified as monophasic variants of *S. Typhimurium* with antigenic formulas S. 4,[5],12:i:-
 b) An isolate was categorised as 'domestic' if the patient did not travel outside Denmark one week prior to the onset of the disease

6.3 Campylobacter

Thermotolerant *Campylobacter* spp. are the most commonly reported causes of gastrointestinal bacterial infections in humans in Denmark as well as in the European Union [EU Summary Report 2015, ECDC/EFSA 2016]. In Denmark, 85-95% of the human campylobacteriosis cases are caused by *C. jejuni*.

Campylobacter are widespread in nature and the most important reservoirs are the alimentary tract of wild and domesticated birds and mammals. Among sporadic human cases, broilers have been identified as the primary source of infection, though other sources such as untreated water and other infected animals are also important.

In 2016, most of the *Campylobacter* isolates for DANMAP was supplied by the mandatory sampling of randomly selected broilers at slaughter (caecum). However, isolates from cattle and from broiler meat were also included. In humans Campylobacteriosis is a notifiable disease, but only a selection of isolates from reported human *C. jejuni* cases are susceptibility tested. Only one isolate per farm, meat sample or human case was included. For details see Chapter 9, Materials and Methods. The susceptibility methods follow EFSAs recommendations and MIC distributions for *C. jejuni* from broilers and cattle, broiler meat and humans are presented in the web annex (Tables A6.6- A6.8).

6.3.1 *C. jejuni* in broilers and domestically produced broiler meat

A total of 178 *C. jejuni* isolates were derived from more than 700 samples from broilers and domestically-produced poultry meat collected throughout the year. This more than doubled the number of samples and isolates from 2015 and thus, generate more precise and reliable estimates in 2016. The isolates originated predominately from broilers (n=160) and much fewer were isolated from meat (n=18).

The level of fully sensitive isolates from broilers and broiler meat was 74%, which was similar to the previous three years.

Resistance to ciprofloxacin (22-23%) and nalidixic acid (21-22%) were most frequently observed (Table 6.2). Since 2007, a slow increase in proportion of resistant isolates has been observed from approximately 10% in 2006 to 23% in 2016. This is mainly due to the increase in resistance to ciprofloxacin. Resistance to ciprofloxacin in broilers is fluctuating from year to year, but has increased from 7% to 22% over the last 10 years, despite little or no use of quinolones in the poultry industry since 2009. The Danish increasing trend of resistance to quinolones is similar to the increasing trend of ciprofloxacin resistance in the EU, but the levels in Denmark still remain much lower than the EU averages of 62-66% of isolates resistant to quinolones [EU Summary Report 2014].

Tetracycline resistance has plateaued around 12% in both broilers and broiler meat after a steady increase peaking in 2013 followed by a drop back to 2008 level. This does not clearly follow the use of tetracyclines in broilers, which increased sharply from 2012 to 2015 and decreased in 2016. Resistance to streptomycin remains low at 4% in broilers and stays within the fluctuations of the last two years between 5% in 2014 and 2% in 2015. Only very low levels of resistance to erythromycin (0.6%) and no resistance to gentamycin were observed in 2016.

6.3.2 *C. jejuni* from imported broiler meat

As in previous years, the levels of resistance to ciprofloxacin (71%), nalidixic acid (69%) and tetracycline (63%) in imported poultry meat were higher than the resistance levels observed in isolates from domestically produced broilers and meat (Table 6.2). In 2015, a decreasing trend in resistance to tetracycline was observed, but this year the trend had reversed to an increase. The increase is most likely due to a change in the proportional contribution of poultry meat samples from four EU countries from 2015 to 2016. In 2016, a larger proportion of sampled imported poultry meat originated from countries with higher levels of resistance to tetracycline [EU Summary Report 2015].

Table 6.2. Resistance (%) in *Campylobacter jejuni* isolates from animals, meat of Danish and imported origin and human cases^(a), Denmark

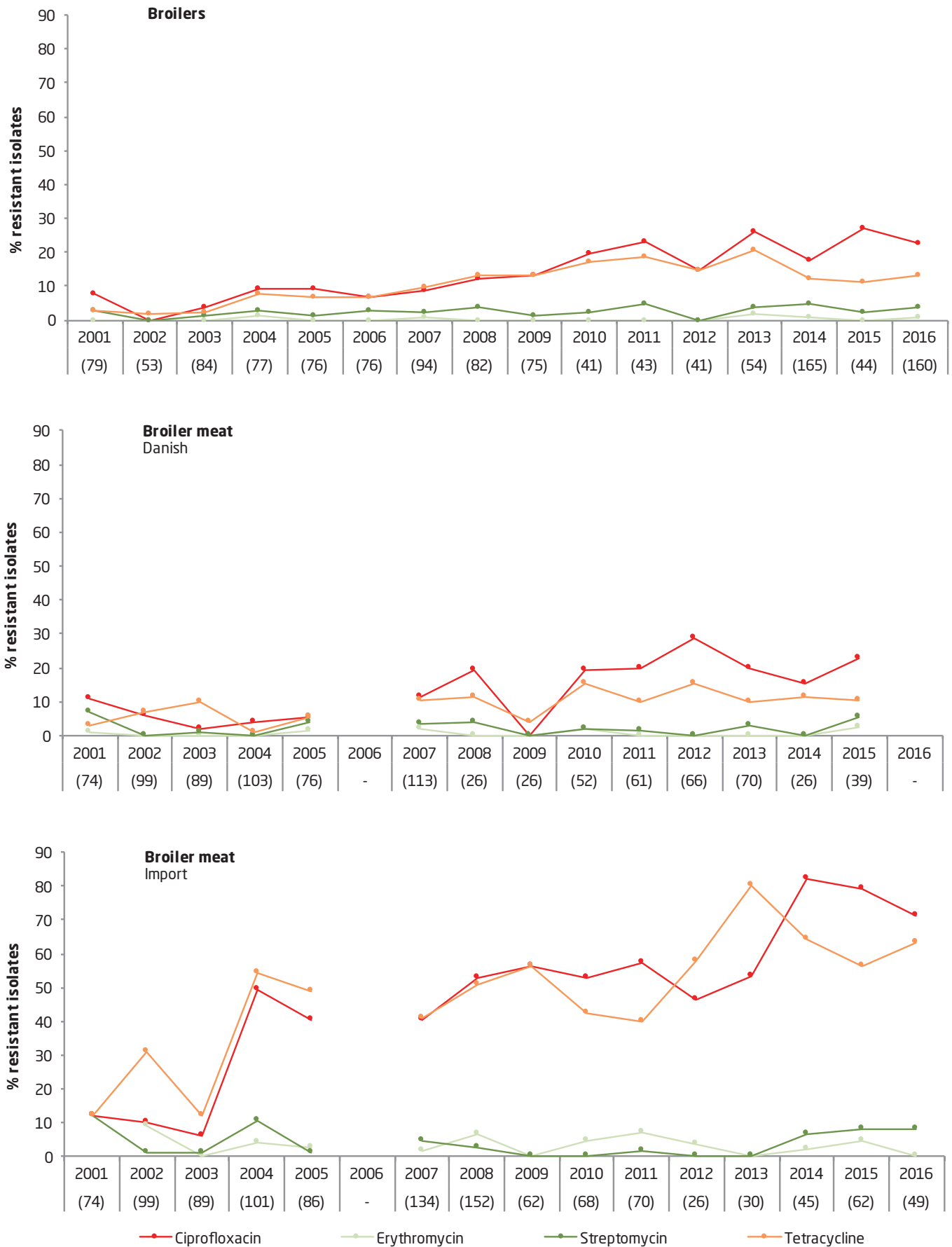
DANMAP 2016

Antimicrobial agent	Cattle	Broilers	Broiler meat		Humans		
	Danish %	Danish %	Danish %	Import %	Domestically acquired %	Travel abroad %	Total %
Tetracycline	13	13	11	63	17	59	23
Erythromycin	0	<1	0	0	1	5	2
Streptomycin	6	4	6	8	3	13	4
Gentamicin	0	0	0	0	<1	10	2
Ciprofloxacin	25	23	22	71	33	80	39
Nalidixic acid	25	21	22	69	33	80	39
Number of isolates	80	160	18	49	241	39	280

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

Figure 6.4. Resistance (%) among *Campylobacter jejuni* from broilers and broiler meat, Denmark

DANMAP 2016



Note: Number of isolates included each year is presented in the parenthesis. The '-' indicate data for application of 2016 cut-offs not available or less than 25 isolates were available

6.3.3 *C. jejuni* in cattle

A total of 82 *Campylobacter* isolates were derived from more than 120 cattle caeca taken from all over Denmark and 80 *C. jejuni* isolates were susceptibility tested. Most of the isolates (70%) were fully sensitive to all antibiotics tested and the remaining isolates were resistant to ciprofloxacin, tetracycline, streptomycin (25%, 13% and 6% of all isolates, respectively) and to various combinations of these (Table 6.2).

Resistance to ciprofloxacin continued to increase, despite nearly zero consumption of fluoroquinolones in cattle in Denmark (Figure 6.5). Initially, quinolone-resistant *C. jejuni* isolates were only found in cattle farms in Southern Jutland, but now the resistance is equally distributed all over the country. It is unknown, whether it is a clonal spread or whether the resistant *C. jejuni* are introduced to cattle from other sources. Resistance to streptomycin increased from 2015, but levels remain low (1% to 6%). A small increase in resistance to tetracycline was also observed and the resistance levels are now almost back to 2001 levels after many years of low levels (Figure 6.5).

6.3.4 *C. jejuni* in humans

Campylobacter continued as the most frequent cause of bacterial intestinal infections in Denmark and a total of 4,677 human laboratory confirmed cases of campylobacteriosis were reported (82 per 100,000 inhabitants) [Annual Report on Zoonoses in Denmark 2016].

In 2016 *Campylobacter* isolates were submitted to Statens Serum Institut (SSI) by four Clinical Microbiological Laboratories covering four geographically dispersed and representa-

tive areas of Denmark and both urban and rural areas were included. The isolates were speciestyped and a total of 280 *C. jejuni* isolates were susceptibility tested. Travel history of the patients was collected, when possible, primarily as part of the information given to the diagnostic laboratory at submission of patient samples. In 2016, priority was given to susceptibility testing more isolates from domestically acquired cases (241 isolates; 86%) compared to isolates from travel-associated cases (39 isolates).

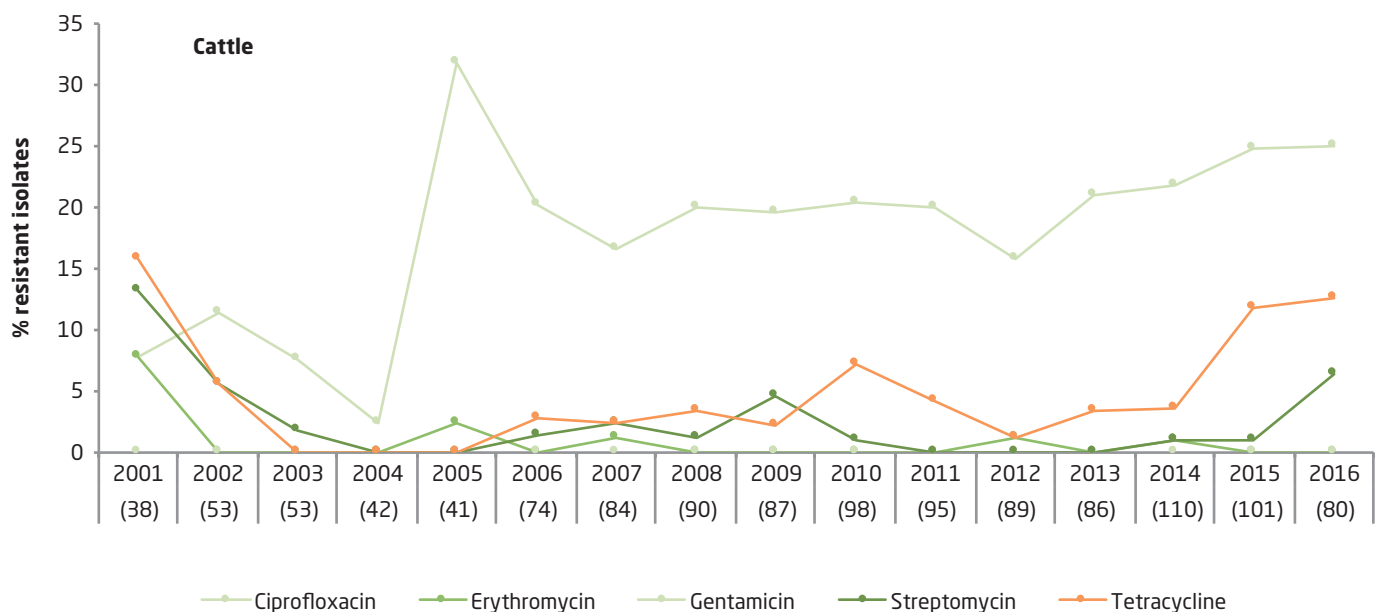
Among the domestically acquired infections, 62% were fully sensitive to all the antimicrobial agents tested, while the percentage of fully sensitive isolates was much lower among isolates from travel associated cases (18%). Similar to previous years, the occurrence of resistance to ciprofloxacin and tetracycline was significantly higher in travel associated *C. jejuni* isolates (80% and 59%, respectively) compared to isolates from domestically acquired infections (33% and 17%, respectively) (Table 6.2 and Figure 6.6).

Among the 123 resistant isolates, the most frequent resistance profile was resistance to ciprofloxacin alone (70/123) or ciprofloxacin in combination with tetracycline (43/123). All isolates resistant to gentamicin (six in total) were multi-resistant, two were acquired domestically and four were travel related. Five isolates were found resistant to erythromycin, three were acquired domestically and two were from a travel associated case.

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Figure 6.5. Resistance (%) among *Campylobacter jejuni* from cattle, Denmark

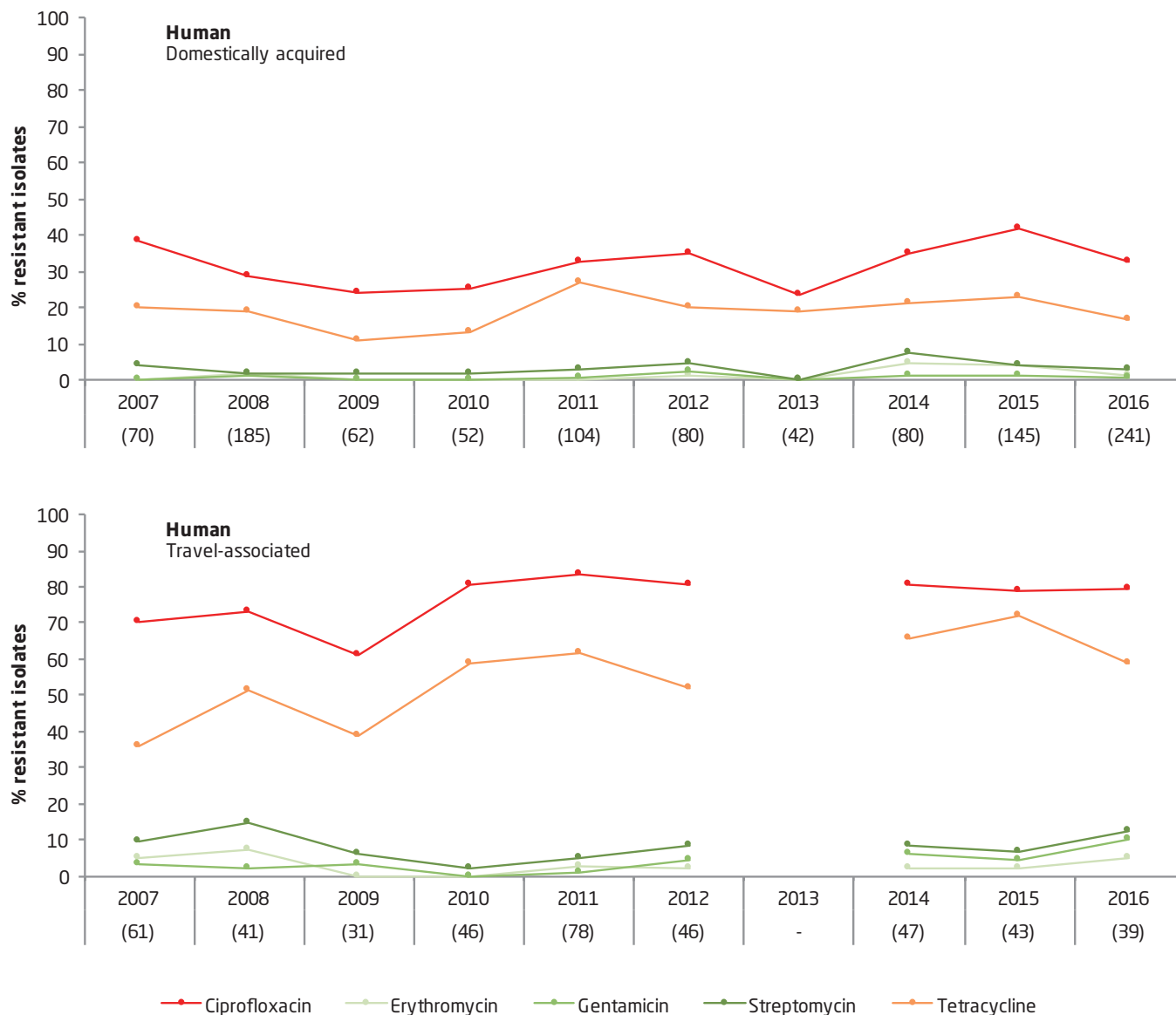
DANMAP 2016



Note: Number of isolates included each year is presented in the parenthesis. The ‘-’ indicate data for application of 2016 cut-offs not available or less than 25 isolates were available

Figure 6.6. Resistance (%) among *Campylobacter jejuni* from humans^(a), Denmark

DANMAP 2016



Note: Number of isolates included each year is presented in the parenthesis. The '-' indicate data for application of 2016 cut-offs not available or less than 25 isolates were available

Textbox 6.1

Resistance in bacteria from diagnostic submissions from pigs

Antimicrobial susceptibility testing of veterinary pathogens takes place at two accredited diagnostic laboratories in Denmark, at DTU National Veterinary Institute and at SEGES: The Danish Pig Research Centre's Laboratory for Pig Diseases (VSP) in Kjellerup. Data on susceptibility of three important veterinary pathogens *Escherichia coli* O149, *Streptococcus suis*, and *Actinobacillus pleuropneumoniae*, were obtained from the routine diagnostic laboratory at Laboratory for Pig Diseases and presented here, whereas the number of isolates of other bacterial pathogens were too small.

The antimicrobial susceptibility testing was carried out using the broth microdilution method with SensiTitre. Since approved clinical breakpoints are not available for most of the drug-bacterium combinations, the results are presented both as MIC distributions, which allows for the reader's own interpretation, and as % resistant isolated according to the clinical breakpoints that are agreed by both DTU National Veterinary Institute and Laboratory for Pig Diseases.

***E. coli* O149**

Enterotoxigenic *E. coli* (ETEC) is one of the most important bacterial causes of diarrhoea in pigs, in particular weaning pigs and the most virulent types are haemolytic, enterotoxin and F4 fimbria positive strains. The MIC distributions and resistance data are shown in Table 1. High resistance levels were recorded for ampicillin, streptomycin, sulphonamides, tetracyclines (ASSuT-resistance pattern also known from *Salmonella*), and trimethoprim. This is in accordance with figures from 2015, and no major differences were recorded compared to 2015 data. Almost 7 out of 10 isolates were resistant to tetracycline, sulphonamides, and streptomycin. Resistance to florfenicol remains at 10% like in 2015, which is higher than most recent figures from 2012 (3%) and 2011 (0%). In view of the current focus on colistin resistance it is notable, that no resistance was recorded to colistin. Resistance to nalidixic acid was recorded in 10% of the isolates, which is in line with the 11% found in 2015, in spite of no use of quinolone for treatment of pigs. The result may suggest the persistence of quinolone resistant strains.

Actinobacillus pleuropneumoniae

Actinobacillus pleuropneumoniae is the cause of a severe pleuropneumonia in pigs with fever, coughing, depression, loss of appetite, and bloody discharge from the nose as main clinical findings. In case of an outbreak, it is important to be able to initiate proper treatment very quickly. Fortunately, *A. pleuropneumoniae* still have a predictable resistance pattern and low resistance to most compounds. MIC distributions and percent resistance are shown in Table 2. Most isolates were resistant to erythromycin, but were susceptible to all other compounds, including penicillin. A single isolate was resistant to tetracycline. Resistance patterns did not differ between serotypes.

Streptococcus suis

Streptococcus suis is alfa-haemolytic streptococcus, which cause several infectious conditions in pigs, including meningitis, arthritis, pneumonia, and septicaemia, and cause losses to the farmers due to increased mortality and veterinary costs. MIC distributions and percent resistant isolates are shown in Table 3. Resistance was high to macrolides (erythromycin) and tetracyclines, but low or zero for most other compounds. An important observation was that resistance to penicillin remains at zero.

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Textbox 6.2

DANMAP detecting emerging and rare resistances

DANMAP reports % isolates resistant to each antimicrobial tested without presenting confidence intervals to extrapolate these to population such as isolates, samples or animals. Normally, the % resistant isolates will be an indication of the mean in the relevant population, because of the carefully designed sampling strategies. However, when DANMAP does not find any resistance to a given antimicrobial agent, the outcome (0%) is harder to interpret. It is tempting to conclude that the resistance is not present in Denmark, but in reality the interpretation is complicated.

To prove that a population is free from a specific disease, pathogen or resistance mechanism is nearly impossible. Firstly, tests are rarely 100% sensitive and may misclassify a few infected individuals and secondly, when testing only some individuals in a population, we are likely to miss infected animals if only few are infected. Instead, a zero-finding should be expressed in the context of the sample size and the level of confidence in the given result. The number of samples or isolates tested for resistance will determine the minimum prevalence that could have been present, without finding at least one resistant isolate or sample with 95% certainty. The minimum prevalence is called the detection limit or the design prevalence. When MIC testing a number of isolates, the correct interpretation of a zero-finding would be: *We can be 95% certain that xx resistance is not present in more than yy% of isolates.*

Ideally, we would like to interpret the DANMAP outcomes in relation to the animal population or food type that was sampled, but since DANMAP only MIC tests one isolate per animal or food, making this leap would be equivalent to sampling one child in a school class for head lice, finding no lice and concluding that the whole class is free of head lice. Thus, we cannot interpret one isolate as representative for one animal/food sample, especially for indicator bacteria, where it is very likely that multiple isolates with different resistance patterns are present in one sample.

Detection limits in DANMAP 2016 - the example: of carbapenem resistance

Carbapenem resistance has not yet been identified in Danish animal or food reservoirs via DANMAP surveillance and the antimicrobial is not used in animals. Resistance is present in low levels in the human reservoir and has been detected in production animals elsewhere in Europe. The risk factors for Danish production animals are not known, but likely introduction routes would be humans, imported animals and imported feed.

Based on the current sampling plans, detection limits of carbapenem resistant bacteria were calculated using a probability formula to substantiate freedom from disease, when considering test sensitivity and a confidence of 95% [1]

Table 1. Detection limits for carbapenem resistance in susceptibility tested (MIC) isolates at 95% confidence, Denmark

DANMAP 2016

Isolates	Number of isolates	Detection limit %	
		Test sensitivity = 100%	Test sensitivity = 85%
<i>S. Typhimurium</i> from pigs	56	5.2	6.1
<i>S. Typhimurium</i> from pork	51	5.7	6.7
Indicator <i>E. coli</i> from chicken	186	1.6	1.9
Indicator <i>E. coli</i> from cattle	121	2.5	2.9
<i>E. coli</i> ESBL from poultry ^[2]	48	6.1	7.1
<i>E. coli</i> ESBL from poultry meat ^[2]	52	5.6	6.6

Note: *E. coli* ESBL isolates from poultry and poultry meat detected by the EURL-AR protocol [2] for detection of cefotaxime-resistant *E. coli* and then tested by MIC

continued ... Textbox 6.2

Table 1 shows that we are 95% confident that carbapenem resistance in *S. Typhimurium* from pigs is less than 5.2% assuming the test sensitivity was 100%. If the test sensitivity of the MIC for meropenem is lower in *S. Typhimurium* e.g. 85%, we are 95% certain that carbapenem resistance is not present in 6.1% or more of *S. Typhimurium* isolates from pigs. Similar results for indicator *E. coli* isolates from different sources are shown in Table 1. Since only one isolate from each sample was susceptibility tested, the estimates apply to carbapenem resistance in the population of bacterial isolates only, not the animals or meats.

Some samples were examined for CPE directly via the EURL-AR protocol [2], which looks for carbapenem resistance directly in the sample by selective media rather than examining just one isolate. This expands our representativeness and the conclusion now applies to the samples e.g. meat or animal rather than only to the bacterial isolates. We were now 95% certain that CPE was not present in 1% or more broiler caeca samples and 1.3% chicken meat samples, if the test was perfect (Table 2). At lower test sensitivity, the detection limits increased to 1.2% and 1.5% respectively.

Understanding surveillance design and data is paramount to interpret its outcomes, as demonstrated by CPE resistance in Denmark. With the increasing prevalence of CPE and other rare resistances outside our borders in the EU, it is important to evaluate the surveillance programme's ability to detect these and align detection levels with control action points.

Table 2. Detection limits for carbapenem resistance in samples at 95% confidence, Denmark

DANMAP 2016

Samples	Number of samples	Detection limit %	
		Test sensitivity = 100%	Test sensitivity = 85%
Broiler samples	298	1,0	1,2
Poultry meat samples	229	1,3	1,5

Note: Findings in poultry and poultry meat examined by EURL-AR protocol [2] for specific detection of carbapenemase producing *E. coli* by direct plating onto selective media

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References

- [1] Cameron & Baldock 1998. A new probability formula for surveys to substantiate freedom from disease *Prev Vet Med.* 1998 Feb 6;34(1):1-17. The calculations were performed online at: www.epitools.ausvet.com.au
- [2] EURL-LA LABORATORY PROTOCOL -Isolation of ESBL-, AmpC- and carbapenemase-producing *E. coli* from fresh meat Version 4: January 2017



7

RESISTANCE IN
INDICATOR BACTERIA

7. Resistance in indicator bacteria



Highlights: In 2016, resistance to erythromycin and tetracycline was observed in 37% and 50% of the *Enterococcus faecalis* isolates from Danish broilers and has been increasing since 2011. For the first time since DANMAP was established ciprofloxacin resistant *E. faecalis* (n=3) was isolated from Danish produced broilers. These three isolates were also resistant to tetracycline, maybe suggesting presence of a clone.

Occurrence of fluoroquinolone resistance among indicator *Escherichia coli* from broilers and of sulfonamide and trimethoprim resistance among indicator *E. coli* from pigs increased from 2015. Approximately half of the indicator *E. coli* from broilers and pigs exhibited resistance to at least one of the antimicrobial agents tested, whereas the proportion of antimicrobial resistant isolates was remarkably low in isolates from Danish cattle.

ESBL/AmpC-producing *E. coli* was recovered from 16% of the samples from broilers, from 23% of the samples from domestically produced broiler meat and from 56% of the samples from imported broiler meat. The occurrence in broiler meat was comparable to the levels observed in 2015. CTX-M-1 was the most common enzyme in ESBL/AmpC-producing *E. coli* from Danish and imported broiler meat, whereas CMY-2 and upregulation of chromosomal AmpC expression were predominant in the isolates from broilers. A wide variety of ESBL/AmpC-producing *E. coli* sequence types (ST) was identified across the three sources. Of concern is the persistence in imported broiler meat of ST131 that harbour CMY-2, because of its potential implications in human infections. Due to the changes in methodology implemented in 2015 to enhance the sensitivity of the detection method, comparative evaluations and temporal trends between years should be done cautiously.

7.1 Introduction

Enterococci are included in the DANMAP programme to monitor resistance in Gram-positive bacteria, while *Escherichia coli* are included as representatives of Gram-negative bacteria. These bacteria species were selected as indicators for occurrence of antimicrobial resistance in the different reservoirs through the food chain for several reasons: they are ubiquitous and present as major commensals in both the animal and human reservoirs; they can acquire antimicrobial resistance as a response to antibiotic selective pressures, and finally they have the potential for transferring resistance to pathogenic bacteria and can cause infection in humans. From enterococci, both *Enterococcus faecium* and *Enterococcus faecalis* were used since these species may acquire resistance to different antimicrobials.

Extended-spectrum beta-lactamase producing (ESBL/AmpC) bacteria exhibiting resistance to 3rd generation cephalosporins are one of the fastest emerging resistance problems in both humans and production animals worldwide. Lately, several studies have found similar ESBL/AmpC genes, plasmids and clones of *E. coli* isolates in animals, meat and human infec-

tions, suggesting a zoonotic link. Furthermore, the occurrence of carbapenemase-producing Enterobacteriaceae (CPE) is an even greater threat to human health, since carbapenems are the last-line antimicrobial agents for treatment of infections caused by multidrug-resistant Gram-negative bacteria.

E. faecalis, *E. faecium* and *E. coli* have been included in the DANMAP programme since 1995, where isolates were recovered for antimicrobial susceptibility testing in samples from broilers, cattle and pigs. Sampling of fresh meat has been performed since 1997. Indicator enterococci and *E. coli* from healthy humans were collected from 2002 to 2005. Results from surveys of ESBL/AmpC-producing bacteria from animals and meat have been presented in DANMAP reports since 2006-2007. Since 2014, sampling and testing of *Enterococcus*, indicator *E. coli* and ESBL/AmpC-producing *E. coli* have been performed according to the EU harmonised monitoring of antimicrobial resistance [Decision 2013/652/EU]. In 2016, most of the sampling for indicator bacteria for DANMAP was allocated to the mandatory sampling of broilers; however, sampling of fattening pigs and cattle were also conducted.

Table 7.1. Resistance (%) among *Enterococcus faecalis* from broilers, Denmark

DANMAP 2016

Antimicrobial agent	Broilers Danish %
Tetracycline	53
Tigecycline	0
Chloramphenicol	2
Ampicillin	0
Erythromycin	37
Gentamicin	0
Ciprofloxacin	3
Vancomycin	0
Teicoplanin	0
Linezolid	0
Daptomycin	0
Number of isolates	119

7.2 Enterococci

For enterococci, DANMAP 2016 includes only randomly collected *Enterococcus faecalis* isolates from healthy broilers (caecum samples) at slaughter. The antimicrobials recommended by EFSA were used for susceptibility testing and MIC distributions and occurrence of resistance among *E. faecalis* from broilers are presented in the web annex (Table A7.1). The changes to the DANMAP sampling regime means that all reservoirs are no longer sampled each year, reducing the ability to spot changes on a yearly basis.

7.2.1 *E. faecalis* from domestically produced broilers

A total of 119 *E. faecalis* isolates from broilers were selected from 621 samples and tested for antimicrobial susceptibility (Table 7.1). Approximately half of the isolates were resistant to tetracycline, 37% were resistant to erythromycin and 3% (n=3) were resistant to ciprofloxacin. Resistance to tetracycline and erythromycin increased in prevalence. (Figure 7.1). Ciprofloxacin-resistant *E. faecalis* from Danish broilers has not previously been observed in the DANMAP and the fact that all three ciprofloxacin-resistant isolates are also resistant to tetracycline, may suggest the presence of a clone.

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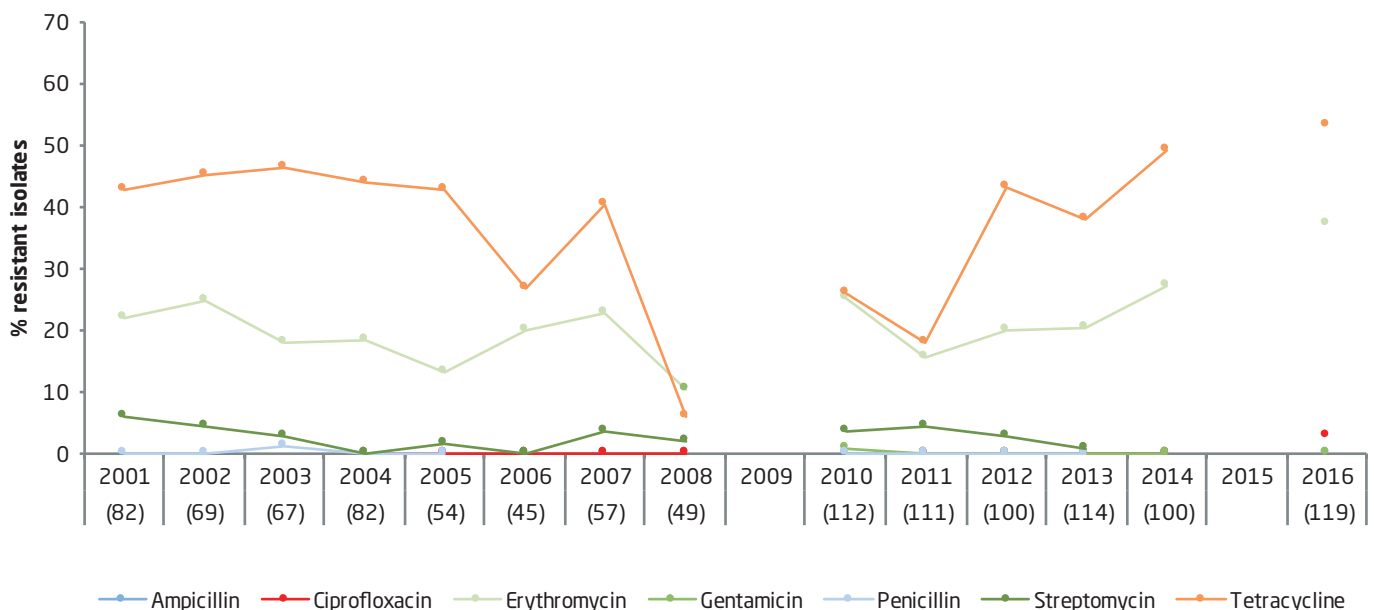
7.3 Indicator *Escherichia coli*

All isolates were randomly collected from healthy pigs, broilers and cattle at slaughter (caecum samples). Only one isolate per farm was included. For details on methodology, see Chapter 9, Materials and Methods. Isolates were not collected from meat samples in 2016.

The antimicrobials recommended by EFSA were used for susceptibility testing and MIC distributions and occurrence of resistance among indicator *E. coli* are presented in the web annex (Table A7.2). These results were obtained using the non-selective isolation procedure. Results obtained by using selective enrichment procedures for detection of cefotaxime-resistant *E. coli* are reported in section 7.4.

Figure 7.1. Resistance (%) among *Enterococcus faecalis* from broilers, Denmark

DANMAP 2016



Note: Number of isolates included each year is presented in the parenthesis. The '-' indicate data for application of 2016 cut-offs not available or less than 25 isolates were available

7.3.1 Indicator *E. coli* from broilers

A total of 186 indicator *E. coli* isolates from broilers were selected from 193 samples tested for antimicrobial susceptibility (Table 7.2). Approximately half (56%) of these were susceptible to all antimicrobials tested. As in previous years, the highest occurrence of resistance was observed to ampicillin, sulfonamide and trimethoprim (21-27%). Resistance to nalidixic acid, ciprofloxacin and tetracycline was moderate (13-16%), whereas resistance to the other compounds in the panel was 1-2% or not detected. Occurrence of nalidixic acid and ciprofloxacin resistance was significantly higher in *E. coli* from broilers compared to *E. coli* from pigs and cattle.

Compared to 2015, an increase in resistance to ciprofloxacin and a decrease in resistance to ampicillin, tetracycline, trimethoprim and sulfonamide were observed (Figure 7.2), but the changes were not statistically significant.

A total of 21 resistance profiles were detected among the 83 resistant isolates. Co-resistance to ampicillin, sulfonamide and trimethoprim (ASuTm resistance profile) was the most common profile found in 19% of the resistant isolates. In addition 24% of the resistant isolates, the ASuTm resistance profile occurred in combination with other resistances.

Resistance to nalidixic acid and ciprofloxacin was observed either alone or together with resistance to other antimicrobial classes (15% and 16% of the resistant isolates, respectively). Eight isolates exhibited co-resistance to fluoroquinolones and ASuTm. One isolate exhibited co-resistance to ampicillin, 3rd generation cephalosporins, fluoroquinolones and tetracycline. Two other fluoroquinolone-resistant isolates exhibited co-resistance to gentamicin either alone or in combination with the ASuTm resistance profile.

Of the three isolates resistant to 3rd generation cephalosporins and clavulanic acid, one was only resistant to these. The other two isolates were co-resistant to ampicillin, tetracycline and sulphonamide, and ampicillin, fluoroquinolones and tetracycline, respectively.

7.3.2 Indicator *E. coli* from pigs

A total of 145 indicator *E. coli* isolates from pigs were selected from 150 samples and tested for antimicrobial susceptibility (Table 7.2). Approximately half (46%) of the isolates from pigs were susceptible to all antimicrobials tested. Resistance to ampicillin, sulfonamide, tetracycline and trimethoprim was common (30-42%), whereas resistance to the other compounds occurred in 5% or less isolates. Colistin, meropenem and tigecycline resistance were not detected, suggesting that the true prevalence of these resistance phenotypes in *E. coli* from pigs is less than 2% (see Textbox 6.2 and Chapter 9, Materials and Methods, section 9.7.3). Occurrence of sulfonamide and tetracycline resistance was significantly higher in *E. coli* from pigs than in *E. coli* from broilers and cattle.

The levels of resistance were comparable to those observed in 2015 apart from an increase in resistance to sulphonamide and trimethoprim (Figure 7.2). The proportion of isolates resistant to trimethoprim was the highest observed in DANMAP since 2008, when the current trimethoprim concentration range was introduced.

A total of 25 resistance profiles were detected. The ASuTm resistance profile was the most common and detected in 33% of the resistant isolates. The majority of isolates with this profile exhibited additional resistance to tetracycline alone or in combination with resistance to other antimicrobials including fluoroquinolones in one isolate and gentamicin in three isolates. Third-generation cephalosporin resistance was found only in two isolates not expressing resistance to any non-beta-lactam antibiotic.

7.3.3 Indicator *E. coli* from cattle

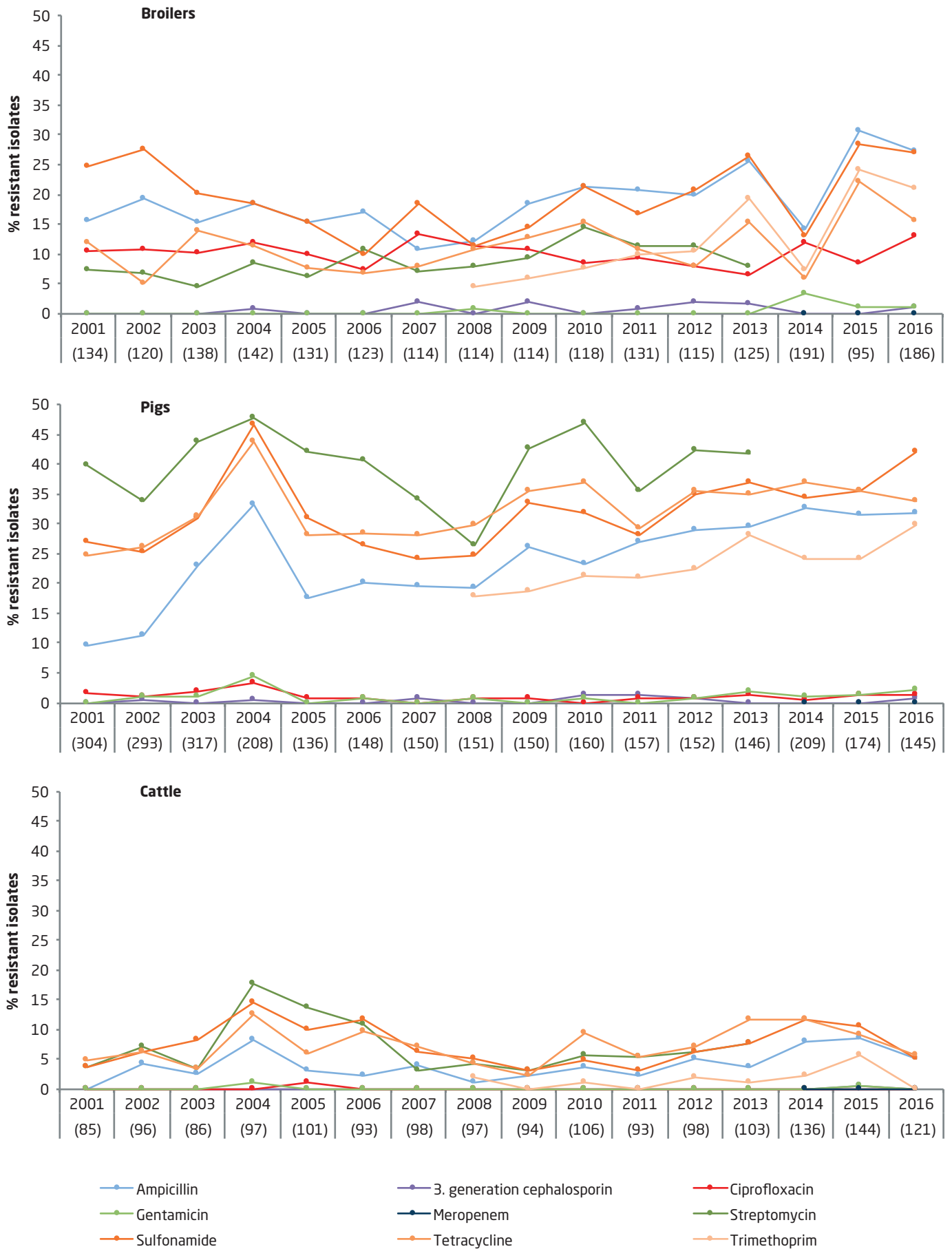
A total of 121 indicator *E. coli* isolates from cattle were selected from 121 samples and tested for antimicrobial susceptibility (Table 7.2). The vast majority of isolates (93%) was susceptible to all tested antimicrobials. Ampicillin, chloramphenicol, sulfonamide and tetracycline resistance levels were low, and no resistance to the remaining antimicrobials tested was detected. Occurrence of resistance in 2016 was comparable or slightly lower than in 2015, except for a statistically significant decrease in trimethoprim resistance. Five resistance profiles were detected. Two profiles consisted of resistance to one antimicrobial only (ampicillin or tetracycline resistance). Resistance to ampicillin, chloramphenicol, sulfonamide and tetracycline was the most common profile of resistance to more than one drug detected in 2% (n=3) of isolates.

Table 7.2. Resistance (%) among *Escherichia coli* from animals, Denmark DANMAP 2016

Antimicrobial agent	Broilers	Cattle	Pigs
	Danish %	Danish %	Danish %
Tetracycline	16	6	34
Tigecycline	0	0	0
Chloramphenicol	2	3	5
Ampicillin	27	5	32
Cefotaxime	1	0	<1
Ceftazidime	1	0	<1
Meropenem	0	0	0
Trimethoprim	21	0	30
Sulfonamide	27	5	42
Azithromycin	0	0	2
Gentamicin	1	0	2
Ciprofloxacin	13	0	1
Nalidixic acid	13	0	1
Colistin	0	0	0
Number of isolates	186	121	145

Figure 7.2. Resistance (%) among *E. coli* from animals, Denmark

DANMAP 2016



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

7.4 Extended spectrum beta-lactamase (ESBL), AmpC and carbapenemase-producing *E. coli*

DANMAP 2016 includes ESBL/AmpC- and carbapenemase-producing *E. coli* (CPE) from broilers at slaughter (caecal samples) and from broiler meat at retail (Danish and imported). Samples were collected randomly and analysed using selective enrichment procedures for detection of cefotaxime-resistant *E. coli* as well as CPE (including strains producing OXA-48-like enzymes) as described in the EURL-AR protocol for detection of ESBL/AmpC- and CPE. Whole genome sequencing (WGS) and in silico bioinformatics tools were used to determine genotypes. For details on methodology, see Chapter 9, Materials and Methods.

7.4.1 ESBL/AmpC and carbapenemase-producing *E. coli* from broilers and domestically produced broiler meat

A total of 298 samples from broilers and 229 samples from domestically produced broiler meat resulted in 48 (16%) and 52 (23%) isolates, respectively (Table 7.3). Most samples were

Table 7.3. Resistance (%) and 3rd gen.-cephalosporin resistance phenotype among ESBL/AmpC-producing *Escherichia coli* from broilers and meat thereof, Denmark DANMAP 2016

Antimicrobial agent	Broiler		Broiler meat
	Danish %	Danish %	Imported %
Tetracycline	56	44	65
Tigecycline	0	0	0
Chloramphenicol	10	10	8
Ampicillin	100	100	100
Cefoxitin	63	35	27
Cefotaxime	100	100	100
Ceftazidime	100	100	97
Cefepime	67	90	97
Meropenem	0	0	0
Ertapenem	0	0	5
Imipenem	0	0	0
Trimethoprim	23	39	30
Sulfonamide	42	73	73
Azithromycin	2	0	0
Gentamicin	8	14	14
Ciprofloxacin	40	15	41
Nalidixic acid	33	15	38
Colistin	0	0	0
ESBL phenotypes	38	65	73
AmpC phenotypes	58	35	24
ESBL/AmpC phenotypes	2	0	3
CPE phenotypes	0	0	0
Other phenotypes	2	0	0
Number of isolates	48	52	37
Number of samples	298	229	66

Note: Isolates recovered by the selective enrichment methods described in the EURL-AR laboratory protocol (October 2015)

also specifically examined for CPE and no CPE isolates were recovered.

Among the 48 ESBL/AmpC-producing *E. coli* isolates from **broilers**, 38% exhibited an ESBL phenotype, 58% exhibited an AmpC phenotype and 2% (n=1) exhibited both ESBL and AmpC phenotype. All isolates were resistant to ampicillin and 3rd generation cephalosporins, and co-resistance was observed i) to tetracycline in more than half of the isolates (56%), ii) to sulfonamides in 42% of the isolates and iii) to trimethoprim in 23% of the isolates. Co-resistance to ciprofloxacin was found in 40% (n=19) of the isolates, and 16 of these were also resistant to nalidixic acid. Only a few isolates were co-resistant to gentamicin (8%, n=4) and azithromycin (2%, n=1). None of the isolates was co-resistant to colistin, tigecycline and carbapenems.

Among the 52 isolates from **domestically produced broiler meat**, the AmpC phenotype (65%) was more common than the ESBL phenotype (35%). The CPE phenotype was not observed. The isolates from domestically produced broiler meat exhibited similar co-resistance patterns and levels of resistance as the isolates from broilers, with the exception of significantly lower occurrence of co-resistance to fluoroquinolones and cefoxitin, and higher occurrence of co-resistance to cefepime and sulfonamides. None of the isolates was co-resistant to azithromycin and carbapenems (Table 7.3).

The occurrence of ESBL/AmpC-producing *E. coli* in domestically produced broiler meat in 2016 was similar to the occurrence in 2015 (23% and 26%, respectively)(Figure 7.3). However, when evaluating the trend over time, it must be taken into account that the presumptive ESBL/AmpC *E. coli* from 2015 were not further confirmed by susceptibility testing, and only 28 (12%) of the ESBL/AmpC phenotypic isolates were verified by WGS. No further historical comparisons could be carried out due to changes in methodology (Decision 2013/652/EU).

7.4.2 ESBL/AmpC and carbapenemase-producing *E. coli* from imported broiler meat

A total of 66 samples from imported broiler meat resulted in 37 (56%) ESBL/AmpC isolates (Table 7.3), which was significantly higher than the 23% positive samples from domestic broiler meat. Most samples (n=63) were also specifically examined for CPE and no CPE isolates were recovered.

The ESBL phenotype (73%) was more common than the AmpC phenotype (24%) among the 37 isolates from **imported broiler meat**. One isolate exhibited both ESBL and AmpC phenotype. Generally, the levels of co-resistance were comparable to those observed in ESBL/AmpC isolates originating from domestically produced broiler meat apart from a higher proportion of isolates co-resistant to fluoroquinolones (Table 7.3). Low level carbapenem (ertapenem) resistance was observed among the ESBL/AmpC-producing isolates.

The occurrence of ESBL/AmpC-producing *E. coli* in imported broiler meat in 2016 was comparable to the 64 % found in 2015 (Figure 7.3). Further historical comparisons were not carried out due to changes in methodology over the last few years.

7.4.3 ESBL/AmpC enzymes detected in *E. coli* isolates from broilers and domestically produced broiler meat

From **broilers**, only 34 of the 48 ESBL/AmpC-producing *E. coli* were sequenced. ESBL/AmpC enzymes were detected in all 34 isolates and one isolate had two different enzymes (Table 7.4).

Among the 34 ESBL/AmpC-producing *E. coli* isolates from **broilers**, CMY-2 (n=12) was the most common enzyme and was detected in isolates belonging to nine sequence types (ST); including ST429 (n=3), ST1286 (n=2) and ST10, ST295, ST371, ST718, ST1056, ST2040, and ST4663 (one isolate each). Additional 11 isolates expressing the AmpC phenotype by upregulation of chromosomal *ampC* expression belonged to ST4663 (n=9), ST23 (n=1) and an unknown ST (n=1). The CTX-M-1-encoding gene was the most common ESBL gene detected in seven isolates (belonging to five STs; ST752 and ST4980 (two isolates each), and ST162, ST1640, and ST1850 (one isolate each). One ST57 isolate carried the gene encoding SHV-12. Four isolates producing TEM-52B/C belonged pairwise to ST115 and ST155 (Figure 7.4).

All 52 ESBL/AmpC-producing *E. coli* isolates from **domesti-**

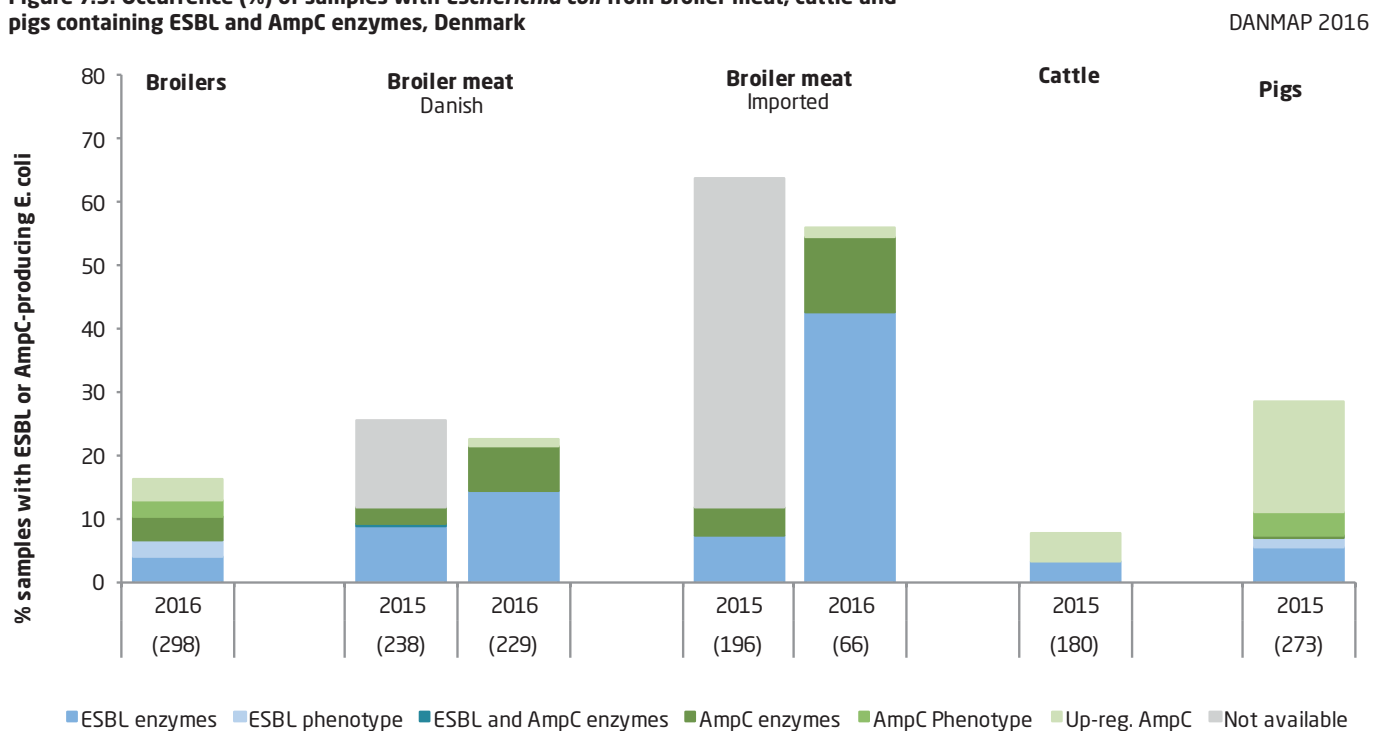
cally produced broiler meat were available for sequencing and ESBL/AmpC enzymes were detected in all strains (Table 7.4). The predominance of CTX-M-1 in isolates in domestic-produced broiler meat seemed to continue in 2016 followed by CMY-2 enzymes, which were the most frequent resistance determinants until 2014 (Table 7.4).

Among the 52 ESBL/AmpC-producing *E. coli* isolates from domestically produced broiler meat, more than half harboured the CTX-M-1-encoding gene (n=22) and belonged to seven STs; ST4980 (n=14), ST295 (n=2), ST57, ST117, ST155, ST351, ST1463, and an unknown ST (one isolate each). CMY-2-encoding gene (n=16) and upregulation of chromosomal *ampC* expression (n=3) were observed in strains belonging to ST429 (n=8), ST10 (n=3), ST155, ST355, ST1011, ST1144, and ST1640 and ST120, ST4512, and unknown ST (one isolate each). All four SHV-12 enzymes belonged to ST57 whereas the seven isolates harbouring the TEM-52B-encoding gene belonged to ST155 (n=6) and ST5183 (Figure 7.4).

Eight STs out of 28 were observed in both broilers and domestically produced broiler meat: ST10, ST57, ST155, ST295, ST429, ST1640, ST4980, and an unknown ST. Three of these STs were linked to one enzyme: ST10 and ST429 associated with CMY-2, and ST4980 associated with CTX-M-1.

Of the 10 different STs associated with CTX-M-1 in domestically produced broiler meat in 2015, only ST295 and ST4980

Figure 7.3. Occurrence (%) of samples with *Escherichia coli* from broiler meat, cattle and pigs containing ESBL and AmpC enzymes, Denmark



Note: Number of tested samples each year is presented in the parentheses. Samples were processed according to the EURL-AR laboratory protocol (October 2015). Occurrence is based on WGS data, supplemented with data from presumptive ESBL/AmpC-producing isolates when not available (Phenotype included when available)

were also found in 2016. In 2015, two isolates of ST295 and three isolates of ST4980 were found. In 2016, two isolates of ST295 and 14 isolates of ST4980 were detected, thus suggesting clones persisting over time. No similarities were observed among STs associated with CMY-2 and upregulation of chromosomal *ampC* in 2015 and 2016.

7.4.4 ESBL/AmpC enzymes detected in *Escherichia coli* isolates from imported broiler meat

All 37 ESBL/AmpC-producing *E. coli* isolates from imported broiler meat were sequenced. ESBL/AmpC enzymes were detected in all strains and six different ESBL/AmpC genes or mutations were identified (Table 7.4).

Among the 37 ESBL/AmpC-producing *E. coli* isolates from imported broiler meat the CTX-M-1-encoding gene (n=22) was the most common and occurred in isolates belonging to 12 STs; three isolates of each of ST10, ST38, and ST212, two isolates of each of ST57, ST93, ST155 and ST515, and one isolate each of ST48, ST746, ST1011, ST4512 and ST5363 (Figure 7.4). One CTX-M-14b isolate belonged to ST1011, and three SHV-12 isolates belonged to ST117, ST1944, and ST57 (one isolate each). CMY-2-producing *E. coli* (n=8) and *E. coli* with upregulation of chromosomal *ampC* expression (n=1) were observed in strains belonging to ST131, ST429, ST1706, ST2701 and unknown STs. As in 2015, the important human pathogen *E. coli* ST131 was observed among the imported broiler meat isolates. Persistence of this *E. coli* clone as well as its increasing occurrence among poultry sources and human infections in other European countries raise some concern. Comparing strains from imported broiler meat and domestically produced broilers and meat thereof, ST429 coding for CMY-2 was present in all three sources.

7.4.5 Perspectives

In general, the most common ESBL/AmpC enzymes identified across the broiler sources were the ESBL enzyme CTX-M-1 and the AmpC enzyme CMY-2. CTX-M-1 was the most common enzyme in *E. coli* from Danish and imported broiler meat. The CMY-2 and upregulation of chromosomal *ampC* expression were the most common genotypes detected in ESBL/AmpC *E. coli* from Danish broilers. In the previous surveys from 2009 to 2013, CMY-2 was the predominant enzyme among the ESBL/AmpC *E. coli* isolates from domestically produced and imported broiler meat. However, in the surveys from 2014 to 2016, CTX-M-1 became increasingly prevalent [DANMAP 2014 and 2015].

Table 7.4. Distribution (%) of ESBL and AmpC enzymes detected in *E. coli* isolates^(b) from animals and meat of Danish and imported origin, Denmark DANMAP 2016

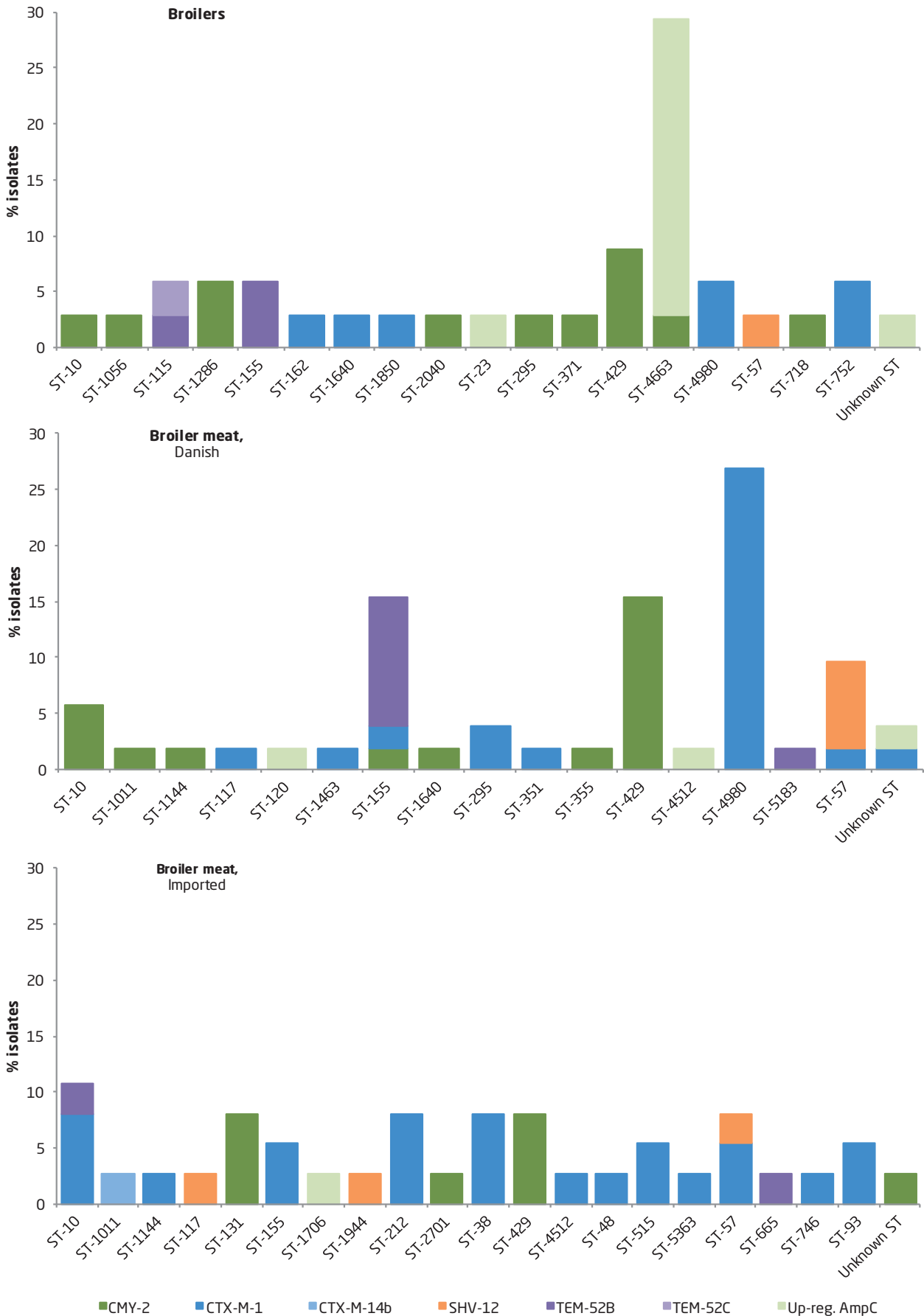
Enzyme	Broilers	Broiler meat	
	Danish %	Danish %	Imported %
CMY-2	34	31	22
CTX-M-1	20	42	59
CTX-M-14b	0	0	3
SHV-12	3	8	8
TEM-52B	9	13	5
TEM-52C	3	0	0
Up-reg. AmpC	31	6	3
Total number of :			
Enzymes	35	52	37
Number of isolates	34	52	37
ESC positive samples ^(a)	48	52	37
Samples tested	298	229	66

Note: Proportion of total number of enzymes. In one isolate from broilers, chromosomal *ampC* upregulation and CMY-2 co-existed (a) Total number of enzymes present. Each enzyme is counted based on the number of strains in which it is present and not based on the type (e.g. CTX-M-1 present in two strains counts as 2) (b) Samples were processed according to the EURL-AR laboratory protocol (October 2015), thus number of samples and number of isolate coincide. Whole genome sequencing was only performed on a selection of the presumptive ESBL/AmpC from broilers (34 of 48 isolates)

Large variation of the *E. coli* STs harbouring the ESBL/AmpC genes or mutations leading to chromosomal *ampC* overexpression was observed across the three sources of isolates. A few STs producing the same ESBL/AmpC enzyme were present in both domestically produced broilers and meat thereof. Similarly, a few identical STs were observed between Danish and imported broiler meat, though the ESBL/AmpC enzymes produced were not always identical. Of concern is the persistence of a low number of *E. coli* ST131 that harbour CMY-2 in imported broiler meat due to its possible role in human infections. This ST type was not found in Danish broilers or meat thereof.

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Figure 7.4. Distribution (%) of ESBL and AmpC enzymes in *E. coli* MLST types in isolates from broilers and broiler meat, Denmark
 DANMAP 2016



Note: Isolates recovered by the selective enrichment method described in the EURL-AR laboratory protocol (October 2015). Whole genome sequencing was applied to ESBL/AmpC isolates from broilers (34 isolates, recovering 35 enzymes), Danish broiler meat (52 isolates) and imported broiler meat (37 isolates)

Textbox 7.1

ESBL/pAmpC-producing *Escherichia coli* - comparison of isolates of animal origin with isolates obtained from human bloodstream infections

Background: ESBL/pAmpC-producing bacteria are widespread in both humans and production animals worldwide. Studies have found similar ESBL/pAmpC genes, plasmids and clones of *E. coli* in animals, meat and human infections, suggesting a zoonotic link.

Materials and methods: ESBL/pAmpC-producing *E. coli* isolates from production animals and meat (section 7.4) obtained in 2015 and 2016 were compared with ESBL/pAmpC-producing *E. coli* isolates from human bloodstream infections (Textbox 8.1) obtained during 2016. For isolates sharing the same combination of ESBL/pAmpC genes and sequence types (STs), clonal relationships were investigated by whole-genome-based SNPs analyses. The combination was further investigated if < 100 SNPs were observed between isolates from animal and human origin. Thus, six ST131 CMY-2-producing *E. coli* isolates were compared with 309 ST131 ESBL/pAmpC-producing *E. coli* isolates obtained from 2015 and 2016 (DANMAP 2015 and Textbox 8.1).

Results: On five occasions, the same combinations of ESBL/pAmpC genes and STs were detected in *E. coli* of human and animal origin; ST131 CMY-2-producing *E. coli* isolates, CTX-M-1-producing *E. coli* belonging to ST1431, ST23 or ST767, and ST12 CTX-M-14-producing *E. coli* isolates. SNP-based comparisons were performed for each of the five combinations.

The ST131 CMY-2-producing *E. coli* isolates represented five isolates from imported broiler meat (three isolates from 2016 and two from 2015) and one human bloodstream isolate from 2016 (Figure A7.1 SNP phylogeny of ST131 CMY-2, web annex). Between the *E. coli* isolate of human origin and the five *E. coli* isolates from broiler meat, a range of 78-206 SNPs were detected. Comparing the six ST131 CMY-producing isolates with the 309 ST131 *E. coli* isolates from bloodstream infections from 2015-2016 in Denmark, a distinct clade with these six CMY-2-producing *E. coli* isolates was observed (Figure A7.2, ST131 and ST12, web annex).

The ST1431 CTX-M-1-producing *E. coli* encompassed one imported broiler meat isolate from 2015 and one human bloodstream isolate from 2016, with 3,756 SNPs detected between them.

The ST23 CTX-M-1-producing *E. coli* represented one isolate from a pig caecal sample from 2015 and one human bloodstream infection isolate, which differed by 3,389 SNPs.

The ST767 CTX-M-1-producing *E. coli* represented one isolate from pork from 2015 and one human bloodstream infection isolate from 2016, which differed by 640 SNPs.

The ST12 CTX-M-14-producing *E. coli* encompassed one pig isolate from 2015 and eight human bloodstream isolates from 2016 (Figure ST12 CTX-M-14, web annex). More than 900 SNPs were detected between the pig isolate and the human isolates.

Discussion and conclusion: ST131 *E. coli* has been the most frequently detected ST among the ESBL/pAmpC-producing *E. coli* isolates from human bloodstream infections in Denmark in the past years, with most of them being CTX-M-15-producers (Textbox 8.1). In 2016, an ST131 CMY-2-producing *E. coli* was observed for the first time causing bloodstream infection in a Danish patient. The pAmpC enzyme CMY-2 has often been detected among *E. coli* of animal origin in Denmark, but only rarely for *E. coli* belonging to ST131. During 2015-2016, five ST131 CMY-2-producing *E. coli* were detected from imported broiler meat by the DANMAP surveillance [DANMAP 2015, section 7.4], and when investigating the clonal relationship to the ST131 CMY-2-producing *E. coli* human bloodstream isolates, the pairwise comparison did not indicate any direct link. However, when broadening the comparison to include all known ST131 *E. coli* cases from bloodstream infections in Denmark during 2015-2016 in a phylogenetic analysis, it appears that the six ST131 CMY-2-producing *E. coli* constitute a sub-clone, thus suggesting a potential zoonotic link between imported broiler meat and bloodstream infections in humans.

SNP analysis did not support indications of any zoonotic link for the other ESBL/pAmpC-producing *E. coli* isolates with the same combination of ST obtained from both human and animal samples (CTX-M-1-producing isolates belonging to ST1431, ST23, or ST767, and CTX-M-14 belonging to ST12), as no similar SNP profiles were detected

The observation of phylogenetically related ST131 CMY-2 *E. coli* from imported meat and from a human bloodstream infection should be noted. However, larger studies are needed to investigate and quantify the possible zoonotic link between ESBL/pAmpC-producing *E. coli* from meat/animals and human severe infections.

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8

RESISTANCE IN HUMAN
CLINICAL BACTERIA

8. Resistance in human clinical bacteria

▶▶▶ **Highlights:** DANMAP 2016 includes resistance data from human invasive infections (isolates from blood and cerebrospinal fluid) reported from the Danish clinical microbiological departments for the last decade. In general, the number of blood isolates has increased during the last ten years, while resistance rates for most invasive species show decreasing trends. This is not the case for the multi-resistant species isolated from other clinical samples, eg carbapenemase-producing organisms (CPO) and methicillin-resistant *Staphylococcus aureus* (MRSA) as well as for vancomycin-resistant enterococci (VRE) from all clinical samples. For all of these, the increasing trends observed during the last years continued (**textboxes 8.1, 8.2, 8.3 and 8.4** and section 8.7).

For ***Escherichia coli***, resistance data for isolates from blood stream infections showed a significant increase in the resistance rates to gentamicin, and cefuroxim for 2007 to 2016. In 2016 the resistance to gentamicin was 6%, ciprofloxacin 11%, cefuroxime 9%, and 3rd generation cephalosporins 7%, respectively. One carbapenemase-resistant and two intermediary resistant *E.coli* isolates were reported. In urine cultures, a significant increase in the proportion of ciprofloxacin *E. coli* isolates was reported since 2007, however with a decrease and stabilization since 2009 reported from both hospital samples and samples from primary health care, respectively.

For ***Klebsiella pneumoniae***, a significant decrease in the resistance rates in blood isolates to gentamicin, ciprofloxacin, and cefuroxim was observed from 2008 to 2016. In 2016 the resistance to gentamicin was 3%, ciprofloxacin 5%, cefuroxime 11%, and 3rd generation cephalosporins 7%, respectively. Three carbapenemase-resistant and one intermediary resistant *K.pneumoniae* isolates were reported. In urine cultures, the proportions of ciprofloxacin-resistant and 3rd generation cephalosporin-resistant *K.pneumoniae* isolates have decreased since 2009.

Enterococci isolates from bloodstream infections were, together with *Streptococcus pneumonia*, the only species where a slight decline in the number of isolates reported was observed for 2016. In general the number of bloodstream infections with *E. faecium* has increased during the last decade from 352 in 2007 to 692 in 2016, while the number of infections with *E. faecalis* has fluctuated slightly but remained more or less stable around 600 (606 in 2016). Thus, the ratio between bloodstream infections with *E. faecalis* and *E. faecium* has changes considerably from 1.6 in 2007 to 0.9 in 2016. The prevalence of vancomycin-resistant *E. faecium* in isolates from bloodstream infections was 7.3% in 2016 compared to 0.6% in 2007. None vancomycin-resistant *E. faecalis* were reported. Linezolid-resistance was reported in one of each species for 2016.

DANMAP 2016 includes for the first time, the numbers of highly resistant ***Acinetobacter spp.*** isolates from human bloodstream infections. During 2012 to 2016 between 69 and 83 *Acinetobacter* blood isolates were reported annually. In this period the resistance rates to ciprofloxacin, gentamicin and meropenem decreased significantly, thus in 2012, ten isolates were resistant to either one or more of the three tested agents (corresponding to 10-12%), in 2016 two isolates were resistant to ciprofloxacin (3%), while none of the invasive isolates were resistant to meropenem. In contrast, 26 carbapenemase-producing *A. baumannii* from other clinical sites (all specimen types) were sent to SSI for wholegenome-sequencing, which is an increase from 19 isolates sent in 2015 (see **textbox 8.2** for further information).

Introduction

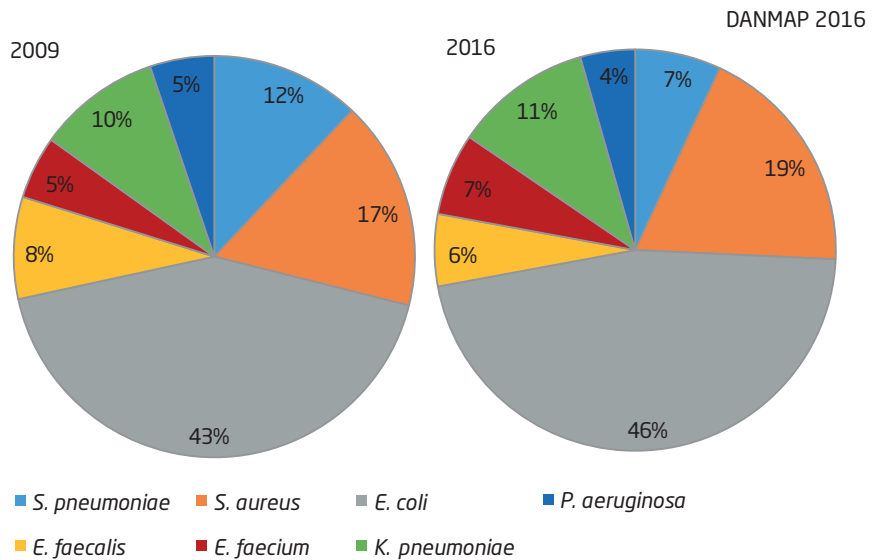
Statens Serum Institut receives data on the resistance in isolates for the seven most frequently occurring species obtained from human invasive infections, either obtained from blood or cerebrospinal fluid. These include: *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterococcus faecium* and *Enterococcus faecalis*. For all species the first isolate per patient per year is reported, which corresponds to the method used by Ears-Net. All reporting is performed by the Danish Clinical Microbiological Departments (DCM) situated at regional hospitals. Data is reported once yearly. Until 2009 all invasive infections were counted in 30-day periods. Since only a very minor part of the patients has recurring periods of bacteraemia within one calendar year, the difference in the number of patients and species between the two methods is negligible and is thus not commented on in this chapter.

In addition to the reporting of data, for several species strains are also sent to the reference laboratory at SSI for descriptive and/or surveillance purposes. For invasive infections with *Streptococcus pneumoniae* surveillance is mandatory. For invasive infections with either *Staphylococcus aureus*, beta-haemolytic streptococci or ESBL-producing *E. coli* as well as for carbapenemase-producing organisms (CPO) and vancomycin-resistant enterococci (VRE) from all clinical sites and samples strains are referred to the reference laboratory on a voluntary basis. Finally, the submission of all clinical strains of methicillin-resistant *S. aureus* (MRSA) and *Neisseria gonorrhoea* is also mandatory.

In 2016, the number of DCM changed from 11 to 10, but in most chapters in this DANMAP report we still refer to 11 DCM, since the reporting on many strains continued as usual from the different geographical settings.

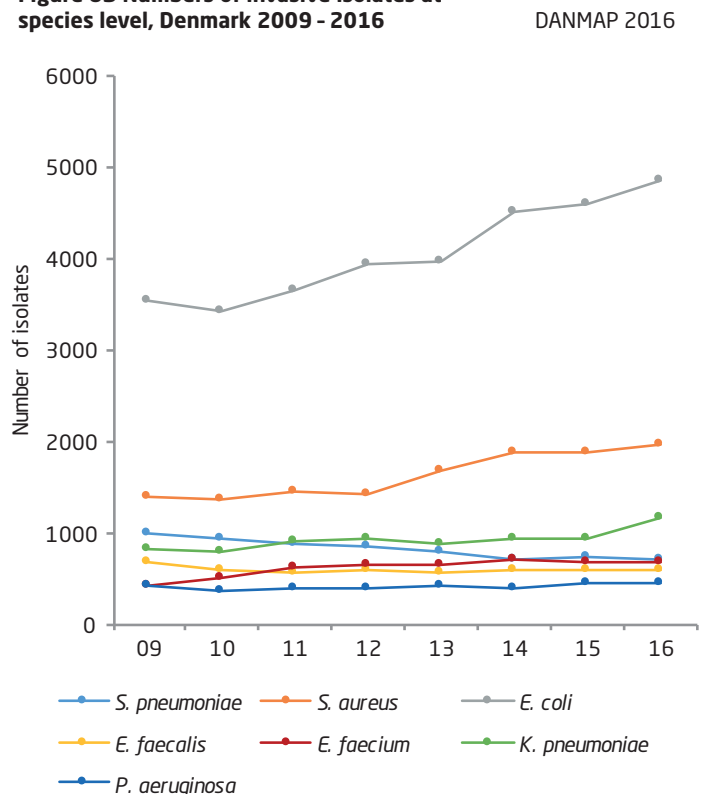
From 2009 until 2014, DANMAP received data from all but one DCM, covering approximately 95% of the population; from 2015 all DCM had joined the reporting thus giving coverage of the total Danish population. From 2009 to 2016, the number of isolates reported increased by 26%, from altogether 8,277 to 10,442 isolates. The biggest increase observed was for *E. faecium* with 65 %, followed by *K. pneumoniae*, *S. aureus* and *E. coli* with 41%, 40% and 37%, respectively. *Ps. Aeruginosa* increased with 7%, while *S. pneumoniae* and *E. faecalis* decreased with - 27% and - 11%, respectively. Figure 8A shows the changes in the distribution of species among the invasive isolates reported for 2009 and 2016, respectively. Figure 8B shows the trends from 2009 to 2016, *E. coli* accounts for almost 50% of the cases. The increases can neither be explained

Figure 8A introduction. Distribution of species in invasive isolates, Denmark 2009 - 2016



through changes in the reporting nor through increases in the population size alone (which increased from 5.5 million to 5.7 million inhabitants corresponding to an increase of 3.6%), but probably also other important factors contribute: demographic changes with a growing population of elderly, changes in hospital workflow with more blood culturing bottles taken per patient and finally changes in logistics with more effective transport of the samples to microbiological laboratories as well as improved culturing systems.

Figure 8B Numbers of invasive isolates at species level, Denmark 2009 - 2016



8.1 *Escherichia coli*

Escherichia coli (*E. coli*) is the most frequent cause of community- and hospital acquired urinary tract infections and of bacteraemia in Denmark. It is part of the normal intestinal flora in both animals and humans, where it comes into close proximity to many other bacterial species. Transferred resistance mechanisms from other bacterial species to *E. coli* are frequently seen. Some *E. coli* contain virulence factors that dispose for gastrointestinal illnesses of varying severity, such as traveller’s diarrhoea and gastrointestinal illness associated with the development of haemolytic uremic syndrome.

As for 2015, DANMAP received resistance data from all 11 Departments of Clinical Microbiology (DCM) in Denmark for 2016, thus representing 100% of the Danish population.

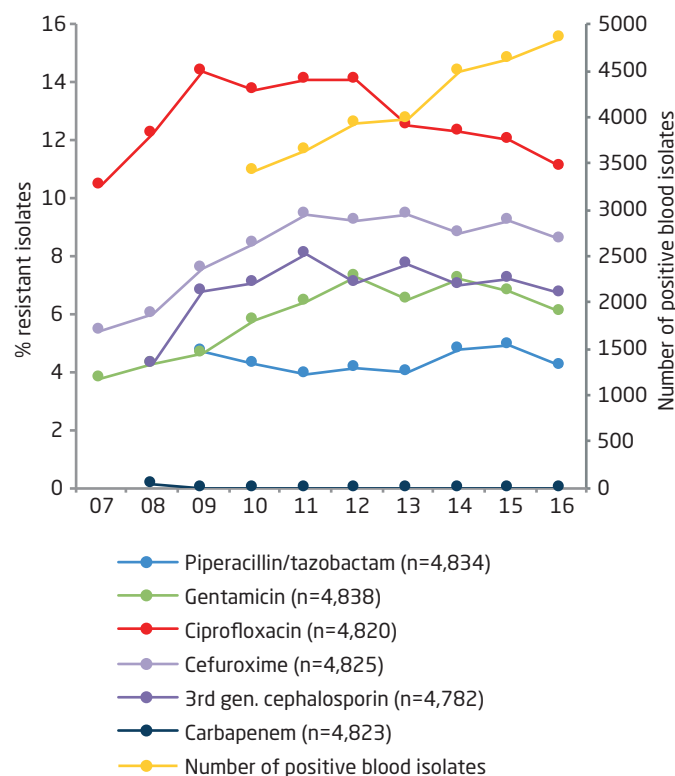
8.1.1 Blood isolates from hospital patients

For 2016, DANMAP received data on the antibiotic susceptibility of 4,841 *E. coli* isolates from blood cultures from all 11 DCM in Denmark. Eight of the 11 DCM routinely tested for mecillinam resistance (> 75% of isolates) and all 11 DCM routinely tested for all the other presented antibiotics (Table 8.1.1). As in previous years resistance testing was mainly performed by disc diffusion. The presented data consist of the reported interpretation results, performed by the DCM and based on the S-I-R system.

A continuous increase in the number of *E. coli* isolates from blood cultures was observed throughout the years (Figure 8.1.1). As discussed in the introduction, this may be caused by several factors such as, demographic changes, improved culturing methods and changes in hospital workflow.

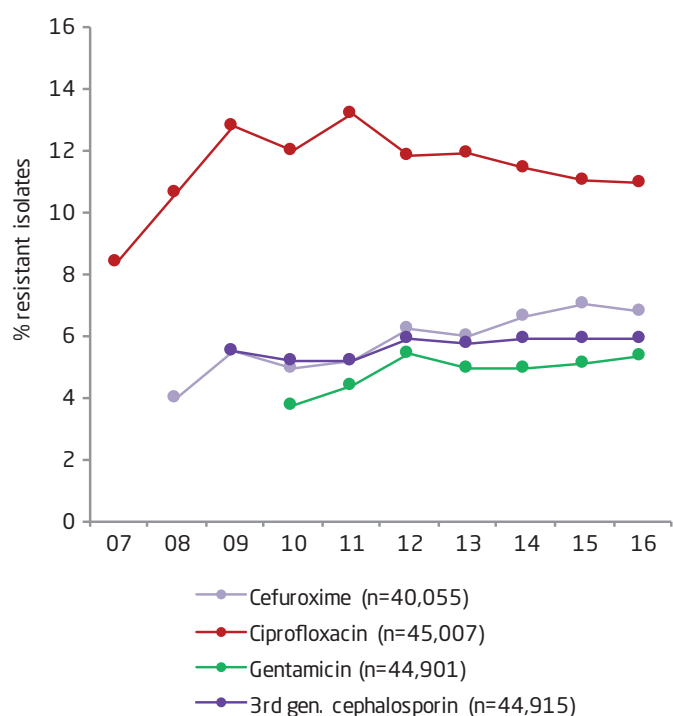
Overall, there was a tendency towards a more stationary resistance level for the last four to seven years (Figure 8.1.1). The same pattern was observed when looking at the concrete numbers of resistant *E. coli* blood isolates instead of the proportional resistances (data not presented). For gentamicin and cefuroxime, resistance has increased significantly since 2007, from 3.9% to 6.1% and 5.7% to 8.6%, respectively (Table 8.1.1). The resistance towards ciprofloxacin was 11% and is now at the same level as in 2007 after the peak of 13% in 2009. This trend mirrors the pattern in consumption, where also a decrease in the overall use of ciprofloxacin has been observed since 2009 (p. 38, table 5.1). Interestingly, the proportion of reported ciprofloxacin resistant invasive *E. coli* isolates varied significantly between some of the DCM, with 27% as the highest resistance level and 4.1% as the lowest resistance level reported. Likewise, there was a significant discrepancy in the reported resistance level towards piperacillin/tazobactam, with the highest proportion being 8.6% and the lowest proportion being <1%. These data are based on the interpretations of the individual DCM’s, where local interpretation rules may apply.

Figure 8.1.1 Resistance (%) in *Escherichia coli* invasive isolates from humans, Denmark DANMAP 2016



Note: The number (n) in parentheses represents the number of isolates tested for susceptibility in 2016

Figure 8.1.2 Resistance (%) in *Escherichia coli* urine isolates from humans in hospitals, Denmark DANMAP 2016



Note: The number (n) in parentheses represents the number of isolates tested for susceptibility in 2016

Table 8.1.1 Resistance (%) in *Escherichia coli* isolates from humans, Denmark 2016

DANMAP 2016

Substance	Blood isolates, hospitals %	Urine isolates, hospitals %	Urine isolates, primary health care %
Ampicillin	45	41 [#]	38 [§]
Mecillinam	13 ^{* ✕}	7 [§]	6 [¶]
Piperacillin/tazobactam	4	3 [#]	4 [#]
Sulfonamide		35 [*]	29 ^{# §}
Gentamicin	6 [¶]	5	4
Ciprofloxacin	11	11 [¶]	10 ^{* ✕}
Cefuroxime	9 [¶]	7	6
3rd generation cephalosporins ^{a)}	7	6	4
Carbapenem	<1	<1	<1
Max. number of isolates tested for resistance to the presented antibiotics	4838	46780	67765

For all isolates, statistical comparison of proportions of resistant isolates between 2015 and 2016 was calculated for all antibiotics. Statistical comparison between 2007 and 2016 of proportions of resistant isolates was calculated for ampicillin, gentamicin, ciprofloxacin and cefuroxime on blood isolates and for ampicillin, mecillinam, sulfonamide and ciprofloxacin on urine isolates.

* Indicates a significant increase from 2015 to 2016

Indicates a significant decrease from 2015 to 2016

✕ Indicates a significant increase from 2007 to 2016

§ Indicates a significant decrease from 2007 to 2016

a) Tested 3rd generation cephalosporins were ceftazidime, ceftriaxone, cefpodoxime and cefotaxime

We assume that especially the interdepartmental variance in piperacillin/tazobactam resistance would be smaller, if a standardized interpretation method was to be used. Still, local differences in resistance levels probably exist, and should be kept in mind in the development of empirical antibiotic guidelines.

The number of carbapenem (including meropenem) resistant *E. coli* isolates is continuously very low (Figure 8.1.1) with only one carbapenem resistant and two intermediary resistant *E. coli* isolates reported in 2016. In 2015, there were one carbapenem resistant and five intermediary resistant *E. coli* (DANMAP 2015). Although still at a low level, the risk of increasing levels of carbapenem resistance in the future is worrisome. A larger proportion of isolates (1.6-2.0% through the years 2014-2016) had combined resistance to 3rd generation cephalosporins, ciprofloxacin, and gentamicin (hereafter referred to as multi-resistant) (Table 8.1.2). Fewer than 75% of all isolates were tested for all three antibiotics before 2014, so the previous level of multi-resistance is not known. Situations may arise in the future in which only colistin, tigecycline and phosphomycin are left as treatment options for patients with severe *E. coli* infections. Susceptibility testing towards these antibiotics are not routinely done in Denmark. One colistin resistant *E. coli* isolate from 2015 was detected through whole genome-sequencing of all referred resistant strains from Danish human blood cultures from 2014 to 2016.

The overall resistance level towards mecillinam was 13%, which was a significant increase compared with 2007 and 2015 (Table 8.1.1). However, some DCM only performed mecillinam resistance testing on very few isolates and others reported resistance data using more restrictive breakpoints than EUCAST. As discussed in DANMAP 2015, even though it is not

optimal for surveillance purposes, more restrictive breakpoints may be sensible in a clinical situation. As in previous years, approximately half of all *E. coli* isolates were resistant towards ampicillin (Table 8.1, DANMAP 2015).

8.1.2 Urine isolates from hospital patients

For 2016, results of resistance testing were submitted to DANMAP from all 11 DCM for altogether 46,865 *E. coli* isolates, cultured in urine samples from hospitalised patients. All 11 DCM routinely tested their *E. coli* urine isolates from hospitalised patients for resistance to mecillinam and gentamicin; 10 DCM routinely tested for resistance to ampicillin, ciprofloxacin, cefuroxime, and 3rd generation cephalosporins; nine DCM routinely tested for piperacillin/tazobactam resistance; eight DCM routinely tested for carbapenem resistance; and six DCM routinely tested for sulfonamide resistance. Overall, the proportions of isolates tested at the different DCM varied for the different antibiotics, from 100% (mecillinam) to 60% (sulfonamide).

Throughout the years, there has been a slight but significant increase in the proportions of resistance towards cefuroxime and gentamicin, from 4.0% in 2008 to 6.8% in 2016 and from 3.7% in 2010 to 5.3% in 2016, respectively (Figure 8.1.2). The proportion of resistance to 3rd generation cephalosporins has not changed since 2009 and was 5.9% in 2016. After a steep increase in the proportion of ciprofloxacin resistance observed from 8.4% in 2007 to 13% in 2011, the proportion of resistance has decreased slightly ever since and reached 11% in 2016. This is still significantly higher compared to the level in 2007 (Table 8.1.1). In contrast to 2015, there was a slight but significant decrease in the proportion of piperacillin/tazobactam resistance (DANMAP 2015, Table 8.1). The reason for this is not known.

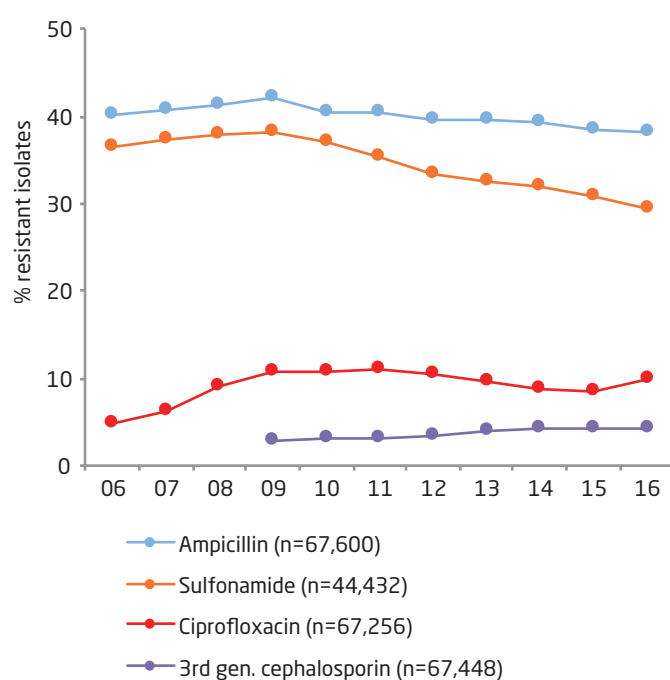
The level of carbapenem resistance is continuously low among urine isolates from hospitalized patients in Denmark but seems to be increasing, with no carbapenem resistant isolates and one intermediary resistant isolate reported in 2014, three carbapenem resistant isolates and 11 intermediary resistant isolates reported in 2015, and eight carbapenem resistant *E. coli* isolates and eight intermediary resistant isolates reported in 2016. The apparent increase is worrisome although a significant difference still cannot be measured ($p = 1.00$, Fischer's exact test).

As previously reported, approx. 40% of the isolates were resistant to ampicillin, although a slight but significant decrease in the resistance level was observed from 2015 to 2016. The proportion of sulfonamide resistance was also continuously high and with a slight but significant increase since 2015. No significant change was observed in the proportion of mecillinam resistance since 2015. However, the resistance proportion has increased significantly since 2007. This is possibly caused by an increased use of mecillinam as first choice treatment of urinary tract infections as recommended by Danish treatment guidelines. Mecillinam is the antibiotic available for oral administration that most *E. coli* isolates are susceptible to.

8.1.3 Urine isolates from primary health care

For 2016, results from antibiotic resistance testing were submitted to DANMAP for 67,798 *E. coli* isolates that were cultured from urine samples from primary health care. One of the DCM does not receive any samples from primary health care, thus results were only submitted from 10 of the 11 DCM in Denmark. All 10 DCM routinely tested their *E. coli* urine

Figure 8.1.3 Resistance (%) in *Escherichia coli* urine isolates from humans in primary health care, Denmark DANMAP 2016



Note: The number (n) in parentheses represents the number of isolates tested for susceptibility in 2016

Table 8.1.2 Combined resistance to 3rd generation cephalosporins, ciprofloxacin, and gentamicin (multiresistance) in blood *Escherichia coli* isolates from humans, Denmark DANMAP 2016

	2014 % (N)	2015 % (N)	2016 % (N)
Resistance	2 (72)	2 (93)	2 (87)
Percentage (no.) of isolates tested for combined resistance (multiresistance) ^{a)}	90 (4039)	88 (4071)	98 (4763)
Total number of blood isolates	4495	4614	4841

^{a)} Tested 3rd generation cephalosporins were ceftazidime, ceftriaxone, cefpodoxime and cefotaxime.

isolates from primary health care for resistance to ampicillin, mecillinam and 3rd generation cephalosporins; seven DCM routinely tested for sulfonamide resistance; five DCM routinely tested for cefuroxime resistance; four DCM routinely tested for resistance to gentamicin and carbapenem and three DCM routinely tested for piperacillin/tazobactam resistance. Overall, the proportion of isolates tested for the presented antibiotics varied from 31% (piperacillin/tazobactam) to 100% (ampicillin and mecillinam). In Denmark many general practitioners perform urine culturing and thus only submit urine samples for culturing at the local DCM in case of e.g. treatment failure or difficulties in interpretation of their locally acquired results from resistance testing. Thus, besides the selection of samples that are tested for the different antibiotics, a selection in the samples submitted to the DCM for culturing occurs.

From 2007 to 2016, a significant increase in the proportion of ciprofloxacin resistant *E. coli* isolates was observed in urine cultures from primary health care (Table 8.1.1). As for resistance levels in urine cultures from hospitalised patients, this increase happened primarily from 2007 to 2009 and resistance levels have remained stable since 2009 (Figure 8.1.3). This coincides with a decrease in the consumption of fluoroquinolones, which has been observed since 2010 (DANMAP 2016, chapter 5). Since 2007 and 2009, respectively, the level of resistance to gentamicin and 3rd generation cephalosporins did not change.

In 2015, four carbapenem resistant and nine intermediary resistant *E. coli* isolates from primary health care were reported. In 2016, there were two carbapenem resistant and seven intermediary resistant *E. coli* isolates. As mentioned earlier, the actual number of resistant isolates may have been higher, as routine carbapenem resistance testing on *E. coli* urine isolates from primary health care was only performed at four DCM in 2016.

Even though a significant decrease in comparison with 2007 was seen, the proportion of resistance to ampicillin and sulfonamide remained continuously high, with approximately a third of all isolates being resistant towards these two antibiotics. Since 2007, a significant increase in the proportion of mecillinam resistant isolates has been observed (moving from 3.9 to 5.6%) (Table 8.1.1). This may also be due to the present recom-

mendations to use mecillinam as a first choice antibiotic in the treatment of urinary tract infections. A discrete but significant decrease (from 4.0 to 3.5%) in the proportion of piperacillin/tazobactam resistance was observed from 2015 to 2016.

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8.2 *Klebsiella pneumoniae*

Klebsiella pneumoniae (*K. pneumoniae*) is part of the gastrointestinal flora in humans. It may cause infections such as urinary tract infections, pneumonia, and blood stream infections.

As for 2015, DANMAP 2016 includes resistance data for *K. pneumoniae* from all 11 Departments of Clinical Microbiology (DCM) in Denmark, thus representing 100% of the Danish population.

8.2.1 Blood isolates from hospital patients

For 2016, DANMAP received resistance results from 1,156 tested blood isolates of *K. pneumoniae*. In total 94%-100% of isolates were tested for the presented antibiotics. Nine of the 11 DCM routinely (>75% of isolates) tested for resistance to all the presented antibiotics (Table 8.2.1). Two of the DCM routinely tested for all the presented antibiotics except mecillinam. As in the previous years, resistance testing was mainly performed by disc diffusion. The presented data consists of the reported interpretation results, made by the DCM and based on the S-I-R system.

Resistance in *K. pneumoniae* from bloodstream infections has been included in DANMAP since 2008. As for many other species the number of bacteremias with *K. pneumoniae* has shown continuous moderate increases in all years, but an unexpected steep increase from 922 reported isolates in 2015 to 1,156 in 2016 (Figure 8.2.1). The cause of this is unknown. A similar steep increase from 2015 to 2016 was not observed for *E. coli* (Figure 8.1.1) or for *S. aureus* (p. 106) and thus cannot be explained by demographic changes or changes in hospital workflow.

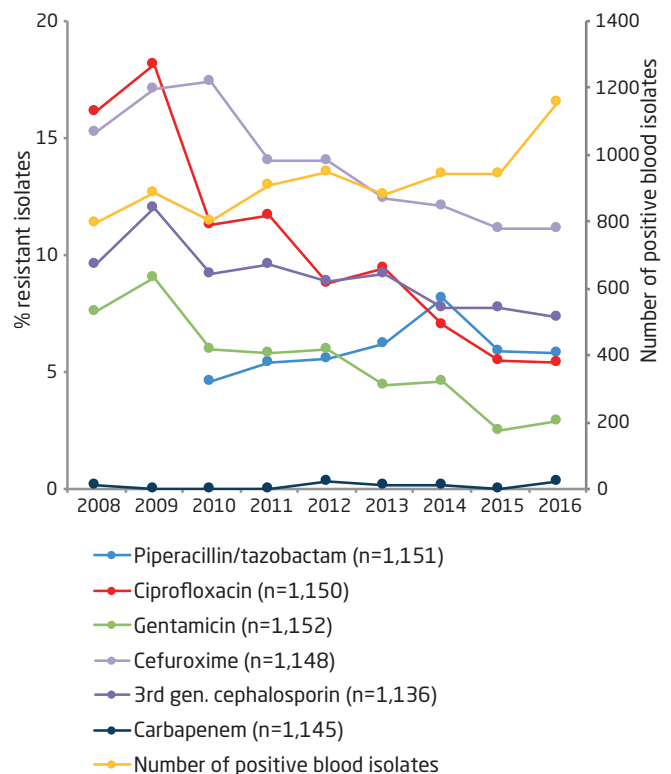
Resistance levels in 2016 were similar to the reported resistance levels in 2015 for all antibiotic classes. Overall the levels of resistance have decreased significantly since 2008 (Figure 8.2.1, Table 8.2.1). Resistance to ciprofloxacin, gentamicin, and 3rd generation cephalosporins increased from 2008 to 2009 and have decreased thereafter to levels below those of 2008. Except for 3rd generation cephalosporins, this decrease was significant (Table 8.2.1). A slight increase in the proportion of piperacillin/tazobactam resistant isolates from 4.6% to 5.8% was observed since 2010. Significant variations between resistance proportions reported from the different DCM were noticed for cefuroxime (spread 0.0%-20%) and piperacillin/tazobactam (spread 1.1%-17%). As for *E. coli* these data are based on the interpretations from the individual DCM. We

expect that especially the interdepartmental variance in piperacillin/tazobactam resistance would be smaller, if a standardized interpretation method was to be used.

The proportion of carbapenem (including meropenem) resistant isolates is continuously very low, with only three resistant and one intermediary resistant isolates in 2016. In 2015, no carbapenem resistant and two intermediary resistant *K. pneumoniae* blood isolates were reported (DANMAP 2015). The proportions of isolates with combined resistance towards 3rd generation cephalosporins, ciprofloxacin, and gentamicin (here after referred to as multi-resistant isolates) have only been reported systematically for at least 75% of blood isolates since 2014. Luckily, the proportions of multiresistant isolates are still relatively low with only 1.6% resistant isolates in 2016 (Table 8.2.2). The preferred antibiotic for treating patients with serious infections caused by multiresistant *K. pneumoniae* is meropenem. Thus, when bearing in mind the potential risk of spread of plasmids that contain genes encoding for carbapenemases in the near future, even 1.6% multi-resistant isolates is worrisome. In case of infection with a combined multi-resistant and meropenem resistant isolate, drugs such as colistin, fosfomycin, and tigecycline can be used for treating serious infections. No systematic surveillance of resistance towards these antibiotics is performed.

Figure 8.2.1 Resistance (%) in *Klebsiella pneumoniae* blood isolates from humans, Denmark

DANMAP 2016



Note: The number (n) in parentheses represents the number of isolates tested for susceptibility in 2016.

Two of the 11 DCM reported results from mecillinam resistance testing on 13% and 26% of their isolates, respectively. All other DCM reported mecillinam resistance results in 91% of isolates, with seven DCM reporting mecillinam resistance results on 98%–100% of their isolates. In total, 8.6% of blood isolates were reported resistant to mecillinam, varying from 2.9% to 31%. However, as with piperacillin/tazobactam we suspect that local interpretation of zone diameters is the cause of these variations. To lower the use of cefuroxime and piperacillin/tazobactam, some DCM recommend treating *K. pneumoniae* blood stream infections with mecillinam, if the isolates are susceptible according to local interpretations.

8.2.2 Urine isolates from hospital patients

For 2016, DANMAP received resistance results from altogether 7467 *K. pneumoniae* isolates cultured in urine samples from hospitalised patients. Results were submitted from all 11 DCM in Denmark. All 11 DCM routinely tested for gentamicin resistance; ten DCM routinely tested for resistance to mecillinam, ciprofloxacin, cefuroxime and 3rd generation cephalosporins; nine DCM routinely tested for resistance to piperacillin/tazobactam and six DCM routinely tested for resistance to sulfonamide.

Overall a decrease in the resistance levels was seen for all the tested antibiotics (Figure 8.2.2). As in blood isolates, a significant decrease in the proportion of ciprofloxacin resistant isolates was seen, from 17% resistant isolates in 2009 to only 6.1% in 2016 (Table 8.2.1). Significant decreases in the proportions of gentamicin and cefuroxime resistance since 2010 (from 7.3% to 3.2% and from 13% to 9.1%, respectively) and in the proportions of 3rd generation cephalosporin resistance since 2009 (from 13% to 6.8%) were also observed.

Table 8.2.2 Combined resistance to 3rd generation cephalosporins, ciprofloxacin, and gentamicin (multiresistance) in *Klebsiella pneumoniae* blood isolates from humans, Denmark

	DANMAP 2016		
	2014 % (N)	2015 % (N)	2016 % (N)
Resistance	3 (26)	1 (9)	2 (18)
Percentage (no.) of isolates tested for combined resistance (multiresistance) ^{a)}	91 (859)	89 (840)	98 (1131)
Total number of blood isolates	943	943	1156

^{a)} Tested 3rd generation cephalosporins were ceftazidime, ceftriaxone, cefpodoxime and cefotaxime.

Four carbapenem resistant isolates and seven intermediary resistant isolates were reported in 2015. In 2016, four carbapenem resistant isolates and six carbapenem intermediary resistant isolates were reported. As not all DCM routinely tested for carbapenem resistance on *K. pneumoniae* urine isolates from hospitalized patients, the carbapenem resistance data is based on a selected population.

In contrast to the previous year (DANMAP 2015), a significant decrease was seen in the level of mecillinam resistance, from 10% mecillinam resistant isolates in 2015 to 7.7% resistant isolates in 2016 (Table 8.2.1). A steep increase in the level of sulfonamide resistance was observed in 2011 (from 27% in 2009 to 33% in 2011) but since then, the level has decreased to the present 17% resistant isolates (Figure 8.2.2).

Table 8.2.1 Resistance (%) in *Klebsiella pneumoniae* isolates from humans, Denmark

DANMAP 2016

Substance	Blood isolates, hospitals	Urine isolates, hospitals	Urine isolates, primary health care
	%	%	%
Mecillinam	9	8 [#] °	9°
Piperacillin/tazobactam	6	6	5
Sulfonamide		17°	19°
Gentamicin	3 [§]	3	2
Ciprofloxacin	5 [§]	6°	6°
Cefuroxime	11 [§]	9	6
3rd generation cephalosporins a)	7	7°	5°
Carbapenem	<1	<1	0
Max. number of isolates tested for resistance to the presented antibiotics	1152	7142	7358

For all isolates, statistical comparison of proportions of resistant isolates between 2015 and 2016 was calculated for all antibiotics. On blood isolates, statistical comparison of proportions of resistant isolates between 2008 and 2016 was calculated for ciprofloxacin, gentamicin, cefuroxime, 3rd generation cephalosporins and carbapenem. For urine isolates, statistical comparison of proportions of resistant isolates between 2009 and 2016 was calculated for for sulfonamide, mecillinam, ciprofloxacin, 3rd generation cephalosporins and carbapenem.

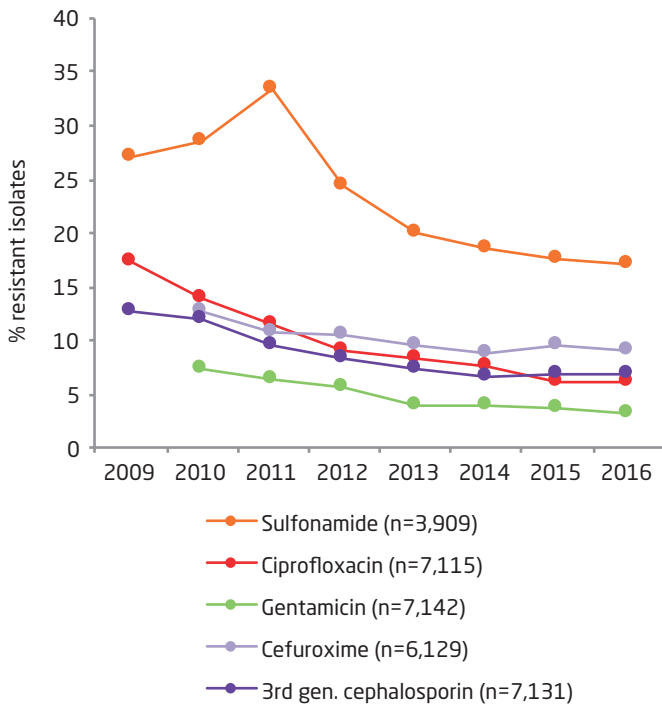
Indicates a significant decrease from 2015 to 2016.

§ Indicates a significant decrease from 2008 to 2016.

° Indicates a significant decrease from 2009 to 2016.

a) Tested 3rd generation cephalosporins were ceftazidime, ceftriaxone, cefpodoxime and cefotaxime.

Figure 8.2.2 Resistance (%) in *Klebsiella pneumoniae* urine isolates from humans in hospitals, Denmark DANMAP 2016



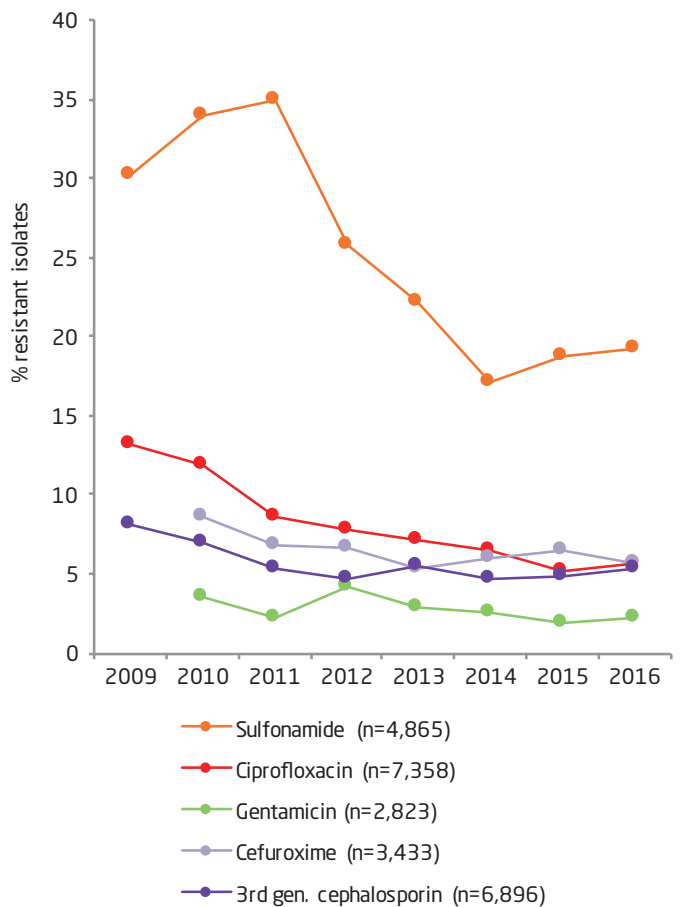
Note: The number (n) in parentheses represents the number of isolates tested for susceptibility in 2016

8.2.3 Urine isolates from primary health care

For 2016, DANMAP received results from antibiotic resistance testing on 7615 *K. pneumoniae* isolates cultured in urine samples from primary health care. Results were only submitted from 10 DCM, as one hospital did not receive samples from primary health care. All 10 DCM routinely tested for ciprofloxacin resistance, nine DCM routinely tested for resistance to mecillinam and 3rd generation cephalosporins, seven DCM routinely tested for sulfonamide resistance, four DCM routinely tested for gentamicin and cefuroxime resistance, three DCM routinely tested for carbapenem resistance and two DCM routinely tested for piperacillin/tazobactam resistance.

The changes in the antibiotic resistance pattern of *K. pneumoniae* that were isolated from blood, hospital urine samples, and urine samples from the primary health care were similar throughout the years. Since 2009, a significant decrease in the resistance levels of ciprofloxacin and 3rd generation cephalosporins was observed, from 13% and 8.1% in 2009 to 5.6% and 5.4% in 2016, respectively (Table 8.2.1 and Figure 8.2.3). Likewise, a significant decrease in the proportions of cefuroxime and gentamicin was observed from 8.6% and 3.5% in 2010 to 5.6% and 2.2% in 2016, respectively (Table 8.2.1, trend not shown).

Figure 8.2.3 Resistance (%) in *Klebsiella pneumoniae* urine isolates from humans in primary health care, Denmark DANMAP 2016



Note: The number (n) in parentheses represents the number of isolates tested for susceptibility in 2016

In 2015, one carbapenem resistant and three intermediary resistant *K. pneumoniae* isolates from primary healthcare urine samples were reported. In 2016, no *K. pneumoniae* isolates were reported resistant or intermediary resistant to carbapenem. As in 2015, the tested population was selected as only three DCM routinely tested for carbapenem resistance.

After a steep increase in the proportion of sulfonamide resistance observed from 30% in 2009 to 35% in 2011, the proportion decreased to 17% in 2014 (Figure 8.2.3). The proportion of sulfonamide resistance has increased slightly since then to the present level of 19% in 2016. The level of both sulfonamide and mecillinam resistance was significantly lower in 2016 than in 2009. All DCM routinely tested for sulfonamide resistance in 2009.

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Textbox 8.1

Characterization of ESBL/pAmpC- and carbapenemase producing *Escherichia coli* from bloodstream infections, 2016 Denmark

Background: The prevalence of third-generation cephalosporin-resistant *Escherichia coli* (3GC-R *Ec*) is still increasing in Europe [EARS-Net report, 2015]. The resistance mechanisms detected for third-generation cephalosporin-resistance in *E. coli* are the production of extended-spectrum beta-lactamases (ESBLs), plasmid-mediated AmpC (pAmpCs) and hyperproduction of the chromosomal AmpC gene due to mutations within the promoter/attenuator region.

The occurrence of 3GC-R among *E. coli* isolated from bloodstream infections in Danish patients was low before 2007, but has since increased, most notably from 2007 to 2011.

The pandemic clone O25b-ST131 is in part responsible for the worldwide spread of ESBL-producing *E. coli*. This clone is strongly associated with the presence of antimicrobial resistance genes, particular CTX-M-15, but other ESBL enzymes and virulence factors as well.

The aim of the present study was to characterize ESBL, pAmpC and carbapenemase-producing *E. coli* from bloodstream infections according to resistance genes and Multilocus Sequence Types (MLSTs).

Material and Methods: During January 2016 through December 2016, all Danish departments of clinical microbiology collected their 3GC-R *Ec* (reported as ceftazidime, ceftriaxone, cefpodoxime or cefotaxime resistance) from bloodstream infections. Furthermore, carbapenemase producing *E. coli* from bloodstream infections were included in the study. The isolates were sent to Statens Serum Institut for further characterization. Only one isolate per patient per year was included in the study.

Since 2014, whole genome sequencing (WGS) and *in silico* bioinformatics analysis have been used for characterization of the genetic background of the ESBL and AmpC phenotypes.

The Bacterial Analysis Pipeline - Batch upload version 1.0 from Center of Genomic Epidemiology (<https://cge.cbs.dtu.dk/services/cge/>) was used for *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/services/MyDbFinder-1.2/>).

Results: Whole genome sequence data were obtained from 317 *E. coli* isolates, compared to 294 in 2015 and 261 in 2014. Genes encoding ESBL, pAmpCs and/or carbapenemase production were detected in 312 isolates in 2016, compared to 275 in 2015. Five isolates were hyperproducers of chromosomal AmpC only; these isolates were not investigated further.

Isolates with ESBL, pAmpC- and carbapenemase production were observed in all the five Danish regions (Table 1).

Demographic data was available for all 312 *E. coli* isolates; 166 (53%) of the patients were men and 146 (47%) were woman.

The average age at diagnosis was 70 years, ranging from below one to 98 years.

Among the 312 isolates, 25 different ESBL, pAmpC and carbapenemase-enzymes were detected (Table 2), including two novel CTX-M- and one novel CMY-variant (CTX-M-127, CTX-M-203 and CMY-154). CTX-M-15 dominated (50%) followed by CTX-M-27, CTX-M-14 and CTX-M-101 (Table 2). Different plasmid-mediated AmpC producers (CMY- and DHA-variants) were detected in 18 isolates (6%). Only one isolate carried a carbapenemase producing gene, OXA-181 together with CTX-M-15 and CMY-2. In several of the *E. coli* isolates more than one gene encoding ESBL/pAmpC and/or carbapenemases were detected (Table 2). Plasmid-mediated colistin resistance genes (*mcr-1*, *mcr-2*, *mcr-3* and *mcr-4*), were not observed in 2016.

In 2016, the 312 *E. coli* isolates belonged to 43 different MLSTs. ST131 was still the most common sequence type (ST), with 177 (57%) of the isolates belonging to this type. Other frequent sequence types were ST38 (7%), ST69 (5%), ST12 (4%), ST1193 (3%), ST405 (2%), and ST410 (2%), whereas the remaining isolates belonged to STs, which only were detected in 1-5 isolates (<1-2% per type) (Table 3).

Among the 177 *E. coli* isolates belonging to ST131, CTX-M-15 (55%) was most common, followed by CTX-M-27 (20%), CTX-M-14 (11%) and CTX-M-101 (8%). The carbapenemase producing OXA-181, CTX-M-15 and CMY-2 isolate belonged to ST410.

Conclusion: From 2015 to 2016, the reported cases of 3GC-R *Ec* increased 13% from 275 to 312 (out of 294 and 317 referred suspected resistant strains, respectively). Comparing the distribution of ESBL/pAmpC, carbapenemases and MLSTs, from the results reported in DANMAP 2015 to 2016, no remarkable changes were observed. As in previous Danish studies of ESBL-producing *E. coli* from bloodstream infections, most of the isolates produced a CTX-M enzyme, with CTX-M-15 being the most predominant enzyme [DANMAP 2009, DANMAP 2011, Roer *et al.* JAC. 2016, Hansen *et al.* Microb. Drug Res. 2014]. Additionally, three novel ESBL-encoding genes were observed; CTX-M-127 (3/312), CTX-M-203 (1/312) and CMY-154 (1/312). Only a minor part (13/312) of the isolates carried CMY-variants.

As observed in the studies by Roer *et al.* and by Hansen *et al.* the worldwide disseminated ST131 clone carrying CTX-M-15 was strongly represented in this study, however other international STs (e.g., ST38, ST405, ST410, ST69 and ST648) related to spread of ESBLs were observed among the Danish *E. coli* isolates.

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Table 1. Distribution of ESBL, pAmpC and Carbapenemase producing *E. coli* from bloodstream infections, Denmark 2016 DANMAP 2016

Region	2014	2015	2016
	Numbers	Numbers	Numbers
The Capital Region of Denmark	110	116	111
The Zealand Region	27	14	36
Region of Southern Denmark	43	45	67
Central Denmark Region	43	59	66
North Denmark Region	22	41	32
Total Numbers	245	275	312

Table 2. Most common ESBL/pAmpC enzymes and carbapenemases detected in *E. coli* from bloodstream infections, Denmark 2016 DANMAP 2016

Enzyme	DANMAP 2014		DANMAP 2015		DANMAP 2016	
	Number	%	Number	%	Number	%
CTX-M-15 ¹	121	49	139	51	157	50
CTX-M-27 ¹	25	10	33	12	44	14
CTX-M-14 ¹	38	16	33	12	40	13
CTX-M-101	12	5	15	5	14	4
CMY-2 ¹	10	4	6	2	10	3
CTX-M-14b	5	2	5	2	9	3
CTX-M-1	10	4	7	3	8	3
CTX-M-3 ¹	4	2	4	1	7	2
CTX-M-55 ¹	8	3	14	5	6	2
Other CMY variants ¹	4	2	10	4	3	1
Other ESBL enzymes	14	6	16	6	27	9
OXA-48-group ¹	3	1	3	1	1	0

¹In some isolates more than one enzyme was detected in 2016

Table 3. Distribution of MLSTs in *E. coli* from bloodstream infections DANMAP 2016

MLST	DANMAP 2014		DANMAP 2015		DANMAP 2016	
	Numbers	%	Numbers	%	Numbers	%
ST131	124	51	135	49	177	57
ST38	18	7	23	8	21	7
ST405	13	5	12	4	7	2
ST410	4	2	11	4	6	2
ST69	10	4	10	4	16	5
ST648	7	3	10	4	5	2
ST12	5	2	9	3	14	4
ST354	1	< 1	4	1	5	2
ST95	5	2	3	1	5	2
ST1193	2	1	5	2	10	3
Other STs ¹	56	23	53	19	46	15

¹ less than 5 isolates per ST in 2016

8.3 *Pseudomonas aeruginosa*

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

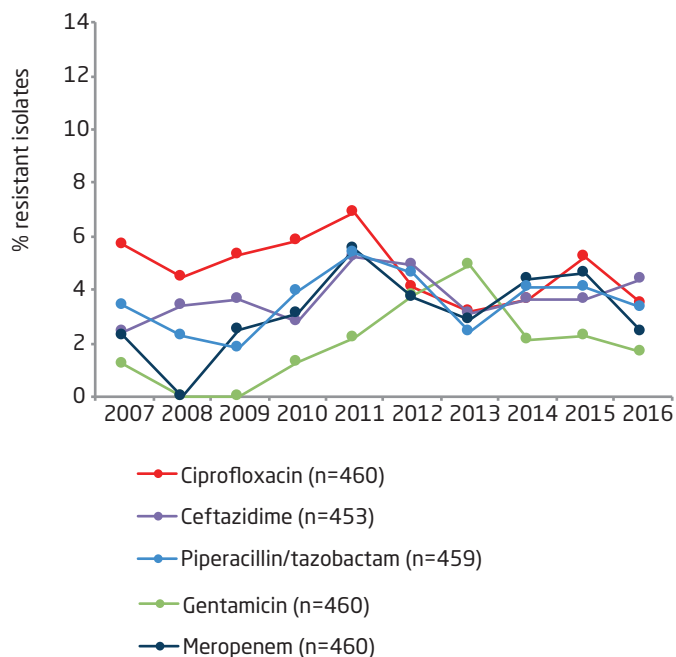
8.3.1 *P. aeruginosa* blood isolates obtained from hospitalised patients

For *P. aeruginosa*, DANMAP 2016 includes data from 11 out of 11 Departments of Clinical Microbiology (DCM), covering the total Danish population. DANMAP received data of 460 *P. aeruginosa* isolates from blood. Resistance levels to all the tested antimicrobial agents was not significantly different from the levels in 2015 (Figure 8.3.1). EARS-Net 2015 reported an increasing resistance rate for the EU/EEA population-weighted mean for piperacillin/tazobactam (18.1% in 2015) and decreasing rates for the EU/EEA population-weighted mean for fluoroquinolones (19.3% in 2015) as for aminoglycosides (13.3% in 2015). Denmark stayed below 5% resistance rates in invasive *P. aeruginosa* isolates in 2016.

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Figure 8.3.1. Resistance (%) in *Pseudomonas aeruginosa* blood isolates from humans, Denmark DANMAP 2016



Note: The number (n) in parentheses represents the number of isolates tested for susceptibility in 2016

8.4 *Acinetobacter* spp.

The genus *Acinetobacter* includes several species and is found widespread in nature, where the different species are present in soil, water and/or animals and humans. In humans *Acinetobacter* can colonize the skin and wounds but may also be the cause of hospital-acquired infections like central line-associated bloodstream infections, nosocomial pneumonia, urinary tract infections and wound infections. Many of the different subspecies are phenotypically alike, and thus identification at species level can be difficult. Spp. belonging to the *A. baumannii* group are considered being the most clinically important. *Acinetobacter* spp. possess an inherent resistance to a broad range of antibiotics because of a low membrane permeability and constitutive expression of efflux systems. The antimicrobial classes which can be used for treatment include some fluoroquinolones, aminoglycosides, carbapenems and colistin. Especially for *A. baumannii* multiresistant clones are widespread. Of worldwide concern are severely war-wounded soldiers infected with multiresistant *A. baumannii*.

Resistance in invasive *Acinetobacter* spp. in Denmark has been monitored and reported to EARS-Net since 2012, but 2016 is the first year where these data are presented in the DANMAP-report.

8.4.1 *Acinetobacter* blood isolates obtained from hospitalised patients

During 2012 to 2016 between 69 and 83 *Acinetobacter* blood isolates were reported yearly. From 2012 to 2014 about 95% of the Danish population were covered in the reporting and since 2015 the total Danish population has been covered.

Most DCM reported on resistance testing to ciprofloxacin and gentamycin throughout the whole period and in 2015 and 2016 most also reported on meropenem resistance. Data are presented in table 8.4.1 and in figure 8.4.1.

In 2012, significantly more *Acinetobacter* blood isolates were resistant towards ciprofloxacin, gentamycin and meropenem (resistance rates 10-12%) than in 2016 (resistance rates 0-3%). For the period in-between no significant changes were observed. Resistance rates varied from 3% to 7% for ciprofloxacin and 0 to 4.4% for gentamycin and meropenem. In EARS-

Net markedly differences in resistance profiles across Europe have been reported with especially high resistance rates reported from the Baltic, southern and south-eastern countries in Europe. For example, in 2015 87% of invasive *Acinetobacter* spp. in Croatia were reported with combined resistance to fluoroquinolones, aminoglycosides and carbapenems, while Sweden reported 3.8% combined resistance.

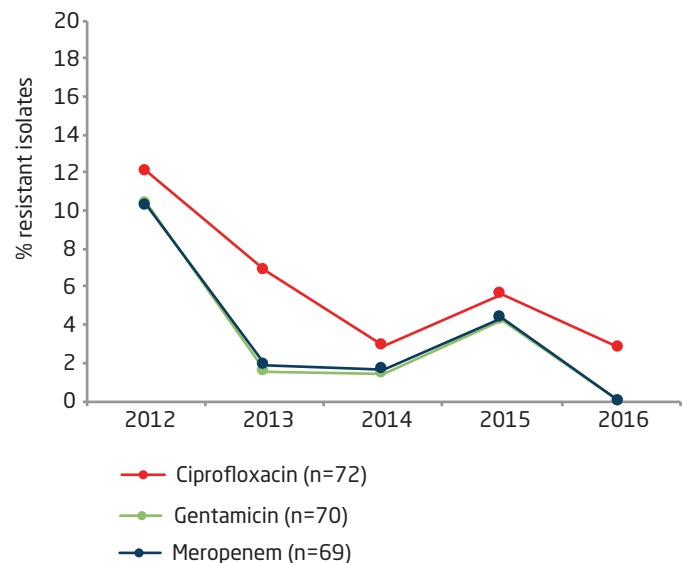
In the Danish invasive *Acinetobacter* spp. from 2016 approximately half of the 72 reported isolates were reported as *Acinetobacter* spp., while the other half consisted of *A. baumannii*, *A. haemolyticus*, *A. johnsonii*, *A. junii* and *A. Iwoffii*. None of the isolates were resistant to meropenem. In contrast, as described in textbox 8.2, 26 carbapenemase-producing *A. baumannii* isolates from all specimen types were detected in Denmark in 2016, which was an increase from 19 detected isolates in 2015. One of these referred 2016 isolates was invasive.

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Figure 8.4.1 Resistance (%) in *Acinetobacter* spp. Blood isolates from humans, Denmark

DANMAP 2016



Note: The number (n) in parentheses represents the number of isolates tested for susceptibility in 2016

Table 8.4.1 Invasive *Acinetobacter* spp, Denmark 2012 - 2016. Number of resistant isolates per year per antibiotic and number of tested isolates per year per antibiotic

DANMAP 2016

Substance	2012		2013		2014		2015		2016	
	Resistant	Tested	Resistant	Tested	Resistant	Tested	Resistant	Tested	Resistant	Tested
Ciprofloxacin	10	83	5	72	2	69	4	71	2	72
Gentamicin	8	77	1	65	1	70	3	71	0	70
Meropenem	6	58	1	52	1	62	3	68	0	69
Max. Number of isolates tested	83		72		69		71		72	

Textbox 8.2

Carbapenemase producing bacteria in Denmark, 2016

Background: Carbapenems comprise one of the only classes of antimicrobial agents that can be used for treatment of infections with multi-resistant Gram-negatives like *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. Treatment options for infections with carbapenem resistant bacteria are often none or suboptimal. Resistance can be caused by the presence of various carbapenemases of which the most frequently occurring are *K. pneumoniae* carbapenemase (KPC), Oxacillinase (OXA), Verona integron-encoded metallo- β -lactamase (VIM), New Delhi metallo- β -lactamase (NDM), and Imipenemase (IMP).

In recent years, Danish Departments of Clinical Microbiology (DCM) have on a voluntary basis submitted carbapenem resistant isolates for verification and genotyping at Statens Serum Institut. The present textbox describes carbapenemase-producing *Enterobacteriaceae* (CPE), carbapenemase-producing *P. aeruginosa* and carbapenemase-producing *Acinetobacter* spp.

During 2016, 115 carbapenemase producing organisms (CPO) were detected from 99 patients. More than one isolate from the same patient were included, if the isolates belonged to different bacterial species and/or if the isolates harboured different carbapenemases. Among the 115 CPO, 94 were from clinical samples and 21 were from screening samples. Nine of the CPOs were from bloodstream infections (eight CPE, and one *A. baumannii*) compared with eight CPO in 2015.

Enterobacteriaceae: In 2016, 82 CPE from 72 patients were detected compared to 63 CPE in 2015 (Figure 1). In 2016, 16 of the patients had been travelling abroad prior to detection of the CPE, 28 of the patient did not report any travel and for the remaining 28 patients no travel information were obtained (Figure 2). Since 2012, an increasing number of cases have been related to spread between patients in Denmark.

Seven isolates of 82 CPE isolates produced both NDM and OXA-48 group enzymes, 44 produced OXA-48-like enzymes and 27 were NDM-producing (Figure 1). Furthermore, two KPC-producing isolates and two VIM-producing isolates were detected.

The NDM-1 producing *C. freundii* outbreak, which started in 2012 in the North Denmark Region, continued in 2016 (Table 1). Until the end of 2016, 14 patients were involved in this outbreak. None of these patients had a prior history of travel noted in their hospital records. The origin of the NDM-1 producing *C. freundii* was unknown. NDM-1 plasmid transfer was also detected to other CPEs with the patients involved in the outbreak [Hammerum *et al.* 2016, J. Antimicrob. Agents, 71:3117-3124].

Besides the NDM-1 *C. freundii* outbreak, possible clonal spread of CPE were detected for NDM-1 producing *K. pneumoniae*, KPC-3 producing *K. pneumoniae*, OXA-181 producing *K. pneumoniae*, NDM-5/OXA-181 *E. coli* and OXA-48 *E. coli* during 2016 (Table 1). In all cases, the CPE isolates had highly similar SNP-profiles, indicating a possible spread between the patients or a common origin. Furthermore, it seems very likely that the increase in OXA-48 producing CPE was due to plasmid transfer, but this was not investigated further.

Table 1. Possible clonal spread of carbapenemase producing *Enterobacteriaceae* (CPE) during 2016, Denmark DANMAP 2016

Year	No. of Patients	Carbapenemase	Species	Region
2012-16	14	NDM-1	ST18 <i>C. freundii</i>	North Denmark Region
2015-2016	4	NDM-1	ST15 <i>K. pneumoniae</i>	Region Zealand, Region of Southern Denmark
2015-2016	2	KPC-3	ST101 <i>K. pneumoniae</i>	Region Zealand
2015-2016	4	OXA-181	ST571 <i>K. pneumoniae</i>	The Capital Region of Denmark
2016	6	NDM-5/OXA-181	ST410 <i>E. coli</i>	The Capital Region of Denmark/Region Zealand
2016	3	OXA-48	ST354 <i>E. coli</i>	Region Zealand /The Capital Region of Denmark

Acinetobacter spp: In 2016, 26 carbapenemase producing *A. baumannii* isolates were detected compared to 19 isolates in 2015. In 2016, 23 OXA-23 producing *A. baumannii* isolates were detected. Furthermore, two OXA-72 producing *A. baumannii* and one OXA-58 producing *A. baumannii* were detected.

P. aeruginosa: In 2016, five VIM-2 producing *P. aeruginosa*, one IMP-7 *P. aeruginosa* and one NDM-1 producing *P. aeruginosa* were detected.

Conclusion: The occurrence of carbapenemase producing bacteria in Denmark is still increasing, a trend worrisome to patients and clinicians. Especially the spread of CPE is of concern, since *Enterobacteriaceae* can be carried in the intestine for a long time without any symptoms of infections, which makes outbreak control difficult.

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Figure 1. Numbers of carbapenemase-producing *Enterobacteriaceae* (CPE)

DANMAP 2016

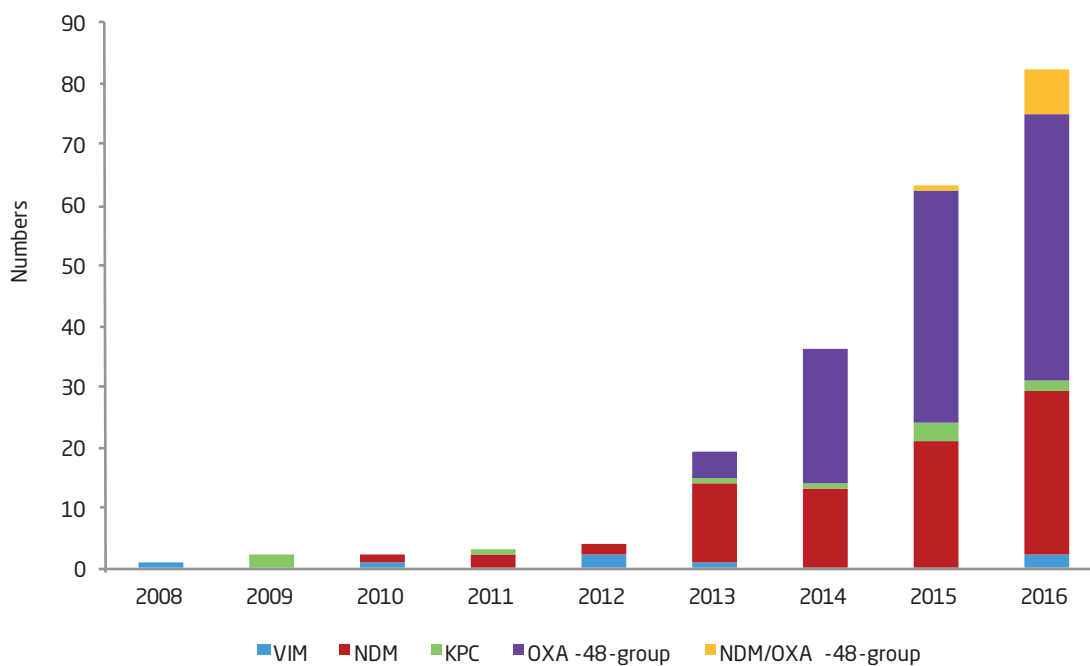
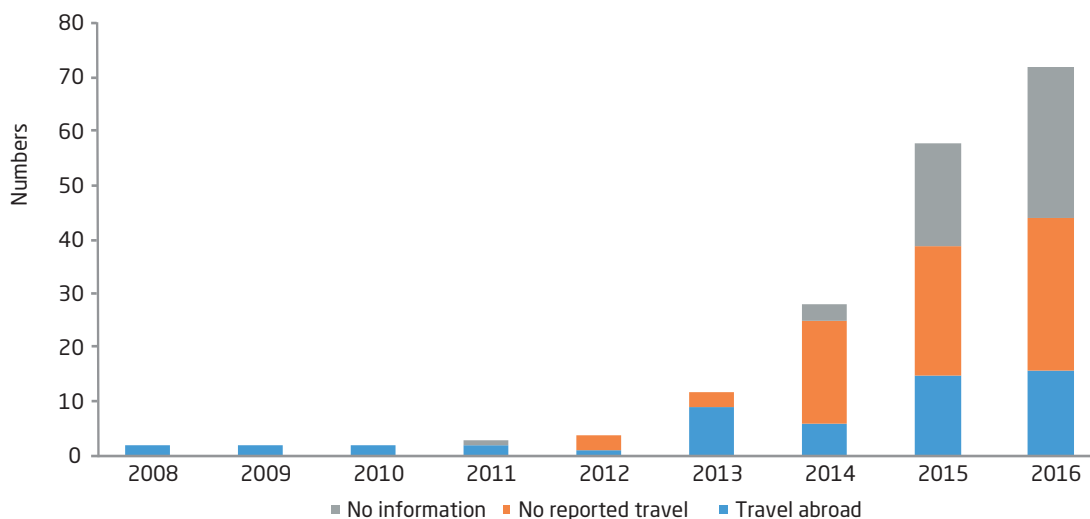


Figure 2. Travel information for patients with carbapenemase-producing *Enterobacteriaceae* (CPE) during 2008-2016

DANMAP 2016



8.5 Enterococci

Enterococci constitute a part of the normal intestinal microbiota in humans and animals and colonise the host in beneficial co-existence. More than 54 species belonging to the genus enterococcus have been described, but the majority of the human infections are caused by *E. faecalis* and *E. faecium*, with considerable differences in susceptibility to the different antimicrobial agents.

Most common clinical infections caused by *Enterococcus* species include: urinary tract infections, bacteraemia and bacterial endocarditis (an inflammation process of the inner tissues of the heart, usually of the valves). As an opportunistic pathogen it causes severe illness in the immunocompromised host. Found in hospital environment, bacteria can lead to colonization or infection of a hospitalized patient. The source of hospital infection is often associated with use of medical supplies, such as catheters, as well as other instruments and medical devices.

Therapy of enterococcal infection is complicated and has limited variations due to a high level of natural antimicrobial resistance. In severe cases enterococcal infections are treated with vancomycin. Combinational therapy based on a synergistic effect of beta-lactam antibiotic (penicillin/ampicillin) with aminoglycoside (gentamicin) or glycopeptide (vancomycin) is required in cases of endocarditis. More recent antibiotics, such as linezolid (oxazolidinone) and daptomycin (lipopeptide) are the only options for treatment of multiresistant pathogen, vancomycin-resistant Enterococcus (VRE), which adapt to persist in the health care facilities. A new antibacterial from the oxazolidinone-class with restrictive indications for usage was introduced to the Danish market in 2015.

All DCM performed susceptibility testing for ampicillin and vancomycin on all strains of *E. faecalis* and *E. faecium* populations isolated from blood cultures. As in previous years, only one DCM investigated all invasive *E. faecalis* and *E. faecium* isolates for occurrence of high-level resistance to gentamicin. More than half of all DCMs (6/11) performed phenotypical susceptibility testing to linezolid for all invasive *E. faecium* isolates. Furthermore, 5/11 laboratories examined all invasive *E. faecalis* isolates for susceptibility to linezolid.

In 2016, a total of 606 *E. faecalis* and 692 *E. faecium* isolates were reported from all DCM, thus representing the entire bacterial populations of invasive *E. faecium* and *E. faecalis* in Denmark.

From 2015 to 2016, the total number of invasive isolates declined slightly from 617 to 606 for *E. faecium* and from 711 to 692 for *E. faecalis*. Throughout the 15-years period the ratio of *E. faecalis* to *E. faecium* dropped markedly from 2.9 in 2002 to 0.9 in 2016. These variations equally can be explained by a significant rise of *E. faecium* infections, as well as by a decrease of *E. faecalis* infections. From 2015 to 2016

the ratio of *E. faecalis* to *E. faecium* remained unchanged (Figure 8.5.1).

Ampicillin-resistance

The national rate of ampicillin-resistance in invasive *E. faecalis* was reported to 1.6 % in 2016. Prevalence of non-susceptibility to ampicillin in *E. faecium* isolates dropped from 95.0 % in 2015 to 91.5 % in 2016, which is the lowest level verified since 2009.

Vancomycin-resistance

Prevalence of transferable vancomycin-resistance in *E. faecium* was 7.3% in 2016, this is a two-fold increase compared to 2015. Rates of resistance to vancomycin increased during the 9-years period from 0.6 % in 2007 to 3.7 % in 2015. The vancomycin-resistant *E. faecium* were part of hospital outbreaks, which is described in textbox 8.3 on VRE (p.102). The level of vancomycin-resistant *E. faecium* was above the level reported to EARS-Net 2015 from the other Nordic countries [EARS-Net 2015]. No vancomycin-resistant *E. faecalis* from bloodstream infections were reported in 2016.

High-level gentamicin resistance

The rate of high-level gentamicin resistance (> 128 mg/L) was biased, due to the small number of analysed isolates. Still in 2016, 11 out of 54 (20%) *E. faecalis* isolates showed high level resistance to gentamicin.

Linezolid-resistance in invasive isolates

In 2016, linezolid-resistance was for the first time reported to DANMAP. Among the tested 417 *E. faecium* and 310 *E. faecalis* isolates, a single linezolid-resistant isolate of each species was detected.

Linezolid-resistance in non-invasive isolates

Due to an increased use of Linezolid at the clinical departments at Rigshospitalet during the later years, since November 2015 all clinical enterococcal strains found resistant to linezolid through routine susceptibility testing with disc diffusion have been confirmed by Etest® (BioMérieux) at the local DCM. There were no findings of linezolid-resistance in invasive isolates. Among the 641 non-invasive *E. faecium* isolates 27 (4 %) were resistant to linezolid in addition to ampicillin. Nine of the 27 *E. faecium* isolates were co-resistant to vancomycin in addition to linezolid and ampicillin. Among the 1,074 non-invasive *E. faecalis* isolates fourteen (1%) were phenotypically resistant to linezolid. Only a few isolates were examined for acquired mechanisms of resistance. In one of these strains presence of transferable linezolid-resistance due to the plasmid-mediated *optrA* gene was found for the first time in Denmark [Vorobieva *et al.* 2017, J Glob Antimicrob Resist., published online]. This finding has led to an increased focus on the use of linezolid in the Hospital.

In conclusion, the two-fold increase of transferable vancomycin-resistance in *E. faecium* blood culture isolates is worrying.

A national surveillance, as well as strict infection control measures shall be continued. Due to the limited options of treatment of VRE-infections, as well as the risk of rapidly acquired resistance to oxazolidinones and lipopeptides, an introduction of a national surveillance to linezolid and daptomycin in clinical enterococcal isolates should be considered.

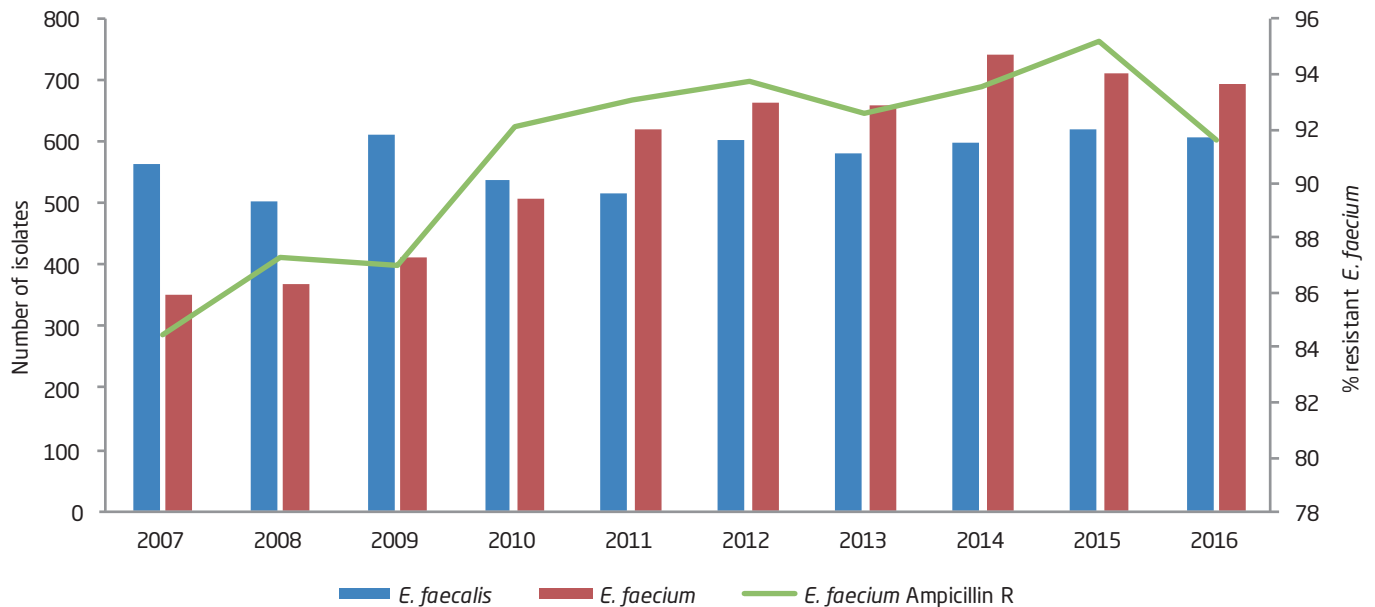
From 2007 to 2009 the presented data covers 75% of the Danish population. From 2010 to 2014 data covers 95% of the Danish population and from 2015 the total Danish population is covered.

Acknowledgements We thank Niels Frimodt-Møller and Jenny Dahl Knudsen from DCM Rigshospitalet for providing the information from the internal data analysis, regarding the rates of resistance to linezolid in non-invasive *E. faecalis* and *E. faecium* isolates obtained at the Department of Clinical Microbiology. We also want to note the kindly assistance of Ute Wolff Sönksen and Anette M. Hammerum with writing the section.

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Figure 8.5.1 Number of bacteremia cases caused by *E. faecalis* and *E. faecium* and rates of resistance to ampicillin (%) in *E. faecium* bloodstream isolates from humans in Denmark, 2007 to 2016

DANMAP 2016



Textbox 8.3

Emergence of clinical ST203-CT859 *vanA E. faecium* in Denmark, 2016

Background: *Enterococcus faecalis* and *Enterococcus faecium* are commensal bacteria in the intestine of humans. *E. faecalis* and *E. faecium* can also cause urinary tract infections (UTI) and fatal infections like sepsis and endocarditis, especially among older and/or chronically ill patients. Enterococci are intrinsically resistant to a number of first-line antimicrobial agents including cephalosporins. Therefore, therapy of enterococcal infections may be difficult. Vancomycin is an important drug for the treatment of severe enterococcal infections, but an increase in the occurrence of vancomycin-resistant enterococci (VRE) has been observed in Denmark and internationally. Many of the VRE are also resistant to ampicillin and gentamicin thus limiting the treatment possibilities. Newer antibiotics such as linezolid and daptomycin can be used for treatment of VRE, but both antimicrobial agents have many side effects.

Surveillance of VRE: Since 2005, Danish Departments of Clinical Microbiology (DCM) have on a voluntary basis, submitted VRE for species identification, genotyping and surveillance to the Antimicrobial Resistance Reference Laboratory at Statens Serum Institut.

In 2012 and 2013, an increase in clinical *vanA E. faecium* isolates were observed (Figure 1 and Figure 2). They were primarily detected at hospitals in the Capital Region, but also from the Zealand Region and the Central Denmark Region. VRE was also detected in the two remaining regions of Denmark, but to a much lower extent (Figure 2). This trend continued in 2014-2016. In 2016, 434 clinical VRE isolates were detected and 88% of these were from hospitals located in the Zealand Region and the Capital Region (Figure 2).

In 2016, 434 clinical VRE were detected compared to 371 clinical VRE in 2015. Most of the clinical VRE were *vanA E. faecium*, which increased with 15%, from 367 in 2015 to 422 in 2016 (Figure 1). Furthermore, two *vanB E. faecium* isolates were detected in 2016, and for the first time two *E. faecium* isolates with both *vanA* and *vanB* were detected. Besides this, seven *vanB E. faecalis* and one *vanA E. faecalis* were detected.

From 2015, all clinical VRE isolates have been whole-genome sequenced. From the WGS data, MLSTs were extracted *in silico*. Core genome MLST (cgMLST) analysis was performed for the vancomycin-resistant *E. faecium* isolates. The majority of the *E. faecium* isolates belonged to three sequence types, ST80, ST117 and ST203, whereas the rest of the isolates belonged to ST17, ST18, ST78, ST294, ST192, ST787 and novel STs (ST1297, ST1298, ST1300, ST1301). The STs were all part of the CC17 complex, which are commonly detected in hospitals outside of Denmark.

In 2016, cgMLST subdivided the 422 *vanA E. faecium* isolates into 42 cluster types (CTs), ST203-CT859 was most prevalent (64%), followed by ST80-CT14 (9%), ST117-CT24 (5%), ST117-CT873 (3%), ST80-CT993 (3%), ST80-CT860 (3%) and ST80-CT866 (2%).

Comparison to the cgMLST.org database, previous studies, and personal communications with neighboring countries revealed that the novel cluster ST203-CT859 emerged in December 2014, and spread to the South of Sweden and the Faroe Islands during 2015 [Hammerum *et al.* 2017]. Antimicrobial. Chemother.]. In 2015, 187 (51%) of the Danish clinical *vanA E. faecium* belonged to ST203-CT859, this increased to 268 (64%) in 2016.

vanA E. faecium isolates belonging to ST80-CT14 were also detected in the Capital Region in 2013 and 2015 (www.cgMLST.org), however the cgMLST database did not contain any non-Danish ST80-CT14 isolates.

In contrast to this, *vanA E. faecium* isolates belonging to ST117-CT24 were detected in Danish hospitals both in 2010 and in 2016, and have been reported from clinical samples from Sweden and Germany as well.

The two *vanB E. faecium* isolates belonged to ST17-CT29 and to ST80-CT880, whereas the two *vanA/vanB E. faecium* isolates belonged to a ST80-CT1064.

Conclusion: The continued increase in *vanA E. faecium* cases in 2016 in Denmark is worrying, and especially the further emergence of ST203-CT859 *vanA E. faecium* needs further investigation. VRE can be carried in the intestine for a long period without any symptoms of infection and likewise persist in the hospital environment, which makes infection control difficult but should include proper cleaning, good hand hygiene, screening for VRE and subsequent isolation of patients.

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Figure 1. Numbers of vancomycin-resistant *Enterococcus faecium* and *Enterococcus faecalis* isolates and *vanA* and *vanB* genes from clinical samples, 2005-2016 Denmark DANMAP 2016

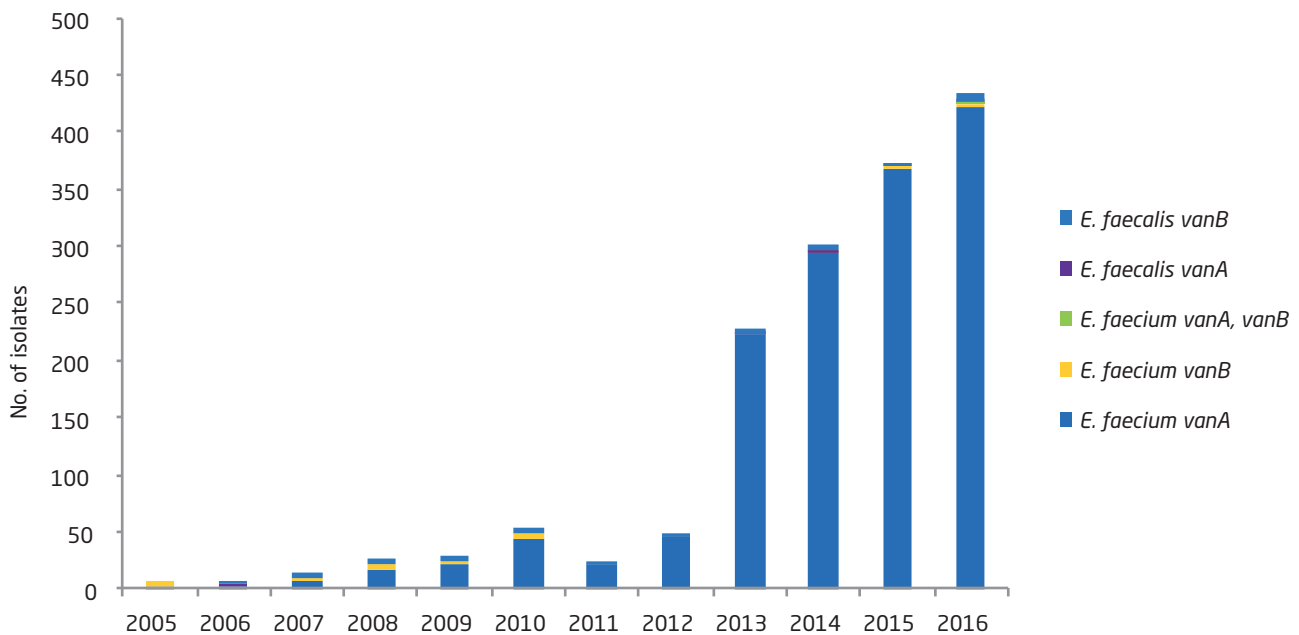
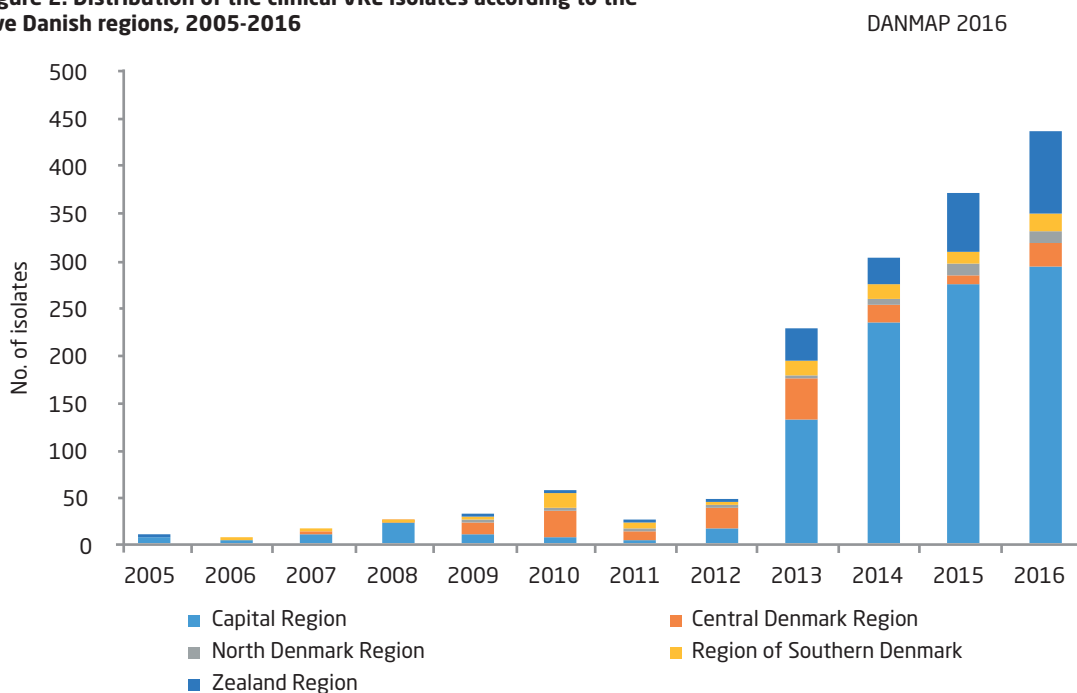


Figure 2. Distribution of the clinical VRE isolates according to the five Danish regions, 2005-2016 DANMAP 2016



8.6 Streptococci

Streptococci include *Streptococcus pneumoniae* (pneumococci), beta-haemolytic streptococci (BHS), and non-haemolytic streptococci (NHS). All streptococci are Gram-positive bacteria that belong to the human nasopharyngeal flora and may in varying degree and frequency be the cause of both common and severe infections. In the following section the surveillance of pneumococci and beta-haemolytic streptococci causing invasive disease is presented.

8.6.1: Pneumococci

Pneumococci are the most common bacterial cause of pneumonia in all age groups but are also a frequent cause of otitis-media and bacterial sinusitis in children. They may also cause severe invasive infections and are among the leading causes of bacteremia, endocarditis and meningitis.

The prevalence of asymptomatic carriage with pneumococci in the nasopharynx varies with age.

Infections with pneumococci are treated with either beta-lactam antibiotics - in Denmark the drug of choice is beta-lactamase sensitive penicillins - or macrolides.

The surveillance of pneumococci causing invasive disease in Denmark occurs through mandatory submission of clinical isolates to Statens Serum Institut (SSI), in order to enable surveillance of vaccine coverage and the prevalent type distribution. At SSI, the isolates are serotyped and tested for antimicrobial susceptibility.

Susceptibility testing of isolates from cases occurring in 2016 was performed on 714 isolates of *S. pneumoniae* found in Danish patients and from either blood (658 isolates), cerebrospinal fluid (56 isolates) or from blood where the patients also had pneumococci in the cerebrospinal fluid (17). The isolates belonged to 39 different serotypes, of which isolates belonging to 18 serotypes showed non-susceptibility (resistant or intermediary resistant) to either penicillin or erythromycin or both (62 isolates, 8.7%).

For penicillin, 44 isolates (6.2%) were non-susceptible of which 3 isolates (0.4%) were resistant. For erythromycin, 34 isolates (4.8%) were resistant (Figure 8.6.1).

For pneumococci, antibiotic susceptibility is closely connected to serotypes. Serotypes are again influenced by vaccines as well as natural fluctuation. For example, serotype 1 was dominant in the years 2009 to 2013 encompassing 17% of all of the received isolates. Serotype 1 has gradually decreased after the introduction of the PCV13 childhood vaccine in 2010, and was at just 0.8% of all of the received isolates in 2016. In contrast, the non-vaccine serotype 8 is now dominant with 21% of the received isolates in the years 2014 to 2016, while in the years 2009 to 2013 it was at 7.4%. When comparing the percentage of non-sensitive isolates between different years, the differences in serotype-distributions should therefore be kept in mind.

All of the received isolates of serotypes 7C, 14 and 9V (n=5) were non-susceptible to either erythromycin or penicillin or both. In contrast, 21 different serotypes (n=470) were always fully susceptible to both of these antibiotics. Serotypes with more than 25% non-susceptibility to erythromycin were 6B, 7C, 9V, 14, 15A, 24F and 33F. Serotypes with more than 25% non-susceptibility to penicillin were: 1, 6B, 7C, 9V, 14, 15A, 17F and 23B.

The levels of penicillin non-susceptibility in Denmark is similar to the levels reported in 2015 to EARS Net by the neighbouring countries Sweden (6.8%), Norway (5.4%) and Germany (6.2%). Correspondingly, the levels of erythromycin non-susceptibility in Denmark is similar to the levels reported in 2015 from Sweden (6.6%) but lower than the levels reported from Norway (10.7%) and Germany (8.1%).

8.6.2: Beta-haemolytic streptococci

Beta-haemolytic streptococci (BHS) can be divided into 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. The rate of asymptomatic throat carriage of GAS is approximately 2%.

Figure 8.6.1 Nonsusceptibility (%) in *Streptococcus pneumoniae* blood and spinal fluid isolates from humans, Denmark

DANMAP 2016

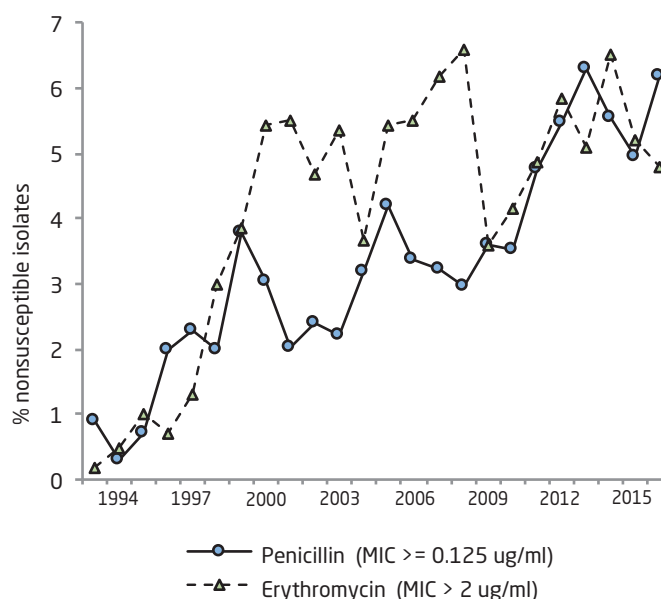


Table 8.6.1. Beta-haemolytic streptococci: Antimicrobial resistance testing results, 2013-2016

DANMAP 2016

BHS	Resistance testing results	2013	2014	2015	2016
GAS	Number of isolates	177	149	154	174
	Erythromycin resistant	5 (2.8%)	1 (0.7%)	3 (1.9%)	9 (5.2%)
	Clindamycin resistant	3 (1.7%)	1 (0.7%)	0 (0%)	1 (0.6%)
	Additionally, inducible clindamycin resistance	1 (0.6%)	0 (0%)	2 (1.3%)	7 (4.0%)
	Susceptible to penicillin, erythromycin and clindamycin	172 (97%)	147 (99%)	151 (98%)	165 (95%)
GBS	Number of isolates	113	139	140	182
	Erythromycin resistant	20 (18%)	31 (22%)	30 (21%)	35 (19%)
	Clindamycin resistant	14 (12%)	16 (11%)	14 (10%)	17 (9.3%)
	Additionally, inducible clindamycin resistance	1 (0.9%)	4 (2.9%)	7 (5.0%)	11 (6.0%)
	Susceptible to penicillin, erythromycin and clindamycin	92 (81%)	107 (77%)	111 (79%)	147 (81%)
GCS	Number of isolates	68	80	103	107
	Erythromycin resistant	3 (4.4%)	3 (3.8%)	8 (7.8%)	9 (8.4%)
	Clindamycin resistant	2 (2.9%)	3 (3.8%)	2 (1.9%)	4 (3.7%)
	Additionally, inducible clindamycin resistance	1 (1.4%)	1 (1.3%)	4 (3.9%)	6 (5.6%)
	Susceptible to penicillin, erythromycin and clindamycin	65 (96%)	76 (95%)	95 (92%)	97 (91%)
GGS	Number of isolates	145	183	183	234
	Erythromycin resistant	17 (12%)	19 (10%)	18 (9.8%)	38 (16%)
	Clindamycin resistant	6 (4.1%)	3 (1.6%)	5 (2.7%)	3 (1.3%)
	Additionally, inducible clindamycin resistance	6 (4.1%)	13 (6.9%)	9 (4.9%)	29 (12%)
	Susceptible to penicillin, erythromycin and clindamycin	128 (88%)	165 (90%)	167 (91%)	196 (84%)

Resistance findings for erythromycin and clindamycin are shown as number (%)

Streptococcus agalactiae (group B streptococci; GBS) may be present in the vaginal flora of 20-25 % of women in the child-bearing age and may therefore cause meningitis and septicaemia 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 non-duplicate invasive isolates (i.e. from blood or cerebrospinal fluid) of BHS submitted in 2016 to the Neisseria and Streptococcus Reference laboratory. Isolates are received from all DCMs in Denmark. It is voluntary to submit isolates of BHS, and not all DCMs submit BHS of all groups.

Infections with BHS are usually treated with penicillins or macrolides. All submitted isolates of BHS group A, B, C and G are therefore tested for susceptibility to penicillin, erythromycin, clindamycin as well as inducible clindamycin resistance.

Table 8.6.1 shows the resistance findings for the years 2013 through 2016. The numbers of submitted isolates of GAS, GBS, and GGS increased substantially in 2016 compared to 2015: GAS, +13%; GBS, +30%, and GGS, +28%, while the number of GCS increased by 4%. All isolates were fully susceptible

to penicillin. The erythromycin resistance rate was virtually unchanged for GBS and GCS, but had increased from 1.9% to 5.2% for GAS and from 9.8% to 16% for GGS. The clindamycin resistance rate showed only minor increases, but the percentage of strains with inducible clindamycin resistance increased for GGS and especially GAS. The percentage of fully susceptible isolates was unchanged for GBS and GCS, but decreased for GAS (from 98 % to 95 %) and in particular for GGS (from 91% to 84%).

Conclusions

The number of submitted isolates increased for all four groups of BHS, in particular for GBS (+28%) and GGS (+30%). An increase of resistance at varying levels was observed for all four groups, being most pronounced for GGS.

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8.7 *Staphylococcus aureus*

Staphylococcus aureus is part of the normal flora of the skin and mucosa in approximately 50% of humans. Some people only carry *S. aureus* intermittently whereas others carry *S. aureus* for longer time. However, in addition *S. aureus* also causes infections ranging from superficial skin infections, i.e. impetigo and boils, to more invasive infections such as post-operative wound infections, infections related to intravenous catheters and prosthetic devices, septic arthritis osteomyelitis, endocarditis and bacteremia. Some of these may have a fulminant progress and are associated with high mortality.

In Denmark, a voluntary surveillance program of all *S. aureus* bacteremia cases was established in 1957. By comparison with the numbers of bacteremia cases registered in the Danish Microbiology Database (MiBa) since 2010, the number of cases reported to SSI has been almost complete (94-97%). Laboratory and clinical notification of methicillin-resistant *S. aureus* (MRSA) has existed since November 2006. At SSI, all isolates are initially tested using a multiplex PCR detecting: the *spa*, *mecA*, *hsd*, *scn* and *pvl* gene (LukF-PV). *spa* is used as *S. aureus* specific marker and for subsequent typing by Sanger sequencing, *mecA* to determine MRSA status, and *scn* and *hsd* as markers for human adaptation and relation to CC398, respectively. PVL has been closely linked to skin abscesses and the very rare condition of severe necrotizing pneumonia. PVL is found both in methicillin-susceptible *S. aureus* (MSSA) and MRSA. Presence of PVL genes has been closely associated with community acquired (CA) MRSA strains, although not all CA-MRSA carry PVL. Isolates positive for *mecA* and the CC398 specific *hsd* fragment but negative for *scn* (human adaptive factor) and *pvl* genes are considered typical livestock MRSA (LA-MRSA) and are not *spa* typed. All others including human-adapted CC398 isolates are *spa* typed. All bacteremia cases

and *mecA* negative presumed MRSA are tested for presence of the *mecC* gene.

A representative selection of bacteremia isolates, every second received in the first half of the year (n=560), were tested for antimicrobial susceptibility against 17 antimicrobials. In addition demographic and epidemiological information is registered. Based on the epidemiological information each case is classified with respect to possible place of acquisition: hospital (HA), community (CA), healthcare-associated with a community onset (HACO), import (IMP) and livestock contact (LA) MRSA. For CA and HACO classification is separated into known and unknown exposure.

8.7.1. Surveillance of bacteraemia

In 2016, 1,981 *S. aureus* bacteremia (SAB) cases corresponding to 34.7 cases per 100,000 inhabitants were reported from the Departments of Clinical Microbiology (DCM) in Denmark. The number of cases have stabilized at this level after a steep increase from approximately 1,500 annual cases before 2014. Forty (2.1%) of the SAB cases were caused by MRSA. Thus, the MRSA frequency among SAB isolates continues to be very low compared to most other countries participating in EARS-Net [EARS-Net 2015]. Seven of the 40 MRSA cases were caused by LA-MRSA CC398 (compared to three in 2015). Four hundred and fifty two (23%) patients died within 30 days of the diagnosis of bacteremia. The mortality for the MRSA bacteremia cases was 15% (n=6). Antimicrobial resistance in SAB isolates from 2005-2016 is presented in Table 8.7.1. The highest frequency of resistance to other antimicrobials than penicillin was observed for fusidic acid (12%), erythromycin (7%), clindamycin (6%) and norfloxacin (4%). Susceptibility to the tested antimicrobial agents was lower for several antimicrobials compared to 2015, resistance to fusidic acid decreased

Table 8.7.1 Resistance (%) in isolates from *S. aureus* bacteraemia cases, Denmark

DANMAP 2016

Antimicrobial agent	2007 %	2008 %	2009 %	2010 %	2011 %	2012 %	2013 %	2014 %	2015 %	2016 %
Methicillin	0.6	1.3	1.6	1.4	1.4	1.2	1.7	2.9	1.5	2.1
Penicillin	78	77	77	75	77	74	76	77	71	71
Erythromycin	4	5	7	5	7	6	7	8	7	7
Clindamycin	3	4	6	4	6	6	6	8	7	6
Tetracycline	2	3	2	3	2	2	3	5	4	3
Fusidic acid	9	9	9	13	13	14	15	15	16	12
Rifampicin	<1	<1	<1	<1	<1	<1	0	<1	<1	<1
Norfloxacin	1	2	2	3	4	4	5	6	6	4
Kanamycin	<1	1	1	1	<1	1	2	2	3	1
Linezolid	nt	0	0	0	0	0	0	0	0	0
Mupirocin	<1	<1	<1	<1	<1	<1	<1	<1	<1	0
Trimethoprim-sulfamethoxazole	nt	nt	nt	nt	<1	1	1	1	<1	<1

Notes: nt = not tested. In chapter 9, table 9.2, the distribution of MICs and resistance for all tested antimicrobial agents are shown.

Table 8.7.2 The ten most prevalent *spa* types demonstrated in SAB and in non-CC398 MRSA cases, Denmark 2016

DANMAP 2016

SAB			MRSA			
<i>spa</i> type	CC group ^{a)}	No. of cases	<i>spa</i> type	CC group ^{a)}	No. of cases	No. causing infections (%)
t002	CC5	87	t304	CC8	223	69 (31)
t127	CC1	80	t223	CC22	212	64 (30)
t230	CC45	80	t002	CC5	188	98 (52)
t084	CC15	78	t127	CC1	135	58 (43)
t091	CC7	61	t008	CC8	105	72 (69)
t012	CC30	61	t019	CC30	96	69 (72)
t008	CC8	53	t044	CC80	84	44 (52)
t021	CC30	39	t437	CC59	46	30 (65)
t015	CC45	38	t005	CC22	39	22 (56)
t701	CC8	37	t657	CC97	36	20 (56)

^{a)} CC = Clonal complex

from 16 % in 2015 to 12% in 2016. Resistance to fusidic acid has otherwise increased steadily from 2007 to 2015 (Table 8.7.1). Typing identified 578 different *spa* types distributed in 28 different CC groups (the ten most prevalent *spa* types representing 31% of the total are presented in Table 8.7.2). The PVL toxin was demonstrated in 22 (1.1%) cases of which six were MRSA. None of cases with a PVL positive MRSA were reported to have pneumonia. The 22 PVL containing isolates belonged to 15 different *spa* types and 7 different CC groups.

Surveillance of MRSA

In 2016, 3,550 new MRSA cases were detected (62.2 per 100,000 inhabitants). This was 20% higher than observed in 2015 in Denmark (Figure 8.7.1). A case was defined as a person found positive for the first time with a specific MRSA strain regardless whether the patient was infected or colo-

nized only. MRSA isolates were confirmed by detection of either the *mecA*, or more uncommonly, the *mecC* gene.

After the number of new cases had stabilized in 2015, the observed increase in 2016 followed the increasing trend registered since 2009. CC398 cases constituted 35% (n=1,297) of new MRSA cases in 2016, of which 1,249 belonged to the LA-MRSA CC398 and 48 to the human adapted PVL-positive variant. The number of LA-MRSA CC398 increased in 2016 compared to 2015, but is at the same level as in 2014. It should be noted, that this trend may be influenced by the fact that only new cases are registered and that many people with livestock contact, in particular pig farmers, have already been tested positive in previous years; therefore, they are not counted as new cases, even though most of them will still be LA-MRSA CC398 carriers.

Figure 8.7.1 Number of methicillin-resistant *Staphylococcus aureus* cases, with a three years moving average, Denmark

DANMAP 2016

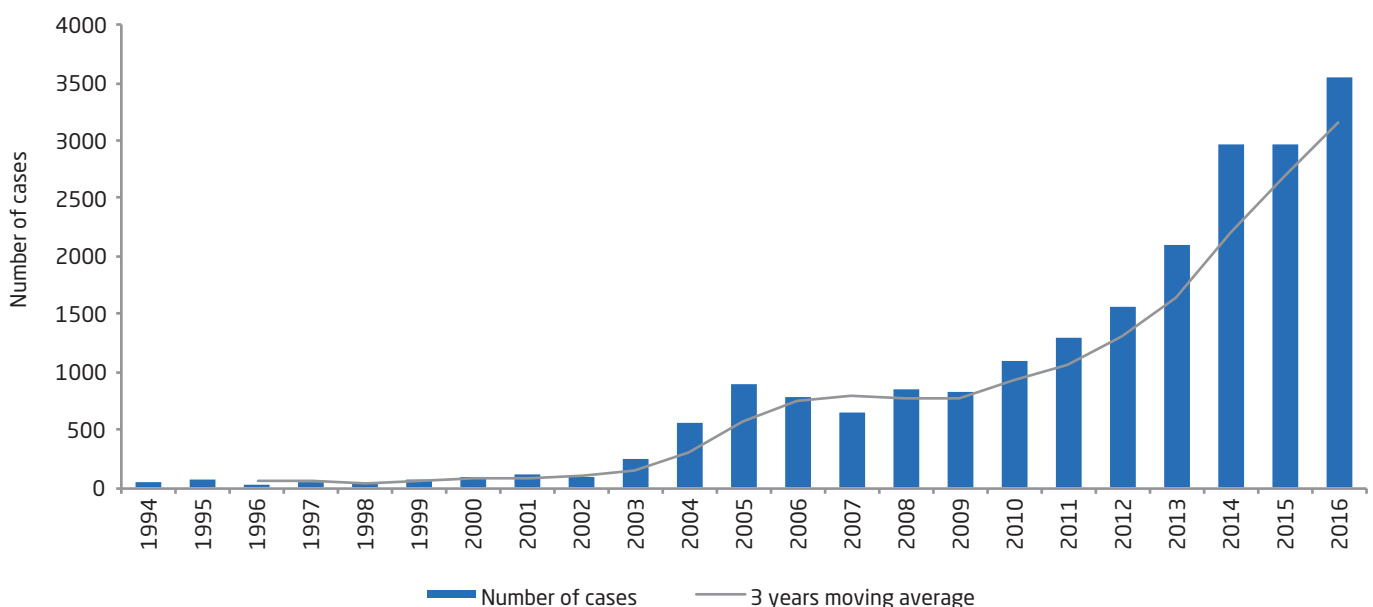


Table 8.7.3 Epidemiological classification of new MRSA cases, Denmark 2016

DANMAP 2016

Epidemiologic classification	Exposure	No. of cases (% of total)	No. (%) of cases with infections
Imported (IMP)		712 (20)	325 (46)
Hospital-acquired (HA)		43 (1)	30 (70)
Health-care associated, community onset (HACO)		211 (6)	
	with known exposure	29	14 (48)
	without known exposure	182	141 (77)
Health care worker		40 (1)	21 (53)
Community-acquired (CA)		1280 (36)	
	with known exposure	674	153 (23)
	without known exposure	606	454 (75)
LA-MRSA CC398		1249 (35)	
	with known exposure	1040	120 (12)
	without known exposure	209	98 (47)
Unknown/missing		15	

Note: Numbers shown in bold are totals

MRSA isolates carrying *mecC* were demonstrated in 45 cases (1.3%) in 2016 (9 in 2009, 21 in 2010, 37 in 2011, 24 in 2012, 41 in 2013, 53 in 2014 and 61 in 2015). Thirty of the cases (67%) had infections at the time of diagnosis. No live-stock contact was reported for the 45 *mecC* cases.

The total number of cases and the number of cases presenting with infection according to epidemiological classification are shown in Table 8.7.3. Most of the cases (80%) were acquired in Denmark. At the time of diagnosis, 38% (n=1,356) of cases had infection, which was similar to 2015 but lower than in previous years due to a much lower fraction of infections among LA-MRSA CC398 cases (n=208, 16%).

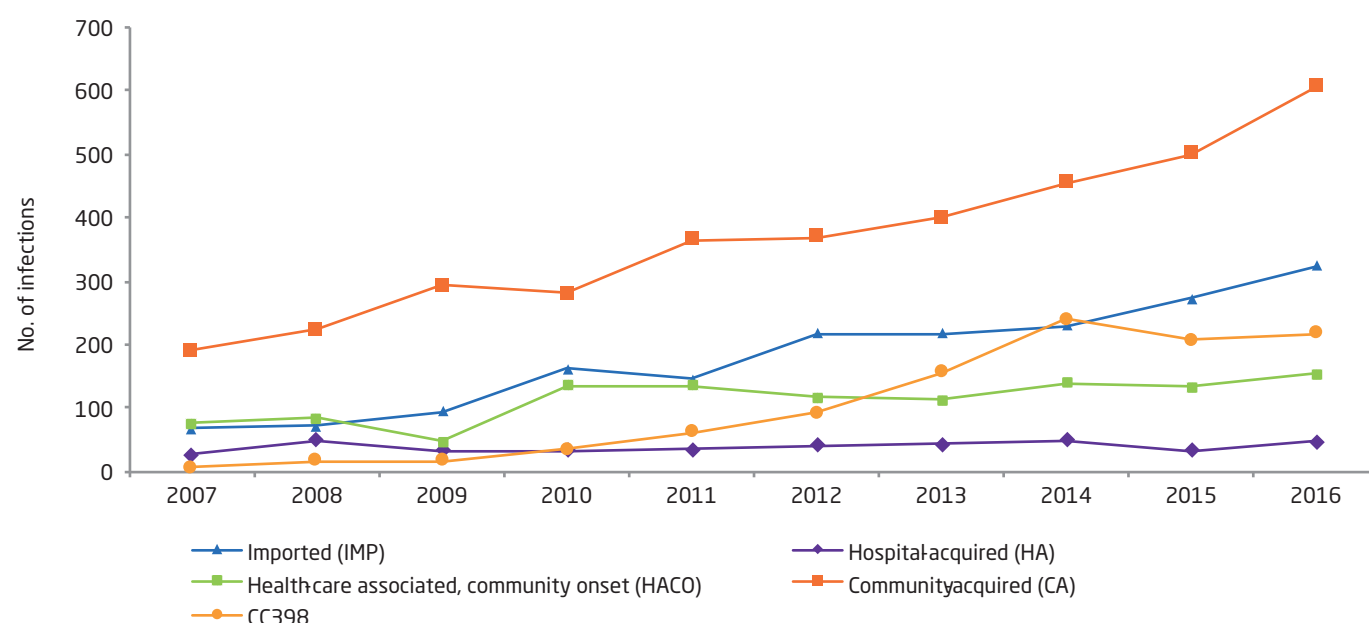
The epidemiological classification of MRSA infections 2007–2016 is shown in Figure 8.7.2. Despite the increasing number of cases, the number of HA-MRSA infections (n=30) and HACO-MRSA infections (n=155) remained low. The number of CA and imported infections continued the increasing trend in 2016 and were by far the two largest groups (n = 607 and n= 325, respectively) while infections caused by LA-MRSA CC398 was similar (n=218) to the number in 2015 (n=208) (Figure 8.7.2). The number of infections among health care workers increased from 31 cases in 2015 to 40 cases in 2016 (Table 8.7.3).

Molecular typing of non-CC398 MRSA isolates

In total, typing revealed 322 different *spa* types not associated with CC398, of which 233 types were associated with clinical infections. The 10 dominating non-CC398 *spa* types in 2016 are shown in Table 8.7.2. They constituted 52% of the total number of non-CC398 MRSA isolates. Among the MRSA causing clinical infections at time of presentation, ten *spa* types constituted 49% of the 1,115 clinical cases with MRSA. These most prevalent *spa* types were t002/CC5 (n=98), t008/CC8 (n=72), t019/CC30 (n=69), t304/CC6/CC8 (n=69), t223/CC22 (n=64) t127/CC1 (n=58), t044/CC80 (n=44), t437/CC59 (n=30), t657/CC1 (n=20) and. PVL was found in 39.5% of isolates related to infections and in 20.8% of isolates from asymptomatic carriers. PVL was predominant in isolates belonging to *spa* types t019 (n=86), t008 (n=81), t002 (n=37), t044 (n=79) and t437 (n=41).

Figure 8.7.2 Number of MRSA infections according to epidemiological classification, Denmark

DANMAP 2016



Resistance among non-CC398 MRSA isolates

Resistance data is presented for non-CC398 isolates in Table 8.7.4. Every second non-CC398 isolate received in the laboratory in 2016 was tested (n=1,184). Human adapted Resistance to at least one, two or three other antimicrobials in addition to β -lactam antibiotics (cefoxitin/penicillin) was demonstrated in 64%, 45% and 31% of non-CC398 cases, respectively.

In Table 9.2, (chapter 9, material and methods) the distribution of MICs and resistance for all tested antimicrobials are shown.

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Table 8.7.4 Resistance (%) in non- CC398 MRSA isolates, Denmark 2015 DANMAP 2016

	% non-CC398
Erythromycin	34
Clindamycin	25
Tetracycline	26
Fusidic acid	18
Rifampicin	1
Norfloxacin	19
Kanamycin	28
Linezolid	<1
Mupirocin	<1
Trimethoprim-sulfamethoxazole	2
Number of tested isolates	1184

Advisory Service for LA-MRSA

This national advisory service was established on July 1, 2014 with the purpose of providing help regarding the handling of livestock associated (LA)-MRSA for citizens, people working with livestock, and relevant institutional bodies in Denmark. LA-MRSA differs from ordinary MRSA in a number of areas, including infection control and relevance for different sectors of health care. The aim of the advisory service is to reduce the spread of livestock-MRSA into the community. This is done by increasing the understanding of the connection between hygiene, carriage and infection risks among people who work with farm animals and thus reduce further transmission to humans without direct contact with domestic animals.

Since 2006, there has been a focused Danish strategy for the prevention of MRSA, with the Danish Health Authority maintaining the guidelines. The strategy is based on a search and destroy principle including i) monitoring MRSA positive cases by clinical microbiology laboratories, ii) mandatory reporting of new MRSA cases and risk factors to SSI iii) typing of new MRSA cases iv) investigating outbreaks and v) providing relevant treatment for persons infected or colonized with MRSA. Transmission in hospitals is prevented by isolation precautions, and healthcare professionals can get advice from regional infection control units. In most regions, patients receive free treatment of carrier status.

The strategy has focused on limiting spread in hospitals but has most likely also helped to limit the number of infected persons in the community.

The Advisory Service is communicating about infection control, medical and social issues related to livestock-MRSA for both employees in pig production, persons who come into contact with domestic animals e.g. craftsmen, other citizens and health professionals. The Advisory Service offers teaching / lectures about livestock-MRSA in farmers associations, educational institutions e.g. agricultural schools, and to health care providers. Evidence-based information is prepared based on a systematic knowledge capture and analysis of data from LA-MRSA positive persons. The Advisory Service is now setting up a mandatory e-learning course for all persons working with live pigs. The course is expected to be completed by the end of 2017.

For more information visit www.ssi.dk/MRSA

The Advisory Service is co-financed by the Ministry of Health and the Elderly and the Ministry of the Environment and Food and is a temporary function that has been provisionally extended until the end of 2018

Textbox 8.4

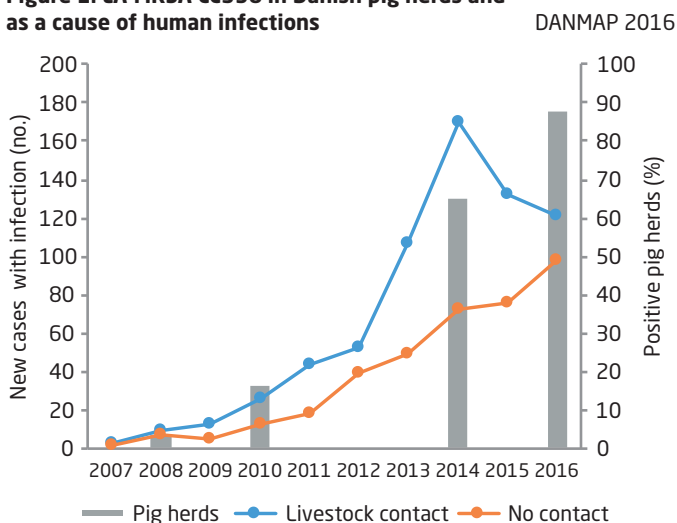
LA-MRSA CC398 in animals and humans

The number of LA-MRSA CC398 infection in people with livestock contact continued to decrease in 2016, from 132 cases in 2015 to 121 cases in 2016. This trend may be due to the fact that new MRSA cases are only registered once - regardless of whether these are healthy carriers or have infections. Thus, many people with livestock contact, in particular pig farmers, have already been tested positive for LA-MRSA CC398 and will not be counted again as new cases, even when being infected with LA-MRSA CC398. In contrast, the number of LA-MRSA CC398 infections in people with no livestock contact, i.e. the general population, continued to increase in 2016, from 73 cases in 2015 to 98 cases in 2016, which seems to follow the increasing prevalence of positive pig herds, as illustrated in Figure 1. This is worrisome because the general population includes a higher proportion of elderly and immunocompromised people with an elevated risk of developing invasive staphylococcal illnesses. In a recent Danish study, Statens Serum Institut investigated the occurrence and origin of bloodstream infections (BSIs) caused by LA-MRSA CC398 between 2010 and 2015 (Larsen et al., Clin Infect Dis., 2017, May 30). The analysis showed that the annual number of LA-MRSA CC398 BSIs increased, peaking in 2014, where LA-MRSA CC398 accounted for 16% (7/44) of all MRSA BSIs, which corresponds to 1.2 cases per 1,000,000 person-years. LA-MRSA CC398 BSIs and associated deaths were more common in people with no livestock contact (10 BSIs and 6 deaths) than in people with livestock contact (7 BSIs and 0 deaths). In addition, whole-genome sequence analysis showed that most of the BSI isolates were closely related to Danish pig isolates. These findings support a causal relationship between the continued spread of LA-MRSA CC398 into the general population and the increasing number of serious infections and deaths.

MRSA in conventional pig herds

In 2016, the Danish Veterinary and Food Administration conducted a screening of conventional pig herds for LA-MRSA. Altogether 221 herds were tested; 57 of these were randomly selected for an estimate of the present prevalence. In addition, herds from two geographic areas (Southeast Zealand and Bornholm), not represented in the 2014 survey (N = 53), and some herds whose MRSA status already was known from the 2014 survey (N = 117, among these 111 production herds and six breeding herds) were included. The purpose of re-testing herds from the 2014 survey was to estimate the proportion of negative herds that had become positive during the 2-year period, and to see if any of the previously positive herds had become negative. The results are presented in Table 1.

Figure 1. LA-MRSA CC398 in Danish pig herds and as a cause of human infections



The overall prevalence of LA-MRSA in the randomly selected herds was 88%, an increase from 68% in 2014. The prevalence was lower in Southeast Zealand (59%) and on Bornholm (62%). In 2014, the proportion of positive herds among herds tested in other parts of Zealand (53%) was also lower than in Jutland (70%) and on Funen (69%).

It is interesting that all six breeding herds were positive for LA-MRSA, including the three herds that were negative in 2014. These herds had become positive although no animals had been introduced, suggesting that LA-MRSA can be introduced via human carriers or other sources. It is also interesting that 62% of the herds, which were negative in 2014, had become positive in 2016.

An important observation is that all herds, which were positive in 2014, were still positive in 2016, suggesting that spontaneous elimination of LA-MRSA from conventional pig herds is unlikely to occur.

A total of 179 LA-MRSA isolates obtained from the 2016 survey were subjected to *spa*-typing. The CC398-associated *spa*-types t034 (n=109) and t011 (n=53) were by far the most prevalent, while nine other *spa*-types and an unknown *spa*-type were found in 1-4 isolates. In one of the breeding farms, two different clones, a CC398 and a CC1 were found.

MRSA in organic and free-range pigs

A survey of organic pig herds carried out by the Danish Veterinary and Food Administration in 2015 revealed that only 6% of the tested herds were positive for LA-MRSA. In 2016, six free-range pig farms (five organic and one conventional) were tested multiple times to examine the population dynamics of LA-MRSA after introduction of positive conventional breeding pigs. Although five of the farms received MRSA-positive conventional breeding pigs, four of them tested MRSA-negative in all sections after 3 months. This indicates that LA-MRSA is less well maintained in free-range pig farms pigs, compared to conventional pig farms.

MRSA in pork

The increased prevalence of LA-MRSA in pigs is mirrored by an increasing prevalence of LA-MRSA in domestically produced pork. Among 163 conventional pork meat samples collected at retail level, LA-MRSA was detected in 78 (48%). This is a marked increase compared to previous sampling in 2009 (5%), 2010 (6%) and 2011 (10%) (Figure 2).

Of the LA-MRSA isolates from conventional pork, 76 belonged to CC398, one was CC9, and CC1, respectively. The CC1 isolate belonged to *spa*-type t127, while the CC9 isolate belonged to t1430. The CC398 isolates were vastly dominated by t034 (n = 46) and t011 (n = 23), while a single isolate belonged to each of the *spa*-types t108, t571, t898, t1255, t2123, t2997 and t4652.

Interestingly, among 97 samples from organic pork, 31 (32%) were LA-MRSA positive with CC398. These were also dominated by *spa*-types t034 (n = 18) and t011 (n = 11), while one isolate was t1255 and t1606, respectively. Considering that only 6% of the organic pig farms were found positive for LA-MRSA in the 2015 survey (see above), which suggests a low prevalence in organic and free range pigs, the observed prevalence of positive organic pork products is surprisingly high. One possible explanation is that cross-contamination from conventional pigs may take place at the slaughterhouse.

Imported pork from conventional production was also sampled and LA-MRSA was detected in 13 of 45 samples (29%). As in the domestically produced meat, CC398 was most common belonging to *spa*-type t034 (n = 5), t011 (n = 3), t11374 (n=1) and t1451. Three isolates belonged to CC9 (t899, t1200 and t1430).

Table 1. Prevalence of pig herds positive for LA-MRSA in 2014 and 2016

DANMAP 2016

Pig herd type		Pig herds positive for LA-MRSA in 2014	Pig herds positive for LA-MRSA in 2016
Slaughter pigs	randomly selected herds	68 % (N = 205) ¹	88 % (N = 57) ²
	geographic area Bornholm	Not tested	62% (N = 26)
	geographic area Southeast Zealand	Not tested	59% (N = 27)
Breeding herds		66% (N = 70)	
	positive in 2014		100% (N = 3)
	negative in 2014		100% (N = 3)
Production herds	positive in 2014		100 % (N = 58)
	negative in 2014		62 % (N = 53)

¹ Geographic areas; Jutland: 70% (N = 147), Funen: 69% (N = 39), Zealand: 53% (N = 19)

² All samples were from herds from Jutland or Funen

Textbox 8.4 continued...

MRSA in mink

In 2016, the National Veterinary Institute conducted a survey of all submissions of mink to the laboratory. The mink were submitted for diagnostic laboratory investigations due to disease outbreaks. Previous studies had revealed that LA-MRSA in mink is most often located on the paws and in the pharynx. A total of 89 submissions were examined and LA-MRSA was found in 30 (34%). This is equivalent to the prevalence found in a survey conducted in 2015 (Hansen et al., *Vet. Microbiol.* 2017;207:44-9). In addition, two cases of methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) were found.

MRSA in bulk tank milk

In November and December 2016, bulk tank milk samples were collected from 236 dairy cattle herds. LA-MRSA was found in seven of these samples (3%). This prevalence is slightly higher than those in 2014 (2%) and 2012 (1.8%), but this is not statistically significant. Of the seven positive samples, six isolates carried the *mecA* gene, while one isolate carried the *mecC* gene. This is the first record of *mecC* in bulk tank milk in Denmark. The six *mecA*-isolates had *spa*-type t034 associated with CC398, whereas the *mecC*-isolate had *spa*-type t843 associated with CC130.

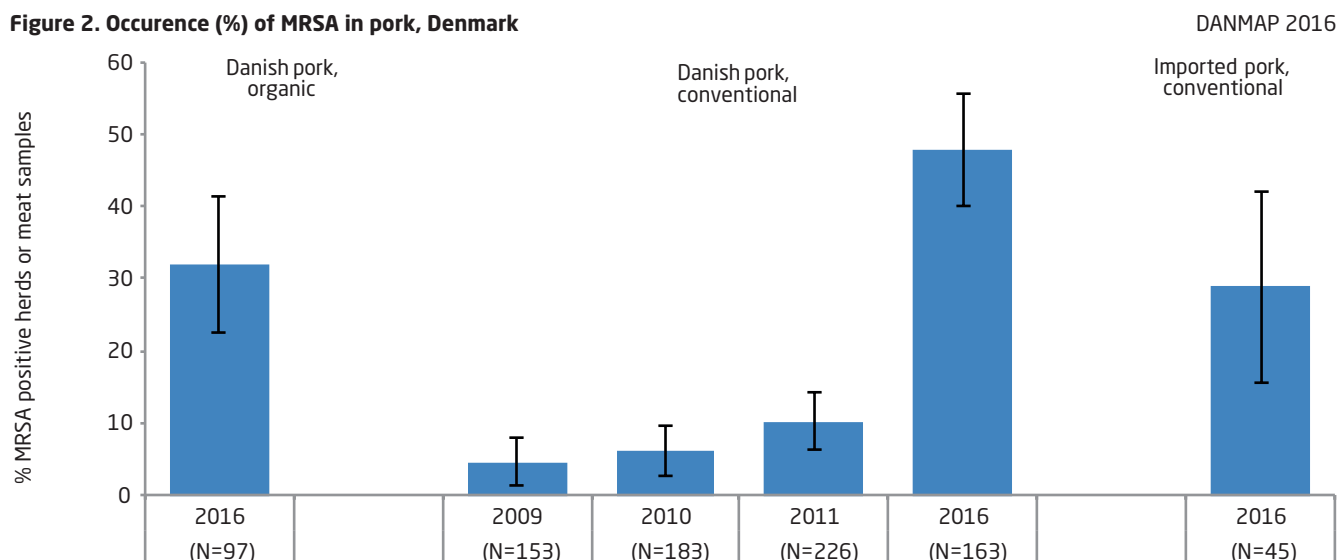
MRSA in horses

Between April and August 2015, University of Copenhagen and Statens Serum Institut performed a screening survey for LA-MRSA in horses (Islam et al., *Front. Microbiol.* 2015;8:543). In total, 17 of 401 horses (4%) from seven of 74 farms (9%) were positive for LA-MRSA, including 14 *mecA*-carrying CC398 isolates with *spa*-types t011 (N = 10) and t034 (N = 4) and three *mecC*-carrying CC130 isolates with *spa*-type t528. Whole-genome sequence analysis showed that the ten CC398 isolates with *spa*-type t011 belonged to a separate horse-adapted sublineage within CC398, whereas the four CC398 isolates with *spa*-type t034 were closely related to CC398 isolates from Danish pigs, suggesting that CC398 is able to spread between different animal species.

Conclusions

The results presented above show that conventional pig farms are still the primary reservoir for LA-MRSA, especially CC398. However, there is a growing body of evidence that LA-MRSA CC398 is spreading to other animal species, such as cattle, mink, and horses, and to people in contact with those animals. In addition, the number of infections, including life-threatening BSIs, among people with no contact to livestock is increasing.

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Figure 2. Occurrence (%) of MRSA in pork, Denmark

Note: Confidence intervals are calculated as 95% binomial proportions presenting Wilson intervals.

8.8 *Neisseria gonorrhoeae*

Background: *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 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 inoculation.

Methods: Since 1962, the Departments of Clinical Microbiology in Denmark have submitted isolates of gonococci to the *Neisseria* and *Streptococcus* Reference (NSR) laboratory at Statens Serum Institut for national surveillance of antimicrobial resistance.

At the NSR laboratory ceftriaxone, ciprofloxacin and azithromycin MICs are determined using the Etest® on chocolate agar incubated at 35 °C in 5% CO₂. The breakpoints used are those defined by EUCAST. Both resistant and intermediary susceptible isolates are categorized as resistant in this report. Penicillinase production is tested for using the Nitrocephin assay.

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

Results and discussion: Most of the received isolates are 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.

In 2016, isolates from 1,493 unique cases of gonorrhoea were received. The annual number has increased considerably from 2011 through 2016 (Figure 8.8.1), partly because the

widespread use of combined nucleic acid amplifications tests for *Chlamydia trachomatis* and *N. gonorrhoeae* has identified unexpected cases of gonorrhoea (followed by culture), and partly due to an increasing incidence of gonorrhoeae, especially among young heterosexual persons, among whom an increasing proportion are women.

The ciprofloxacin resistance rate was 18% in 2016 (29% in 2015 and 46% in 2014), thus showing a steady decline since the peak of 75% in 2009 (Figure 8.8.1). The percentage of strains producing penicillinase was 7 % in 2016. It has fluctuated between 22% in 2005 and 8% in 2015. Azithromycin resistance was found in 1% of the isolates and intermediary susceptibility in 6% (1% and 10%, respectively, in 2015).

Ceftriaxone resistant gonococci (MIC > 0.125 mg/L) as well as ceftriaxone treatment failure in patients with gonorrhoea have occurred in several countries during recent years. During 2003 through 2009 the proportion of isolates with ceftriaxone MIC \geq 0.008 mg/L gradually increased from 40% to nearly 75% (Figure 8.8.2), but during recent years this shift has nearly reversed (44% in 2014 and 17% in 2015). Thus, there is no evidence of emerging ceftriaxone resistance in Denmark.

The National Board of Health issued guidelines in 2015 for the diagnosis and treatment of sexually transmitted infections. A combination of high dose ceftriaxone (500 mg i.m.) and azithromycin (2 g) is recommended for the treatment of gonorrhoea. Ciprofloxacin 500 mg p.o. may be used for treatment if the strain is fully susceptible.

In a subset of 111 isolates, resistance against cefixime (MIC > 0.125 mg/L) was 0% in 2016 (0% in 2015). Cefixime is an oral cephalosporin that has never been used in Denmark. Resistance against spectinomycin was 0% in 2016 as well as in 2015.

Conclusions: The incidence of gonorrhoea is increasing substantially. The ciprofloxacin resistance rate continues to decrease, concomitantly with an increasing proportion of gonorrhoeae cases being among heterosexual patients and among females. Although resistance problems are still not present in Denmark, the emerging ceftriaxone treatment failures in other countries underlines the importance of maintaining the centralised national surveillance of antimicrobial resistance in gonococci.

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Figure 8.8.1 The number of submitted gonococcus isolates from males and females, and the percentage of isolates with ciprofloxacin resistance, azithromycin resistance, and penicillinase production, Denmark, 2003-2016

DANMAP 2016

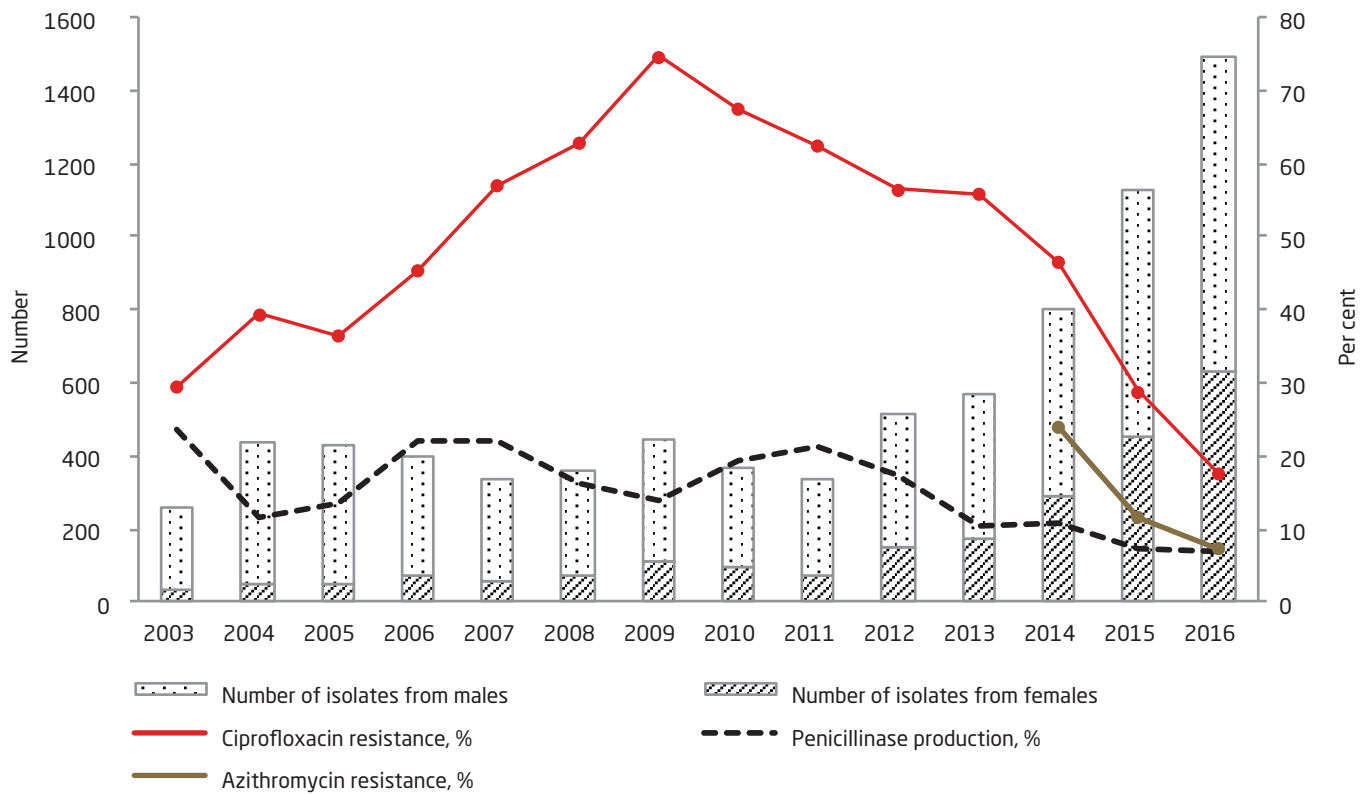
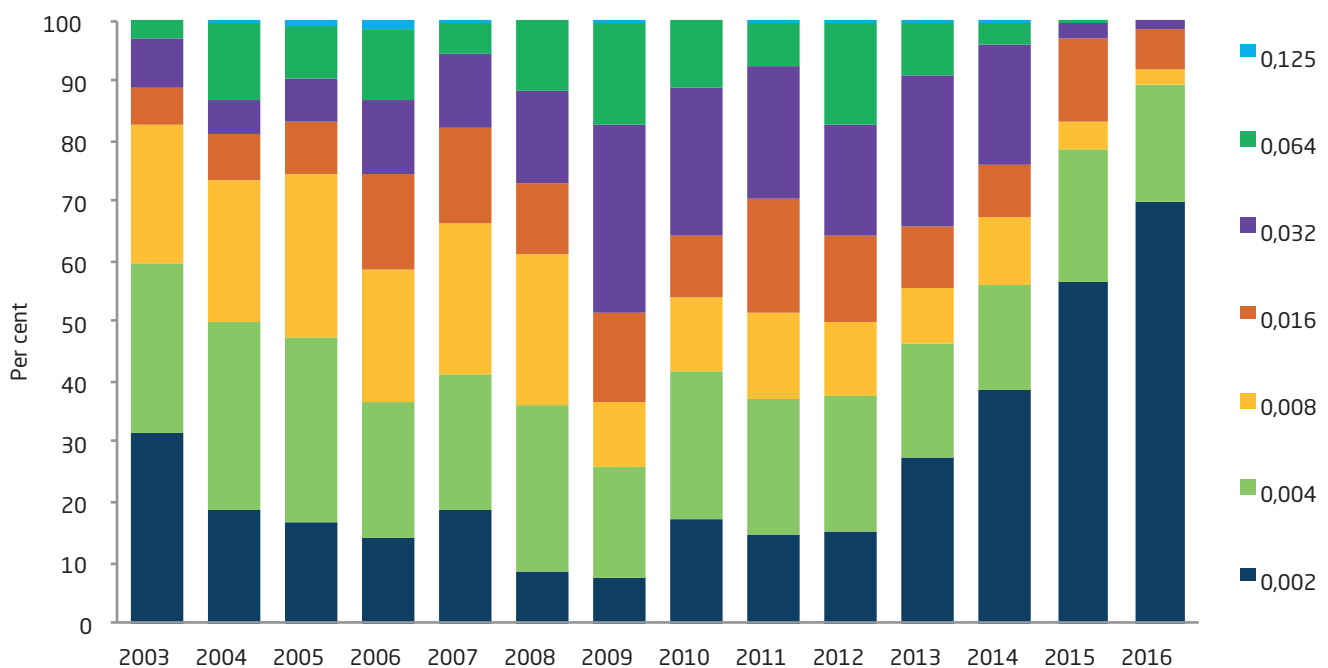


Figure 8.8.2 Distribution of ceftriaxone MIC (mg/L) values in gonococci, Denmark, 2003-2016

DANMAP 2016



Textbox 8.5

Clostridium difficile surveillance

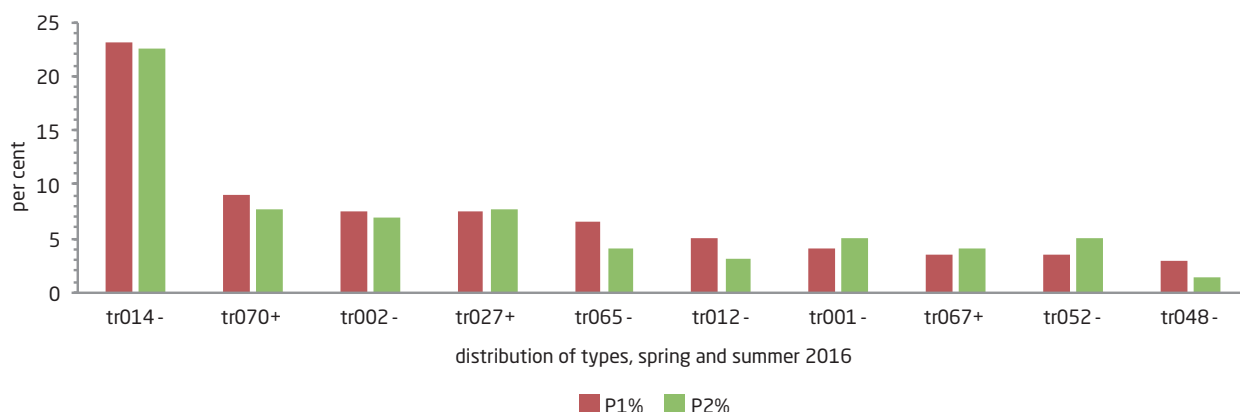
C. difficile is an important human pathogen and the primary cause of pseudomembranous colitis and antibiotic-associated diarrhoea. Since the beginning of the millennium, *C. difficile* has caused large outbreaks in hospitals worldwide, coinciding with the emergence of epidemic strains such as PCR-ribotypes RT027 and RT078 associated with increased incidence, severity, mortality and recurrence. The main virulence factors of pathogenic *C. difficile* are the toxins TcdA and TcdB, but in addition, *C. difficile* 027 and other important clones also produce a binary toxin (CDT).

In Denmark, more than 5,000 *C. difficile* infections are diagnosed each year; the majority of these are hospital-acquired. In recent years, more than 1,000 isolates have been submitted annually from the regional hospital laboratories to Statens Serum Institut for typing and further characterization. Since 2013, Statens Serum Institut has replaced PCR-ribotyping with tandem-repeat sequence typing (TRST) [1] for the routine typing of *C. difficile*. There is a good correlation between the two methods; e.g. RT027, RT078, RT023, RT066 and RT014 correspond to TRST types tr027, tr070, tr016, tr067 and tr014, respectively. Due to the severity of disease related to the multi-resistant and epidemic *C. difficile* 027 clone, many laboratories use diagnostic methods that detect specific genetic markers for this clone incl. the binary toxin. Since 2012, the guidelines for submission of isolates have focused on the isolates harboring the binary toxin gene. Therefore, approximately 90% of the isolates typed at Statens Serum Institut in recent years were binary toxin positive, mainly tr027, tr070, tr016 and tr067. While several successful measures, like hygiene, patient isolation and efficient diagnosis have reduced the number of *C. difficile* 027 infections, other important clones seem to increase.

In order to improve the future surveillance of *C. difficile*, a study was carried out in 2016 with the objectives of determining the distribution of types and antimicrobial resistance profiles among all toxigenic *C. difficile*, irrespective of the presence of the binary toxin. Each of the 10 clinical microbiological laboratories in Denmark participated by submitting all toxigenic strains or samples in a 2-week period in the spring and again in the autumn. The isolates were typed by TRST, the toxin profile was determined by PCR and the antimicrobial resistance profile was determined for a selection of the isolates. A total of 417 isolates were characterized. Of these, 72.2% were positive only for *tcdA* and *tcdB*, while 23.8% were additionally positive for the binary toxin. The distribution of the 10 most prevalent TRST types in the spring (P1) and autumn (P2) period is shown in the figure, indicating similar distribution in the two different periods. Among the 10 most prevalent types, seven did not contain

Figure 1. Clostridium difficile. Percentage of the 10 most prevalent TRST types in unbiased surveillance project in spring (P1, n=199) and autumn (P2, n=218) in 2016

DANMAP 2016



Textbox 8.5 continued...

the binary toxin. Among all types, tr014 (binary toxin negative) was the most prevalent at both the national level and at each of the five Danish regions. Preliminary antimicrobial resistance testing showed that tr014 isolates are generally susceptible, whereas tr027 are resistant to moxifloxacin and erythromycin and in varying degree resistant to clindamycin and rifampicin.

The role of the binary toxin has been a controversial subject as some studies have shown increased mortality among patients infected with binary toxin positive strains, while other studies have not confirmed this [2-5]. In this study, 30 days mortality was 13.2% and 12.9% among patients with and without the binary toxin, respectively. Based on this study, we recommend that future surveillance should include the binary toxin negative isolates for two reasons; several highly prevalent types of binary toxin negative isolates are dominating the Danish reservoir and the binary toxin negative isolates are associated with 30 days mortality similar to the binary toxin positive isolates. Consequently, the surveillance of *C. difficile* has been changed. Starting in 2017, the clinical laboratories are referring all diagnosed toxigenic *C. difficile* samples in two periods of one month to Statens Serum Institut for characterization. In addition, samples related to suspected outbreaks or severe clinical manifestations can also be referred. The new surveillance scheme will enable detection of all toxigenic types, including emerging types and new resistant clones.

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Acknowledgment:

We wish to thank the ten Clinical Microbiology Departments in Denmark for participating in the project

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9

MATERIALS AND METHODS



9. Materials and methods

9.1 General information

For the DANMAP 2016 report, population sizes and geographical data were obtained from Statistics Denmark [www.dst.dk] and data on general practitioners from the Danish Medical Association [www.laeger.dk].

The epidemiological unit for pigs and cattle was defined as the individual farm level, meaning that only one isolate per bacterial species per farm was included in the report. The same epidemiological unit was applied for broilers, except for *Salmonella* where the individual flock of broilers was defined as the epidemiological unit. 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 patient per year was included.

Unless stated otherwise, all differences and temporal trends were tested for statistical significance ($p < 0.05$) using Chi-square tests (see Section 9.7.3).

9.2 Data on antimicrobial consumption

An overview of all antimicrobial agents used for humans and animals in Denmark is presented in Table 3.2.

9.2.1 Antimicrobial usage for animals

In Denmark, all antimicrobial agents used for treatment are available only on prescription. Until 2007, antimicrobial agents were only sold by pharmacies or as medicated feed from the feed mills. However, from April 2007, the monopoly was suspended and private companies (four in 2016) were given license to sell prescribed veterinary medical products for animals, when following strict guidelines, identical to those applied by pharmacies. Furthermore, in 2007 price setting of antibiotic was liberalised, which allowed for discounts to veterinarians, when buying larger quantities.

A pharmacy or company either sells the medicines to veterinarians for use in the practice or for re-sale to farmers, or sells the medicines directly to the animal holder on presentation of a prescription. By law, veterinarians are allowed only very small profits on sale of medicine (5%), to limit the economic incentive to overprescribe.

In 2016, 98% of antimicrobial agents were purchased through pharmacies and the drug trading companies, while 2% were purchased from the feed mills. These numbers did not include prescribed zinc oxide from the feeding mills for the pigs. For cattle, 43% of antimicrobial agents used in 2016 were

purchased from pharmacies, whereas 10 years ago where more than 80% of the antimicrobial agents used in cattle was purchased through the veterinarian. In aquaculture, more than 80% is purchased through the feed mills.

Data registration

In addition, data on coccidiostatics as feed additives (non-prescription) and antimicrobial growth promoters (not in use since 2000) were also collected by VetStat, providing an almost complete register of all antimicrobial agents used for animal in Denmark each year. In very rare instances medicines are prescribed on special license and will not be included in VetStat (i.e. medicines not approved for marketing in Denmark).

Data on all sales of veterinary prescription medicine from the pharmacies, private companies, feed mills and veterinarians are sent electronically to a central database called VetStat, which is hosted by the Danish Veterinary and Food Administration. Prior to 2001, all data on antimicrobial sales were derived from pharmaceutical companies. Veterinarians are required by law to report all use of antibiotics and prescriptions for production animals to VetStat monthly. For most veterinarians, the registration of data is linked to the writing of invoices. The electronic registration of the sales at the pharmacies is linked to the billing process and stock accounts at the pharmacy, which ensures a very high data quality regarding amounts and identity of drugs. Data are transferred daily from pharmacies to The Register of Medicinal Product Statistics at SSI and on to VetStat also daily. However, VetStat does not have any validation on data entry and slight typing errors from vets may occur.

The VetStat database contains detailed information about source and consumption for each prescription item: date of sale, identity of prescribing veterinarian, source ID (identity of the pharmacy, feed mill, or veterinarian practice reporting), package identity code and amount, animal species, age-group, disease category and code for farm-identity (CHR Danish Central Husbandry Register). The package code is a unique identifier, relating to all information on the medicinal product, such as active ingredient, content as number of unit doses (e.g. number of tablets), package size, and code of the antimicrobial agent in the Veterinary Anatomical Therapeutic Chemical (ATCvet) classification system.

Knowledge of the target animal species enables the presentation of consumption data in "defined animal daily doses" a national veterinary equivalent to the international defined daily doses (DDDs) system applied in the human field [www.whocc.no].

Methods

DANMAP reports usage of antimicrobials in different animal populations and in veterinary and human sectors. To allow for quantitative comparison in the different populations, the quantity of antimicrobials used, their potency, their formulation, the route of administration, and the age of the animals (where relevant) are accounted for by generating Defined animal daily doses (DADDs).

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 for each antimicrobial agent, administration route and animal species and when appropriate, also age group. 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 basic principles for the DADD are similar to the principles previously described for the ADD [DANMAP 2011, DANMAP 2012]. The DADD is based on the VetStat ADDs, but re-defined for each group of antimicrobial agents, adding adjustments for each combination of active compound, administration route, formulation, according to following principles:

1. A DADD group is defined for each antimicrobial agent by administration route, pharmaceutical form and animal species; and when appropriate also age group;
2. Minor inconsistencies have been corrected e.g. due to rounding of numbers;
3. Approved dosage for the most widely used antimicrobial products were given priority above dosage for products that are rarely used;
4. Approved dosage for older products within the group were maintained as the common DADD even if a new product is approved with a higher dosage;

5. If the dosage for a group with large variation in approved dosages of the products, the dosages provided by "The Veterinary Formulary" [British Veterinary Association 2005, 6th edition] were applied;
6. Dosages may vary within active compound and administration route, if different dosages have been approved for different age group/indication or formulation.

When principle 3 and 4 are conflicting, principle 5 is applied.

Denominator

Trends in antimicrobial consumption in pigs are presented in DADD per 1,000 animals per day (DAPD). 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 body-weight and the average lifespan in each age group. In 2016, DAPD calculations were carried out for pigs only.

Due to a relative high number of pigs exported around 30 kg; an adjusted measure of consumption per pig was calculated. The adjustment is based on the assumption that pigs exported at 30 kg, on average, received the same amount of antimicrobial agents before export, as other pigs from farrowing to 30 kg. Antimicrobial use per pig produced (adjusted) is calculated as: $[DADDs + DADDw + (1+Q)*DADDf] / (\text{biomass-days-total} + Nw*5,800(\text{kg*days}))$, where

DADDs = amount of antimicrobial agents used in sows;

DADDw = amount of antimicrobial agents used in weaners;

DADDf = amount of antimicrobial agents used in finishers;

Q is the proportion of weaning pigs exported around 30 kg.

Nw = number of pigs exported at 30 kg bodyweight,

Nw*5,800 is the number of biomass days the exported pigs would have contributed to the live biomass if not exported.

Estimation of live biomass of animals

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.

DAPD - DADD per 1,000 animals per day

The number of DADDs administered to an animal species during a year (in thousands) divided by the number of standard animals at risk per day. The number of standard animals at risk per day takes into account species differences in average body-mass and life-span. When relevant, the numbers of DADDs and standard animals at risk are estimated for specific age groups, or simply as number of doses (DADDs) used to treat one kg of animal divided with the total estimated biomass (in tonnes).

DAPD is a statistical measure, providing a rough estimate of the proportion of animals treated daily with an average maintenance dose of 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. The DAPD is also referred to as the treatment proportion. In principle, the metric DAPD is parallel to the metric used in pharmaco-epidemiology for the human sector, Defined daily dose per 1,000 inhabitants per day (DID), see Section 9.2.2.

Pig production. The estimation was based on the number of pigs produced, including exports at different ages [Statistics Denmark; Danish Agriculture and Food Council], productivity data for each year [Danish Agriculture and Food Council, 2016] and census data for breeding animals [Statistics Denmark, 2016]. The average weight and life span for the growing animals (piglets, weaners and finishers) were estimated from the annual productivity numbers. 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 could not be 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.

Broiler (*Gallus gallus*). The live biomass was estimated based on number of broilers produced [Statistics Denmark; Danish Agriculture and Food Council], an average live weight at slaughter of 1.97 kg after an estimated average life span of 30 days. The mean live biomass per broiler is assumed to be half of the weight at slaughter.

Turkey production. The live biomass is estimated based on the number of turkeys produced [Statistics Denmark; Danish Agriculture and Food Council] and an average live weight at slaughter of 21 kg for male turkeys and 11 kg for hens after an estimated average life span of 20 weeks and 15.5 weeks, respectively [Danish Agro; S. Astrup, personal communication]. The estimated mean live biomass per turkey is assumed to be half of the weight at slaughter.

Fur animals. The live biomass of mink is estimated from production data [Statistics Denmark, Copenhagen Fur] and the average weight at pelting was 2.45 kg [Kopenhagen Fur]. The progeny live for approximately 7 months. The biomass for the breeding animals (female) was estimated based on census data and an assumed average live weight of 2 kg.

Pet animals. Only dogs and cats are taken into account, as the other population sizes are negligible in Denmark, and relatively rare in veterinary practice. The population is based on census data [Statistics Denmark, 2000] estimating 650,000 cats and 550,000 dogs. The number of dogs in Denmark has been relatively stable during the last ten years [Danish Dog register, 2012]. The average live weight for cats and dogs were estimated to 4 kg and 20 kg, respectively (based on pedigree registration data).

Aquaculture. The estimation is based on data from the Danish AgriFish Agency (Ministry of Environment and Food) on produced amounts in each subtype of production, and information on the typical lifespan and entrance and exit body weights, and were calculated in cooperation with Danish Aquaculture [N.H. Henriksen, Danish Aquaculture].

9.2.2 Antimicrobial consumption in humans

Data on consumption of antibacterial agents in humans were obtained from The Register of Medical the Department of Data Delivery and Medicinal Product Statistics, at Statens Serum Institut. The Register receives monthly reporting of consumption from all pharmacies in Denmark, including hospital pharmacies. Data from primary health care sector have been collected since 1994, whereas data on consumption in hospitals are available from 1997.

Only somatic hospitals were included, when the consumption was measured by occupied bed-days and admissions. Data from private hospitals and clinics, psychiatric hospitals, specialised non-acute care clinics, rehabilitation centres and hospices were excluded from DANMAP (representing approximately 3% of the antimicrobial consumption at hospitals and of the number of bed-days).

In the primary health care in Denmark, all antibacterial agents for human use are prescription-only medicines and are sold by pharmacies in defined packages. Each package is uniquely identified by a code, which can be related to package size, content as number of unit doses (e.g. number of tablets), content as number of Defined Daily Doses (DDDs), code of the antimicrobial agent in the Anatomical Therapeutic Chemical (ATC) classification system, and producer. In addition, the following information is collected for each transaction: social security number (CPR number) of the patient, code identifying the prescribing physician, date and pharmacy of the transaction, and information regarding reimbursement of cost, if applicable. For hospital data we have information of the type of department and hospital but no information on the individual consumption level.

Before 2012, data from hospitals on certain infusion substances was obtained directly from the hospital pharmacies. From 2013 onwards all data from hospitals are reported to and delivered by The Register of Medicinal Product Statistics at SSI.

The present report includes data on the consumption of "anti-infectives for systemic use", or group J01, of the 2014 update of the ATC classification, in primary health care and in hospitals as well as consumption of per oral and rectal preparations of metronidazole (P01AB01) and oral preparations of vancomycin (A07AA09). As recommended by the World Health Organization (WHO), consumption of antibacterial agents in primary health care is expressed as DIDs, i.e. the number of DDDs per 1,000 inhabitants per day (DDD/1,000 inhabitant-days). Consumption in primary health care is also reported as the number

of packages per 1,000 inhabitants. Consumption of antibacterial agents in hospitals is expressed as DIDs, for comparison with primary health care, and DBDs, the number of DDDs per 100 occupied beds per day (DDD/100 occupied bed-days)

Since antimicrobial consumption expressed as DDD/100 occupied bed-days does not necessarily reflect changes in hospital activity and production, consumption in hospitals is also presented as DAD (the number of DDDs per 100 admitted patients).

The number of occupied bed-days is calculated as the date of discharge minus the date of admission (minimum one day), and the number of admissions is counted as one admission whenever a patient is admitted to a specific ward (i.e. one patient can be registered as admitted multiple times if transferred between wards during the same hospital stay). Data on the number of occupied bed-days (or patient-days) and number of admissions in each hospital were obtained from the National Patient Registry at the National Board of Health [www.sst.dk].

9.3 Collection of bacterial isolates

9.3.1 Animals

The sampling for DANMAP has changed markedly since the legislation regarding the EU harmonised monitoring of antimicrobial resistance in zoonotic and commensal bacteria came into force in 2014 [Decision 2013/652/EU]. The legislation requires, in addition to national *Salmonella* control programmes, sampling of broilers and fattening turkeys in even years (2014-2020) and sampling of fattening pigs and cattle < 1 year in odd years (2015-2019). In 2016, most of the sampling for DANMAP was allocated to the mandatory sampling of broilers (examined for *Campylobacter jejuni*, indicator *E. coli* and ESBL/AmpC *E. coli*) however, additional sampling of fattening pigs (examined *Salmonella* and indicator *E. coli*) and cattle < 1 year (examined *Campylobacter* and *E. coli*) were also carried out (Table 9.1).

Meat inspection staff or abattoir personnel at the slaughter houses collected caecal samples from healthy pigs, cattle (< 1 year) and broilers. The samples were collected throughout

Table 9.1. Legislative and voluntary sampling plans^(a) under national control programmes and EU harmonised monitoring that contribute isolates to DANMAP 2016

Bacteria	Origin of isolates	Legislative sampling frequency (2013/652/EU)	DANMAP 2016
			Number of samples in 2016
<i>Salmonella spp.</i>	On-farm samples from laying hens, broilers and fattening turkeys (breeder and production flocks)	Annually	4290 flocks
	Caecal samples from fattening pigs		707 animals
	Carcasses of broilers	Annually	203 carcasses
	Carcasses of fattening pigs and cattle <1 year	Annually	15075 carcasses
	Imported broiler meat and pork, ready for retail		274 batches
<i>Campylobacter jejuni</i>	Caecal samples from broilers	Even years	735 animals
	Caecal samples from cattle <1 yr		121 animals
	Danish and imported broiler meat ready for retail		276 batches
Indicator <i>E. coli</i>	Caecal samples from broilers	Even years	193 animals
	Caecal samples from fattening pigs and cattle <1 yr		150 pigs and 121 cattle <1yr
Specific monitoring of ESBL/AmpC-producing <i>E. coli</i>	Caecal samples from broilers	Even years	298 animals
	Fresh broiler meat at retail	Even years	295 samples
<i>Enterococcus spp.</i>	Caecal samples from broilers, fattening pigs or cattle <1 yr		621 broilers
MRSA	Samples from animals		227 animals
	Samples from fresh meat		259 samples

Note: Only a selected number of the obtained isolates, from each source, were speciated and susceptibility tested. Thus, data in this table should not be used for reporting prevalences.

a) All animal samples originate from different flocks/herds. All food samples originate from different slaughter batches, batches of Danish or imported meat ready for retail or packages of fresh meat at retail

2016, and the sampling was stratified per slaughterhouse by allocating the number of samples from domestically produced animals collected per slaughterhouse proportionally to the annual throughput of the slaughterhouse. For broilers, four intact caeca from each flock were pooled into one sample. For pigs and cattle, samples contained 30-100 g caecal material from a single animal. All samples were processed at the Danish Veterinary and Food Administration's (DVFA) laboratory in Ringsted.

Prior to 2014, DANMAP data also included isolates from the national *Salmonella* surveillance programme in pigs, where serological surveillance at slaughter identified high risk herds for on-farm sampling. *Salmonella* from layers, broilers, turkeys and cattle are not included in DANMAP 2016 due to low numbers of isolates available from the national surveillance [Annual Report on Zoonoses in Denmark, 2016].

9.3.2 Meat

The EU harmonised monitoring requires, in addition to national *Salmonella* control programmes, sampling of broiler meat in even years (2014-2020) and sampling of pork and beef in odd years (2015-2019). In 2016, broiler meat was examined for *Salmonella*, *C. jejuni* and ESBL/AmpC *E. coli* (Table 9.1).

ESBL/AmpC *E. coli* isolates originate from samples taken throughout the year by the regional DVFA officers. Samples consist of packages of chilled or frozen broiler meat collected in Danish wholesale and retail outlets according to the sampling framework laid down by Decision 2013/652/EU. Products with added saltwater or other types of marinade were excluded and the packages were selected without pre-selecting based on the country of origin. 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 product (minimum of 200 g) was collected and all samples were processed at the DVFA laboratory.

The *Campylobacter* isolates from broiler meat originated from the national control programme: Intensified control of *Salmonella* and *Campylobacter* in fresh meat based on a case-by-case risk assessment. Sampling of broiler meat ready for retail, e.g. located in cold stores, slaughterhouses, cutting and processing facilities, but also at catering companies or at border control posts. The number of batches to be controlled by each regional DVFA control unit is proportional to the number of establishments in the Region and the country. A 100 gram sample is cut from packages of meat, where 10 gram is used for analysis. From each batch, 12 packages of meat are tested and analysed as single samples. All samples were processed at the DVFA laboratory.

The *Salmonella* isolates from pork originate from the national control programme at the slaughterhouses, where the carcasses are swabbed in four designated areas (the jaw, breast, back and ham) after min. 12 hours of chilling (covering 10x10cm). The numbers of swabs collected depend on the slaughterhouse capacity. All samples were processed at Industry laboratories. Isolates from all *Salmonella* positive samples were sent to DTU food, where one isolate per sample were serotyped and susceptibility tested.

Salmonella from broiler meat and beef (incl. imported meat) are not included in DANMAP 2016 due to low numbers of isolates available from the national surveillance [Annual Report on Zoonoses in Denmark, 2016].

9.3.3 Humans

S. Typhimurium and C. jejuni. Antimicrobial susceptibility was performed on human faecal isolates submitted to Statens Serum Institut (SSI). *Salmonella* isolates were submitted from all DCM in Denmark and *Campylobacter* isolates were submitted from Departments of Clinical Microbiology (DCM) covering three geographical regions: Northern Jutland, Funen and Zealand. Exact figures of the proportion tested and the sampling strategy for the different species can be found in Sections 6.1 and 6.2. As in previous years, SSI collected information on travel history through phone interviews. Patients were asked about the date of disease onset and whether they had travelled abroad within a seven-day period prior to the onset of disease. Furthermore, patients who had been abroad were asked about their destinations. Cases were categorised as "domestically acquired" if the patients had not travelled within the week prior to the onset of disease.

Staphylococcus aureus. Blood isolates are referred on a voluntary basis by all DCM's to the Staphylococcus reference laboratory at SSI. Detection of Methicillin-resistant *S. aureus* (MRSA) is a notifiable condition in Denmark and therefore all MRSA isolates from all sample types are sent to the reference laboratory.

Invasive Streptococcus pneumoniae. Invasive pneumococcal disease is a notifiable disease in Denmark, and therefore all invasive isolates nationwide are sent to SSI for identification or confirmation as well as susceptibility testing and typing.

Invasive Streptococcus pyogenes (group A streptococci) and group B, C and G streptococci. Isolates are submitted to SSI on a voluntary basis. Only isolates from blood and spinal fluid are included in the DANMAP report.

E. coli, K. pneumoniae, P. aeruginosa, Acinetobacter spp., E. faecium and E. faecalis. Data are provided from all DCM's on all isolates from either blood samples (*E. coli*, *K. pneumoniae*, *P. aeruginosa*, *Acinetobacter spp.*, *E. faecium* and *E. faecalis*) or urine samples (*E. coli* and *K. pneumoniae*).

No samples were collected from healthy humans.

9.4 Microbiological methods

9.4.1 Animals and meat

Salmonella was isolated according to the methods issued by the NMKL [NMKL No. 187, 2007] or Annex D, ISO 6579 [ISO6579:2002/Amd 1:2007]. Serotyping of suspect colonies by slide agglutination according to the White-Kauffmann-Le Minor Scheme in combination with "The molecular approach" developed by CDC [Fitzgerald et al, 2007. J Clin Microbiol. 45:3323-34; McQuiston et al, 2011. J Clin Microbiol. 49: 565-573]. Only one isolate per serotype was selected from each herd, flock or slaughter batch (food sample).

Campylobacter from broilers, cattle and broiler meat was isolated and identified according to the methods issued by the NMKL [NMKL No. 119, 2007] with the following exceptions: 1. Use of direct spread on to selective agar for the broiler and cattle samples and 2. Use of pre-enrichment in Bolton broth for the cattle samples. Only one *C. jejuni* isolate per broiler flock, cattle herd or per batch of fresh meat was selected.

Indicator E. coli from broilers, pigs and cattle was isolated by direct spread of caecal sample material onto violet red bile agar and incubated for 24h at 44°C. Presumptive *E. coli* was identified using TBX agar incubated o/n at 44°C. Only one indicator *E. coli* isolate per broiler flock was selected.

For Isolation of **ESBL-, AmpC- and carbapenemase-producing E. coli** from caecal samples, the EURL-AR laboratory protocol was applied (October 2015) [<http://www.eurl-ar.eu/233-protocols.htm>]. Only one ESBL-, AmpC- and carbapenemase-producing *E. coli* isolate per broiler flock or meat sample was selected.

Enterococcus from broilers was isolate from an adequate amount of caecal material suspended in 2 ml BPW. The suspension was streaked onto Slanetz Bartley agar and incubated 48h at 44°C. Two colonies resembling typical *E. faecalis* were sub-cultivated on blood agar. Species identification was done by rtPCR-assay. Only one isolate of *E. faecalis* was selected per broiler flock.

9.4.2 Humans

Salmonella isolates were serotyped by slide agglutination according to the Kauffman-White Scheme.

Campylobacter. Species identification was performed using a species-specific PCR assay [Klena et al. 2004. J Clin Microbiol. 42: 5549-5557].

Staphylococcus aureus. Species confirmation and typing was performed by sequencing of the *S. aureus* specific *spa* gene [Harmsen et al. 2003. J Clin Microbiol. 41: 5442-5448]. *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). For MRSA isolates, presence of the *mecA* or *mecC* methicillin

resistance genes was confirmed by PCR [Larsen et al. 2008. Clin Microbiol Infect. 14: 611-614; Stegger et al. 2012. Clin Microbiol Infect. 18: 395-400]. For all isolates, presence of *lukF-PV* gene (PVL) was demonstrated by PCR [Larsen et al. 2008. Clin Microbiol Infect. 14: 611-614; Stegger et al. 2012. Clin Microbiol Infect. 18: 395-400].

Invasive Streptococcus pneumoniae. Serotype identification of invasive *S. pneumoniae* was performed by latex agglutination (ImmuLex™ Pneumotest Kit, SSI Diagnostica, Hillerød, Denmark) and further with factor specific antisera by the Neufeld Quellung test (SSI Diagnostica, Hillerød, Denmark).

Invasive Streptococcus pyogenes (group A streptococci), group B, C and G streptococci. Identification of group was performed by latex agglutination (Streptococcal Grouping Reagent, Oxoid, Roskilde, Denmark).

9.5 Susceptibility testing

Antimicrobial susceptibility testing of **Salmonella, Campylobacter, E. coli, Enterococcus and human clinical Staphylococcus aureus** was performed as microbroth dilution MIC with Sensititre (Trek Diagnostic Systems Ltd.) Inoculation and incubation procedures were in accordance with the CLSI guidelines [Clinical and Laboratory Standards Institute, USA] and the European standard ISO 20776-1:2006. The isolates were susceptibility tested in accordance with in Decision 2013/652/EU.

Relevant quality control strains were used: *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212 and *Campylobacter jejuni* ATCC 33560.

Most of the isolates from animals and meat were susceptibility tested at the DVFA laboratory. Only *Salmonella* from the surveillance of fresh meat were tested at DTU National Food Institute. Isolates were stored at -80°C until susceptibility testing. *Salmonella, Campylobacter and Staphylococcus aureus* isolates of human origin were tested at SSI. MIC-testing at DTU National Food Institute and the DVFA laboratory in Ringsted is accredited by DANAK (the national body for accreditation).

Invasive Streptococcus pneumoniae from humans.

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 Müller-Hinton agar (Müller-Hinton plate, 5% blood, 20 mg beta-NAD, SSI Diagnostica, Hillerød, Denmark). Penicillin and erythromycin MICs were determined using STP6F plate, Sensititre (Trek Diagnostic Systems Ltd.) as recommended by the manufacturer. All breakpoints used were as defined by the EUCAST. Both fully and intermediary resistant isolates were defined as resistant.

Invasive *Streptococcus pyogenes* (group A), group B, C and G streptococci from humans. 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, Roskilde, Denmark) on Müller-Hinton agar (Müller-Hinton plate, 5% blood, 20 mg beta-NAD, SSI Diagnostica, Hillerød, Denmark). Isolates were also tested for inducible clindamycin resistance. For non-susceptible streptococci the MIC was determined with E-test (Biomérieux), with either benzylpenicillin, erythromycin or clindamycin on Müller-Hinton agar. The breakpoints used were as defined by the EUCAST. Both fully and intermediary resistant isolates were categorized as resistant.

***E. coli*, *K. pneumoniae*, *P. aeruginosa*, *Acinetobacter spp.*, *E. faecium* and *E. faecalis* from humans.** Since November 2015, all Danish DCM used the EUCAST terminology with the EUCAST breakpoints and the EUCAST disk diffusion method for most species. Interpretation mainly follows EUCAST principles but for some DCM local interpretation rules are applied on the susceptibility in specific species, primarily from invasive infections (fx susceptibility to mecillinam in *E. coli* obtained from blood samples).

Data on antimicrobial resistance from private hospitals and clinics and from psychiatric hospitals were excluded. All submitting laboratories participate in national and international quality assurance collaborations such as the United Kingdom National External Quality Assessment Schemes (NEQAS).

9.6 Whole genome sequencing

Whole genome sequencing (WGS) and in silico bioinformatics tools were used to detect the genetic background of the ESBL/AmpC- and carbapenemase-producing *E. coli* phenotypes identified. The strains were sequenced using the Illumina HiSeq platform followed by de novo assembly and prediction of antimicrobial resistance genes, virulence genes, plasmid replicons and MLST using the online freely available in silico bioinformatics tool; Bacterial Analysis Pipeline - Batch Upload (version 1.0) from Center of Genomic Epidemiology (www.genomicepidemiology.org; <https://cge.cbs.dtu.dk/services/all.php>) [Thomsen et al, 2016; PLoS One 21;11(6):e0157718]. The sequences of isolates negative for all ESBL-, AmpC- and carbapenemase-encoding genes were investigated for promoter mutations compatible with up-regulation of chromosomal ampC expression by the use of the ResFinder tool (version 3.0). All genome sequences were submitted to the European Nucleotide Archive (ENA).

9.7 Data handling

9.7.1 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 electronically transferred to DTU National Food Institute. At the DTU National Food Institute, data were harmonised and one isolate per epidemiological unit was selected for reporting.

Sampling details and laboratory results from the national *Salmonella* control programmes were stored at DVFA (*Salmonella* database), whereas serotyping and results from the susceptibility testing were stored in the DTU Laboratory system. Data were collated at DTU and validated by DVFA. All data were combined in one Oracle database at isolate level (9i Enterprise Edition®). The database contained all antimicrobial data reported in DANMAP or to EFSA since 2007 (partial dataset from 2001-2006).

Variables for animal samples include bacterial species, subtype where applicable, date of sampling, species of animal and herd identifier. For each meat isolate, variables include food type, bacterial species, date of sampling, date of examination and country of origin whenever possible. MIC values were retained as continuous variables in the database, from which binary variables were created using the relevant ECOFF from 2016 for all years. Since 2007, data are interpreted by EUCAST epidemiological cut-off values (ECOFFs) 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.

All handling, validation and analysis of results were carried out using Microsoft Excel and SAS® Software, SAS Enterprise Guide 6.1.

9.7.2 Human

***Salmonella* and *Campylobacter*.** Data on *Salmonella* and *Campylobacter* infections are stored in the Danish Registry of Enteric Pathogens (SQL database) 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.

***Staphylococcus aureus*.** 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 colonization/active screening (i.e. surveillance samples to detect nasal, throat, gut or skin colonization), 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 12 months as either health-care associated with community onset (HACO) or

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 2016

Antimicrobial agent	<i>Salmonella</i>		<i>E. coli</i>		<i>E. faecalis</i>		<i>C. jejuni</i>		<i>S. aureus</i>
	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	ECOFF µg/ml
Ampicillin	>8	>8	>8	>8	>4	>8			
Azithromycin	>16 ^(a)		>16 ^(a)						
Cefepime	>0.125 ^(a)	>4	>0.125 ^(a)	>4					
Cefotaxime	>0.5	>2	>0.25	>2					
Cefoxitin	>8		>8						>4
Ceftaroline									>1
Ceftazidime	>2	>4	>0.5	>4					
Ceftobiprole					9				>1
Chloramphenicol	>16	>8	>16	>8	>32				
Ciprofloxacin	>0.064	>0.064	>0.064	>0.5	>4		>0.5	>0.5	>1
Clindamycin									>0.25 ^(c)
Colistin	>2 ^(b)	>2	>2	>2					
Daptomycin					>4				>1
Ertapenem	>0.064	>1	>0.064	>1					
Erythromycin					>4		>4	>4	>1
Fusidic acid									>0.5
Gentamicin	>2	>4	>2	>4	>32		>2		>2
Imipenem	>1	>8	>0.5	>8					
Kanamycin									>8
Linezolid					>4	>4			>4
Meropenem	>0.125	>8	>0.125	>8					
Mupirocin									>1
Nalidixic acid	>16		>16				>16		
Norfloxacin									>4
Penicillin									>0.125
Rifampicin									>0.03
Streptomycin							>4		
Sulfamethoxazole/ Trimethoprim									>0.5
Sulfonamide (Sulfamethoxazole)	>256		>64						
Teicoplanin					>2	>2			
Tetracycline	>8		>8		>4	>0.5	>1	>2	>1
Tigecycline	>1	>2	>1	>2	>0.25	>0.25			
Vancomycin					>4	>4			>2

Note: EUCAST epidemiological cut-off values (ECOFFs) and EUCAST clinical breakpoints listed unless noted

a) The EUCAST ECOFF (>2) for colistin was applied for *S. Typhimurium* and other serotypes, except for *S. Enteritidis* and *S. Dublin* where ECOFF >8 was applied according to investigations presented in DANMAP 2011

b) No current EUCAST ECOFF is available, apply complementary interpretative thresholds as suggested by EFSA [EFSA Supporting publication 2017:EN-1176]

c) Inducible clindamycin resistance is included

community-acquired (CA). Health-care associated risk factors included prior hospitalizations, 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.

***Streptococcus pneumoniae*, *Streptococcus pyogenes* (group A streptococci), group B, C and G streptococci.**

Data on susceptibility testing of isolates were stored as MICs in a Microsoft® Access database linked to a SQL server at SSI. Analysis including selection of isolates from blood and spinal fluid samples and removal of duplicate isolates was performed in Microsoft® Access.

***Streptococcus pyogenes* (group A streptococci), group B, C and G streptococci.** Data on susceptibility testing of isolates were stored as inhibition zone diameters and if indicated also MICs in a Microsoft® Access database linked to a SQL server at SSI.

***E. coli*, *K. pneumoniae*, *P. aeruginosa*; *Acinetobacter spp.*, *E. faecium* and *E. faecalis*.** All DCM in Denmark provided data on resistance levels in *E. coli*, *K. pneumoniae*, invasive *P. aeruginosa*, invasive *Acinetobacter spp.*, invasive *E. faecium* and invasive *E. faecalis* isolates. Data were extracted from the following laboratory information systems:

ADBakt (Autonik AB, Skoldinge, Sweden) for the DCM at Hvidovre, Herlev and Aalborg Hospitals.

MADS (DCM Skejby Hospital, Aarhus, Denmark) for the DCM at Rigshospitalet, Slagelse/Region Zealand, Odense, Sønderborg, Esbjerg, Vejle, Herning/Viborg, and Aarhus (Skejby) Hospitals.

Resistance data on the first isolate per patient per year were included. Generally, resistance data were excluded if susceptibility to a certain antimicrobial agent was tested on only a selected number of isolates.

9.7.3 Statistical tests

Significance tests of differences between proportions of resistant isolates were calculated using SAS®Software, SAS Enterprise Guide 6.1 using univariable Chi-square, or Fisher's Exact Tests as appropriate.

All changes and differences yielding $p < 0.05$ were commented on in the text, whereas the remaining data was visualised in figures or tables only.

Some types of resistances were looked for, but not found by the DANMAP surveillance system, yielding a prevalence of zero. It is not possible for surveys to prove freedom from diseases or resistances in populations, but with a defined confidence, surveys can identify the maximum possible prevalence given that the survey failed to find any positives. This maximum prevalence was calculated for the report using 95% confidence and assuming a perfect test by a probability formula to substantiate freedom from disease via online epitools. ausvet.com.au (Cameron and Baldock 1998, Prev. Vet. Med).

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10

TERMINOLOGY

List of abbreviations

AGP	Antimicrobial growth promoter
ATC	Anatomical Therapeutic Chemical Classification System
ATCvet	Anatomical Therapeutic Chemical Classification System for veterinary medicines
BSI	Blood Stream Infection (in humans)
CA	Community-acquired
CC	Clonal complex
CDI	Clostridium difficile infections
CHR	Central Husbandry Register
CI	Confidence interval
CNS	Central nervous system
COHA	Community onset hospital-acquired
CPE	Carbapenemase producing Enterobacteriaceae
CPO	Carbapenemase producing organisms
CPR	Danish Civil Registry, register for social security numbers
DAD	Defined Daily Doses per 100 admissions
DADD	Defined animal daily dose
DAPD	Defined animal daily dose per 1,000 animals per day
DBD	Defined Daily Doses per 100 occupied bed-days
DCM	Department of Clinical Microbiology
DDD	Defined Daily Dose
DID	Defined Daily Doses per 1,000 inhabitants per day (DDD/1000 inhabitant days)
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
ESBL	Extended spectrum beta-lactamase
GI	Gastrointestinal
GP	General Practitioner
HAI	Hospital-acquired infections
HAIBA	Hospital-acquired infections database
HLGR	High-level gentamicin resistance
HOHA	Hospital onset hospital-acquired
MIC	Minimum inhibitory concentration
MRSA	Methicillin-resistant Staphylococcus aureus
N	Number of samples
n	Number of isolates tested for antimicrobial susceptibility
OIE	World Organisation for Animal Health
RFCA	Regional Veterinary and Food Control Authorities
SAB	<i>Staphylococcus aureus</i> bacteremia
SEGES	Knowledge Centre for Agriculture
S-I-R	interpretation scale for results from resistance testing noting "susceptible-intermediary-resistant"
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
WGS	Whole-genome sequencing
WHO	World Health Organization

Glossary

Anatomical Therapeutic Chemical (ATC) classification.

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]. Antimycobacterial agents are not included. In the section of 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 usually 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).

Broiler. A type of chicken raised specifically for meat production. In Denmark, the average weight after slaughter is 1.51 kg.

Central Husbandry Register (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 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). In contrast to the ADD previously used in DANMAP, the DADD has not been defined for each antimicrobial agent, administration route and animal species but at product level. In DANMAP 2012, the DADD replaces the ADD (as defined in VetStat), which had been used since DANMAP 2003. For more details, see Chapter 9, Materials and Methods. The DADDs used in DANMAP 2015 are presented in the web annex.

DADD per 1000 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 life-span. 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 (Section 4.3 and Chapter 9, Materials and Methods).

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/indexdatabase/).

DDD per 1000 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 account of hospital activity (discharges). 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 indicates that 1% of the population on average gets a certain treatment daily. In figure presented as DDD/1000 inhabitant-days.

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.

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 antimicrobial classes. The number of antimicrobial classes and antimicrobial agent included therein depend on the test panel for each bacterium.

Pets or pet animals. Dogs, cats, birds, mice, Guinea pigs and more exotic species kept at home for pleasure, rather than one 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



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