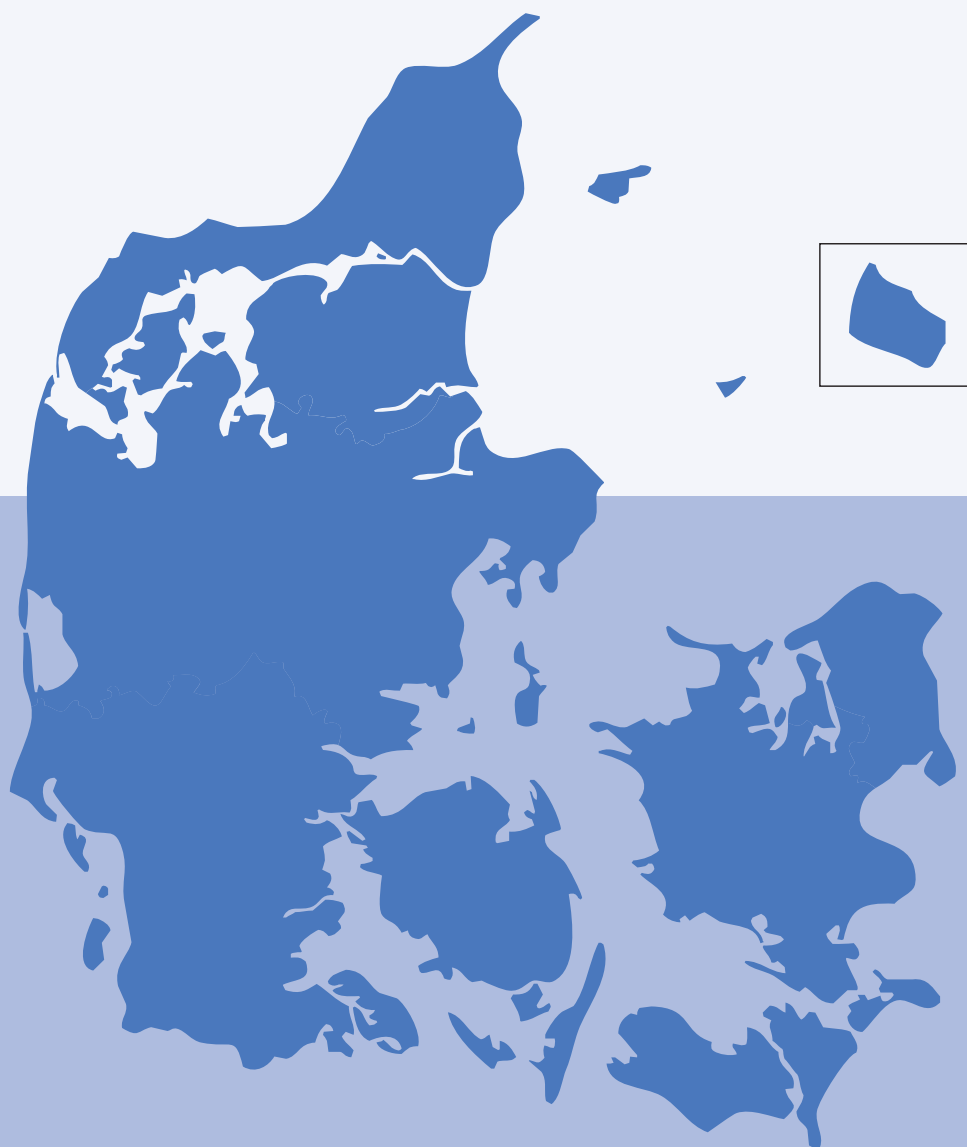




DANMAP 2015

DANMAP 2015 - Use of antimicrobial agents and occurrence
of antimicrobial resistance in bacteria from food animals,
food and humans in Denmark



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DANMAP 2015

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Printing: STEP

DANMAP 2015 - November 2016
ISSN 1600-2032

Text and tables may be cited and reprinted only with reference to this report: DANMAP 2015
- Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food
animals, food and humans in Denmark. ISSN 1600-2032

The report is available from www.danmap.org

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 2015. 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 2015

DANMAP 2015 - Use of antimicrobial agents and occurrence
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1. Editorial

Welcome to DANMAP 2015, a 20-year anniversary edition of the Danish surveillance programme of antimicrobial resistance and consumption in humans and animals. The report includes the annual surveillance and a jubilee chapter presenting the main milestones and experiences from the last 20 years. The jubilee chapter also outlines suggestions on monitoring of antimicrobial resistance (AMR) in the future. The layout of the report has changed slightly compared to previous years: Each chapter commences with a short introduction to most important findings and highlights of 2015. Most chapters also include the most significant findings in their area from the last 20 years. This has made the main summary obsolete.

DANMAP was developed making the most of a collaborative spirit between stakeholders and with a common understanding of AMR as a serious health threat requiring a One Health approach to counter - because humans, foods and animals constitute overlapping reservoirs of antimicrobial resistance.

The driver for DANMAP was the concern that use of the antimicrobial growth promoter avoparcin posed a risk for occurrence of vancomycin resistant enterococci (VRE) in hospitals. These concerns led to intensive research and the Danish ban on avoparcin in 1995, eventually followed by termination of the use of all antimicrobial agents for growth promotion in the entire EU from 2006. You can read more about the collaboration and its impacts on the Danish and international animal and human welfare in the jubilee chapter on page 9.

A circle closes, a new one begins. VRE has re-appeared on the health agenda, since 2013 in the form of hospital outbreaks in the Capital Region of Denmark and in the Region of Zealand. This report describes whole-genome sequencing of VREs, which points towards a niche in the hospital environment, where certain subtypes of these bacteria thrive (textbox 8.3).

The biggest emerging threat we are facing is the occurrence of carbapenemase-producing Enterobacteriaceae (CPE) and other carbapenemase-producing organisms (CPO) in Danish hospitals (textbox 8.2). Spread is not only restricted to specific species but also includes their resistance genes, which move between the different bacterial species with ease. As for the VRE there is no known or suspected animal or food reservoir of the CPE and CPO in Denmark. Although only sporadic cases and outbreaks have been reported, the numbers are increasing at a frightening speed, and spread not only within departments but between hospitals has been observed. This demands an intensification of all efforts, if we are to control the situation. While some departments have begun screening, others have not yet encountered CPE in their patients. Thus a new surveillance programme for these resistance genes is needed, if we want to stop the spread in time. The questions are: How do we both extend and restrict the screening to cover all relevant patients

and departments in a timely national surveillance programme? And should screening procedures, for the first time, include hospital staff at exposed departments? - A decision to carry out such screening would be in conflict with the protection of the individual worker and will demand ethical discussions as well.

Once again, we need to draw on our experience and knowledge from 20 years of collaboration and research. Here we benefit from our experience with methicillin-resistant *Staphylococcus aureus* (MRSA). During the last eight years, livestock-associated MRSA (LA-MRSA) has been DANMAP's biggest One Health encounter. The knowledge gained from an extensive surveillance and research programme on MRSA has enabled definition and control of some of the health problems that arise from transmissible resistant bacteria. It also supports development of targeted efforts for protection of the vulnerable population at hospitals. An example is the newest version of "The National Guidelines on Prevention of Transmission of MRSA" published by the Danish Health Authority.

Characterization of possible zoonotic transmission links of *Escherichia coli* and their many resistance mechanisms has proven more difficult and challenging than for MRSA. In Denmark, no specific direct transmission route from food to humans has been identified and we suspect a complex link to exist between animals and humans. This link might only consist of transmission of the resistance genes and not necessarily the bacterial strains. Several attempts have been made to establish these possible transmission routes and the latest attempt is described in textbox 7.3.

It is important to remember that most surveillance programmes have gaps and primarily find what they are designed to look for. The task is to discover new or unknown resistances in a timely manner, which requires special surveillance designs. One of the newest examples is the discovery of the *mcr-1* gene conferring resistance to colistin. Historically, DANMAP has not had focus on colistin consumption and resistance in animals as the drug was rarely used in humans, though it had been reintroduced due to the occurrence of CPE. The finding of the *mcr-1* genes in Chinese meat and humans in August 2015, with a possible high rate of transfer even between different species, was alarming (Liu et al, 2015). Using the existing ESBL-surveillance data from DANMAP, Denmark was the first country outside of China to identify *mcr-1* among sequences from human blood and animal indicator *E.coli* isolates in November 2015 [Hasman et al, 2015]. Since then, it has been described by most European countries in human and animal isolates, some of which date back as early as the 1980s.

In the last 20 years, technology, methodology and interpretation of antimicrobial resistance identification have developed

massively. Today, fast track systems such as whole-genome sequencing present a wide variety of opportunities for the future. Interpretation and standardization of these massive data files will be the challenge over the next decade. It is important to keep in mind that the discovery of a resistance gene not necessarily renders that specific strain resistant to the antimicrobial. We therefore need to keep qualified resistance testing methods like broth dilution for determination of phenotypic colistin resistance in bacteria and ensure robust and representative comparative studies, before we choose to abandon classic and standardized methods.

There are several lessons to be learned from the emerged mobile colistin resistance:

Firstly, surveillance has never been more important - and no national surveillance and control system, no matter how good, is adequate in the face of a global AMR threat. We need global interactions and interventions, also in the fight for a more prudent use of antimicrobial agents in both veterinary and human medicine.

Secondly, it is clear that minor antimicrobials may suddenly rise to prominence. This applies to colistin but may apply to other antimicrobial classes as well. An example is the use of coccidiostats, which is monitored by VetStat, but has not been reported since 2004 as we have focused on prescription antimicrobials. In DANMAP 2015, data on the use of coccidiostats are shown in textbox 4.1.

We believe that AMR levels in Denmark would have been significantly higher without the detailed research and monitoring of antimicrobial consumption in food animals and in humans. This has provided significant tools for timely decision-making for authorities and other stakeholders. In other words, DANMAP provides data for action.

Today we acknowledge the need for new and better surveillance tools and especially the need for a more timely dissemination of the results to medical and veterinary professionals. In this respect, other countries with younger models and systems might be of inspiration to us. As we face a pandemic and extremely costly threat from AMR [O'Neill, 2016] we need to reinvent our model of "data for action".

We are thankful for all the collaboration, formal and informal, that has developed and stabilised through the years, in Denmark and around the world. We would like to thank all contributors to this edition of DANMAP without whom, the report would not exist.

On the threshold to the next twenty years, we would like to ask: The times they are a-changing - are we able to keep up?

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References

- Lui YY, et al. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. *The Lancet Infectious Diseases*. Retrieved 2015-11-19
- Hasman H et al. Detection of mcr-1 encoding plasmid-mediated colistin-resistant *Escherichia coli* isolates from human bloodstream infection and imported chicken meat, Denmark 2015. *Euro Surveill*. 2015;20(49). doi: 10.2807/1560-7917.ES.2015.20.49.30085.
- O'Neill J. Tackling drug-resistant infections globally: Final report and recommendations 2016. Wellcome Trust, and HM Government, United Kingdom. <http://amr-review.org/>.

Acknowledgements

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

- the meat inspection staff and the company personnel at the participating slaughterhouses for collecting samples from animals at slaughter. Without their careful recording of the animals' farm of origin, the results would be less useful;
- the Laboratory of Swine Diseases, the Danish Agriculture and Food Council, Kjellerup, and the DTU National Veterinary Institute for making isolates of animal pathogens available to the programme;
- the staff of the Regional Veterinary and Food Control Authorities for collecting food samples and isolating bacteria;
- the Department of Medication Statistics and Research Support at SSI (formerly the Danish Medicines Agency) for collecting and transmitting data on veterinary consumption of antimicrobial agents from the pharmacies;
- the Danish Veterinary and Food Administration for collecting and transmitting data on veterinary consumption of antimicrobial agents from VetStat, including statistics on consumption measured in tonnage; and
- the Danish Agriculture and Food Council for cooperation regarding the estimation of live biomass of production animals.

Statens Serum Institut would like to thank the following:

- the staff of the Neisseria and Streptococcus Typing Unit at SSI;
- the staff of the Foodborne Pathogens Unit at SSI;
- the staff of the Staphylococcus Laboratory at SSI;
- the staff of the Antimicrobial Resistance Reference Laboratory and Surveillance Unit at SSI;
- Maja Laursen from the Department of Data Delivery and Medicinal Product Statistics and Erik Villadsen, both at Danish Health Data Authority.
- the Departments of Clinical Microbiology and the DANRES group - Danish Study Group for Antimicrobial Resistance Surveillance - for providing data on resistance in bacteria from human clinical samples;

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2

**DANMAP - A 20 YEAR
PERSPECTIVE**



2. DANMAP – a 20 year perspective

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Introduction

DANMAP – the Danish Antimicrobial Resistance Monitoring and Research Programme – was a reaction to concerns about how antimicrobials were used in food animal production in Denmark in the mid-1990s and what the consequences might be for human and animal health. It came in the wake of a national food crisis caused by a steep increase in human salmonellosis incidence that could be traced back to domestic food animal production. At the same time Europe was struggling with the BSE crisis that originated in the UK.

Based on an idea of a One Health perspective – the purpose of which is to unite methods and knowledge and share surveillance data between sectors for a better health for humans and animals – the first samples for DANMAP were collected in 1995 and published in an interim report in 1996. The One Health paradigm was a pioneer concept and the specific design used in the DANMAP programme was state-of-the-art at the time. The design has only changed slightly over the past 20 years. It is, however, becoming quite clear that antimicrobial resistance is no longer an issue that can be handled adequately at the local regional or national level. Today, the world faces a global antimicrobial resistance pandemic that might exert devastating effects on both human and animal health. A British report on antimicrobial resistance estimates that the costs due to increased illness and deaths and a subsequent fall in productivity amount to 100 trillion dollars by 2050 (O'Neill, 2016). So, while local measures to control antimicrobial resistance are indispensable, they are not sufficient in the face of a pandemic.

As DANMAP celebrates 20 years of existence, we describe some highlights and key elements from the programme and present a perspective on where to go from here.

Antimicrobial growth promoters

Antimicrobial agents were included in feed for food animals as early as the late 1940s in order to improve growth and reduce production costs. In these early days the positive effects observed – initially when feeding waste from production of tetracyclines to chickens – may partly have been due to the improvement of low quality feed in general, but the procedure

of including sub-therapeutic concentrations of antimicrobials in animal feed was soon integrated as an established practice in several countries [Moore, 1946; Dibner, 2005].

Following the introduction of the first antimicrobial agents for human clinical treatment, resistance very rapidly emerged. However, as several new antimicrobial classes were discovered in the late 1940s and early 1950s, this was not considered a major problem. During the 1960s, bacteria resistant to several antimicrobial classes were discovered, while emerging treatment failures in humans gave the first warnings that resistance might outrace the discovery of new antimicrobials.

In food animals high levels of resistance were observed, to some extent related to the use of antimicrobial growth promoters (AGPs). In 1969, the Swann Committee in the United Kingdom considered the available evidence and clearly concluded that the use of AGPs selected for resistance that was transferable between bacterial species and could cause problems for human health [Swann et al, 1969]. The report recommended that antimicrobial agents used for therapy should not be used for growth promotion. Following this, a number of antimicrobial agents were banned for use as AGPs in the UK. After the UK entered the European Union (EU) the ban was implemented in the other European Union member states. In other countries, including the USA, similar recommendations were issued by the authorities, but restrictions were never implemented.

One important thing, however, appears to have been overlooked, namely that different antimicrobial agents belonging to the same drug class will select for the same antimicrobial resistance mechanisms. Furthermore, it was not considered that other antimicrobial classes already being used as AGPs might become important for human treatment as new resistance mechanisms emerge. Consequently, a number of new antimicrobial agents were approved and used as AGPs in the EU and other countries in the 1970s and 1980s.

Discussions of potential problems associated with this continued use surfaced from time to time, but it was not until the mid-1990s, when new studies found new evidence, that larger scale interventions occurred.

Resistance to glycopeptides

In 1993 enterococci resistant to the glycopeptide antimicrobial vancomycin were isolated from food animals in England and soon after in 1994 in Germany [Bates et al]. This was surprising because no vancomycin or other glycopeptides was approved for therapy in food animals. However, the glycopeptide avoparcin had been used for several years as an AGP.

In Denmark, we first became aware of this in December 1994 and, based on a small survey of organic and conventional broiler flocks, it was in January 1995 suggested that occurrence of vancomycin resistant enterococci (VRE) was associated with the use of avoparcin [Aarestrup, 1995]. The use of antimicrobial agents for food animals - including therapeutic use - had been an issue of increasing concern in Denmark since the early 1990s. The new findings resulted in much scientific and public attention and already in April the Danish agricultural organizations decided to voluntarily stop the use of avoparcin. This move was followed by a ban imposed by the Minister of Agriculture in May 1995.

Within the European Union AGPs were approved centrally by the European Commission's Scientific Committee on Animal Nutrition (SCAN). Denmark was allowed to ban avoparcin only if new information was able to show the danger to human health. Initial research, conducted within a short time-frame in Denmark, had clearly demonstrated that the use of avoparcin selected for occurrence of VRE, that these bacteria were found in and were transferable through food, and that the responsible gene was transferable. However, the direct consequences for human health were difficult to prove definitely.

The Danish ban was highly controversial and not supported by the European Commission's Scientific Committee on Animal Nutrition. However, the ban was upheld by the European Council of Ministers and as additional scientific evidence accumulated, similar restrictions were implemented by other countries, e.g. by Germany in January 1996. In 1997 a general EU ban was implemented.

These initial findings prompted a number of initiatives in Denmark and elsewhere. Research into the potential consequences of other AGPs was initiated and all use of AGPs has been banned by EU member states since 2006. In addition, it was realized that one of the reasons that these problems had passed undiscovered for so many years was the complete lack of any monitoring programmes. Thus, the DANMAP programme was established, first including samples from food animals only in 1995 and extended to food and humans in 1996.

Escherichia coli - the ideal sentinel organism

Escherichia coli is a common member of the intestinal and faecal flora of most vertebrates including humans, although outnumbered 1000 : 100 : 1 by many Gram-positives and anaerobes in the gut flora. Usually *E. coli* lives in happy collaboration with the other bacteria in the gut, without causing

harm to its host. However, if it gains access to the urinary tract or other organ systems or worse, to the blood stream, *E. coli* can be a deadly encounter for the host. This opportunistic pathogen is the most commonly found bacterial agent in bacteraemia or septicaemia, as it is present in around 30% of all positive blood cultures in human patients in most of the world. It is also by far the most common pathogen found in urinary tract infections (UTI), one of the most frequent occurring bacterial infections in humans both in primary care and in hospitals. In addition, diarrhoeagenic *E. coli* dominates among aetiologies for bacterial diarrhoeal diseases, not least traveller's diarrhoea. The bacterium is very versatile and can carry a broad range of virulence factors on its chromosome or on several different plasmids in the same cell. The virulence factors help the bacterium adhere to, penetrate and survive in epithelial cells, spread in tissues, gain access to blood vessels, escape the immune system, etc.

E. coli is a potential zoonotic pathogen, although not in the same strict sense as non-typhoid *Salmonella enterica*, which within certain limits is an obligatory pathogen for humans, causing infections via intake of food. Being ubiquitous in most production animals and their environment including wastewater and vegetable products, *E. coli* is commonly introduced into the human gut on a daily basis and often in vast amounts. Most humans carry between 1 - 6 different clones of *E. coli* in the gut at all times [Nielsen et al, 2014], meaning that there is a constant flow and replacement of these bacteria. Furthermore, these *E. coli* may interact with other Gram-negative bacteria in the gut and exchange genetic information.

From the viewpoint of antimicrobial susceptibility, the naïve *E. coli* strain is susceptible to most antimicrobials with anti-Gram-negative activity. It is therefore relatively easy to treat when infections occur, even with sulfonamide, the first chemotherapeutic antibiotic drug introduced in the 1930's. On the other hand, *E. coli* can easily mutate or take up foreign DNA by all mechanisms, i.e. transformation, transduction and conjugation, the latter being by far the most common.

E. coli is an ideal bacterial organism to study because of the relationship between antimicrobial use and the development of antimicrobial resistance. This is due to *E. coli*'s uptake and presence in the heavily populated gut flora with availability of millions of resistance genes, its ease in developing resistance towards the most important antimicrobial classes, and the fact that all antimicrobials administered systemically will reach the gut either directly from the stomach or indirectly via blood to the intestines and their lumen.

E. coli in DANMAP

When planning DANMAP, the aim was to encompass antimicrobial resistance in important pathogenic bacteria that were present in production animals, meat and in humans. It was therefore obvious that *E. coli* would have a pivotal role. Since the first DANMAP report in 1996, antimicrobial resistance

has been monitored in *E. coli* both as commensals sampled from poultry, pigs and cattle, and meat products from these animals (so-called *indicator* strains), as well as pathogens from diagnostic samples from production animals (*diagnostic animal* strains) and from blood and urine from infected humans (*diagnostic human* strains). Furthermore, within the frame of the DANMAP programme *E. coli* has been followed as commensal in gut flora from various cohorts of healthy humans (military recruits, nurses, random healthy volunteers etc.) and from environmental samples from wastewater and sludge. (See chapter 8.1. for more information on the *E. coli* from clinical submissions and textbox 7.2 for sampling from healthy humans during the years).

Collections of preserved *E. coli* isolates from all of the above origins have since been used in numerous research projects. Various aspects of the bacterium have been studied: virulence genes, antimicrobial resistance genes, resistance fitness-cost, *E. coli* clonal types and their possible spread from animals directly or via food to humans, in most cases taking into account antimicrobial use in animals and in humans. As one example, for the purpose of determining the role of animal derived *E. coli* strains in urinary tract infections in humans, a large DANMAP collection of *E. coli* strains from cattle, pigs, poultry and meat products from these animals was used and compared to a collection of *E. coli* isolates from healthy humans and human urinary tract infections. Comparison of virulence genes, phylotypes, pulse-field gel electrophoresis and antimicrobial susceptibility demonstrated complete overlap between animal or meat and humans for some isolates and, in general, antimicrobial resistance markers corresponded among all groups, which lead to the conclusion that *E. coli* UTI in humans can be a zoonosis [Jakobsen et al, 2012].

Antimicrobial resistance monitoring - caveats

With respect to using monitoring of antimicrobial resistance levels in *E. coli* as a basis for understanding the relationship to antimicrobial consumption, for use in policies, intervention purposes or for treatment guidelines, respectively, it is extremely important to take into account the origin of isolates, i.e. the sampling strategy.

In animals and food, antimicrobial resistance in *E. coli* has been evaluated in isolates from samples obtained using randomized sampling strategies - collecting samples from healthy animals at slaughter or fresh meat at wholesale and retail outlets. In contrast, the treating veterinarian or physician mostly obtains clinical samples of *E. coli* from infected animals or humans. This results in a tendency towards a skewed prevalence of antimicrobial resistance, since GPs may tend to submit samples from problematic patients only and not from those who respond to first-hand empiric antimicrobial treatment. This was shown in a study, where general practitioners were asked to submit all *E. coli* samples from patients with urinary tract infections irrespective of whether these were uncomplicated or compli-

cated, i.e. recurrent infections or infections not responding to first-line antimicrobials [Kern et al, 2002].

The study demonstrated that *E. coli* from the urine of patients with uncomplicated urinary tract infections showed resistance levels about 50% lower than isolates from complicated cases e.g. resistance towards ampicillin 20% versus 40%, sulfonamide 20% versus 40%, and trimethoprim 10% versus 20%, respectively [Kern et al]. The higher resistance levels correspond to those levels usually reported in DANMAP reports in *E. coli* from urinary tract infections from primary care and hospitals as well as from bacteraemia cases (See all DANMAP reports from 1996 - 2015, chapter 8 on clinical resistance in humans). Several investigations from Danish clinical microbiology laboratories also showed, that the first *E. coli* isolate from a patient usually is more susceptible than later isolates from the same patient (Thøger Gorm Jensen, Henrik Schønheyder, Jens Kjølseth Møller, personal communication). All the above adds a cautionary note for the use of antimicrobial resistance monitoring data for research and policy purposes.

Relationship between antimicrobial use and resistance

Even so, DANMAP has amply demonstrated a relationship between antimicrobial use and antimicrobial resistance levels with *E. coli* as the indicator bacterium both in production animals and in humans. Figure 1 illustrates in rough terms the relationship between antimicrobial consumption by species and the resistance levels in *E. coli* from poultry, cattle and pigs when taking into account the size of the biomass treated. Diagnostic samples show the highest resistance levels. Among indicator *E. coli*, resistance levels are clearly highest in pigs, which have the highest antimicrobial use both for treatment and meta-prophylaxis.

An example from humans is illustrated in Figure 2, which shows ciprofloxacin use in primary health care and ciprofloxacin resistance in *E. coli* from urine samples in primary care. Both sets of data are from 2000 - 2015 (modified from this year's report). There is a clear correlation association between the increase in use and concomitant increase in resistance from 2000 - 2009, after which a levelling off in the resistance level reflects a levelling off in the consumption from 2009 and onwards. One of the reasons for the rapid increase in ciprofloxacin use during the '00's was a significant reduction in price of ciprofloxacin after the market was opened to generic ciprofloxacin in 2002 (DANMAP report 2003).

These examples of a relationship between antimicrobial use and resistance led to policy changes both in the veterinarian and the human field, e.g. a general warning against widespread use of ciprofloxacin in primary care in Denmark was issued by the Danish Health Authority (Sundhedsstyrelsen) in 2012.

Novel antimicrobial resistance mechanisms

As new resistance mechanisms have emerged in the world, DANMAP has been used to evaluate the impact of these resistance determinants in all fields in Denmark. The occurrence of extended spectrum beta-lactamase (ESBL) in *E. coli* was reported from Europe already in the 1980s, but did not reach Denmark on a larger scale until around 2005.

The first ESBL-positive *Klebsiella* isolates from humans were reported in 1998 [Hansen et al, 1998]. A prospective study of isolates from blood cultures at Herlev Hospital in 2003 found three isolates (0.8%) of 380 consecutive *E. coli* and *Klebsiella* spp. [Kjerulf et al, 2008]. The first ESBL-positive isolates in food animals was a *Salmonella Heidelberg* isolate from a boar in 2003 imported from Canada [Aarestrup et al, 2004]. Since then, several studies have been conducted to evaluate the importance of ESBL-producing *E. coli* in animals and humans. Resistance to 3rd generation cephalosporins in *E. coli* as a marker for ESBL-production was introduced in DANMAP 2009. While CTX-M-15 has been the dominating genotype among *E. coli* and *Klebsiella* spp. in humans (around 50-70% of all ESBLs) this type has rarely been found in Danish production animals, indicating that little transfer of these types occur from Danish

husbandry to humans. Resistance towards 3rd generation cephalosporins has been around 5-6% in *E. coli* from urine cultures from 2010 - 2015 although there is a slight increasing tendency in resistance in urines from primary health care. The lack of steep increases in resistance seems to indicate that policy changes in response to surveillance and research, e.g. recommendations on reduced use of cephalosporins and other broadspectrum antibiotics in antimicrobial guidelines, have had an impact, probably associated with implementation of measures on infectious disease control.

The new fluoroquinolone resistance gene, *qnr*, was reported for the first time in Denmark in a collaborative veterinary and human research project under DANMAP in 2007 [Cavaco et al, 2007].

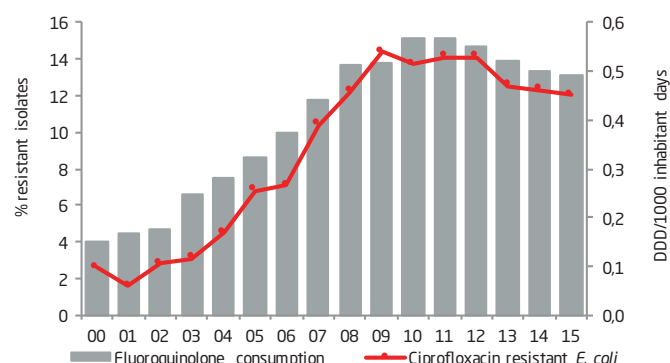
The new global threat from carbapenemase-producing Enterobacteriaceae (CPE) has also been recognised in the Danish community since 2008. This is now under intense scrutiny at Statens Serum Institut (SSI), which offers whole-genome sequencing on all submitted carbapenemase-producing bacteria from clinical laboratories. Until 2014, most of the cases were related to travel abroad, but from 2014 for many cases no

Figure 1. Resistance (%) in indicator and diagnostic *Escherichia coli* from pigs, cattle and broilers, Denmark



Note. Example of relationship between antimicrobial resistance in *E. coli* found from healthy pigs, cattle and broilers (indicator *E. coli*) and from diagnostic samples (pigs and cattle) from 2001-2008. The total antimicrobial consumption and total meat/milk production for 2008 [DANMAP 2008] is also presented.

Figure 2. Resistance (%) in *Escherichia coli* from urine samples and fluoroquinolone consumption, data from primary health care, Denmark



relation to travel could be established [DANMAP report 2014 and Textbox 8.2 in DANMAP 2015]. A long-term outbreak (more than 4 years) with NDM-1 producing *Citrobacter freundii* and further spread to other Enterobacteriaceae has been reported (Hammerum et al, 2016). The cooperation between the clinical microbiology departments and SSI via the so-called DANRES-network seems to assure that all laboratories screen for meropenem-resistance in Enterobacteriaceae and other selected Gram-negative pathogens in all clinical samples and submit suspected isolates to SSI for epidemiological surveillance. Carbapenem use in hospitals has also been restricted via the Danish Health Authority's 2012 warning.

In 2015, a transferable colistin resistance, encoded by the *mcr-1* gene, was reported from China. Also in Denmark the *mcr-1* gene was detected from the DANMAP 2014 ESBL-producing *E. coli* collections from both veterinary and human origins [Hasman et al, 2015] and phenotypical resistance testing for colistin is now offered at the reference laboratory at Statens Serum Institut. So far no phenotypically resistant strains have been found.

Monitoring antimicrobial consumption

For many years Denmark has had a restrictive approach to the use of antimicrobials. In human medicine there were never any over-the-counter sales of antimicrobials. Rather, they are available only through licensed pharmacies on prescription from a medical doctor or dentist. Pharmaceutical companies are free to set any price for their products. However, in Denmark there is a high degree of transparency in price structures, with the mark-up by pharmacies defined through regulation. Particularly, medical professionals do not derive any significant part of their income from the sale of antimicrobials, i.e. there is no financial incentive to overprescribe.

The supply infrastructure for antimicrobials in the agricultural sector closely reflects this. Since 1995 veterinarians in Denmark have earned their income from offering specialized

diagnostic services, and treatment, not from the sale of medicines. The mark-up for veterinarians dispensing antimicrobials directly to the animal owner, is by regulation non-profit.

Veterinary use of antimicrobials

The legal infrastructure underpinning the supply chain is of key importance for the implementation of VetStat, the Danish system for monitoring the use of antimicrobials in food animals at species and herd level. Monitoring of antimicrobial usage was part of the original DANMAP brief from 1995.

In 1995, data on the use of antimicrobials in the veterinary field were available from the Danish Medical Statistics and - for extemporaneous mixtures (magistral formulas) - from production pharmacies and wholesalers. It was clear, however, that consumption of therapeutics had increased sharply during the second half of the 1980s and the early 1990s to the extent that the National Pig Producers' Association became concerned. They formed a stakeholder committee to analyse the situation and in a report from November 1995 [N.E. Rønn, ed., 1995] concluded that use of antimicrobials in pigs had increased by 91% between 1986 and 1994, in particular as oral medication, mainly tetracycline. The committee was of the opinion that the increased consumption largely represented overuse and was not justified by disease patterns or increased production. The report describes a number of drivers contributing to the increase, among them the difficulty of monitoring by the authorities the compliance of farmers and veterinarians with existing legislation.

The intention to implement VetStat was announced in September 1998 and the system went live on 1 August 2000. The system was designed as an add-on to the national farm register (CHR) which contains data on all farms in Denmark, including animal species present on the farm and their numbers (or a surrogate measure, eg. the number of pen places). This provides denominator information for reporting of use. The objective of VetStat was to provide close to real-time data on all use of prescription medicines in production animals, at farm and species level (cattle, small ruminants, pigs, poultry, aquaculture, fur animals and other). Also, the target age group is recorded as is the reason for prescribing.

The collection of data for VetStat is shown in Figure 3. In 2015, approximately 95% of consumption was represented by prescriptions that were processed at pharmacies. At the pharmacy, the prescription is processed electronically and relevant data extracted and sent to The Danish Health Data Authority. Data are forwarded to VetStat on a daily basis for production animals. Use by veterinary practitioners account for about 3% of the total use. Data on this are harvested automatically when veterinarians invoice their clients. The data are sent to VetStat via the Dairy Associations database (for historic reasons). Finally, about 2% of the antimicrobial consumption is in the form of medicated feed. Medicated feed is produced by

feed mills on the basis of a veterinary prescription and data are reported directly to VetStat. Medicated feed is used mainly in aquaculture.

The only exception to the rule that antimicrobials are prescription-only is the use of coccidiostats. The consumption of coccidiostats is reported by feed mills to VetStat.

Combining information contained within CHR about herd size and type with data on the antimicrobial prescribed, including recommended dosages, allows VetStat to monitor total usage as Animal Defined Dosages [Jensen et al, 2004]. This metric is highly attractive in terms of permitting comparison of usage over time, even when patterns of use of antimicrobials with different potency vary over time. It also makes it possible to show which part of production is associated with a particularly high use of antimicrobials, eg. use in weaner pigs is much higher than in older age groups. In addition, relevant changes in dispensation form (eg. parenteral vs. oral) can be extracted from the database.

Interventions in the animal sector

Data on the use of antimicrobials have been reported annually in the DANMAP reports (www.DANMAP.org) and have formed the basis for a number of interventions by stakeholders – primarily the relevant authorities, but also the animal industry associations.

Figure 4 shows a timeline on interventions to reduce antimicrobial consumption. Among the most notable in the veterinary field are

- **Implementation of Veterinary Advisory Service Contracts (VASC)**

The first statutory order on VASC in herds of cattle and pigs came into force in 1995. Since then, the concept has been improved and extended several times. In 2010, VASC became mandatory for large herds of cattle and pigs as well as mink farms. The VASC results in frequent veterinary advisory visits and provides the farmer with extended treatment possibilities. The most important aims of the VASC are to focus on advice and prevention of illness rather than treatment, to optimize the use of antimicrobials in order to minimize antimicrobial resistance and to improve animal welfare.

- **Stop for use of antimicrobial growth promoters**

Since 2006, use of AGPs has been illegal within the EU. DANMAP has helped document the effect of the ban on antimicrobial resistance.

- **Prudent use guidelines in pigs and cattle**

Official guidelines on the use of antimicrobials in pigs and cattle have been available since 1996. They were initially developed by the National Veterinary Laboratory (now DTU

Figure 3. Harvesting data on antimicrobial consumption in animals for VetStat

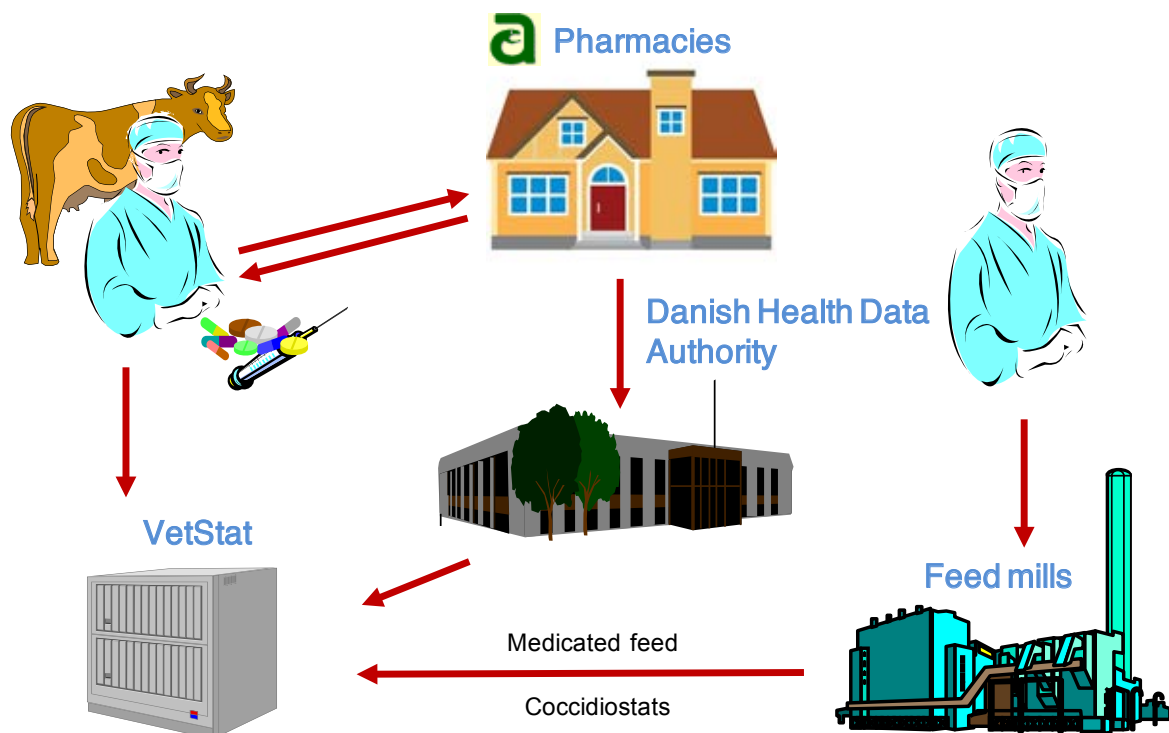
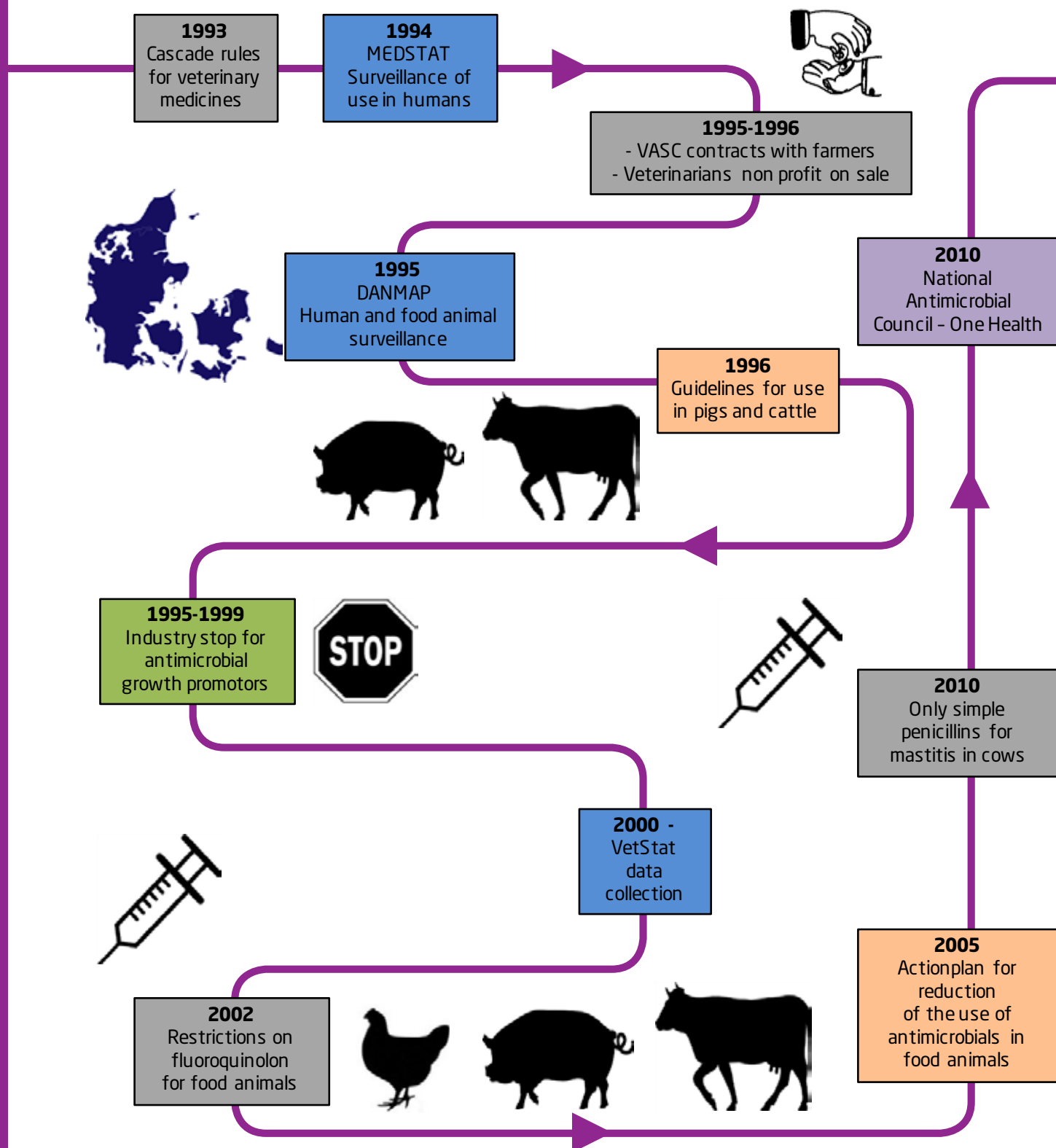


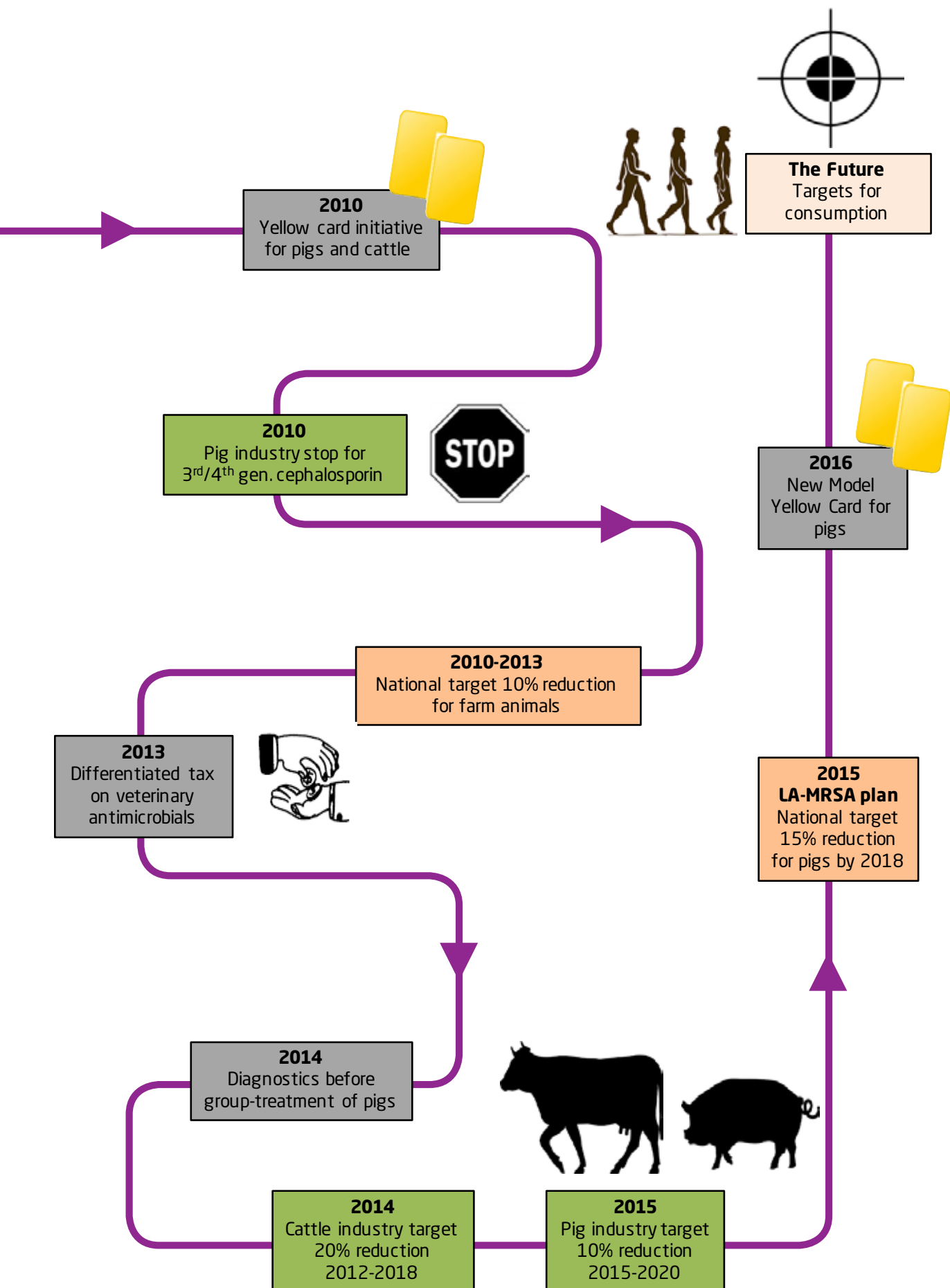
Illustration: Erik Jacobsen

Note: All antimicrobials used for animals must be prescribed by veterinarians and most prescriptions are processed at pharmacies (98% of the total use). Of the antimicrobials processed at pharmacies, 3% are administered directly by the veterinarians and 95% are distributed via the pharmacies. In addition, 2% of the total use, are distributed via medicated feed.

Figure 4. Timeline illustration activities concerning antimicrobial use in humans and animals, Denmark



Note: The colours illustrate activities related to legislation (■), monitoring (■), Council established by the Ministry of health (■), voluntary actions taken by industry (■) and national guidelines and targets (■). Illustration: Elisabeth Okholm Nielsen



National Veterinary Institute). Since 2005 the guidelines have been updated by the veterinary authorities, in collaboration with university experts and stakeholders.

- **Benchmarking herds according to use of antimicrobials – the Yellow Card initiative**

VetStat data are used to rank pig herds in a standardized way according to their use of antimicrobials, calculated as Animal Daily Doses (ADDs). In 2010, the veterinary authorities launched the Yellow Card initiative. The initiative was a response to an increase in use of antimicrobials in pig production since 2001. In brief, the authorities set threshold limits for use of antimicrobials in weaners, grower pigs and adult pigs. If over a 9-month period a farm exceeds a threshold, the veterinary authorities may issue an injunction, requiring the animal owner to bring down consumption. More details about the Yellow Card system may be found in the DANMAP 2010 report (www.DANMAP.org).

- **Industry initiatives to stop use of 3rd and 4th generation cephalosporins**

In 2009, the Danish pig industry, in response to concerns about extended spectrum cephalosporin resistance raised by DANMAP, decided to stop using 3rd and 4th cephalosporins in pigs, except as last resort antimicrobials. Since 2011, VetStat has been able to document that the industry intervention has been effective, with a total use of cephalosporins in pig production close to zero. In 2014, the Danish dairy cattle association made a similar decision, which it also showing some effect.

Human consumption of antimicrobials

Until 1991 the use of antimicrobials among humans was reported once a year through the Association of Medicine Importers (Medicinimportøreningen, MEDIF) and the Association of Danish Medicinal Factories (Foreningen af danske Medicinfabriker, MEFA) and was based on the whole sales to the pharmacies. From the end of the 1980's sales data became more and more unreliable since there was an increasing amount of parallel imported drugs which were not covered by this registration. Since 1994, one year before the establishment of the DANMAP programme, sales have been recorded in the Register of Medicinal Product Statistics at the Danish Health Data Authority based on the information delivered by all pharmacies. Because drug sales is mandatory to report the total sales in Denmark is covered. For the primary sector data have through all DANMAP years been detailed and covered the number of prescriptions, the size and number of packages as well as age and gender of the patient. These data are reported from each pharmacy and thus make it possible also to follow the sales of antimicrobials in different regions. For hospitals the sales have covered the amount of antimicrobials delivered to the different departments, but no information on the patient level has been available. Also, in the beginning the consumption of antimicrobials at hospitals was difficult to establish, since multipackages and magistral formulas were

not clearly marked, which made it impossible to get information on the precise drug, ATC group, strength, package size and dosages.

Today surveillance of consumption is possible through several publically available sources:

WWW.MEDSTAT.dk provides aggregate statistics on medicine sales in the Danish primary health care sector since 1996 and the hospital sector since 1997. Data are available as total sales for all antimicrobial substances by Region. Prescription sales are additionally aggregated by sex and age. Sales at package-level are available for both sectors.

Other statistics on primary sector sales (www.esundhed.dk) show indications and specialty of the prescriber (e.g. dermatologist or dentist) as well as the patient's age and gender. The number of prescriptions (indicating the number of treatment courses) as well as number of Defined Daily Doses (DDD) per prescription is shown among other variables.

The website www.Ordiprax.dk is a closed online system with user access that provides general practitioners with feedback on their prescribing practice by enabling comparison with other practices. One measure is number of DDDs per 1000 patients.

Improved surveillance will shortly be implemented on hospital antimicrobial use related to the individual patient. This will make it possible to establish the total use per patient independent of sector. Access to data from the shared medicine card will provide higher data quality on indications and the dosage and duration of treatment described. The shared medicine card also makes available information on prescriptions that have not been redeemed.

Important milestones in the human consumption

As mentioned above Denmark has always had a restrictive policy on the consumption of antimicrobials. In human medicine this becomes manifest through a general agreement on using penicillins whenever appropriate

Focus on the appropriateness of antimicrobial use might have arisen due to an outbreak with methicillinresistant *Staphylococcus aureus* in Danish hospitals in the 1960's, also described from other European hospitals [Rosdahl and Knudsen, 1991]. Measures against their spread included treatment of carriers, isolation of patients and restraint in the use of antibiotics. The prevalence of MRSA declined again in the beginning of the 1970's, but in Denmark the surveillance of Staphylococcal strains continued and over the years the surveillance was broadened to also include *streptococcus pneumoniae*, gonococci and other important human pathogen bacteria.

This might have become the cornerstone not only of the Danish surveillance programme but also to a restrictive antibiotic policy and demands among many other things the availability

of microbiological diagnostics combined with a close collaboration between the clinical microbiologist and the treating clinician.

Microbiological diagnosis is also performed in the primary sector, where most GP's perform microscopy and culturing of urines for an identification and resistance testing of the infectious agent in their UTI patients. The system is surveilled through mikap (www.mikap.dk), a system developed for quality assurance of microbiology on regional level. Again, this also includes close collaboration with the local clinical microbiological laboratory and includes advice on how diagnostics are to be performed.

National guidelines on the use of medicine have existed for many years, published through the Danish Medicinal Information A/S, a daughter company of the Danish Pharmaceutical Industry. Information on the use of the different drugs is written by Danish experts and includes a chapter on the use of antibiotics for the different organ systems and infection types that is restrictive and follows the mentioned rule on using penicillins whenever possible.

Also regional and local guidelines on the consumption of antibiotics have been developed over the years, often in collaboration between the clinical microbiologist and specialists in infectious diseases and in some situations in response to the occurrence of new resistant mechanisms and strains. An example of this is the antibiotic intervention group from Bispebjerg Hospital, where the occurrence of ESBL-producing *Klebsiella pneumoniae* was worrisome and resulted in an antibiotic programme abandoning the use of cephalosporins in all acutely ill patients combined with reduction of all fluoroquinolones and encouragement to the use of penicillins if possible (see Textbox 8, Danmap 2010).

As mentioned earlier the Council of Antibiotics was established in 2010 and a national action plan on the use of antimicrobials was issued the same year.

This plan has been supported by the National Health Authorities who on a regular basis publish guidelines for the rational use of medicine (www.irf.dk) which also covers the use of antimicrobials for specific infectious diseases.

New guidelines on prescribing antibiotics in primary health care and hospitals from the Danish Health and Medicines Authority were issued in 2012. These recommend the reduced use of cephalosporins and define rules for the consumption of fluoroquinolones in the Primary health sector as well as restricted use of these and the carbapenems at Danish Hospitals (see Textbox 3, DANMAP 2012).

In addition the Danish Council of Ethics published a recommendation on the ethical use of antibiotics in Danish context in January 2014.

Initiatives directed towards the education of the Danish population on a more rational use of antibiotics cover the participation in the yearly European Antibiotic Awareness Day and the establishment of a homepage containing the material of the campaign as well as more information on the subject of bacterial infections and how and when to treat them. Denmark has also participated in developing a Danish model of the e-Bug system, where school material for teachers and students is developed, aiming at the education of future generations. The e-Bug system is funded by the Directorate-General for Health and Consumers of the European Commission (see Textbox 2, DANMAP 2011).

Current initiatives include the development of National guidelines on a more rational use of antimicrobials developed in collaboration between the five Danish Regions and medical specialists covering the different disease topics. Also most hospitals are working on developing local guidelines on the implementation of antibiotic stewardship based on local resistances and pathogenic strains (see textbox 5.1 DANMAP 2014)

Visions for the surveillance of antimicrobial drug resistance

From a One Health perspective the DANMAP monitoring programme has been innovative in setting up the first programme in the world to monitor antimicrobial consumption and resistance in farm animals, meat products and humans. The annual report has been a model for many other monitoring programmes internationally. The foundation DANMAP provides for research within the field of antimicrobial resistance is exemplary. Furthermore, DANMAP is more than data and a report: It is a network and collaboration between various groups from different disciplines and institutions, all striving towards the common goal of fighting the threat to humankind posed by antimicrobial resistance.

The design of DANMAP in 2016 largely remains a solution to the challenges that were identified 20 years ago. With the realization that the occurrence of antimicrobial resistance in one country is affected by the global antimicrobial resistance pandemic, and with the technological breakthroughs in recent years, there is considerable potential for improvement. DANMAP was not designed as a rapid monitoring and reporting system. Thus, the annual reports present data that are a snapshot in time and may not reflect the most recent trends, new emerging types of resistance or current outbreaks. Furthermore, economic considerations dictate that the number of samples that can be included in the monitoring programme is limited, and accordingly the sensitivity, in terms of detecting new types of resistance, is limited.

Real-time monitoring of AMR in human health care - the MiBa system

Hence, in order to create data that the industry, researchers, clinicians and decision makers can act on, there is a need to

complement DANMAP with a timely system for surveillance of antimicrobial drug resistance. This can be done in the form of automated and electronic surveillance where users can access data on the internet and create tables and graphs. With current information and communication technology, such a system is in principle not difficult to build. It has many advantages, including the possibility of adding algorithms for outbreak detection, setting flags for emergence of certain drug-bug combinations, and many other tools for decision support and quality assurance.

Challenges emerging from limitations of the Linnean taxonomy

Bacteria are prokaryotic microorganisms that traditionally are classified in a Linnean hierarchical family tree (species, genus, family, tribe, order), as described by Carl von Linné. In many respects, this hierarchical system is insufficient in modern microbiology. In the context of antimicrobial resistance, the fact that drug resistance often is carried by mobile genetic elements constitutes a challenge, because these elements can be exchanged between different species and thereby do not obey hierarchical rules.

For example, the same or very closely related extended spectrum beta-lactamase genes can be found in many different gram negative species due to genetic exchange. Furthermore, some of these species may have other characteristics, for example virulence traits that are also distributed across species or subtypes. Moreover, with the current development in typing and classification, including the use of whole genome sequencing and a focus on a dynamic bacterial evolution with exchange of genetic elements, a data model based solely on the Linnean system is outdated for the purpose of surveillance and reporting of test results.

In real life, in the medical sector different departments of clinical microbiology manage this situation differently. In test reports to the requesting clinicians, some departments will give the result of an "MRSA" in the species-field in the IT system, almost as if MRSA was a bacterial species in its own right. In contrast, others prefer to answer "growth of *Staphylococcus aureus*" as the result and then make a text remark that it is an MRSA. These text remarks are rarely standardized. Furthermore, the antimicrobial resistance pattern included in clinical microbiology reports is often restricted to include drugs relevant for treatment.

The lack of a common data model for exchanging digital information about specific resistance mechanisms is one of the bottlenecks for implementing electronic surveillance for antimicrobial resistance. To develop electronic surveillance, we need to extend the currently used data model based on the Linnean classification.

The implementation of flexible property tables in MiBa

Broadly speaking, we define properties as characteristics of bacteria that can be measured by various methods (e.g. by de-

fined genotypic and phenotypic methods) and at various levels of detail (e.g. a Minimum inhibitory concentration (MIC) value or an overall interpretation as "resistant" against a drug). These can be expressed in various ways (from a gene sequence to an overall conclusion, e.g. an ESBL producing organism or a Multi-locus sequence typing (MLST) type). Bacteria can have a very large number of properties, and the same properties can be found across the Linnean family tree. Joining flexible property tables to the traditional tables of microbiological findings is a solution to the limitations of the current data model. The new data model for microbiological reporting in Denmark will also provide opportunities for sending other types of information along with the result produced by the clinical microbiology department, e.g. patient data or exposure information.

From 2010, all reports from Danish clinical microbiology departments have been stored in a national database called MiBa. At present, only limited and unstructured data on antimicrobial resistance are stored in the database, for the reasons mentioned above. Currently, MiBa is suitable for general microbial surveillance, but not for detailed surveillance of antimicrobial resistance. However, flexible property tables have been developed and are currently being implemented [Voldstedlund et al, 2014]. With a consensus about the use and content of these tables, the vision of electronic and timely antimicrobial resistance is getting within reach.

The eRES and MiBAlert projects

In anticipation of the implementation and use of property tables, the next steps include the development of a system for AMR surveillance. In the beginning, priority will be given to the major indicators and bacteria including MRSA, VRE, ESBL and CROs. However, other types of resistance will also be included in a stepwise manner. Thereby, it will be possible to describe and analyse trends and distributions of resistance in an interactive and timely manner, and develop tools for risk assessment and action.

One example of a very specific tool for action is the MiBAlert project. MiBAlert is a tool directed toward healthcare personnel who access a patient's electronic health record as well as those further involved in the care and treatment of the patient. It is based on a web service using data from MiBa. The alert is generated automatically whenever the health record of a patient is accessed and can thus provide timely information about for example previous positive cultures for eg. VRE, MRSA, CPE and multi-drug-resistant enterobacteriae. This will enable hospital staff to implement timely measures such as isolation of patients and will also assist in the selection of antimicrobial treatment.

MiBAlert is currently implemented in the Capital Region of Denmark and is expected to be implemented in other regions as well. Experiences from the Capital Region show that MiBAlert was one of the helpful components in a bundle approach to control a VRE outbreak in Copenhagen. MiBAlert

is an example of how the same data that are used for national surveillance can be applied in the encounter between a patient and the hospital where they are being treated.

Real-time sampling of populations for AMR

Current surveillance of antimicrobial resistance (AMR) is mainly based on passive reporting of clinical diagnoses and phenotypic laboratory results for specific pathogens. Even utilizing the potential of whole genome sequencing, this procedure leads to significant time-delays and a narrow pathogen spectrum that does not capture all relevant AMR genes, where the bulk may be present in the commensal bacterial flora.

Although Denmark was the first country in the world to establish integrated - One Health - surveillance in 1995, the coverage of bacterial species is extremely limited and does not include a screening programme for healthy humans. Among food animals sentinel bacteria (*E. coli* and enterococci) are monitored, but again only an extremely narrow spectrum and the current programme with a single isolate per farm has very low sensitivity.

From a surveillance point of view, sewage is an attractive matrix because it combines material from a large and mostly healthy population, which would otherwise not be feasible to monitor. In addition, sampling this matrix requires no informed consent from providers of samples, consequently avoiding most ethical concerns. For food animals, combined slaughterhouse samples could also be used to obtain samples from a large number of farms simultaneously.

Next generation sequencing (NGS) based diagnostics provide both cost-efficient sample analysis and rapid turnaround. NGS generates millions of DNA reads from a sample, where each target is sequenced according to the abundance within the sample. Metagenomics benefit from the ability to quantify thousands of targets in a single sample without requiring a priori knowledge of which genes are present, including additional features of interest, such as presence of pathogens and virulence genes.

Through a number of recent projects [www.genomicepidemiology.org, [www.compare-europe.eu], we have developed an online method for identifying all known resistance genes, which has been intensively accessed from more than 70 countries [Zankari et al, 2012]. We have also shown that NGS is superior to phenotypic testing for susceptibility testing [Zankari et al, 2014]. Genotyping using whole genome sequencing is a realistic alternative to surveillance based on phenotypic antimicrobial susceptibility testing [Hasman et al, 2014] and it is feasible to identify resistance through metagenomic sequencing of clinical samples. We have recently shown that metagenomic read mapping can be used to quantify AMR genes from airplane septic tanks from international flights [Nordahl Petersen et al, 2015], which demonstrates the potential of enabling real-time monitoring of the AMR load in a country. In

addition, we have demonstrated that metagenomics is superior to conventional methods for resistance surveillance at the pig herd level [Munk et al, 2016] and proven the feasibility of rapidly sharing data with the global research community.

Through further development and use of the technology, it is the expectation that in the future it will be possible to establish a novel supplement to the current DANMAP programme, where data on AMR genes are also collected from sewage and slaughterhouses and analysed in real-time, and the data shared by researchers and the general public.

Such surveillance will provide an important sentinel function and provide a concurrent baseline, informing decision makers about needs for adaptation of reservoir specific monitoring efforts.

Selected references

Aarestrup, F.M. Occurrence of Glycopeptide Resistance among *Enterococcus faecium* Isolates from Conventional and Ecological Poultry Farms. Microbial Drug Resistance, 1(3): 255-257, 1995

Aarestrup F.M, Hasman H., Olsen I. and Sørensen G. International Spread of *bla*_{CMY-2}-Mediated Cephalosporin Resistance in a Multiresistant *Salmonella enterica* Serovar Heidelberg Isolate Stemming from the Importation of a Boar by Denmark from Canada, AAC 48: 1916-7, 2004.

Aarestrup F.M., Hasman H., Jensen L.B. Resistant *Salmonella* Virchow in Quail Products. Emerg Infect Dis. 2005 Dec; 11(12): 1984-1985

Bates J., Jordens J. and Griffiths, T. Farm animals as a putative reservoir for vancomycin-resistant enterococcal infection in man. J. Antimicrob. Chemother.(1994)34(4):507-514.

Cavaco L.M., Hendriksen R.S. and Aarestrup F.M.. Plasmid-mediated quinolone resistance determinant qnrS1 detected in *Salmonella enterica* serovar Corvallis strains isolated in Denmark and Thailand. J Antimicrob Chemother 2007; 59: 804-5

Dibner J.J. and Richards J.D. Antibiotic Growth Promoters in Agriculture: History and Mode of Action. Poultry Science 84:634-643, 2005

Hammerum, A.M, Hansen, F., Nielsen, H.L., Jakobsen, L., Stegger, M., Andersen, P.S., Jensen, P., Nielsen, T.K., Hansen, L.H., Hasman, H., Fuglsang-Damgaard, D. Use of WGS data for investigation of a long-term NDM-1-producing *Citrobacter freundii* outbreak and secondary in vivo spread of blaNDM-1 to *Escherichia coli*, *Klebsiella pneumoniae* and *Klebsiella oxytoca*. Journal of Antimicrobial Chemotherapy. 10.1093/jac/dkw289

Hansen D.S., Sirot D., Kolmos H.J., Extended spectrum beta-lactamases in Danish *Klebsiella* isolates. Ugeskr Laeger 160: 2261-2, 1998

Hasman H., Saputra D., Sicheritz-Ponten T., Lund O., Svendsen C.A., Frimodt-Møller N., Aarestrup F.M. Rapid whole-genome sequencing for detection and characterization of microorganisms directly from clinical samples. *J Clin Microbiol.* 2014 Jan;52(1):139-46].

H. Hasman, A.M. Hammerum, F. Hansen, R.S. Hendriksen, B. Olesen, Y. Agersø, E. Zankari, P. Leekitcharoenphon, M. Stegger, R.S. Kaas, L.M. Cavaco, D.S.Hansen, F.M. Aarestrup, R.L. Skov. Detection of *mcr-1* encoding plasmid-mediated colistin-resistant *Escherichia coli* isolates from human bloodstream infection and imported chicken meat, Denmark 2015. *Eurosurveillance*, Volume 20, Issue 49, 10 December 2015.

Jakobsen L., Garneau P., Bruant G., Harel J., Olsen S.S., Porsbo L.J., Hammerum A.M., Frimodt-Møller N. Is *Escherichia coli* urinary tract infection a zoonosis? Proof of direct link with production animals and meat. *Eur J Clin Microbiol Infect Dis.* 2012 Jun;31(6):1121-9

Jensen, V.F., Jacobsen, E. and Bager F. Veterinary antimicrobial-usage statistics based on standardized measures of dosages. *Prev Vet Med.* 2004 Jul 16; 64 (2-4): 201-15

Kern M.B., Klemmensen T., Frimodt-Møller N., Espersen F. Susceptibility of Danish *Escherichia coli* strains isolated from urinary tract infections and bacteraemia, and distribution of *sul* genes conferring sulphonamide resistance. *J Antimicrob Chemother* 2002, 50: 513-6

Kjerulf A., Hansen D.S., Sandvang D., Hansen F., Frimodt-Møller N. The prevalence of ESBL-producing *E. coli* and *Klebsiella* strains in the Copenhagen area of Denmark. *APMIS* 2008; 116: 118-24.

Moore, P. R., A. Evenson, T. D. Luckey, E. McCoy, E. A. Elvehjem, and E. B. Hart. 1946. Use of sulphasuccidine, streptothricin and streptomycin in nutrition studies with the chick. *J. Biol. Chem.* 165:437-441

Munk, Patrick; Andersen, Vibe; de Knecht, Leonardo; Jensen, Marie; Knudsen, Berith; Lukjancenko, Oksana; Mordhorst, Hanne; Clasen, Julie; Agersø, Yvonne; Folkesson, Anders; Pamp, Sünje; Vigre, Håkan; Aarestrup, Frank. A sampling and metagenomic sequencing-based methodology for monitoring antimicrobial resistance in swine herd *Journal of Antimicrobial Therapy*, Accepted 2016

Nordahl Petersen T., Rasmussen S., Hasman H., Carøe C., Bælum J., Schultz A.C., Bergmark L., Svendsen C.A., Lund O., Sicheritz-Pontén T., Aarestrup F.M. Meta-genomic analysis of

toilet waste from long distance flights; a step towards global surveillance of infectious diseases and antimicrobial resistance. *Sci Rep.* 2015 Jul 10;5:11444

Nielsen KL, Dynesen P, Larsen P, Frimodt-Møller N. Faecal *Escherichia coli* from patients with *E. coli* Urinary Tract Infection (UTI) and Healthy Controls who Have Never Had UTI. *J Med Microbiol.* 2014, 63:582-9

O'Neill J., Tackling drug-resistant infections globally: Final report and recommendations 2016. Wellcome Trust, and HM Government, United Kingdom

Rosdahl V T and Knudsen A M. The Decline of Methicillin Resistance Among Danish Staphylococcus Aureus Strains. *Infection Control & Hospital Epidemiology*, Volume 12, Issue 2 February 1991, pp. 83-88

Rønn, N.E. (ed). Vurdering af antibiotikaforbruget i dansk svineproduktion (Evaluation of the use of antimicrobials in Danish pig production). Report, the Federation of Danish Pig Producers and Slaughterhouses, 1995.

Schmidt M., Hallas J., Laursen M., Friis S. Data Resource Profile: Danish online drug use statistics (MEDSTAT). *International journal of Epidemiology* (in print).

Swann M.M., Baxter K.L., Field H.I., et al. Report of the Joint Committee on the use of Antibiotics in Animal Husbandry and Veterinary Medicine. HMSO; London: 1969.

Steg H., Bager F., Jacobsen E. and Thougard A. VETSTAT - the Danish system for surveillance of the veterinary use of drugs for production animals. *Preventive Veterinary Medicine*, 2003, 57, 3, 105-115.

Voldstedlund M., Haarh M., Mølbak K.; MiBa Board of Representatives. The Danish Microbiology Database (MiBa) 2010 to 2013. *Euro Surveill.* 2014;19(1).

Zankari E., Hasman H., Cosentino S., Vestergaard M., Rasmussen S., Lund O., Aarestrup F.M., Larsen M.V. Identification of acquired antimicrobial resistance genes. *J Antimicrob Chemother.* 2012 Nov;67(11):2640-4.].

Zankari E., Hasman H., Kaas R.S., Seyfarth A.M., Agersø Y., Lund O., Larsen M.V., Aarestrup F.M. Genotyping using whole-genome sequencing is a realistic alternative to surveillance based on phenotypic antimicrobial susceptibility testing. *J Antimicrob Chemother.* 2013 Apr;68(4):771-7.]



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 bacteria isolated 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 2015, 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 2015.

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 2015 (www.dst.dk). The distribution of the population, which could potentially have received antimicrobial treatment in 2015, is shown in Figure 3.2, together with the five healthcare regions and the 11 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. From 1995 to 2015, there has been an overall decrease in the number of turkeys, cattle and dairy cows produced pr. year. In contrast, the number of pigs produced has increased remarkably over the same period of

time. Also, the production of milk and marine fish has increased over the past two decades.

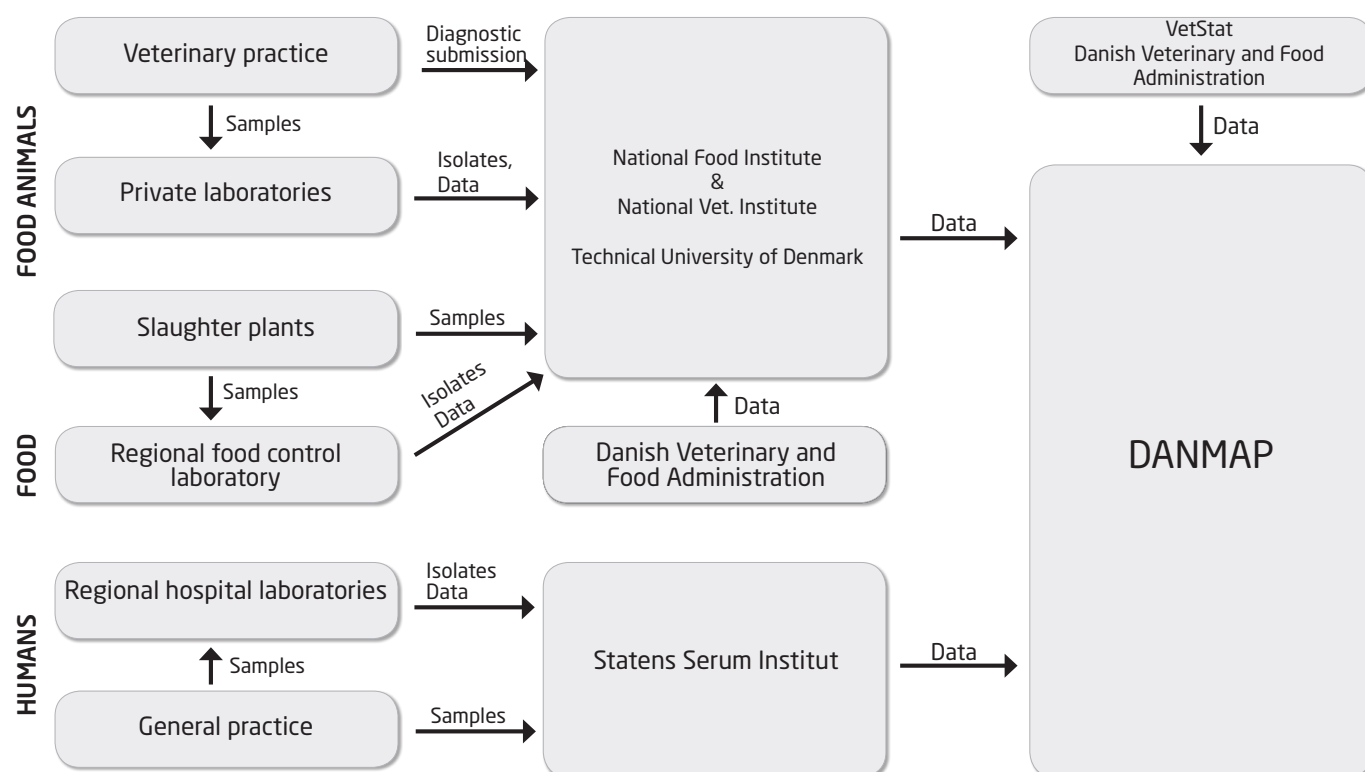
In 2015, the number of pigs produced was approximately 3% higher than in 2014 and the number of fattening pigs (15–50 kg) exported increased by 17% from 2014 to 2015. Since 2004, the total exports of fattening pigs have increased by more than six-fold.

From 2014 to 2015, the number of cattle slaughtered decreased by 6%, while the number of dairy cows remained almost unchanged and the amount of milk produced continued to increase (up 3%).

The number of broilers produced decreased by approximately 1% from 2014 to 2015, and approximately 16% of the broilers produced in 2015 were exported for slaughter. The production of turkeys has fluctuated considerably over the past decade but remained at almost the same level in 2014 and 2015. 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.

Figure 3.1. Organisation of DANMAP

DANMAP 2015



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 treating bacterial infections in humans. An antimicrobial agent is considered critically important if it is the only compound, or one of a limited number compounds, available to treat serious human disease. Fluoroquinolones, 3rd and 4th generation cephalosporins, macrolides and glycopeptides are among these critically important antimicrobial agents [AGISAR, 3.revision, WHO 2011]. 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.

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 11 Departments of Clinical Microbiology (DCM) in Denmark

DANMAP 2015

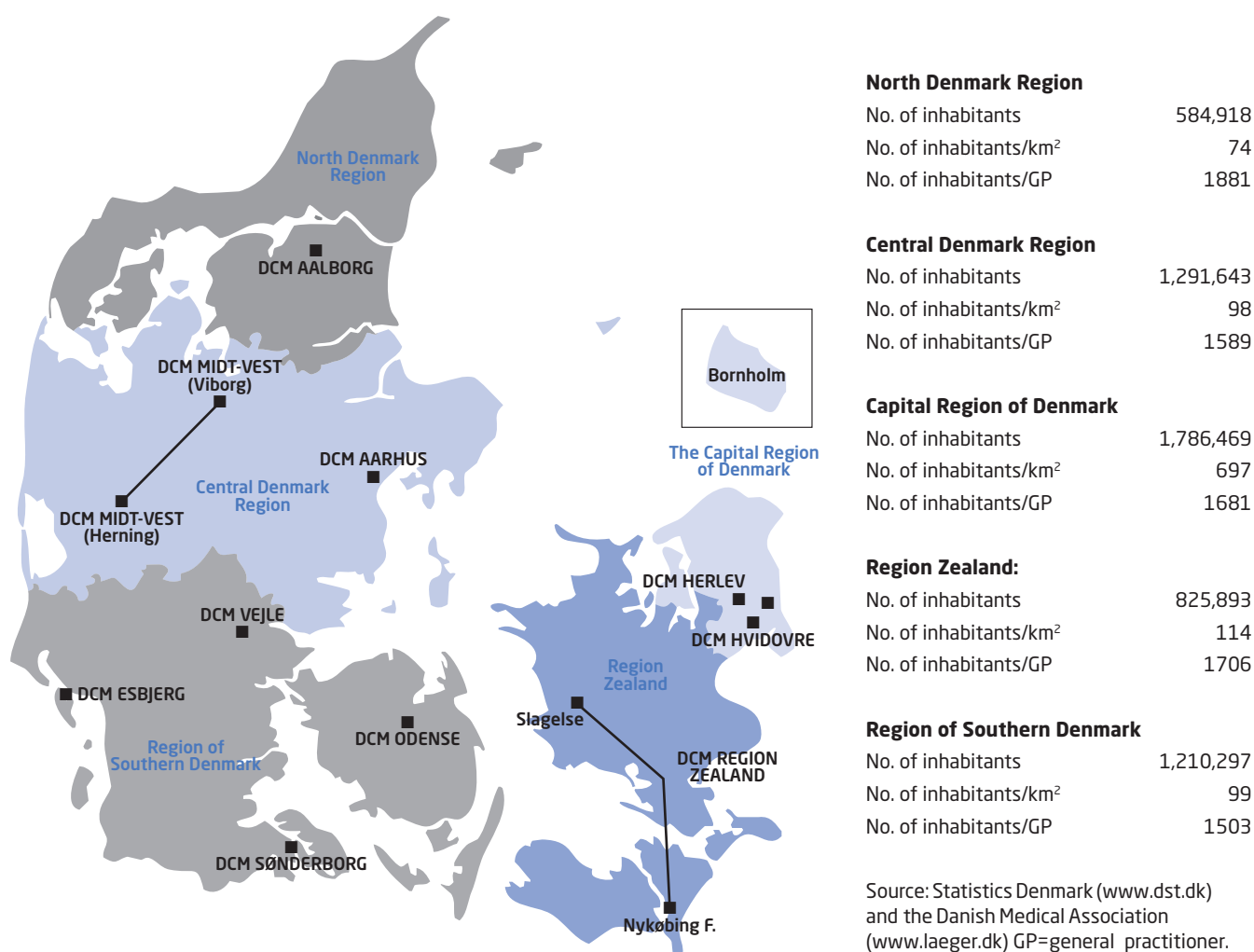


Table 3.1. Production of food animals and the production of meat and milk, Denmark

DANMAP 2015

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	1,000 heads	Export 1,000 heads ^(c)	mill. kg	Fresh water	Marine
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	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	522	135	561	5270	30874	12314	1954	30	11
Diff.(d)	-1%	-1%	1%	0	-6%	-6%	<1%	3%	3%	17%	2%	0	0

Source: Statistics Denmark (www.dst.dk) and The Danish AgriFish Agency. Production data for farmed fish was not available for 2015. 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 2015 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 2014 to 2015

Table 3.2. Antimicrobial agents registered for systemic and veterinary intramammary therapeutic use in animals and humans, Denmark 2015

DANMAP 2015

ATC / ATCvet codes ^(a)	Therapeutic group	Antimicrobial agents within the therapeutic groups	
		Animals	Humans
S01AA / J01AA / QJ01AA,QJ51AA	Tetracyclines	Chlortetracycline, doxycycline, oxytetracycline	Doxycycline, lymecycline, oxytetracycline, tetracycline, tigecycline
S01AA01 / QJ01BA	Amphenicols	Florfenicol	Chloramphenicol
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, incl. 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	Cefoperazone, ceftiofur, cefovecin	Cefotaxime, ceftazidime, ceftriaxone
J01DE / QJ51DE	Fourth-generation cephalosporins	Cefquinome	Cefepime
J01DF	Monobactams		Aztreonam
J01DH	Carbapenems		Meropenem, ertapenem
J01DI	Fifth-generation cephalosporins		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 sulfamethoxazol/trimethoprim	Sulfadiazine/trimethoprim, sulfadoxine/trimethoprim, Sulfamethoxazole/trimethoprim	
J01FA / QJ01FA	Macrolides	Spiramycin, tylosin, tilmicosin, tylvalosintartrat, tulathromycin, gamithromycin, tildiprocin	Erythromycin, roxithromycin, clarithromycin, azithromycin, telithromycin
J01FF / QJ01FF	Lincosamides	Clindamycin, lincomycin	Clindamycin
J01FG / 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, fosfomycin
QJ01XQ	Pleuromutilins	Tiamulin, valnemulin	
QP51AG04	Antiprotozoals, sulfonamides	Sulfaclozine	
Not in ATCvet ^(b)	Oligosaccharides	(Avilamycin)	
Not in ATCvet ^(b)	Flavofosfolipols	(Flavomycin)	

a) ATCvet codes start with a Q

b) Animal growth promoters used before 1999 are listed in parentheses

c) Intestinal 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: In 2015, the overall use of antimicrobials for animals decreased by 4%. This was mainly caused by a 5% decrease in antimicrobial usage in the pig industry, which is the dominant user of antimicrobials for animals in Denmark. The use of tetracycline, aminoglycosides and pleuromutilins for pigs has decreased consistently over the last three years. In the poultry production (other than turkeys), the use of antimicrobials increased by 184% in 2015, due to several serious disease outbreaks in the broiler production. In contrast, the use for turkeys decreased by 41%. The fur animal industry also experienced serious disease outbreaks in 2015, which is reflected in a 23% increase antimicrobial usage.

The use of critically important antimicrobials remained low in the pig production, and other food production animals. In companion animals, however, the use of critically important antimicrobials remained relatively high compared with other species, but has decreased over the last three years. It is, however, of some concern that the use of colistin in pigs has increased, especially from 2014 to 2015. Colistin is of increasing importance as a last resort antimicrobial in human medicine.

For the first time since DANMAP 2004, the use of coccidiostats is also presented in this report; the total use of coccidiostats in Denmark has increased steadily from 2008 to 2015.

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].

Overall, the antimicrobial consumption for both humans and animals has increased since the late 1990s. Figure 4.1 shows the total antimicrobial consumption in animals and humans since 1994 and 1997, respectively. Increases in antimicrobial consumption for animals can partly be explained by the increase in pork production, which constitutes approximately 86% of the meat production in Denmark (Table 3.1), however, risk management measures to reduce consumption have also played a role.

The prescription patterns were clearly influenced by implemented legislation (Figure 4, chapter 2). For example, the decrease in antimicrobial consumption after 1994 was likely the result of the following important actions: 1) limitation of veterinarians 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 agent use per pig [DANMAP 2010]. Effects from other parts of the legislation may be less obvious, must also be considered, when interpreting the veterinary prescription patterns.

Official veterinary guidelines regarding the selection of antimicrobial agents for pigs and cattle, have existed 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 National Veterinary Institute DTU, National Food Institute DTU, the Practising Veterinarians Organization, university experts, the Danish Association of the Veterinary Pharmaceutical Industry and the Danish Agriculture and Food Council. 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, 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.

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 medicine is available by prescription only, and since 2001, data on all medicine prescribed for use in animals, including vaccines, have been collected in a national database (VetStat). Data on consumption of coccidiostatic agents (non-prescription) and antimicrobial growth promoters (no longer permitted), are also collected in VetStat.

Consumption data used in DANMAP 2015 were extracted from VetStat by the Danish Veterinary and Food Administration (DVFA) in March (zinc oxide) and October 2016. National Food Institute DTU carried out no further validation of the received data. Furthermore, data concerning use of coccidiostats were also obtained from VetStat (July 2016) and these are presented in Textbox 4.1

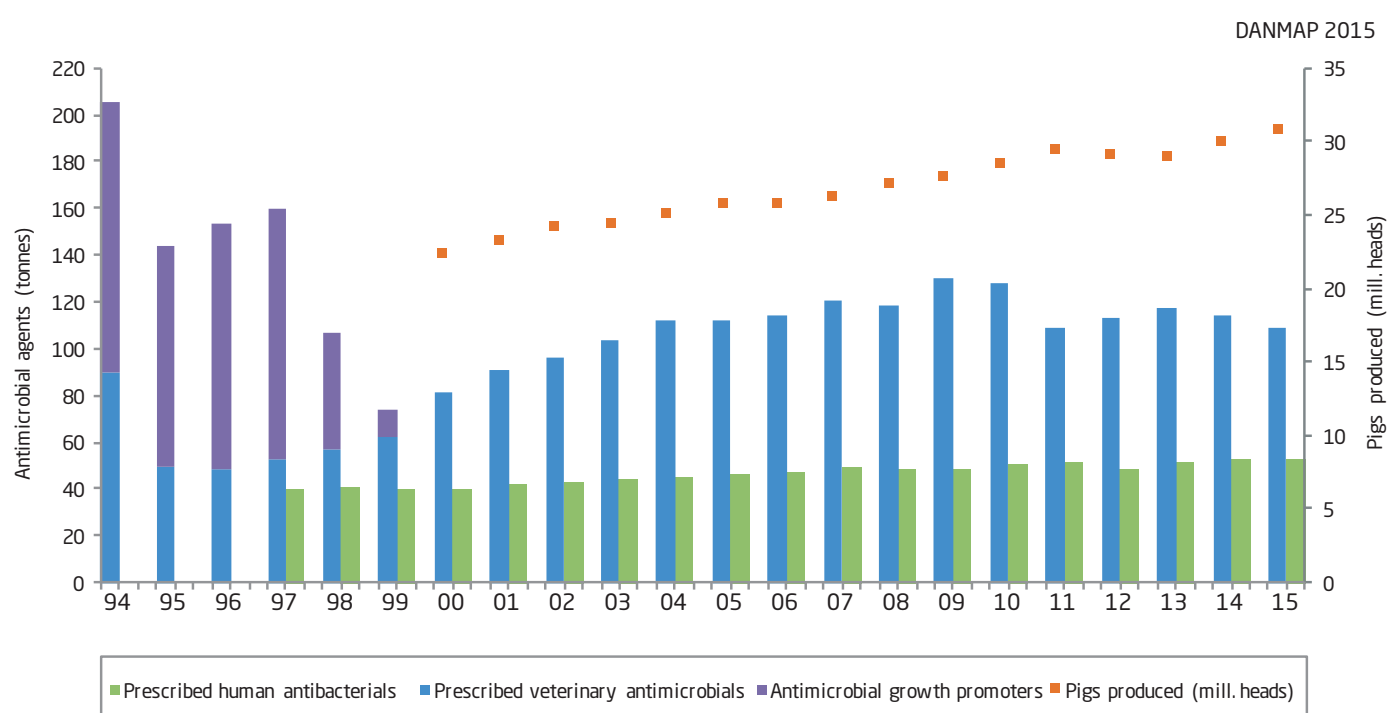
4.1.2 Methods

Metrics of antimicrobial consumption are numerous, each with its own advantages and limitations. Therefore, the selection of measures to monitor must depend on the purpose and the available information.

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

Figure 4.1. Prescribed antimicrobial agents for humans, and for all animal species, including the number of pigs produced, Denmark



Sources: Human therapeutics: The Danish Medicines Agency. 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 2004-2015 are based on data extracted from VetStat.

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 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 veterinary consumption, 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 e.g. cattle and chicken.

DAPD, or estimated treatment proportion, is a statistical measure that provides a rough estimate of the proportion of

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

DANMAP 2015

ATCvet code	QJ01B	QJ01G	QJ01D	QJ01MA	QJ01MB	QJ01FF	QJ01FA	QJ01XQ	QJ01CE	QJ01CA	QJ01E	QJ01AA			
Therapeutic group	Amphenicols	Aminoglycosides	Cephalosporins	Fluoroquinolones	Other quinolones	Lincosamides	Macrolides	Pleuromutilins	Penicillins, beta-lactamase sensitive	Penicillins, others ^{a)}	Sulfonamides and trimethoprim	Tetracyclines	Others	Total 2014	Total 2015
<i>Pigs, total</i>	338	4435	1	0	0	1987	10157	7878	16744	8059	7172	23902	825	86020	81499
-Sows and piglets	251	1806	0	0	0	448	516	802	8619	3648	5226	1955	97	24140	23366
-Weaners	72	2444	1	0	0	725	6675	3065	1872	3538	1749	14142	710	36131	34992
-Finishers	15	186	1	0	0	815	2966	4012	6253	874	197	7805	18	25749	23141
<i>Cattle, total</i>	563	647	70	0	3	4	228	0	7919	918	1062	1720	16	13048	13151
-Intramammaries		32	59			2			245	158	7		1	497	503
-Cows and bulls (excl intramammaries)	9	286	10	0	3	1	113	0	7207	567	893	1155	1	10377	10245
-Calves < 12 mdr	549	308	1	0	0	1	113	0	388	183	152	531	15	2008	2241
-Heifers and steers	5	21	0			0	2		79	10	10	34	0	166	162
<i>Poultry, total</i>	4	258	0	1	0	129	114	0	184	500	445	796	7	1548	2440
Poultry incl. broilers, layers and other poultry	3	251	0	1	0	125	37	0	119	387	444	561	2	679	1931
Turkeys	1	8				4	77		65	113	1	235	5	869	509
<i>Other production animal species</i>	311	375	0	0	1005	188	645	0	1	2221	2705	681	14	9318	8147
Fur animals	0	375	0	0	0	188	645	0	1	2216	1056	681	14	4202	5177
Aquaculture	311	0	0	0	1005	0	0	0	0	5	1650	1	0	5116	2970
<i>Companion animals</i>	0	191	164	16	0	89	51	1	824	808	1624	197	34	4088	3999
Horses	0	10	3	4	0	0	0	0	762	144	1076	28	0	2071	2027
Pets(b)	0	181	161	12	0	89	51	1	62	664	548	169	34	2017	1972
<i>Total</i>	1217	5907	235	17	1008	2398	11194	7880	25673	12505	13009	27297	897	114023	109235

Note: Only the ATCvet group contributing mostly to the antimicrobial group is mentioned. Combination drugs are divided into active compounds

a) Penicillins with extended spectrum, cloxacillin and amoxicillin/clavulanic acid

b) Antimicrobials used for companion animals: DVFA has allocated kg active compound to the appropriate target species (horses/pets) based on knowledge of which products are used for the particular species

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

In 2015, the total veterinary consumption of antimicrobial agents, including agents used for companion animals, amounted to 109.2 tonnes active compound (Table 4.1), representing a 4% decrease compared with 2014.

In 2015, the antimicrobial use for pigs, cattle and poultry comprised 75%, ~12%, 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 5% 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

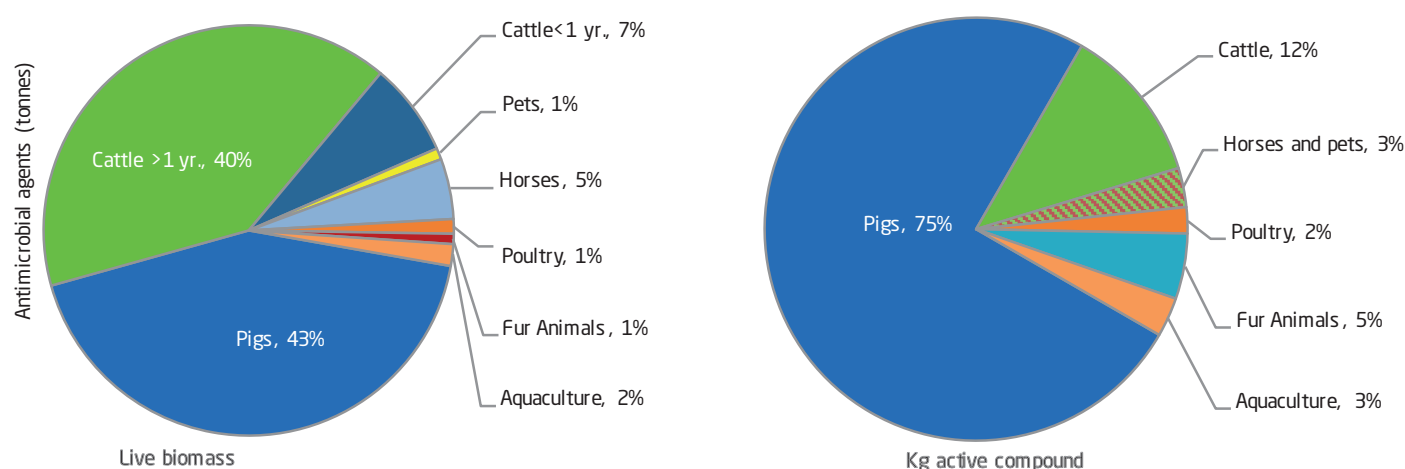
of live biomass. However, the vast proportion of cattle biomass consists of dairy cows, which have very low consumption of antimicrobial agents compared with growing animals.

Historically, the overall consumption, measured as kg active compound, was 47% lower in 2015 compared with 1994. In contrast, the total meat production increased by 15% during this period (Table 3.1 and Figure 4.1). 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 also driven mainly by an increase in consumption in pigs and should be seen in the context that the number of pigs produced increased 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 2015 it was 16% lower than in 2009.

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

DANMAP 2015



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

4.3 Antimicrobial consumption by animal species

4.3.1 Antimicrobial consumption in pigs

For this issue of DANMAP, updated data from 2004-2015 were extracted from VetStat and all measures for the antimicrobial usage in pigs were recalculated for all years, using the updated dataset.

In 2015, the total antimicrobial consumption in pigs was 81.5 tonnes active compound (Table 4.1), a decrease of 4.5 tonnes (5%) compared with 2014.

The treatment proportions (DAPD) in the pig population overall and by age group are presented in Figure 4.3 and in the web annex (Table A4.1).

The DAPD of the total population should reflect the trends in selection pressure in the population. Due to the differences in treatment proportion between age groups, the DAPD of the total population is therefore affected by changes in population structure. For example, an increased export of live pigs at 30 kg would, in itself, cause an increase in DAPD for the remaining population, since the DAPD for finishers is relatively low. Any

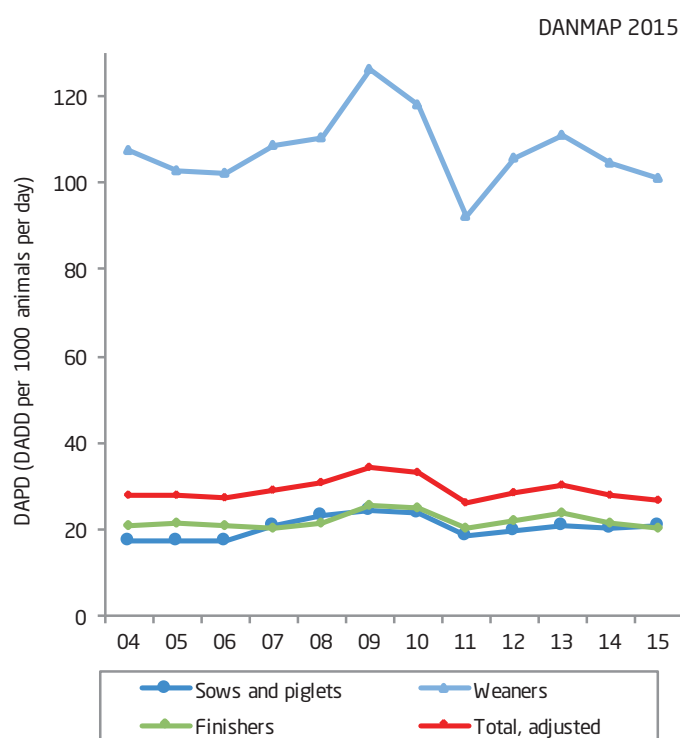
changes in export and productivity must therefore be taken into account to get a true impression of the antimicrobial consumption pattern and selection pressure in the pig production.

Also, the treatment proportion (DAPD) is much higher in the weaning pigs, compared with finishers and sows (Figure 4.3). However, the biomass of the weaning pigs is very small (7.5-30 kg, 4 weeks), compared with the finishers (31-107 kg, 12 weeks) and the sows.

In 2015, the antimicrobial consumption in pigs, measured in DAPD decreased by 4% to approximately 27 DAPD (Figure 4.3) when adjusted for changes in export. Overall the number of pigs produced in 2015 increased by 3%, and the number of pigs exported increased by 17% (Table 3.1).

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). The reductions in antimicrobial usage were also associated with increasing use of vaccines (DANMAP 2014) and a slight temporary decrease in productivity in some herds. However, disease outbreaks did not increase [Danish Veterinary Bulletin no. 6, 2012]. Measured in DAPD, the antimicrobial consumption in pigs in 2015 was 22% lower than in 2009, when adjusted for changes in export (Figure 4.3).

Figure 4.3. Antimicrobial consumption in the pig production, and the distribution on age groups, Denmark



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

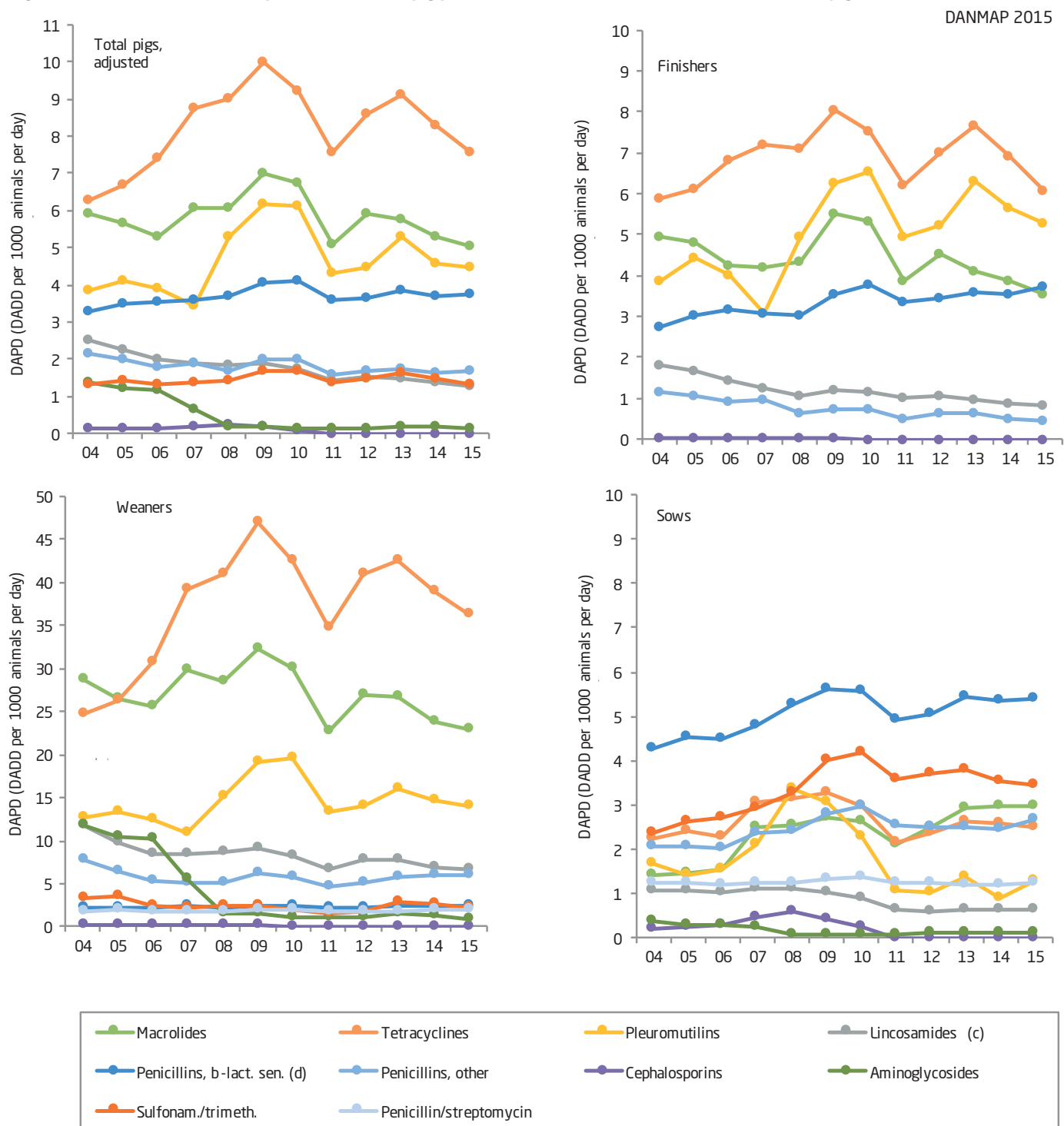
The DAPD is calculated as the number of standard doses for one kg animal divided by the estimated live biomass in the age group at the total population (in tonnes).

Within the different age groups, the DAPD decreased in weaners and finishers, but remained at approximately the same level for sows and piglets. The decrease was primarily in the use of tetracyclines and macrolides (Figure 4.4). Tetracycline has been one of the most commonly used antimicrobials in the Danish pig production for a decade (Figure 4.4). They are almost entirely administered orally, and particularly used for treatment of gastrointestinal disease in weaning pigs and finishers. The overall use of tetracyclines in the pig production has fluctuated since 2009, but 2015 saw the lowest DAPD levels since 2006. Measured in DAPD the use of tetracyclines has been reduced by 9% since 2014 and by 24% since 2009.

In 2014 the Danish pig producers committed themselves to reduce the consumption of tetracyclines by 50% by the end of 2015. However, this goal has yet to be achieved.

While the overall consumption of antimicrobials for pigs has decreased consistently since 2009, the use of colistin for pigs has increased markedly from 409 kg in 2009 to 825 kg in 2015, mainly in weaners. The consumption of colistin in the different age groups of pigs, measured in DAPD, is shown in Figure 4.5. Although the use of colistin constitutes a very small fraction of the overall antimicrobial consumption in pigs (1% in 2015), the increase is concerning due to One Health implications, as polymyxins, to which colistin belongs, have found a role for treatment of carbapenemase resistant infections in human medicine.

Figure 4.4. Antimicrobial consumption^(a) in the total pig production^(b), and in finishers, weaners, sows and piglets, Denmark



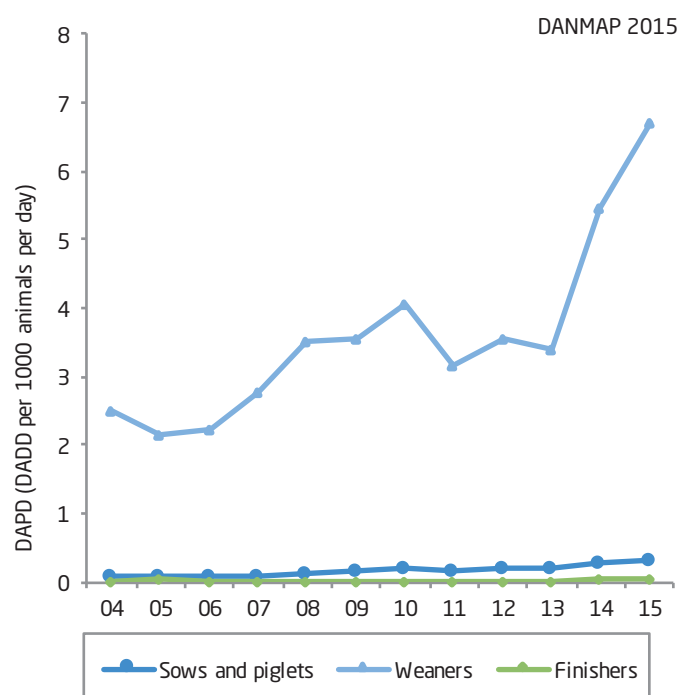
Note: Amphenicols, colistin, fluoroquinolones, intramammaries, gynecologicals and topical drugs are not included in the figure.

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 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 4.5. Consumption of colistin in finishers, weaners, sows and piglets, Denmark

Of critically important antimicrobial agents, no flouroquinolones were used in 2015, and the use of cephalosporins decreased to approximately 1 kg.

Consumption of zinc oxide and zinc in the pig production

Zinc is a critical trace element for animals and humans. For use of zinc in pig production, please see Textbox 4.3 in DANMAP 2014. Zinc is relevant in the context of DANMAP

because its use may select for resistance in some bacteria, including MRSA. Figure 4.6 shows the use of medical zink in Danish pig production. Piglets must be fed a sufficient amount of zinc every day, since the immediately available plasma and body zinc pool is very limited. Since the feed intake is very low right after weaning, the concentration of zinc in compound feedstuffs and mixed diets must be increased to counterbalance this. Two weeks after weaning, the feed intake has increased to a normal level and the lower dietary zinc inherent in the compound feedstuffs is sufficient. The most commonly used product is zinc oxide (ZnO) which contains 80% zinc.

The use of zinc oxide prescribed by veterinarians has increased over the last decade. From 2005 to 2011 a three-fold increase in use of zinc and zinc oxide was reported to VetStat, whereas the number of pigs produced in Denmark increased by 14%. Since 2011, the consumption has been relative stable at approximately 500 tonnes of zinc oxide (ZnO) equivalent to 400 tonnes of zinc (Zn).

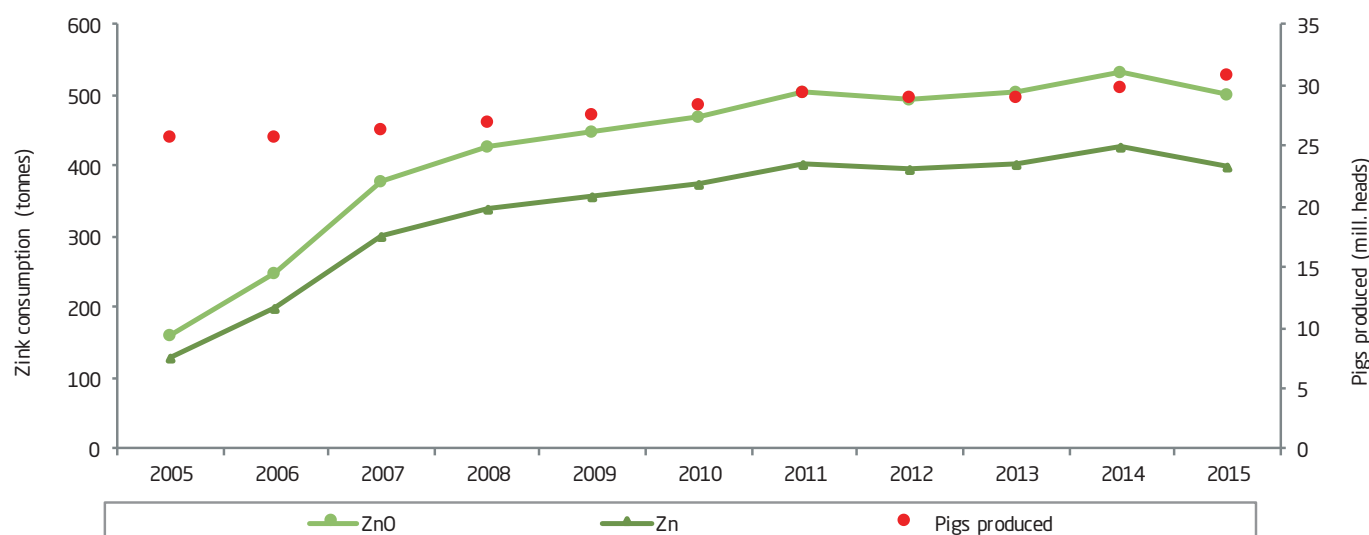
4.3.2 Antimicrobial consumption in cattle

In 2015, the overall consumption of antimicrobials in cattle remained at approximately 13 tonnes, similar to 2014. The production of veal and beef remained more or less at the same level from year to year, while the 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 2015. Approximately 10 kg of cephalosporins were used systemically, which represents a 66% reduction since 2014, when the dairy association decided to phase out its use, and

Figure 4.6. Consumption (tonnes) of zinc oxide (ZnO) and zinc (Zn) in the pig production, Denmark 2004-2015

DANMAP 2015



Note: In addition to medicinal ZnO, Zn is added to all compound feeding stuffs for pigs, up to a level of 150 ppm.

an 85% reduction compared since 2008, when cephalosporin consumption was at its peak (Figure 4.7).

The majority of antimicrobials administered parenterally 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 decreased again in 2015 (Table 4.2). In 2010, the Danish Cattle Association (Agriculture and Food Council) introduced the "milk quality campaign". The goal of the campaign is to reduce treatment of clinical mastitis by 50%, mainly through a reduction of treatment of subclinical mastitis, but also by increased monitoring of cell counts to determine the need for treatment. Order (DK) 785/2010 provides legal regulations of use of antimicrobial agents for mastitis in cattle (recommending using narrow spectrum penicillins). Furthermore, 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. Since then, the overall use of intramammary treatment, measured in DADDs, has been reduced by 4%. The relative proportion used for drying-off versus therapeutic treatment has shifted markedly from 22% versus 78% in 2010 to 55% versus 45% in 2015 (Table 4.3).

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

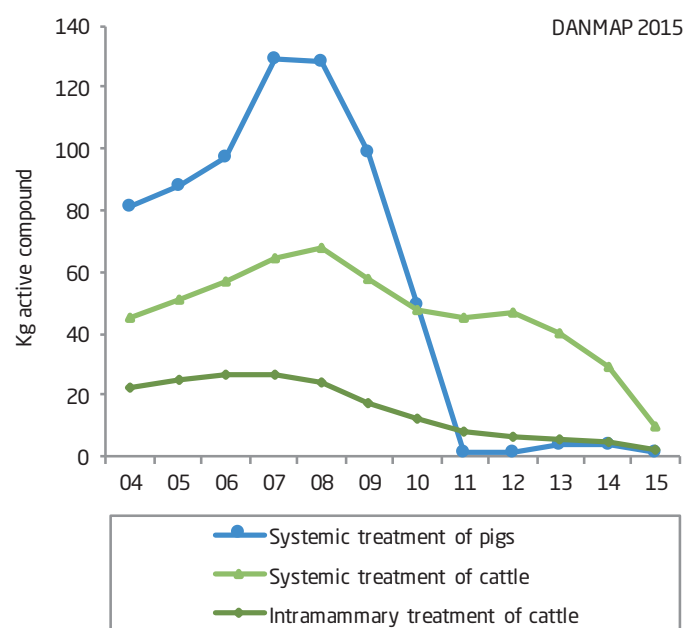


Table 4.2. Use of antimicrobial agents for intramammary application in cattle, Denmark

DANMAP 2015

	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
Doses per antimicrobial class	DADDs (1000's)										
Penicillins ^(a)	201	211	211	236	282	314	318	324	311	317	293
Aminoglycoside-benzylpenicillin combinations ^(b)	130	104	101	101	110	93	48	47	58	90	135
Cephalosporins, 1st generation	103	98	89	85	89	89	99	105	111	113	95
Cephalosporins, 3rd and 4th generation	110	124	127	112	76	51	34	30	24	21	10
Others ^(c)	21	20	16	15	14	12	9	8	0	0	1
Total	566	558	544	549	570	559	508	514	504	541	535
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

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 2015

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

Note: 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. One product used for both indications is included as half drying off and half therapeutic treatments.

4.3.3 Antimicrobial consumption in poultry

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

In 2015, the total consumption of antimicrobials in poultry (all species) was 2,440 kg active compound, an increase of 58% compared with 2014. This is the highest amount recorded since the DANMAP programme began.

For the first time since 2004, we also show the use of coccidiostats in poultry production (Textbox 4.1). The coccidiostats do not belong to groups of antimicrobial compounds presently used in human medicine, i.e. their use is not seen as posing a hazard to consumers.

The consumption of antimicrobials could not be precisely differentiated to the different sectors of the poultry production on the basis of the information entered into VetStat. Therefore, consumption for turkeys was identified 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.

Danish broiler farms have a very high level of biosecurity and the antimicrobial consumption in broiler production has generally been low compared with other species. Accordingly, a few disease outbreaks in some farms can markedly affect and cause considerable fluctuations in the national consumption statistic.

From the late 2014 throughout 2015 the broiler industry experienced several serious disease outbreaks caused by *E. coli* or other infections, associated with high mortality and increased health problems in the affected flocks. Many of the affected flocks and offspring from affected parent flocks were treated with a combination of lincosamides and spectinomycin (linco-spectin). During 2015, investigative work was carried out by the industry to determine the cause of the increase in the infections. The source of the problem was identified and control measures were implemented, (personal communication: J. Dahl and M. Nielsen Blom, Danish Agriculture and Food Council).

The antimicrobial consumption in poultry (excl. turkeys), increased by 22% already in 2014 and this was followed by a further increase of 184% in 2015, from 679 kg in 2014 to 1,931 kg. The increase included several classes of antimicro-

bials. For broilers, amoxicillin has been the most commonly used antimicrobial agent for more than a decade. However, in 2015 the consumption was also driven by increases in the consumption of tetracyclines, macrolides, lincosamides, and others (Table 4.1).

In the turkey flocks, antimicrobial consumption decreased markedly in 2015 compared with 2014, representing the first decrease since 2012. It may be explained by several changes in the turkey production in 2015. A new hybrid of birds, less prone to arthritis, was introduced and the vaccination strategy against Turkey Rhino Tracheitis virus was improved. A shift in type of antimicrobial agents towards using penicillins rather than macrolides was also observed (personal communication: S. Astrup, PoultryVet). These changes are reflected by an overall reduction in antimicrobial consumption of 41%, representing a decrease in the use of macrolides from 222 kg in 2014 to 77 kg in 2015 and an increase in the use of beta-lactamase sensitive penicillins from 39 kg to 65 kg.

4.3.4 Antimicrobial consumption in aquaculture, fur animals and companion animals

The antimicrobial consumption in aquaculture decreased by 42% to 2,970 kg in 2015 compared with 2014 (Table 4.1). Measured in kg active compound sulphonamide/trimethoprim comprised 56%, quinolones 34% and amphenicols 10%, respectively.

Antimicrobial consumption in aquaculture is mostly influenced by the summer temperatures. During the colder summers in 2011 and 2012, consumption was very low compared to previous years. In the following years (2013 and 2014) the summer months were extraordinarily warm, which lead to increased occurrence of bacterial infections and an increase in antimicrobial consumption. Furthermore, in recent years the aquaculture industry has focused on developing new and better vaccines and improving 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 in 2015 [personal communication: N. H. Henriksen, Danish Aquaculture].

Historically, the use of antimicrobial agents in mink production has increased steadily from 1,948 kg in 2004 to 5,177 kg in 2015. During the same period, the production of mink has also increased significantly from 12.5 million in 2004 to 17.8 million in 2015 (Source: Copenhagen Fur). In recent years, the industry focussed on strategies to reduce the antimicrobial consumption, by increasing the quality of

feed and improving animal welfare leading to a decrease in antimicrobial consumption in 2013 and 2014 of 11% and 14% respectively. However, in 2015 the consumption of antimicrobial agents increased by approximately 23% to 5,177 kg active compound, mainly due to secondary bacterial infections associated with the biggest outbreak of plasmocytosis in the history of Danish mink production. The outbreak affected 260 farms, which is approximately 20-25% of the total production. Furthermore, there was a higher occurrence of pneumonia caused by *Pseudomonas Aeruginosa* and more parvovirus outbreaks than in the previous years. An extensive testing programme has been launched by the industry to test for and to help prevent similar large disease outbreaks in the future (personal communication: Tina Struve). The consumption includes several antimicrobial agents, but was driven very mostly by penicillins, sulphonamide/thymethoprim and macrolides (Table 4.1). Use of fluoroquinolones and cephalosporins in fur animal production has been close to zero for more than a decade.

The information available on antimicrobial consumption in companion animals (horses and household pets) is not as detailed as for production animals.

A large proportion of antimicrobials used for companion animals are prescribed for treatment of chronic or recurrent disease, mainly dermatitis. Particularly the consumption of critically important antimicrobial agents could pose a risk to owners of diseased dogs that are frequently treated.

In 2015, the use of fluoroquinolones for use in household pets was 12 kg, compared with 9 kg in 2014. The amount of fluoroquinolones constituted 71% the total veterinary use (measured in kg) of fluoroquinolones in 2015, with the remainder used almost exclusively for horses. Similarly, household pets accounted for a significant proportion (161 kg or 69%) of the use of cephalosporins used in animals.

Over the past three years the consumption of fluoroquinolones for use in household pets has fluctuated from 9-12 kg, while the use of cephalosporins has been reduced from 211 kg in 2014 to 161 kg in 2015. During the same period we have observed a slight shift in use of the different antimicrobial classes. This may be an effect of treatment guidelines issued by Danish Veterinary Association in November 2012, recommending that use of critically important antimicrobials should be reduced as much as possible.

Birgitte Borck Høg and Leonardo de Knecht

Textbox 4.1

Consumption of coccidiostats in poultry in Denmark, 2008-2015

Background: Coccidiosis is one of the most important poultry diseases worldwide, and left unattended, it is a seriously limiting factor for intensive production of broilers on litter. Coccidia are highly host specific protozoa. Most coccidia in poultry belong to the genus *Eimeria* and grow and multiply intracellularly in epithelial and subepithelial cells, usually in the intestines. They have a direct life cycle. Oocysts are passed with feces to the environment where they sporulate and are ingested by the host. The life cycle in the gut takes about 7 days, followed by sporulation in 1-2 days under optimal conditions, resulting in hundreds of thousands of oocysts produced from one single ingested oocyst. The sporulated oocysts are resistant to most environmental conditions and can survive for months or even years outside a host, which is a key factor in the epidemiology of coccidiosis. Under field conditions, day old chickens are exposed to low doses of coccidia and develop immunity that will be life long as there is a frequent low-grade exposure to infection. Clinical outbreaks of coccidiosis are characterized by sudden onset of diarrhoea and elevated mortality. Even more important are subclinical infections that impair growth and feed conversion, causing considerable production losses.

Coccidiostats: Anticoccidial agents were introduced in the 1940s and have been crucial in controlling coccidiosis especially during the evolution of the broiler industry. The coccidiostatic effects enable development of protective immunity. Eleven different coccidiostats have been authorised for use in the EU; both ionophores and non-ionophores. Ionophores (monensin, narasin, lasalocid, salinomycin, maduramicin) have been widely used since the early 1970s. Ionophores are fermentation products that affect ion transport across cell membranes in target- and non-target species. Ionophores have antibacterial activity, and there are toxic interactions between some ionophores and some antibiotics. Non-ionophore (chemical) coccidiostats have no antibacterial effect. These drugs are thiamine analogues (amprolium), carbanilides (nicarbazin), and triazinone derivatives (diclazuril, toltrazuril). The environmental impact of coccidiostats needs further investigation.

Consumption: The total use of coccidiostats in Denmark increased steadily from 2008 to 2015 and salinomycin has been the dominating anticoccidial drug used in Denmark throughout this period (Figure 1, Table 1). The use of a combination of narasin and nicarbazin has been increasing since 2009. Since 2014 narasin also increased, mirroring that this drug is used after the first 25 days of the broiler growing period, as the withdrawal time is zero days. The lower but rather constant consumption of monensin and lasalocid comes from the turkey producers that use monensin in winter periods and lasalocid in summer. Since 2012, lasalocid has also been used in feed for gamebirds.

Figure 1. Consumption (tonnes) of the most frequently used coccidiostats in poultry, Denmark

DANMAP 2015

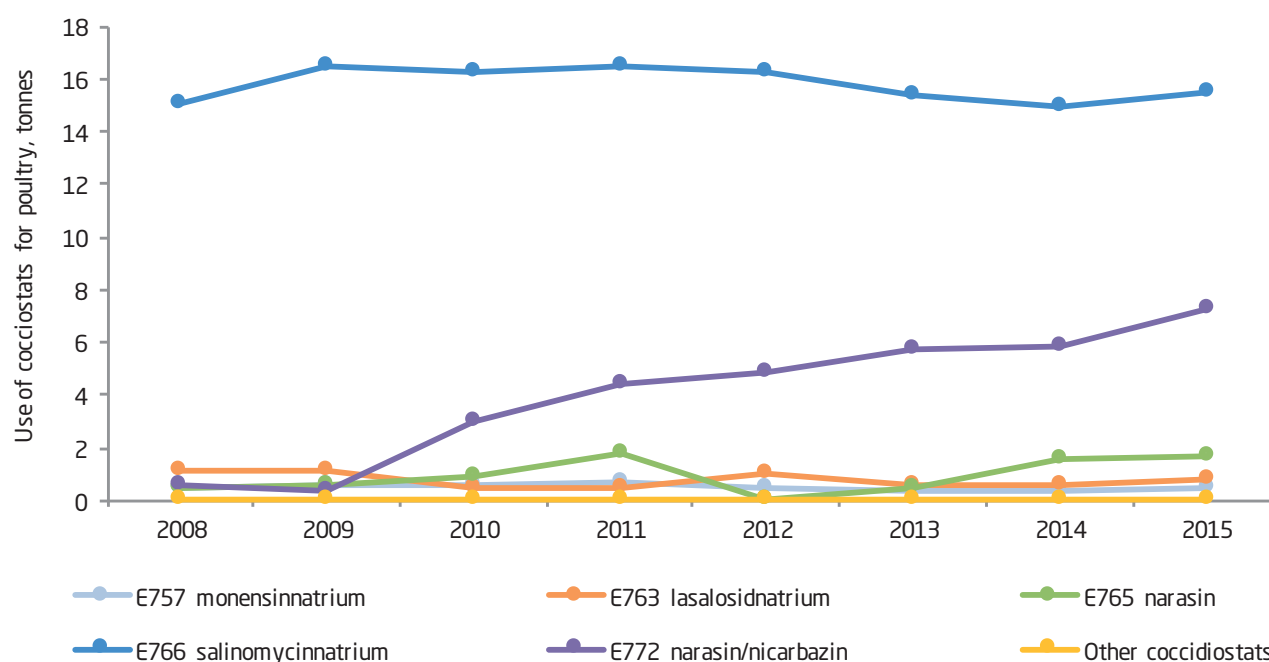


Table 1. Total consumption (kg) of coccidiostats in poultry, Denmark 2008-2015

DANMAP 2015

E-number	Name	2008	2009	2010	2011	2012	2013	2014	2015
E25	Halofuginon	1							5
E26	Salinomycin sodium	4							
E712	Flavofosfolid		1		1		0	6	
E757	Monensin sodium	489	640	622	679	503	327	347	441
E761	Metiklorpindol/metylbenzoat				7		4	0	
E763	Lasalosisodium	1177	1178	539	497	1060	547	585	770
E764	Halofuginon			1					0
E765	Narasin	499	617	872	1853		490	1568	1717
E766	Salinomycin sodium	15050	16429	16274	16528	16263	15367	14958	15457
E768	Nicarbazin		3	3	8		1	2	8
E769	Nifursol	4	6						
E770	Maduramicin ammonium							26	6
E771	Diclazuril	0	1	7	1				0
E772	Narasin/nicarbazin	603	348	2994	4448	4922	5743	5853	7324
E773	Semduramicin sodium							1	
51772	Narasin/Nicarbazin								16
Total		17827	19221	21311	24023	22748	22479	23347	25728

Resistance: The efficacy of many coccidiostats has been reduced by drug resistance in coccidia - by selection, which happens at various speeds. Development of resistance has been slow for ionophores compared to chemical drugs. In order to avoid resistance, drug programmes involving changing compounds during the growing period ("shuttle programs"), using combinations of ionophore and chemical coccidiostats, or changing drugs from one rotation to the next ("switching or rotation programs") for an entire broiler operation have been advocated. There is currently no scientific evidence to suggest that shuttle programs delay the emergence of resistance.

In recent years, there has been focus on the risk of promoting antimicrobial resistance from poultry that have been fed ionophores. In 2015, The Panel of Animal Feed of the Norwegian Scientific Committee for Food Safety evaluated the risk of development of antimicrobial resistance with the use of coccidiostats in poultry diets. They concluded that enterococci from poultry fed with narasin, monensin and salinomycin may become resistant to these agents, while the non-ionophore coccidiostats do not induce resistance in bacteria. However, data are lacking concerning the broader environmental impact of residues of coccidiostats.

Alternatives / future perspectives: In a recent position paper, the Federation of Veterinarians of Europe recommends that coccidiostats should be under veterinary prescription and be included in the ESVAC monitoring system. Live vaccines have been produced from various *Eimeria* strains, attenuated by rapid passage in vivo, selecting "precocious strains" that are non-pathogenic but still immunogenic. These vaccines are used in Denmark as alternative to coccidiostats in all non-caged replacement pullets, and in the rapidly growing production of organic broilers. Price and availability limits a wider use. A recombinant vaccine has been developed for breeders in order to confer antibody-mediated passive immunity to the broiler offspring. Further research may lead to development of subunit, recombinant and DNA based vaccines of relevance for future coccidiosis control.

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Textbox 4.2

Effect of new legislation on antimicrobial treatment of groups of pigs

Background: In June 2014, the Danish Veterinary and Food Administration (DVFA) introduced new legislation on antimicrobial treatment of groups of pigs. The rules apply to treatment of diarrheal or respiratory disease administered through feed or water. The aim is to achieve a more prudent use of antimicrobials in the pig production to support the goal to maintain a low level of antimicrobial resistance in Denmark. The legislation was introduced as part of the Second Veterinary Action Plan, covering initiatives 2013-2016 [BEK 534 of 27/05/2014].

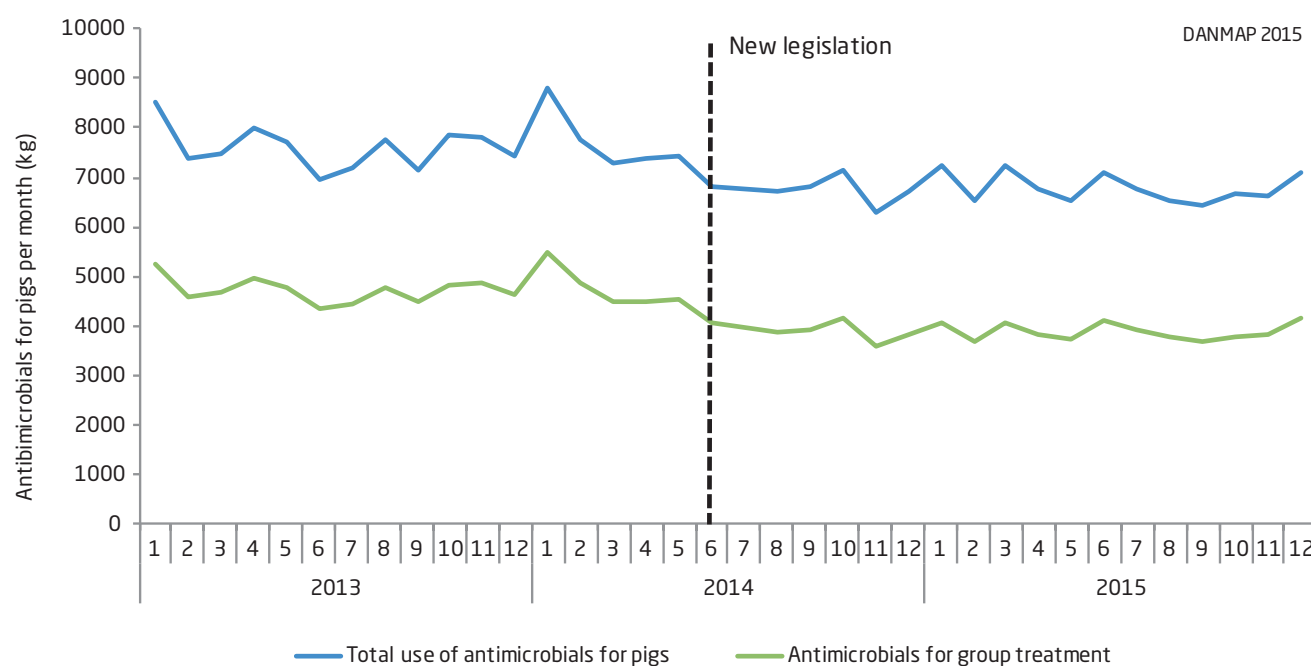
The legislation introduced the following limitations: Antimicrobials for group treatment may only be prescribed in connection with a consultation on the farm, and the veterinarian must verify the clinical diagnosis by laboratory testing. The prescription-period for antimicrobials for treatment of groups of pigs was also shortened compared to the general prescription rules.

Results: Since June 2014, antimicrobial use for group treatment has decreased by 17%, and the percentage of antimicrobials used for group treatment has been reduced from 62% to 57%. However, the decline in consumption of antimicrobials is probably mostly attributable to the reduction of the threshold levels of the Yellow Card Initiative in the same period. The effects of the legislation and reduction of threshold level cannot be distinguished clearly. A DVFA enforcement review in 2015 showed that veterinarians in general comply with the new rules.

Elisabeth Okholm Nielsen

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Figure 1. Prescription of antimicrobials per month (kg) in total and for group treatment of pigs, Denmark



5

ANTIMICROBIAL CONSUMPTION IN HUMANS



5. Antimicrobial consumption in humans



Highlights: In 2015, the total consumption of antimicrobials in humans was 18.5 defined daily doses per 1000 inhabitants per day (DID), very similar to the consumption in 2014 (18.58 DID).

Penicillins remained the most frequently used antimicrobial agent in both primary health care (65%) and in hospital care (51%). Within this drug group large changes in consumption have taken place in the last decade (from 2006 to 2015): In the primary sector betalactamase sensitive penicillins decreased by 19% (5.40 DID to 4.33 DID), while, simultaneously, combination penicillins increased by 1200% (0.12 DID to 1.42 DID). In the hospital sector betalactamase sensitive penicillins decreased by 27% (45.26 DBD to 30.52 DBD) and combination penicillins increased by approximately 600% (7.77 DBD to 54.14 DBD). In 2015 combination penicillins constituted 9% of the total antimicrobial consumption in the primary sector and 17% in the hospital sector, which makes them the largest antimicrobial drug group consumed in the hospital sector.

In 2015, the consumption of critically important antimicrobials was as follows at hospitals: Fluoroquinolones continued to constitute 9% of the consumption (9.30 DBD), cephalosporines constituted 15% (11.47 DBD), an increase in the proportional consumption compared to 2014 (11%, The proportional consumption of cephalosporins at hospitals increased and in 2015 they constituted 15% of the hospital consumption, corresponding to 11.47 DBD. 2nd generation cephalosporins make the largest share of the cephalosporins used at hospitals (10.37 DBD in 2015). In the primary sector the consumption of cephalosporins is close to zero. The consumption of carbapenems, mainly meropenem, has increased drastically during the last five years, in 2015 it constituted 4% of the total consumption of antimicrobials at hospitals.

The past decade has seen three marked changes in the overall amounts of antimicrobials consumed:

- (1) The number of DDDs per patient has increased from 17.9 DDD/patient to 21.8 DDD/patient (22%).
- (2) The number of DDDs per 1,000 inhabitants increased from 5,584.5 in 2006 to its peak of 6,387.5 in 2011 (14%), but declined to 5,913 in 2015, yielding a total increase of 6% during the decade.
- (3) The number of antimicrobial prescriptions per 1,000 inhabitants increased until 2011 but has since declined, showing a total decrease from 587.47 in 2006 to 511.46 in 2015 (- 13%).

During the past twenty years, the total consumption of antimicrobials increased by 38% (from 13.60 DID in 1996 to 18.50 DID in 2015). This increase happened primarily from 2000 to 2011 with large increases in 2006 - 2007 and 2009 -2010. As for the last decade, changes in the primary sector were mainly due to changes in the consumption of the different penicillins: the beta-lactamase resistant penicillins increased from 0.30 DID to 1.38 DID (360%), penicillins with extended spectrum increased from 2.50 DID to 3.61 DID (44%) and combination penicillins increased from zero to 1.42 DID. The increase in penicillins with extended spectrum was primarily due to changes in the treatment of urinary tract infections from sulfonamides (from 0.40 DID to 0.18 DID) to pivmecillinam (0.30 DID to 2.38 DID). The beta-lactamase sensitive penicillins were the only group of penicillins, where the consumption did not continue to increase through all 20 years but decreased since 2007, yielding a total reduction from 4.50 DID to 4.30 DID (- 4%).

Also at hospitals, notable changes in the consumption of penicillins were observed for the twenty-year period: while beta-lactamase sensitive penicillins increased slightly (8.02 to 10.03 DBD, 25%), the consumption of the other three penicillin groups increased steeply, penicillins with extended spectrum by 52% (11.21 to 17.01 DBD), beta-lactamase resistant penicillins by 120% (4.44 to 9.80 DBD) and combination penicillins by > 1000% (0.03 to 17.90 DBD). Increases were also observed for cephalosporins (3.99 to 10.40 DBD), fluoroquinolones (1.46 to 9.30 DBD) and carbapenems (0.36 to 4.10 DBD). While the consumption of the former two has stabilized or slightly decreased since 2010, the consumption of carbapenems, mainly meropenem, has continued to increase.

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. Sales are reported through the pharmacies which, for the primary sector 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 the hospital sector sales are available through the hospitals pharmacy and contain information on the amount and formulations of antimicrobials delivered to the different departments. Thus for both sectors quite detailed information is achieved but specifications on the exact amount actually used are missing as are informations regarding the indication for the specific treatment and the condition of the patient. For both sectors work is in progress that will ensure these more specific treatment data in the future.

Recording has been performed since 1994 - for the primary sector this included all sales from the beginning, for the hos-

pital sector the first recordings were uncomplete and did not include sales from multipackages and marginal products. These data have been included since January 1997. For more information on the registration of the human consumption please see chapter 2 "Danmap - a 20 year perspective".

As for previous reports the consumption in the primary sector corresponds to all antimicrobials delivered upon prescriptions from the general practitioner, medical specialists and dentists as well as prescriptions handed out at hospitals to patients upon discharge. For the hospital sector only data from public somatic hospitals are included - data from psychiatric hospitals, hospices and rehabilitation centers were omitted since they contribute a large proportion of bed-days but presumably much lower consumption of antimicrobials and thus might skew the data.

The term 'antimicrobial agents' covers in this chapter all systemic antibacterial agents for human use listed in the Anatomical Therapeutic Chemical (ATC) Classification under the code J01. The only other antibacterials included are metronida-

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



zole (ATC code P01AB01) and vancomycin (ATC code A07AA09), since these constitute important systemic antibacterial treatment as well. Their consumption has been included since DANMAP 2014 and tables and figures were updated ten years back. Tuberculostatica, antiviral and antifungal drugs are not included.

5.2 Total consumption (Primary Health Care and Hospital Care)

In 2015 the total consumption of antimicrobials in both sectors was 18.50 defined daily doses per 1000 inhabitants per day (DID), close to the total consumption of 2014, where a total of 18.58 DID was registered (Figure 5.1). This corresponds to 52,691kg active compound consumed in 2015 (table A5.1 in web annex).

The total consumption calculated over 20 years shows no big variations for the first five years of registration from 1996 (13.40 DID) till 2000 (13.63 DID), although there was a single top with a 5% higher consumption in 1998. From 2000 onwards, continuously increasing trend was observed until the year 2011, where the consumption peaked with 19.31 DID, an overall increase of 42%. Since 2011 the consumption has levelled off, primarily due to minor decreases in the primary sector, corresponding to a total decline of 4.2% over the past four years. Generally, the total consumption in the 20 year period increased by 38%, from 13.40 DID in 2006 to 18.50 DID in 2015.

Primary health care accounts for approximately 90% of the consumption, thus significant changes in prescription here will markedly influence the total consumption.

Among the Scandinavian countries, the Danish consumption during the past 20 years was generally the lowest during the 1990s. However, continuously increasing trends in Denmark and simultaneously decreasing trends in Sweden, resulted in Denmark having the highest consumption of the three countries in 2014 and Sweden the lowest (www.ecdc.europa.eu/en; Data & Tools, ESAC-database). For Norway the consumption fluctuated between 16 and 18 DID during the 20 years, showing decreasing trends for the last three years. In 2015 the total consumption of antimicrobials was 13.6 DID in Sweden and 18,8 DID in Norway. The Norwegian national action plan from 2015 aiming for a 30% reduction in the total consumption ((with 2012 as the reference point) will expectantly result in a much lower consumption in the years to come.

At the European level, Denmark reports a comparatively low consumption of antimicrobial agents, specifically characterized by the proportionally high consumption of penicillins (ESAC-Net 2014).

Table 5.1a. Consumption of antimicrobial agents for systemic use in primary health care (DDD/1000 inhabitant-days), Denmark, 1996 - 2005

DANMAP 2015

ATC group ^{a)}	Therapeutic group	Year									
		1996	1997	1998	1999	2000	2001	2002	2003	2004	2005
J01AA	Tetracyclines	1.07	0.98	0.98	0.93	0.98	0.99	1.04	1.07	1.17	1.28
J01CA	Penicillins with extended spectrum	2.50	2.39	2.39	2.29	2.29	2.47	2.51	2.52	2.63	2.79
J01CE	Beta-lactamase sensitive penicillins	4.50	4.57	4.81	4.48	4.69	4.91	5.00	5.07	5.20	5.28
J01CF	Beta-lactamase resistant penicillins	0.30	0.34	0.40	0.48	0.52	0.65	0.77	0.85	0.92	0.97
J01CR	Combinations of penicillins, including beta-lactamase inhibitors	0.00	0.02	0.03	0.02	0.02	0.03	0.04	0.05	0.06	0.08
J01D	Cephalosporins and other β -lactam antibiotics	0.00	0.02	0.03	0.02	0.02	0.03	0.03	0.02	0.02	0.03
J01EA	Trimethoprim and derivatives	0.30	0.30	0.32	0.32	0.33	0.35	0.36	0.38	0.41	0.44
J01EB	Short-acting sulfonamides	0.40	0.41	0.41	0.38	0.38	0.36	0.36	0.36	0.36	0.35
J01EE	Combinations of sulfonamides and trimethoprim, including derivatives	0.10	0.08	0.04	0.03	0.04	0.03	0.02	0.03	0.00	0.00
J01FA	Macrolides	1.90	2.03	2.28	2.17	2.02	2.10	2.15	2.13	2.23	2.41
J01FF	Lincosamides	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
J01GB	Aminoglycosides	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.00	0.01
J01MA	Fluoroquinolones	0.20	0.22	0.23	0.20	0.15	0.17	0.18	0.25	0.28	0.33
J01XA	Glycopeptides	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
J01XB	Polymyxins	0.00	0.03	0.02	0.03	0.03	0.02	0.02	0.02	0.02	0.02
J01XC	Steroid antibacterials (kombination fusidic acid)	0.00	0.02	0.02	0.02	0.02	0.01	0.01	0.01	0.01	0.01
J01XE	Nitrofurantoin derivatives (nitrofurantoin)	0.04	0.35	0.36	0.36	0.38	0.39	0.41	0.42	0.43	0.45
J01XX	Other antibacterials (methenamine >99%)	0.50	0.46	0.43	0.40	0.36	0.33	0.34	0.32	0.30	0.28
J01XD and P01AB*	Nitroimidazole derivatives (metronidazole)	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
J01+P01AB	Antibacterial agents for systemic use (total)	12.20	12.24	12.76	12.15	12.21	12.85	13.26	13.53	14.06	14.96

a) From the 2015 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 Primary Health Care

5.3.1 Total consumption in primary health care

In 2015 the consumption in the primary sector at 16.46 DID was almost the same as in 2014, (16.40 DID), (Table 5.1 a and b). Since 2006 the total consumption increased by 6% (15.45 DID to 16.46 DID). During the last 20 years the increase was 34%, showing gradual increases from 12.20 DID in 1996 to the peak of 17.34 DID in 2011 and lately reaching a plateau with the consumption of 2015 being close to that reported in 2009, (Figure 5.1b).

The trends in the consumption of leading antimicrobials for the last decade are shown in figure 5.2. Beta-lactamase sensitive penicillins continued being the antimicrobial class with the highest consumption. They are the only antimicrobial class with an overall decreasing trend for the past decade, decreasing from 5.40 DID in 2006 to 4.33 in 2015, a decline of 19%. Macrolides also show decreasing trends for the 10 year period, from 2.31 DID in 2006 to 1.77 DID in 2015, the decrease being interrupted from 2010 to the beginning of 2012 due to a doublem, nationwide *Mycoplasma pneumoniae* epidemic. A new *Mycoplasma* epidemic was registered in the autumn of 2015, lasting until the spring of 2016; this will most likely be reflected in the consumption patterns for 2016.

Most notable increases were shown for penicillins with extended spectrum and combination penicillins; these increased from

2.95 to 3.61 DID (22%) and from 0.12 to 1.42 DID (1200%), respectively. Such increases may reflect changes in treatment recommendations for specific infections; for the penicillins with extended spectrum the change is probably due to shifts in the treatment of urinary tract infections from sulfamethizole to pivmecillinam as the prime drug. This change caused the number of DDDs to decrease from 4 g to 0.6 g per day, but simultaneously the duration of treatment increased. Likewise the change from phenoxymethylpenicillin to amoxicillin with

Figure 5.1.b Consumption in Primary Health Care 1996 - 2015, DID

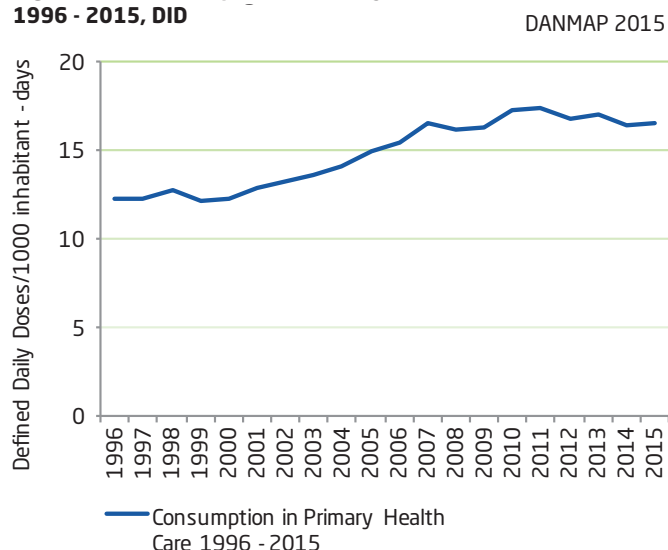


Table 5.1b. Consumption of antimicrobial agents for systemic use in primary health care (DDD/1000 inhabitant-days), Denmark, 2006 - 2015

DANMAP 2015

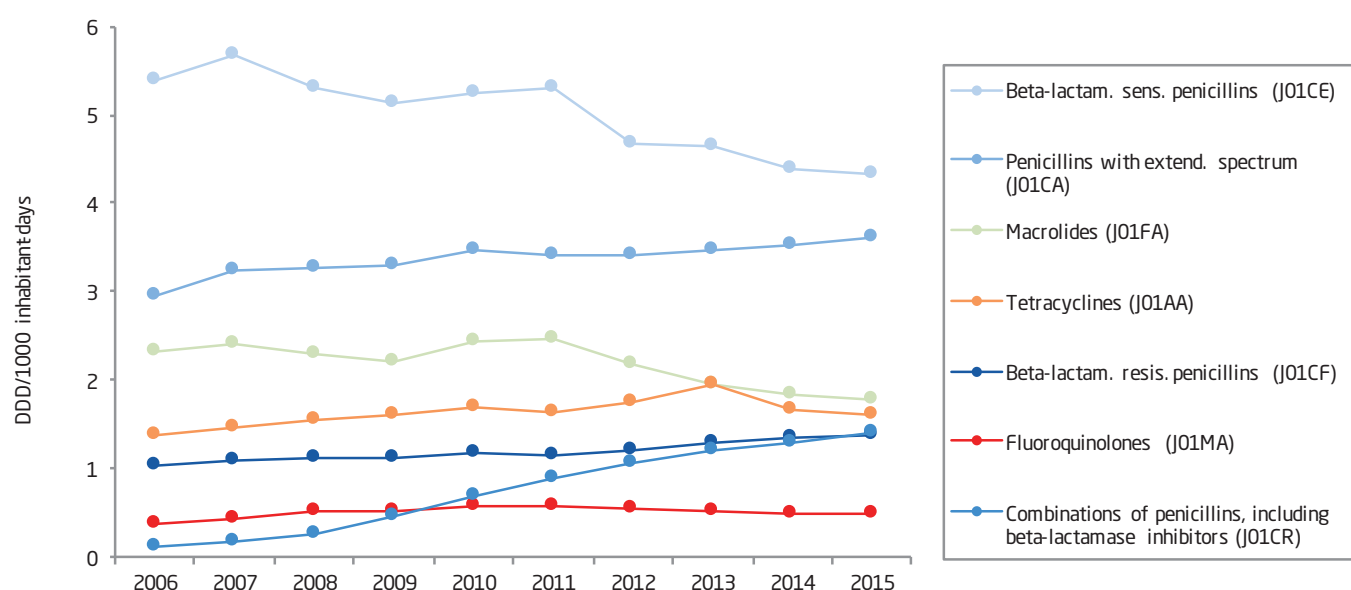
ATC group ^{a)}	Therapeutic group	Year									
		2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
J01AA	Tetracyclines	1.38	1.48	1.54	1.61	1.69	1.64	1.76	1.96	1.66	1.61
J01CA	Penicillins with extended spectrum	2.95	3.25	3.26	3.29	3.47	3.41	3.40	3.48	3.53	3.61
J01CE	Beta-lactamase sensitive penicillins	5.40	5.67	5.30	5.12	5.25	5.31	4.68	4.65	4.38	4.33
J01CF	Beta-lactamase resistant penicillins	1.05	1.09	1.12	1.13	1.17	1.14	1.21	1.30	1.36	1.38
J01CR	Combinations of penicillins, including beta-lactamase inhibitors	0.12	0.19	0.27	0.45	0.68	0.89	1.05	1.22	1.30	1.42
J01D	Cephalosporins and other β -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	0.47	0.49	0.49	0.48	0.51	0.50	0.52	0.53	0.55	0.56
J01EB	Short-acting sulfonamides	0.35	0.31	0.28	0.27	0.26	0.24	0.22	0.22	0.21	0.18
J01EE	Combinations of sulfonamides and trimethoprim, including derivatives	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.01	0.00	0.00
J01FA	Macrolides	2.31	2.42	2.28	2.21	2.44	2.47	2.19	1.93	1.79	1.77
J01FF	Lincosamides	0.02	0.02	0.03	0.03	0.04	0.04	0.04	0.05	0.05	0.05
J01GB	Aminoglycosides	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01
J01MA	Fluoroquinolones	0.37	0.44	0.51	0.52	0.57	0.57	0.55	0.52	0.50	0.49
J01XA	Glycopeptides	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
J01XB	Polymyxins	0.02	0.02	0.02	0.02	0.02	0.03	0.02	0.02	0.02	0.02
J01XC	Steroid antibacterials (kombination fusidic acid)	0.01	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01
J01XE	Nitrofurantoin derivatives (nitrofurantoin)	0.46	0.47	0.47	0.49	0.51	0.50	0.49	0.49	0.48	0.45
J01XX	Other antibacterials (methenamine >99%)	0.27	0.26	0.27	0.26	0.27	0.26	0.25	0.24	0.24	0.25
J01XD and P01AB*	Nitroimidazole derivatives (metronidazole)	0.22	0.23	0.24	0.27	0.28	0.28	0.28	0.28	0.28	0.28
J01+P01AB	Antibacterial agents for systemic use (total)	15.45	16.45	16.15	16.22	17.21	17.34	16.75	16.95	16.40	16.46

a) From the 2015 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

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

DANMAP 2015



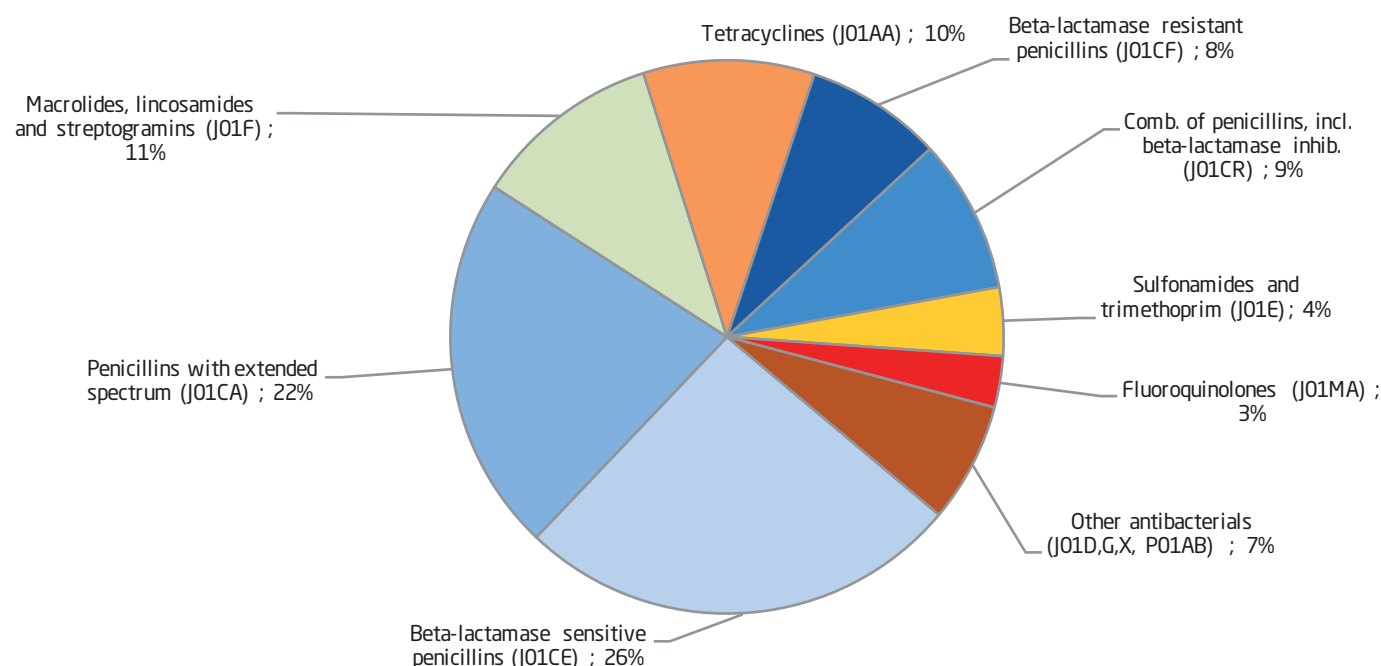
clavulanic acid for the treatment of respiratory tract infections led to changes in DDD from 2g to 1g per day but probably no noteworthy changes in the recommended duration of treatment

The 2015 distribution of the leading antimicrobial classes is shown in figure 5.3. Beta-lactamase sensitive penicillins represented the largest therapeutic group of antimicrobial agents consumed, constituting 26% of the consumption. Penicillins in general (including combinations with beta-lactamase inhibitors) accounted for 65% of the total consumption.

Macrolides accounted for 11%, tetracyclines for 10%, sulfonamides and trimethoprim for 4%, nitrofurantoin and Fluoroquinolones for each 3% (Figure 5.3). A decade ago, in 2006, beta-lactamase sensitive penicillins accounted for 35%, penicillins with extended spectrum for 19% and macrolides for 15%. Tetracyclines accounted for 9%, beta-lactamase resistant penicillins for 8%, sulfonamides and trimethoprim for 6%, nitrofurantoin for unchanged 4%, fluorquinolones for unchanged 3% and combination penicillins for only 1% (DANMAP 2006, not shown).

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

DANMAP 2015



5.3.2 Initiatives on prudent use of antimicrobials in primary health care

It is assumed that patient demands for receiving antibiotic treatment may play an important role in the increasing total consumption of antimicrobial drugs as well as causing changes observed in the consumption of individual antimicrobial agents. National campaigns aimed at the population have been launched to target this problem, anchored on the citizen-centered homepage "www.antibiotikaellerej.dk" (see also DANMAP 2012).

Another focus on the reduction of antimicrobials is improving diagnostics and point-of-care-tests and their applicability in primary health care, both at the GP and in nursing homes and other institutions. It is generally believed that the amount of antimicrobials consumed will be reduced consequently. Also the Danish Health Authority has in recent years focused on issuing guidelines on the diagnostics and treatment of specific infectious diseases. An example is the guidelines on diagnostics and treatment of otitis media, issued in 2015. Recently, guidelines on the prescription of antibiotics for odontal processes and infections were issued.

Data on prescription habits for the different medical specialties are not included in this report, but are crucial for future efforts

on a more prudent use of antimicrobial agents. Further investigations on these will be undertaken over the next years.

5.3.3 Measures at treated patient level

In 2015, each treated patient received 21.8 DDDs per year, which is slightly higher than in 2014 (21.5 DDDs). The same applies to the DDDs per package, which increased from 9.9 in 2014 to 10.2 in 2015 (Table 5.2). During the last decade the number of DDDs per patient per year increased by 22% and the DDDs per package by 17%, while the number of packages per patient remained quite stable around 2.1. The number of patients treated decreased by 13%, from 310.3 to 270.6 patients per 1000 inhabitants per day (Table A5.2 in web annex).

The most pronounced changes in DDD per patient since 2006 were observed for the following drug classes: increases were seen for beta-lactamase resistant penicillins (+ 34%), tetracyclines (+ 26%), combination penicillins (+ 25%), cephalosporins (+ 25%) and for penicillins with extended spectrum (+ 24%) (Table 5.2 and Table A5.3 in web annex).

Reductions in DDD per patient were observed for trimethoprim, short-acting sulfonamides and lincosamides with - 11%, - 2% and - 19%, respectively.

Table 5.2. Number of DDDs and packages per treated patient among leading groups of antimicrobial agents in primary health care, Denmark

DANMAP 2015

ATC group ^{a)}	Therapeutic group	Indicator	Year									
			2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
J01AA	Tetracyclines	DDD / patient	40.9	43.0	44.4	45.2	45.9	44.0	47.6	51.6	49.9	51.7
		Packages / patient	1.9	2.0	2.0	2.0	2.0	1.9	2.1	2.1	2.1	1.9
		DDD / package	21.0	22.0	22.7	22.7	22.7	22.6	23.1	25.5	23.8	27.8
J01CA	Penicillins with extended spectrum	DDD / patient	14.2	14.4	14.7	14.8	14.9	14.8	16.1	16.7	17.2	17.6
		Packages / patient	1.6	1.6	1.6	1.6	1.6	1.6	1.7	1.7	1.7	1.7
		DDD / package	8.9	9.0	9.2	9.2	9.0	9.2	9.7	10.0	10.0	10.3
J01CE	Beta-lactamase sensitive penicillins	DDD / patient	11.5	11.7	11.8	11.8	11.8	11.8	11.8	12.0	11.9	12.1
		Packages / patient	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4
		DDD / package	8.0	8.2	8.2	8.4	8.4	8.4	8.4	8.5	8.5	8.8
J01CF	Beta-lactamase resistant penicillins	DDD / patient	13.0	13.4	13.7	13.9	14.2	13.8	15.5	16.4	17.1	17.4
		Packages / patient	1.5	1.5	1.5	1.5	1.5	1.4	1.6	1.7	1.8	1.8
		DDD / package	8.6	8.7	9.0	9.1	9.3	9.6	9.7	9.4	9.7	9.5
J01CR	Combinations of penicillins, incl. beta-lactamase inhibitors	DDD / patient	19.3	19.1	19.9	20.4	21.1	21.9	22.3	22.6	23.2	23.6
		Packages / patient	1.8	1.6	1.6	1.5	1.5	1.6	1.6	1.6	1.6	1.6
		DDD / package	10.7	11.7	12.4	13.3	13.7	14.1	14.3	14.3	14.3	15.1
J01FA	Macrolides	DDD / patient	12.6	12.4	12.5	12.5	12.2	11.5	12.4	12.6	12.8	12.5
		Packages / patient	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.6	1.6	1.6
		DDD / package	8.3	8.1	8.1	8.1	8.1	7.9	8.0	8.0	7.9	7.7
J01MA	Fluoroquinolones	DDD / patient	10.3	10.6	11.0	11.2	11.2	11.5	11.7	11.8	11.9	12.0
		Packages / patient	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
		DDD / package	6.9	7.0	7.5	7.6	7.6	7.7	7.8	7.8	7.9	7.9
J01	Antibacterial agents for systemic use (total)	DDD / patient	17.9	17.3	18.9	19.2	19.6	19.4	20.6	21.3	21.5	21.8
		Packages / patient	2.0	1.9	2.1	2.1	2.1	2.1	2.1	2.1	2.2	2.1
		DDD / package	8.7	8.9	9.1	9.3	9.3	9.3	9.7	9.9	9.9	10.2

a) From the 2015 edition of the Anatomical Therapeutic Chemical (ATC) classification system

For tetracyclines, both the number of DDDs per patient and DDDs per package have continuously been higher than for any other antimicrobial group, primarily due to the fact that tetracyclines are commonly used for acne treatment with higher dosages given in treatments of typically three, but up to six months.

Figure 5.4 shows the trends in consumption for the number of packages per 1,000 inhabitants per year compared to the treated patients per 1,000 inhabitants per year and the total consumption of DDDs per 1,000 inhabitants per day for the last decade, when measured against the consumption 15 years ago. Although the trends parallel each other, DDDs per 1,000 inhabitants per day remained above 110% through all ten years shown, compared to the index year 2001. From 2006 until 2011 increasing trends were observed with a peak of 123% in 2011, followed by gradual decreases. Development in the number of treated patients and the number of packages show almost equal lines, starting at 102% and 100%, respectively, fluctuating around 100% in the period from 2006 to 2011 and, following distinct decreases, ending at 89% and 92%, respectively.

When comparing the consumption of DDD per 1,000 inhabitants to the number of prescriptions and users per 1,000 inhabitants once more changes in the consumption of DDDs are noted: the number of DDDs increased from 2006 to 2011 (5,585 to 6,388 DDD/1,000 inhabitants) and slowly declined thereafter, reaching 5,913 DDD/1,000 inhabitants in 2015. The number of users and prescriptions paralleled each other in trends without noteworthy changes in the first six years, but with minor declines through the past four years. Overall

there was a decrease through the decade from 313 to 274 users/1,000 inhabitants and 587 to 511 prescriptions/1,000 inhabitants, respectively (Figure 5.5).

Table 5.3 shows the number of prescriptions per 1,000 inhabitants for leading antimicrobial agents in primary health care from 2006 to 2015. In 2015 530.62 prescriptions per 1,000 inhabitants were redeemed at pharmacies, a decrease of 12% from the 603.60 receptions per 1,000 inhabitants in 2006. Generally, the number of prescriptions decreased by 24% from 2006 to 2015 (171.29 to 130.07 prescriptions per 1,000 inhabitants), compared to the 19% decrease in DID shown in figure 5.2. For betalactam sensitive penicillins and combination penicillins fluctuations in the number of prescriptions followed fluctuations in DIDs. However, it is generally clear that changes in consumption measured as DIDs do not necessarily mirror changes in the number of prescriptions.

For the penicillins with extended spectrum the number of prescriptions increased from 2006 until 2010, from 75.78 to 85.04 prescriptions per 1,000 inhabitants, but declined again to 74.88 prescriptions per 1,000 inhabitants in 2015. During the same time, the consumption measured in DID gradually increased, from 2.95 DID in 2006 to 3.61 DID in 2015.

For the beta-lactamase resistant penicillins the number of prescriptions increased from 2006 to 2011, (29.38 -30.34 prescriptions per 1,000 inhabitants) but decreased the next four years to 28.85 prescriptions per 1,000 inhabitants in 2015. This change in trends was not reflected in the consumption of DID, that seemed almost unchanged but with a slight increase over the decade, from 1.05 DID in 2006 to 1.38 DID in 2015.

Figure 5.4. Indicators of antimicrobial consumption (J01) in primary health care, Denmark

DANMAP 2015

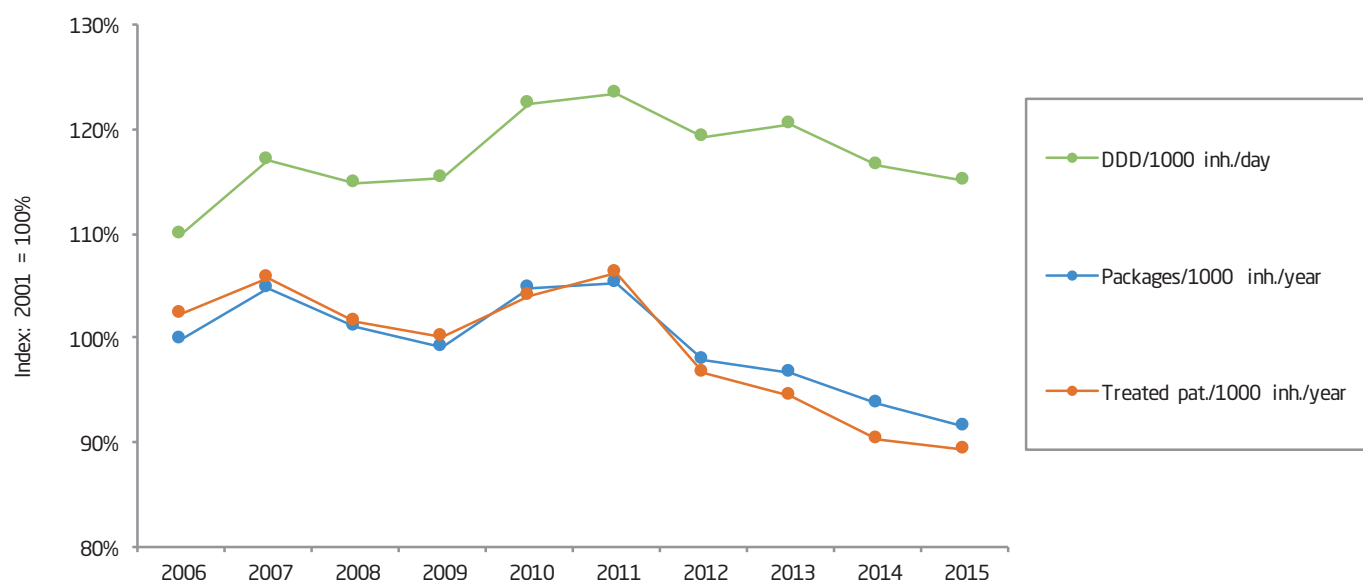
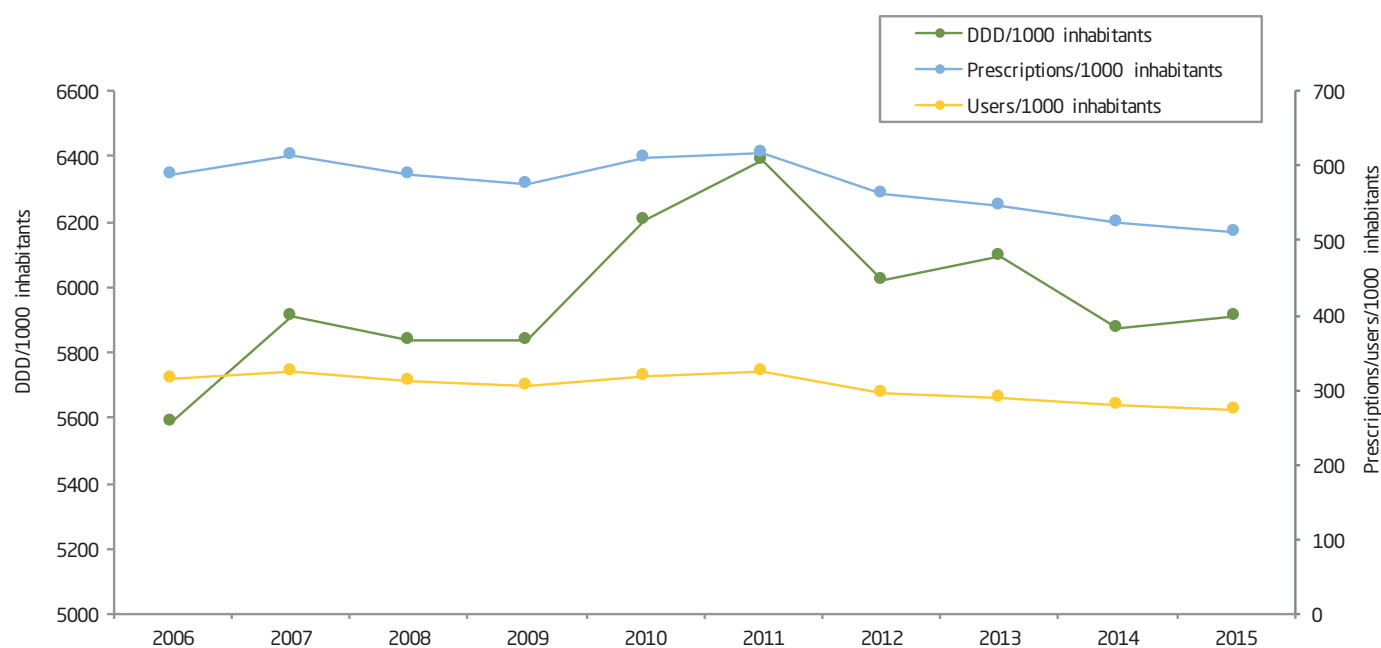


Figure 5.5. Indicators of antimicrobial consumption (J01) in primary health care, Denmark

DANMAP 2015



Such changes in DID that are not reflected in the number of prescriptions and vice versa probably reflect alterations in consumption within the same antimicrobial class. For instance there may be increases in DDD according to new treatment recommendations or clinical guidelines - this happens when the dosage or the daily intake increases (e.g. from 200mg to 400mg per dosage or from three to four times daily).

A concurrent decrease in DDDs may be caused by changes in the recommended treatment duration (e.g. from seven days treatment to five days). Finally, changes in the application or preference of the different drugs may change over time - for

instance, when a specific drug is used for infections other than the primary indication.

Table 5.4 shows the total consumption of antimicrobial classes at a regional level for the last five years, 2011 to 2015. All five regions show decreases for both the DDD per 1,000 inhabitants per day and for the number of prescriptions per 1,000 inhabitants per day. In 2015 the Region of Zealand had the highest consumption, when measured in DDD per 1,000 inhabitants per day, while the Central Denmark Region and the Northern Denmark Region had the lowest consumption, 16.9 DID and 15.2 DID, respectively. The Region Zealand had

Table 5.3. Number of prescriptions per 1,000 inhabitants for leading antimicrobial agents in primary health care, Denmark

DANMAP 2015

ATC group ^(a)	Therapeutic group	Year									
		2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
J01AA	Tetracyclines	12.25	12.52	12.73	13.02	13.44	13.66	13.53	13.85	12.20	11.32
J01CA	Penicillins with extended spectrum	75.78	82.03	81.27	81.07	85.04	84.19	77.29	76.09	75.32	74.88
J01CE	Beta-lactamase sensitive penicillins	171.29	177.07	164.38	158.72	162.81	164.34	145.50	142.16	134.79	130.07
J01CF	Beta-lactamase resistant penicillins	29.38	29.67	29.89	29.87	30.02	30.34	28.50	29.07	29.24	28.85
J01CR	Combinations of penicillins, including beta-lactamase inhibitors	2.27	3.57	4.95	8.02	11.70	14.95	17.34	19.71	20.52	22.03
J01E	Sulphonamides and trimethoprim	36.56	33.62	30.49	29.51	29.31	27.63	26.47	26.15	24.65	22.45
J01FA	Macrolides	67.03	71.38	66.84	64.44	72.67	78.75	64.72	56.15	51.38	51.75
J01MA	Fluoroquinolones	13.09	15.19	17.05	16.87	18.45	18.10	17.24	16.04	15.30	15.04
J01XX	Other antibacterials (methenamine >99%)	7.70	7.17	7.43	7.67	7.53	7.74	7.54	7.48	7.16	7.35
J01XD and P01AB	Nitroimidazole derivatives (metronidazole)	13.77	14.44	15.32	16.28	16.73	16.90	16.86	16.51	16.31	16.47
J01 (incl. P01)	Antibacterial agents for systemic use (total)	603.60	630.08	606.26	595.28	628.78	638.08	582.69	565.16	542.53	530.62

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

DANMAP 2015

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

also the highest number of prescriptions redeemed per 1,000 inhabitants, while the Central Denmark Region had the lowest, 574.4 and 494.5 prescriptions per 1,000 inhabitants, respectively. Interestingly Region Zealand has the highest amount and proportion of phenoxymethylpenicillin consumed in the country (data not shown). Thus the regional differences in consumption may resemble differences in local recommendations and treatment guidelines as well as in custome.

5.3.4. Penicillins

Penicillins account for 2/3 of all antimicrobial consumption in primary health care, but while this has remained unchanged since 2006 individual changes in the consumption of the different groups of penicillin have been observed. The consumption of penicillins in total increased slightly in 2015 (10.74 DID) compared to 2014 (10.58 DID). Increases have been noticed for all years in the last decade except for the year 2013 to 2014, where no apparent increase was observed. From 2006 to 2015 the consumption of penicillins increased by 13% (corresponding to a total of 9.51 DID in 2006), (table 5.1).

20 year trends of the consumption of leading penicillins are shown in figure 5.6. The consumption of beta-lactamase sensitive penicillins was almost unchanged from 2014 to 2015, with a slight decrease of 1%. Small increases in consumption were observed for beta-lactamase resistant penicillins (1.5%) and penicillins with extended spectrum (2.2%). Marked increases were seen for 'combination penicillins' (9.2%). The consumption of these three groups has risen considerably over the past decade, with particular increases for beta-lactamase resistant penicillins from 1.05 DID to 1.38 DID (31%), penicillins with extended spectrum from 2.95 DID to 3.61 DID (22%) and the combination penicillins with betalactamase inhibitors from 0.12 DID to 1.42 DID (1183%). For the 20-year period beta-lactamase sensitive penicillins decreased with 3.8%,

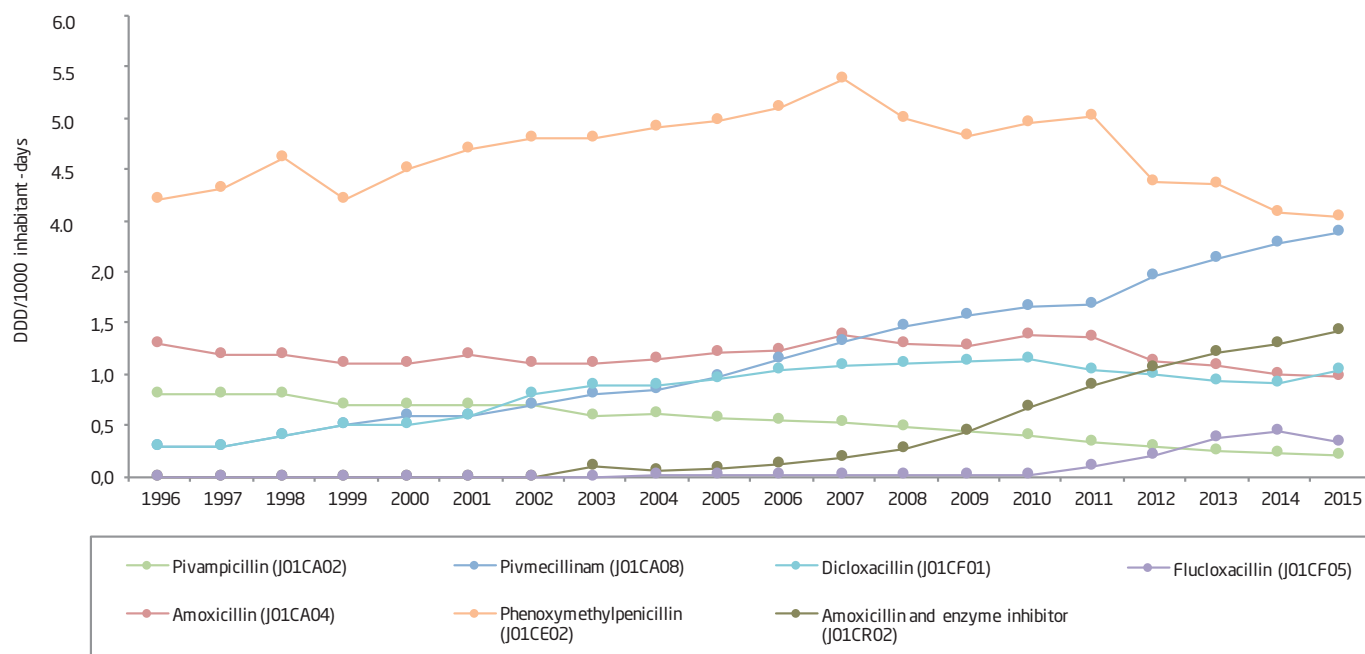
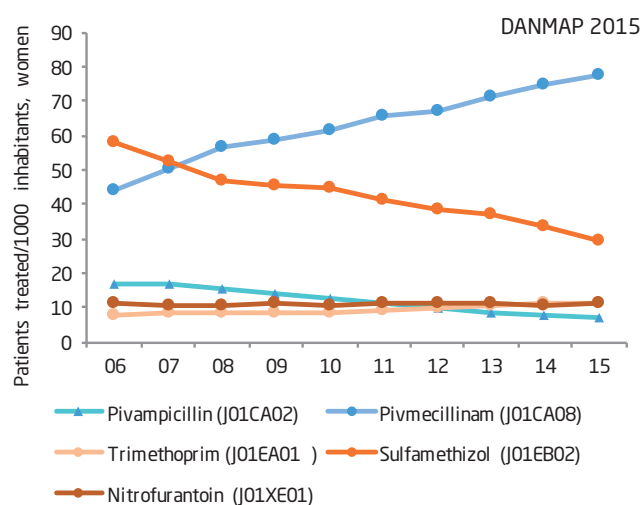
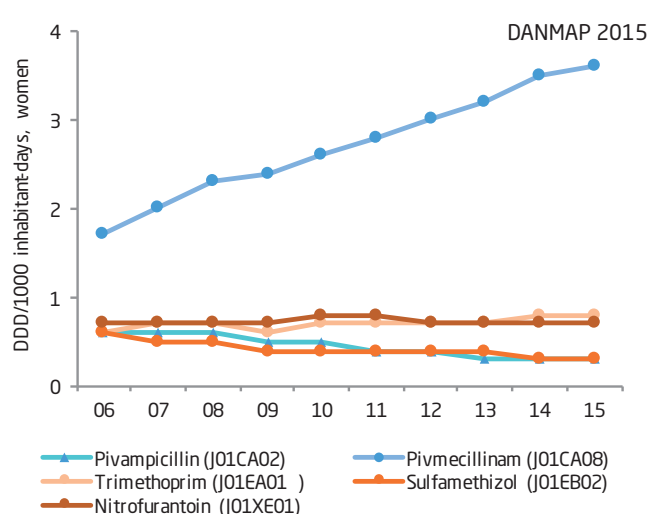
penicillins with extended spectrum increased with 44% and beta-lactamase resistant penicillins increased with 360%. The increases in percent for the combination penicillins cannot be estimated for the 20-year period since the consumption in 1996 was registered as being zero.

Consumption at the substance-level for the different penicillin groups is shown in figure 5.6. As mentioned above phenoxymethylpenicillin is the most consumed penicillin. In a 20-year chart trends in the consumption become very clear, for phenoxymethylpenicillin showing increasing trends for the first eleven years (from 4.50 DID in 1996 to 5.67 DID in 2007) and an almost equivalent but steeper decrease onwards to 2015 (4.33 DID). For all other penicillins the trends over the 20-year period were more stable, pivmecillinam and amoxicillin with clavulanic acid showing parallel increasing trends, pivmecillinam from the first registration in 2000, amoxicillin with clavulanic acid from 2005 and onwards. Also Flucloxacillin showed increasing trends paralleling the others from 2010 until 2014; this drug was introduced to the Danish market in 2011 following delivery problems and production cessation of the oral mixture for dicloxacillin. In 2015 a reduction in consumption was observed.

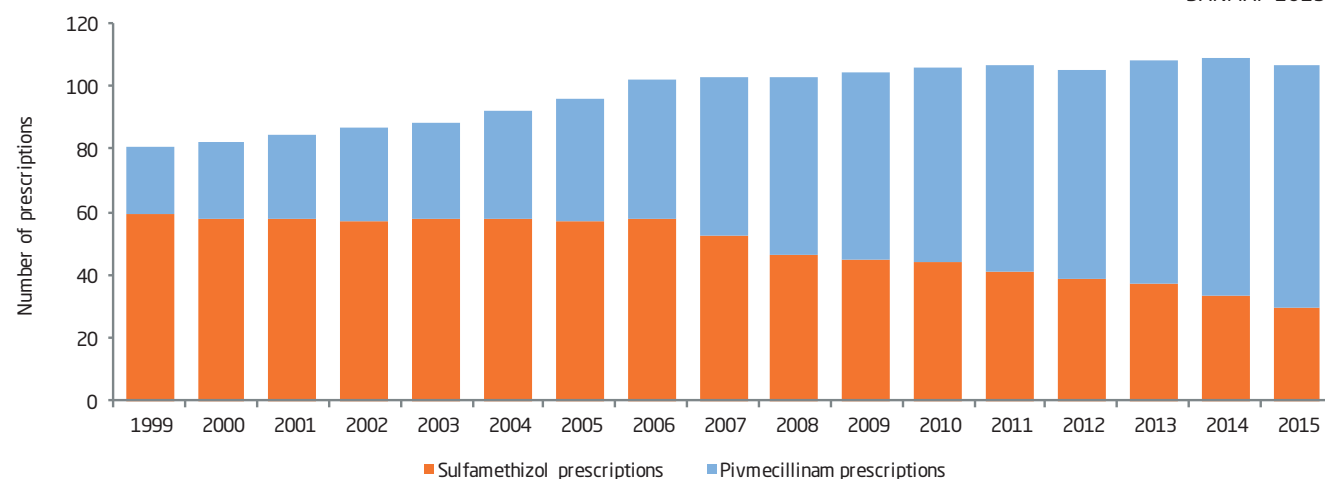
Both pivmecillinam, pivampicillin and amoxicillin belong to the penicillins with extended spectrum; amoxicillin and pivampicillin slowly declined over the last twenty years, while pivmecillinam increased steeply. The increase of pivmecillinam is due to changes in the recommended treatment of urinary tract infections (UTI), where sulfamethizole used to be the prime drug, see figure 5.7 - 5.9 for the treatment of UTI in women, 1999-2015.

Figure 5.6. Consumption of leading penicillins in primary health care, Denmark

DANMAP 2015


Figure 5.7 Female urinary tract infections treated per 1,000 inhabitants in primary health care, Denmark

Figure 5.8 Consumption of antimicrobial agents by female urinary tract infections in primary health care, Denmark, DID

Figure 5.9, number of prescriptions for women treated for urinary tract infection, 1999 - 2015

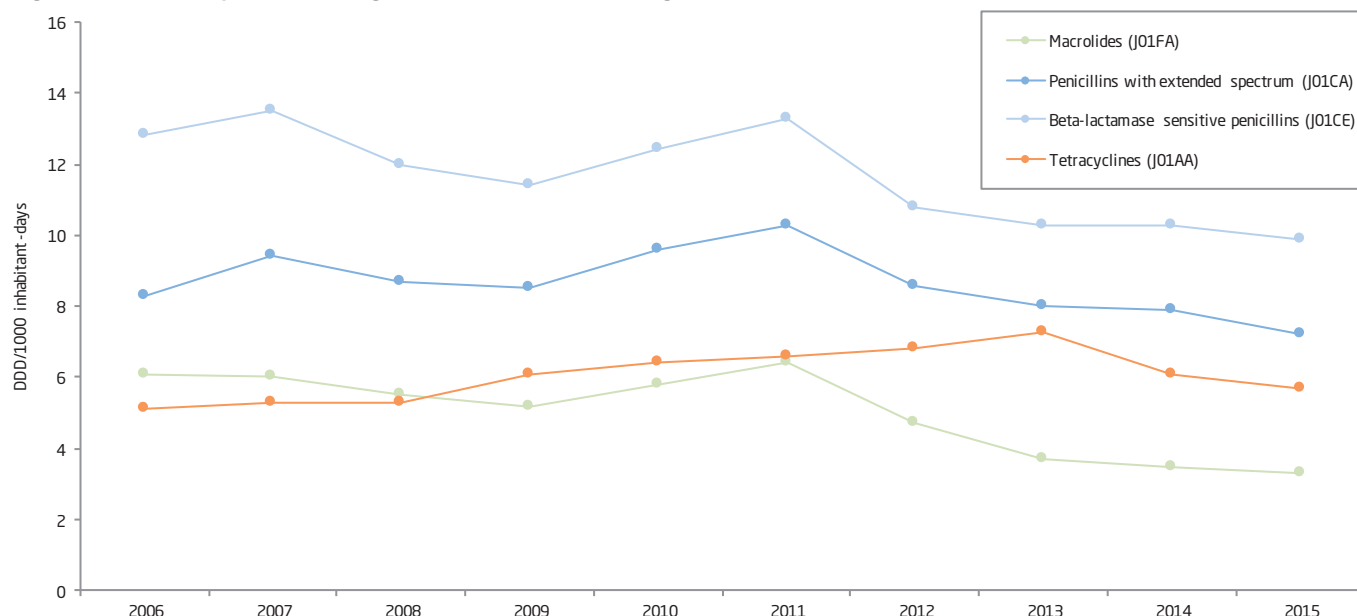
DANMAP 2015



Note: the figure includes only consumption of the two main drugs: sulfamethizole and pivmecillinam

Figure 5.10. Consumption of leading antimicrobials in children aged 0 - 18, Denmark

DANMAP 2015



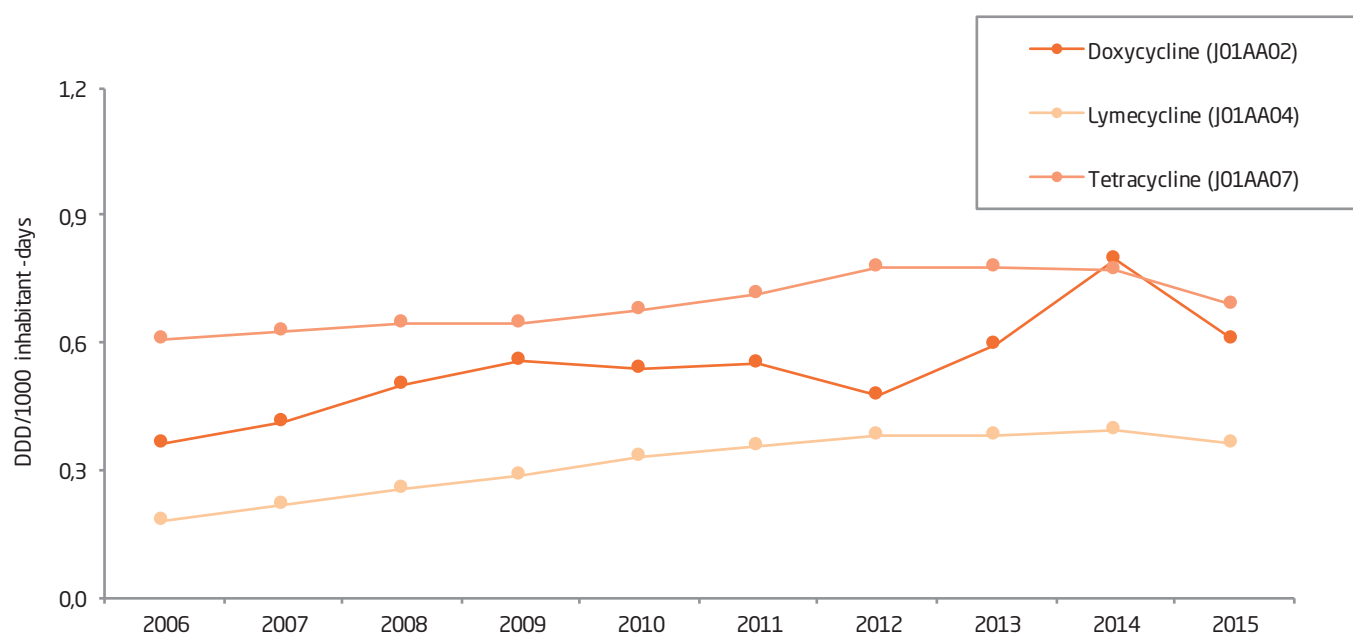
5.3.5 Consumption of antimicrobials among children/youngsters (0 - 18 years)

Since 2013 there has been extra focus on the consumption of antimicrobials in children and teenagers. Children account, together with the elderly, for a relatively high proportion of penicillins consumed. In addition, adolescents have the highest consumption of tetracyclines - most likely due to the treatment of acne. The total consumption during the last decade decreased from 32.3 DID to 26.1 DID.

Figure 5.10 shows consumption of the four leading antimicrobial groups in children and teenagers during the last decade. For the penicillins and macrolides peaks are observed in 2007 and 2011, mirroring the trends observed for the total consumption of antimicrobials in the primary sector. They also reflect the occurrence of the avian Flu pandemic in the winter 2006 to 2007 and the *Mycoplasma pneumoniae* epidemic from autumn 2010 to spring 2012. In 2015 the betalactamase sensitive penicillins accounted for 9.9 DID, penicillins with extended spectrum for 7.2 DID and macrolides for 3.3 DID.

Figure 5.11. Consumption of tetracyclines in primary health care, Denmark

DANMAP 2015



Inspired from initiatives performed at STRAMA in Sweden regarding the antimicrobial use in children the national campaign on use of antibiotics launched on the European Awareness Day in 2012 focused on viral versus bacterial infections in children. It was followed by a campaign focusing on “red ear” (otitis media) in 2013.

In 2013, following steady increases in the consumption of tetracyclines suspected to be related to increasing systemic treatment of acne teenagers, it was decided to further investigate the possible causes behind the increase (Kuhn et al, 2016). Encouragingly, new data from 2014 showed a sudden unexpected decrease. This was most likely related to the raised public awareness of antibiotics as the cause of unwanted resistances in general and on the appearance of livestock-associated MRSA in humans and speculations on a possible link to the use of tetracycline in the animal husbandry (see chapter 8.5 and MRSA textbox).

5.3.6 Tetracyclines

For the second year in a row the consumption of tetracyclines decreased, though less marked, from 1.66 DID to 1.61 DID (3%) (Figure 5.11). The decrease was observed for lymecycline and tetracycline, while doxycycline increased. Since 2006 the consumption has altogether increased with 17% (from 1.38 DID).

5.4 Hospital Care

5.4.1 Introduction

The antimicrobial consumption for hospital care reported was related only to bed-days and admissions in public somatic hos-

pitals. Specialized hospitals (psychiatric hospitals, hospices and rehabilitation centers) were not included as they might skew the data due to contribution of a large proportion of bed-days and admissions but only a small proportion of antimicrobial consumption (approximately 3%). 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 has changed notably: more people are admitted to somatic hospitals while the average length of stay is considerably shorter (Figure 5.12, Table A5.4 and A5.5 in web annex). Selection pressure for the emergence of antimicrobial resistance increases with increasing hospital activity and thus the selection pressure has increased considerably from 2006 to 2015.

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

In 2015, the consumption of antimicrobial agents in somatic hospitals was 103.02 DBD, a minor decrease of 1.3% from 2014 (104.30 DBD), but the first decrease in a decade following continuous increases (from 67.71 DBD in 2006), (Table 5.5).

Since 2006, the consumption increased with 52%. This reflects a combination of the described increased hospital activity and decreased number of hospital bed-days with an increase in DDDs. In 2015 combination penicillins, for the first time since recording of the antimicrobial consumption was begun in 1997, represented the largest group of antimicrobials consumed

Figure 5.12. Number of bed-days and admissions in somatic hospitals, Denmark

DANMAP 2015

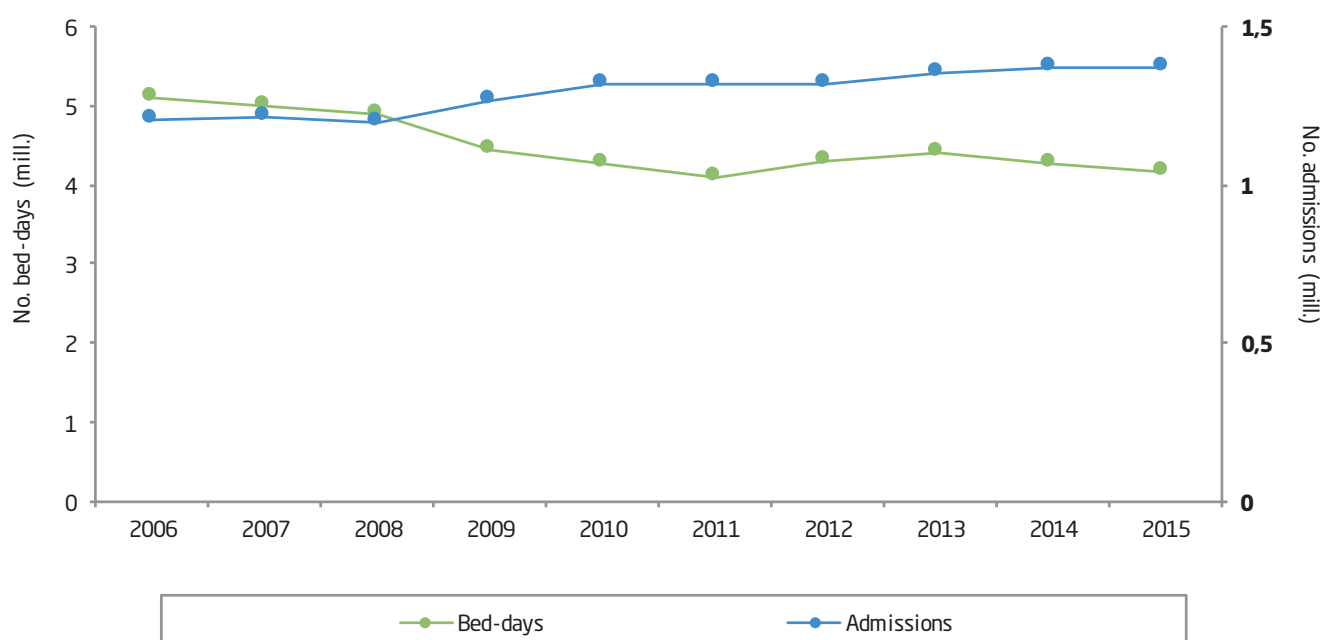


Table 5.5 Consumption of antibacterial agents for systemic use in somatic hospitals (DDD/100 occupied bed-days), Denmark

DANMAP 2015

ATC group ^(a)	Therapeutic group	Year										
		1997	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
J01AA	Tetracyclines	0.34	0.39	0.63	0.78	1.04	1.09	1.18	1.58	1.52	1.73	1.86
J01CA	Penicillins with extended spectrum	11.21	13.00	13.42	13.96	15.37	14.61	14.41	14.90	15.06	16.40	17.01
J01CE	Beta-lactamase sensitive penicillins	8.02	10.67	10.79	9.98	9.90	9.49	9.32	10.13	10.13	10.31	10.03
J01CF	Beta-lactamase resistant penicillins	4.44	6.51	6.70	6.81	7.40	7.71	7.30	8.37	9.07	9.57	9.80
J01CR	Combinations of penicillins, incl. beta-lactamase inhibitors	0.03	1.83	2.95	4.00	5.65	7.13	8.51	12.00	13.64	16.04	17.80
J01DB	First-generation cephalosporins	0.13	0.14	0.13	0.18	0.13	0.13	0.13	0.12	0.11	0.06	0.04
J01DC	Second-generation cephalosporins	3.99	9.38	12.31	13.32	15.76	16.21	16.14	14.15	12.32	11.68	10.40
J01DD	Third-generation cephalosporins	0.50	0.83	1.03	1.25	1.42	1.26	1.39	1.07	1.08	1.02	1.06
J01DF	Monobactams	0.06	0.00	0.04	0.07	0.06	0.09	0.19	0.15	0.14	0.06	0.03
J01DH	Carbapenems	0.36	1.38	2.13	2.70	3.15	4.02	4.16	3.86	4.02	4.09	4.10
J01EA	Trimethoprim and derivatives	0.42	0.42	0.44	0.44	0.44	0.36	0.36	0.38	0.41	0.52	0.46
J01EB	Short-acting sulfonamides	1.29	0.75	0.34	0.35	0.35	0.33	0.25	0.20	0.20	0.18	0.15
J01EE	Combinations of sulfonamides and trimethoprim, incl. derivatives	0.44	2.12	1.52	1.95	2.28	3.04	4.11	3.33	4.21	4.70	5.04
J01FA	Macrolides	3.45	2.83	3.08	3.06	3.42	3.52	3.69	3.56	3.39	3.88	4.56
J01FF	Lincosamides	0.13	0.31	0.35	0.41	0.50	0.47	0.53	0.62	0.64	0.65	0.57
J01GB	Aminoglycosides	3.38	1.81	1.79	1.64	1.56	1.71	1.91	2.14	2.13	1.61	1.68
J01MA	Fluoroquinolones	1.46	6.74	8.16	9.53	10.71	10.44	10.70	10.02	9.77	9.88	9.30
J01XA	Glycopeptides	0.21	0.56	0.63	0.68	0.99	1.07	1.24	1.29	1.29	1.15	1.07
J01XB	Polymyxins	0.04	0.12	0.05	0.05	0.07	0.10	0.09	0.09	0.16	0.27	0.25
J01XC	Steroid antibacterials (fusidic acid)	0.25	0.28	0.28	0.26	0.31	0.34	0.27	0.23	0.22	0.23	0.16
J01XD	Imidazole derivatives	1.42	2.78	2.62	3.27	3.84	3.93	4.19	4.16	4.08	4.48	4.25
J01XE	Nitrofurantoin derivatives (nitrofurantoin)	0.37	0.29	0.28	0.29	0.36	0.31	0.33	0.34	0.38	0.36	0.32
J01XX05	Methenamine	0.18	0.11	0.09	0.10	0.09	0.08	0.10	0.09	0.09	0.07	0.09
J01XX08	Linezolid	0.00	0.20	0.16	0.21	0.22	0.22	0.32	0.32	0.35	0.34	0.43
J01XX09	Daptomycin	0.00	0.00	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.03	0.04
P01AB01	Metronidazole		2.13	2.20	2.43	2.91	2.99	3.12	2.89	2.70	2.48	2.05
A07AA09	Vancomycin		2.11	2.19	2.43	2.93	2.96	3.12	2.93	2.75	2.55	0.47
J01	Antibacterial agents for systemic use (total)	42.13	67.71	74.33	80.14	90.87	93.67	97.08	98.94	99.88	104.34	103.02

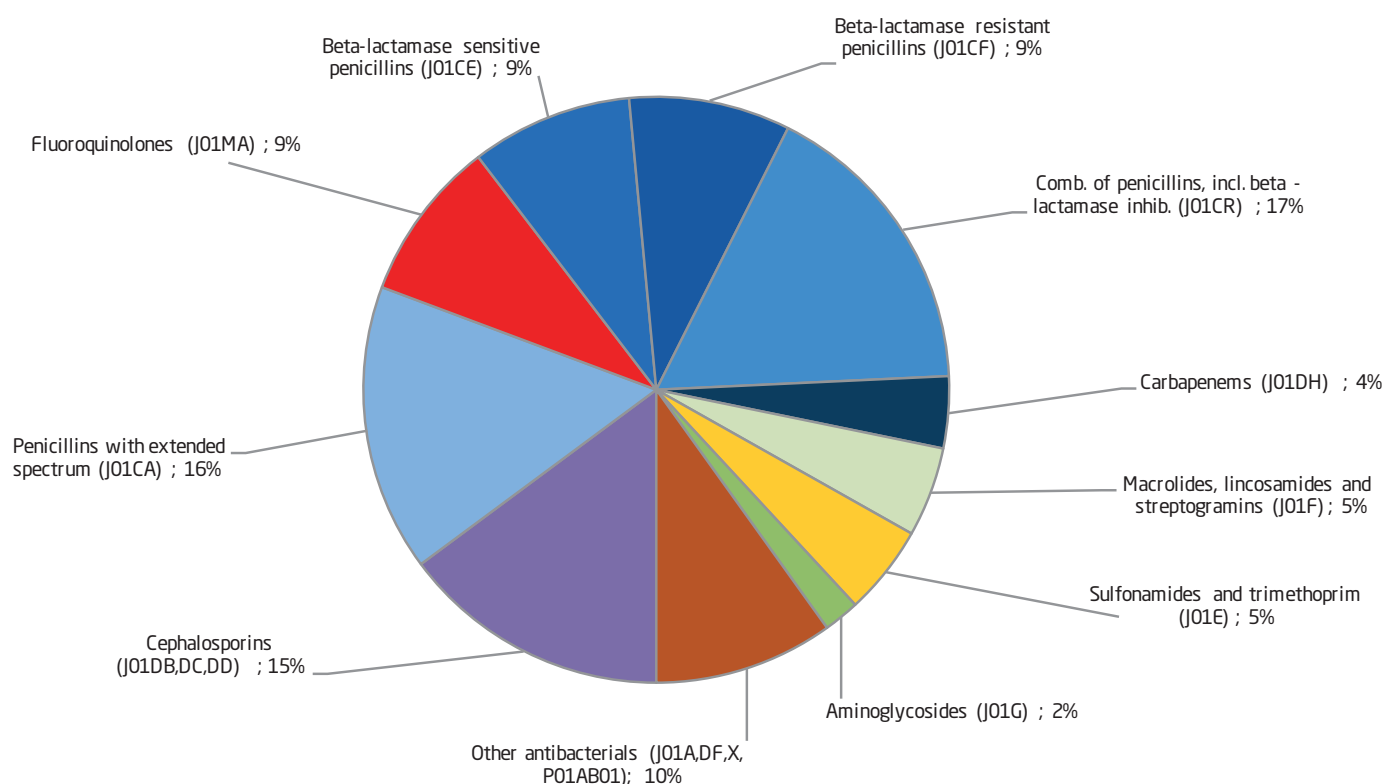
(17%), closely followed by penicillins with extended spectrum (16%). Penicillins in general accounted for 51% of the total consumption. Cephalosporins (15%) and fluoroquinolones (9%) were also still among the most commonly consumed antimicrobial agents in hospitals (Figure 5.13). The proportional consumption of cephalosporins increased from 11% in 2014.

It is important to note delivery problems for the following intravenously administered antimicrobials: meropenem, vancomycin, clarithromycin and mecillinam. Whether the shortage of these drugs was a consequence of increasing consumption is not known, but may very well be the case. The following additional sales to hospitals were reported from wholesalers: 290 DDD for meropenem, 3,121 DDD for vancomycin, 316 DDD for clarithromycin and 958 DDD for mecillinam. These numbers were not included in the figures and tables since it is

not known how much of the extra deliverances actually were consumed. The additional sales amount only for around 0.1% of the total consumption and thus hardly had any impact on the total amounts of antimicrobials consumed. However, when there is shortage of important antimicrobial classes, changes in consumption of the other classes might happen as well, especially in hospital departments. The shortage of both meropenem and mecillinam thus very likely caused consequential increases in the consumption of piperazillin with tazobactam and probably also in gentamicin and cephalosporins. These drugs are all important in the treatment of sepsis and complicated urinary tract infections, as are meropenem and mecillinam. For vancomycin it is more difficult to estimate whether other antimicrobials might have been affected by the deliverance shortage, since alternatives in treatment very much depend on the infectious disease to be treated. For clarithro-

Figure 5.13. Distribution of the total consumption of antimicrobial agents in somatic hospitals, Denmark

DANMAP 2015



mycin other macrolides may have been used for substitution.

For most antimicrobial classes and drugs the trends in consumption observed over the past decade continued (Figure 5.14 and table 5.5). From 2014 to 2015 the following changes were observed: increases were seen for combination penicillins (from 16.04 DBD to 17.80 DBD, 11%), for macrolides (from 3.88 DBD to 4.56 DBD, 18%) and less marked for penicillins with extended spectrum (3.7%), beta-lactamase resistant penicillins (2.4%) and 3rd generation cephalosporins (3.9%). Increases were also observed for the rarely used antimicrobial classes tetracyclines (from 1.73 DBD to 1.86 DBD, 8%) and linezolid (from 0.34 DBD to 0.43 DBD, 27%). These two drugs are primarily used in the treatment of complicated staphylococcal infections and the slight but noteworthy increase might reflect the increasing occurrence of staphylococcal bacteremia's (see chapter 8.6 for information on staphylococcal infections).

Decreases were observed for 2nd generation cephalosporins (from 11.68 DBD to 10.40 DBD, - 11%), trimethoprim derivatives and short-acting sulfonamides (- 12% and - 17%, respectively) and for beta-lactamase sensitive penicillins (from 10.31 DBD to 10.03 DBD) - a minor decrease of - 2.7%, but in accordance with decreasing trends during the last ten years. The delivery shortage of vancomycin clearly showed in consumption, decreasing from 2.55 DBD to 0.47 DBD, (- 82%).

Special focus should be paid to the consumption of cephalosporines, where a decrease was observed from altogether 12.76 DBD in 2014 to 11.50 DBD in 2015. Despite this decrease, the proportion of cephalosporins among the leading antimicrobial classes used at hospitals increased from 11% in 2014 to 15% in 2015. It is alarming that the changes observed in the use of cephalosporins during the last five years have not been bigger in spite of multifaceted efforts on more prudent use of antimicrobials at hospitals. Although a significant reduction in the consumption of 2nd generation cephalosporins is observed from 2010 and onwards, (from 16.21 DBD in 2010 to 10.40 DBD in 2015), this decrease would have been expected to be more pronounced. Most hospitals changed their recommendations from the use of cefuroxime for patients suffering from sepsis or urinary tract infection to either piperacillin with tazobactam or mecillinam, and cefuroxime as the prime drug in acutely ill patients was more or less omitted. Cefuroxime still plays an important role as a first line drug in antibiotic prophylaxis for different surgical interventions and for patients suffering from nephrological diseases as well as constituting an alternative to beta-lactams for patients with suspected penicillin allergy. With a population where approximately 10% render themselves to be allergic to penicillin, this might lead to overuse of more broadspectrum antibiotics. It is thus important that patients suspected of having penicillin allergy are diagnosed, if possible through their GP or in ambulatory care, before

Figure 5.14. Total somatic hospital consumption (DBD) by leading groups of antimicrobial agents (J01), Denmark

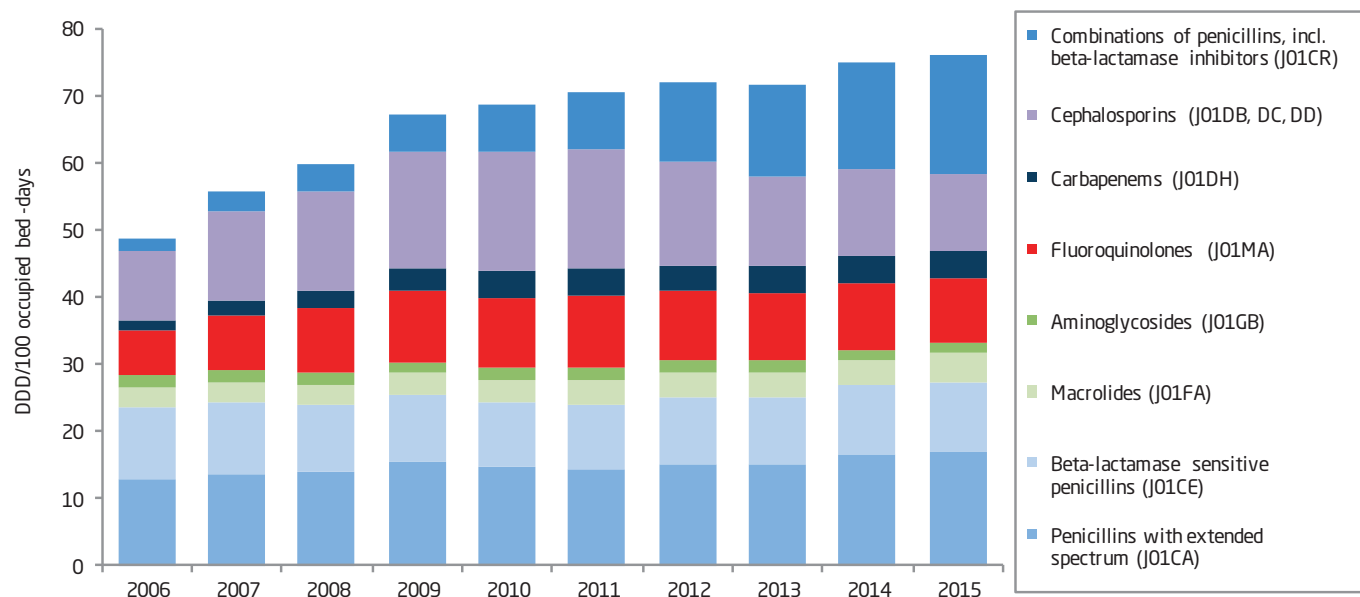


Table 5.6. Consumption of antimicrobial agents for systemic use in somatic hospitals (DDD/100 admitted patients), Denmark

ATC group ^(a)	Therapeutic group	Year									
		2006	2007	2008(b)	2009	2010	2011	2012	2013	2014	2015
J01AA	Tetracyclines	1.67	2.59	3.19	3.63	3.55	3.66	5.15	4.97	5.37	5.65
J01CA	Penicillins with extended spectrum	55.13	55.39	57.18	53.76	47.46	44.77	48.60	47.90	50.95	51.75
J01CE	Beta-lactamase sensitive penicillins	45.26	44.55	40.90	34.61	30.83	28.98	33.04	33.13	32.03	30.52
J01CF	Beta-lactamase resistant penicillins	27.60	27.64	27.89	25.86	25.04	22.71	27.30	29.64	29.73	29.82
J01CR	Comb. of penicillins, incl. beta-lactamase inhibitors	7.77	12.17	16.37	19.74	23.15	26.47	39.14	44.60	49.81	54.14
J01DB	First-generation cephalosporins	0.60	0.55	0.72	0.46	0.43	0.41	0.40	0.37	0.20	0.13
J01DC	Second-generation cephalosporins	39.76	50.81	54.55	55.12	52.65	50.19	46.17	40.27	36.29	31.53
J01DD	Third-generation cephalosporins	3.53	4.24	5.10	4.98	4.10	4.33	3.50	3.53	3.18	3.22
J01DF	Monobactams	0.00	0.18	0.27	0.21	0.29	0.60	0.48	0.45	0.19	0.08
J01DH	Carbapenems	5.86	8.78	11.08	11.01	13.07	12.55	12.60	13.14	12.70	12.50
J01EA	Trimethoprim and derivatives	1.78	1.81	1.80	1.56	1.17	1.11	1.23	1.33	1.62	1.40
J01EB	Short-acting sulfonamides	3.18	1.41	1.43	1.21	1.09	0.78	0.63	0.62	0.55	0.46
J01EE	Comb. of sulfonamides and trimethoprim, incl. derivatives	8.98	6.28	7.98	7.96	9.88	12.79	10.87	13.76	14.53	15.32
J01FA	Macrolides	12.01	12.70	12.53	11.97	11.45	11.47	11.61	11.08	12.04	13.90
J01FF	Lincosamides	1.31	1.46	1.69	1.74	1.52	1.63	2.01	2.09	2.03	1.75
J01GB	Aminoglycosides	7.68	7.39	6.71	5.45	5.56	5.95	6.99	6.97	5.01	5.11
J01MA	Fluoroquinolones	28.58	33.66	39.04	37.45	33.92	33.30	32.67	31.96	30.69	28.28
J01XA	Glycopeptides	2.38	2.61	2.77	3.48	3.47	3.87	4.20	4.22	3.58	3.25
J01XB	Polymyxins	0.53	0.22	0.21	0.24	0.32	0.28	0.30	0.54	0.85	0.77
J01XC	Steroid antibacterials (fusidic acid)	1.19	1.17	1.05	1.09	1.12	0.85	0.76	0.71	0.71	0.50
J01XD	Imidazole derivatives	11.81	10.83	13.39	13.43	12.76	13.03	13.55	13.33	13.93	12.93
J01XE	Nitrofurantoin derivatives (nitrofurantoin)	1.24	1.17	1.19	1.27	1.01	1.02	1.12	1.25	1.13	0.98
J01XX05	Methenamine	0.46	0.38	0.43	0.31	0.27	0.32	0.28	0.30	0.22	0.29
J01XX08	Linezolid	0.86	0.68	0.84	0.76	0.72	0.99	1.04	1.14	1.05	1.31
J01XX09	Daptomycin	0.00	0.03	0.06	0.06	0.07	0.05	0.06	0.07	0.10	0.12
P01AB01	Nitroimidazole derivatives (metronidazole)	9.02	9.10	9.99	10.20	9.72	9.70	9.44	8.83	7.72	6.24
A07AA09	Intestinal anti-infectives (vancomycin)	8.95	9.10	9.96	10.20	9.73	9.71	9.54	9.00	7.92	1.43
J01	Antibacterial agents for systemic use (total)	287.1	306.9	328.3	317.8	304.3	301.9	322.7	325.2	324.1	313.38

a) From the 2015 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

acute disease demands quick action with no time for allergic investigation.

The consumption of carbapenems was 4.10 DBD in 2015, almost unchanged to 2014 (4.09 DBD) in spite of the described shortage of the drug. In 2006 the consumption was 1.38 DBD, an increase of 297% for the decade. Thus in spite of recommendations from the National Board of Health on the reduced use of carbapenems and delivery problems for meropenem during the summer of 2014 and in 2015 no apparent decrease in the consumption of meropenem was observed.

5.4.3 Other measures of somatic hospital consumption DDD per 100 admissions (DAD)

Because of the observed changes in the number of hospital bed-days over time, the consumption of antimicrobials in Danish hospitals may also be measured in relation to admissions (i.e. DDD per 100 admissions, DAD). When expressed as DAD, the total consumption of antimicrobial agents in somatic hospitals showed a decrease from 2014 to 2015 (from 324.1 DAD to 313.4, - 3.3%) (Table 5.6).

Among the leading individual antimicrobial groups, increases for the year 2014 to 2015 were observed for 'combination penicillins' (8.7%), penicillins with extended spectrum (1.6%) and macrolides (15%).

Decreases were observed for most other antimicrobials: beta-lactamase sensitive penicillins (-4.6%), 2nd generation cephalosporins (-13%), fluoroquinolones (- 7.9%) and to a lesser extent for carbapenems (-1.6%).

As observed for the consumption measured in DBD, increases were also observed for the rarely used tetracyclines and linezolid (5.2% and 26%, respectively), while vancomycin showed a marked decrease (-82%).

During the past decade, DAD increased by 9.1%; an increase primarily driven by a higher number of DDDs but counterbalanced by an increase in the number of hospital admissions.

When compared to the consumption in primary health care, and thus calculated in DID, the consumption of antimicrobial agents in somatic hospitals decreased from 2.18 DID in 2014 to 2.04 DID in 2015. During the past decade, the DIDs consumed at hospitals have increased by 13% (1.81 DID in 2006), (Table A5.4 in web annex).

5.5. Comments on 20 years surveillance of consumption

While the consumption kept rising through the beginning of the new century, so were the occurrences of resistance in human pathogen bacteria.

Already in 2000, time series analysis were introduced in DANMAP for measuring the effect of interventions on the control of the antimicrobial consumption. In 2004 a detailed analysis of changes in consumption of the different anti-

microbial classes were given (Figur 5.15); these show the same trends observed for 2015, namely shifts from consumption of the most narrow spectrum classes to more broad spectrum classes. In 2007 the barometer of antibacterials was introduced to give a more timely and appropriate picture of the consumption by reporting quarterly changes in chosen antimicrobials classes. Unfortunately it has not been possible to maintain these additional surveillance functions.

Despite these different attempts on measuring the antimicrobial consumption, no significant effect on neither consumption nor development in resistance were observed. Among emerging resistances in clinical human bacteria were the outbreak of a multiresistant *Klebsiella pneumoniae* at Hillerød hospital in Northern Zealand in 2008, the almost simultaneously increasing occurrence of *Clostridium difficile* in the Capital Region of Denmark, followed by occurrence of the first NDM-1 positive strain of *Klebsiella pneumoniae* as well as a hospital outbreak of vancomycin resistant enterococci at Skejby hospital in 2010 (see DANMAP 2009 to 2011 for information).

Resistance outbreaks certainly would have been much bigger if no awareness on the consumption and constant surveillance had taken place. The simultaneously increasing resistances in other European countries combined with the described outbreaks were alarming and functioned as a trigger for the initiation of several efforts happening on local regional hospital levels. Among these were strong efforts to reduce the consumption of cephalosporins and fluoroquinolones. National recommendations regarding more prudent use of antimicrobials were issued by the National Health Authorities in 2012. These give restrictions on the use of cephalosporins and fluoroquinolones for both health sectors and for the use of carbapenems at hospitals (see DANMAP 2012).

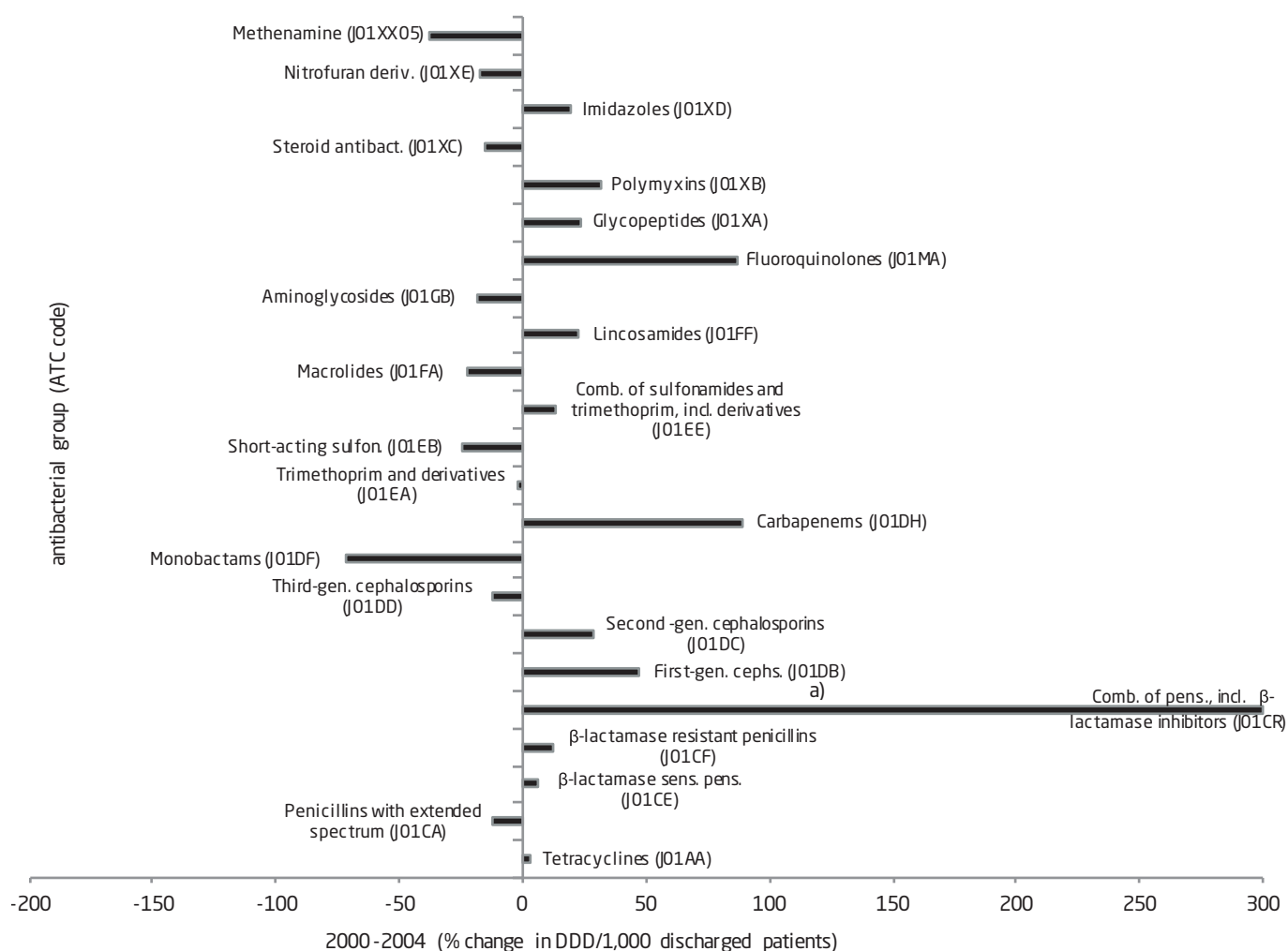
Even though the primary sector accounts for almost 90% of the antimicrobial consumption it has proven much more difficult to establish good and stable systems of antimicrobial stewardship here. Comprehensive research is undertaken these years investigating on the use of diagnostic tools as well as supporting the implementation of rational antimicrobial use.

The Danish system with detailed surveillance of consumption combined with the surveillance of occurring resistance has so far proven to prevent steep increases in these. Future efforts must continue to focus on prudent use, which not only includes lowering the total consumption but applying the achieved knowledge on the complex interplay between consumption of specific antimicrobial drugs and emergence of resistance in every way. Here a special focus must be paid on the consumption of fluoroquinolones and other more broad spectrum antimicrobials in the primary sector.

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Figure 5.15. Historical figure AB2 from DANMAP 2004. Changes in hospital consumption of antibacterials for systemic use between 2000 and 2004, Denmark.

DANMAP 2015



a) The consumption of J01CR increased by 681% from 4.9 to 38.7 DDD/1,000 discharged patients.

Textbox 5.1

Incidence of *Neisseria gonorrhoeae*, multiresistant bacteria and consumption of antimicrobial agents in Greenland

Background: Greenland has a population of 55,984 inhabitants (January 2015) 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: Qeqqa (Sisimiut and Maniitsoq), Disko (Aasiaat, Kangaatsiaq, Qeqertarsuaq and Qasigiannnguit), Avannaa (Ilulissat, Uummannaq, Upernavik and Qaanaaq), Sermersooq (Nuuk, Paamiut/Ivittuut, Tasiilaq and Illoqqortoormiut), and Kujataa (Qaqortoq, Nanortalik and Narsaq).

There are several smaller hospitals and health care centres in the five health regions. The largest hospital, Dronning Ingrid's Hospital, is situated in Nuuk (182 beds). Around 15-16,000 persons are admitted to hospital once or several times a year. The hospital clinics are used for open access for patients from the primary health care. In Nuuk a large health care centre has combined functions as medical clinic, emergency room and primary health care with doctors and nurses. The settlements have nursing stations (supervised by doctors via phone or telemedicine and doctors visiting three to four times a year). Medication on prescription is free of charge. Patients with specific/serious diseases that can not be treated at Dronning Ingrid's Hospital are transferred to Denmark or Iceland for further treatment (e.g. hemodialysis, cancer treatment, brain surgery etc.).

***Neisseria gonorrhoeae*:** Gonorrhoea has been a major health challenge in Greenland for several decades. In 2015, 1,582 new cases of gonorrhoea were reported in Greenland, corresponding to an incidence of 2.825 per 100,000 inhabitants. Ten years ago, in 2005 the incidence was markedly lower (915 per 100,000 inhabitants) so this sexually transmitted infection is an increasing problem in Greenland. In Denmark and other European countries resistance to ciprofloxacin in *Neisseria gonorrhoeae* isolates increased rapidly more than fifteen years ago and treatment of gonorrhoea has therefore changed to ceftriaxone and, more recently, to ceftriaxone with azithromycin for infections with unknown susceptibility pattern. This increase in quinolone resistance was not seen in Greenland until recently. The first reports of ciprofloxacin-resistant *N. gonorrhoeae* came in the beginning of 2014 with an overall resistance rate of 31% for 2014 increasing to 55.9% in 2015. Resistance to ceftriaxone in gonococci has not yet been seen in Greenland.

Since 2011 the diagnosis of gonorrhoea in Greenland has been performed on urine samples with nucleic acid amplification tests (NAATs) and all hospitals/health care clinics in Greenland submit their NAATs to The Central Laboratory at Dronning Ingrid's Hospital in Nuuk. This test is fast and automated, and the number of individuals tested increased from 17,000 in 2010 to 19,000 in 2015. However, it does not allow antimicrobial susceptibility testing.

Surveillance for antimicrobial resistance in gonococci is performed at the Primary Health Care Center in Nuuk where efforts are made to achieve samples for culture of *N. gonorrhoeae* from all NAAT positive men. Due to logistic difficulties in getting samples to Nuuk for culture, surveillance of antimicrobial resistance is not performed routinely outside of Nuuk.

The sudden increase in resistance to ciprofloxacin led to a change in the treatment regimen. Whereas ciprofloxacin was the first line treatment of gonorrhoea until October 2014 the treatment regimen was changed to a single dose of 500 mg ceftriaxone intramuscularly in combination with 2 g azithromycin orally, in accordance with the recently updated European guideline on this topic.

Resistant bacteria: From 2004 to 2015, 22 patients have been diagnosed with methicillin-resistant *Staphylococcus aureus* (MRSA), 70 patients with ESBL-producing Enterobacteriaceae, one patient with vancomycin-resistant enterococci (VRE), and 47 out of 119 patients with *Clostridium difficile* infection had the 027 type.

The number of patients with MRSA has increased during this last year due to outbreaks with two different strains of MRSA in two towns. In Aasiaat there was an outbreak during the late 2014 to 2015 consisting of seven persons (four children and three adults) with the same MRSA-strain not previously seen in Greenland. Six of the seven persons were from the same family and one child was from the same kindergarten as two of the other children and found by the screening of all 70 children and employees in the kindergarten. The first child diagnosed with MRSA had a clinical middle-ear-infection. All the others were only colonized with MRSA (nose or throat or both). It is still a mystery where this MRSA-strain (t902 CC22) came from because this strain has not yet been seen in Denmark and none of the family members have been travelling or admitted to

continued ... Textbox 5.1

hospitals abroad. The other outbreak consisted of two persons (a couple) in Maniitsoq and the husband was a cancer-patient who was treated for a long period of time in a Danish hospital. When the patient was referred back to Maniitsoq Hospital he had a wound infection with MRSA (t002 CC5) and his wife became colonized in the nose with the same strain. Close family contacts and a few nurses were examined for MRSA but none of these had MRSA. Both outbreaks illustrates the fact that MRSA is mainly spread by close contact in families, and rarely to other contacts in the community.

In spite of outbreaks in Denmark with VRE, so far only one patient has been diagnosed with VRE in Greenland. This was a five-months-old child being colonized in the rectum with VRE after admission to a Danish hospital. The mother of the child and other close family members were examined but none of these were colonized with VRE and no transmission was seen at the ward.

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. Since 2011, there has been an increasing problem with *C. difficile* infections (mainly type 027) in the hospitals, and transmission within the country has occurred. A project focusing on identification of risk factors for acquisition of *C. difficile* infections, mapping of *C. difficile* types and prevention strategies will be performed in the future.

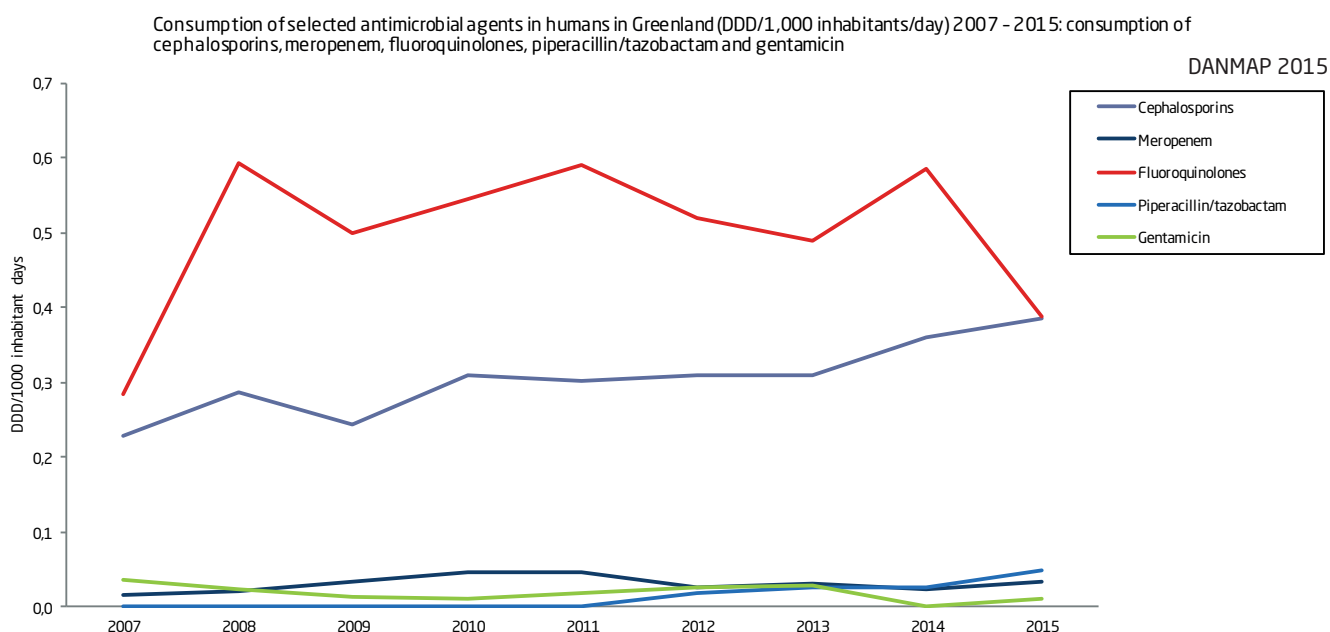
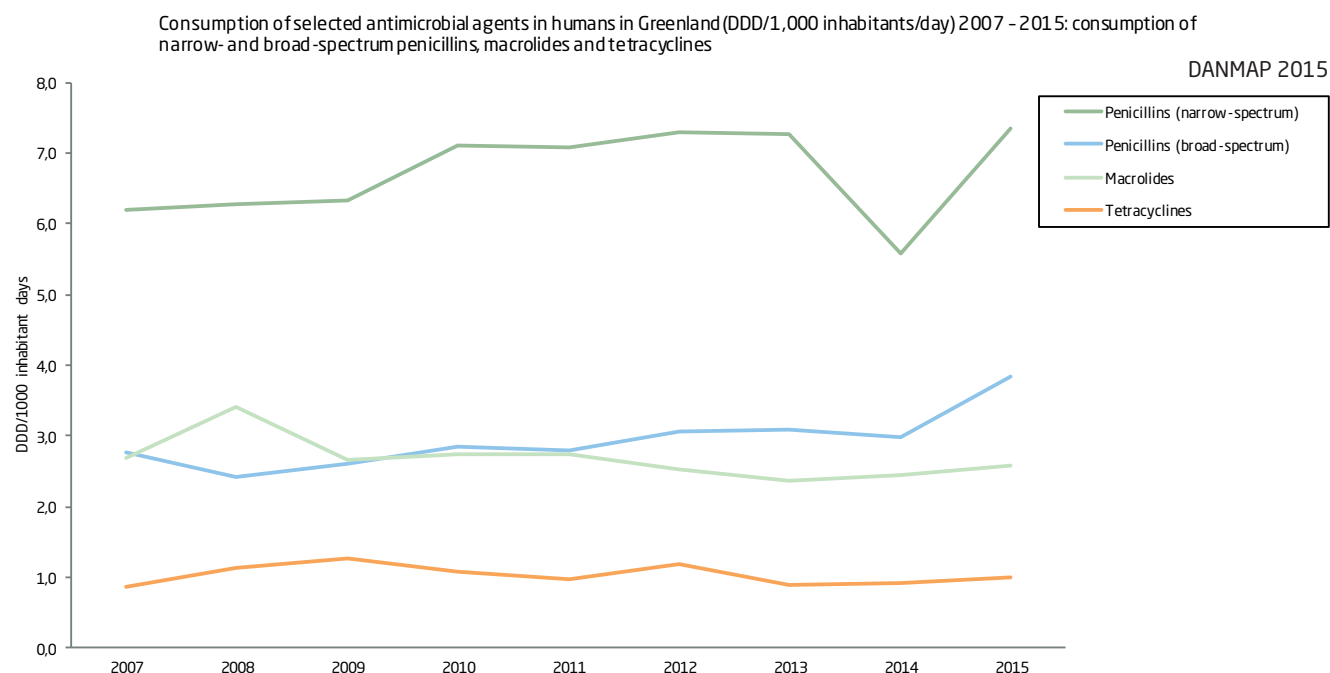
Consumption of antimicrobial agents: All antimicrobial agents in Greenland are purchased and disseminated from the National Pharmacy. Figure 1 shows the total purchase of selected antimicrobial agents in DDD per 1,000 inhabitants per day (DID) from 2007 to 2015. From 2007–2013, an increase of narrow-spectrum (18%) and broad-spectrum penicillins (12%) has been seen, but from 2013 to 2014 a decrease of 23% and 4%, respectively, has occurred. An increase was seen again from 2014 to 2015 of 31% and 28%, respectively. From 2014 to 2015 a minor increase in broad-spectrum antimicrobial agents such as macrolides (5%), tetracyclines (6%), and cephalosporins (7%) has been seen. Meropenem increased 36% whereas fluoroquinolones decreased 34% from 2014 to 2015. The largest increases from 2014 to 2015 were seen in piperacillin-tazobactam (81.5%), and in gentamicin (500%).

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, where teaching on prescription and antibiotic audits are performed on a yearly basis) a continued increase in purchase of piperacillin-tazobactam has been seen. A remarkable increase was also seen for gentamicin. Due to the change in treatment of gonorrhoea a decrease in purchase of fluoroquinolones was seen. It is – however – a bit worrying that the purchase of meropenem has increased again after a decrease last year.

Continued focus in Greenland on the use of broad-spectrum antimicrobial agents – both in hospitals and in primary health care – and on the incidence of sexually transmitted infections, e.g. gonorrhoea, as well as on multiresistant bacteria is very important in the future.

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Figure 1. Consumption of selected antimicrobial agents in humans in Greenland (DDD/1,000 inhabitants/day) 2007-2015: (a) consumption of narrow- and broad-spectrum penicillins, macrolides and tetracyclines; and (b) consumption of cephalosporins, meropenem, fluoroquinolones, piperacillin/tazobactam and gentamicin. (Note: Narrow-spectrum penicillins include benzylpenicillin, phenoxymethylpenicillin and dicloxacillin, and broad-spectrum penicillins include ampicillin, pivampicillin, amoxicillin and amoxicillin with enzyme inhibitor).



Textbox 5.2

Continuous focus on antimicrobial resistance and usage of antimicrobials in the Faroe Islands

Background: The Faroe Islands (FI) consists of 18 islands, inhabited by approx. 49,000 inhabitants, 19,000 of whom live in the capital Tórshavn. The main hospital (Landssjúkrahúsið, 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 secondary care provided by the hospital. 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. The healthcare staff constitutes a stable workforce but consultants, mainly from Denmark, perform specialized treatment where the number of patients is too small to support full-time specialist employment.

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; the latter were derived from another administrative source than earlier, which is the reason why hospital data in this report only go as far back as 2011 and are not fully consistent with data supplied in Danmap reports 2012 and 2013.

Resistant microorganisms. Since the first case of MRSA in 2004, a total of 47 cases of MRSA have been identified (27 with infection and 20 carriers). From 2006–2015, 46 ESBL-producing Enterobacteriaceae (*Escherichia coli* and *Klebsiella pneumoniae*) have been detected. Since April 2015, vancomycin-resistant enterococci (VRE) have been an increasing problem – especially at LS.

Study of VRE in the FI and antibiotic usage. A retrospective case-control study was performed consisting of 247 patients divided into VRE-negative (n=168) and VRE-positive (n=79). The aim of the study was to determine if the use of broad- and/or narrow-spectrum antibiotics influenced the risk of being colonized with VRE. Data regarding patients' VRE status (positive vs negative), the use of antibiotics two calendar months prior to a positive- or negative rectal swab, administration time, gender and age were collected. The data regarding antibiotics usage were divided into two groups consisting of broad- and narrow-spectrum antibiotics and were measured in how many days the patients were exposed to antimicrobial agents. The length of administration of antibiotics was named antibiotic pressure. Acquisition of VRE colonization was effected by broad- and/or narrow-spectrum antibiotics. The VRE-positive patients had received significantly more antibiotics in several of the antibiotic classes, 2 calendar months prior to a positive swab test.

Figure 1. Consumption of selected antibiotics (narrow and broad-spectrum) at LS 2011-15 (DDD/100 bed-days)

DANMAP 2015

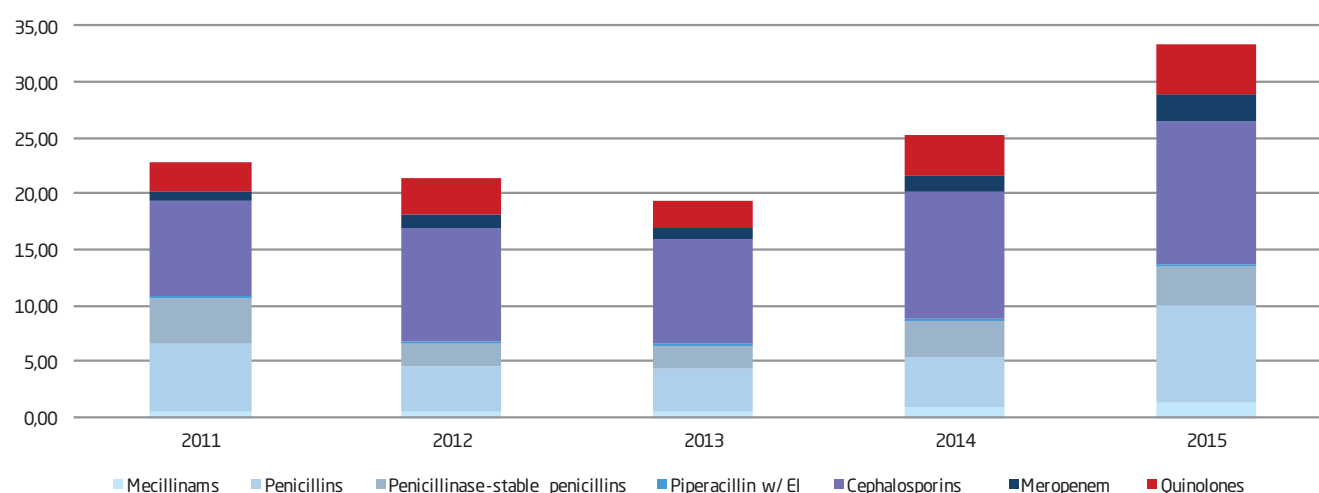
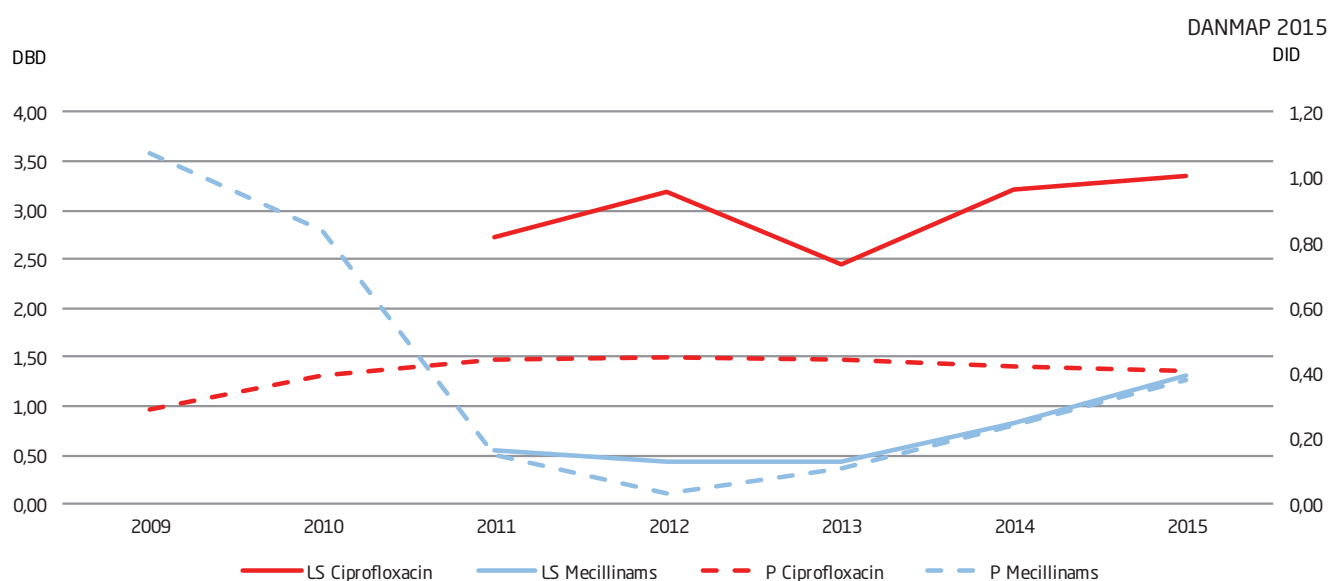
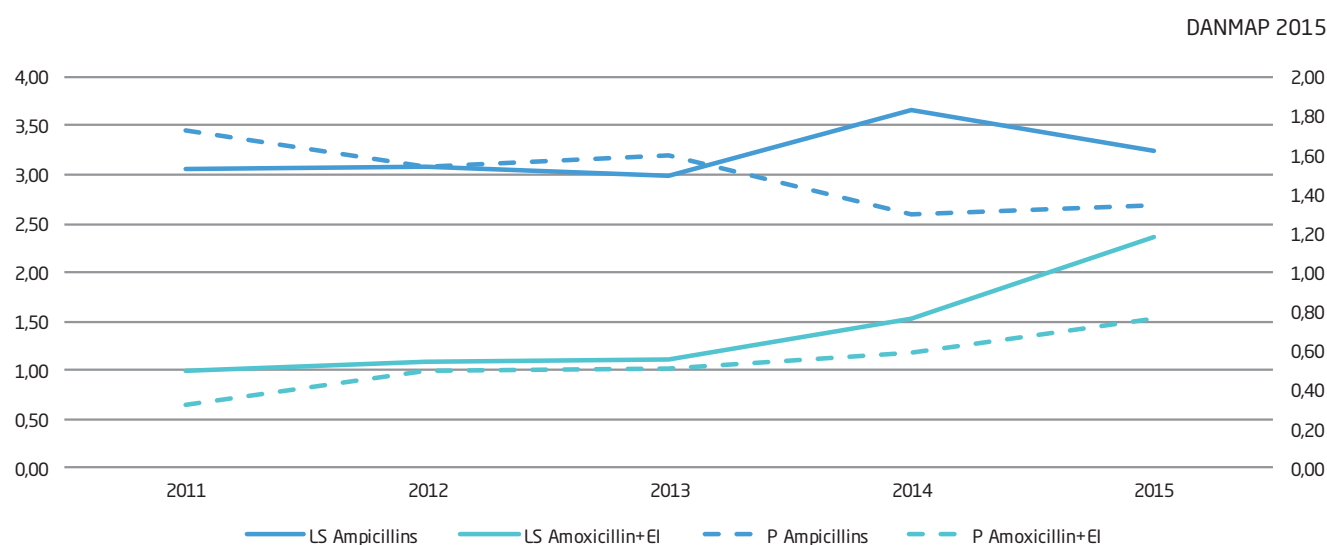


Figure 2. Consumption of ciprofloxacin and mecillinams LS (hospital, DBD) and P (primary healthcare, DID)

Data from this study were provided by the following authors: H. Djurhuus, A. E. Lisberg, M. Stegger, W. Smith, and S. Gaini.

Antimicrobial consumption at LS. Total antimicrobial consumption was 50.11 DDD/100 bed-days (DBD), similar to the use in 2014 but representing a 24.5 % increase compared to 2013. Of particular concern in 2015 is a further increase in the use of three broad-spectrum antimicrobials: Cefuroxim (9 % increase compared to 2014 and 33 % compared to 2013), ciprofloxacin (4 % increase compared to 2014 and 36 % compared to 2013), and meropenem (61 % increase compared to 2014 and 162 % compared to 2013), especially in the light of the current VRE prevalence. See details in figure 1 and 2.

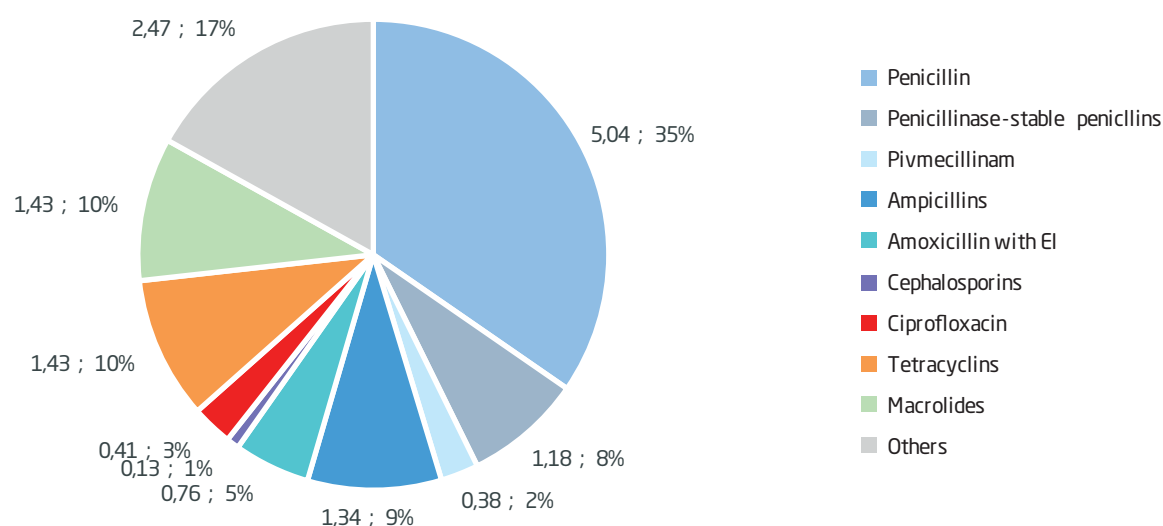
It is, however, noteworthy to see that the use of mecillinams is increasing. Although still at a low level due to an earlier ban (1.32 DBD in 2015), it increased by 59 % compared to 2014 and 214 % compared to 2013. Besides, the use of penicillinase-

Fig. 3. Consumption of ampicillins vs. amoxicillin w/ enzyme inhibitor LS (hospital, DBD) and P (primary healthcare, DID)

continued ... Textbox 5.2

Figure 4. Consumption of antimicrobials in primary healthcare, 2015 (DDD/1,000 patients/day; %)

DANMAP 2015



sensitive penicillin increased in 2015 (89 % increase compared to 2014 and 119 % compared to 2013), as well as penicillinase-stable penicillin (14 % increase compared to 2014 and 77 % compared to 2013). See details in Figure 1 and 2.

Another striking observation, both in hospital and primary healthcare, is the increase in the use of amoxicillin with enzyme inhibitor (EI), shown in Figure 3. The cause of this increase remains to be elucidated.

Antimicrobial consumption in primary healthcare. Total antimicrobial consumption in 2015 was 14.57 DDD/1,000 inhabitants/day (DID) which represented an increase compared to 2014 (13.92 DID) but was similar to earlier years. Narrow-spectrum antimicrobials made up 55 % of the use in 2015; penicillinase-sensitive penicillin constituted 35 % of total DID. The share of broad-spectrum antimicrobials was 14 %, among these the use of tetracyclins has decreased since 2013 but still constitutes 10 % of the total DID. A further development in the use of pivmecillinam and ciprofloxacin is anticipated. See details in Fig. 2 and 4.

Conclusion. Antimicrobial consumption is still increasing, at LS as well as in primary healthcare. Annual antibiotic audits have been in place since 2011. Implementation of antibiotic stewardship and increased focus on adherence to general infection control precautions are the next steps in the effort to reduce development and spreading of antimicrobial resistance in LS and in primary healthcare.

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6

RESISTANCE IN
ZOO NOTIC BACTERIA

6. Resistance in zoonotic bacteria



Highlights: The occurrence of monophasic *Salmonella* Typhimurium, which are often multi-resistant, has increased in pigs, pork and among isolates from human infections over the past decade leading to the current high levels of resistance to ampicillin, sulfonamide and tetracycline (50-80%). Previously, human cases associated with travel had significantly higher levels of resistance compared to domestic cases; however in 2015 the monophasic clone resulted in similar levels of resistance for many antimicrobial agents. An exception being resistance to quinolones that was higher among isolates from the travel-related human cases. As a result of pig producers' highly restrictive use of antibiotics critical for human treatment, fluoroquinolone (ciprofloxacin) resistance has been absent from *S. Typhimurium* from pigs and domestically produced pork for several years. Resistance to 3rd generation cephalosporins and carbapenems have remained very low in *S. Typhimurium* from domestic cases and absent among the *Salmonella* isolates from Danish pigs and pork.

Over the last 15 years, resistance to fluoroquinolone and tetracycline has slowly increased among *Campylobacter jejuni* from broilers; however most isolates remain fully sensitive. The level of fluoroquinolone resistance in *Campylobacter jejuni* continues to be higher among isolates from imported broiler meat compared with isolates from Danish broilers.

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 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 2015 [www.food.dtu.dk].

Zoonotic bacteria (*Salmonella* Typhimurium, *Salmonella* Enteritidis, *Campylobacter jejuni* and *Campylobacter 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.

6.1 *Salmonella*

Salmonella is the second most important zoonotic bacterial pathogen in Denmark and can have a severe impact on both animal production and human health [Annual Report on Zoonoses in Denmark 2015].

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 the consumption of contaminated eggs or poultry meat, whereas *S. Typhimurium* cases are mostly associated with the consumption of contaminated pork, beef or poultry meat.

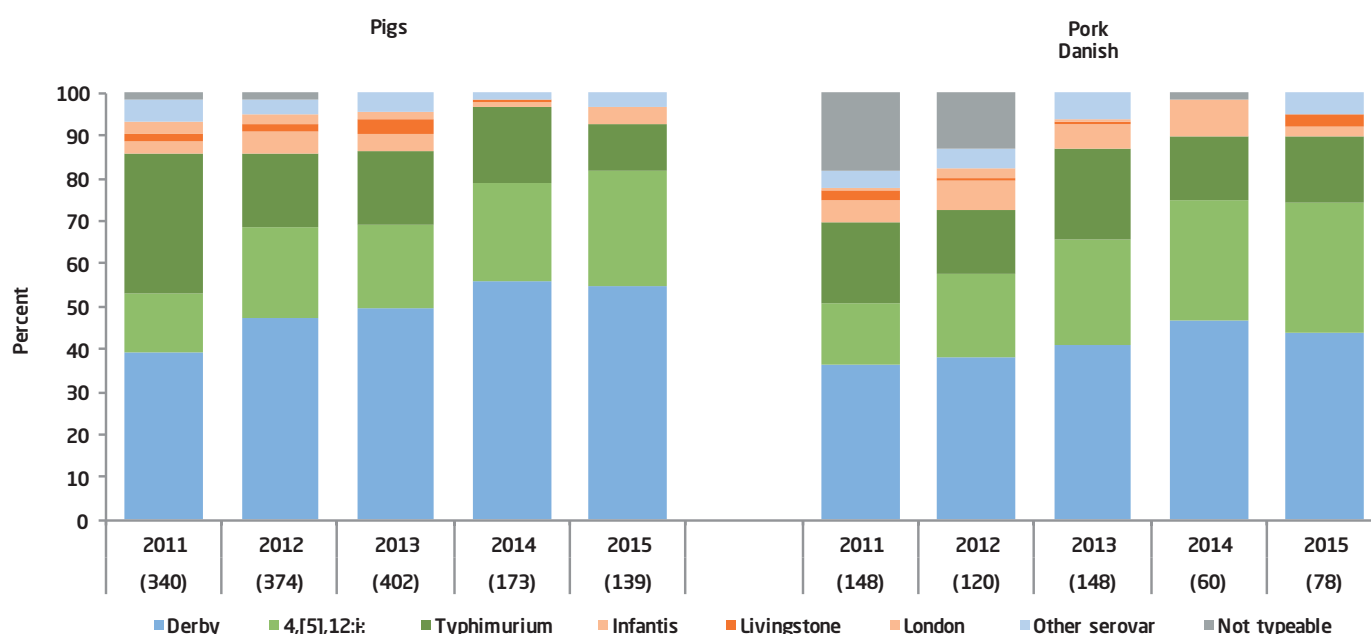
Salmonella isolates for DANMAP 2015 were derived from national surveillance and control programmes. Pig isolates originate from slaughterhouses, where representative samples of healthy pigs (caecum samples) and pork (carcass swabs) are sampled each year. Salmonellosis is a notifiable disease and isolates from all reported human *S. Typhimurium* cases are susceptibility tested. Only one isolate per farm, meat sample or human case was included. For details see Chapter 9, Materials and Methods.

Salmonella from domestic broilers, layers and cattle as well as some other types of meat including imports are also monitored in Denmark each year. However, they do not contribute to DANMAP 2015, as only few isolates were found and thus, fall below the inclusion threshold for DANMAP of 15 isolates per population. These 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 2015.

The DANMAP report primarily presented resistance among *S. Typhimurium*. Resistance in all *Salmonella* serotypes detected

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

DANMAP 2015



The number of isolates varies between years (pigs: n = 139-402, Danish pork: n = 60-148). Before 2011, not all serotypes were susceptible tested. Number of isolates included each year is presented in the parentheses.

by the national control programme for pigs and Danish pork is however presented for 2011 and onwards; the year when 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.

In 2014 the panel for susceptibility testing was changed to follow the recommendations of EFSA by excluding apramycin, ceftiofur, florfenicol, neomycin, streptomycin and spectinomycin and including ceftazidime, meropenem and tigecycline instead.

MIC distributions for isolates from pigs, pork and humans are presented in the web annex (Tables A6.1 - A6.5).

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

In total, 803 samples from pigs caeca (pigs) and 15,905 pig carcass swabs (pork) yielded 217 *Salmonella* isolates tested for antimicrobial resistance.

As in the previous years, *S. Typhimurium* (including the monophasic variants) and *S. Derby* were the most common serotypes isolated from Danish pigs and pork in 2015; representing 92% of all the isolates (Figure 6.1). The majority of *S. Derby*

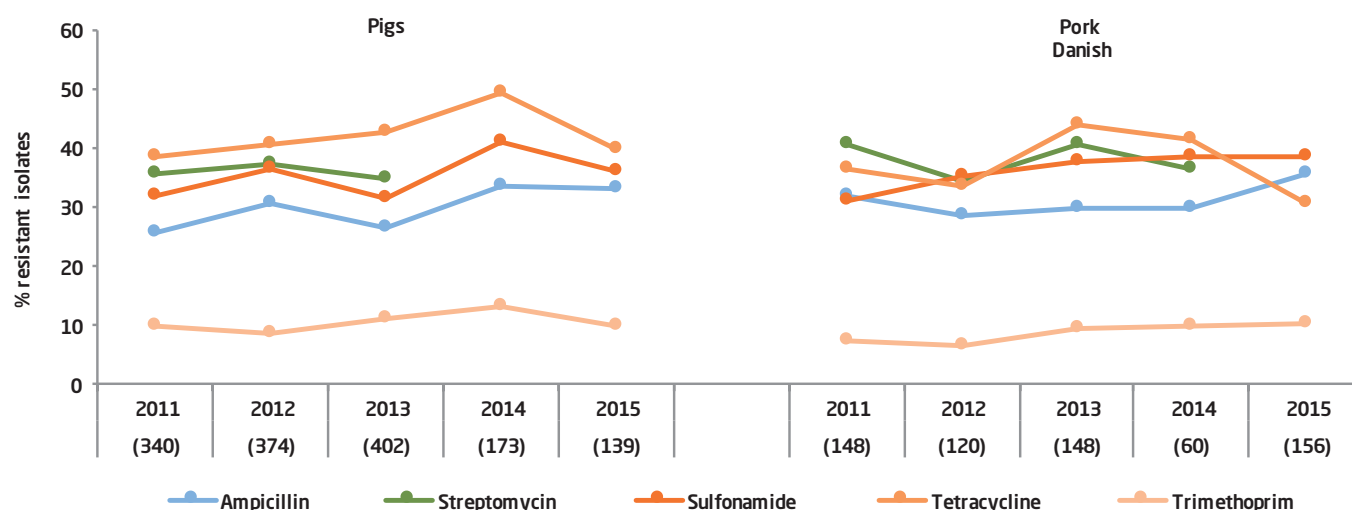
isolates from Danish pigs and pork were fully sensitive to all antimicrobial agents tested in opposition to *S. Typhimurium*, where only 15% of isolates exhibited full sensitivity. The serotype distribution has remained fairly constant in Denmark in many years suggesting a stable endemic situation for *Salmonella* serotypes in the pig population with no rare outbreak

Table 6.1. Resistance (%) among *Salmonella* isolates (all serotypes) from pigs and pork, Denmark DANMAP 2015

Antimicrobial agent	Pigs	Pork
	Danish %	Danish %
Tetracycline	40	31
Tigecycline	0	0
Chloramphenicol	6	5
Ampicillin	33	36
Cefotaxime	0	0
Ceftazidime	0	0
Meropenem	0	0
Trimethoprim	10	10
Sulfonamide	36	38
Azithromycin	1	1
Gentamicin	4	0
Ciprofloxacin	1	0
Nalidixic acid	1	0
Colistin	0	0
Number of isolates	139	36

Figure 6.2. Resistance (%) in all *Salmonella* serotypes from pigs and Danish pork, Denmark

DANMAP 2015



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

strains in 2015. Furthermore, the serotype distribution in the Danish pork is similar to the distribution among isolates from pigs suggesting that *Salmonella* usually arrives at the slaughterhouse with the live pigs rather than reside in the slaughter houses.

Resistance to at least one antimicrobial agent was found in 68 (49%) of the isolates from pigs and in 39 (50%) of isolates from pork. As in the previous years, the highest levels of resistance were found to ampicillin, sulfonamide and tetracycline (Table 6.1). Among the resistant isolates, isolates were primarily co-resistant to ampicillin, sulfonamide and tetracycline (ASuT resistance profile) with further resistance up to three more antibiotics.

The level of resistance to tetracycline was lower in 2015 than in 2014 in isolates from pigs and pork, but no significant difference was observed among changes in resistance to other antimicrobial agents (Figure 6.2).

None of the *Salmonella* isolates from pigs or pork were resistant to cephalosporins (cefotaxime and ceftazidime) or carbapenems (meropenem).

Even though *S. Derby* is very common among pigs, only few human *S. Derby* cases (N = 13) were reported in Denmark in 2015 [www.ssi.dk]. The majority of the *S. Derby* isolates from pigs were fully sensitive (72%) or resistant to tetracycline only (17%, data not presented), and the overall occurrences of resistance among *S. Derby* in pigs and Danish pork were lower than observed among the *S. Typhimurium* isolates only. Quinolone resistance was found in two *S. Derby* isolates; one resistant to nalidixic acid and one to ciprofloxacin. The quinolone resistance was also additional to an ASuT-profile in both isolates.

Table 6.2. Resistance (%) among *Salmonella* Typhimurium^(a) isolates from pigs, Danish pork and human cases^(b), Denmark

DANMAP 2015

Antimicrobial agent	Pigs	Pork	Human cases		
	Danish %	Danish %	Domestically acquired %	Travel abroad reported %	Total %
Tetracycline	66	50	55	74	61
Tigecycline	0	0	2	3	2
Chloramphenicol	11	8	12	24	16
Ampicillin	74	72	65	67	64
Cefotaxime	0	0	2	14	6
Ceftazidime	0	0	2	11	5
Meropenem	0	0	0	0	0
Trimethoprim	19	17	14	11	13
Sulfonamide	83	72	66	76	68
Azithromycin	2	3	0	3	1
Gentamicin	8	0	1	11	3
Ciprofloxacin	0	0	2	20	7
Nalidixic acid	0	0	2	8	3
Colistin	0	0	2	2	2
Number of isolates	53	36	129	66	231

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

6.1.2 *S. Typhimurium* in pigs, domestically produced and imported pork

A total of 89 *S. Typhimurium* (including monophasic strains) were isolated from Danish pigs and pork. Of these 27 were generic *Typhimurium* and 62 monophasic, which continued the historic increasing trend of the proportion of monophasic isolates, illustrating the spread of these novel serotypes.

The level of fully sensitive *S. Typhimurium* isolates in both pig and pork in 2015 remained similar to the level in 2014 (17% and 20%, respectively). No important differences in resistance levels were found between *S. Typhimurium* isolates from pig and pork (Table 6.2) and resistance to the tested antimicrobial agents was similar to levels reported in 2014.

As in the previous years, the highest resistance levels were observed to ampicillin, tetracycline and sulfamethoxazole. The occurrence of ASuT multi-resistance has been increasing among *S. Typhimurium* from pigs since 2010, partly due to an increase in the occurrence of the monophasic variants of *S. Typhimurium*, which often carry the ASuT multi-resistance (Figure 6.3). This year 63% of the monophasic isolates carried ASuT mostly in combination with resistance to one or more antibiotics, whereas the ASuT-profile only was seen in 32% of the generic *Typhimurium* isolates. The increasing occurrence of multi-resistant monophasic *S. 4,[5],12:i:-* is also happening in Europe [Hopkins et al. 2010. Eurosurveillance 3:1]. This illustrates how spread of antimicrobial resistance among *Salmonella* can be highly influenced by the spread a multi-resistant clones highlighting the multifactorial causes of global antibiotic resistance.

Tetracyclines, beta-lactamase sensitive penicillins, pleuromutilins and macrolides (mainly Tylosin and Tilmicosin) are the main antimicrobial agents used for weaners and pigs in Denmark (Figure 4.4). The use of tetracycline and to some extent macrolides increased from 2004 to 2009, where after the consumption was reduced over the following years. However these changes in usages of tetracycline and macrolides is not reflected in the observed levels of resistance in *S. Typhimurium* from pigs and domestically produced pork (Figure 6.3) - as the trends is dominated by the increasing occurrence of monophasic *S. 4,[5],12:i:-* clones.

None of the *S. Typhimurium* isolates from pigs or pork were resistant to quinolones (ciprofloxacin or nalidixic acid), cephalosporins (cefotaxime and ceftazidime) or carbapenems (meropenem); and the use of flouroquinolones and cephalosporins in the Danish pig production has been very low for at last decade (web annex, Table A4.2). Two *S. Typhimurium* (one of these monophasic) isolates were resistant to azithromycin (assuming the cutoff MIC>16), one originating from live pigs and one from pork. In both cases, the isolates also carried an ASuT-profile.

6.1.3 *Salmonella* in humans

In 2015, *Salmonella* continued to be the second most frequent

cause of bacterial intestinal infections in Denmark. A total of 925 human laboratory-confirmed cases of salmonellosis was reported (16.3 cases per 100,000 inhabitants). The most common serotypes were *S. Typhimurium* (including the monophasic variants) and *S. Enteritidis* with 4.1 and 4.6 cases per 100,000 inhabitants, respectively [Annual report on Zoonoses in Denmark 2015].

6.1.4 *S. Typhimurium* in humans

S. Typhimurium, including the monophasic variants, was the second most common serotype among the human cases (233 cases), *S. Enteritidis* being the most common (259 cases). The monophasic variants represented half the *S. Typhimurium* cases (117 monophasic versus 116 generic *Typhimurium*). Available isolates were susceptibility tested (n = 231).

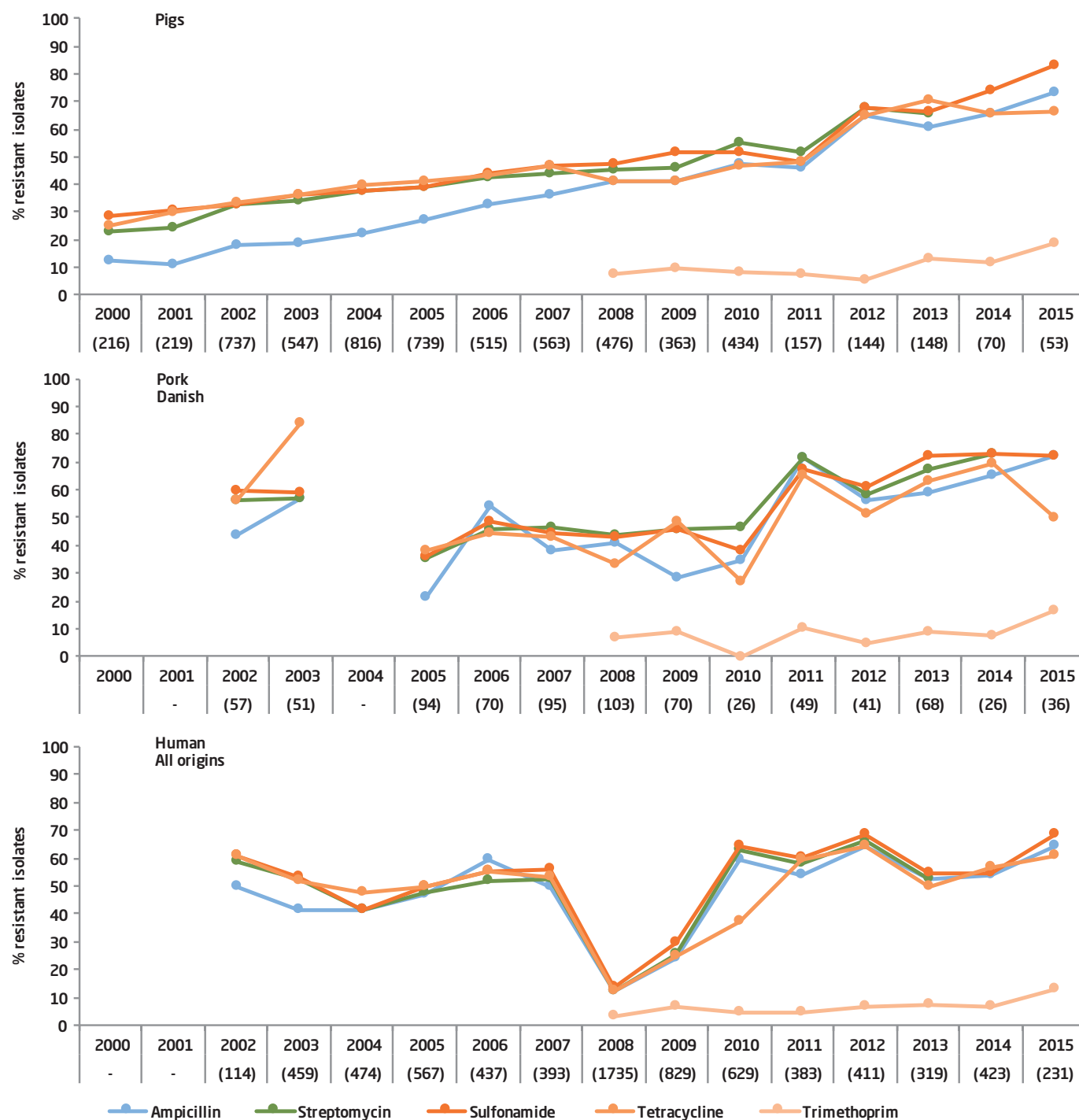
Information on travel history was collected through phone interviews, and among the reported human *S. Typhimurium* isolates included in DANMAP 2015, 29% of the cases were categorised as travel associated, whereas 56% most likely had acquired their infection in Denmark (Table 6.2). In 2015, only one outbreak with the monophasic variant of *S. Typhimurium* was detected and only 6 human cases were considered 'outbreak-related'. In contrast to previous years, domestically acquired human cases includes sporadic as well as out-break related cases (Table 6.2).

Again this year, high levels of resistance to ampicillin, sulfonamide, and tetracycline were observed. Resistance to ampicillin was observed at the same level (65-67%) among isolates from domestic and travel-associated cases (Table 6.2). For domestically acquired cases the level of ampicillin resistance had increased significantly from 2014 to 2015 (from 54% to 65%). Resistance to sulfonamide and tetracycline increased significantly in travel associated cases from 2014 to 2015, where 76% and 74% were resistant in 2015 respectively. Overall, 48% of all *S. Typhimurium* isolates carried the ASuT multi-resistance profile (n = 112) alone or in combination with other antimicrobials. Comparison with previous years showed that the proportion of isolates resistant to these antimicrobial agents have stabilised at this fairly high level. The level of resistance to trimethoprim increased in domestically acquired cases from 2014 to 2015 (6% to 14%, including both sporadic and outbreak related cases) reaching the same level as for travel-associated cases (11% in 2015).

There is a tendency for higher levels of resistance among travel-associated isolates compared with domestic infections in 2015. Both ciprofloxacin and nalidixic acid resistance were higher in isolates from travel-related cases (20% and 8% respectively) compared with isolates from domestic cases (2% for both antibiotics). Resistance to both tetracycline and gentamicin were also significantly higher in travel-associated cases (74% and 11% respectively) when compared to domestically acquired cases (55% and 1% respectively). Finally, resistance to chloramphenicol increased significantly in both

Figure 6.3. Resistance (%) in *Salmonella* Typhimurium in (a) pigs, pork and human cases (b), Denmark

DANMAP 2015



Note: Number of isolates included each year is presented in the parenthesis. The '-' indicate data for application of 2015 cut-offs not available or less than 25 isolates were included.

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

b) Includes all susceptibility tested isolates irrespective of known origin.

domestically and travel associated cases from 2014 and 2015 and resistance to chloramphenicol in the travel associated cases (24%) were also significantly higher in 2015 when compared to the domestically acquired cases (12%).

Regarding resistance to antimicrobial agents critical for treatment of human infections, resistance to 3rd generation cephalosporins (cefotaxime and/or ceftazidime) was found in 11 *S. Typhimurium* isolates (5% of cases); from 8 travel-related cases and 3 domestic cases. The resistance being significantly higher in isolates from travel-related cases (12%) when compared to isolates from domestic cases (2%).

Among all *S. Typhimurium* isolates tested between 2002 and 2015, there is a decline in resistance to all antimicrobials tested in the years 2008 and 2009 (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. Resistance to spectomycin, chloramphenicol and florfenicol has declined between 2002 and 2015, due to a general decline of the *S. Typhimurium* DT104 clone holding resistance to these antimicrobials. Resistance to ampicillin, sulfonamide, streptomycin and tetracycline are generally high over the 14 year period as the declining DT104 clone was replaced by a new emerging variant of monophasic *S. Typhimurium*; both clones possessing resistance to ampicillin, sulfonamide, streptomycin and tetracycline.

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6.2 *Campylobacter*

Thermotolerant *Campylobacter* are the most commonly reported causes of gastrointestinal bacterial infections in humans in Denmark as well as in the European Union [EU Summary Report 2014, ECDC/EFSA 2015]. The species most commonly associated with human infections is *C. jejuni*, but other species may also cause infections. 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 also exist, e.g. water from untreated water sources and other infected animals.

For *Campylobacter*, DANMAP 2015 includes randomly collected isolates from broilers and cattle at slaughter (caecum samples) and from broiler meat ready for retail (meat samples). Campylobacteriosis is a notifiable disease, however only a random 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.

In 2014 the panel for susceptibility testing was changed to follow the recommendations of EFSA by excluding chloramphenicol. MIC distributions for *C. jejuni* from broilers and cattle, broiler meat and humans are presented in the web annex (Tables A6.6 - A6.8).

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

A total of 286 samples from broilers and domestically produced meat resulted in 83 *C. jejuni* isolates, which were tested for antimicrobial resistance. This was much lower than the 191 isolates in 2014 where monitoring of poultry was mandatory in the EU.

The level of fully sensitive *C. jejuni* isolates in both broilers and Danish broiler meat in 2015 (Table 6.3) remained similar to 2014 (68% and 74%, respectively). Among the resistant isolates, resistance to ciprofloxacin and nalidixic acid is dominant (21 of 24 isolates, in total). For both broilers and Danish broiler meat, resistance levels in *C. jejuni* isolates were comparable to 2014.

However, over the last 15 years the proportion of isolates from Danish broilers fully sensitive to the six antimicrobial agents included in the current test panel has decreased from around 90%, as a result of an increase in resistance to tetracycline and quinolones (Figure 6.4) - following the trend in Europe even though at a slower rate [EU Summary Report 2014, ECDC/EFSA 2015].

From 2002 to 2011, a steady increase in resistance to ciprofloxacin was observed in *C. jejuni* isolates from broilers and Danish broiler meat; even after 2009 where the poultry industry stopped using fluoroquinolones in the broiler production. However, during the last five years, resistance to ciprofloxacin has fluctuated reaching 27% in 2015 (Figure 6.4). The EU average 2014-level of ciprofloxacin and tetracycline resistance among *C. jejuni* from broiler was 66% and 54%, respectively [EU Summary Report 2014, ECDC/EFSA 2015].

In contrast to the overall increasing occurrence of quinolone resistant *C. jejuni* isolates from broilers, the increase has discontinued for tetracycline resistance over the last two years (Figure 6.4). The consumption of antimicrobial agents in Danish broilers is generally low, but tetracycline has been one of the most commonly used antimicrobial agents in Danish broilers over the last decade. Consumption of tetracyclines, sulphonamide/trimethoprim, aminoglycosides and lincosamides increased considerably in 2015 due an increased occurrence of *E. coli* or other infections, associated with high mortality and increased health problems in the affected flocks. However, this is not reflected in the resistance patterns in *C. jejuni* isolates from broilers or broiler meat in contrast to increased resistance in indicator *E. coli* (Figure 7.3).

As in most of Europe, resistance to macrolides (erythromycin), aminoglycosides (gentamicin and streptomycin) and chloramphenicol have remained at a very low level.

6.2.2 *C. jejuni* from imported broiler meat

As in previous years, the levels of resistance quinolones (79%) and tetracycline (56%) in imported poultry meat (N=62) were higher than the levels of observed in isolates from domestically produced broilers (Table 6.3).

Most of the imported broiler meat tested originated from EU Member States, and the trends in resistance among the DANMAP isolates reflect the general increase in resistance to tetracycline and quinolones (Figure 6.4). Interestingly, the decline in tetracycline resistance observed among the isolates from Danish broilers is also observed among the isolates from imported broiler meat (from 80% in 2013 to 56% in 2015).

6.2.3 *C. jejuni* in cattle

A total of 138 *Campylobacter* spp. isolates were derived from 183 cattle ceaca from all of Denmark, and a total of 101 *C. jejuni* isolates were susceptibility tested.

Most of the isolates (68%) were fully sensitive to the six antimicrobials included in the test panel, and the remaining isolates were resistant to quinolones (25%) and/or tetracycline (12%). One isolates were resistant to streptomycin only (Table 6.3).

Over the last 15 years the proportion of fully sensitive isolates has varied between 66% and 95%. From 2004 to 2005 resistance to quinolones increased from 2% to 32%, however only 27 isolates were tested in 2005 (Figure 6.5). Despite continued low consumption of fluoroquinolones in cattle, resistance to fluoroquinolones (ciprofloxacin) among *C. jejuni* from cattle, resistance levels varied between 16% and 22% from 2006 to 2014. Initially, quinolone-resistant *C. jejuni* isolates came from

cattle farms in Southern Jutland, but has now spread to other parts of Denmark.

Most years, resistance to tetracycline has been below 5%. In *C. jejuni* from cattle, however, tetracycline resistance increased from 2014 to 2015, reaching the highest level of tetracycline resistance reported in *C. jejuni* from Danish cattle since 2002 (Figure 6.5). This is not a reflection of an increase in the use of tetracyclines in cattle, which remained at the same level as in 2014.

6.2.4 *C. jejuni* in humans

In 2015, *Campylobacter* continued as the most frequent cause of bacterial intestinal infections in Denmark. A total of 4,348 human laboratory confirmed cases of campylobacteriosis were reported (77 per 100,000 inhabitants) [Annual report on Zoonoses in Denmark 2015].

A random selection of the *Campylobacter* isolated from stool samples in three of five geographical regions were submitted to SSI for species identification and susceptibility testing. In 2015, 188 *C. jejuni* isolates were susceptibility tested.

Information on travel history was collected through phone interviews, and among the tested isolates, 23% were from travel-associated cases and 77% were domestically acquired. Among the domestically acquired infections, 54% 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 (9%). The occurrence of resistance to ciprofloxacin and tetracycline continued to be significantly higher in travel associated *C. jejuni* isolates (79% and 72%, respectively) compared to isolates from domestically acquired infections (42% and 23%, respectively) (Table 6.3).

Among the resistant isolates, most were resistant to ciprofloxacin and nalidixic acid often in combination with tetracycline

Table 6.3. Resistance (%) in *Campylobacter jejuni* isolates from animals, meat of Danish and imported origin and human cases(a), Denmark

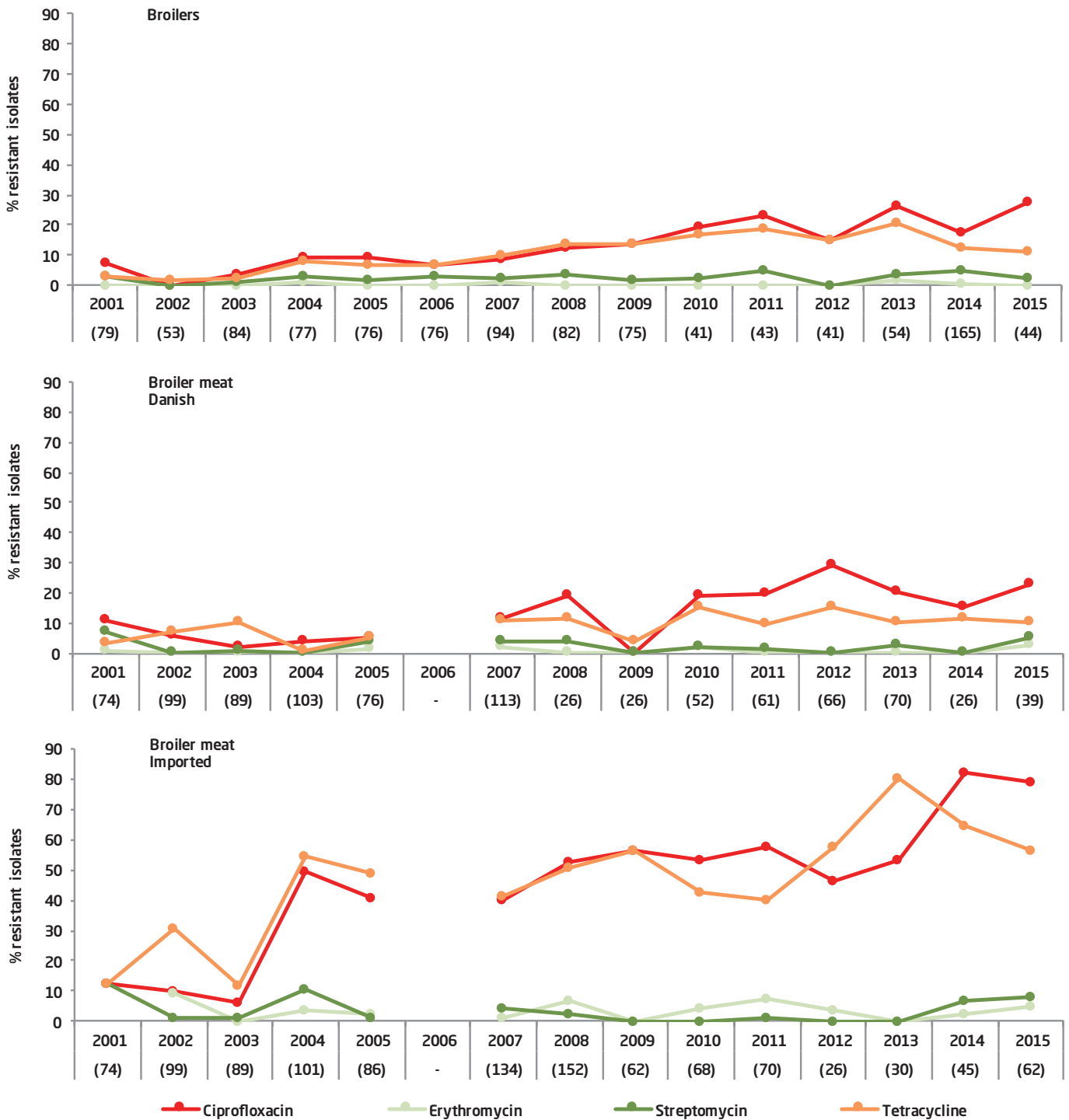
DANMAP 2015

Antimicrobial agent	Cattle	Broilers	Broiler meat	Humans			Total %
	Danish %	Danish %	Danish %	Import %	Domestically acquired %	Travel abroad %	
Tetracycline	12	11	10	56	23	72	34
Erythromycin	0	0	3	5	4	2	4
Streptomycin	1	2	5	8	4	7	5
Gentamicin	0	0	0	0	1	5	2
Ciprofloxacin	25	27	23	79	42	79	51
Nalidixic acid	25	23	23	76	42	79	51
Number of isolates	101	44	39	62	145	43	188

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

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

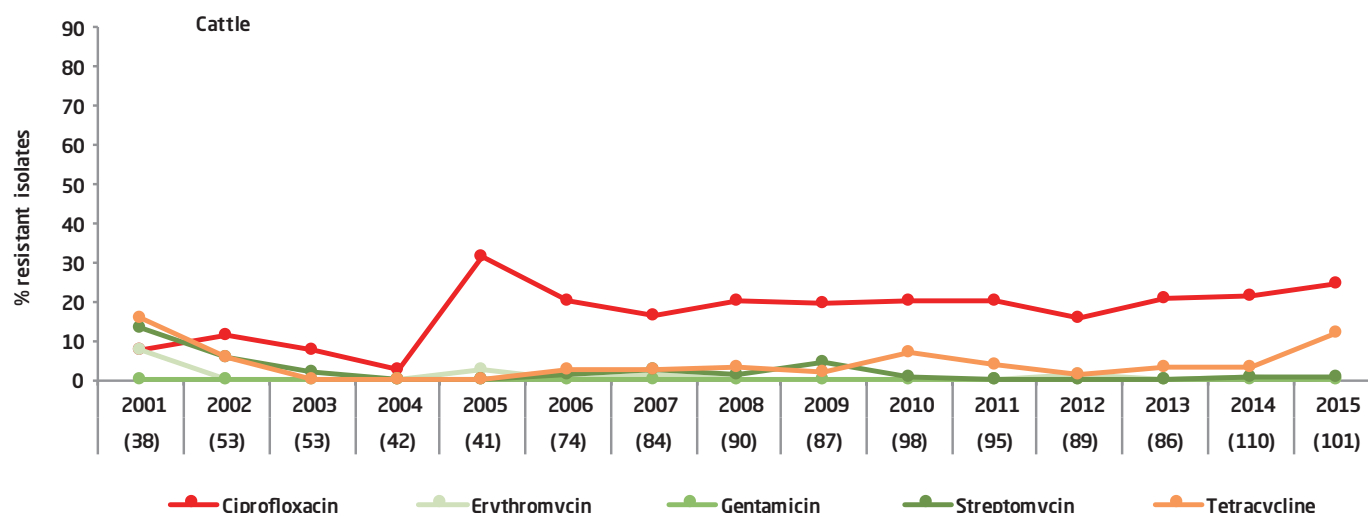
DANMAP 2015



Note: Number of isolates included each year is presented in the parenthesis. The '-' indicate data for application of 2015 cut-offs not available or less than 25 isolates were included.

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

DANMAP 2015



Note: Number of isolates included each year is presented in the parenthesis. The '-' indicate data for application of 2015 cut-offs not available or less than 25 isolates were included

(26 of 43 among domestic cases and 30 of 61 travel associated cases). All isolates resistant to gentamicin (four in total) were multi-resistant, two were acquired domestically and two were travel related. Seven isolates were found resistant to erythromycin, all in combination with at least one other antimicrobial agent, six were acquired domestically and one was from a travel associated case.

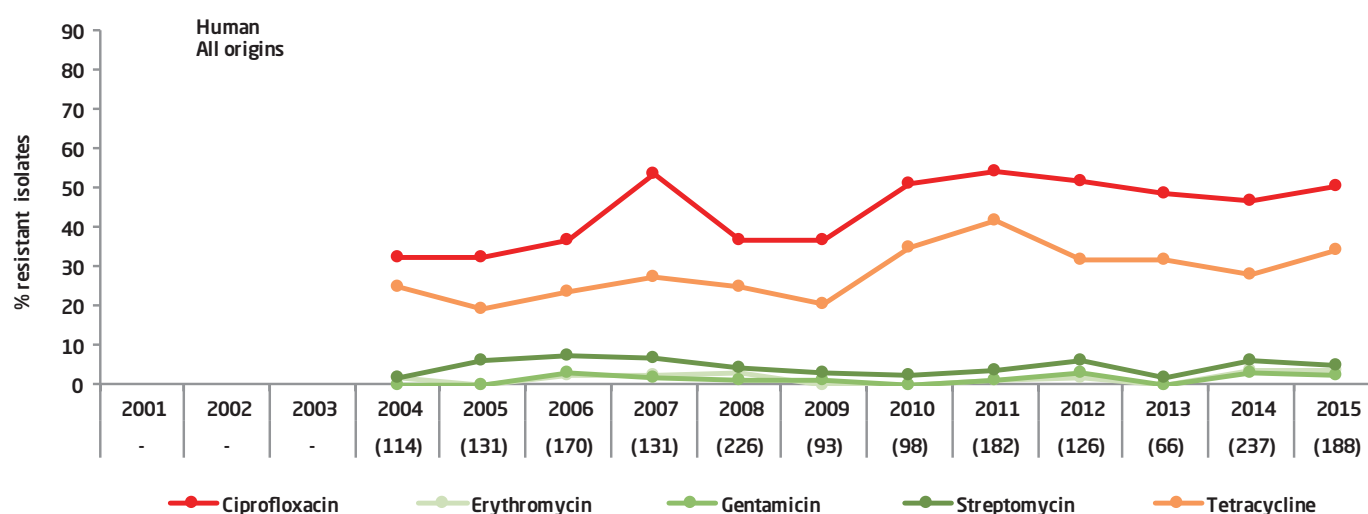
Among all isolates tested from 2004-2015 resistance to chloramphenicol did almost not exist, and only 1% of the tested isolates were found resistant in three separate years. Resistance to erythromycin and gentamicin were below 5%

and streptomycin resistance was below 10%; resistance in all isolates tested for these three antimicrobials were quite stable over the 12 years included. Resistance to ciprofloxacin, nalidixic acid and tetracycline is fluctuating over the 12 years with a slight increasing tendency. For ciprofloxacin and nalidixic acid, the percentage of resistant isolates seen were from 32% to 54% with an average of 44% and tetracycline resistance was seen in 19% to 42% of the isolates tested with an average of 29% (Figure 6.6).

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Figure 6.6. Resistance (%) in *Campylobacter jejuni* from human cases^{a)}, Denmark

DANMAP 2015



Note: Number of isolates included each year is presented in the parenthesis. The '-' indicate data for application of 2015 cut-offs not available or less than 25 isolates were included.

a) Includes all susceptibility tested isolates irrespectively of known origin.

Textbox 6.1

Resistance in bacteria from diagnostic submissions from pigs

Surveillance of antimicrobial resistance in veterinary pathogens is important as support for practicing veterinarians in prescribing effective drugs for treatment. Since 2013, the DANMAP report has not presented data on antimicrobial resistance from diagnostic submissions from animals, unlike SVEDRES and NORM-VET. This has mainly been due to lack of availability of isolates. Most routine diagnostic services on production animals take place at private, commercial laboratories, with no or limited tradition for storing bacterial isolates for research or surveillance.

However, in 2015 the Danish Veterinary and Food Administration set aside resources to initiate an investigation of specified organisms from production animals. The focus in 2015 was on bacteria from pigs. Isolates and MIC data were obtained from SEGES, The Danish Pig Research Centre's Laboratory for Pig Diseases (VSP) in Kjellerup. *Escherichia coli* O149, *Streptococcus suis*, and *Actinobacillus pleuropneumoniae* are among the most important pathogenic bacteria in pigs and were analysed for antimicrobial resistance.

The antimicrobial susceptibility testing was carried out using the broth dilution method with SensiTitre. The clinical breakpoints are a combination of Clinical and Laboratory Standards Institute (CLSI) breakpoints if available, and those routinely used at VSP Kjellerup and DTU - Vet.

***E. coli* O149**

Enterotoxigenic *E. coli* (ETEC) is a major cause of diarrhoea in pigs and *E. coli* containing the haemolytic, F4 fimbria and enterotoxin producing serotype O149 are the most virulent for pigs. Although systematic vaccination and breeding against resistance to the F4 fimbria has somewhat reduced the problems with this type in the first weeks after birth as well as post weaning, it remains the most prevalent type of ETEC in Danish pigs. Percent resistant isolates are shown in Table 1. Resistance levels are high for ampicillin, sulphonamides, streptomycin, and tetracyclines (ASSuT - resistance pattern also known from *Salmonella*), but also for spectinomycin and trimethoprim. This was not unexpected as similar resistance levels were also recorded in 2012 and the preceding 10 years (DANMAP 2012, p.104). An increase in the number of isolates resistant to florfenicol compared to 2012 is not statistically significant, but considering the increase in use of florfenicol in recent years, this development may be relevant to follow. Two isolates were resistant to ceftiofur (3rd generation cephalosporin), and these two isolates were also the only isolates that were resistant to amoxicillin with clavulanic acid and cefotaxime, strongly suggesting that these isolates were ESBL. Resistance to nalidixic acid was recorded in 11 % of the isolates although quinolones have not been used for pigs since 2002. Notably, no isolates were resistant to colistin.

Streptococcus suis

Streptococcus suis is the cause of a plethora of infections in pigs, such as meningitis, arthritis, pneumonia, and septicaemia, and causes losses to the farmers due to increased mortality and veterinary costs. Percent resistant isolates are shown in Table 1. Most notably, no isolates were resistant to simple penicillin. Resistance was highest to macrolides and tetracyclines, but low or zero for most other compounds.

Actinobacillus pleuropneumoniae

Actinobacillus pleuropneumoniae is the cause of a severe pleuropneumonia in pigs. This disease has a rapid onset in pig farms and spreads through the farm. Symptoms include fever, coughing, depression, loss of appetite, bloody discharge from the nose, and pathological findings include fibrinous pleuritis and pericarditis and haemorrhagic and necrotizing pneumonia. A rapid diagnosis and initiation of proper treatment is essential. Fortunately, there is little resistance to this organism, and apart from erythromycin, resistance was zero or very low to all other compounds (Table 1). Notably, all isolates were susceptible to simple penicillin, although this is a Gram negative organism. Most of the isolates belonged to the serotypes O2 and O6, but no difference in resistance patterns was recorded between serotypes.

In 2016 the AMR surveillance is extended to include bacteria from cattle, poultry, mink.

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continued ... Textbox 6.1

Table 1. Resistance (%) among clinical isolates from pigs, Denmark

Antimicrobial agent	<i>E. coli</i> O149 %	<i>Actinobacillus</i> <i>pleuropneumoniae</i> %	<i>Streptococcus</i> <i>suis</i> %
Tetracycline	64	0	47
Chloramphenicol	29	-	0
Florfenicol	10	0	0
Ampicillin	40	0	-
Penicillin	-	0	0
Amoxicillin/clavulanic acid	4	-	-
Ceftiofur	4	0	-
Trimethoprim	51	-	2
Sulfonamide	70	-	19
Sulfa-trimethoprim	-	0	0
Erythromycin	-	100	67
Streptomycin	65	-	47
Gentamicin	2	-	0
Neomycin	6	-	-
Apramycin	2	-	-
Tulathromycin	-	ND	-
Tilmicosin	-	3	-
Ciprofloxacin	0	0	0
Nalidixic acid	11	-	-
Colistin	0	-	-
Tiamulin	-	0	19
Spectinomycin	45	0	26
Number of isolates	53	70	43

Note: Some of the *E. coli* 149 isolates were not tested for Chloramphenicol (n=45), Florfenicol (n=52), Ampicillin (n=52), Amoxicillin/clavulanic acid (n=45), Trimethoprim (n=51), Streptomycin (n=52), Apramycin (n=48), Ciprofloxacin (n=52) and Nalidixic acid (n=46). Proportion of tulathromycin resistant isolates is not calculated (ND)



7

**RESISTANCE IN
INDICATOR BACTERIA**

7. Resistance in indicator bacteria



Highlights: Resistance to tetracycline and erythromycin was observed at moderate to high levels in *Enterococcus faecalis* from Danish broilers and pigs as well as in meat thereof. During the last years, resistance levels in enterococci isolated from domestically produced broiler meat has increased and now approaches resistance levels found in imported meat. From 2014 to 2015, tetracycline resistance among enterococci from domestically produced broiler meat increased; an increase that is probably related to the increased use of tetracycline in the poultry production. For *E. faecalis* from pigs, levels of resistance to tetracycline, erythromycin and gentamicin has been declining to levels equal to or lower than 15 years ago. Resistance levels have consistently been lower in *E. faecalis* from domestically produced pork compared to isolates from the Danish pigs. Resistance to tetracycline is also lower in *E. faecalis* from domestically produced pork compared with isolates from imported pork.

Most of the *Enterococcus faecium* from domestically produced beef and broiler meat, as well as from imported beef, were fully sensitive to all antimicrobial agents included in the test panel. Resistance to tetracycline, ampicillin, erythromycin and ciprofloxacin was lower in *E. faecium* from domestically produced broiler meat compared with isolates from imported broiler meat.

The occurrence of resistance to ampicillin, tetracycline, sulfonamides and trimethoprim increased significantly in **indicator *Escherichia coli*** from broilers compared to 2014. This is most likely linked to the increased use of antimicrobial agents in the broiler production. In *E. coli* from Danish broilers, pigs and meat thereof, approximately half of the isolates exhibited resistance to at least one of the antimicrobial agents tested, while the proportion of antimicrobial resistant isolates was remarkably low in isolates from Danish cattle and beef. As in previous years, proportions of antimicrobial resistant *E. coli* were generally higher in imported meat than in domestically produced meat independent of the meat type (broiler meat, pork, beef).

Third-generation cephalosporin-resistant (ESBL/AmpC) *E. coli* were observed in pigs, cattle and meat thereof. The occurrence in pigs was higher than in 2009-2013, mainly due to a high frequency of samples containing *E. coli* with AmpC upregulation. We consider that the changes in methodology implemented in 2015 may have had an important impact on the observed results.

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. In the early 90ties, focus was especially on resistance in Gram-positive bacteria, since most growth promoters used then targeted Gram-positive bacteria. The chosen 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 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 due to natural resistance to used antimicrobial agents such as flavomycin and streptogramins and observed differences in levels of resistance.

E. faecalis, *E. faecium* and *E. coli* have been included in the DANMAP programme since 1995, where isolates were recovered for susceptibility testing in samples from broilers, cattle and pigs. Sampling of fresh meat was initiated from 1997. Indicator enterococci and *E. coli* from healthy humans was collected from 2002 to 2005. Results from surveys of ESBL-producing bacteria from animals and meat have been presented in DANMAP reports since 2006-2007.

7.1 Enterococci

For enterococci, DANMAP 2015 includes randomly collected *Enterococcus faecalis* isolates from healthy pigs (caecum samples) and *E. faecalis* and *E. faecium* from domestically produced and imported broiler meat, pork and beef sold at wholesale and retail outlets (meat samples). Only one isolate per farm or meat sample was included. For details on methodology, see Chapter 9, Materials and Methods.

In 2014, the panel for susceptibility testing was changed to follow the recommendations of EFSA by excluding kanamycin, penicillin, salinomycin and streptomycin, but including daptomycin.

MIC distributions for *E. faecium* and *E. faecalis* from animals and meat are presented in the web annex (Tables A7.1 – A7.3).

7.1.1 *E. faecalis* from domestically produced broiler meat

From a total of 217 samples of domestically produced broiler meat, 48 *E. faecalis* isolates was recovered and tested for antimicrobial resistance (Table 7.1). Enterococci were not collected from broilers in 2015.

As in 2014, approximately half of the *E. faecalis* isolates from **domestically produced broiler meat** were fully sensitive to the antimicrobial agents included in the test panel (not includ-

ing quinupristin/dalfopristin). Among the resistant isolates all were tetracycline-resistant, representing a significant increase compared to 2014 (48% versus 35% of all isolates). Increasing resistance levels were observed for several bacterial species isolated from Danish broilers and broiler meat in 2015, and may likely be related to the increase in usage of antimicrobial agents for broilers in 2015 (Table 4.1). Resistance to tetracycline and erythromycin in *E. faecalis* from Danish broilers and broiler meat has increased over the last five years; reaching levels for isolates from domestically produced broiler meat similar to what was observed in 2002-2004 (Figure 7.1). There are no data to verify if the increase in resistance levels in Danish broilers continued in 2015.

However, none of the *E. faecalis* isolates from domestically produced broiler meat were resistant to fluoroquinolones or vancomycin in 2015, and resistance levels to these antimicrobial agents among *E. faecalis* from Danish broilers and meat hereof, has been close to zero for the last 15 years.

7.1.2 *E. faecalis* from pigs and domestically produced pork

A total of 703 samples from pigs and domestically produced pork resulted in 160 *E. faecalis* isolates, which were tested for antimicrobial resistance.

Generally, the level of resistance is higher in *E. faecalis* from **pigs** compared to isolates from broilers. In 2015, the level of *E. faecalis* isolates from pigs that were fully sensitive to the antimicrobial agents included in the test panel (not including quinupristin/dalfopristin) was slightly higher than observed in 2014 (25% versus 15%).

As in previous years, most of isolates from pigs were resistant to tetracycline and resistance to erythromycin was also

Table 7.1. Resistance (%) among *Enterococcus faecalis* from animals and meat of Danish and imported origin, Denmark DANMAP 2015

Antimicrobial agent	Broiler meat		Beef		Pigs	Pork	
	Danish %	Imported %	Danish %	Imported %	Danish %	Danish %	Imported %
Tetracycline	48	58	12	22	72	14	44
Tigecycline	0	0	0	0	0	0	0
Chloramphenicol	2	0	0	2	15	4	0
Ampicillin	0	0	0	0	0	0	0
Erythromycin	25	33	2	2	35	6	11
Gentamicin	0	1	0	0	10	2	4
Ciprofloxacin	0	5	0	0	0	0	0
Vancomycin	0	0	0	0	0	0	0
Teicoplanin	0	0	0	0	0	0	0
Linezolid	0	0	2	0	0	0	0
Daptomycin	0	0	0	0	0	0	0
Number of isolates	48	78	57	59	40	120	27

common (Figure 7.2). Most of the isolates were resistant to erythromycin, chloramphenicol and/or gentamicin (3 of 4 isolates) was also resistant to tetracycline. The levels of resistance were comparable with those from 2014, and resistance levels have generally been very stable over the last 15 years. An exception to this is resistance to erythromycin, which slowly increased until 2012, where after the resistance declined reaching levels equal to what was observed in 2001 (Figure 7.2).

Resistance to tetracycline, chloramphenicol, erythromycin and gentamicin in *E. faecalis* from **domestically produced pork** was significantly lower than in the isolates from pigs. The majority (86%) of the isolates from pork were fully sensitive to the tested antimicrobial agents (not including quinupristin/dalfopristin). These differences in resistance levels between isolates from Danish pigs and domestically produced pork have been apparent since the start of the DANMAP monitoring programme (Figure 7.2); however no explanation has been documented.

7.1.3 *E. faecalis* from domestically produced beef

From a total of 132 samples of domestically produced beef, 57 *E. faecalis* isolates was recovered and tested for antimicrobial resistance (Table 7.1). Enterococci were not collected from cattle in 2015.

In *E. faecalis* from **domestically produced beef**, most of the isolates (88%) were fully sensitive to the antimicrobial agents included in the test panel (not including quinupristin/dalfopristin). All resistant isolates (n=7) were resistant to tetracycline, and one of these isolates was also resistant to erythromycin and linezolid (Table 7.1). Levels of antimicrobial resistances were similar to those from 2014.

7.1.4 *E. faecalis* from imported meat

During 2015, a total of 164 *E. faecalis* isolates, from 345 processed samples from imported broiler meat, pork and beef, were tested for antimicrobial resistance (Table 7.1).

The proportion of isolates fully sensitive to the tested antimicrobial agents (not including quinupristin/dalfopristin) were lower among *E. faecalis* from imported broiler meat (38%), pork (55%) and beef (78%) compared to the isolates from domestically produced meat.

Generally, the levels of resistance observed in *E. faecalis* from imported meat were comparable to 2014. Resistance to tetracycline and co-resistance to tetracycline and erythromycin was most commonly observed (Table 7.1). Among all *E. faecalis* tested, ciprofloxacin resistance was only detected in isolates from imported boiler meat (n = 4).

Previously, higher levels of resistance to tetracycline, erythromycin and streptomycin (as well as several other compounds) have been observed in *E. faecalis* from **imported broiler meat** compared to isolates from domestically produced broiler meat. However, resistance in *E. faecalis* from imported and domestically produced broiler meat has, in recent years, approached similar levels, and no significant differences were observed in 2015 (Figure 7.1).

Resistance to tetracycline was higher in *E. faecalis* from **imported pork** compared with isolates from domestically produced pork. This tendency has been observed for years (Figure 7.2) with a small increase in levels of tetracycline resistance for imported pork meat.

As observed in most years, resistance levels are similar in *E. faecalis* from **imported beef** and domestically produced beef. Tetracycline resistance has only been found significantly higher in isolates from imported beef in 2012 and 2014.

Figure 7.1. Resistance (%) in *Enterococcus faecalis* from broilers and broiler meat, Denmark

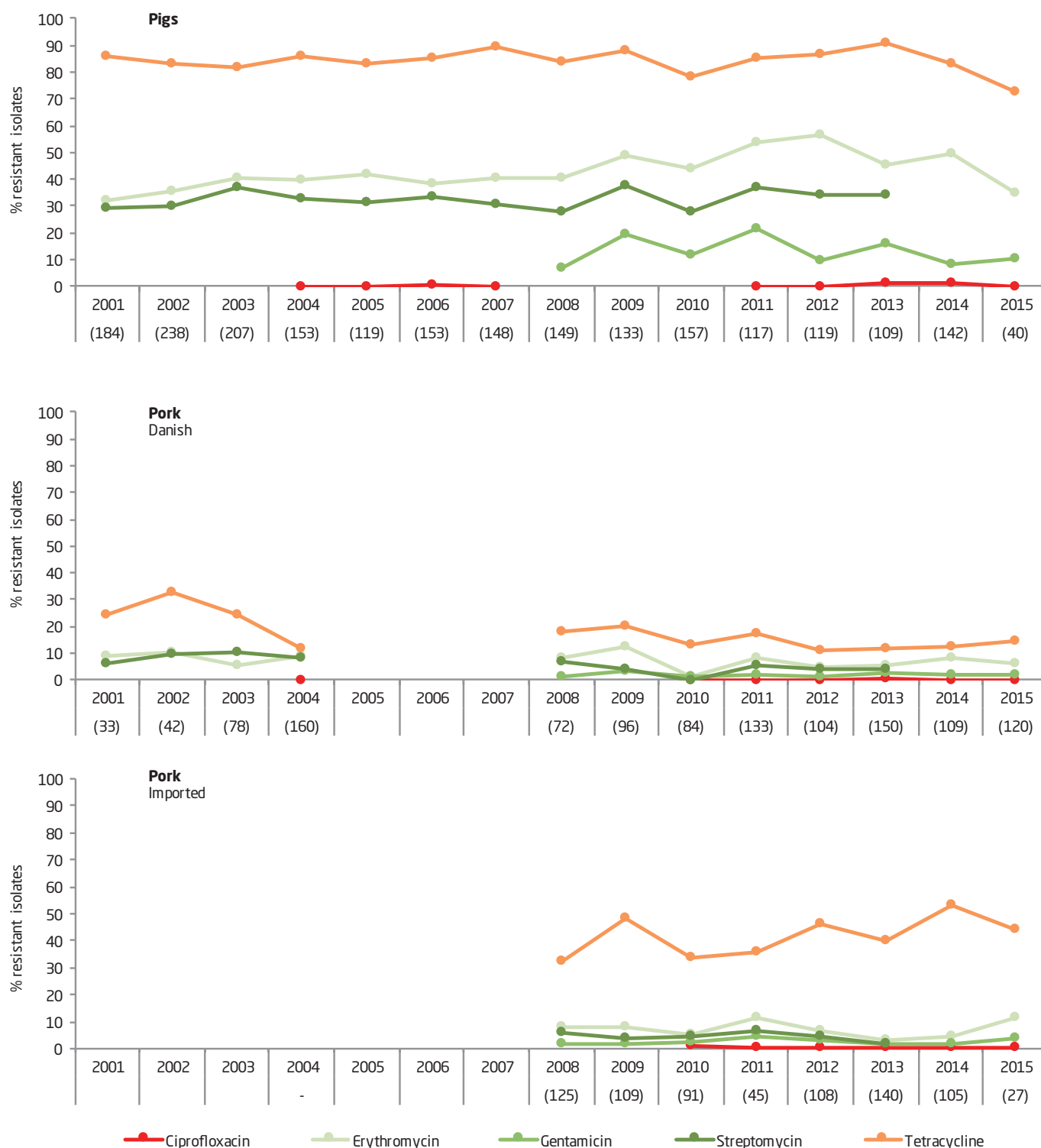
DANMAP 2015



Note: Number of isolates included each year is presented in the parenthesis. The '-' indicate data for application of 2015 cut-offs not available or less than 25 isolates were included. No isolates from broilers in 2015.

Figure 7.2. Resistance (%) in *Enterococcus faecalis* from pigs and pork, Denmark

DANMAP 2015



Note: Number of isolates included each year is presented in the parenthesis. The '-' indicate data for application of 2015 cut-offs not available or less than 25 isolates were included.

7.1.5 *E. faecium* from domestically produced meat

During 2015, a total of 543 processed samples from domestically produced broiler meat, pork and beef resulted in 216 *E. faecium* isolates, which were tested for antimicrobial resistance (Table 7.1). Since only 12 *E. faecium* isolates were recovered from domestically produce pork, these data are not presented.

In the *E. faecium* isolates from domestically produced broiler meat and beef, most of the isolates were fully sensitive to all antimicrobial agents included in the test panel (75% and 88%, respectively). Among the resistant isolates tetracycline and erythromycin resistance was most common, whereas a few isolates were resistant to ampicillin (n = 6 from broiler meat), gentamicin (n = 1 from broiler meat and beef, respectively) and daptomycin (n=2 from broiler meat, n = 1 from beef). Resistance to quinupristin/dalfopristin and ciprofloxacin was only detected in broiler meat (n = 7 and n = 1, respectively) (Table 7.1) All six ampicillin resistant isolates from broiler meat were also resistant to tetracycline and erythromycin.

The levels of tetracycline resistance in domestically produced broiler meat increased from 2014 to 2015 (Figure 7.3). This increase is very likely linked to the general increase in the use of antimicrobial agents for poultry in Denmark in 2014 and 2015, especially increased use of tetracyclines (Table 4.1).

7.1.6 *E. faecium* from imported meat

During 2015, a total of 345 processed samples from imported broiler meat, pork and beef resulted in 103 *E. faecium* isolates, which were tested for antimicrobial resistance (Table 7.2). Only six *E. faecium* isolates were recovered from imported pork, and data are therefore not presented.

Resistance levels were low among *E. faecium* from **imported beef**, and 79% were fully sensitive to all antimicrobial agents included in the test panel. Among isolates from **imported broiler meat** 37% were fully sensitive.

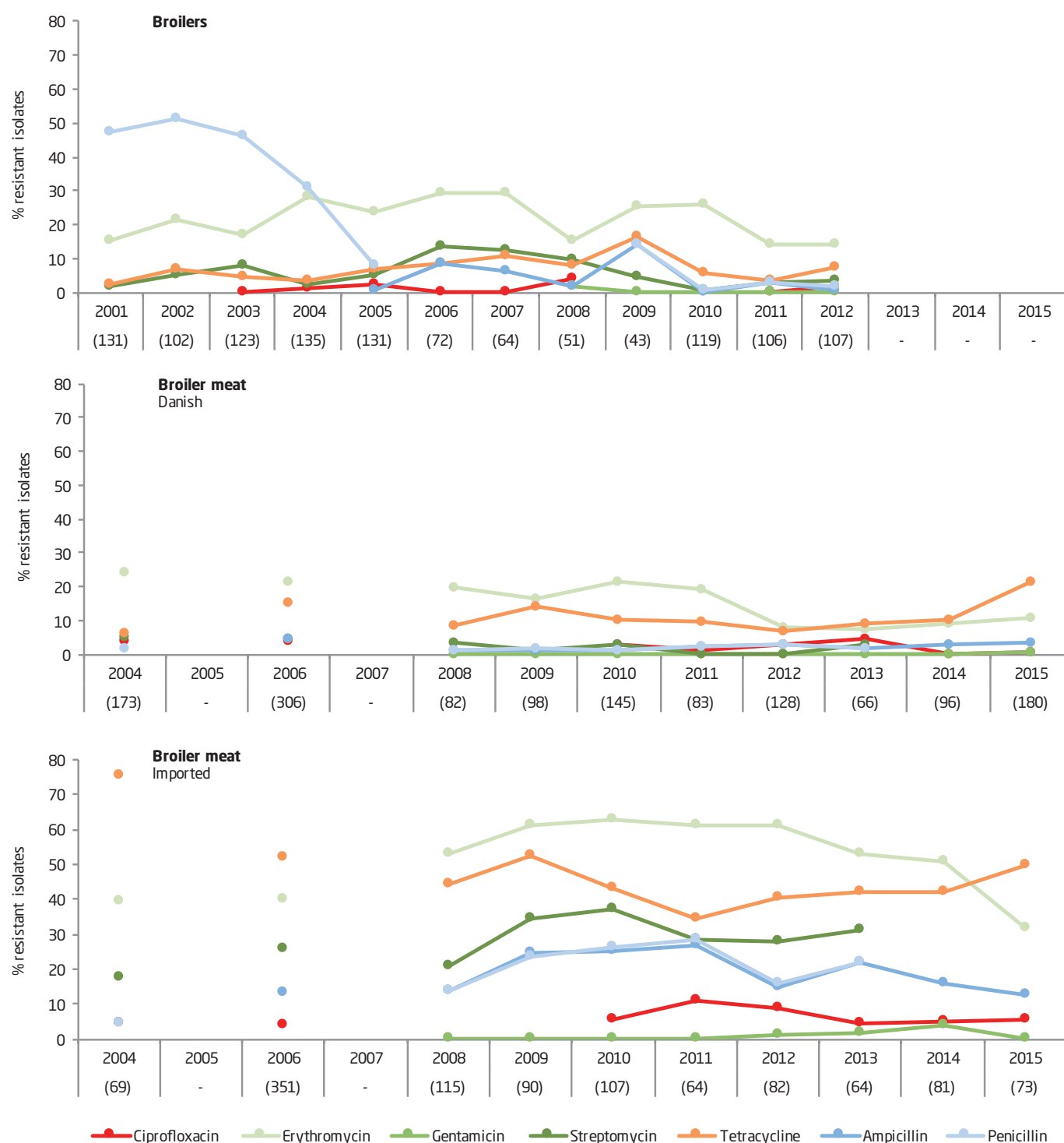
As in 2013 and 2014, resistance to tetracycline, ampicillin, erythromycin and ciprofloxacin was significantly more frequent in *E. faecium* from imported broiler meat than in isolates from domestically produced broiler meat (Figure 7.3). Overall, 10% of the *E. faecium* isolates from imported broiler meat were resistant to more than three tested antimicrobials; all but one were resistant to tetracycline, erythromycin and ampicillin. In isolates from imported beef, one isolate was resistant to tetracycline, erythromycin and ampicillin. When comparing data from 2014 and 2015, only resistance levels to erythromycin in imported broiler meat decreased significantly.

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Table 7.2. Resistance (%) among *Enterococcus faecium* from meat of Danish and imported origin, Denmark

DANMAP 2015

Antimicrobial agent	Broiler meat		Beef	
	Danish %	Imported %	Danish %	Imported %
Tetracycline	22	49	8	17
Tigecycline	0	0	0	0
Chloramphenicol	0	0	0	0
Ampicillin	3	12	0	4
Erythromycin	11	32	4	8
Quinupristin/dalfopristin	4	4	0	0
Gentamicin	1	0	4	0
Ciprofloxacin	1	5	0	0
Vancomycin	0	0	0	0
Teicoplanin	0	0	0	0
Linezolid	0	0	0	0
Daptomycin	1	0	4	4
Number of isolates	180	73	24	24

Figure 7.3. Resistance (%) in *Enterococcus faecium* from broilers and broiler meat, Denmark

Note: Number of isolates included each year is presented in the parenthesis. The '-' indicate data for application of 2015 cut-offs not available or less than 25 isolates were included.

7.2 Indicator *Escherichia coli*

For indicator *E. coli*, DANMAP 2015 includes randomly collected isolates from healthy pigs, broilers and cattle at slaughter (caecum samples) and from domestically produced and imported broiler meat, pork and beef sold at wholesale and retail outlets (meat samples). Only one isolate per farm or meat sample was included. For details on methodology, see Chapter 9, Materials and Methods.

In 2014 the panel for antimicrobial susceptibility testing was changed to follow the recommendations of EFSA by excluding apramycin, ceftiofur, florfenicol, neomycin, streptomycin and spectinomycin and replacing them with ceftazidime, meropenem and tigecycline. The MIC distributions and occurrence of resistance among indicator *E. coli* are presented in the web annex (Tables A7.4 and A7.5).

7.2.1 Indicator *E. coli* from broilers and domestically produced broiler meat

A total of 396 processed samples from broilers and domestically produced meat resulted in 309 *E. coli* isolates, which were tested for antimicrobial resistance. The number of isolates was much lower compared to previous years as, according to the EU legislation, antimicrobial susceptibility testing of *E. coli* isolates from chickens was not mandatory in 2015.

Approximately half (56%) of indicator *E. coli* isolates from **broilers** were susceptible to all antimicrobials tested. High occurrence of resistance was detected towards ampicillin, tetracyclines, trimethoprim and sulfonamides, low occurrence of resistance was detected towards quinolones (Table 7.3). The most common co-resistance profiles were resistance to ampicillin and sulfonamides in combination with trimethoprim alone (n=9) and with trimethoprim and tetracyclines (n=7).

Fluoroquinolone resistance was observed in eight isolates from broilers and in five (62%) of these isolates, fluoroquinolone resistance was exhibited together with resistance to other antimicrobials (ampicillin, azithromycin, chloramphenicol, gentamicin, sulfonamides, tetracyclines and trimethoprim in various combinations). Occurrence of fluoroquinolone-resistant *E. coli* was significantly higher in broilers compared to pigs and cattle.

No resistance to cefotaxime, ceftazidime, colistin, meropenem and tigecycline was detected in isolates from broilers (Table 7.3). Nevertheless, by using a pre-enrichment procedure, cefotaxime-resistant *E. coli* were detected, as reported in Textbox 7.1.

Compared to 2014, a significant increase in resistance to ampicillin, tetracyclines, trimethoprim and sulfonamides was observed. These are the highest proportions of isolates resistant to ampicillin, tetracyclines and sulfonamides detected in

DANMAP since 2001 (Figure 7.4), whereas the proportion of isolates resistant to trimethoprim was the highest detected in DANMAP since 2007 (data not presented). Due to changes in the range of tested trimethoprim concentrations, trimethoprim data of earlier DANMAP iterations cannot be used for comparison.

The use of antimicrobial agents increased considerably in 2014 and 2015 due to *E. coli* or other infections in a large number of broiler flocks, thus it appears that the occurrence of antimicrobial resistance generally correlates with antimicrobial use. The use of tetracyclines, penicillins (other), sulfonamides and trimethoprim has increased steadily in broilers since 2012, whereas increase in the use of penicillins (b-lactamase sensitive), aminoglycosides and lincosamides was observed since 2014 (Section 4.3). Fluoroquinolones have not been used in the Danish broiler production from 2010 to 2014, and were used in very small amounts in 2015, which likely explains the low occurrence of fluoroquinolone resistance detected. Similarly, very low occurrence of chloramphenicol resistance is likely linked to limited use of amphenicols since 2010.

Among *E. coli* isolates from Danish **broiler meat**, the occurrence of susceptibility and resistance paralleled that observed in broilers. Thus, 54% of isolates were susceptible to all antimicrobials, and high proportions of isolates showed resistance towards ampicillin, tetracyclines, trimethoprim and sulfonamides. Low occurrence of resistance was detected towards quinolones and very low occurrence of resistance was detected towards cefotaxime (0.4%), gentamicin and chloramphenicol (Table 7.3). No resistance to azithromycin, ceftazidime, colistin, meropenem and tigecycline was detected.

The proportion of broiler meat isolates susceptible to all antimicrobials diminished compared to 2014, when it was 70%. The occurrence of resistance towards ampicillin, tetracyclines and trimethoprim was significantly higher than that observed in domestically produced broiler meat in 2014 (Figure 7.4). Overall, in 2015, the occurrence of resistance to the different antimicrobial agents for which data are available over time, were the highest observed since 2004, with the notable exception of 3rd generation cephalosporins, for which the highest occurrence of resistance was observed in 2011 and has been decreasing since then (Figure 7.4).

7.2.2. Indicator *E. coli* from pigs and domestically produced pork

During 2015, processing of a total of 403 samples from pigs and domestically produced meat resulted in 231 *E. coli* isolates, which were tested for antimicrobial resistance.

Among the *E. coli* from **pigs**, high occurrence of ampicillin, tetracycline, trimethoprim and sulfonamide resistance was detected (Table 7.3), and only 47% of the isolates were suscepti-

ble to all tested antimicrobial agents. Among isolates displaying resistance to one antimicrobial agent ($n = 27$), tetracycline resistance was by far the dominant phenotype detected in 14 (52%) isolates, whereas among the isolates resistant to more than one antimicrobial agent ($n = 66$), the most frequent phenotype was ampicillin, tetracycline, trimethoprim and sulfonamide resistance detected in 19 (29%) isolates. Resistance to cefotaxime, ceftazidime, meropenem and tigecycline was not observed (Table 7.3). Nevertheless, by using a pre-enrichment procedure, cefotaxime-resistant *E. coli* were detected, as reported in section 7.3 and Textbox 7.1.

The proportions of isolates resistant to ampicillin, tetracyclines, trimethoprim and sulfonamides remained at levels comparable to those observed in 2014. Ciprofloxacin and colistin resistances were detected in one isolate each in 2015 but were not observed in 2014. Although no drastic fluctuations over time were observed, the proportions of isolates resistant to ampicillin, tetracyclines and sulfonamides were among the highest detected in all DANMAP iterations, with the exception of the levels observed in 2004 (Figure 7.5).

Among the *E. coli* isolates from domestically produced **pork**, 63% were susceptible to all antimicrobial agents tested. High occurrence of resistance to ampicillin, tetracyclines, trimethoprim and sulfonamides was detected, whereas resistance to the other tested antimicrobial agents was not observed (Table 7.3). The most common resistance profiles were as observed among isolates from pigs: tetracycline resistance ($n=7$) and co-resistance to ampicillin, tetracyclines, trimethoprim and sulfonamides ($n=5$). Overall, occurrence of resistance was

slightly lower in *E. coli* from pork than in *E. coli* from pigs (Table 7.3), though this difference was statistically significant only for sulfonamide-resistant isolates.

Occurrence of resistance to ampicillin, tetracyclines, trimethoprim and sulfonamides declined compared to 2014 (Figure 7.5, only statistical significant for sulfonamide). The proportions of ampicillin- and tetracycline-resistant *E. coli* were the lowest observed since 2008, whereas proportions of sulfonamide and trimethoprim resistance were comparable to those observed in 2010, being in both years the lowest levels ever observed in DANMAP.

7.2.3 Indicator *E. coli* from cattle and domestically produced beef

During 2015, a total of 379 processed samples from cattle and domestically produced meat resulted in 199 *E. coli* isolates, which were tested for antimicrobial resistance.

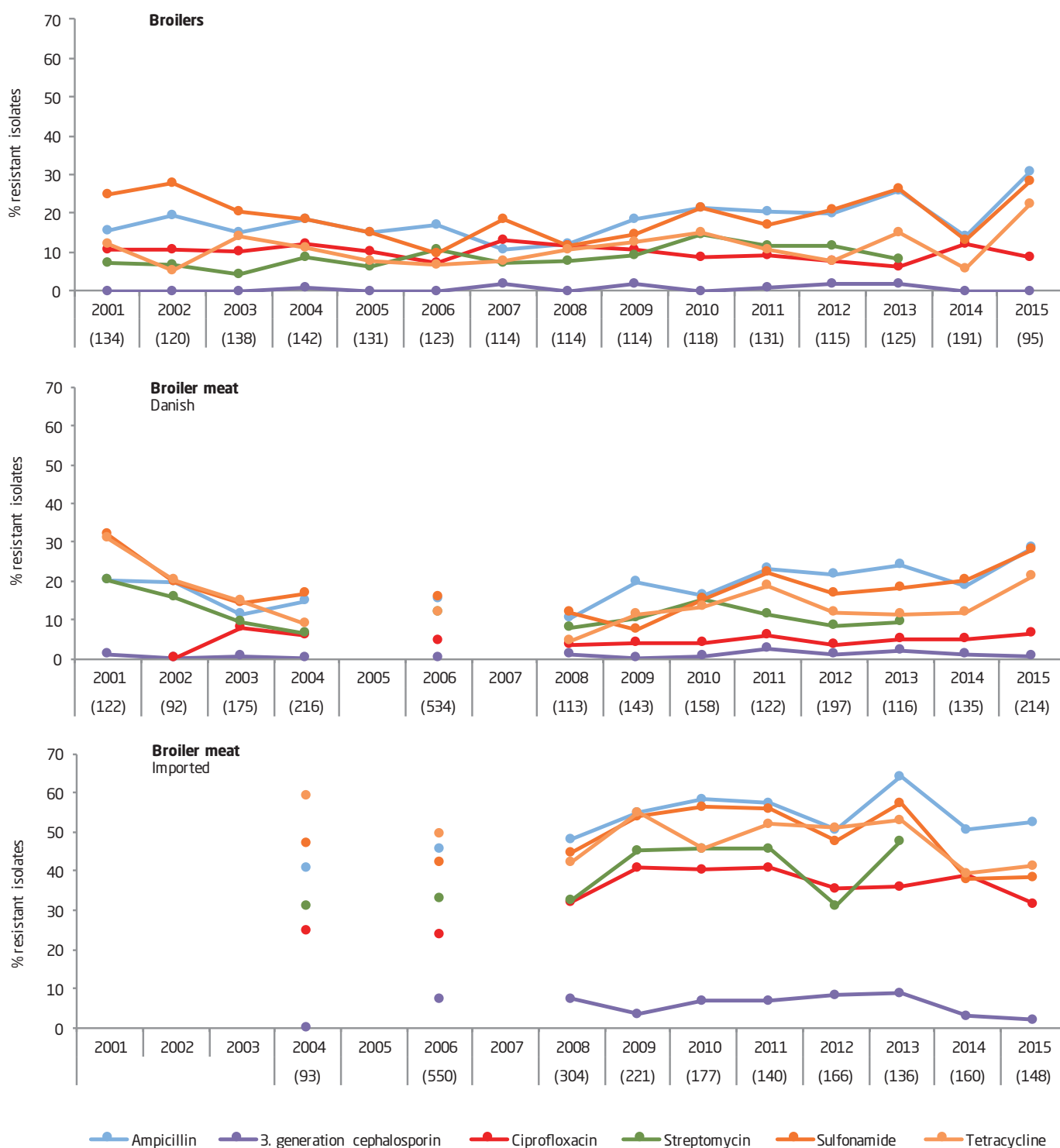
Among *E. coli* from **cattle**, the majority of isolates (87%) were susceptible to all tested antimicrobial agents, and mainly low occurrence of resistance to ampicillin, chloramphenicol, sulfonamides, tetracyclines and trimethoprim was observed (Table 7.3). The majority of resistant isolates (17 out of 19) exhibited co-resistance to antimicrobials belonging to two or more classes, but there was no dominant resistance profile. No resistance to azithromycin, ceftazidime, meropenem, colistin, nalidixic acid, ciprofloxacin and tigecycline was observed. Results on occurrence of cefotaxime-resistant *E. coli* obtained by a pre-enrichment procedure are reported in section 7.3 and Textbox 7.1.

Table 7.3. Resistance (%) among *Escherichia coli* from animals and meat of Danish and imported origin, Denmark DANMAP 2015

Antimicrobial agent	Broilers	Broiler meat		Cattle	Beef		Pigs	Pork	
	Danish %	Danish %	Imported %	Danish %	Danish %	Imported %	Danish %	Danish %	Imported %
Tetracycline	22	21	41	9	9	11	36	25	27
Tigecycline	0	0	0	0	0	0	0	0	0
Chloramphenicol	2	3	7	6	4	8	2	0	0
Ampicillin	31	29	53	8	5	17	32	23	27
Cefotaxime	0	<1	2	1	0	3	0	0	0
Ceftazidime	0	0	2	0	0	3	0	0	0
Meropenem	0	0	0	0	0	0	0	0	0
Trimethoprim	24	23	37	6	0	6	24	18	13
Sulfonamide	28	28	39	10	7	14	36	19	27
Azithromycin	1	0	1	0	0	0	1	0	0
Gentamicin	1	1	1	1	0	3	1	0	0
Ciprofloxacin	8	7	32	0	0	6	1	0	0
Nalidixic acid	8	7	29	0	0	6	1	0	0
Colistin	0	0	1	0	0	0	1	0	0
Number of isolates	95	214	148	144	55	36	174	57	15

Figure 7.4. Resistance (%) in *Escherichia coli* from broilers and broiler meat, Denmark

DANMAP 2015



Note: Number of isolates included each year is presented in the parenthesis. The '-' indicate data for application of 2015 cut-offs not available or less than 25 isolates were included.

Figure 7.5. Resistance (%) in *Escherichia coli* from pigs and pork, Denmark

DANMAP 2015



Note: Number of isolates included each year is presented in the parenthesis. The '-' indicate data for application of 2015 cut-offs not available or less than 25 isolates were included.

Figure 7.6. Resistance (%) in *Escherichia coli* from cattle and beef, Denmark

DANMAP 2015



Note: Number of isolates included each year is presented in the parenthesis. The '-' indicate data for application of 2015 cut-offs not available or less than 25 isolates were included.

These results are in line with those observed since 2001, although very minor fluctuations were observed over time. Increasing occurrence of ampicillin and trimethoprim resistance was observed since 2008, whereas sulfonamide and tetracycline resistance increased from 2008 to 2014 (Figure 7.6).

Also among *E. coli* isolates from domestically produced **beef**, most isolates (89%) were susceptible to all antimicrobials, and resistance paralleled that observed in cattle. Comparable low levels of resistance have been observed over the last 15 years (Figure 7.6).

7.2.4 Indicator *E. coli* from imported meat

During 2015, a total of 362 processed samples from imported broiler meat, pork and beef resulted in 199 *E. coli* isolates, which were tested for antimicrobial resistance. As in previous years, the levels of resistance were significantly higher in imported broiler meat than in broiler meat produced in Denmark, a trend that is no longer apparent for Danish vs. imported pork (Table 7.3).

Among the *E. coli* from **imported broiler meat**, only 29% were susceptible to all tested antimicrobial agents. Occurrence of resistance was very high for ampicillin, high for nalidixic acid, ciprofloxacin, sulfonamides, tetracyclines and trimethoprim, moderate for cefotaxime, ceftazidime and chloramphenicol, and low for azithromycin, colistin and gentamicin (Table 7.3). No resistance to tigecycline and meropenem was observed (Table 7.3). Ampicillin resistance was present in the majority of isolates displaying a multidrug resistance profile, and was shared among 27 antimicrobial resistance profiles. Ampicillin resistance was often combined with resistance to sulfonamides, trimethoprim and tetracyclines in various combinations and generally in association with resistance to additional antimicrobials. Fluoroquinolone resistance was exhibited by 40 isolates either alone ($n = 7$) or in combination with resistance to other antimicrobials. Co-resistance to ampicillin, nalidixic acid, ciprofloxacin, tetracycline, sulfonamides and trimethoprim was observed in nine (11%) isolates.

Compared to 2014, occurrence of ampicillin, sulfonamides and tetracycline resistance slightly increased and occurrence of cefotaxime and ciprofloxacin resistance decreased (Figure

7.4). In the period 2008-2015, the proportions of ampicillin-resistant isolates ranged between 48% (2008) and 64% (2013), thus the level detected in 2015 (53%, Table 7.3) represented a fluctuation similar to what observed previously (Figure 7.4). The proportions of ciprofloxacin and tetracycline resistant isolates were comparable to those observed in 2008, being in both DANMAP iterations the lowest levels observed in the period 2008-2015 (Figure 7.4). The proportion of cefotaxime and sulfonamide resistant isolates were the lowest detected in DANMAP since 2008. In section 7.3 and Textbox 7.1, results on occurrence of cefotaxime-resistant *E. coli* obtained by a pre-enrichment procedure are reported.

Among *E. coli* isolates from **imported pork**, 60% were susceptible to all tested antimicrobial agents. A high proportion of isolates showed resistance to ampicillin, sulfonamides and tetracyclines, whereas a moderate proportion of isolates showed resistance to trimethoprim (Table 7.3). No resistance to the other antimicrobial agents was observed, however the number of isolates was very limited ($n = 15$, Table 7.3). The proportion of isolates susceptible to all antimicrobial agents increased compared to 2014 (when it was 48%) and the proportion of isolates displaying resistance to any antimicrobial agent decreased compared to 2014 and overall was the lowest recorded in DANMAP since 2008 (Figure 7.5). An exception was resistance to 3rd generation cephalosporins for which no resistance was observed also in 2009-2010 and 2013-2014 (Figure 7.5).

The majority (81%) of *E. coli* isolates from **imported beef** was susceptible to all tested antimicrobial agents. Ampicillin, sulfonamide and tetracycline resistance occurred in a moderate proportion of isolates, whereas cefotaxime, ceftazidime, chloramphenicol, gentamicin, nalidixic acid, ciprofloxacin and trimethoprim resistance occurred in a low proportion of isolates (Table 7.3). No resistance to azithromycin, colistin, meropenem and tigecycline was observed (Table 7.3). Only minor differences were observed compared to 2014, however the proportions of isolates resistant to the different antimicrobials were the highest (ampicillin and sulfonamides) or close to the highest (tetracyclines) observed in DANMAP since 2008 (Figure 7.6).

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

DANMAP 2015 includes randomly collected ESBL/AmpC *E. coli* isolates from healthy pigs and cattle at slaughter (caecum samples) and from domestically produced and imported broiler meat, pork and beef sold at wholesale and retail outlets (meat samples). Only one isolate per farm or meat sample was included. Antimicrobial susceptibility testing was only performed on isolates from pigs, cattle and meat thereof in line with European Commission Decision 2013/652/EU on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria.

The samples were collected, and isolates were recovered, identified and tested for antimicrobial susceptibility in accordance with Decision 2013/652/EU. It is important to note that the changes in methodology introduced by Decision 2013/652/EU most likely increased the recovery of ESBL-, AmpC- and carbapenemase-producing *E. coli* in 2015. Thus, caution should be taken when evaluating the occurrence of ESBL/AmpC *E. coli* in samples from 2015 and when comparing these data with results from previous years. For details on methodology, see Textbox 7.1 and Chapter 9, Materials and Methods.

In *E. coli*, an ESBL phenotype is defined by i) resistance to cefotaxime or ceftazidime and ii) susceptibility to ceftazidime and iii) synergy with clavulanic acid (synergy being defined as a ≥ 3 twofold concentration decrease in a MIC for either cefotaxime or ceftazidime tested in combination with clavulanic acid versus the MIC of the agent when tested alone. E.g. MIC FOT : FOT/CL or TAZ : TAZ/CL ratio ≥ 8). Differently, an AmpC-phenotype is characterized by i) resistance to cefotaxime or ceftazidime and ii) resistance to ceftazidime and iii) no synergy with clavulanic acid. A carbapenemase (CPE) phenotype is defined by resistance to meropenem.

The MIC distributions and occurrence of resistance among ESBL/AmpC *E. coli* are presented in the web annex (Tables A7.6 and A7.7).

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

Using the pre-enrichment procedure, cefotaxime-resistant *E. coli* were detected from **pigs** (29% of samples), domestically produced pork (2% of samples) and imported pork (2% of samples) (Table 7.4). The majority of isolates of domestic origin displayed an AmpC-phenotype, and only 12 of 82 isolates displayed an ESBL-phenotype (Textbox 7.1 for further discussion). No carbapenemase phenotype was detected.

Among the ESBL/AmpC-producing *E. coli* isolates from pigs, resistance to gentamicin ($n = 6$) or chloramphenicol ($n = 1$) occurred in a low proportion of isolates. Azithromycin resistance was observed in three isolates using the tentative cut-off of MIC $> 16 \mu\text{g/ml}$, and no resistance to ciprofloxacin was detected (Table 7.4).

A low number of ESBL/AmpC-producing *E. coli* isolates ($n = 4$) was isolated from domestically produced **pork**. In these isolates, co-resistance patterns and levels were similar to those observed in ESBL/AmpC-producing *E. coli* from Danish pigs. Only one ESBL/AmpC-producing *E. coli* strain was isolated from imported pork. This isolate exhibited an ESBL phenotype together with resistance to ciprofloxacin, sulfonamides, tetracyclines and trimethoprim (Table 7.4). The carbapenemase phenotype was not found in either domestically produced or imported pork.

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

Using the pre-enrichment procedure, cefotaxime-resistant *E. coli* were detected from **cattle** (8% of samples), domestically produced beef (1% of samples) and imported beef (4% of samples). Half of the isolates displayed an AmpC phenotype, and slightly less than the remaining half of the isolates displayed an ESBL phenotype (Table 7.4 and Textbox 7.1 for further discussion). One isolate did not fall within the definitions of AmpC and ESBL phenotype (other phenotypes) and no carbapenemase phenotype was observed.

Among the ESBL/AmpC-producing *E. coli* isolates from cattle, co-resistance to gentamicin and chloramphenicol occurred in a low number of isolates, whereas co-resistance to sulfonamides, tetracyclines and trimethoprim was slightly more common (Table 7.4). None of the isolates displayed resistance to azithromycin, colistin, nalidixic acid, ciprofloxacin and tigecycline (Table 7.4).

Following the same trend observed for general indicator *E. coli* (Table 7.3), occurrence of resistance to sulfonamides, tetracyclines and trimethoprim was lower among ESBL/AmpC *E. coli* from cattle compared to ESBL/AmpC *E. coli* from pigs (Table 7.3 and 7.4).

Only two ESBL-producing *E. coli* were isolated from domestically produced **beef**. Such isolates did not show resistance to antimicrobials other than beta-lactams (Table 7.4). No AmpC- and carbapenemase-producing *E. coli* were isolated from domestically produced beef.

Six ESBL- producing *E. coli* were isolated from imported beef (Table 7.4). Co-resistance to chloramphenicol (n = 1), ciprofloxacin (n = 2) and gentamicin (n = 2) was observed in those isolates (Table 7.4). No AmpC- and carbapenemase-producing *E. coli* were isolated from imported beef.

All ESBL/AmpC -producing *E. coli* were susceptible to colistin, nalidixic acid and tigecycline (Table 7.4). Colistin susceptibility indicates that none of the isolates harboured either the newly discovered colistin-resistance genes (*mcr-1* and *mcr-2*) or the chromosomal mutations associated with colistin resistance (PmrA and PmrB) [Agersø et al, 2011; Foodborne Pathog Dis. 9(4):367-9] [Hasman et al 2015; Euro Surveill. 20(49)] [Xavier et al, 2016; Euro Surveill. 21(27)]. The fact that the isolates from imported beef were susceptible to nalidixic acid and resistant to ciprofloxacin (MIC compatible with low level resistance) indicates presence of *qnr* genes [Jacoby 2005; Clin Infect Dis.41 Suppl 2:S120-6].

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Table 7.4. Resistance (%) and 3rd generation cephalosporin-resistance phenotype among ESBL/AmpC-producing *Escherichia coli* from cattle, pigs and meat thereof, Denmark

DANMAP 2015

Antimicrobial agent	Cattle	Beef		Pigs	Pork	
	Danish %	Danish %	Imported %	Danish %	Danish %	Imported %
Tetracycline	14	0	50	54	75	100
Tigecycline	0	0	0	0	0	0
Chloramphenicol	7	0	17	1	25	0
Ampicillin	100	100	100	100	100	100
Cefoxitin	57	0	0	74	50	0
Cefotaxime	100	100	100	100	100	100
Ceftazidime	93	100	100	97	100	100
Cefepime	43	100	100	37	75	100
Meropenem	0	0	0	0	0	0
Ertapenem	0	0	0	0	0	0
Imipenem	0	0	0	0	0	0
Trimethoprim	14	0	33	49	75	100
Sulfonamide	14	0	50	56	75	100
Azithromycin	0	0	0	4	0	0
Gentamicin	7	0	33	8	0	0
Ciprofloxacin	0	0	33	0	25	100
Nalidixic acid	0	0	0	0	0	0
Colistin	0	0	0	0	0	0
ESBL phenotypes	43	100	100	24	50	100
AmpC phenotypes	50	-	-	74	50	-
CPE phenotypes	-	-	-	-	-	-
Other phenotypes	7	-	-	1	-	-
Number of isolates	14	2	6	78	4	1
Number of samples	180	149	166	273	239	50

Textbox 7.1

Extended-spectrum beta-lactamase (ESBL)/AmpC- and carbapenemase-producing *Escherichia coli* from animals and meat

Background: Extended-spectrum beta-lactamase (ESBL/AmpC) -producing 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 infections, suggesting a zoonotic link [Vieira et al, 2011; Foodborne Pathog Dis 8(12):1295-301] [de Been et al, 2014; PLOS Genetics 10:12] [Börjesson et al, 2016; Emerg Infect Dis 22:634] [Hartung et al. 2016; Appl Environ Microbiol 82:4705]. Furthermore, the occurrence of carbapenemase-producing Enterobacteriaceae (CPE) is an even greater threat to human medicine, since carbapenems are the last line antimicrobial agents for treatment of infections caused by multidrug-resistant Gram-negative bacteria. Carbapenemase-producing *E. coli* and *Salmonella* have recently been discovered in the primary production of pigs in Germany [Fisher et al, 2016; j.vetmic.2016.04.026, Epub ahead of print], thus it cannot be excluded that carbapenemase-producing *E. coli* and *Salmonella* entered the German food production chain. To date, only ESBL/AmpC -producing bacteria have been found by the Danish surveillance of antimicrobial resistance in food and food-producing animals with no signs of carbapenem-resistant bacteria in either meat or food-producing animals in Denmark.

Cephalosporin and carbapenem resistance can be selected by usage of cephalosporins and/or carbapenems as well as by antimicrobial usage in general, through co-selection. In July 2010, the use of cephalosporins in the Danish pig production was discontinued, whereas it is still applied for systemic and intramammary treatment in cattle [Agersø et al, 2013; J Antimicrob Chemother. 68(3):569-72]. Cephalosporins have not been used in the Danish broiler production for at least a decade, but were used in the foreign production of grandparent animals of the Danish broilers before May 2012 [DANMAP 2012]. At present, carbapenems are not licensed for use in food-producing animals in Denmark or other parts of the world [EFSA Journal 2013; 11(12):3501].

The 12th of November 2013, the European Commission issued the Implementing Decision 2013/652/EU on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria. The decision lays down specific requirements regarding isolation, identification and antimicrobial susceptibility testing for monitoring ESBL-, AmpC- and carbapenemase-producing *E. coli* in certain food-producing animals and meat, which should be performed according to the most recent version of the protocol of the European Union Reference Laboratory for Antimicrobial Resistance [www.eurl-ar.eu]. These specific requirements also indicate that poultry samples are monitored in even years and pigs and cattle samples in odd years.

Materials and methods: From January to December 2015, samples were collected for monitoring ESBL-, AmpC- and carbapenemase-producing *E. coli* according to the Decision 2013/652/EU. Caecal samples from pigs and cattle as well as meat samples of pork and beef were collected from all regions of Denmark according to the regulation. In addition, meat samples from duck and broiler meat were collected in relation to the national surveillance. Table 1 presents number of samples collected. For details on sampling, see chapter 9, Materials and Methods. There was no monitoring of ESBL/AmpC-producing *E. coli* from broilers in 2015.

One gram of caecal material and 25 gram of meat material were pre-enriched in 9 ml and 225 ml of non-selective peptone water without an antibiotic supplement, respectively, and were incubated at 37 °C for 18-22 hours. After this pre-enrichment, the samples were inoculated onto MacConkey agar plates supplemented with 1µg/ml cefotaxime and incubated at 44 °C for 18-22 hours. To specifically isolate carbapenemase producing *E. coli* (including strains producing only OXA-48-like enzymes), a loop-full of 10 µl of the pre-enriched sample was inoculated onto selective agar. Colonies with the expected *E. coli* morphology were inoculated onto non-selective agar plates and incubated at 37 °C for 18-22 hours and stored for antimicrobial susceptibility testing following the European Commission Implementing Decision 2013/652/EU.

It is important to note that the changes in methodology introduced by Decision 2013/652/EU most likely increased the recovery of ESBL-, AmpC- and carbapenemase-producing *E. coli* in 2015. Thus, caution should be taken when comparing the

continued ... Textbox 7.1

occurrence of ESBL/AmpC *E. coli* in samples from 2015 with data from previous years. As a consequence, statistical analysis to describe temporal trends in occurrence of ESBL/AmpC -producing *E. coli* in pigs, cattle and meat thereof could not be performed. It is noteworthy to mention that no comparative evaluation between the two methodologies applied in 2009-2014 and 2015 has been conducted.

Whole genome sequencing (WGS) and *in silico* bioinformatics tools were used to detect the genetic background for the ESBL and AmpC 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 a specific script executed via MyDb-Finder (version 1.1, <https://cge.cbs.dtu.dk/services/all.php>). All genome sequences were submitted to the European Nucleotide Archive (ENA).

Results: Proportion of samples from animals and meat positive for ESBL/AmpC-producing *E. coli* are reported in paragraph 7.3 and only genotypic data are presented here. No carbapenemase-producing *E. coli* (including strains producing only OXA-48-like enzymes) were identified by screening the samples on selective agar.

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

Among the 78 ESBL/AmpC-producing *E. coli* isolates from pigs, five different ESBL/AmpC genes or mutations were identified.

Table 1. Distribution (%) of ESBL/AmpC and CPE enzymes detected in *Escherichia coli* isolates from animals and meat of Danish and imported origin, Denmark

DANMAP 2015

Enzyme	Broiler meat		Ducks	Cattle	Beef		Pigs	Pork	
	Danish %	Imported %	Imported %	Danish %	Danish %	Imported %	Danish %	Danish %	Imported %
CARB-2	3	-	-	-	-	-	-	-	-
AmpC upregulation	7	-	25	57	-	-	75	40	-
CMY-2	20	39	50	-	-	-	2	-	-
CTX-M-1	70	43	25	21	50	-	20	40	-
CTX-M-14	-	-	-	7	-	100	2	-	-
CTX-M-15	-	-	-	-	33	-	-	20	100
CTX-M-32	-	-	-	-	17	-	-	-	-
CTX-M-55	-	-	-	-	-	-	2	-	-
SHV-2	-	4	-	-	-	-	-	-	-
SHV-12	-	4	-	-	-	-	-	-	-
TEM-52B	-	4	-	14	-	-	-	-	-
TEM-84	-	4	-	-	-	-	-	-	-
Total number of :									
Enzyme count (a)	30	23	8	14	6	2	64	5	1
ESBL/AmpC positive samples (b)	61	125	11	14	6	2	78	4	1
Meat samples tested	238	196	111	180	166	149	273	239	50

Note: Proportion of total number of enzymes. In three isolates AmpC upregulation enzymes was found as well as CTX-M-15 (Danish pork), CMY-2 or CARB-2 (Danish broiler meat).

(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 Danish broiler meat (28 out of 60 isolates), imported broiler meat (23 out of 125 isolates), imported duck (8 out of 11 isolates) and pigs (63 out of 78 isolates).

The most common ESBL type was CTX-M-1 (n = 13), but CTX-M-14 (n = 1) and CTX-M-55 (n = 1) were also observed. Among the AmpC enzymes, CMY-2 was identified in one isolate. The occurrence of isolates producing these four enzymes is comparable to previous years (web annex, Table A7.8). Surprisingly, the majority (n = 48) of isolates displayed an AmpC phenotype linked to upregulation of chromosomal *ampC* expression (Table 1). The occurrence of samples containing *E. coli* with upregulated chromosomal *ampC* expression changed from 1% in 2013 to 18% in 2015. Monitoring from 2009 to 2013 only recovered *E. coli* with upregulation of chromosomal *ampC* expression in 1-4% of samples from pigs (Figure 1).

The 13 strains producing CTX-M-1 were associated to ten different sequence types (ST); including ST23, ST75, ST88 (n = 2), ST101 (n = 3), ST117, ST295, ST367, ST3564, ST4373, and ST4580. The strains producing CTX-M-14, CTX-M-55, and CMY-2 enzymes belonged to ST88, ST2952 and ST58, respectively. The upregulated AmpC's belonged to 13 STs, where ST23 (n = 17) and ST88 (n = 12) (Figure 2) constituted the majority.

The ESBL/AmpC-producing *E. coli* strain isolated from imported pork harboured the CTX-M-15-encoding gene, whereas the isolates from domestically produced pork harboured genes encoding CTX-M-1 (n = 2) and CTX-M-15 (n = 1) and exhibited mutations leading to upregulation of chromosomal *ampC* (n = 2, Table 1). Monitoring of domestically produced and imported pork has been performed since 2009 and in all years ESBL/AmpC-producing *E. coli* was observed in less than 5% of the samples, mainly in association with CTX-M-1 enzymes (web annex, Table A7.8)

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

Among the 14 sequenced ESBL/AmpC-producing *E. coli* isolates from cattle, three ESBL types were detected, and each strain produced one enzyme only. Most of the strains (n = 8) displayed an upregulation of chromosomal *ampC* and belonged to five STs: ST441 (n = 3), ST392 (n = 2), ST155, ST56, and ST1611 (Table 1, Figure 2). The remaining six strains displayed CTX-M-1 (n = 3)-, CTX-M-14 (n = 1)-, and TEM-52B (n = 2)-encoding genes. The three strains harbouring the CTX-M-1-encoding gene belonged to three MLSTs: ST58, ST362, and ST647. The two TEM-52B-producing strains belonged to ST10 (Figure 2).

ESBL/AmpC-producing *E. coli* from domestically produced and imported broiler meat

In 2015, whole genome sequencing was only performed on a selection of the presumptive ESBL/AmpC isolates from domestic-produced broiler meat. A total of 28 out of 61 isolates were sequenced. Among these isolates, four ESBL/AmpC types were detected and two isolates produced two enzymes simultaneously (Table 1). A total of 23 out of 125 isolates from imported broiler meat were sequenced. Six ESBL/AmpC variants were detected, and each strain produced one enzyme only. Therefore, proportions of samples containing ESBL/AmpC -producing *E. coli* could not be calculated for Figure 1.

From 2009 to 2011, a significant increase in AmpC-producing *E. coli* was observed in the domestic-produced broiler meat (Figure 1). However, after discontinuation of the use of 3rd generation cephalosporins in grandparent animals exported to the Danish broiler production, the occurrence of AmpC-producing *E. coli* in broiler meat significantly decreased [DANMAP 2014]. As in 2014, the ESBL/AmpC phenotype in isolates from domestic-produced broiler meat was mainly linked to CTX-M-1 (n = 21, Table 1) and, in only a few isolates, to CMY-2 (n = 6).

The CTX-M-1-encoding gene was linked to 10 STs; ST10, ST88 (n = 5), ST752 (n = 5), ST162, ST295 (n = 2), ST770, ST1147, ST1640, ST4980 (n = 3), and an unknown ST. CMY-2-encoding genes and upregulation of chromosomal *ampC* expression were observed in strains belonging to ST38, ST101, ST154, ST2309 and unknown STs (Figure 2). The CARB-2-encoding gene was detected in three isolates from domestic-produced broiler meat belonging to ST101. In 2014, most of the ESBL/AmpC -producing *E. coli* from domestically produced broiler meat belonged to ST752 (n=7) and ST181 (n=3), and mainly harboured the CTX-M-1-producing gene [DANMAP 2014].

CTX-M-1-encoding gene was detected in 43% (n = 10) of the ESBL/AmpC -producing *E. coli* from imported broiler meat, and all these strains exhibited different MLSTs except for two strains belonging to ST752. The remaining MLSTs were: ST117, ST602, ST1431, ST1640, ST2070, ST2197, ST4243, and one unknown ST (Figure 2). Interestingly, ST752 was also represented among strains harbouring the CTX-M-1-encoding gene from Danish broiler meat. Four MLSTs were associated with the remaining ESBLs detected in imported broiler meat: ST115 (SHV-12), ST69 (SHV-2), ST2607 (TEM-52B), and ST10 (TEM-84) (Table 1). The nine AmpC-producing strains from imported broiler meat (all CMY-2 producers) belonged to seven STs; ST23, ST117, ST131 (n = 2), ST354 (n = 2), ST355, ST665, and ST2040 (Figure 2). In 2014, ESBL/AmpC-producing *E. coli* from imported broiler meat also belonged to a multitude of different STs, mainly associated to genes encoding CTX-M-1 and CMY-2 enzymes [DANMAP 2014].

continued ... Textbox 7.1

Conclusion: In 2015, changes in the methodology for detection of ESBL/AmpC -producing *E. coli* were enforced by EU to enhance the sensitivity of the detection method. In the previous studies, ESBL/AmpC-producing *E. coli* were recovered from 1 g caecal and 5 g meat after selective enrichment in MacConkey broth with ceftriaxone (1 µg/ml). Due to the change in methodology, comparing results over years to assess temporal trends should be done with great caution. Unfortunately, no comparative evaluation between past and present methodologies was conducted to assess the effect inferred by the change in methodologies.

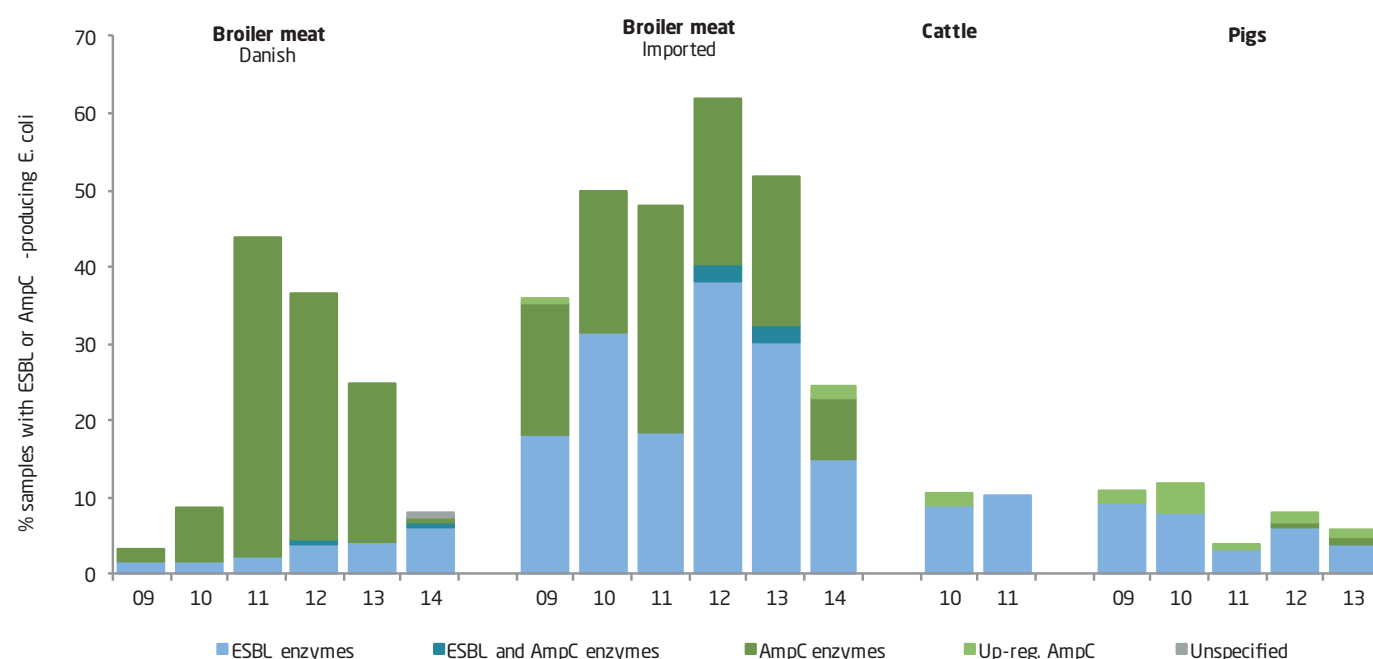
No of carbapenem-resistant bacteria was observed in the samples from meat or food-producing animals, and the overall proportions of the samples positive for ESBL / AmpC were similar to the levels detected in previous years from all sources - except for pigs.

The observed level of ESBL/AmpC -producing *E. coli* in pigs, however, was higher than in previous years. The increase was linked to high occurrence of *E. coli* with upregulation of chromosomal *ampC* expression not previously observed among pigs. This increase might have been caused by the changes in methodology enforced by EU to enhance the sensitivity of the detection method. Theoretically, increased consumption of antimicrobials may also contribute to an increased occurrence of AmpC-producing *E. coli* among Danish pigs. However, this does not seem to be the case, since the total antimicrobial use for weaners and finishers has declined over the last five years, and consumption of fluoroquinolones and cephalosporins, described as a risk factor for carriage of ESBL/AmpC -producing *E. coli* in humans, has been very low for more than a decade.

Similar to previous years, ESBL/AmpC -producing *E. coli* was only isolated from a few samples of domestically produced and imported pork and beef. The occurrence of ESBL/AmpC -producing *E. coli* from cattle was also relatively low. Only a subset of presumptive ESBL/AmpC-producing isolates from domestically produced and imported broiler meat was investigated (by WGS) in 2015, thus calculation of distribution of ESBL/AmpC types among samples cannot be compared with previous DANMAP iterations. Nevertheless, CTX-M-1 was by far the most common ESBL enzyme in *E. coli* from Danish broiler meat, and only a few isolates produced CMY-2.

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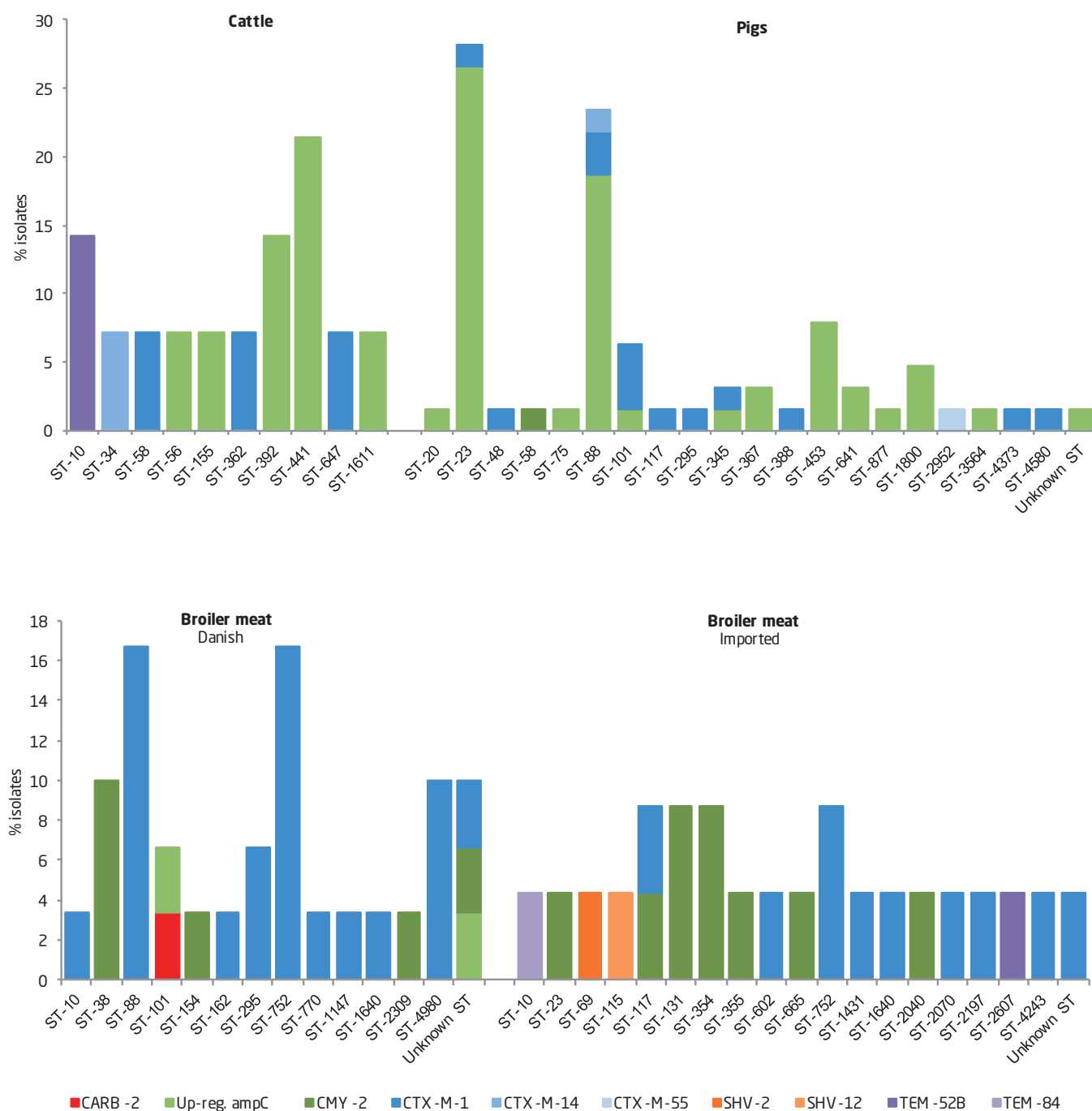
Figure 1. Occurrence (%) of samples with *Escherichia coli* from broiler meat, cattle and pigs containing ESBL and AmpC enzymes, Denmark DANMAP 2015



Note: Isolates recovered by the selective enrichment methods (See previous DANMAP reports). Distribution is based on WGS data, and faecal samples were collected from pigs and cattle between 2009 and 2013. During the period 2009-2014, *E. coli* isolates containing ESBL or AmpC enzymes were recovered from none or less than 2% of the samples of beef (ESBL enzymes, n=7) and pork (ESBL enzymes, n=23; AmpC enzymes, n=2). Data from 2015 is not included in the figure, as the selective enrichment methods was changed [EURL-AR laboratory protocol, October 2015]

Figure 2. Distribution (%) of ESBL, AmpC and CPE enzymes in *Escherichia coli* MLST types in isolates from pigs, cattle and broiler meat, Denmark

DANMAP 2015



Note: Isolates recovered by the selective enrichment methods described in the EURL-AR laboratory protocol (October 2015). Whole genome sequencing was applied ESBL/AmpC suspect isolates from cattle (14 isolates), pigs (63 isolates, recovering 64 enzymes), Danish broiler meat (28 isolates, recovering 30 enzymes) and imported broiler meat (23 isolates)

Textbox7.2

Indicator bacteria from humans - historical review

Escherichia coli as indicator of resistance in humans

In some of the years since the DANMAP programme was established, indicator *E. coli* were collected from either rectal swabs or faecal samples from different groups of people, theoretically exposed to different resistant bacteria through their work, or as representatives of the "normal" healthy Danish population. In 1997, 50 isolates from 60 pig producers, 41 isolates from 58 abattoir workers, 50 isolates from 57 nurses and 88 isolates from 112 army recruits were susceptibility tested. In 2008, again a collection of 75 *E. coli* isolates from 84 army recruits was tested. In the five-year period from 2002 to 2006, a surveillance of stool samples from healthy human volunteers in the community was conducted (NorMat). Subjects selected for participation were found through the Danish Civil Register system (CPR) with a selection algorithm based on the age and gender distribution of the total Danish population (DANMAP 2002, Appendix 1). In total 48 to 111 *E. coli* isolates from 57 to 125 human volunteers were tested.

Figure 1. Resistance (%) to ampicillin, ciprofloxacin, gentamicin and 3rd generation cephalosporins in human *E. coli* clinical isolates and from the intestinal flora of different population groups

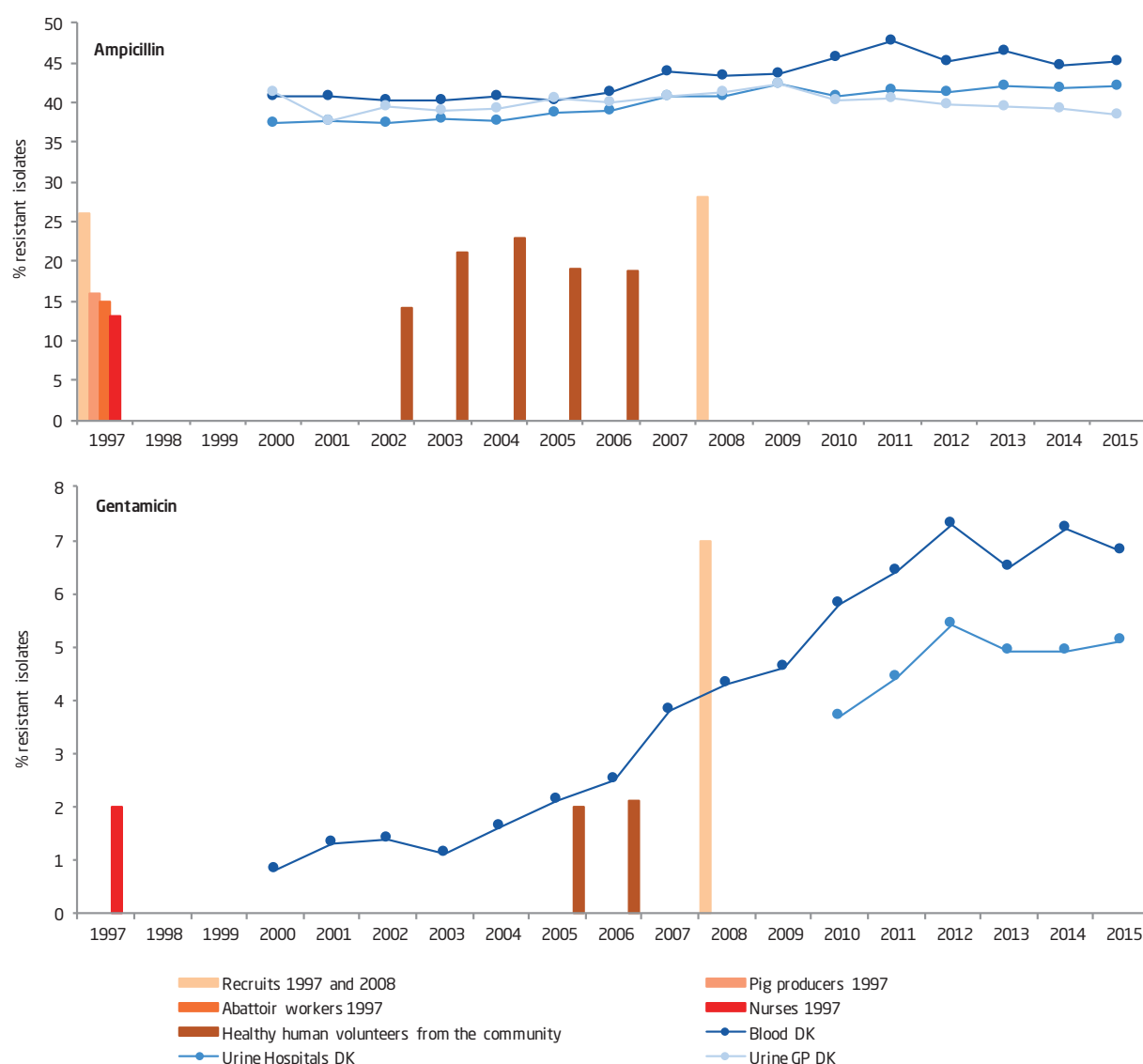
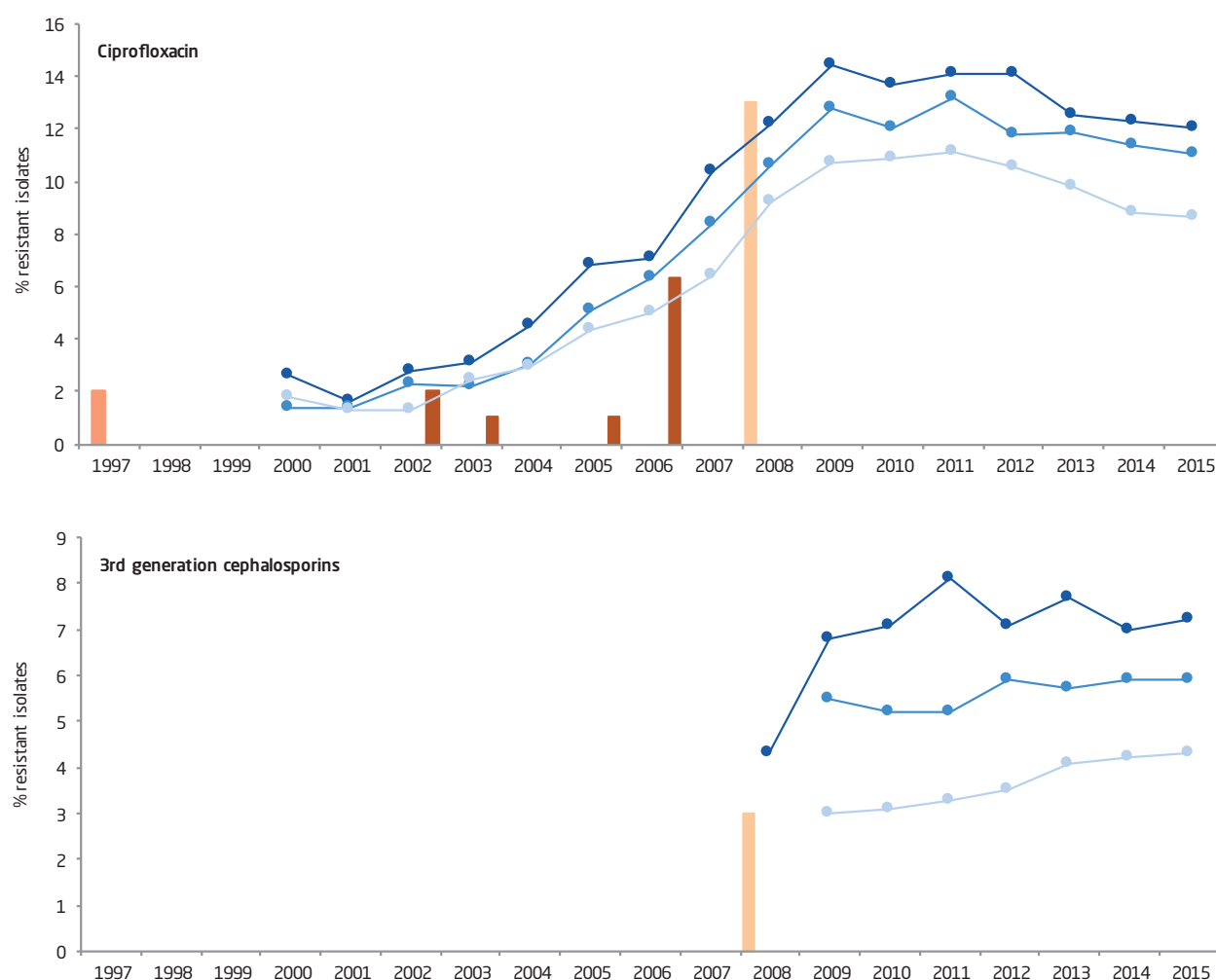


Figure 1 presents the resistance data from these indicator samplings together with the clinical *E. coli* from blood and urines. For ampicillin the resistance profile of indicator bacteria stayed well below that of the clinical isolates. This was not the case for ciprofloxacin and gentamicin. In 1997, 2% of *E. coli* from pig producers harboured ciprofloxacin resistance, while none of the *E. coli* isolates from abattoir workers, nurses or army recruits were ciprofloxacin resistant. Also, the clinical *E. coli* from 2000 to 2003 and the *E. coli* from healthy human volunteers in the community in 2002 to 2005 harboured low or no ciprofloxacin resistance, but in both groups of isolates there was an increase to 5-7% resistance in 2006. In 2008 army recruits posed 13% ciprofloxacin resistance, which was in the high end but similar to the prevalence in clinical isolates. For gentamicin the same applies as for ciprofloxacin. It is surprising though, that *E. coli* from army recruits in 2008 had so relatively high gentamicin resistance. Reporting of resistance to 3rd generation cephalosporin started in 2008/2009 for clinical *E. coli* isolates, where it was around 4%. For *E. coli* isolated from healthy human volunteers in the community, 3rd generation cephalosporin resistance was reported in the five-year period from 2002 to 2006 and there were none resistant isolates, while *E. coli* isolates from army recruits in 2008 harboured 3% 3rd generation cephalosporin resistance.

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Note: Ciprofloxacin resistance was not observed in *E. coli* from recruits, abattoir workers and nurses in 1997 or in *E. coli* from healthy human volunteers from the community in 2004. Gentamicin resistance was not observed in *E. coli* from recruits, abattoir workers and pig producers in 1997 or in *E. coli* from healthy human volunteers from the community in 2002, 2003 and 2004. None of the *E. coli* from healthy volunteers (2002-2006) were resistant to 3rd generation cephalosporins. *E. coli* from recruits, pig producers, abattoir workers and nurses were not tested towards 3rd generation cephalosporin susceptibility in 1997

Textbox 7.3

Comparison of ESBL/AmpC-producing *Escherichia coli* isolates from Danish and imported meat with *E. coli* isolates obtained from human bloodstream infections

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

Materials and methods: ESBL/AmpC-producing *E. coli* isolates from meat (Textbox 7.1) obtained in 2014 and 2015 and ESBL/AmpC-producing *E. coli* isolates from human bloodstream infections (Textbox 8.1) obtained during 2015 were compared for clonal relationship by whole-genome-based SNPs analysis, if sharing the same ESBL/AmpC gene and belonging to the same sequence types (STs). The CGE web tool CSI-phylogeny version 1.3 [<https://cge.cbs.dtu.dk/services/CSIPhylogeny/>] was used to call SNPs between the isolates to infer the phylogeny [Kaas *et al.* 2014, PLoS ONE 2014; 9(8): e104984]. For each comparison a reference genome with identical STs was used.

Results: The same combinations of ESBL/AmpC genes and STs were detected from *E. coli* isolates of human origin and *E. coli* isolates from meat in three occasions; CMY-2-producing *E. coli* isolates belonging to ST38 and ST354, and CTX-M-1-producing isolates belonging to ST131. Three SNP comparisons were produced for each of the tree combinations (Table A7.9, web annex). The CMY-2-producing *E. coli* isolates belonging to ST38 represented five isolates of veterinary origin from 2015 (three isolates from Danish broiler meat and two from imported duck meat) and one human bloodstream isolate from 2015 (Figure ST38 CMY-2, Table A7.9, web annex). More than 6,000 SNPs were detected between the *E. coli* isolate of human origin and *E. coli* isolates from meat.

The CMY-2-producing *E. coli* isolates belonging ST354 encompassed one imported broiler meat *E. coli* isolate from 2014, two imported broiler meat *E. coli* isolates from 2015 and one human bloodstream *E. coli* isolate from 2015 (Figure ST354 CMY-2, Table A7.9, web annex). More than 2,680 SNPs were detected between the human isolate and the broiler meat isolates. One ST131/CTX-M-1-producing *E. coli* isolate from Danish broiler meat from 2015 was compared with three ST131/CTX-M-1-producing *E. coli* isolates from human bloodstream infections (Figure ST131 CTX-M-1, Table A7.9, web annex). More than 1,640 SNPs were detected between the broiler isolate and the human isolates.

Discussion and conclusion: CTX-M-15, CTX-M-14, CTX-M-27 were the most frequently detected enzymes among the ESBL/AmpC-producing *E. coli* isolates from human bloodstream infections in 2015, whereas CTX-M-1 and CMY-2-variants were detected to a lesser extent (Textbox 8.1). None of the *E. coli* isolates from Danish and from imported meat contained any known carbapenemase gene. Therefore, we conclude that presently fresh meat available on the Danish market seems to be a minor source of the ESBL/AmpC-producing bacteria causing bloodstream infections in humans, and not a source of carbapenemase-producing bacteria causing human infections in Denmark.

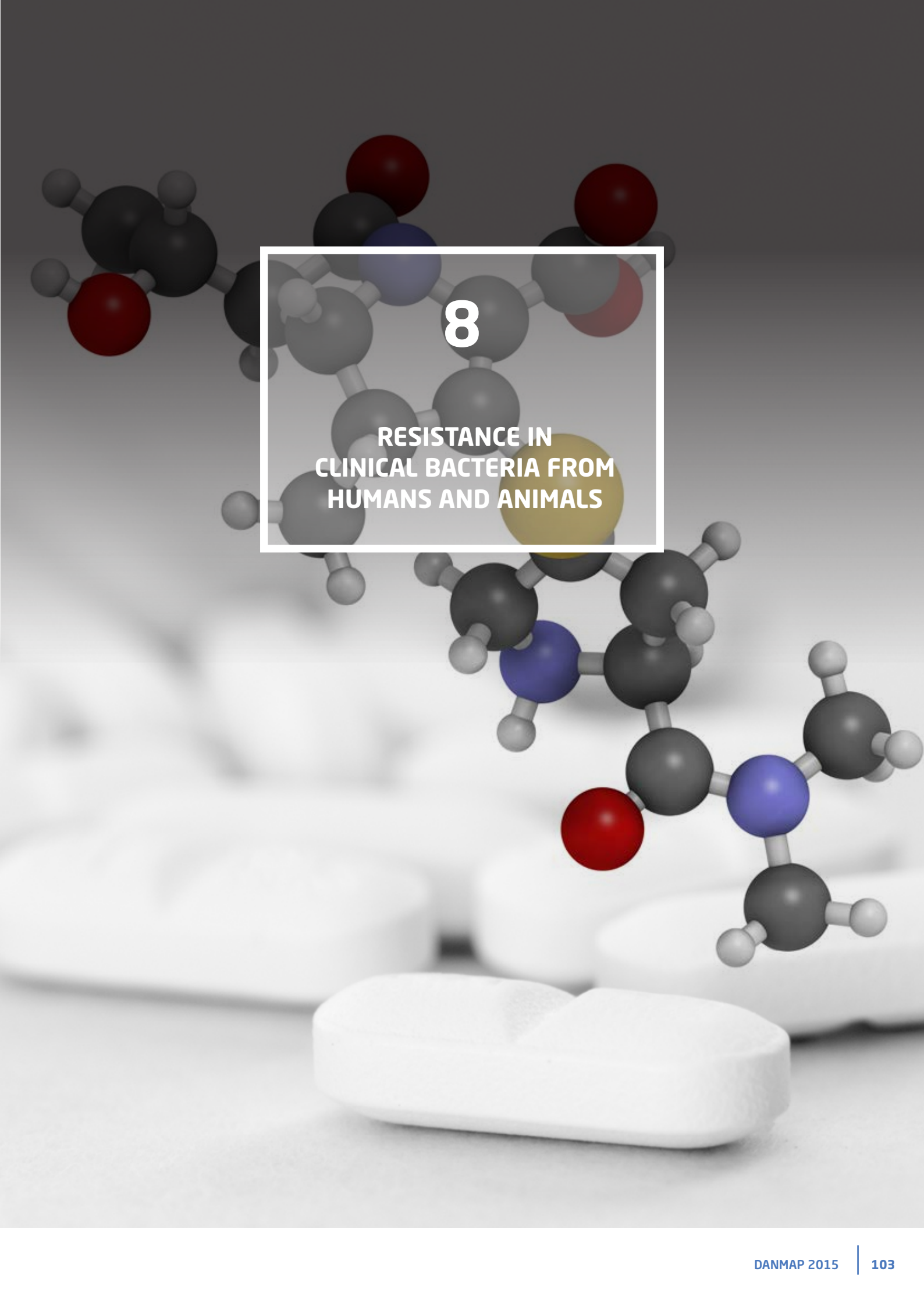
In 2015, similar SNP profiles were not detected between ESBL/AmpC-producing *E. coli* from human bloodstream infections and ESBL/AmpC-producing *E. coli* isolates from meat. From this limited dataset, we did not detect any indication of zoonotic clonal spread of ESBL/AmpC-producing *E. coli*.

Besides clonal spread, genes encoding ESBL/AmpC enzymes can spread among bacteria of animal and of human origin by horizontal plasmid transfer. Plasmid comparison was not performed in the present study. However, similar plasmids with ESBL/AmpC-encoding genes have been detected from broilers and humans in Denmark, in Sweden and in the Netherlands. [de Been *et al.* 2014 PLOS Genetics 10:12; Börjesson *et al.* 2016 Emerging Infectious Diseases 22:634; Hartung *et al.* 2016 Applied and Environmental Microbiology 82:4705]. Further monitoring, which also could include ESBL/AmpC-producing *E. coli* isolates from urinary tract infections, and larger studies are needed to investigate and quantify the possible zoonotic link between ESBL/AmpC- and carbapenemase-producing *E. coli* from meat/animals and human severe infections.

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8

RESISTANCE IN CLINICAL BACTERIA FROM HUMANS AND ANIMALS

8. Resistance in clinical bacteria from humans and animals



Highlights: DANMAP 2015 is the first DANMAP report with data on resistance in blood isolates referred from all Danish DCM, thereby covering all of the Danish population.

E. coli from bloodstream infections is the only clinically important human bacteria, where resistance has been continuously reported through all 20 years. In 2015, no significant changes in resistance were reported for *E. coli* isolates obtained from bloodstream infections: resistance to ciprofloxacin was 12%, cefuroxime 9%, 3rd generation cephalosporins 7 % and gentamicin 9%, respectively. One carbapenemase-resistant and four intermediary resistant *E. coli* isolates were reported. During the last decade steady increases in resistance occurred for *E. coli* from bloodstream infections, most pronounced for 2006 to 2011, since levelling off. Over the last twenty years the resistance to ampicillin increased from 35% to 45% for *E. coli*, also here, the most pronounced changes happened from 2006 to 2011.

During the last twenty years, the resistance levels have continuously increased for *E. coli* obtained from urinary tract infections from hospitalized patients. Whereas decreases for most antimicrobial classes were observed for *E. coli* isolates obtained from patients with urinary tract infections from primary healthcare.

For ***K. pneumoniae*** isolates obtained from bloodstream infections in 2015, gentamicin resistance decreased to 2.5% (4.6% in 2014). Resistance to most antimicrobials has steadily decreased since 2009. In 2015, 11% of the *K. pneumoniae* isolates were resistant to cefuroxime, 8% to 3rd generation cephalosporins and 6% to ciprofloxacin. The only antimicrobial with increasing resistance since registration was begun in 2009 was piperacillin-tazobactam. In 2015, 8% of the *K. pneumoniae* isolates from blood were resistant to piperacillin-tazobactam. None carbapenem-resistant and two intermediary resistant *K. pneumoniae* isolates were reported in 2015.

For *K. pneumoniae* isolates obtained from urins from patients with urinary tract infections resistance to ciprofloxacin decreased from 8% to 6% in urinary isolates from hospitals and from 7% to 5% in urinary isolates from primary health care.

In 2015, whole-genome sequencing was performed on 275 ESBL- and/or carbapenemase-producing *E. coli* isolates from blood and on additional 59 carbapenemase-producing Enterobacteriaceae (CPE), 19 carbapenemase-producing *A. baumannii* and nine carbapenemase-producing *Pseudomonas* spp. (Textboxes 8.1 and 8.2). The occurrence of these carbapenemase-producing bacteria in Denmark is increasing, a trend worrisome to patients and clinicians. Especially the spread of CPE worries due to their ability to be carried in the gut for a long time.

Staphylococcus aureus bacteremia's accounted for 1,973 cases, the third year in a row where the number of cases was higher than the previous year. Twenty-nine (1.5%) of these were caused by MRSA and three of them by MRSA CC398. The mortality was 23% for the methicillin susceptible cases and 24% for the cases with MRSA. Resistance towards erythromycin, clindamycin, fusidic acid and norfloxacin has been steadily increasing in the last decade. In 2015 it was 7%, 7%, 16% and 6%, respectively. For the first time in recent years, the number of new MRSA cases (colonized or infected patients) stabilized with 2,971 cases compared to 2,965 cases in 2014.

In 2015, a further increase of vancomycin-resistant enterococci in the Capital Region and the Region of Zealand was noted, reaching a total of 372 clinical isolates, through typing characterized as belonging primarily to sequence types (ST), ST80, ST117 and ST203, (Textbox 8.3).

2015 was also the year with the highest incidence of drug-resistant *Mycobacterium tuberculosis*, with five multidrug-resistant tuberculosis (TB) cases and the second case with extensively-drug-resistant TB ever noticed in the country. Even though the global incidence of TB is declining, the emergence of drug-resistant strains is challenging, also in Denmark (Textbox 8.6).

8.1 *Escherichia coli*

Escherichia coli is part of the normal intestinal flora in both humans and animals but also one of the most common causes of infections. In humans, *E. coli* is the most frequent cause of community- and hospital-acquired urinary tract infections and of bacteremia. *E. coli* also accounts for the majority of travel-related diarrhea. It further occasionally causes meningitis in newborns.

For *E. coli*, DANMAP 2015 includes data on resistance referred from all 11 Departments of Clinical Microbiology (DCM), thus for the first time covering the total Danish population. These data cover all blood and urinary isolates from hospitals and a selected number of urines from primary health care.

Blood isolates from hospital patients

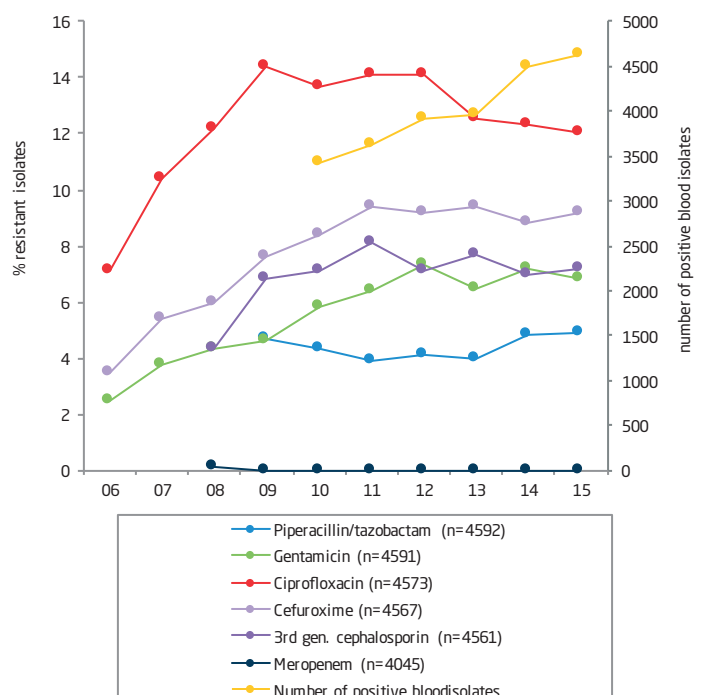
DANMAP received data on the antimicrobial susceptibility of 4,618 *E. coli* isolates from blood. As in previous years resistance testing was primarily performed by disc diffusion and the referred data consisted of interpretation of resistance results based on the S-I-R system.

A minor increase in the total number of *E. coli* isolates from blood was observed from 2014 to 2015 (Figure 8.1). The increase corresponds to the increase in contribution of the last DCM to DANMAP 2015. The increasing tendency of the total number of *E. coli* isolates from blood, registered since 2010 and commented in DANMAP 2014, did not continue in 2015. Still an increase in the number of bacteremias might continue to some extent in the future caused by demographic changes with an ageing population, since the elderly are at the highest risk of bacteremia and have the highest incidence. Increasing trends in the rates of bacteremias seem not only to resemble demographic changes but to be a combination of several factors like advances in health care with improved treatment options as well as lowered thresholds for taking blood cultures and improvement in blood culturing systems.

No significant changes in occurrence of antimicrobial resistance were observed in 2015 compared to 2014 (Figure 8.1 and Table 8.1) For interpretation of mecillinam resistance testing results, more restrictive breakpoints than EUCAST breakpoints are used for blood isolates by some DCM and interpretation rules have changed over time as discussed in DANMAP 2014. This makes comparison of results from resistance testing for mecillinam over time not valid. Therefore in Table 8.1 the number is presented, but without any comparison to former years. If referral of inhibition zone diameters is to be used in

Figure 8.1. Resistance (%) in *Escherichia coli* blood isolates from humans, Denmark

DANMAP 2015



Note: The number (n) in parentheses represents the number of isolates tested for susceptibility in 2015

Table 8.1. Resistance (%) in *Escherichia coli* isolates from humans, Denmark

DANMAP 2015

Substance	Blood isolates, hospitals %	Urine isolates, hospitals %	Urine isolates, primary health care %
Ampicillin	45	42	39 [#]
Mecillinam	10 ^{b)}	8 *	5
Piperacillin/tazobactam	5	4 *	4
Sulfonamide		32	31 [#]
Gentamicin	7	5	4
Ciprofloxacin	12	11	9
Cefuroxime	9	7 *	5
3rd generation cephalosporins ^{a)}	7	6	4
Meropenem	<1	<1	<1
Max. number of isolates tested	4595	46572	61047

*) An asterisk indicates a significant increase from 2014 to 2015

#) A number sign indicates a significant decrease from 2014 to 2015

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

b) Mecillinam resistance from blood isolates is reported without data from Skejby as in 2014 but no significance testing have been done

the years to come, a more stringent surveillance will be possible, independent of local interpreting rules that make sense in the clinical situation for treatment of a specific infection but are not harmonized for national surveillance purposes.

One carbapenem (meropenem) resistant and five intermediary resistant *E. coli* blood isolates were reported in 2015. In 2014, there were none carbapenem resistant *E. coli* blood isolates. This might reflect the general and worrisome increase in carbapenem resistant enterobacteriaceae observed in 2015 commented in textbox 8.2.

In the 10-year period from 2006 to 2015, resistance in *E. coli* blood isolates has altogether increased steadily, the increase being most pronounced from 2006 to 2011, since showing a slight stagnation for most antibiotics. For ciprofloxacin a decrease is observed from its peak of 14% resistance in 2009 to 2012, to 12% resistance in 2015, but still far from the 7% resistance in 2006. This development parallels the trends in total antimicrobial consumption, which showed pronounced increase until 2010 with a peak of 19.25 DID and since a slow decrease to a total of 18.50 DID in 2015 giving a drop in total consumption of 0.75 DID (Figure 5.1). Resistance to piperacillin/tazobactam has been surveilled since 2009. In this 7-year period a rather constant resistance profile has been observed, with 4.7% resistance in 2009, dropping to 4.0% resistance in 2010 to 2013 and then moving back up to 5 % resistance in 2014 and 2015. The trend for piperacillin/tazobactam is observed closely since changes in the consumption owing to a shift from cefuroxime as most used antibiotic in the treatment of *E.coli* infections and sepsis in hospitalized patients to piperacillin/tazobactam have resulted in an increase of the consumption of piperacillin/tazobactam in Danish hospitals from 2008 and onward (Figure 5.11).

Urine isolates from hospital patients

DANMAP received data on 46,723 *E. coli* isolates from urine in hospitalized patients.

Not all DCM tested for all antibiotics reported here. (Table 8.1 and Figure 8.2) For at least 82% of isolates all 11 DCM reported data on mecillinam and ciprofloxacin susceptibility, while 10 DCM reported data on ampicillin, gentamycin and cefuroxime susceptibility. Data on piperacillin/tazobactam, 3rd generation cephalosporins and meropenem susceptibility was reported by 8 DCM and sulfamethizole susceptibility was reported by 7 DCM.

There was a slight but significant increase in resistance to mecillinam, piperacillin/tazobactam and cefuroxime from 7.2% to 7.7%, 3.5% to 3.9% and 6.6% to 7.0% respectively compared to 2014.

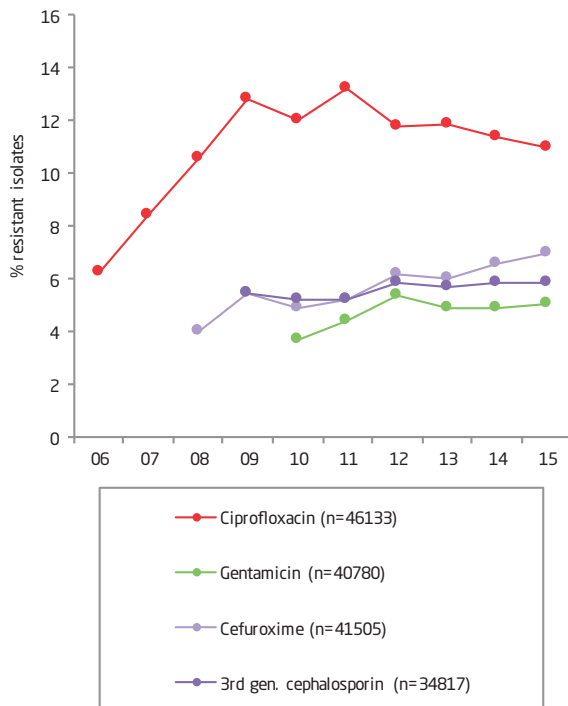
In 2015, three carbapenem (meropenem) resistant and 11 intermediary resistant *E. coli* isolates from hospital urine were reported. For comparison, there was none meropenem resistant and one meropenem intermediary resistant *E. coli* isolate from hospital urine in 2014. As already mentioned there was a worrisome increase in carbapenem resistance in general among Gram negative pathogens in 2015, which is addressed further in textbox 8.2.

Urine isolates from primary health care

DANMAP received data on 61,073 *E. coli* isolates from urine from patients in primary health care.

In Denmark culturing and susceptibility testing of urines from primary health care varies substantially between the different regions. Many GP's perform local testing and thus primarily urines from patients with known resistance problems are sent to the DCM at the regional hospital. Other GP's use their local

Figure 8.2. Resistance (%) in *Escherichia coli* urine isolates from humans in hospitals, Denmark DANMAP 2015



Note: The number (n) in parentheses represents the number of isolates tested for susceptibility in 2015

DCM more extensively and frequently. The amount of culturing and susceptibility testing performed at the different DCM will vary accordingly. All DCM reported data on mecillinam susceptibility and almost all reported data on ampicillin, ciprofloxacin and 3rd generation cephalosporin susceptibility, while 7 DCM reported data on sulfamethizole, 6 reported data on cefuroxime, 5 reported data on gentamycin and meropenem susceptibility and 4 DCM reported data on piperacillin/tazobactam susceptibility.

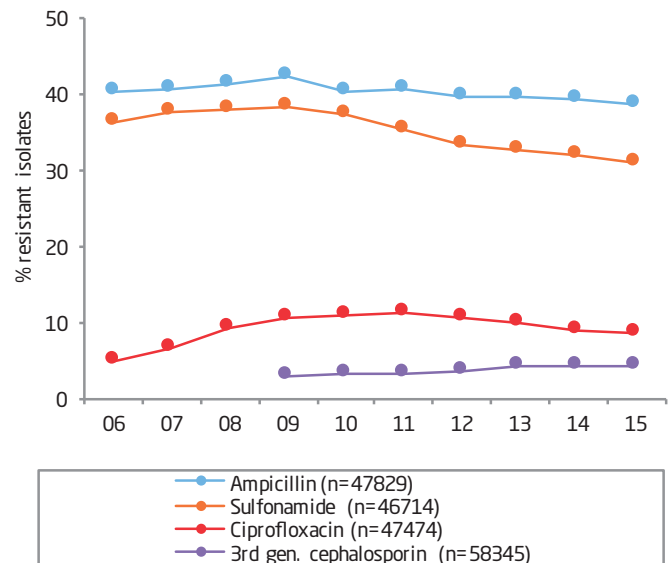
In 2015 there was a small decrease in ampicillin and sulfonamide resistance compared to 2014. Otherwise no significant changes when comparing data from 2015 to 2014 were found. (Table 8.1 and Figure 8.3)

In 2015, four carbapenem (meropenem) resistant and nine intermediary resistant isolates were reported from *E. coli* urine from primary health care. In 2014 those numbers were two and one, respectively. Routine meropenem testing was performed at five DCM in 2015.

20 years of *E. coli* surveillance

The surveillance of *E. coli* has been part of all 20 years in DANMAP's history and thus will be covered more extensively in this chapter. In the beginning, invasive *E. coli* infections were reported by two DCM: SSI and Aarhus. These two laboratories covered 16% of the Danish population. The surveillance expanded during the following 9 years and from 2004 until

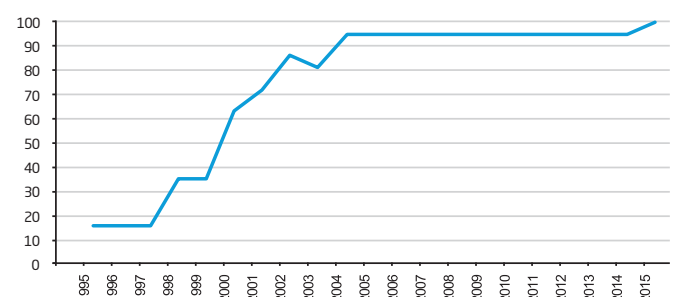
Figure 8.3. Resistance (%) in *Escherichia coli* urine isolates from humans in primary health care, Denmark DANMAP 2015



Note: The number (n) in parentheses represents the number of isolates tested for susceptibility in 2015

2014 all DCM, except one, participated. DANMAP 2015 is the first DANMAP report with data from all Danish DCM, thereby covering 100% of the Danish population (Figure 8.4). During the first years of DANMAP, it was essential to take precautions in the comparison of data from year to year and from reporting department to reporting department as well as it was necessary to generalize some of the observations. This was because of quite different and changing methods in the susceptibility testing and because of the rather small number of participating DCMs. From 2011 on, all DCMs except one agreed upon using the same method as a standard in the susceptibility testing following EUCAST principles and standards. From November 2015 all Danish DCM followed EUCAST principles.

Figure 8.4 Surveillance of invasive *E. coli* - percentage of the Danish population covered DANMAP 2015



In general, the data reported from clinical human samples have become increasingly consistent and adequate during the years.

Still carefulness in interpretation and comparison of data is important. For the year 2015, the DCMs reported zone diameters as well as interpretations of antimicrobial resistance which made it possible to compare local interpretations with EUCAST interpretational breakpoints. From the differences in these, it is clear, that different rules for reporting results of the susceptibility of the isolate to the clinicians still exist. For the antibiotics mostly affected, this is commented below in the relevant sections. In addition, differences between patient populations covered by the different DCMs can influence the comparability of resistance prevalence in-between DCMs. Furthermore, for each pathogen, DANMAP counts resistance data on the first isolate per patient per year per DCM. Thereby transportation of chronically ill patients in-between hospitals or regions can influence the results, since the patient might be counted twice. It seems more appropriate instead to compare clinical departments of the same medical specialty, for example hematology, across the DCMs, which we foresee could be done more readily in the future with databases like the MiBa II and eRES (see the jubilee chapter for more information).

When looking at invasive *E. coli* during all 20 years of reporting (Figure 8.5) data stem from four DCM covering 1995 to 1999. For two of the DCMs data were not included in the first DANMAP reports but those data were included later. From 2000 and onwards data covered at least 63% of the Danish population, and since then an average value of resistance for Denmark is presented.

The trend for ampicillin resistance is a crawling increase over the 20 years with resistance of around 35% of invasive *E. coli* in 1995 to 1998, around 40% from 2000 to 2006 and from 2007 and onwards the ampicillin resistance in *E. coli* is around 45%. The earliest EARS-Net data are from 1999 with Norway reporting 27% resistance in 584 *E. coli* isolates and Germany reporting 36% resistance in 166 *E. coli* isolates. The increasing trend has been the same in the European countries with most countries in 2014 showing 50-60% resistance and a few reaching above 70% resistance, while the Nordic countries stay below 45% [EARS-Net 2014]. In this 20 year period the consumption of penicillins with extended spectrum in total increased significantly in Denmark mainly driven by a steep increase in the consumption of (piv)mecillinam, while the consumption of (piv)ampicillin decreased both in primary care and in the hospital sector. Thus there is no direct link between the occurrence of ampicillin resistance and the consumption of the drug class.

For ciprofloxacin resistance, a steep increase in resistance was observed, from 2-3 % around year 2000 to 14% in the years 2009 to 2012. For the last three years, a small decrease was observed, with 12% resistance in 2015 (Figure 8.6). The same pattern was observed for *E. coli* isolated from urine both from hospitalized patients and from the primary health care sector. Ciprofloxacin was appointed special focus in Denmark's action plan on antimicrobial resistance in 2010 and one of the three antimicrobials with recommendations on restricted use by the Danish Health Authorities in 2012 showing a slight decrease in consumption observed since 2011 (Figure 5.2).

Figure 8.5. Resistance (%) in *Escherichia coli* blood isolates from humans, Denmark, 20 years of surveillance, incl. ampicillin.

DANMAP 2015

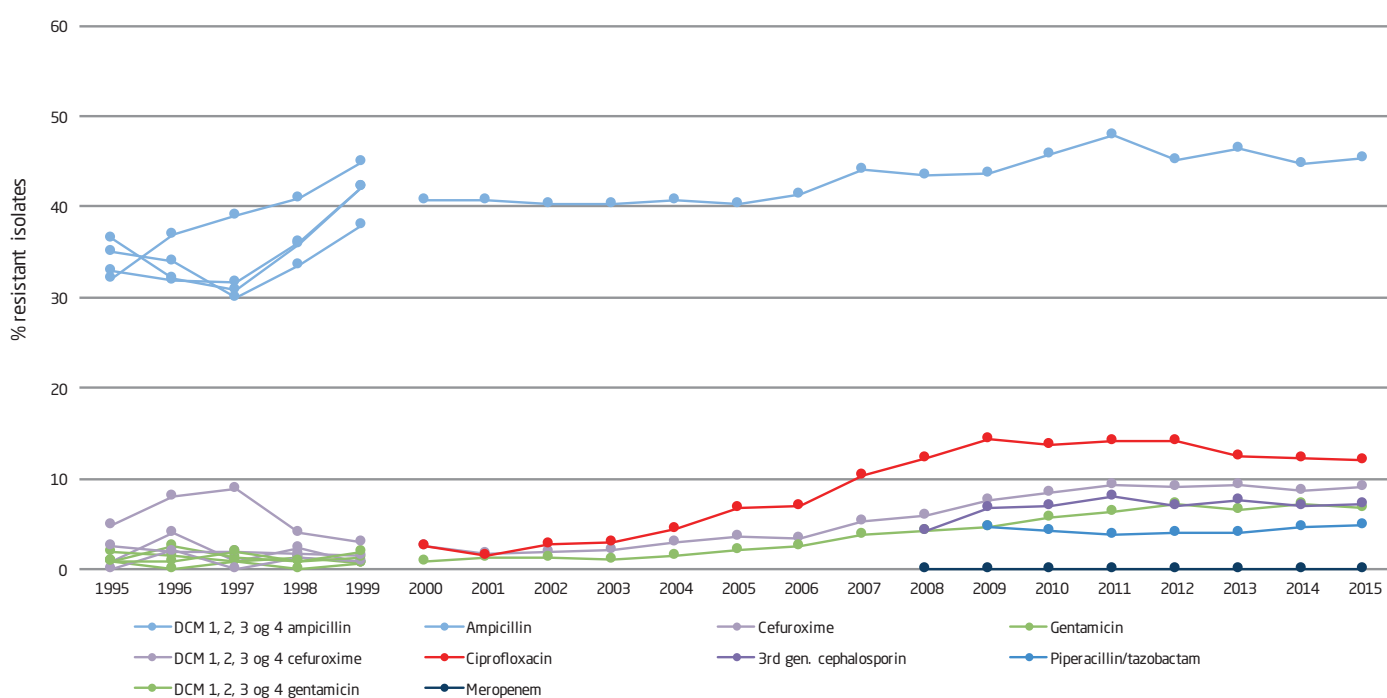
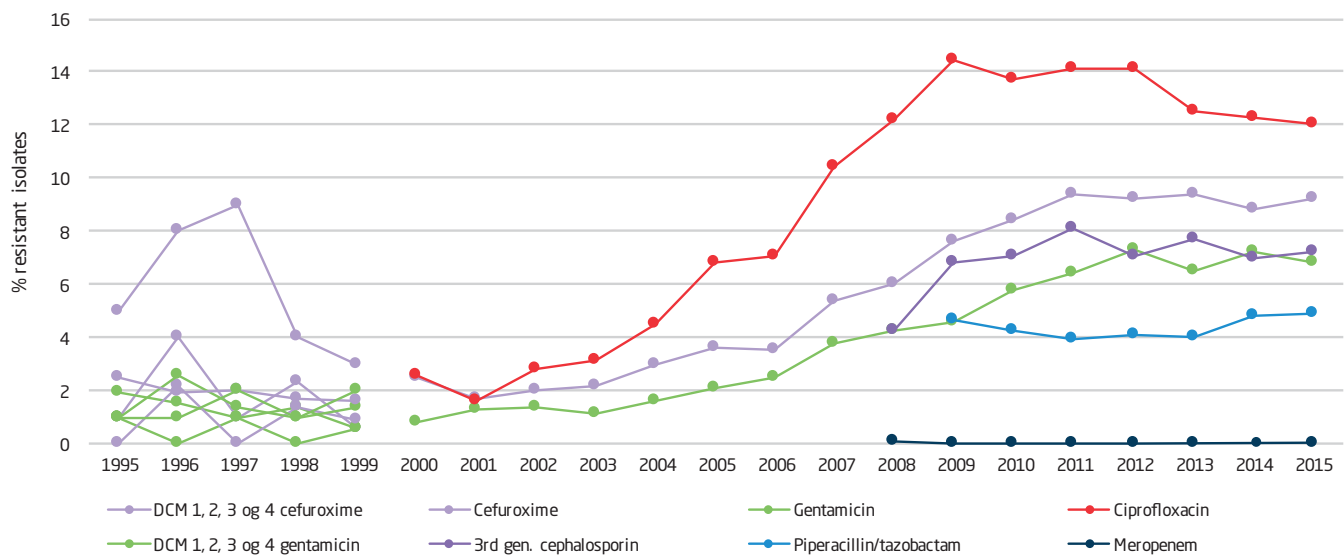


Figure 8.6 Resistance (%) in *Escherichia coli* blood isolates from humans, Denmark, 20 years of surveillance, excl. ampicillin

DANMAP 2015



Gentamycin and cefuroxime (figure 8.6) show a similar trend with an increase in resistance continuing until around 2011 and thereafter reaching a stabilization, but without a following decreasing trend in resistance as observed for ciprofloxacin resistance in *E. coli*. For cefuroxim one DCM, (Skejby), reported relatively high resistances in 1995 to 1998 with a peak of 9% resistance in 1997. Whether this had a methodologically explanation or any other explanations is yet to be elucidated.

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8.2 *Klebsiella pneumoniae*

Klebsiella pneumoniae is part of the normal intestinal flora in humans but can also be the cause of urinary tract-, respiratory tract-, and bloodstream infections. Many of these infections are hospital acquired and can become life threatening, especially if the strains are resistant to antimicrobial agents.

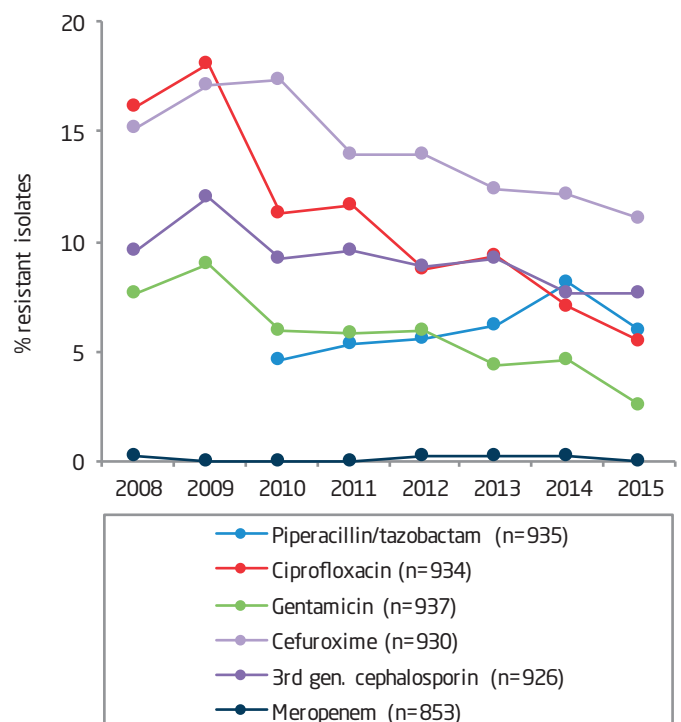
For *K. pneumoniae*, DANMAP 2015 includes data on resistance referred from all 11 Departments of Clinical Microbiology (DCM).

Blood isolates from hospital patients

Statens Serum Institut has since 2006 received data on the antimicrobial susceptibility of *K. pneumoniae* isolates from blood, and following a sudden increase in resistance (owing to an outbreak with the so called *epi-K.pn* in 2007) data were included in the DANMAP report from 2008 and onwards. In 2015, DANMAP received data on 943 *K. pneumoniae* isolates.

Figure 8.7. Resistance (%) in *Klebsiella pneumoniae* blood isolates from humans, Denmark

DANMAP 2015



Note: The number (n) in parentheses represents the number of isolates tested for susceptibility in 2015.

In general, the level of antimicrobial resistance decreased since its peak in 2009. This is probably due to clonal shifts in the infectious strains happening over the years. Piperacillin/tazobactam is the only antimicrobial with continuously slowly increasing resistance from its first registration in the DANMAP report 2010 until 2014 (Figure 8.7). This is concordant to an increased use of this agent due to a change in the Danish antibiotic policy preferring piperacillin/tazobactam for 2nd and 3rd generation cephalosporins that happened in the years 2008 and onwards and might have lessened the selection pressure from cephalosporins at Danish hospitals. In 2015 there was a non-significant decrease ($P=0,061$) in piperacillin/tazobactam resistance, from 8% in 2014 to 6% in 2015 (Table 8.2 and Figure 8.7).

The year 2015 is the first year where the DCM reported data on zone diameter (not shown) as well as interpretations of resistance. From this it became clear that rules for interpreting resistance in enterobacteriaceae are not completely the same in Denmark. Some DCM report as tested according to the measured zone diameter, others have different local (expert) rules. This might for example be inferring all ESBL producing isolates as fully or intermediary resistant towards any beta-lactams or combinations of a penicillin with a beta-lactamase inhibitor, no matter of the measured zone diameters. According to EUCAST expert rules, it should be "*reported as tested and enclosed a warning on uncertain therapeutic outcome for infections other than urinary tract infections*" (version 2.0 available from 29 Oct, 2011). These varying local interpretations developed for helping in the choice of the clinically most relevant treatment might interfere with surveillance analysis when interpreting trends for the different drugs e.g. piperacillin/tazobactam resistance. A more stringent surveillance of AMR is expected in the future with reporting of zone diameters rather than, sometimes, individual interpretations that are meaningful in treatment of the patient in a clinical situation but not in harmonized national surveillance.

The only significant change in 2015 compared to 2014 is aminoglycoside resistance, which decreased from 4.6% in 2014 to 2.5% in 2015. This decrease is not paralleled by a decrease in aminoglycoside consumption at hospitals (table 5.5).

Resistance to 2nd generation cephalosporins (cefuroxime) was 11% and resistance to 3rd generation cephalosporins was 8%. The resistance to 3rd generation cephalosporins is the highest reported level in EARS-Net among the Nordic countries (which in 2014 were reported below 6% for the other Nordic countries), but remains lower than the occurrence reported by most other European countries. [EARS-Net 2014].

In 2015, none carbapenem (meropenem) resistant and two intermediary resistant *K. pneumoniae* blood isolates were reported. These data cover the first *K. pneumoniae* isolate per patient per year and might thus be different from the number of resistant isolates referred to the reference laboratory for further resistance analysis. In 2014, 3 carbapenem (meropenem) resistant and one intermediary resistant isolates were reported. This is divergent from the general trend for 2015, where an increase in carbapenem resistance in enterobacteriaceae and non-fermenters is observed. (Textbox 8.2) The level of resistance to ciprofloxacin, which decreased insignificantly from 7.0% in 2014 to 5.5% in 2015, came to the same lower level as in the other Nordic countries. This is the first time since the reporting of resistance in *K. pneumoniae* from blood isolates began in 2008.

Urine isolates from hospital patients

DANMAP received data on 7175 *K. pneumoniae* isolated from urine in hospitalized patients.

Resistance levels to all the reported antibiotics were unchanged compared to 2014, except for ciprofloxacin where a decrease from 8% to 6% was observed. (Table 8.2 and Figure 8.8)

In 2015, carbapenem (meropenem) resistance was reported in four and intermediary resistance in seven *K. pneumoniae* urine isolates from hospitalized patients. Not all DCM performed routine susceptibility testing for meropenem, thus the number of these strains represents a selected population.

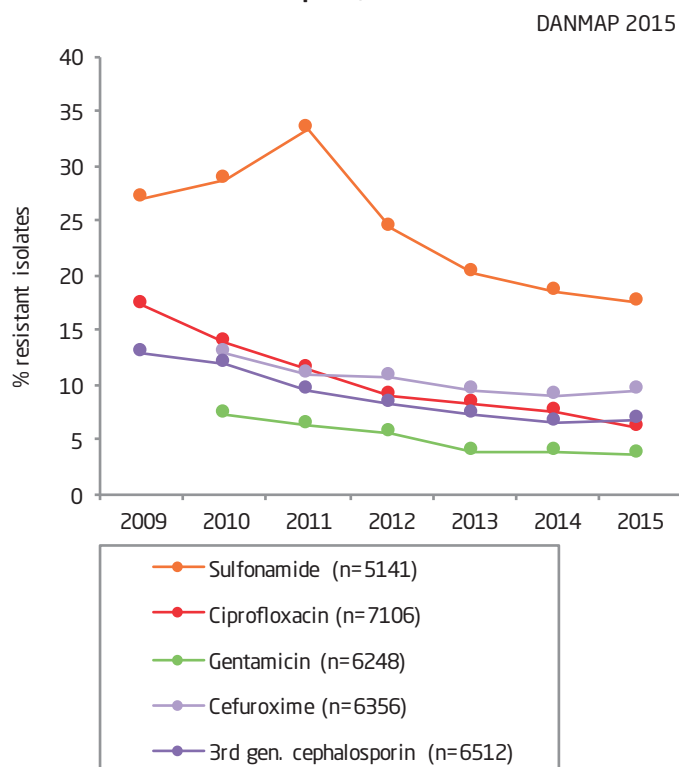
Urine isolates from primary health care

DANMAP received data on the antimicrobial susceptibility of 6372 *K. pneumoniae* isolates from urine from patients in primary health care.

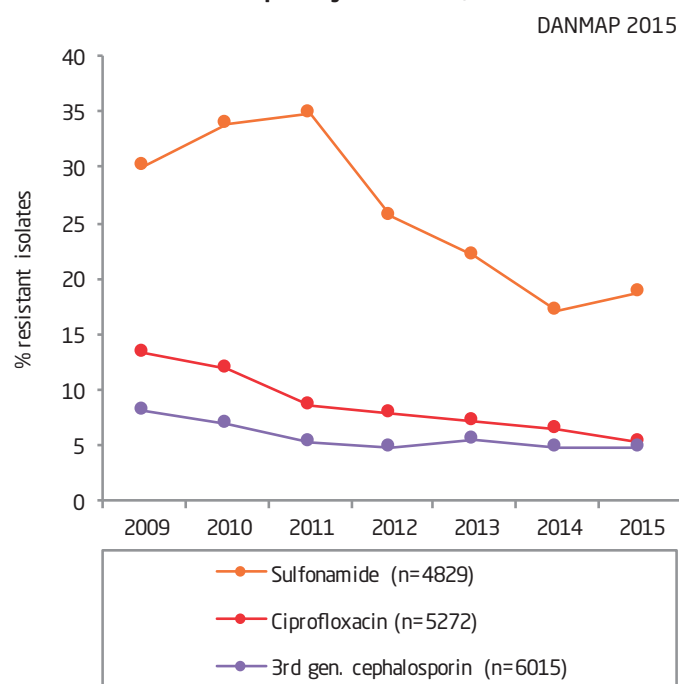
In 2015, there was a significant decrease in resistance towards ciprofloxacin from 7% to 5% compared to 2014. (Table 8.2 and Figure 8.9) The overall trend, as in *K. pneumoniae* from hospital urines and blood, the level of antibiotic resistance has been decreasing compared to 2009.

In 2015, one carbapenem (meropenem) resistant and three intermediary resistant *K. pneumoniae* urine isolates from patients in primary health care were reported. As for the hospital urine isolates, this number represents a selected population, since meropenem is only routinely tested for in urine samples from primary health care by four DCM

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Figure 8.8. Resistance (%) in *Klebsiella pneumoniae* urine isolates from humans in hospitals, Denmark

Note: The number (n) in parentheses represents the number of isolates tested for susceptibility in 2015

Figure 8.9. Resistance (%) in *Klebsiella pneumoniae* urine isolates from humans in primary health care, Denmark

Note: The number (n) in parentheses represents the number of isolates tested for susceptibility in 2015

Table 8.2. Resistance (%) in *Klebsiella pneumoniae* isolates from humans, Denmark

DANMAP 2015

Substance	Blood isolates, hospitals	Urine isolates, hospitals	Urine isolates, primary health care
	%	%	%
Mecillinam	9	10	9
Piperacillin/tazobactam	6	6	
Sulfonamide		18	19
Gentamicin	3#	4	
Ciprofloxacin	6	6#	5#
Cefuroxime	11	10	
3rd generation cephalosporins a)	8	7	5
Meropenem	<1	<1	<1
Max. number of isolates tested	937	7106	6015

*) An asterisk indicates a significant increase from 2014 to 2015

#) A number sign indicates a significant decrease from 2014 to 2015

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

Textbox 8.1

Characterization of ESBL/AmpC-producing and carbapenemase producing *Escherichia coli* from bloodstream infections, 2015

Background: Third-Generation cephalosporin-resistant *Escherichia coli* (3GC-R *Ec*) is increasing in Europe [EARS-Net report, 2014]. The extended-spectrum cephalosporin-resistance in *E. coli* can be due to production of extended-spectrum beta-lactamases (ESBLs), plasmid-mediated AmpC (pAmpCs) or constitutive overexpression of the chromosomal *ampC* gene due to mutations within the promoter/attenuator region.

The worldwide spread of ESBL-producing *E. coli* is in part due to the spread of the pandemic clone O25b-ST131. Evidently, this clone is strongly related to the presence of antimicrobial resistance genes, including CTX-M-15 and other ESBL enzymes, as well as virulence factors.

Before 2007, the occurrence of 3GC-R *Ec* was low among *E. coli* isolated from bloodstream infections in Danish patients. However, the rate of resistance among invasive *E. coli* in Denmark has increased from 2.5% in 2006 to 7.2% in 2015.

Carbapenemase producing *E. coli* are also of concern (see Textbox 8.2 Carbapenemase producing bacteria in Denmark, 2015).

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

Material and Methods: During January 2015 through December 2015, 10 out of the 11 Danish departments of clinical microbiology collected all 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. Only one isolate per patient was included in the study.

The isolates were sent to Statens Serum Institut for further characterization.

Genomic DNA was extracted from the isolates (DNeasy Blood and Tissue Kit, QIAGEN, Copenhagen, Denmark). Fragment libraries were constructed using the Nextera Kit (Illumina, Little Chesterford, United Kingdom) followed by 251-bp paired end sequencing (MiSeq, Illumina) according to the manufacturer's instructions. The paired-end Illumina data was assembled using CLC bio's Genomic Workbench 8.0.

The ResFinder web server, (www.genomicepidemiology.org, version 2.1) was used to identify acquired ESBL (including plasmid AmpCs) and carbapenemase genes from the assembled WGS data.

The MLST web server (www.genomicepidemiology.org, version 1.9) was used for detection of MLST-profiles. For isolates with no ESBL genes detected, the sequences were investigated for mutations presumed to up-regulate chromosomal AmpC by the use of CLCbio Genomic Workbench.

Results: Whole genome sequence data were obtained from 294 *E. coli* isolates. Genes encoding ESBL production (including pAmpCs) and/or carbapenemase production were detected in 275 isolates. Nineteen isolates were hyper AmpC producers only; these isolates were not investigated further.

The distribution of the 275 isolates with genes encoding ESBL and carbapenemase production in relation to the five Danish regions is shown in Table 1.

One hundred-sixty-six (61%) of the patients were men and the average age at diagnosis was 70 years (ranging from below one to 98 years).

Among the 275 isolates, 20 different ESBLs (including pAmpCs) and carbapenemases were detected (Table 2). CTX-M-15 dominated (51%) followed by CTX-M-14, and CTX-M-27 (Table 2). Different variants of CMY were detected in 16 (6%) isolates. Three isolates produced carbapenemases; one OXA-48 and two OXA-181. In several isolates more than one gene encoding ESBLs and/or carbapenemases were detected (Table 2). Additionally, the plasmid-mediated colistin resistance gene, *mcr-1*, was found in an isolate together with CMY-2 and CTX-M-55 [Hasman *et al.* Eurosurveillance 2015].

The 275 isolates belonged to 48 different MLSTs. ST131 was the most common sequence type (ST), 135 (49%) of the isolates belonged to this type. Other major types were ST38 (8%), ST405 (4%), ST410 (4%), ST69 (4%), ST648 (4%), and ST12 (3%), whereas the rest of the isolates belonged to STs, which only were detected in 1-5 isolates (<1-2% per type) (Table 3).

Among the 135 isolates belonging to ST131, CTX-M-15 (56 %) was most common, followed by CTX-M-27 (20%), CTX-M-101 (11%) and CTX-M-14 (7%). The two isolates producing OXA-181 and CMY-2 belonged to ST410, whereas the isolates producing OXA-48 belonged to ST38.

Conclusion: From 2014 to 2015, the reported cases of 3GC-R *Ec* slightly increased from 245 to 281 (261 and 294 including hyper AmpC producers). Comparing the distribution of ESBLs (including pAmpCs), carbapenemases and MLSTs, from the present study to the results reported in DANMAP 2014, 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, DANMAP 2014, Hansen *et al.* Microb. Drug Res. 2014]. Only a minor part of the isolates carried plasmid mediated AmpC (CMY variants).

As observed in DANMAP 2014 and in Hansen *et al.* the worldwide disseminated CTX-M-15 ST131 was strongly represented in this study, but the Danish *E. coli* isolates also belonged to other international STs (e.g., ST38, ST405, ST410, ST69 and ST648) related to spread of ESBLs. The finding of three *E. coli* isolates with OXA-48 group enzymes (OXA-48 and OXA-181) from bloodstream infection is of concern.

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Table 1. Distribution of ESBL and Carbapenemase producing *E. coli* from bloodstream infections, Denmark 2015 DANMAP 2015

Region	2014	2015
	Numbers	Numbers
The Capital Region of Denmark	110	116
The Zealand Region	27	14
Region of Southern Denmark	43	45
Central Denmark Region	43	59
North Denmark Region	22	41
Total Numbers	245	275

Table 2. ESBL enzymes and carbapenemases detected in *E. coli* from bloodstream infections DANMAP 2015

Enzyme	2014		2015	
	Number	%	Number	%
CTX-M-1	10	4%	7	3%
CTX-M-14 ¹	38	16%	33	12%
CTX-M-14 ^b	5	2%	5	2%
CTX-M-15 ¹	121	49%	139	51%
CTX-M-24 ¹	1	< 1%	5	2%
CTX-M-27 ¹	25	10%	33	12%
CTX-M-55 ¹	8	3%	14	5%
CTX-M-10 ¹	12	5%	15	5%
CMY-21	10	4%	6	2%
Other CMY variants ¹	4	2%	10	4%
Other ESBL enzymes	17	7%	15	5%
OXA-48-group ¹	3	1%	3	1%

¹In some isolates more than one enzyme was detected in 2015

Table 3. Distribution of MLSTs in *E. coli* from bloodstream infections DANMAP 2015

MLST	2014		2015	
	Numbers	%	Numbers	%
ST131	124	51%	135	49%
ST38	18	7%	23	8%
ST405	13	5%	12	4%
ST410	4	2%	11	4%
ST69	10	4%	10	4%
ST648	7	3%	10	4%
ST12	5	2%	9	3%
ST10	0	0%	6	2%
ST1193	2	1%	5	2%
Other STs ¹	62	25%	54	20%

¹ less than 5 isolates per ST in 2015

Textbox 8.2

Carbapenemase producing bacteria in Denmark, 2015

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), and 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 to the Antimicrobial Resistance Reference Laboratory at Statens Serum Institut. The present textbox describes carbapenemase-producing Enterobacteriaceae (CPE), carbapenemase-producing *P. aeruginosa* and *Acinetobacter* spp.

During 2015, 91 carbapenemase producing bacteria were detected from 85 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. In 2015, eight of the carbapenemase isolates were from bloodstream infections (four CPE, three *P. aeruginosa* and one *A. baumannii*) compared with seven carbapenemase-producing bacteria (all CPE) in 2014. In many cases, the sources of the carbapenemase producing bacteria were unknown or related to spread between patients in Denmark, which was different compared to the previous years, where most of the cases were related to travel abroad.

Enterobacteriaceae: In 2015, 63 CPE (from 59 patients) were detected compared to 36 CPE in 2014 and 31 CPE during 2008–2013 (Figure 1). Thirty-eight of the 63 CPE isolates harboured OXA-48-like genes. Twenty-one of the 63 CPE isolates were NDM-producing and one isolate produced both NDM and OXA-181. Furthermore, three KPC-producing isolates were detected.

The NDM-1 producing *C. freundii* outbreak, which started in 2012 in the North Denmark Region, continued in 2015. Until the end of 2015, ten 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 [Hammerum *et al.* J. Antimicrob. Agents (2016); published online].

During 2015, NDM-1 producing *K. pneumoniae* were detected from three patients at a hospital in the Region of Southern Denmark. The isolates had highly similar SNP-profiles, indicating a possible spread between the patients or a common origin. None of the patients had travelled recently, and the origin of the NDM-1 producing *K. pneumoniae* was unknown.

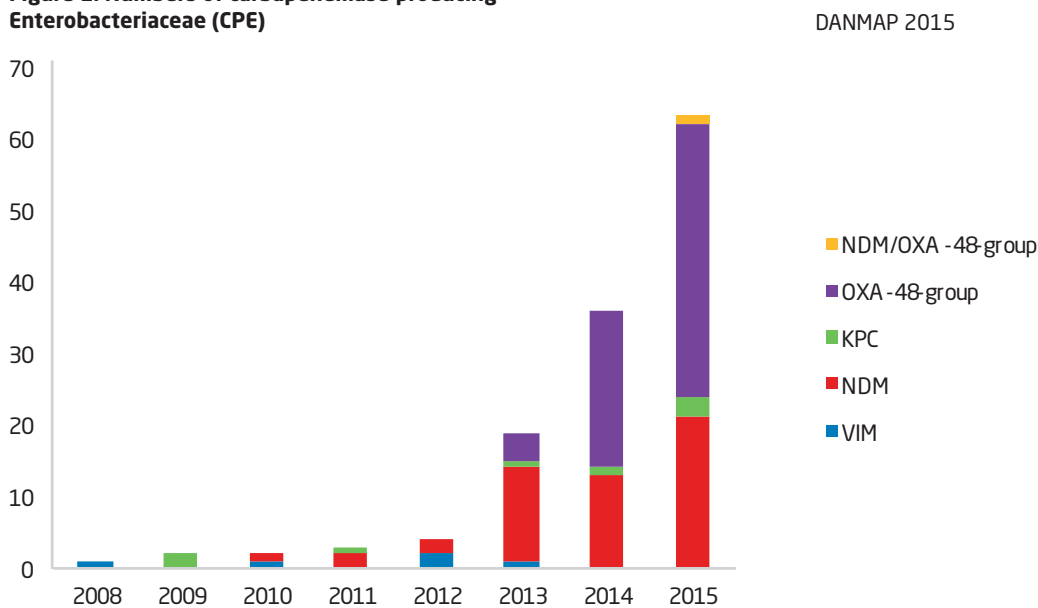
Acinetobacter spp.: In 2015, 17 OXA-23 producing *A. baumannii* isolates were detected. Furthermore, two NDM-1 producing *A. baumannii* were detected.

P. aeruginosa: In 2015, seven VIM-producing *P. aeruginosa* isolates were detected. Furthermore, one IMP-producing *P. aeruginosa* and one IMP-producing *Pseudomonas putida* were detected.

Conclusion: The occurrence of carbapenemase-producing bacteria in Denmark is 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)



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

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 the most 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 (e.g. ciprofloxacin and levofloxacin), aminoglycosides (e.g. gentamicin, tobramycin and amikacin), some beta-lactams (piperacillin-tazobactam, ceftazidime, and carbapenems) and colistin.

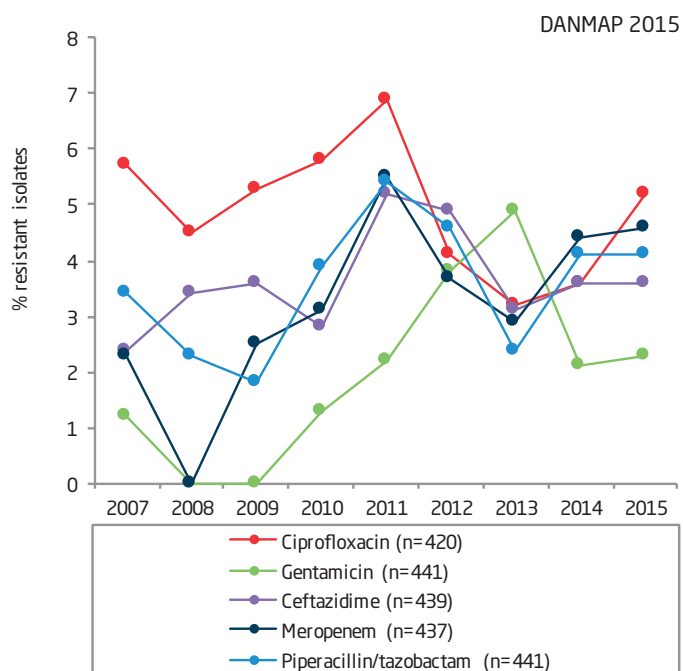
P. aeruginosa blood isolates obtained from hospitalised patients

For *P. aeruginosa*, DANMAP 2015 includes data from 11 out of 11 Departments of Clinical Microbiology (DCM), covering the total Danish population. DANMAP received data of 443 *P. aeruginosa* isolates from blood. Resistance levels to all the tested antimicrobial agents were not significantly different from the levels in 2014 (Figure 8.10). Gentamycin stayed at the same level as in 2014 and the increasing trend in resistance from 2009 to 2013 did not seem to be sustained. Overall the resistance profile of the *P. aeruginosa* isolates from blood has been highly variable both when combining results from all reporting DCM, and when looking at each DCM individually from year to year. This might be explained by natural fluctuation in the numbers of isolates. The occurrence of resistance to fluoroquinolones, carbapenems, ceftazidime and piperacillin/tazobactam remained at the same level or lower as reported to EARS-Net 2014 by the Nordic countries [EARS-Net 2014].

Meropenem resistance was observed for 4,6% (n=20) of the *P. aeruginosa* isolates in 2015. A Danish study of *P. aeruginosa* carbapenem non-susceptible isolates from 2011 showed that carbapenemases were present in a minority of the isolates (7%) [Hansen *et al.* 2014 Microb. Drug Res. 20: 22-9]. As in previous years, putative carbapenemase producing *P. aeruginosa* isolates were sent on a voluntary basis from the DCM to SSI for national surveillance on carbapenemase producing bacteria, including not only isolates from bloodstream infections but also from other origins. In 2015, six VIM-producing *P. aeruginosa* isolates were detected from six different patients. Furthermore, two NDM-producing *P. aeruginosa* were detected from two patients. [Textbox 8.2].

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Figure 8.10. Resistance (%) in *Pseudomonas aeruginosa* blood isolates from humans, Denmark



Note: The number (n) in parentheses represents the number of isolates tested for susceptibility in 2015

8.4 Enterococci

Enterococci are part of the normal intestinal flora of both humans and animals but might also cause infections. Important clinical infections caused by *Enterococcus* species include urinary tract infections, bacteraemia and bacterial endocarditis. *E. faecalis* and *E. faecium* can cause life-threatening infections in humans, especially in the hospital environment. The naturally high level of antimicrobial resistance found in enterococci makes infections difficult to treat. Antimicrobial therapy for serious enterococcal infections requires the use of synergistic combinations of a cell-wall-active agent such as penicillin (ampicillin) with an aminoglycoside (gentamicin) or a glycopeptide (vancomycin).

For *E. faecalis* and *E. faecium*, data from 11 of the 11 DCM were obtained, representing the total Danish population.

***Enterococcus faecium* and *Enterococcus faecalis* blood isolates obtained from hospitalised patients**

DANMAP received data on the antimicrobial susceptibility of 711 *E. faecium* isolates and 617 *E. faecalis* isolates from blood. Not all laboratories tested for susceptibility to the same antimicrobial agents.

As for the last 10 years, most of the *E. faecium* isolates from bloodstream infections were ampicillin resistant. In 2015, 95% of the *E. faecium* isolates were resistant to ampicillin compared to 94% in 2014. Figure 8.11 shows the trend for ampicillin resistance in *E. faecium* from 2002 through 2015. In 2002, 65% of the reported *E. faecium* isolates were resistant to ampicillin followed by an increase to around 87% in the period 2005–2009. The increase observed for ampicillin resistant *E. faecium* in Danish hospital has been attributed to the clonal spread of

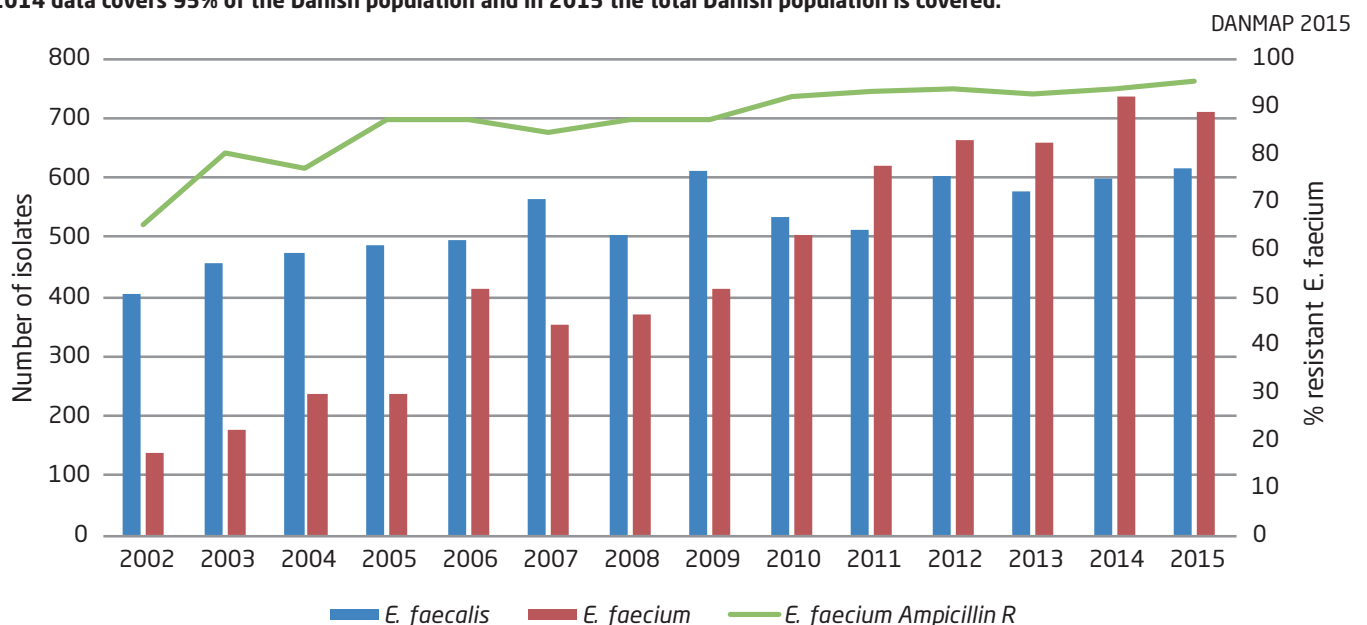
Clonal Complex 17 *E. faecium* isolates [Lester *et al*]. Antimicrob. Chemother, 2008, 1203–1206]. The increase in ampicillin resistant *E. faecium* has continued and has been above 90% since 2010. Also the proportion of *E. faecium* to *E. faecalis* from Danish Hospital blood isolates has continued its increase with the numbers of *E. faecium* blood isolates exceeding the number of *E. faecalis* blood isolates since 2011. Figure 8.11 shows both trends. The increase in vancomycin resistant *E. faecium*, (described in textbox 8.3), still constitutes only a minor fraction, 3.7% in 2015 of the *E. faecium* isolates from bloodstream infections. In 2015, 0.2% of the *E. faecalis* blood isolates were vancomycin resistant. The level of vancomycin resistant *E. faecium* was above the level reported to EARS-Net 2014 by the other Nordic countries [EARS-Net 2014].

Treatment with fluoroquinolones, cephalosporins or carbapenems has been described as a risk factor for development of an *E. faecium* infection. An increasing consumption of these antimicrobial agents has been observed in hospitals in Denmark during the last decade. The antimicrobial selection pressure in a hospital environment might be a reason for the high level of ampicillin resistant *E. faecium* as a cause of bloodstream infections.

Only one of the DCMs (Aalborg) tested all enterococcal blood isolates for high-level gentamicin resistance (HLGR). Among the tested *E. faecalis* isolates in 2015, 25% were HLGR, whereas 75% of the tested *E. faecium* isolates were HLGR. This is a insignificant change compared to 2014 with respectively 30% and 68% HLGR.

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Figure 8.11. Ampicillin resistance (%) in *E. faecium* blood isolates from humans, Denmark and the proportion of *E. faecium*/*E. faecalis* isolates. From 2002 until 2009 the figure presents data reported from DCM covering 75% of the Danish population. From 2010 till 2014 data covers 95% of the Danish population and in 2015 the total Danish population is covered.



Textbox 8.3

Further increase in occurrence of clinical vancomycin-resistant enterococci in Danish hospitals in 2015

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. Severe enterococcal infections with penicillin-resistant enterococci are primarily treated with vancomycin, but recently 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 are associated with severe side effects.

Surveillance of VRE: Since 2005, Danish Departments of Clinical Microbiology (DCM) have on a voluntary basis, submitted VRE for species identification, genotyping (*vanA*, *vanB* and *vanC*) and surveillance to the Antimicrobial Resistance Reference Laboratory at Statens Serum Institut. From 2015, all clinical VRE isolates have been whole-genome sequenced.

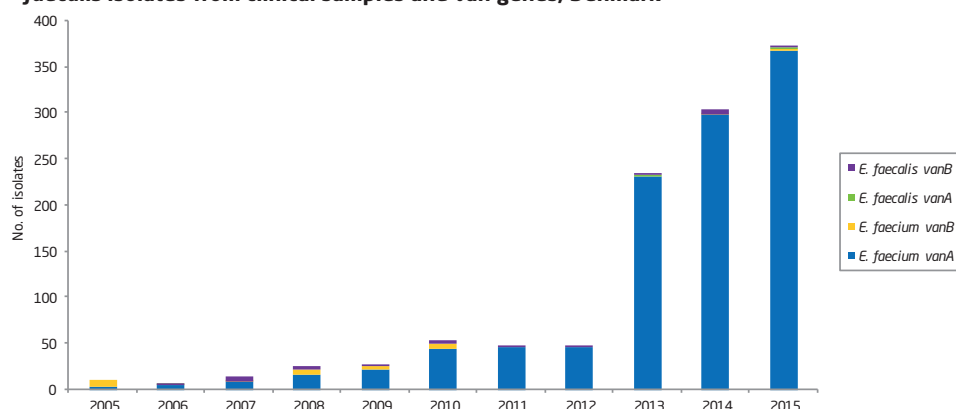
In 2012 and 2013, an increase in clinical *vanA E. faecium* isolates were observed (Figure 1). They were primarily detected at hospitals in the Capital Region, but also from The Zealand Region and the Central Denmark Region. VRE was detected in the two remaining regions of Denmark too, but to a much lower extent. Typing of VRE isolates from 2012 and 2013 showed spread of several *vanA E. faecium* clones both within hospitals and between hospitals [Pinholt *et al.* 2015, J. Antimicrob. Chemother, 70:2474-82]. This trend continued in 2014 and 2015, to a total of 372 clinical VRE isolates in 2015. Most of the clinical VRE were *vanA E. faecium*, which increased from 294 in 2014 to 368 in 2015 (Figure 1). In total, 89% of the *vanA E. faecium* isolates were from hospitals located in The Zealand Region and The Capital Region. The majority of the *vanA E. faecium* isolates belonged to three sequence types; ST80 (33%), ST117 (10%) and ST203 (51%). Whereas the rest of the isolates belonged to ST18, ST192 and novel STs (1-6 isolates per STs). The STs were all part of the CC17 complex, which are commonly detected in hospitals outside of Denmark. None of the *vanA E. faecium* isolates belonged to STs linked to animal production (e.g. CC6).

The number of VREs detected from bloodstream infections in 2015 (n=30) was at the same level as in 2014 with one *vanA E. faecalis*, one *vanB E. faecium* and 28 *vanA E. faecium* reported.

Conclusion: The continued increase in *vanA E. faecium* cases in 2015 in Denmark is worrying. 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 from clinical samples and van genes, Denmark DANMAP 2015



8.5 Streptococci

Streptococci include *Streptococcus pneumoniae* (pneumococci), beta-haemolytic streptococci (BHS), and non-haemolytic streptococci (NHS). The prevalence of asymptomatic carriage with pneumococci in the nasopharynx varies with age. NHS are part of the normal commensal flora of the mouth and upper respiratory tract, skin and intestine in humans.

Pneumococci may cause common and less severe infections such as otitis media and sinusitis, and more severe and invasive infections such as pneumonia, bacteraemia, endocarditis and meningitis.

BHS of group A cause tonsillitis, otitis media, wound infections, but also more severe infections: e.g., bacteraemia, necrotizing myofasciitis, and rarely meningitis. BHS of group B may be present in the vaginal flora of 20-25% of women in the childbearing age and may therefore cause meningitis and septicaemia in the newborn. These infections also occur in elderly or immuno-compromised patients. BHS of groups C and G predominantly cause soft-tissue infections and sometimes bacteraemia. Approximately 10% of humans are asymptomatic throat carriers of BHS of any group. NHS may cause invasive infections, the most severe being endocarditis and cerebral abscesses – in risk of these are fx persons who undergo dental treatment.

This report presents data on resistance in non-duplicate invasive isolates (i.e. from blood or cerebrospinal fluid) of pneumococci and BHS submitted in 2015 to the Neisseria

and Streptococcus Reference laboratory. Isolates are received from all DCMs in Denmark. It is mandatory to submit invasive isolates of pneumococci in order to enable surveillance of vaccine coverage and the prevalent type distribution, while it is voluntary to submit isolates of BHS. Non-invasive isolates are infrequently received but are not included in this report. There is no surveillance of NHS.

Infections with pneumococci and BHS are usually treated with penicillins or macrolides. All submitted pneumococci and group A, B, C and G BHS were therefore tested for susceptibility to penicillin and erythromycin. Moreover, all BHS were tested for susceptibility to clindamycin and for inducible clindamycin resistance (Table 8.3).

Streptococcus pneumoniae

Susceptibility testing was performed on 750 isolates of *S. pneumoniae* found in either blood (706 isolates) or cerebrospinal fluids (44 isolates) from Danish patients. The isolates belonged to 42 different serotypes, of which isolates belonging to 18 serotypes showed non-susceptibility (resistant or intermediary resistant) to either penicillin or erythromycin or both (54 isolates, 7.2%).

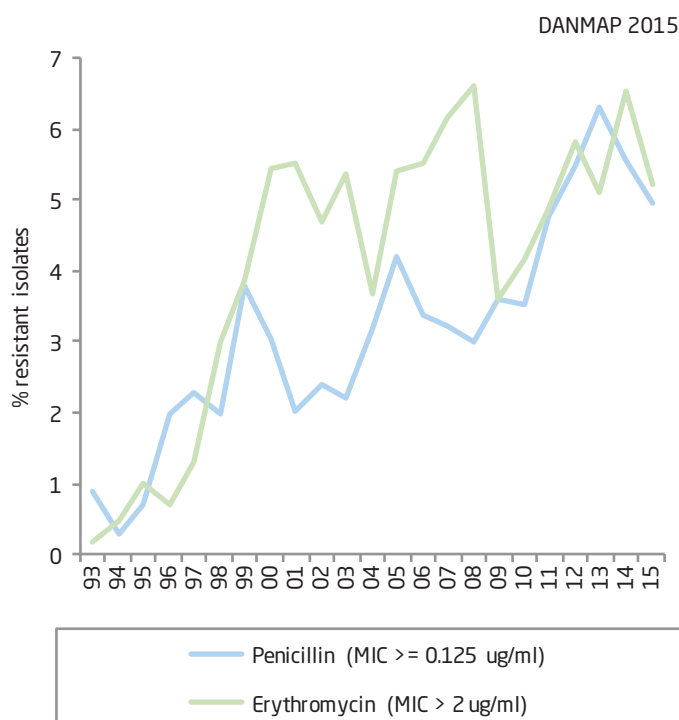
For penicillin, 37 isolates (4.9%) were non-susceptible of which 2 isolates (0.3%) were resistant. For erythromycin, 39 isolates (5.2%) were resistant.

For pneumococci, antibiotic susceptibility is closely connected to serotypes. For example, all three of the received invasive isolates of serotype 14 were non-susceptible to penicillin, and similarly all three of the received invasive isolates of serotype 9V were resistant to erythromycin. Conversely, all of the 23 received isolates of serotype 1 were fully susceptible to both penicillin and erythromycin.

With the introduction of pneumococcal vaccines in 2007 (PCV7) and 2010 (PCV13) in Denmark, the serotype-distribution was influenced. The total susceptibility-profile of Danish invasive pneumococcal isolates have therefore been influenced as well. Serotypes covered by the PCV7 vaccine has diminished dramatically, and while 41% of the isolates reported to DANMAP were PCV7 serotypes in 2006, in 2015 this had decreased to 3%. Some serotypes not included in the vaccines are increasing in incidence, while others fluctuate through the years. In 2015, the predominant serotype found was serotype 8 (164 isolates), of which 3 isolates were non-susceptible to penicillin, erythromycin or both. Serotype 8 was dominant in 2014 as well, while in 2013 serotype 1 was dominant with 100% susceptible isolates (more information on vaccine-coverage and serotypes can be found in the EPI-NEWS newsletters at www.ssi.dk).

The levels of penicillin and erythromycin non-susceptibility in Denmark were similar to the levels reported in 2014 to EARSNet by the neighbouring countries Norway, Sweden,

Figure 8.12. Non-susceptibility (%) in *Streptococcus pneumoniae* blood and spinal fluid isolates from humans, Denmark



Germany, and the United Kingdom. Many other European countries reported considerably higher levels of resistance in 2014 [EARS-Net 2014].

The numbers of submitted isolates of BHS of group A, B and G were virtually unchanged in 2015 compared to 2014, while the number of group C had increased from 80 to 103, thus continuing the tendency from 20013 to 2014.

All BHS isolates were fully susceptible to penicillin.

The prevalence of resistance to erythromycin and clindamycin showed only minor variation compared to previous years. Susceptibility findings for erythromycin and clindamycin are shown in Table 8.3.

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Table 8.3 Beta-haemolytic streptococci: Susceptibility testing results

DANMAP 2015

BHS group	Susceptibility testing results	2013	2014	2015
A	Number of isolates	177	149	154
	Erythromycin resistant, number (%)	5 (2.8%)	1 (0.7%)	3 (1.9%)
	Clindamycin resistant, number (%)	3 (1.7%)	1 (0.7%)	0 (0%)
	Additionally, inducible clindamycin resistance, number (%)	1 (0.6%)	0 (0%)	2 (1.3%)
	Susceptible to penicillin, erythromycin and clindamycin, %	172 (97%)	99%	151 (98%)
B	Number of isolates	113	139	140
	Erythromycin resistant, number (%)	20 (18%)	31 (22%)	30 (21%)
	Clindamycin resistant, number (%)	14 (12.3%)	16 (11%)	14 (10%)
	Additionally, inducible clindamycin resistance, number (%)	1 (0.9%)	4 (2.9%)	7 (5.0%)
	Susceptible to penicillin, erythromycin and clindamycin, %	92 (81%)	77%	111 (79%)
C	Number of isolates	68	80	103
	Erythromycin resistant, number (%)	3 (4.4%)	3 (3.8%)	8 (7.8%)
	Clindamycin resistant, number (%)	2 (2.9%)	3 (3.8%)	2 (1.9%)
	Additionally, inducible clindamycin resistance, number (%)	1 (1.4%)	1 (1.3%)	4 (3.9%)
	Susceptible to penicillin, erythromycin and clindamycin, %	65 (96%)	95%	95 (92%)
G	Number of isolates	145	183	183
	Erythromycin resistant, number (%)	17 (11.7%)	19 (10.1%)	18 (9.8%)
	Clindamycin resistant, number (%)	6 (4.1%)	3 (1.6%)	5 (2.7%)
	Additionally, inducible clindamycin resistance, number (%)	6 (4.1%)	13 (6.9%)	9 (4.9%)
	Susceptible to penicillin, erythromycin and clindamycin, %	128 (88%)	90%	167 (91%)

8.6 *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 invasive infections such as post-operative wound infections, infections related to intravenous catheters and prosthetic devices, bacteremia, osteomyelitis, endocarditis and septic arthritis.

In Denmark, a voluntary surveillance programme of all *S. aureus* bacteremia cases was established in 1957. Laboratory and clinical notification of Methicillin-resistant *S. aureus* (MRSA) has existed since November 2006. At SSI, all isolates are typed by *spa* typing, susceptibility testing is performed for 17 antimicrobials and the presence of the gene *lukF-PV* is determined. *lukF-PV* codes for a cytotoxin (Panton-Valentine leucocidin, PVL), which has been closely linked to skin abscesses and the very rare condition of severe necrotizing pneumonia. PVL is found both in MSSA and MRSA, for MRSA PVL has been closely associated with community acquired MRSA strains. 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-associated MRSA (CC398). For CA and HACO classification is separated into known and not known exposure.

Surveillance of bacteraemia

In 2015, 1,973 *S. aureus* bacteremia cases corresponding to 36.71 cases per 100,000 inhabitants were reported from the

Departments of Clinical Microbiology (DCM) in Denmark. For the third year in a row, the number of cases was higher than in the previous year (ca. 1,500 annual cases). 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%). The increasing number of cases the last 3 years may therefore reflect a change in practice in the hospitals, with either more samples taken, improved diagnostics or both as well as a real increase in occurrence. Twenty-nine (1.5%) of the bacteremia cases were caused by MRSA. This is a decline compared to 2014 but at the same level as in previous years, and still very low compared to most other countries participating in EARS-Net [EARS-Net 2014]. Three of the 29 MRSA cases were caused by CC398 (8 MRSA CC398 in 2014). Four hundred and fifty two (23%) patients died within thirty days of the onset of bacteremia. The mortality for the MRSA bacteremia cases was 24% (n=7). Antimicrobial resistance in *S. aureus* bacteremia isolates from 2005-2015 is presented in Table 8.4. The highest frequency of resistance to other antimicrobials than penicillin was observed for fusidic acid (16%), erythromycin (7%), clindamycin (7%) and norfloxacin (6%). Susceptibility to all tested antimicrobial agents was at the same level as in 2014, but resistance to erythromycin, clindamycin, fusidic acid and norfloxacin has increased steadily since 2005 (Table 8.4). Typing identified 567 different *spa* types distributed in 25 different CC groups (the ten most prevalent *spa* types representing 33% of the total are presented in Table 8.5). The PVL toxin was demonstrated in 25 (1.3%) cases of which five were MRSA. The 25 PVL containing isolates were distributed over 22 different *spa* types and 10 different CC groups.

Table 8.4. Resistance (%) in isolates from *S. aureus* bacteraemia cases, Denmark

DANMAP 2015

Antimicrobial agent	2006 %	2007 %	2008 %	2009 %	2010 %	2011 %	2012 %	2013 %	2014 %	2015 %
Methicillin	1.4	0.6	1.3	1.6	1.4	1.4	1.2	1.7	2.9	1.5
Penicillin	80	78	77	77	75	77	74	76	77	71
Erythromycin	5	4	5	7	5	7	6	7	8	7
Clindamycin	4	3	4	6	4	6	6	6	8	7
Tetracycline	3	2	3	2	3	2	2	3	5	4
Fusidic acid	10	9	9	9	13	13	14	15	15	16
Rifampicin	<1	<1	<1	<1	<1	<1	<1	0	<1	<1
Norfloxacin	2	1	2	2	3	4	4	5	6	6
Kanamycin	1	<1	1	1	1	<1	1	2	2	3
Linezolid	nt	nt	0	0	0	0	0	0	0	0
Mupirocin	0	<1	<1	<1	<1	<1	<1	<1	<1	<1
Trimethoprim-sulfamethoxazole	nt	nt	nt	nt	nt	<1	1	1	1	<1

Notes: nt = not tested. In web annex table A8.1 the distribution of MICs and resistance for all tested antimicrobial agents are shown.

Table 8.5. The ten most prevalent spa types demonstrated in SAB and in non- CC398 MRSA cases, Denmark 2015

DANMAP 2015

SAB			MRSA			
spa type	CC group ^(a)	No. of cases	spa type	CC group ^(a)	No. of cases	No. causing infections (%)
t127	CC1	96	t002	CC5	173	94 (54)
t084	CC15	89	t127	CC1	148	75 (51)
t002	CC5	84	t008	CC8	122	79 (65)
t230	CC45	82	t304	CC8	112	46 (41)
t091	CC7	60	t019	CC30	90	60 (67)
t012	CC30	57	t223	CC22	87	37 (43)
t015	CC45	53	t044	CC80	48	22 (46)
t021	CC30	46	t437	CC59	44	31 (70)
t701	CC8	45	t032	CC22	40	21 (53)
t008	CC8	36	t843	CC130	38	21 (55)

a) CC = Clonal complex

Surveillance of methicillin-resistant *S. aureus*

In 2015, 2,972 new MRSA cases were detected (52.1 per 100,000 inhabitants). This was at the same high level as observed in 2014 in Denmark (Figure 8.13). 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 colonized only. MRSA isolates were confirmed by detection of either the *mecA* or more uncommonly, the *mecC* gene.

In 2015, the number of new MRSA cases stabilized for the first time since 2009. CC398 cases constituted 39% (n=1,173) of new MRSA cases in 2015, which is a slight decrease compared to 2014 (Table 8.6). This decrease was expected to occur

at some point, since the intensive screening of people with contact to livestock introduced in the MRSA guidelines in 2012 has detected many CC398 cases in previous years, thus reaching a saturation in this sub-population.

MRSA isolates carrying *mecC* were demonstrated in 61 cases (2.1%) in 2015 (9 in 2009, 21 in 2010, 37 in 2011, 24 in 2012, 41 in 2013 and 53 in 2014). Thirty-nine of the cases (64%) had infections at the time of diagnosis. Only two possible livestock contacts were registered for the 61 *mecC* cases.

The total number of cases and the number of cases presenting with infection according to epidemiological classification are

Figure 8.13. Number of methicillin-resistant *Staphylococcus aureus* cases, with a three years moving average, Denmark

DANMAP 2015

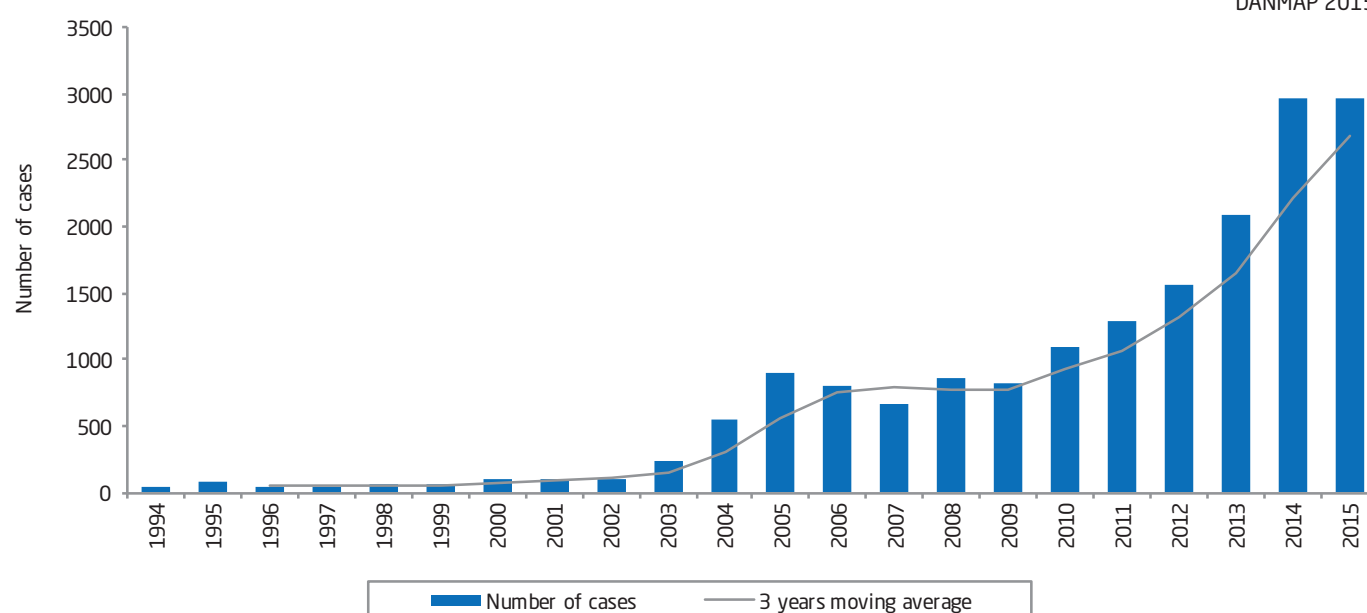
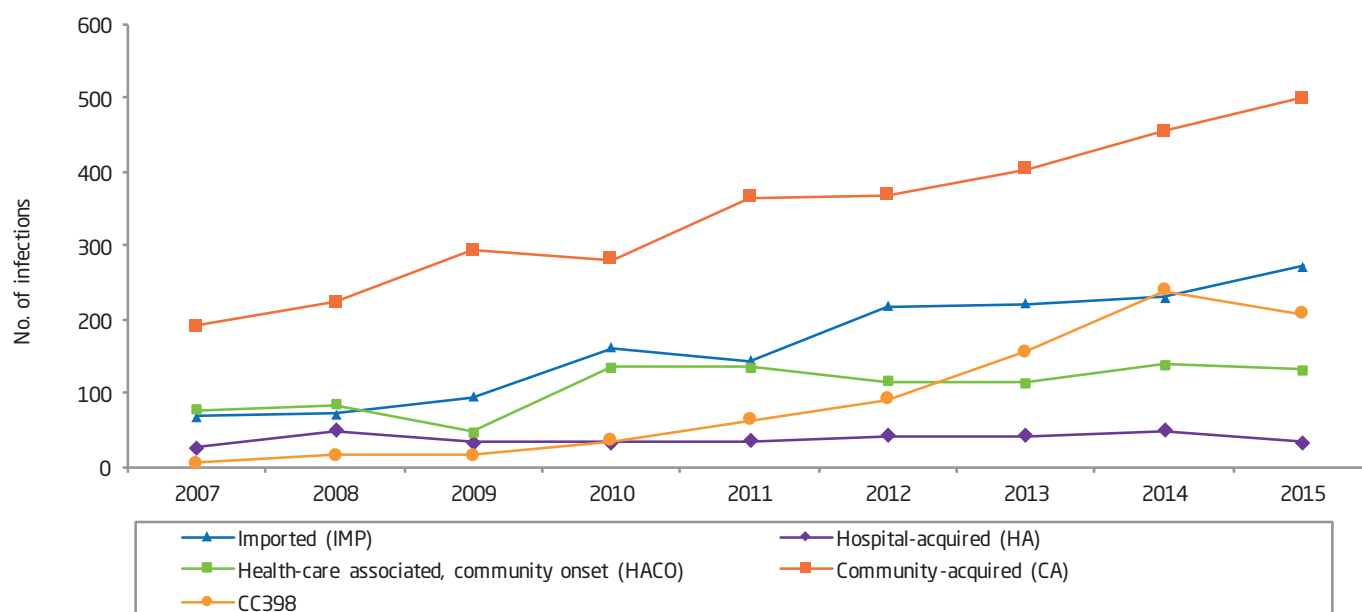


Figure 8.14. Number of MRSA infections according to epidemiological classification, Denmark

DANMAP 2015



shown in Table 8.6. Most of the cases (83%) were acquired in Denmark. At the time of diagnosis, 39% (n=1,147) of cases had infection, which was similar to 2014 but lower than in previous years due to a much lower fraction of infections among CC398 cases (n=208, 18%).

The epidemiological classification of MRSA infections 2007-2015 is shown in Figure 8.14. Despite the overall increasing total number of cases, the number of hospital acquired infections (n=25) and the number of healthcare-associated with community onset (HACO) infections (n=141) were at stable very low levels. The number of CA and imported infections

continued the increasing trend in 2015 and were by far the two largest groups (n = 500 and n= 273, respectively) while infections caused by CC398 decreased in 2015 (n=208) from 240 cases in 2014 (Figure 8.14).

Molecular typing of the MRSA strains

In total, *spa* typing revealed 293 different strain types not including isolate belonging to CC398 of which 204 types were associated with clinical infections. The single most abundant *spa* type detected was t034 (n=237). The 10 dominating non-CC398 *spa* types isolated in 2015 are shown in Table 8.5. They constituted 50% of the total number of non-CC398

Table 8.6. Epidemiological classification of new MRSA cases, Denmark

DANMAP 2015

Epidemiologic classification	Exposure	No. of cases (% of total)	No. (%) of cases with infections
Imported (IMP)		500 (17)	273 (55)
Hospital-acquired (HA)		40 (1)	25 (63)
Health-care associated, community onset (HACO)		171 (6)	
	with known exposure	16	5 (31)
	without known exposure	155	128 (83)
Health care worker		31 (1)	8 (26)
Community-acquired (CA)		1057 (36)	
	with known exposure	516	79 (15)
	without known exposure	541	421 (78)
CC398		1173 (39)	
	with known exposure	982	131 (13)
	without known exposure	140	77 (55)
	project sampling	51	0 (0)

Note: Numbers shown in bold are totals

Table 8.7. Resistance (%) in MRSA isolates, Denmark 2015

DANMAP 2015

	%	
	non-CC398	CC398
Erythromycin	37	43
Clindamycin	29	87
Tetracycline	24	100
Fusidic acid	19	1
Rifampicin	<1	0
Norfloxacin	21	29
Kanamycin	32	7
Linezolid	0	0
Mupirocin	<1	0
Trimethoprim-sulfamethoxazole	4	1
Number of tested isolates	1242	68

In web annex table A8.2 the distribution of MICs and resistance for all tested antimicrobials are shown.

MRSA isolates. Ten *spa* types constituted 45% of the 1,147 clinical infections with MRSA. These most prevalent *spa* types causing clinical infections at time of presentation were t002/CC5 (n=94), t008/CC8 (n=79), t127/CC1 (n=75), t019/CC30 (n=60), t304/CC8 (n=46), t034/CC398 (n=46), t223/CC22 (n=37), t437/CC59 (n=31), t657/CC1 (n=25) and t044/CC80 (n=22). The PVL encoding gene *lukF-PV* was demonstrated in 34.6% of the infections and in 12.5% of the asymptomatic carriers and most often in relation to isolates with *spa* types t008 (n=98), t019 (n=88), t002 (n=60), t044 (n=47) and t127 (n=39).

Resistance among MRSA isolates

Resistance data is presented in Table 8.7 and is divided into two categories: CC398 and other. CC398 isolates were typically resistant to tetracycline (100%) and clindamycin (87%) with high levels of resistance to erythromycin and norfloxacin (43% and 29%, respectively). Resistance to fusidic acid and kanamycin was higher among non-CC398 (19% vs 1% and 32% and 7%, respectively). Resistance to at least 1, 2 or 3 other antimicrobials in addition to β -lactam antibiotics (cefoxitin/penicillin) was demonstrated in 64%, 45% and 31% of non-CC398 the cases, respectively.

Andreas Petersen, Robert L. Skov and Anders Rhod Larsen

Staphylococcus aureus surveillance, 1996-2015

Staphylococcus aureus has been surveyed in Denmark since 1957 and has thus been included in DANMAP since its initiation. From its very beginning *S. aureus* bacteremia (SAB) surveillance has been based on a voluntary submission of all *S. aureus* bacteremia isolates from the Clinical Microbiology Departments and registration of clinical epidemiological data. This information together with antimicrobial susceptibility testing, typing results and isolates has been curated by Statens Serum Institut. A comparison of submitted isolates to SSI in the period 2010-15 with SAB cases registered in the National Microbiology Database (MiBa) showed 95-97% concordance in the period.

The number of SAB cases has increased throughout the period and has now reached almost 2,000 annual cases. Most of these cases are in elderly people who often suffer from one or more comorbidities. The mortality rate decreased steadily from almost 50 % in the 1960s to around 20% in 1990 but has since remained stable.

During the surveillance period, the methodology has changed due to technical developments. Until the late 1990s strains were typed using phage typing, this was followed by a DNA restriction analysis pulse field gel electrophoresis, which again was replaced by sequencing of the *spa* gene in 2006. This method has the great advantage that it easily can be compared to what is done in other laboratories. Tablet and disk diffusion has been used until 2013, where it was replaced by microbroth dilution to determine the MIC values for different panels of antimicrobials. MRSA was common among SAB isolates in the period 1965- 75 with frequencies of 15-25%. In 1967, MRSA accounted for 46% of SAB cases in hospitalized patients. This was primarily due to the success of isolates belonging to phage type 83A complex, which were often also resistant to tetracycline, streptomycin and erythromycin. These clones were replaced by other less resistant types of *S. aureus* and from the mid- 1970s the frequency of MRSA among SAB isolates decreased and has remained below 2% among SAB cases, except in 2014 where it reached 2.9%.

A complete MRSA surveillance was established on a voluntary basis in 1988, improved with a systematic collection of clinical information since 1999, and ultimately became notifiable in November 2006. Figure 8.13 shows the number of new MRSA cases registered in the period 1994-2015. Until 2001, less than 100 new cases were registered annually. The following increase has mostly been driven by the emergence of community associated (CA-) MRSA, which depicted a completely new era in MRSA evolution and epidemiology since MRSA had previously been restricted to hospital settings.

The first Danish CA-MRSA (European CA-MRSA CC80-IV) cases were reported in 1997. These were followed by emergence of other CA-MRSA clones, most notably the South West Pacific (ST30-IV) and USA300 (ST8-IV), named after the geographic epicenters of their emergence. Import of these clones has occurred with travelers, migrants and refugees and has subsequently led to secondary transmissions in the local communities. The CA-MRSA clones share molecular characteristics, which may be of

Of importance for the success of these clones outside hospital settings were: Smaller *SCCmec* elements (carrying the *mecA* gene, responsible for the MRSA phenotype) type IV and V compared to the *SCCmec* II and III found in the older hospital clones; and carriage of the Pantone-Valentine leukocidine (PVL) genes. The smaller *SCCmec* elements IV and V in CA-MRSA may be an adaptation to the environment outside hospitals with less need for additional resistance genes and the PVL genes may increase infectivity in the skin and thereby transmission.

Despite of the increasing number of CA-MRSA in Denmark, infection control procedures has ensured a low level in hospitals. The annual number of patients acquiring MRSA infections in hospital has remained below 50 cases since 2006.

Very few larger hospital outbreaks have been noted with the exception of the ST22-IV outbreak in Vejle County that went on for more than 4 years between 2002-2006. This outbreak peaked in 2005 and accounted a total of 514 cases during the 4 years.

However, as seen in Figure 8.13 the decline in MRSA following the "Vejle outbreak" was only temporary, since the numbers of imported cases, CA-MRSA as well as spread into health care facilities has continued.

A new wave in the evolution of MRSA appeared with the emergence of MRSA in livestock (LA-MRSA). The spread of this clone among livestock was very fast with 3% of pig herds being positive in 2008, 15 % in 2011 and 68% in 2014. The first human cases with LA-MRSA (CC398) occurred in 2004 and the incidence has increased dramatically in the years after, peaking in 2014 with 1,277 new cases. In 2012, a revision of the MRSA guidelines from the Danish Health Authorities, depicted screening of people with livestock contact at hospital admissions. Consequently, CC398 cases are more often found by active screening than from clinical samples (20%) compared to other MRSA types (40-50%). The cases most often have documented contact to pigs (85-89%). The human to human transmission appears to be less effective for this clone compared to others, which may be due to its adaptation to livestock as host, a feature that may be linked to the absence of the human immune modulatory genes (*scn*, *sak* and *chp*). Furthermore, the predominant *SCCmec* element found in CC398 is a type IV variant (5C2&5) that is comparable large to the old hospital type II and III elements probably making CC398 less fit to survive in the community.

Despite the steady increase in MRSA in the surveillance period, the frequency has remained low compared to other European countries (EARSSnet.org) and most importantly the number of infections in hospitalized patients low. This success in keeping a low frequency of MRSA in Denmark, the other Nordic countries (www.nordicmrna.org) and in the Netherlands is most likely a result of the "search and destroy" policy in the hospitals in combination with a general rational antibiotic policy.

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Textbox 8.4

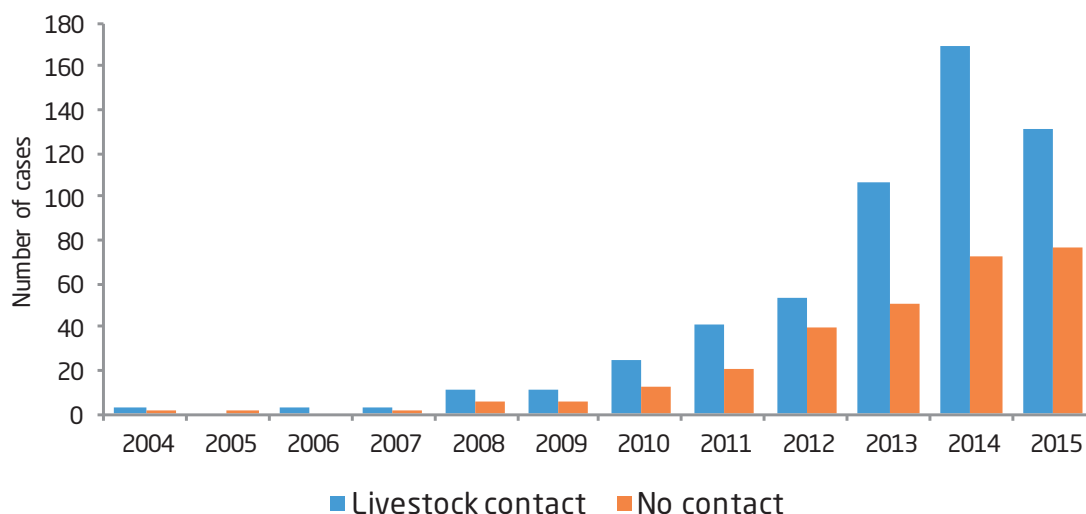
LA-MRSA CC398 in people with no livestock contact and in various animal populations

LA-MRSA CC398 in people with no livestock contact

Danish pigs constitute a rapidly expanding reservoir of LA-MRSA CC398 and occupational contact to pigs is known to be a major source of MRSA in livestock workers where they may also cause infections (Textbox 8.5, DANMAP 2014). In a recent Danish study, Statens Serum Institut analyzed patient information and MRSA strain typing results collected between 1999 and 2011 to investigate the emergence and early epidemiology of LA-MRSA CC398 in humans [Larsen et al. 2015. Euro. Surveill. 20;pii=30021]. The study included 148 LA-MRSA CC398 cases where infections were registered. The incidence increased by ca. 66% per year from the first cases in 2004 to 2011 (from 0.093 to 1.1 per 100,000 person-years). Approximately one-third of the new infections per year were observed in people with no livestock contact, of whom the vast majority lived in rural areas with livestock production. Figure X shows that this proportion of LA-MRSA CC398 infections in people with no livestock contact has remained stable when analyzing data from 2012 to 2015 (Statens Serum Institut, unpublished data). Only seven of the 148 documented LA-MRSA CC398 infections during 2004-2011 occurred in Copenhagen. Thus, the risk of acquiring a LA-MRSA CC398 infection was higher in rural areas compared to other areas of Denmark, including Copenhagen. However, LA-MRSA CC398 only accounted for a small proportion of all MRSA infections in the period, and the overall incidence in people without direct or indirect exposure to animals was not statistically different between urban and rural municipalities. These findings support that LA-MRSA CC398 is able to spread from animal farms into the surrounding community most likely via livestock workers, whereas the low number of LA-MRSA CC398 infections in urban areas of Denmark suggests that foodborne transmission does not play a major role in the LA-MRSA CC398 epidemiology. In 2012, screening of livestock workers at hospital admissions was amended to the MRSA guidelines from The Danish Health Authority. Consequently, many livestock workers have been tested and found LA-MRSA CC398 positive in the years after and non-tested persons in this subpopulation has diminished. This may explain the decrease in incidence observed in 2015, as only new MRSA cases are reported. Since asymptomatic carriage of CC398 MRSA in livestock workers is not treated, the accumulated numbers of MRSA positive livestock workers has most likely increased during the years and thereby the potential source for secondary transmissions. Despite of that, the incidence of CC398 MRSA among the general population has not increased in 2015, which indicates that secondary transmissions are limited. This limitation could be due to constraints of the CC398 MRSA in the competition with other *S. aureus* in the environment outside the stables or in the human host, which needs to be investigated in more details.

Figure 1. Number of new patients with LA-MRSA CC398 infection per year.

DANMAP 2015



LA-MRSA CC398 in various animal populations

During 2015 the Danish Veterinary and Food Administration conducted a series of screenings for LA-MRSA in different production animal populations, poultry, veal calves, mink, and organic pigs. In the poultry herds, which comprised broilers, layers, and turkeys, all in all 54 herds, 25 choanal swab samples were collected and analyzed in pools of 5. In veal calves, 25 animals were sampled in each of 50 herds by nasal swab samples, which also were analyzed in pools of 5. A total of 64 organic pig herds were also sampled, 5 pools of 5 nasal swabs from each herd. Finally, an investigation of 50 mink farms was conducted. These farms were all sampled during the pelting season so that the animals could be sampled immediately after euthanasia. On each farm, 25 animals were sampled by pharyngeal swabs. Findings are shown in Table 1.

Table 1. LA-MRSA in various production animals

DANMAP 2015

Production animal	Number of herds sampled	Number (%) LA-MRSA positive herds
Poultry	54	1 (2%)
Organic pigs	64	4 (6%)
Veal calves	50	5 (10%)
Mink	50	8 (16%)

The single poultry herd that was found positive was a turkey flock. The finding indicates that LA-MRSA is not common in Danish poultry production. Among the organic pig herds, only 6 % were positive for LA-MRSA, as opposed to the 63-68 % positive conventional herds that was found in a screening in 2014. The reasons for this marked difference are essentially unknown but presently under investigation, as it may provide important knowledge about the epidemiology of LA-MRSA in pigs.

Sixteen percent of the mink farms were found positive for LA-MRSA. However, there are indications from other investigations that the proportion of positive farms is in fact higher, and that LA-MRSA may also be found in the mink feed (Hansen et al., unpublished). LA-MRSA was detected in 5 of the 50 veal calf farms, corresponding to 10 %. In another investigation in Danish veal production carried out by DTU and SEGES in 2015, 2 out of 17 herds were found positive, but out of 697 sampled animals, only 4 were found positive, suggesting a low within-herd prevalence. LA-MRSA is therefore concluded to be a minor issue in Danish veal production.

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8.7 *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 colonization after 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. Further, conjunctivitis may occur in newborns after transmission from an infected mother during labour and rarely in adults following direct inoculation.

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

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

As part of NSR's participation in ECDC's surveillance of sexually transmitted infections since 2009, approximately 110 gonococcus isolates are investigated per year for susceptibility to an expanded panel of antimicrobial agents. In addition to ceftriaxone, ciprofloxacin and azithromycin, this panel includes cefixime and in selected years also spectinomycin, and gentamicin. The latter two drugs were not included in the surveys in 2014 and 2015.

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

In 2015, isolates from 1127 unique cases of gonorrhoea were received. The annual number has increased considerably from 2011 through 2015, presumably because the widespread use of combined PCR-testing for *Chlamydia trachomatis* and *N. gonorrhoeae* has identified unexpected cases of gonorrhoea (followed by culture), but possibly also due to an increasing incidence of gonorrhoeae.

The ciprofloxacin resistance rate was 46% in 2014 and 29% in 2015, thus showing a steady decline since the peak of 75% in 2009 (Figure 8.15). The percentage of strains producing penicillinase was 8% in 2015. It has fluctuated between 22% in 2005 and 11% in 2014 and 2013.

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. No cases from Denmark have ever been reported. During 2003 through 2009 the proportion of isolates with MIC ≥ 0.008 mg/L gradually increased from 40% to nearly 75% (Figure 8.16), 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.

In 2014, azithromycin resistance including intermediary resistance (MIC > 0.5 mg/L) in gonococci was 36% while in 2015 it was 11% (Table 8.8). 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.

Resistance against cefixime (MIC > 0.125 mg/L) was 6% in 2014, i.e. a decrease compared to 2013, and further decreased to 0% in 2015. Cefixime is an oral cephalosporin that has never been used in Denmark.

Conclusions: The incidence of gonorrhoea is increasing substantially. Although resistance problems are so far 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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Table 8.8. Resistance rates (%) against azithromycin, cefixime and spectinomycin in gonococci, Denmark 2009-2015 DANMAP 2015

Antimicrobial agent	2009	2010	2011	2012	2013	2014	2015
Azithromycin	46	23	12	12	45	36	11
Cefixime	15	18	21	11	9	6	0
Spectinomycin	0	0	0	0	0	NT	NT
Number of isolates	119	111	126	114	111	108	1127

NT = not tested

Figure 8.15. Ciprofloxacin resistance and penicillinase production in gonococci, Denmark 2003-2015

DANMAP 2015

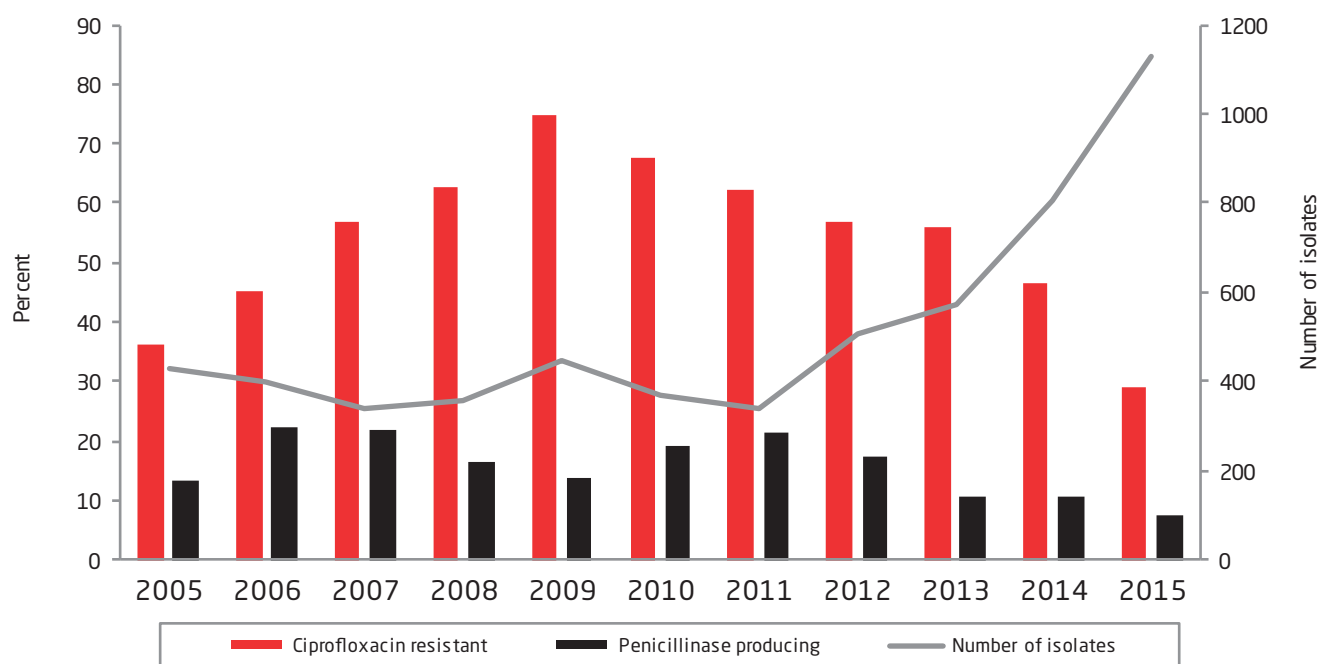
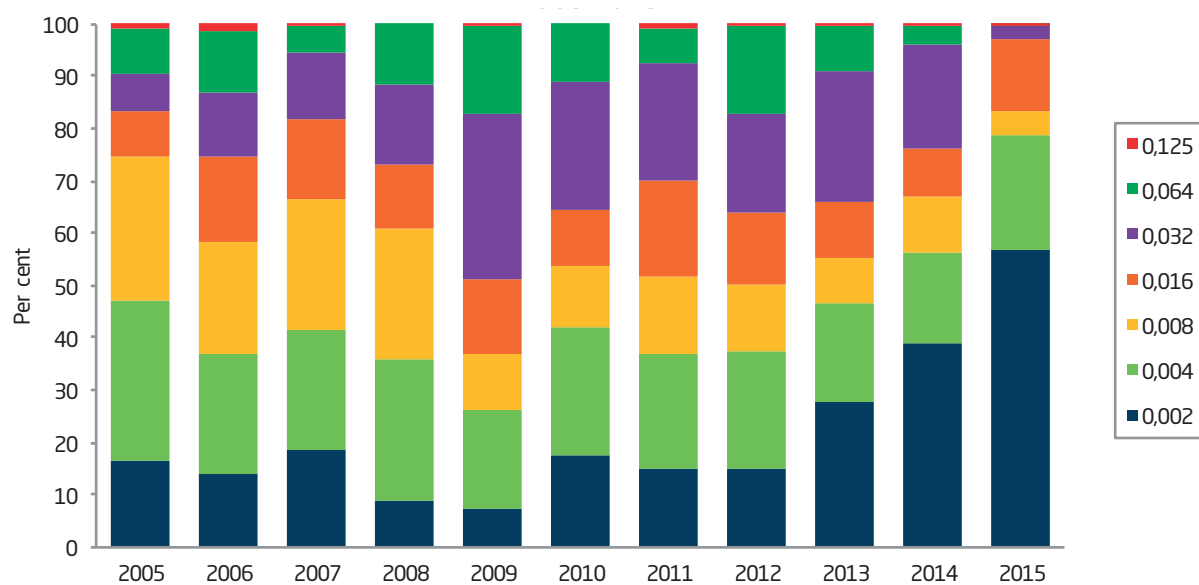


Figure 8.16. Distribution of ceftriaxone MIC values in gonococci, Denmark 2003-2015

DANMAP 2015



Textbox 8.5

Increasing rates of drug-resistance in *Mycobacterium tuberculosis* isolates in Denmark

Background: In recent years, the global incidence of tuberculosis (TB) has declined. However, the emergence of drug-resistant *Mycobacterium tuberculosis* (Mtb) strains challenges TB control in many parts of the world, especially Eastern Europe, Asia and Southern Africa (https://extranet.who.int/sree/Reports?op=vs&path=/WHO_HQ_Reports/G2/PROD/EXT/MDRTB_Indicators_map). Multidrug-resistant (MDR) TB is caused by Mtb resistant to at least rifampin and isoniazid, the two most important drugs for TB treatment. Extensively-drug-resistant (XDR) TB is a form of MDR-TB with additional resistance to any of the fluoroquinolones (e.g. moxifloxacin) and to at least one of three injectable second-line drugs (amikacin, capreomycin or kanamycin). By now, XDR-TB has been reported in 105 countries worldwide [1]. Mtb drug resistance is caused by spontaneous mutations in genes during the bacterial multiplication, which confer resistance to the drugs at different frequencies. Exposure to a single drug, or suboptimal drug concentrations, provides a selective environment favouring drug-resistant bacteria and the development of MDR/XDR-TB [2]. Previously, MDR-TB has only been identified in approx. 0.5% of all culture-verified TB-cases in Denmark, corresponding to approx. two cases per year, and until recently in 2013, XDR-TB had not been seen. In comparison, in the WHO European Region, the prevalence of MDR-TB among 88,732 new pulmonary TB cases tested was 18.4% in 2014 [3].

Methods: In Denmark, all clinical samples from suspected TB patients have been cultured at the International Reference Laboratory of Mycobacteriology, Statens Serum Institut (SSI). At first, stained using auramine-rhodamine followed by fluorescence microscopy. Then, cultured in liquid medium (BACTEC MGIT 960 System, BD) and solid medium (Löwenstein-Jensen). If culture positive, Mtb has been identified by MTBC TBc ID (BD) and GenoType MTBC (Hain Lifescience) and phenotypic susceptibility tests have been performed by the modified proportion method on liquid media in MGIT 320/960 Systems (BD) supplemented by genotypic susceptibility tests with GenoType MTBDR_{plus} and GenoType MTBDR_{sl} (Hain Lifescience).

Results: Five MDR-TB cases and 1 XDR-TB case were detected in Denmark in 2015 among approx. 358 notified cases, corresponding to 1.7% MDR/XDR-TB in total. The XDR-TB case was Russian-born and found to be resistant to 13 out of 15 tested antibiotics (!) In addition, 1 Somali-born MDR-TB case was resistant to all fluoroquinolones, and 1 Syrian-born MDR-TB case was resistant to 2 out of 3 tested injectable second-line drugs. Thus, these two cases were “pre-XDR”. Two of the 3 other MDR-TB cases were from Somalia whereas one was from Denmark. Additionally 13 cases (3.6%) were isoniazid mono-resistant.

Discussion: The 2015 drug-resistance figures for Mtb are the highest ever registered in Denmark with 5 MDR-TB-cases plus the only second XDR-TB case in the country; the first was registered in 2014. In addition, two of the MDR-TB cases were “pre-XDR” with additional problematic resistance. Although the total figures are small, we have to be prepared for an increasing number of MDR/XDR-TB cases in Denmark in the future. It is important to remind clinicians to be aware of drug-resistant tuberculosis and remember to send all specimens for culturing, among others, to secure sensitive, fast and correct susceptibility testing, performed through a combination of phenotypic- and genotypic test methods. The sensitivity of genotypic tests only is low [4] as well as the positive predictive value [5] and genotypic tests are only available for a limited number of drugs [2].

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[1] <http://who.int/mediacentre/factsheets/fs104/en/>

[2] Domínguez J, Boettger E, Cirillo D, Cobelens F, Eisenach K, Gagneux S, Hillemann D, Horsburgh R, Molina-Moya B, Niemann S, Tortoli E, Whitelaw A, Lange C, TBNET and RESIST-TB networks. Clinical implications of molecular drug resistance testing for *Mycobacterium tuberculosis*: a TBNET/RESIST-TB consensus statement. *Int J Tuberc Lung Dis* 2016;20(1):24-42.

[3] European Centre for Disease Prevention and Control/WHO Regional Office for Europe. Tuberculosis surveillance and monitoring in Europe 2016. Stockholm: European Centre for Disease Prevention and Control, 2016.

[4] Arentz M, Sorensen B, Horne DJ, Walson JL. Systematic review of the performance of rapid rifampicin resistance testing for drug-resistant tuberculosis. *PLoS One* 2013; 8(10): e76533

[5] Tomasichio M, Theron G, Pietersen E, Streicher E, Stanley-Josephs D, van Helden P, Warren R, Dheda K. The diagnostic accuracy of the MTBDR_{plus} and MTBDR_{sl} assays for drug-resistant TB detection when performed on sputum and culture isolates. *Scientific Reports* 2016. 6:doi:10.1038/srep17850

9

MATERIALS AND METHODS



9. Materials and methods

9.1 General information

For the DANMAP 2015 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 9.6.3).

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.

Antimicrobial usage for animals in Denmark

In Denmark, all antimicrobial agents used for treatment were available on prescription only. Antimicrobial agents were only sold by pharmacies or as medicated feed from the feed mills until 2007. From April 2007, the monopoly was suspended and private companies (four in 2015) were given license to sell prescribed veterinary medical products for animals, when following strict guidelines, identical to pharmacies. Furthermore in 2007, price setting of antibiotic was liberalised, which allowed for discounts e.g. to veterinarians, when buying larger quantities.

The 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 2015, 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, the majority of antimicrobial agents 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 antimicrobials 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 consumption of antimicrobials in different animal populations and in veterinary and human sectors. To allow for quantitative comparison in the different population, the quantity of antimicrobials used, their potency, their formulation, the route of administration, the age of the animals (where relevant) and the population sizes are accounted for by generating 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 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 life-span in each age group. In 2015, 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, and $Nw*5,800$ is the number of biomass days the exported pigs would have contributed to the live biomass if not exported.

9.2.2 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.

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] and census data for breeding animals [Statistics Denmark]. The average weight and life span for the growing animals (piglets, weaners and finishers) were estimated from the productivity number. 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

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 9.2.3

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.

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 (NaturErhvervstyrelsen) 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 [NH Henriksen, Danish Aquaculture].

9.2.3 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 the 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 a 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 calculated as one admission whenever a patient is admitted to one specific ward (one patient can be registered as admitted multiple times if transferred between wards during one 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

Meat inspection staff or abattoir personnel at the slaughter houses collected caecal samples from healthy pigs, cattle (< 1 year) and broilers according to sampling framework laid down by Decision 2013/652/EU. The samples were collected once a month throughout 2015, 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 caecum material from a single animal. All samples were sent to the Food Administrations (DVFA) laboratory in Ringsted and examined for indicator *E. coli* and ESBL, AmpC and carbapenemase-producing *E. coli*. In addition, samples from broilers and cattle were examined for *Campylobacter*, and samples from pig were examined for *Salmonella* and *Enterococcus faecalis*. *Salmonella* serotyping was performed at DTU National Food Institute.

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 2015 due to low numbers of isolates available from the national surveillance [Annual Report on Zoonoses in Denmark, 2015].

9.3.2 Meat

The regional DVFA officers collected packages of chilled or frozen broiler meat, pork and beef in Danish wholesale and retail outlets according to sampling framework laid down by Decision 2013/652/EU. The samples were collected two to four times a month throughout the year. (No samples were collected in January and July). 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 establishment 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 examined for indicator *E. coli*, *Enterococcus faecalis*, *Enterococcus faecium* and ESBL, AmpC and carbapenemase-producing *E. coli* at the DVFA laboratory.

The *Salmonella* isolates originate from the national programme at the slaughterhouses, where the carcass 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. If > 30.000 animals are slaughtered/year, 5 animals/day are pooled to one sample. If between 30.000 and 10.000 animals are slaughtered/year, 5 animals are sampled every second months (single samples). If between 10.000 and 1.000 animals are slaughtered/year, 5 animals are sampled every six months (single samples). If less than 1.000 animals are slaughtered/year, no samples are taken. All samples were processed at Industry laboratories. Isolates from all *Salmonella* positive samples were sent to DTU National Food Institute, where one isolate per sample were serotyped. *Salmonella* from broiler meat and beef, as well as imported meat are not included in DANMAP 2015 due to low numbers of isolates available from the national surveillance [Annual Report on Zoonoses in Denmark, 2015].

The *Campylobacter jejuni* isolates originated from the national control programme: Intensified control of *Salmonella* and *Campylobacter* in fresh meat based on a case-by-case risk assessment. Sampling was carried out every week by the regional DVFA officers, and included Danish and imported fresh 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. Sampling includes products with added saltwater but excludes products with other types of marinade. 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. Establishments where DVFA recently have deemed controlled batches of meat unsafe may receive additional control visits.

The Samples were examined for *Campylobacter* at the DVFA laboratory.

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. All blood isolates were referred to the Staphylococcus reference laboratory at SSI on a voluntary basis. In November 2006, methicillin-resistant *S. aureus* (MRSA) became a notifiable disease in Denmark and it became mandatory to submit all MRSA isolates to the reference laboratory.

Invasive *Streptococcus pneumoniae*, *Streptococcus pyogenes* (group A streptococci), group B, C and G streptococci. 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 group A, B, C and G streptococcal isolates are referred to SSI on a voluntary basis. Traditionally, only isolates from blood and spinal fluid are included in the DANMAP report.

***E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *E. faecium* and *E. faecalis*.** Data were provided on all clinical isolates recorded from either blood samples (*E. coli*, *K. pneumoniae*, *P. aeruginosa*, *E. faecium* and *E. faecalis*) or urine samples (*E. coli* and *K. pneumoniae*) submitted for susceptibility testing to the DCM at the following hospitals: Rigshospitalet, Hvidovre, Herlev, Region Zealand, Odense, Sønderborg, Esbjerg, Vejle, Herning/Viborg, Aarhus and Aalborg. No samples were collected from healthy humans.

9.4 Microbiological methods

9.4.1 Animals and meat

Salmonella from pigs and meat 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 CD [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 or type of meat.

Campylobacter from broilers, cattle and meat was isolated according to the methods issued by the NMKL [NMKL No. 119, 2007]. Identification was performed by microscopy and rtPCR assay. [Mayr *et al.* 2010. J Food Prot. 72(2):241-50]. 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 from a 5 gram of sample added to 45 ml of MacConkey broth and incubated o/n at 44°C. The suspension was streaked onto violet red bile agar and incubated for 24 h at 44°C. Presumptive *E. coli* was identified using TBX agar incubated o/n at 44°C. For Isolation of **ESBL-, AmpC- and carbapenemase-producing *E. coli***, the EURL-AR laboratory protocol was applied [October 2015, see also Textbox 7.1]. Only one indicator *E. coli*, ESBL-, AmpC- and carbapenemase-producing *E. coli* isolate per broiler flock, pig herd, cattle herd or meat sample was selected.

Enterococcus from pigs was isolate from an adequate amount of caecal material suspended in 2 ml BPW. The suspension was streaked onto Slanetz Bartley agar and incubated 48 h 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. faecium* and *E. faecalis* was selected

per meat sample and one *E. faecalis* isolate per pig herd was selected.

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 groups were performed by latex agglutination (Streptococcal Grouping Reagent, Oxoid, Roskilde, Denmark).

9.5 Susceptibility testing

Antimicrobial susceptibility testing of ***Salmonella*, *Campylobacter*, indicator *E. coli*, *Enterococcus* and *Staphylococcus aureus*** was performed as microbroth dilution MIC with Sensititre (Trek Diagnostic Systems Ltd., East Grinstead, UK). 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 are susceptibility tested in accordance with in Decision 2013/652/EU.

The 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., East Grinstead, UK) 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-resistant streptococci was performed with 1 unit penicillin G discs, 15 µg erythromycin discs and 2 µg clindamycin discs (Oxoid, Roskilde, Denmark), respectively, on Müller-Hinton agar (Müller-Hinton plate, 5% blood, 20 mg beta-NAD, SSI Diagnostica, Hillerød, Denmark). Isolates were simultaneously tested for inducible clindamycin resistance. Non-sensitive streptococci were tested further with the respective E-tests (Biomérieux), 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 defined as resistant.

***E. coli*, *K. pneumoniae*, invasive *P. aeruginosa*, invasive *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.

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 Data handling

9.6.1 Animals and meat

For the samples examined at the DFVA laboratory, sampling details and laboratory results were stored in the DFVA Laboratory system. Following validation by DFVA, data were electronically transferred to DTU National Food Institute. Sampling details and laboratory results for *Salmonella* in pork were stored in the DTU Laboratory system. At the DTU National Food Institute, data were harmonised and one isolate per epidemiological unit was selected for reporting. 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 and place 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 2015 for all years. Calculations of multi-resistance are not included in DANMAP 2015.

Table 9.1 presents the interpretation of MIC-values used for any combination of bacteria and antimicrobial agent. Since 2007, data are interpreted by EUCAST epidemiological cut-off values (ECOFFs) with a few exceptions described in Table 9.1. In general, if ECOFFs were re-validated and changed by EUCAST during the past year, all data presented from previous years were interpreted using the changed ECOFFs.

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 binomial proportion confidence intervals.

All handling, validation and analysis of results were carried out using Microsoft Excel and SAS® Software, SAS Enterprise Guide 6.1.

9.6.2 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 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 includ-

ed 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 SSL. Analysis including selection of isolates from blood and spinal fluid samples and removal of duplicate isolates was performed in Microsoft® Access.

***E. coli*, *K. pneumoniae*, invasive *P. aeruginosa*, invasive *E. faecium* and invasive *E. faecalis*.** All eleven DCM in Denmark provided data on resistance levels in *E. coli*, *K. pneumoniae*, invasive *P. aeruginosa*, 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.

9.6.3 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.

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Table 9.1. Interpretation criteriae for MIC-testing by EUCAST epidemiological cut-off values (blue fields) and the corresponding EUCAST clinical breakpoints (white fields)

DANMAP 2015

	<i>Salmonella</i>		<i>E. coli</i>		<i>E. faecium</i>		<i>E. faecalis</i>		<i>C. jejuni</i>	
Antimicrobial agent	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	Clinical breakpoint µg/ml
Ampicillin	>8*	>8*	>8*	>8*	>4*	>8*	>4*	>8*		
Azithromycin										
Cefepime			>0.125*							
Cefotaxime	>0.5*	>2*	>0.25*	>2*						
Cefoxitin	>8*		>8*							
Ceftazidime	>2*	>4*	>0.5*	>4*						
Chloramphenicol	>16*	>8*	>16*	>8*	>32*		>32*			
Ciprofloxacin	>0.064*	>0.064*	>0.064*	>1*	>4*		>4*		>0.5*	>0.5*
Colistin	>2*/8 ^(a)	>2*	>2*	>2*						
Daptomycin					>4*	>4*	>4*	>4*		
Ertapenem	>0.064*		>0.064*							
Erythromycin					>4*		>4*		>4*	>8*
Imipenem	>1*		>0.5*							
Gentamicin	>2*	>4*	>2*	>4*	>32*	>4*	>32*	>4*	>2*	
Linezolid					>4*		>4*			
Meropenem	>0.125*	>8*	>0.125*	>8*						
Nalidixic acid	>16*		>16*						>16*	
Quinupristin/dalfopristin					>4 ^(c)	>4*			>4*	
Streptomycin										
Sulfonamide	>256 ^(b)		>64*							
Teicoplanin					>2*	>2*	>2*	>2*		
Tetracycline	>8*		>8*		>4*	>0.5*	>4*	>0.5*	>1*	>2*
Tigecycline	>1*	>2*	>1*	>2*	>0.25*		>0.25*			
Trimethoprim	>2*	>4*	>2*	>4*						
Vancomycin					>4*	>4*	>4*	>4*		

Note: * indicates EUCAST epidemiological cut-off values (ECOFFs) and EUCAST clinical breakpoints.

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 for sulfamethoxazole, so the previous cutoff (>256) was maintained.

c) The EUCAST ECOFF (>1) was not applied for quinupristin/dalfopristin (tradename synergid) according to investigations presented in DANMAP 2006.

10

TERMINOLOGY

List of abbreviations

AGP	Antimicrobial growth promoter
ATC	Anatomical Therapeutic Chemical Classification System
ATCvet	Anatomical Therapeutic Chemical Classification System for veterinary medicines
CA	Community-acquired
CC	Clonal complex
CDI	<i>Clostridium difficile</i> 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 <i>Staphylococcus aureus</i>
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
SEGES	Knowledge Centre for Agriculture
SSI	Statens Serum Institut
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 section of 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 has been used since DANMAP 2003. For more details, see Chapter 10, Materials and Methods. The DADDs used in DANMAP 2015 are presented in the web annex.

DADD per 1,000 animals per day (DAPD). Trends in veterinary consumption, both within and across species, are presented in DAPD, allowing for comparison between sectors and adjusting 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 1,000 inhabitants per day (DID). Consumption in both primary health care, hospital care and the overall total consumption is presented in DID, allowing for comparison between sectors and for illustration of the consumption in hospital care without taking 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/1,000 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.

Intramammaria. 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.

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 bio-masses of Danish pets used for estimating veterinary consumption only include dogs and cat.

Piglet. The newborn 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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