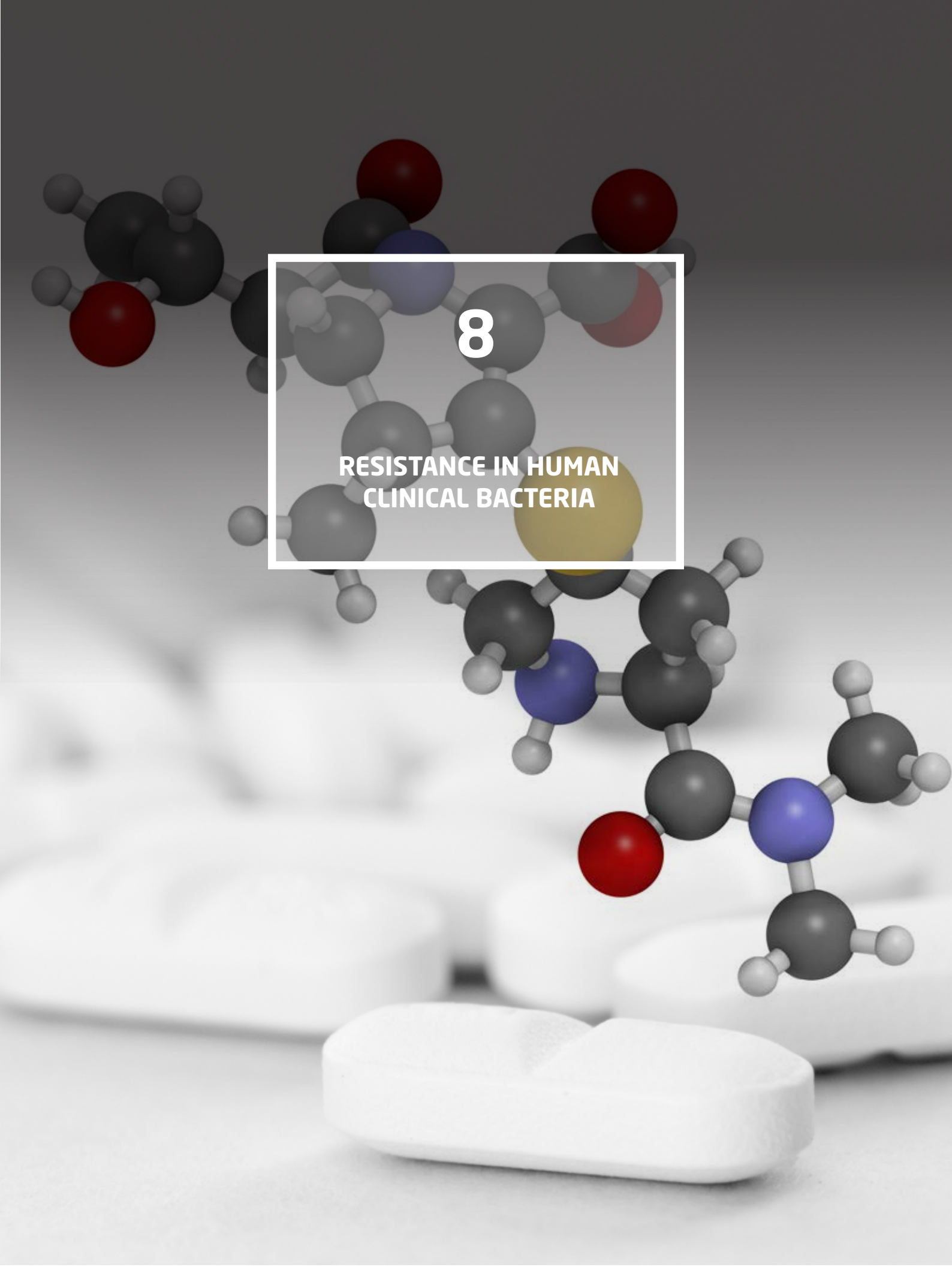


8

RESISTANCE IN HUMAN CLINICAL BACTERIA

8. Resistance in human clinical bacteria

Highlights: In DANMAP 2018, the Danish Microbiology Database (MiBa) has been used as primary data source for monitoring prevalence and resistance in several human clinical bacteria (Textbox 8.1).

Increasing trends in the number of invasive cases continued for the majority of the surveilled species in 2018 (section 8.1).

Blood infections with ***Staphylococcus aureus*** (subsection 8.3.8) have increased gradually from 1400 cases in 2010 to nearly 2300 cases in 2018. In 2018 1.6% of these were methicillin resistant (MRSA). The prevalence of **LA-MRSA CC398**-postive pig farms and the number of LA-MRSA CC398 infections in the general population seem to have reached a maximum (Textbox 8.2).

Resistance rates to 3rd generation cephalosporins have increased slowly during the past 10 and five years in urinary ***Escherichia coli*** (subsection 8.2.1). In invasive *E. coli* no significant increase in 3rd generation cephalosporin resistance was observed. A significant increase in the prevalence of the **ESBL** enzyme, CTX-M-15 (subsection 8.3.1), was found in 3rd generation cephalosporin resistant *E. coli* from bloodstream infections for 2018.

Mecillinam resistance in urinary cases of ***Klebsiella pneumoniae*** (subsection 8.2.2) remained in 2018 at a high level of 16-17%. Thereby the resistance rates to the standard per oral treatment against urinary tract infections with pivmecillinam are high. This places a pressure on the usage of ciprofloxacin, for which the resistance rate also increased from 2017 (5.4-7.6%) to 2018 (6.4-8.9%).

Increasing resistance towards ciprofloxacin was also observed for ***Neisseria gonorrhoeae*** (subsection 8.3.9) – reaching a level of 40% in 2018 (28% in 2017 and 18% in 2016). No isolates were ceftriaxone resistant.

For invasive ***Enterococcus faecium*** cases (subsection 8.2.5), continued escalation of vancomycin-resistance was observed (12 % compared to 7.1% in 2017). Detection and spread of **VVE** (vancomycin-variable enterococci), as part of this steep increase, is described in subsection 8.3.3.

In 2018, an overall 44% increase of submitted **carbapenemase-producing organisms** (CPO) (subsection 3.2.1) was observed compared to 2017 (177 CPO from 160 patients compared to 123 CPO from 115 patients). Several outbreaks with carbapenemase-producing enterobacteriacae (CPE) were observed during 2018. In September 5th 2018 the Danish Health Authority made CPO notifiable.

8.1 Introduction

In Denmark all hospitals and general practitioners are serviced by 10 departments of clinical microbiology (DCMs) located at hospitals in the five regions of Denmark. The national surveillance of resistance in human clinical bacteria is based on either data from routine diagnostics performed at the 10 departments of clinical microbiology (DCMs) in Denmark or on resistance and typing results from isolates received at the reference laboratories at SSI for further characterisation. Isolates are received either based on a mutual agreement of voluntary submission of specific species and/or types of resistances or as part of a mandatory surveillance program of diseases made notifiable by the Danish Health Authority (Table 8.1).

Table 8.1 Summary of species/types, sampling and sources of national resistance surveillance in isolates from humans, 2018

DANMAP 2018

Routine diagnostics from all 10 DCMs in Denmark. Data are directly identified and extracted from MiBa	
Species	**Sampling**
Escherichia coli *Klebsiella pneumoniae*	First isolate per patient per year from blood or cerebrospinal fluid, from urine in hospitalised patients and from urine from primary health care
Pseudomonas aeruginosa *Acinetobacter species* *Enterococcus faecalis* *Enterococcus faecium*	First isolate per patient per year from blood or cerebrospinal fluid
Voluntary submission of isolates to the refererance laboratories at SSI	
Species or type	**Sampling**
Staphylococcus aureus Beta-haemolytic streptococci	One isolate per patient per episode from blood or cerebrospinal fluid
Neisseria gonorrhoeae	One isolate per patient per episode from all sample sites
Third-generation cephalosporin resistant *Escherichia coli*	First isolate per patient within 12 months from blood
Vancomycin-resistant enterococci	First isolate per patient within 12 months irrespective of sample site (excluding screening samples)
Enterococci with exceptional phenotype (e.g. linezolid, daptomycin and tigecycline R)	First isolate per patient within 12 months irrespective of sample site (clinical and screening samples)
All bacterial species with other exceptional phenotypes (e.g. acquired colistin resistance)	One isolate per patient per episode irrespective of sample site
Mandatory submission of isolates to the reference laboratories at SSI	
Species or type	**Sampling**
Carbapenemase-producing organisms	First isolate per patient within 12 months irrespective of sample site (clinical and screening samples)
Methicillin-resistant *Staphylococcus aureus*	First isolate from all new cases of MRSA postive patients irrespective of sample site (clinical and screening samples)
Streptococcus pneumoniae	One isolate per patient per episode from blood or cerebrospinal fluid
Haemophilus influenzae serotype b, Hib	All invasive isolates

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

8.1.1 Surveillance based on MiBa data

The surveillance of resistance in invasive isolates of *Escherichia coli, Klebsiella pneumoniae, Enterococcus faecalis, Enterococcus faecium, Pseudomonas aeruginosa* and *Acinetobacter* species and urine isolates of *E. coli* and *K. pneumoniae* is based on data from routine diagnostics from the DCMs in Denmark. Surveillance has been performed since 1995 – in the very beginning based on reporting from two DCMs, but quickly joined and supported by most DCMs in Denmark. From 2009 to 2014, DANMAP received data from all but one DCM resulting in a coverage of approx. 95% of the population. Since 2015, all DCMs participate in the program resulting in a 100% population coverage. In 2018, all these data were extracted directly from the Danish Microbiology Database (MiBa) (https://miba.ssi.dk/Service/English.aspx). A description of MiBa and the usage and validation of MiBa-data is given in Textbox 8.1. Due to the quality of data in MiBa, this register-based surveillance gives the best achievable monitoring of prevalence of resistance in the surveilled species. Materials and methods are described in chapter 9.

8.1.2 Surveillance based on data from the reference laboratories

In addition to the MiBa-based monitoring of resistance a surveillance program exists, based on submission of specified strains to the reference laboratories at SSI. Isolate-based surveillance gives the opportunity to further characterise strains and resistance mechanisms and type the isolates; since 2015-2016, this has been mainly performed by the use of whole genome sequencing (WGS). Voluntary submission of specified strains has existed since 1957; beginning with the submission of all strains of *Staphylococcus aureus* from bloodstream infections. The submission of invasive beta-haemolytic streptococci is also voluntary, while invasive *Streptococcus pneumoniae*

and *Haemophilus influenzae* serotype b (Hib) are mandatory to submit. The detection of methicillin-resistant *S. aureus* (MRSA) and *Neisseria gonorrhoeae* from all clinical sites is notifiable but the submission of the isolated strains of MRSA is mandatory, while the submission of isolated strains of *N. gonorrhoeae* is voluntary. In addition, the DCMs voluntarily submit isolates of ESBL-producing *E. coli* from blood and vancomycin-resistant enterococci (VRE) from all clinical sites, based on a mutual agreement to survey the development and spread of these often multiresistant bacteria at Danish hospitals. The Danish Health Authority made carbapenemase-producing organisms (CPO) notifiable, and submission of all clinical and screening isolates irrespective of sample site has been mandatory as of 5th September 2018. Before that, CPO was submitted on a voluntary basis.

8.1.3 Surveillance of invasive cases

A key function in the monitoring of antimicrobial resistance for DANMAP is to surveil the number of resistant bacteria in invasive cases (blood and cerebrospinal fluid). This is harmonised with the monitoring performed by the European Antimicrobial Resistance Surveillance Network (EARS-Net). Figure 8.1 presents total numbers of invasive cases in Denmark from 2009 to 2018 for the bacterial species included in the surveillance programmes for both DANMAP and EARS-Net. Excluded from the figure is *Acinetobacter* species – these have only been registered since 2012 in DANMAP and the number of cases are low (55 to 70 cases annually). For all registered species, the following case definitions applies: The first sample, by date of sample collection, of each given bacterial species per unique patient per year of observation. Duplicates within the year of observation from the same patient are removed.

Since 2010, the total number of registered invasive cases increased by 44% (from 8,021 to 11,589 cases). The largest increase observed was for *S. aureus* (68%). The only species with an overall decreasing number of cases was *S. pneumoniae* (-16%). From 2017 to 2018 the number of invasive *S. pneumoniae* increased for the first time in the decade.

Figure 8.2a shows the number of invasive cases per 100,000 inhabitants in Denmark per year for 2010 to 2018. During this period, the Danish population increased by 4.5% (from 5,534,738 to 5,781,190 inhabitants). Figure 8.2b shows the number of blood cultures taken per 100,000 inhabitants per year for the same period. In addition, the number of unique patients being blood cultured per 100,000 inhabitants per year is shown. This demonstrates that the number of unique patients with at least one blood culture taken per year has increased dramatically from approximately 2,060 patients per 100,000 inhabitants in 2010 to approximately 2,940 patients per 100,000 inhabitants in 2018 (an increase of 43%). The total number of blood cultures taken (as registered with a unique sample ID in MiBa) per 100,000 inhabitants has increased even more (52%). Thus, on average more patients have more blood cultures taken each year.

Figure 8.1 Number of submitted invasive isolates for each species under surveillance, Denmark DANMAP 2018

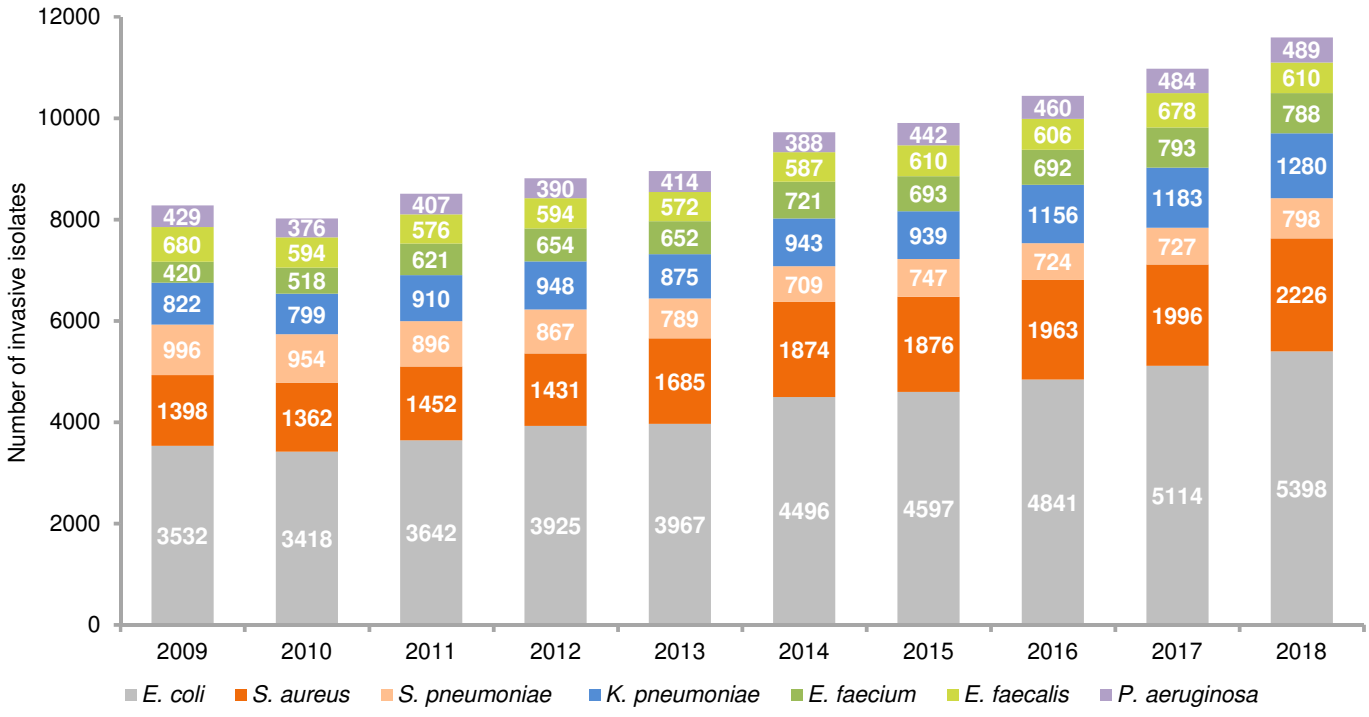
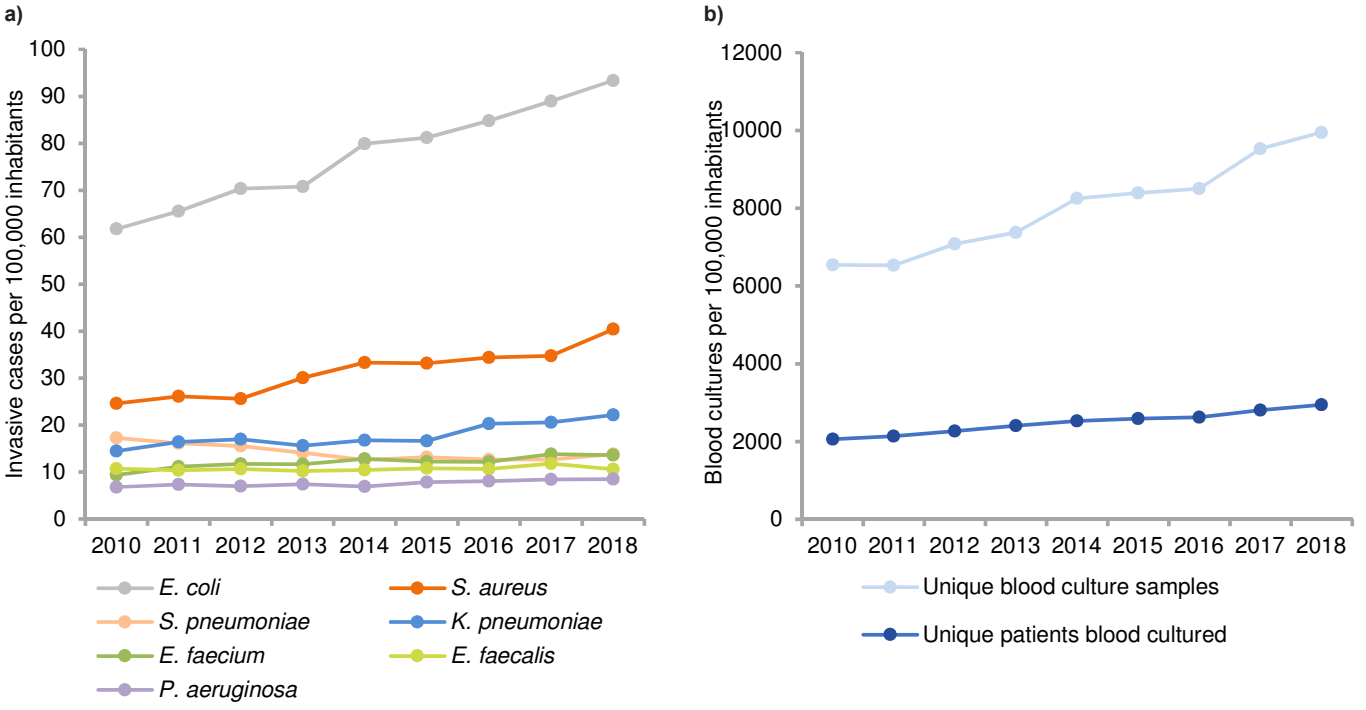


Figure 8.2 Invasisve cases and blood cultures taken per 100,000 inhabitants, Denmark DANMAP 2018



Changes in hospital workflow, improved culturing methods and demographic changes with a growing population of elderly and chronically ill or immunocompromised patients may explain some of the observed changes. The increasing number of invasive infections is of concern to a health care system that is under pressure. It demands fast and effective antibiotic treatment, while simultaneously increasing the risk for the development and selection of resistant bacteria due to a higher consumption of antimicrobials. These resistant bacteria can then be spread in hospital environments with fragile patient populations underlining the need for a health care system with firmly established infection prevention and control. The importance of proper diagnostics combined with a rational use of antibiotics, reserving the most broad-spectrum antibiotic classes to the patients with multiresistant infections is underlined as well.

The next sections in this chapter present the individual results for the species and/or resistance types under surveillance.

Textbox 8.1

MiBa, the Danish Microbiology Database – now prepared for use in national AMR-surveillance

MiBa is a milestone in the development of a national digital surveillance system for infectious diseases in Denmark and an example of how surveillance systems can be an integrated part of national healthcare infrastructure.

MiBa is a nationwide, automatically updated database containing all test results from all departments of clinical microbiology (DCMs) in Denmark..

The objectives of MiBa are:

- To provide access for healthcare professionals to microbiological test results from all of Denmark for patients in their care
- To provide the foundation for a flexible, timely and complete national surveillance of all laboratory confirmed infectious diseases and microorganisms
- To serve as a shared resource for research
- To ensure automatic transfer of data to other databases monitoring e.g. AMR data and hospital acquired infections
- To aid informed decision making on the treatment of individual patients as well as development of local and national antibiotic guidelines

MiBa is integrated into regional electronic health record systems, providing overview and access at a national level to microbiological test reports for relevant health care personnel. This is important e.g. when patients are moved between hospitals and regions or between different sectors with in the health care system. The patients themselves and doctors outside hospital settings, for instance general practitioners, have access via the national health portal www.sundhed.dk.

MiBa is also a primary data source for national surveillance of laboratory confirmed infectious diseases and microorganisms. The development of a fully automated MiBa-based surveillance system is a complex, ongoing process, which includes standardisation of data-coding, clarification of concepts and a close collaboration between stakeholders, in particular all Danish DCMs.

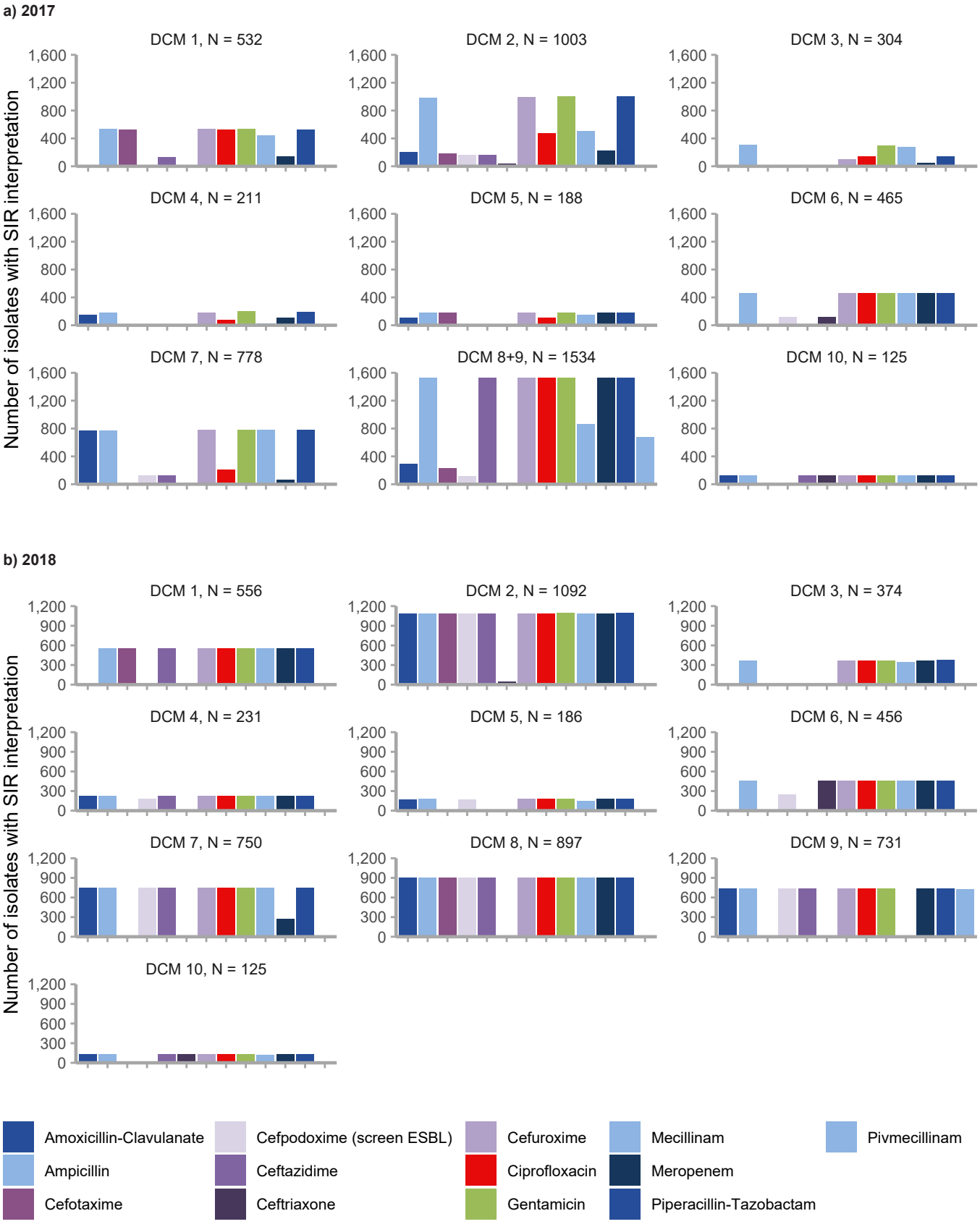
When MiBa was launched in 2010, only information relevant for patient treatment was included in the test reports. The copy of the report transferred to MiBa from the local laboratory information system (LIS) was identical to the report sent to the clinician for diagnostic information and treatment purposes. Denmark has a longstanding tradition for selected reporting of susceptibility results supporting local antibiotic treatment guidelines. Hence, not complete laboratory data, but only the antibiogram of relevance to the clinician's choice of antimicrobial treatment was transferred to MiBa.

In 2017, a new and expanded standard data-transfer protocol was developed and implemented, allowing more complete laboratory data and specifically data relevant for surveillance purposes to be included in the copy transferred from the local LIS to MiBa. From 2018 and onwards, practically all tests for AMR performed in the departments of clinical microbiology have been included in the copy of the report transferred to MiBa, enabling a comprehensive national surveillance of AMR. These "extended" AMR-data are not visible in the version of the report accessed by the clinician. This new and expanded data model for microbiology in Denmark allows MiBa to be used for DANMAP and to form the future basis for national AMR surveillance.

Figure 1 shows examples of antibiograms from human invasive *E. coli* analysed at the Danish DCMs available from MiBa before and after the implementation of the new protocol. It demonstrates clearly, how AMR-data are now transferred systematically and almost completely to MiBa.

Figure 1 Number of invasive *E. coli* (from unique patients) identified in MiBa for, a) 2017 and b) 2018, from the 10 DCMs in Denmark, and the available parts of their antibiograms

DANMAP 2018



continued ... Textbox 8.1

Data for DANMAP used to be reported manually and non-standardized from the DCMs to SSI - a time consuming, cumbersome task demanding active reporting from the labs and data cleaning at SSI. In DANMAP 2018, MiBa was used as the primary data source for monitoring reported resistance in clinical bacteria in humans (calculations and Figures in section 8.2).

To ensure a smooth and safe shift from the old to the new surveillance system, a comprehensive validation of the data, focusing on completeness and correctness, was performed:

1. Was it possible to extract complete national data from MiBa to fulfil the purposes of the surveillance as usual?
2. Were there any mismatches between MiBa data and data delivered from the individual DCM – and can mismatches be explained?

Selected results are presented in Figure 2 (A full dataset of the comparisons is available online).

In general, MiBa data were identical or nearly identical to the DCM data. When differences turned up, we were able to identify the individual cases and explain the cause of the difference.

Most differences could be explained by:

- The data provided by the individual DCM from their own laboratory information system differed a bit in the criteria for in-/exclusion. Examples of this would be: Inclusion of other specimen than blood, e.g. peritoneal fluid collected in a blood culture flask or inclusion of a specimen, identified by DNA detection or microscopy but never cultured
- Differences in the way of sorting and selecting, when only data from the first isolate per patient per sample type per year were supposed to be included
- Missing data from the dataset provided by the individual DCM
- Some S-I-R interpretations were not registered in MiBa, even though they were present in the data provided by the DCMs

In the process of clarifying mismatches we obtained new insight in details of the data, which is important when MiBa-data on AMR are processed automatically. The fact that the selection criteria can be standardised across all DCMs when performed centrally will further increase data quality. Such standardisations and the principles for interpretation of data will be decided in close collaboration with the DCMs in near future.

This new MiBa based reporting will provide timely, harmonised and cleaned AMR data, ready for analysis, with a tremendous reduction in workload. This will lead the way to online publishing of AMR data in (close to) real time.

In conclusion, MiBa data (for surveillance purposes) were as comprehensive as the combined data provided individually from each DCM. Basing DANMAP and national AMR surveillance on MiBa reduces workload considerably, increases data quality and allows real time analysis of AMR data. The next step is to implement standardised search queries and refined algorithms for automatic data processing. The next goal is to develop a full automatic online visualisation tool for national AMR surveillance data in close collaboration with the DCMs.

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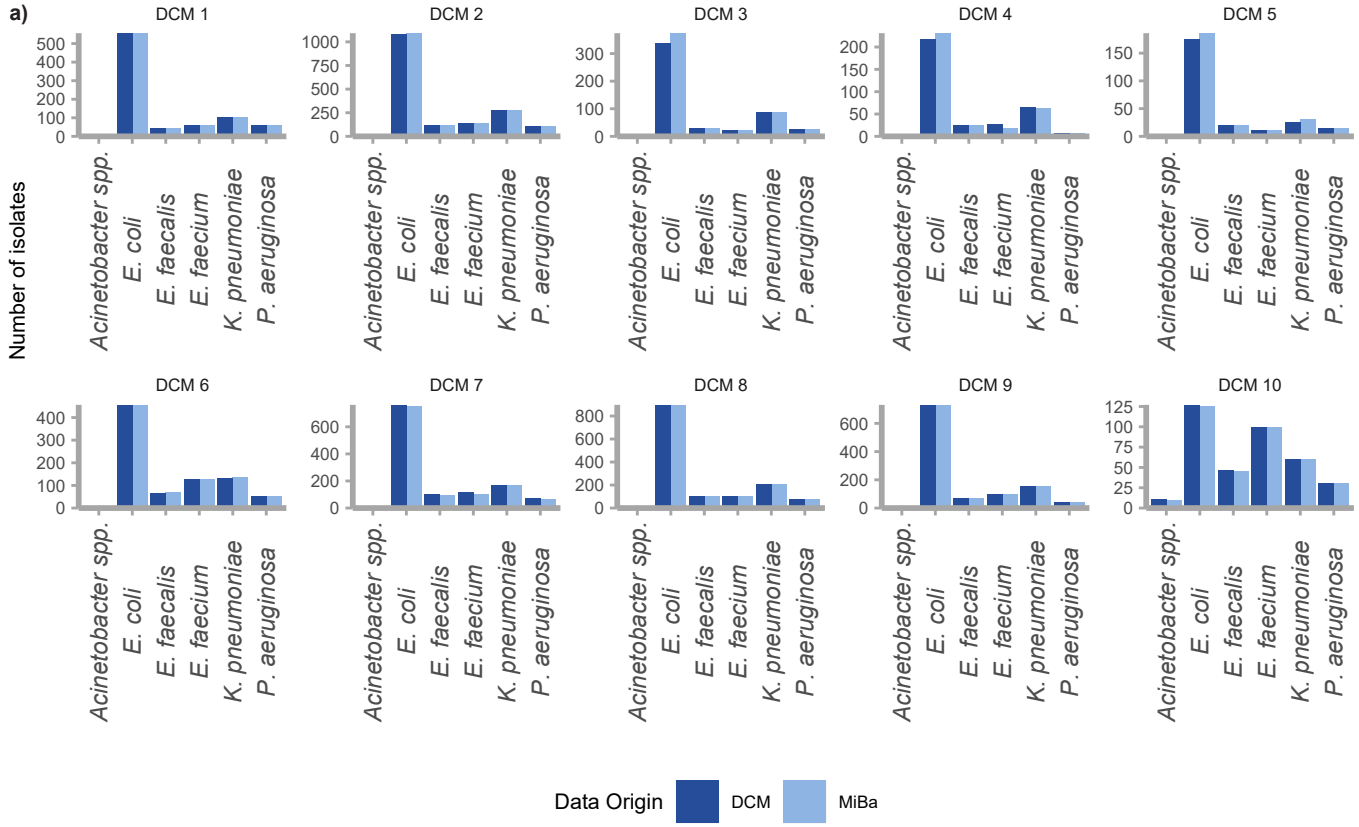
For further information: Marianne Voldstedlund, mav@ssi.dk and Sissel Skovgaard, sisk@ssi.dk

Figure 2 Comparing MiBa data on invasive isolates with data reported from the DCMs (2018) DANMAP 2018



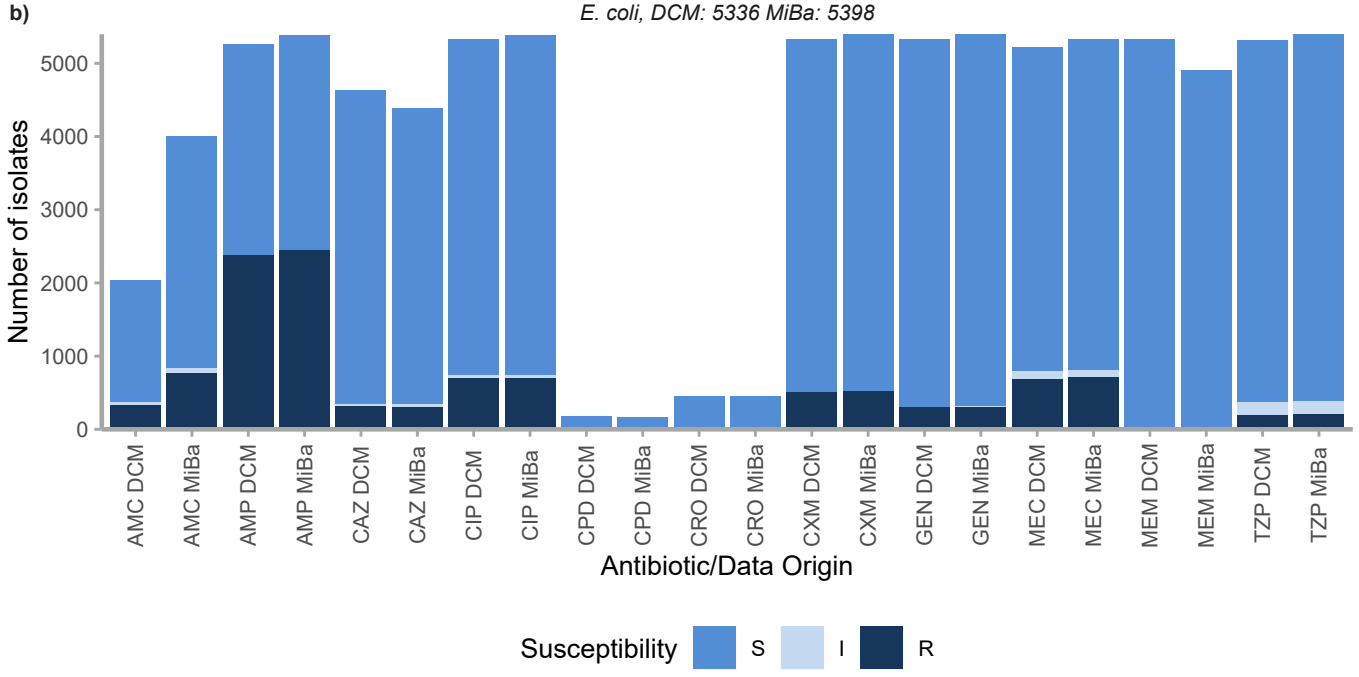


Figure 2a) Comparisons of total numbers of invasive isolates subdivided on species-level and on individual DCM. 2b) Comparisons of S-I-R interpretations of invasive *E. coli* isolates for all 10 DCMs added together
Glossary: AMC = Amoxicillin/Clavulanate, AMP = Ampicillin, CAZ = Ceftazidime, CIP = Ciprofloxacin, CPD = Cefpodoxime, CRO = Ceftriaxone, CXM = Cefuroxime, GEN = Gentamicin, MEC = Mecillinam, MEM = Meropenem, TZP = Piperacillin/Tazobactam

8.2 Surveillance based on MiBa data

8.2.1 *Escherichia coli*

Escherichia coli (*E. coli*) is by far the most frequent cause of community- and hospital acquired urinary tract infections and of bacteraemia in Denmark accounting for 55%, 45% and 21%, respectively, of all registered positive cultures in MiBa for 2018. It is part of the normal intestinal flora in both animals and humans, where it comes into close proximity to many other bacterial species. Transferred resistance mechanisms from these to *E. coli* are thus frequently seen as is the development of resistance through mutations. Since *E. coli* is the most frequent cause of urinary tract infections and bacteraemia, it is also one of the bigger drivers of antibiotic use.

Invasive cases from hospitals

For 2018, altogether 5,398 unique patients with invasive *E. coli* isolates from all departments of clinical microbiology (DCMs) in Denmark, were identified in MiBa. All 10 DCMs routinely (>75% of isolates) registered antimicrobial susceptibility interpretations to ampicillin, ciprofloxacin, piperacillin/tazobactam, gentamicin, cefuroxime and mecillinam.. In addition, nine DCMs routinely registered antimicrobial susceptibility to 3rd generation cephalosporins and meropenem and seven routinely registered antimicrobial susceptibility to amoxicillin/clavulanic acid. Tested 3rd generation cephalosporins were either ceftazidime, ceftriaxone or cefpodoxime, while the tested carbapenem was meropenem for all DCMs in 2018. Resistance testing was mainly performed by disc diffusion. The presented data consist of the registered interpretation results, performed by the DCMs, based on the S-I-R system. Zone diameters have also been registered since 2015 and will be commented on in specific cases.

Resistance rates for all tested antimicrobials, presented as a national mean for each antibiotic class, are summarized in Table 8.2. In Figure 8.3 rates of resistance are shown for the past decade – here data are presented as a national mean, when at least six DCMs have registered routine testing. Time trends and significance levels of these, based on the resistance rates five and ten years back, respectively, are presented in Figure 8.3c. Test results for mecillinam resistance in invasive *E. coli* are excluded from Figure 8.3, since the S-I-R interpretation rules for the individual DCM differ and/or vary over time, making comparison of the results difficult and time trends unreliable.

A continuous increase in the number of invasive *E. coli* cases was observed throughout the years, from 3,426 cases in 2010 to 5,398 cases in 2018. This corresponds to 61.8 cases and 93.4 cases per 100,000 inhabitants respectively and an increase of 51%.. Simultaneously, the total number of blood cultures taken also increased steeply with 52% per 100.000 inhabitants (subsection 8.1.3).

In 2018, the proportion of ciprofloxacin resistant strains (13.0%) was comparable to 2017 (12.8%) after a marked increase from 2016 to 2017. This increase mainly reflected a change in the interpretation of S-I-R more than a true epidemiologic change, since new EUCAST breakpoints for ciprofloxacin were implemented in most of the Danish DCMs as of January 2017.

For cefuroxime resistance in invasive *E. coli* significant increasing trends were observed for the past decade as well as for the past five years. For 3rd generation cephalosporins in invasive *E. coli* there is an increasing trend in resistance rates from 2017 to 2018, but the five year trend analysis shows no significance.

Table 8.2 *Escherichia coli*. Resistance (%) in isolates from humans, 2018 DANMAP 2018

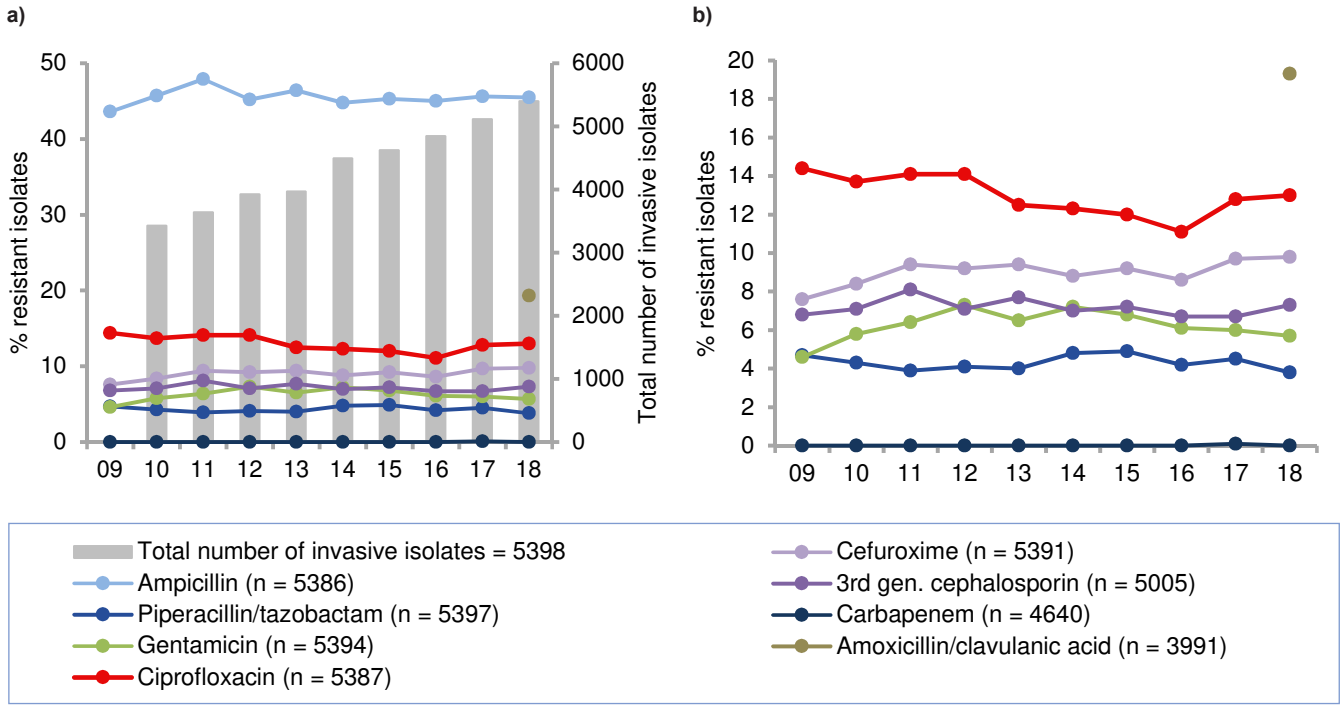
	Invasive isolates, hospitals %	Urine isolates, hospitals %	Urine isolates, primary health care %
Ampicillin	45	42	37
Mecillinam	13	7.4	5.1
Piperacillin/tazobactam	3.8	3.5	2.9*
Amoxicillin/clavulanic acid	19	12*	6.3*
Sulfonamide		31*	28
Trimethoprim		25	23
Nitrofuratoin		1.2*	0.8*
Gentamicin	5.7	4.7	3.8*
Ciprofloxacin	13	11	8.1
Cefuroxime	9.8	7.2	5.6*
3rd generation cephalosporins	7.3	6.4	4.9
Carbapenem	0.0	0.0	0.0*
Max. number of isolates tested for resistance to the presented antibiotics	5397	47828	80703

The presented resistance rates are means of the resistance rates determined by the individual DCMs. Included are results from all DCMs where > 75% of the isolates in each antibiotic/sample group were susceptibility tested. The * markes where less than 6 (out of totally 10 DCMs) tested a sufficient percentage of their samples. For carbapenems in urines from primary health care this were only two DCMs

Resistance to piperacillin/tazobactam and gentamicin both showed significant decreasing trends for the past five years. For more details see Figure 8.3.

The number of carbapenem resistant invasive *E. coli* isolates remained very low with two carbapenem resistant and four intermediate susceptible strains in 2018.The level of multi-resistant (combined resistance to 3rd generation cephalosporins, ciprofloxacin and gentamicin) invasive *E. coli* remained at around 2% (Table 8.3). For colistin none of the invasive *E. coli* were registered resistant. However, colistin resistance is not tested for routinely.

Figure 8.3 *Escherichia coli*. Resistance (%) in invasive isolates from humans, Denmark DANMAP 2018



c)

Substance	Time trends (Cochran-Armitage test)	
	10 years (2009 - 2018)	5 years (2014 - 2018)
Ampicillin	↑ p = 0.46	↓ p = 0.23
Piperacillin/tazobactam	↓ p = 0.1	↓ p = 0.4
Gentamicin	↑ p = 0.17	↓ p = 0.0002
Ciprofloxacin	NA	NA
Cefuroxime	↑ p = 0.001	↑ p = 0.03
3rd generation cephalosporins	↓ p = 0.08	↑ p = 0.42
Carbapenem	↑ p = 0.1	↑ p = 0.31

a) Resistance results and the total numbers of invasive isolates are presented. b) Resistance rates excluding ampicilin. c) Time trends and significance levels for the past five and 10 years, respectively (Cochran-Armitage test).
The number (n) in parentheses represents the numbers of isolates tested in 2018. NA = Not applicable.

Table 8.3 *Escherichia coli*. Combined resistance to 3rd generation cephalosporins, ciprofloxacin, and gentamicin (multiresistance) in invasive isolates from humans, Denmark DANMAP 2018

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

Urinary cases from hospitals

For 2018, altogether 47,914 unique patients with *E. coli* isolates, cultured in urine samples from hospital patients from all DCMs in Denmark, were identified in MiBa. 3,946 unique patients with *E. coli* isolates, cultured in urine samples were excluded because it was not possible to categorise them as hospital or primary urines. However for 1,273 of these samples the patients were already represented in either hospital or primary care or both, with other *E. coli* urine cultures in 2018.

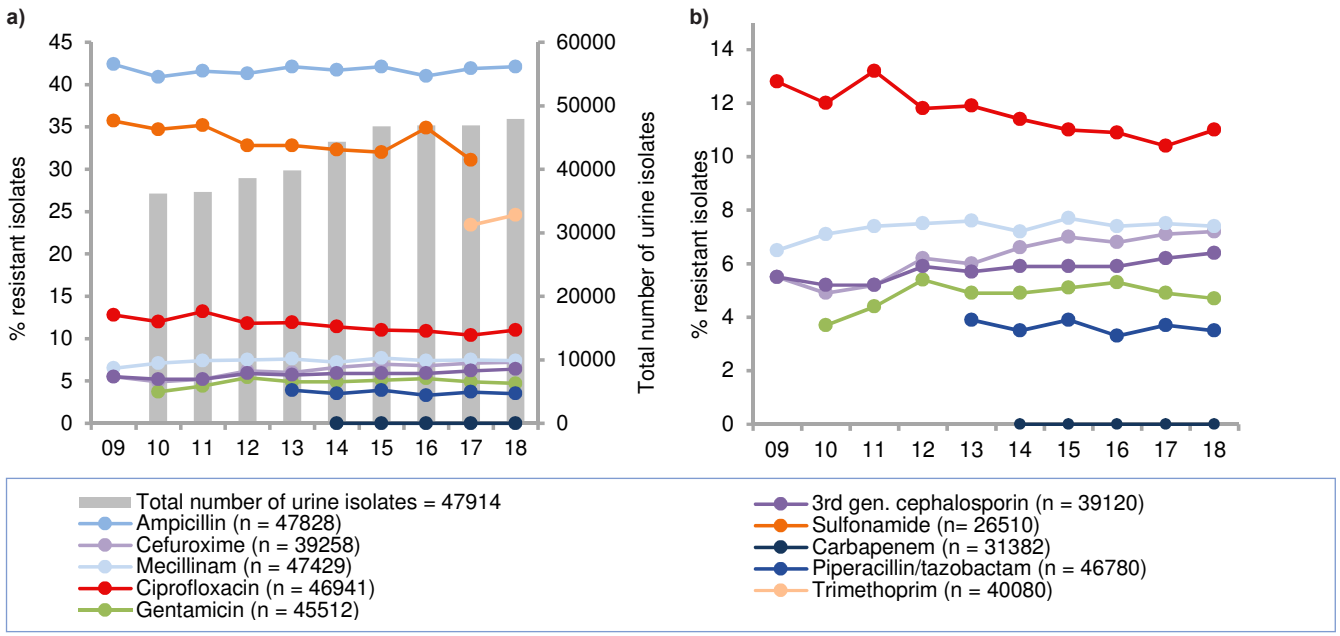
All 10 DCMs routinely (>75% of isolates) registered antimicrobial susceptibility interpretations to ampicillin, mecillinam, piperacillin/tazobactam and ciprofloxacin. In addition nine DCMs routinely registered antimicrobial susceptibilities to gentamicin and eight DCMs to cefuroxime and trimethoprim. Seven DCMs routinely registered antimicrobial susceptibilities to 3rd generation cephalosporins, six DCMs to carbapenem and five DCMs to amoxicillin/clavulanic acid and sulfonamide. Four DCMs routinely registered antimicrobial susceptibilities to nitrofurantoin.

In Table 8.2. resistance results for all tested antimicrobials are summarised together with the results from the invasive isolates as a national mean for each antimicrobial. In Figure 8.4, rates of resistance are presented as a national mean when at least six DCMs have registered routine testing for a given drug. Time trends and significance levels, based on the resistance rates for the past five and 10 years respectively, are presented in Figure 8.4c.

Time trend analysis revealed that mecillinam, cefuroxime and 3rd generation cephalosporins all had significantly increasing resistance rates when looking back 10 years. Looking back five years increases were no longer significant for mecillinam, while cefuroxime and 3rd generation cephalosporin resistance rates were still increasing. For more details see Figure 8.4.

In 2018, 24 carbapenem resistant and 20 intermediate susceptible *E. coli* urine isolates from hospital patients were registered.

Figure 8.4 *Escherichia coli*. Resistance (%) in urine isolates from humans in hospitals, Denmark DANMAP 2018



c)

Substance	Time trends (Cochran-Armitage test)	
	10 years (2009 - 2018)	5 years (2014 - 2018)
Ampicillin	↑ p = 0.32	↑ p = 0.16
Mecillinam	↑ p = 4.578e-10	↑ p = 0.22
Piperacillin-tazobactam		↓ p = 0.32
Sulfonamide	↓ p < 2.2e-16	↓ p = 3.633e-05
Gentamicin		↓ p = 0.05
Ciprofloxacin	NA	NA
Cefuroxime	↑ p < 2.2e-16	↑ p = 0.0005
3rd generation cephalosporins	↑ p = 1.052e-14	↑ p = 0.0009

a) Resistance rates and total numbers of urine isolates are presented, b) Resistance rates excluding ampicillin, sulfonamide and trimethoprim,
c) Time trends and significance levels for the past five and 10 years respectively
The number (n) in parentheses represents the numbers of isolates tested in 2018. NA = Not applicable

Urinary cases from primary health care

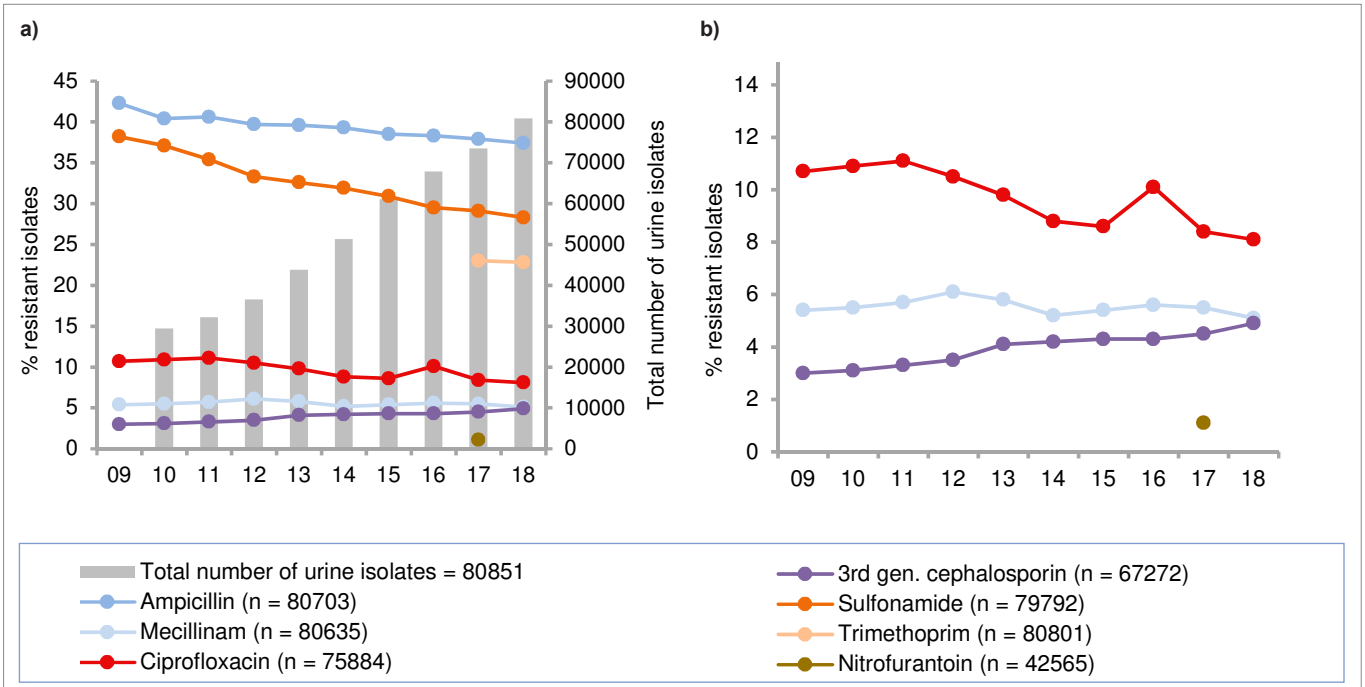
For 2018, altogether 80,851 unique patients with *E. coli* isolates, cultured in urine samples from primary health care, from nine DCMs in Denmark, were identified in MiBa. The general practitioners in Denmark are all serviced by those nine DCMs except for a few GPs in the Capital Region of Denmark whom are serviced by one private laboratory.

All nine DCMs routinely (>75% of isolates) registered antimicrobial susceptibility interpretations to ampicillin, mecillinam, sulfonamide and trimethoprim. In addition eight DCMs routinely registered antimicrobial susceptibilities to ciprofloxacin, seven DCMs to 3rd generation cephalosporins and five DCMs to nitrofurantoin. Four DCMs routinely registered antimicrobial susceptibilities to amoxicillin/clavulanic acid, gentamicin and cefuroxime, three DCMs to piperacillin/tazobactam and two DCMs to carbapenem.

As for the results from invasive isolates and isolates from hospital urines resistance results for all tested antimicrobials are shown as national means in Table 8.2. In Figure 8.5, rates of resistance are presented as a national mean when at least six DCMs have registered routine testing. Time trends and significance levels, based on the resistance rates for the past five and 10 years respectively, are presented in Figure 8.5c.

As for the number of invasive isolates, a steep increase (175%) in registered *E. coli* isolates cultured from urine samples from primary health care has been observed since 2010. Data extractions from MiBa for the same period show a steep increase in the total number of submitted urines for culturing as well. In 2010 between 164,000 and 206,000 urine samples were submitted to the DCMs from the primary sector, compared to 455,500 urine samples in 2018, representing an increase between 120% and 178%. The imprecision in the numbers extracted from MiBa is caused by, in some cases, difficulties in categorisation of hospital and primary care urines.

Figure 8.5 *Escherichia coli*. Resistance (%) in urine isolates from humans in primary health care, Denmark DANMAP 2018



c)

Substance	Time trends (Cochran-Armitage test)	
	10 years (2009 - 2018)	5 years (2014 - 2018)
Ampicillin	↓ p < 2.2e-16	↓ p = 2.914e-11
Mecillinam	↓ p = 0.0006	↑ p = 2.282e-08
Sulfonamide	↓ p < 2.2e-16	↓ < 2.2e-16
Ciprofloxacin	NA	NA
3rd generation cephalosporins	↑ p < 2.2e-16	↑ p = 1.12e-08

a) Resistance results and total number of urine isolates are prensented, b) Resistance rates excluding ampicillin, sulfonamide, trimethoprim
c) Time trends and significance levels for the past five and 10 years respectively
The number (n) in parentheses represents the numbers of isolates tested in 2018. NA = Not applicable

Time trend analysis revealed that resistance to 3rd generation cephalosporins have increased significantly both for the past decade and for the past five years. Mecillinam resistance rates have increased for the past five years. Sulfonamide and ampicillin resistance rates have decreased both for the past decade and for the past five years. Ciprofloxacin resistance rates must be interpreted with caution because of changed EUCAST break points, but the trend seems decreasing. For more details see Figure 8.5.

In 2018, eight carbapenem resistant and two intermediate susceptible *E. coli* isolates from primary health care urinary cases were registered. As noted, registering of carbapenem susceptibility results in urine samples from PHC is only routinely done at two of nine DCMs. However, since carbapenem resistant isolates are often multiresistant, most DCMs recognise them and perform additional testing.

Conclusion

A substantial increase in the total number of invasive and of primary health care urinary *E. coli* cases were observed since 2010. Within the same time period, a corresponding increase in the total numbers of blood cultures taken and urinary samples registered from the primary sector occurred. The number of urinary *E. coli* cases from hospitals showed less increase as did the total number of urine samples registered from hospitals. It could be that at least part of the increase in the number of *E. coli* cases was due to an increased number of cultures taken.

A trend for all three categories of *E. coli* isolates were the increasing cephalosporin resistance rates for both the past decade and the past five years. An exception was the resistance to 3rd generation cephalosporins in invasive *E. coli*, which showed no increases in resistance rates for the past ten and five years, but an increase was observed from 2017 to 2018. See also subsection 8.3.1 for genetic characterisation of ESBLs from bloodstream infections. An additional analysis of zone diameters for cefuroxime in invasive *E. coli*, registered since 2015, revealed the same upward trend, and thereby the increase was not caused by changes in interpretations. In Europe, an increase in resistance to 3rd generation cephalosporins in invasive *E. coli* was observed (EU/EEA population-weighted mean 14.9%). The majority of these (almost 90%) were ex-tended-spectrum beta-lactamase (ESBL)-positive [EARS-Net annual report, 2017]. When the number of ESBL-positive *E. coli* isolates increases, this can lead to increased use of broader spectrum antimicrobials and thereby a vicious circle selecting for even more resistance can start. Whenever possible, a deescalation to narrow spectrum antimicrobials therefore is necessary.

Although still at a relatively low level, an increase in total numbers of carbapenem resistant isolates was observed in *E. coli* urine isolates from hospitals in 2018. The risk of further increasing levels of carbapenem resistance in the future is worrisome.

It is positive to note that the resistance rates for piperacillin/tazobactam remained low and that time trends were stable. Also for gentamicin, the resistance rates were rather low and time trends even decreasing for the past five years. In urine isolates, mecillinam resistance rates were still relatively low and resistance to nitrofurantoin was rare in *E. coli*. There have been no reports of pan-resistant *E. coli* in Denmark yet.

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8.2.2 *Klebsiella pneumoniae*

Klebsiella pneumoniae (*K. pneumoniae*) is capable of colonising the gastrointestinal and respiratory tract in humans, especially in hospitalised patients. It may cause infections such as urinary tract infections, severe pneumonia and blood stream infections – the latter especially in patients with indwelling devices - and may give rise to nosocomial outbreaks. *K. pneumoniae* rather easily acquires and is able to transfer plasmid-borne resistance traits. *K. pneumoniae* is the fourth most common species in blood cultures, constituting 5% of positive blood cultures registered in MiBa in 2018. It is the third most common species in urine from hospitals, constituting 8% of positive urine cultures in 2018 and the second most common species in urine from primary health care, constituting 6% of positive urine cultures in 2018.

Invasive cases from hospitals

In 2018, altogether 1,280 unique patients with invasive *K. pneumoniae* isolates from all departments of clinical microbiology (DCMs) in Denmark, were identified in MiBa. All 10 DCMs routinely (>75% of isolates) registered antimicrobial susceptibility interpretations to ciprofloxacin, piperacillin/tazobactam, gentamicin and cefuroxime in MiBa. In addition, nine DCMs routinely registered antimicrobial susceptibilities to mecillinam, 3rd generation cephalosporins and meropenem and seven routinely registered antimicrobial susceptibilities to amoxicillin/clavulanic acid. Tested 3rd generation cephalosporins were either ceftazidime, ceftriaxon or cefpodoxime, while the tested carbapenem was meropenem for all DCMs in 2018. Resistance testing was mainly performed by disc diffusion. The presented data consist of the registered interpretation results, performed by the DCMs, based on the S-I-R system. Direct zone diameters have also been registered since 2015 and will be commented on in specific cases.

Resistance results for 2018 for all tested antimicrobials, presented as a national mean for each antimicrobial class, are summarised in Table 8.4. In Figure 8.6 rates of resistance are shown for the past decade – here data are presented as a national mean, when at least six DCMs have registered routine testing. Time trends and significance levels, based on the resistance rates for the past five and ten years respectively, are presented in Figure 8.6b. Test results for mecillinam resistance in invasive *K. pneumoniae* are excluded from Figure 8.6, since the S-I-R interpretation rules for the individual DCM

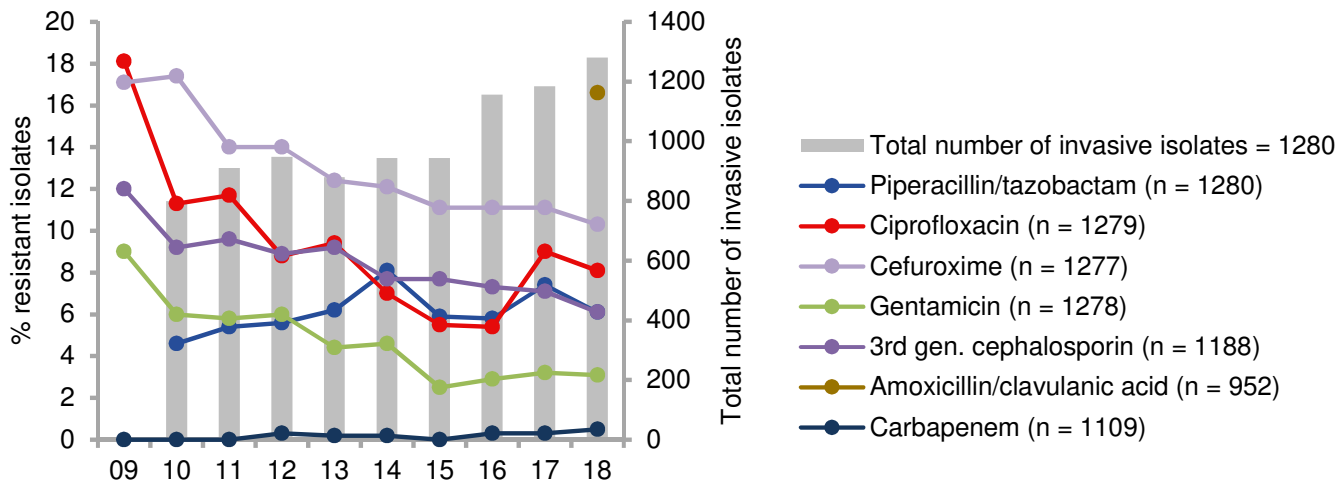
Table 8.4 *Klebsiella pneumoniae*. Resistance (%) in isolates from humans, Denmark DANMAP 2018

	Invasive isolates, hospitals %	Urine isolates, hospitals %	Urine isolates, primary health care %
Mecillinam	18	17	16
Piperacillin/tazobactam	6.1	8.7	7.4*
Amoxicillin/clavulanic acid	17	17	12*
Sulfonamide		22*	25
Trimethoprim		26	26
Nitrofuratoin		14*	15*
Gentamicin	3.1	3.2	2.2*
Ciprofloxacin	8.1	8.9	6.4
Cefuroxime	10	9.5	5.3*
3rd generation cephalosporins	6.1	6.8	5.3
Carbapenem	0.5	0.2	0.0*
Max. number of isolates tested for resistance to the presented antibiotics	1280	8030	9222

The presented resistance rates are means of the resistance rates determined by the individual DCM. Included are results from all DCMs where > 75% of the isolates in each antibiotic/sample group were susceptibility tested. The * markes where less than 6 (out of totally 10 DCMs) tested a sufficient percentage of their samples. For carbapenems in urines from primary health care this were only two DCMs.

Figure 8.6 *Klebsiella pneumoniae*. Resistance (%) in invasive isolates from humans, Denmark DANMAP 2018

a)



b)

Substance	Time trends (Cochran-Armitage test) 10 years (2009 - 2018)	5 years (2014 - 2018)
Piperacillin/tazobactam		↓ p = 0.29
Gentamicin	↓ p =4.095e-16	↓ p = 0.11
Ciprofloxacin	NA	NA
Cefuroxime	↓ p = 1.703e-07	↓ p = 0.11
3rd generation cephalosporins	↓ p = 3.452e-07	↓ p = p = 0.08
Carbapenem	↑ p = 0.01	↑ p = 0.09

a) Resistance results and the total number of invasive isolates are presented. b) Time trends and significance levels for the past five and 10 years, respectively (Cochran-Armitage test). The number (n) in parentheses represents the numbers of isolates tested in 2018

differ and/or vary over time, making comparison of the results difficult and time trends unreliable.

As for invasive *E. coli* cases, continuous increases in the number of invasive *K. pneumoniae* cases was observed throughout the years, from 799 cases in 2010 to 1,280 cases in 2018. This corresponds to 14.4 cases and 22.1 cases per 100,000 inhabitants respectively, and an increase of 53%. Simultaneously, the total number of blood cultures taken also increased steeply with 52% per 100.000 inhabitants (commented on in subsection 8.1.3).

Graphs and time trend analysis revealed that resistance rates have decreased markedly over the past 10 years for gentamicin, ciprofloxacin, cefuroxime and 3rd generation cephalosporins, but with lesser or insignificant decreases for the past five years (Figure 8.6). The increased ciprofloxacin resistance rate in 2017 compared to 2016 mainly reflected a change in interpretation of S-I-R more than a true epidemiologic change because of a new EUCAST breakpoints for ciprofloxacin implemented in most Danish DCMs as for January 2017. The downward trend for ciprofloxacin resistance in invasive *K. pneumoniae* seems to have continued when comparing 2018 to 2017 were the same breakpoint have been used.

For carbapenem resistance in invasive *K. pneumoniae* a very small but significant increase was observed for the past 10 years. Total numbers of meropenem resistant invasive isolates are low, but slowly increasing with seven resistant and one intermediary resistant invasive *K. pneumoniae* isolates in 2018 compared to three and none in 2017. The level of multiresistant (combined resistance to 3rd generation cephalosporins, ciprofloxacin and gentamicin) invasive *K. pneumoniae* remained at around 2% (Table 8.5). For colistin none of the invasive *K. pneumoniae* were registered resistant. However, colistin resistence was not tested for routinely.

Urine cases from hospitals

For 2018, altogether 8,047 unique patients with *K. pneumoniae* isolates, cultured in urine samples from hospitalised patients from all DCMs in Denmark, were identified in MiBa. 564 unique patients with *K. pneumoniae* isolates, cultured in urine samples were excluded because it was not possible to categorise them as hospital or primary care urines. However 198 of those patients were already represented in either hospital or primary care or both, with other *K. pneumoniae* urine cultures in 2018.

All 10 DCMs routinely (>75% of isolates) registered antimicrobial susceptibility interpretations to mecillinam, piperacillin/tazobactam and ciprofloxacin in MiBa. In addition nine DCMs routinely registered antimicrobial susceptibilities to gentamicin and trimethoprim, eight DCMs to cefuroxime, seven DCMs to 3rd generation cephalosporins, six DCMs to carbapenem and amoxicillin/clavulanic acid, five DCMs to sulfonamide and three DCMs to nitrofurantoin.

Resistance results for all tested antimicrobials are summarised together with the results from the invasive isolates as a national mean for each antimicrobial in Table 8.4. In Figure 8.7, rates of resistance are presented as a national mean when at least six DCMs have registered routine testing. Time trends and significance levels, based on the resistance rates for the past five and 10 years, respectively, are presented in Figure 8.7c.

As for the number of invasive cases, an increase (40%) in urinary *K. pneumoniae* cases from hospitals has been observed since 2010. Data extractions from MiBa for the same period show a smaller increase of only 5.9% to 23% in the total number of submitted hospital urine cultures - the imprecision is due to uncertainty regarding the correct categorisation of some urines as hospital or primary care samples.

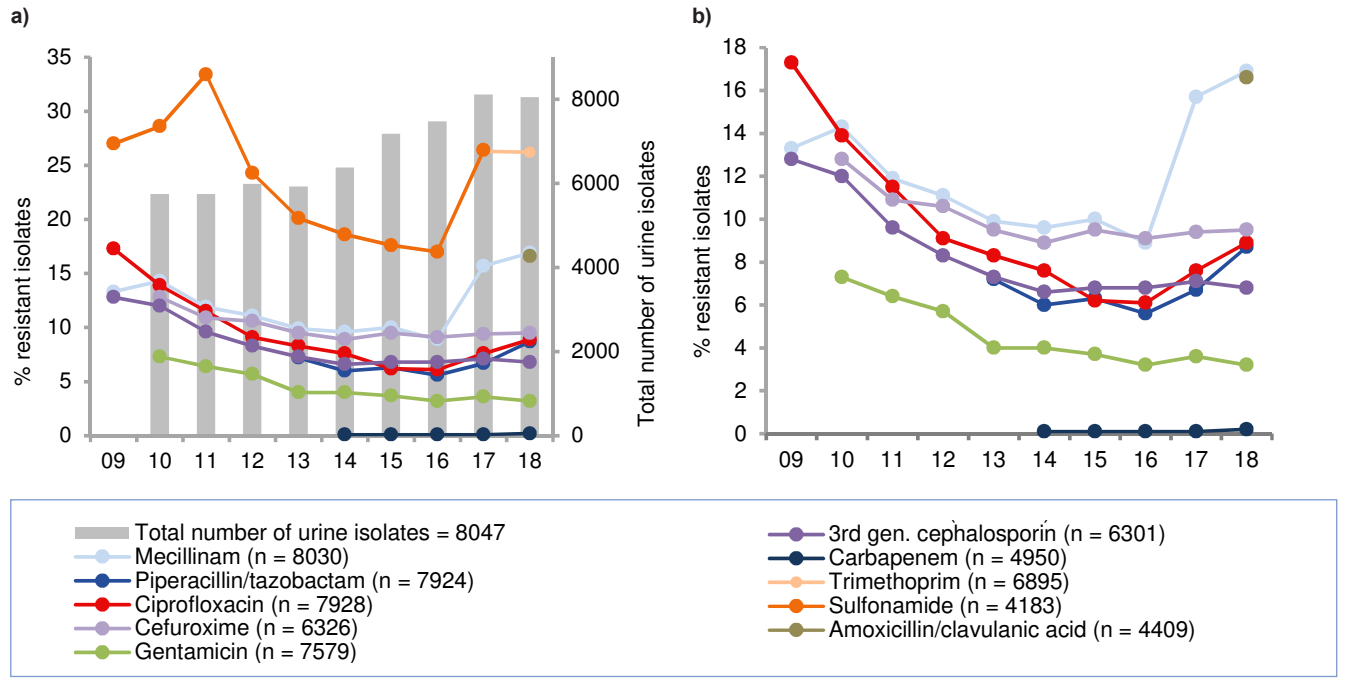
In 2018, a further increase in the resistance to mecillinam was observed in urine isolates from hospitals. The very steep increase observed in 2017 is thereby, at least, confirmed in 2018. In 2018 also a rather steep increase in resistance to piperacillin/tazobactam and ciprofloxacin was observed. For ciprofloxacin the increase in resistance must be interpreted with caution because of the change in EUCAST breakpoints, but from 2017 to 2018 it should be the same breakpoints used. The only downward trend in resistance for the past five years was for gentamicin. For more details see Figure 8.7.

In 2018, 19 carbapenem resistant and six intermediate susceptible *K. pneumonia* isolates from hospital urinary cases were registered.

Table 8.5 *K. pneumoniae*. Combined resistance to 3rd generation cephalosporins, ciprofloxacin, and gentamicin (multiresistance) in invasive isolates from humans, Denmark DANMAP 2018

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

Figure 8.7 *Klebsiella pneumoniae*. Resistance (%) in urine isolates from humans in hospitals, Denmark DANMAP 2018



c)

Substance	Time trends (Cochran-Armitage test) 10 years (2009 - 2018)	5 years (2014 - 2018)
Mecillinam	↑ p = 3.486e-08	↑ p < 2.2e-16
Piperacillin/tazobactam		↑ p = 3.662e-14
Gentamicin		↓ p < p = 0.02
Ciprofloxacin	NA	NA
Cefuroxime		↑ p = 0.18
3rd generation cephalosporins	↓ p < 2.2e-16	↑ p = 0.68

a) Resistance results and total number of urine isolates are presented, b) Resistance rates excluding sulfonamide. c) Time trends and significance levels for the past five and ten years respectively. The number (n) in parentheses represents the numbers of isolates tested in 2018

Urinary cases from primary health care

For 2018, altogether 9,227 unique patients with *K. pneumoniae* isolates, cultured in urine samples from primary health care, from nine DCMs in Denmark, were identified in MiBa. The general practitioners in Denmark are all serviced by those nine DCMs except for a few GPs in the Capital Region of Denmark whom are serviced by one private laboratory.

All nine DCMs routinely (>75% of isolates) registered antimicrobial susceptibility interpretations to mecillinam, sulfonamide and trimethoprim in MiBa. In addition eight DCMs routinely registered antimicrobial susceptibilities to ciprofloxacin, seven DCMs to 3rd generation cephalosporins, four DCMs to amoxicillin/clavulanic acid, gentamicin and cefuroxime, three DCMs to piperacillin/tazobactam and nitrofurantoin and two DCMs to carbapenem.

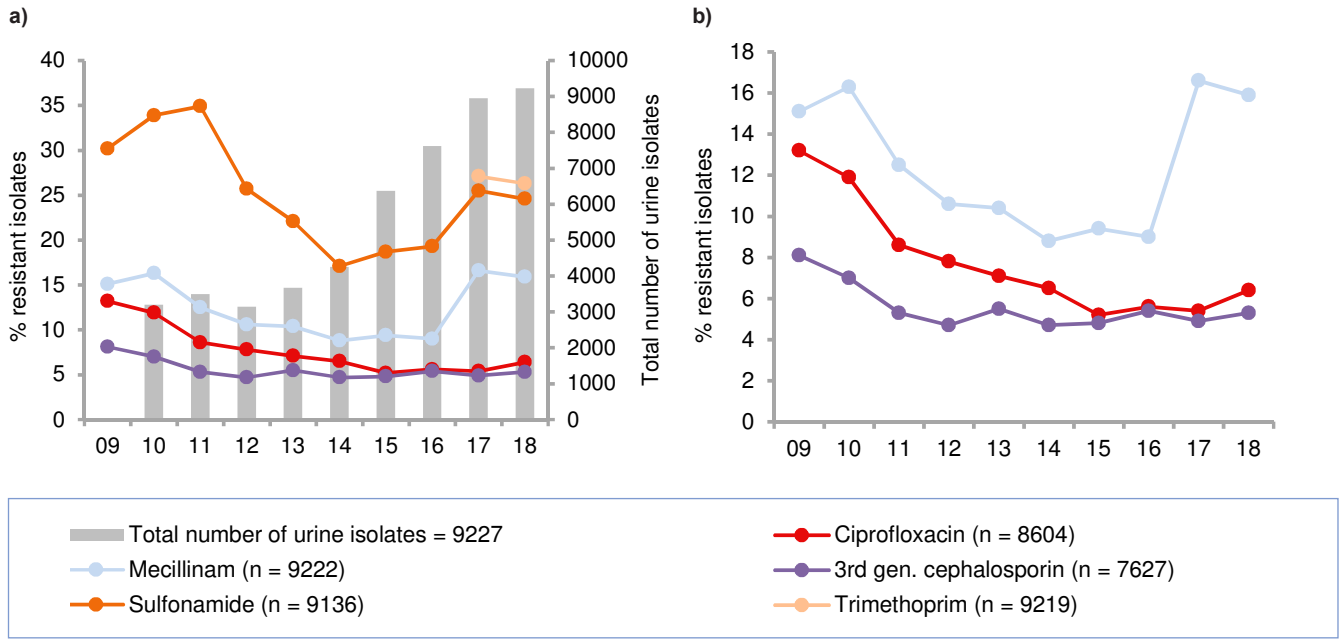
As for the results from invasive isolates and isolates from hospital urines resistance results for all tested antimicrobials are shown as national means in Table 8.4. In Figure 8.8, rates of resistance are presented as a national mean when at least six DCMs have registered routine testing. Time trends and significance levels, based on the resistance rates for the past five and ten years respectively, are presented in Figure 8.8c.

Also the number of urinary *K. pneumoniae* cases from primary health care, saw a very steep increase (189%) since 2010. As mentioned in subsection 8.2.1 *E. coli* in urine from primary health care, the increase in the total number of urine samples submitted to the DCMs from the primary sector, was between 120% and 178%.

In 2018, no further increase in the resistance to mecillinam was observed in urine isolates from primary health care compared to 2017, but still a very steep increase when comparing 2018 to 2016. For more details see Figure 8.8.

Four carbapenem resistant and two intermediate susceptible isolates were registered in 2018.

Figure 8.8 *Klebsiella pneumoniae*. Resistance (%) in urine isolates from humans in primary health care, Denmark DANMAP 2018



c)

Substance	Time trends (Cochran-Armitage test) 10 years (2009 - 2018)	5 years (2014 - 2018)
Mecillinam	↑ p = 1.876e-05	↑ p < 2.2e-16
Sulfonamide	↓ p < 2.2e-16	↑ p < 2.2e-16
Ciprofloxacin	NA	NA
3rd generation cephalosporins	↓ p = 3.069e-05	↓ p = 0.25

a) Resistance rates and total numbers of urine isolates are presented. b) Resistance rates excluding sulfonamide and trimethoprim. c) Time trends and significance levels for the past five and nine years, respectively (Cochran-Armitage test). The number (n) in parentheses represents the numbers of isolates tested in 2018

Conclusion

As for *E. coli*, the continuing increase in total numbers of *K. pneumoniae* cases is worrisome and needs to be further investigated. Especially worrisome is the relatively high resistance rates in *K. pneumoniae* to antimicrobials with per oral formulations. Rates of resistance to mecillinam, amoxicillin/clavulanic acid, sulfonamide, trimethoprim and nitrofurantoin between 12% and 26% can lead to more usage of ciprofloxacin. Campaigns, with focus on the unnecessary treatment of asymptomatic bacteriuria in Denmark, can hopefully contribute to counteract the problem. In *E. coli*, which counts for half of all urinary tract infections, the susceptibility rates to antimicrobials with per oral formulations are more feasible.

The significant increase in resistance in *K. pneumoniae* to mecillinam is planned to be further investigated in the future.

The small but increasing levels of carbapenem resistance in *K. pneumoniae* also worries. Often these isolates carry several resistance mechanisms. Despite the worrying tendencies, the proportion of *K. pneumoniae* with combined resistance to 3rd generation cephalosporins, ciprofloxacin and gentamicin is not increasing, which is encouraging. In the southern and south-eastern part of Europe high levels of carbapenem resistance as well as combined resistance to 3rd generation cephalosporins, ciprofloxacin and gentamicin is truly problematic [EARS-Net annual report, 2017].

There have been no reports of pan-resistant *K. pneumoniae* in Denmark yet.

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8.2.3 *Pseudomonas aeruginosa*

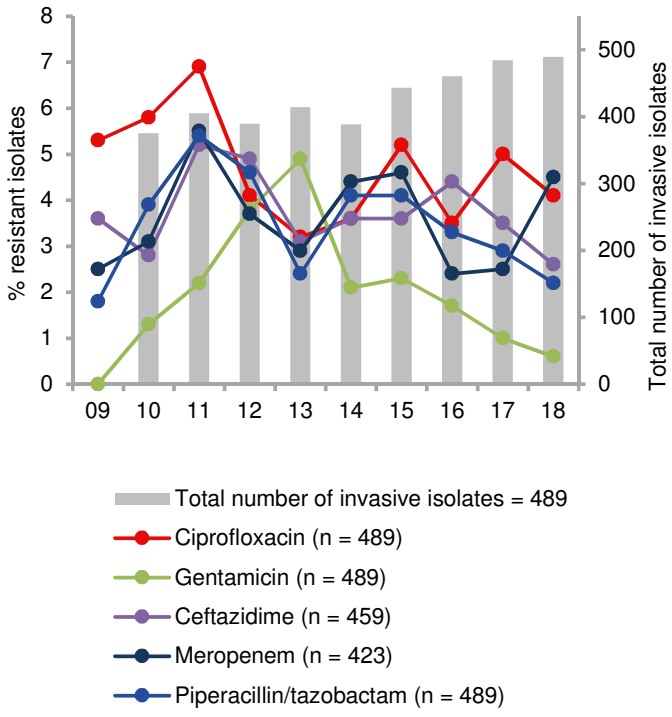
Pseudomonas aeruginosa is an opportunistic pathogen causing relatively rare but significant disease in humans. *P. aeruginosa* typically infects the pulmonary tract, urinary tract, burns, wounds, and can cause bloodstream infections as well. It is a relatively frequent colonizer of medical devices (e.g.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

Invasive cases from hospitals

In 2018, altogether 489 unique patients with invasive *P. aeruginosa* isolates from all departments of clinical microbiology (DCMs) in Denmark, were identified in MiBa. All 10 DCMs routinely (>75% of isolates) registered antimicrobial susceptibility interpretations to ciprofloxacin, piperacillin/tazobactam and gentamicin. Nine DCMs routinely registered antimicrobial susceptibilities to ceftazidime and meropem in MiBa. Resistance testing was mainly performed by disc diffusion or E-test. The presented data consist of the registered interpretation results, performed by the DCMs, and are based on the S-I-R system.

Data are presented in Figure 8.9.

Figure 8.9 *Pseudomonas aeruginosa*. Resistance (%) in invasive isolates from humans, Denmark DANMAP 2018



The number (n) in parentheses represents the numbers of isolates tested in 2018

One invasive isolate of *P. aeruginosa* identified in MiBa for 2018 was registered colistin resistant with an MIC of 3 mg/L.

Conclusion

Regarding resistance in invasive *P. aeruginosa* the situation in Denmark is quite stable and with relatively low prevalence. EARS-Net 2017 reported a small decreasing trend in resistance in the EU/EEA population-weighted mean for all antimicrobial groups under surveillance during the period 2014-2017. EU/EEA population-weighted mean in 2017 were for ceftazidime 14.7%, fluoroquinolones 20.3%, aminoglycosides 13.2%, piperacillin/tazobactam 18.3% and carbapenems 17.4%. Large inter-country variations are reported between south-east Europe and north Europe [EARS-Net annual report, 2017]. Denmark was below 5% resistance proportions for all antibiotics under surveillance in invasive *P. aeruginosa* isolates in 2018.

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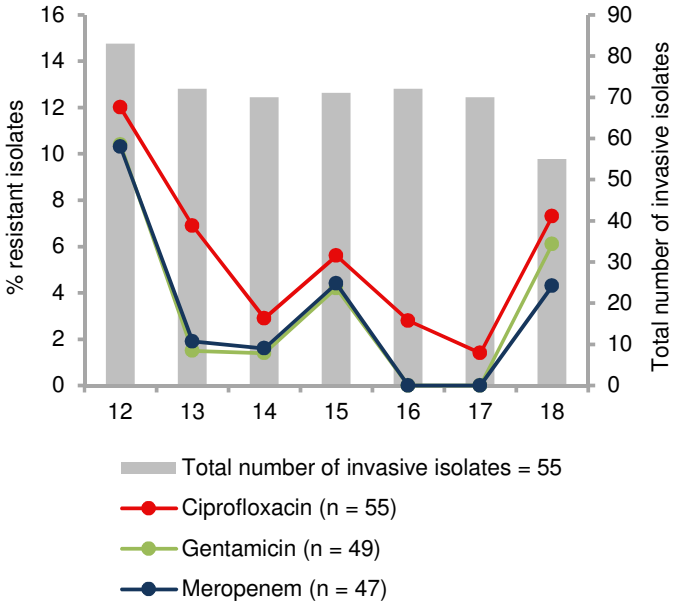
8.2.4 *Acinetobacter* species

The genus *Acinetobacter* includes several species and is found widespread in nature, in soil, water and/or animals and humans. In humans *Acinetobacter* can colonize the skin and wounds but may also be the cause of hospital-acquired infections like central line-associated bloodstream infections, nosocomial pneumonia, urinary tract infections and wound infections. Many of the different subspecies are phenotypically alike, and thus identification at species level can be difficult. Species belonging to the *A. baumannii* group are considered as the most clinically important. *Acinetobacter* species possess an inherent resistance to a broad range of antimicrobials because of a low membrane permeability and constitutive expression of efflux systems. The antimicrobial classes which can be used for treatment include some fluoroquinolones, aminoglycosides, carbapenems and colistin. For especially *A. baumanii*, multiresistant clones are widespread in the hospital environment in many south- and east European countries, where they cause problems with outbreaks in fragile patient subpopulations at e.g. intensive care units. Of worldwide concern are severely war-wounded soldiers colonised or infected with multiresistant *A. baumannii*.

Figure 8.10 *Acinetobacter* spp. Resistance (%) in invasive isolates from humans, Denmark DANMAP 2018



The numbers (n) in parentheses represents the number of isolates tested in 2018

Invasive cases from hospitals

In 2018, 55 unique patients with *Acinetobacter* species invasive isolates from eight departments of clinical microbiology (DCMs) in Denmark, were identified in MiBa. For two DCMs, there were not identified any invasive *Acinetobacter* species in MiBa in 2018. All eight DCMs routinely (>75% of isolates) registered antimicrobial susceptibility interpretations to ciprofloxacin, and seven to meropenem and gentamicin. Resistance testing was mainly performed by disc diffusion. The presented data consist of the registered interpretation results, performed by the DCMs, and are based on the S-I-R system.

Data are presented in Table 8.6 and in Figure 8.10.

Compared to the previous six years, less invasive *Acinetobacter* species were registered in 2018. Two isolates (both *A. baumanii)* had combined resistance to ciprofloxacin, gentamicin and meropenem. None of the invasive *Acinetobacter* species were reported colistin resistant. However, colistin resistance is not tested for routinely.

Conclusion

In general, low total numbers of invasive *Acinetobacter* species is registered in Denmark, as well as low total numbers of resistant invasive *Acinetobacter* species. In EARS-Net, markedly differences in resistance profiles across Europe have been reported with more than 50% of the isolates being resistant to at least one of the three surveilled antimicrobials (fluoroquinolones, aminoglycosides and carbapenems). Particularly the Baltic and southern and south-eastern countries of Europe reported on problems with high resistance and the most common was combined resistance to fluoroquinolones, aminoglycosides and carbapenems. The northern countries reported between 0% and 3.4% combined resistance in 2017 [EARS-Net annual report, 2017].

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Tabel 8.6 *Acinetobacter* spp. Tested and resistant invasive isolates DANMAP 2018

	2012		2013		2014		2015		2016		2017		2018	
	res.	n	res.	n	res.	n	res.	n	res.	n	res.	n	res.	n
Ciprofloxacin	10	83	5	72	2	69	4	71	2	72	1	70	4	55
Gentamicin	8	77	1	65	1	70	3	71	0	70	0	70	3	49
Meropenem	6	58	1	52	1	62	3	68	0	69	0	67	2	47
Total number of invasive isolates	84		72		72		71		72		70		55	

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

8.2.5 Enterococci

Enterococci constitute a part of the normal intestinal microbiota in humans and animals. More than 54 species belonging to the genus enterococcus have been described, but the majority of the human infections are caused by *E. faecalis* and *E. faecium*. Most common clinical infections include urinary tract infections, bacteraemia and bacterial endocarditis. Enterococci are inherently resistant to many groups of antimicrobials and thereby get a selective advantage in e.g. hospitalised patients under antibiotic treatment where they can lead to colonisation or infection. The source of hospital infection is often associated with the use of medical supplies, such as catheters, as well as other instruments and medical devices. Use of antimicrobials in these patients increases the risk for an enterococcal infection.

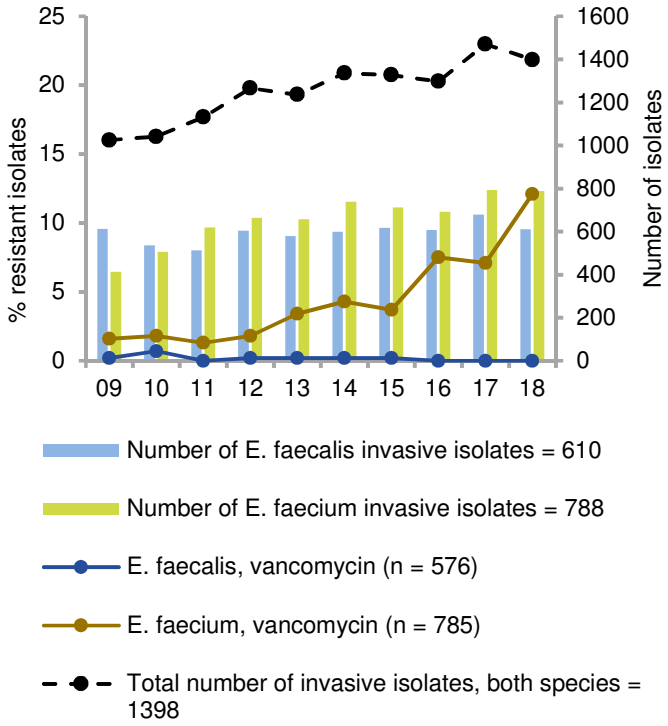
Therapy of enterococcal infections may be challenging. For *E. faecium*, were the vast majority are ampicillin resistant, severe infections are treated with vancomycin. Antimicrobials, such as linezolid and daptomycin are options for treatment of the multiresistant, vancomycin-resistant enterococcus (VRE). Combinational therapy based on a synergistic effect of beta-lactam antimicrobials (penicillin/ampicillin) with an aminoglycoside (most often gentamicin) or glycopeptide (vancomycin) is required in cases of endocarditis.

Invasive cases from hospitals

For 2018, altogether 610 unique patients with invasive *E. faecalis* isolates and 788 unique patients with invasive *E. faecium* isolates from all departments of clinical microbiology (DCMs) in Denmark, were identified in MiBa. For *E. faecalis*, all 10 DCMs routinely (>75% of isolates) reported antimicrobial susceptibility interpretations to ampicillin. In addition, nine DCMs routinely reported antimicrobial susceptibilities to vancomycin, five DCMs to linezolid, four DCMs to gentamicin (high-level resistance to gentamicin), two DCMs to teicoplanin and one DCM to tigecycline. For *E. faecium*, all 10 DCMs routinely (>75% of isolates) reported antimicrobial susceptibility interpretations to ampicillin and vancomycin in MiBa. In addition, six DCMs routinely reported antimicrobial susceptibilities to linezolid, four DCMs to gentamicin (high-level resistance to gentamicin), two DCMs to teicoplanin and one DCM to tigecycline. Resistance testing was mainly performed by disc diffusion. The presented data consist of the registered interpretation results, performed by the DCMs, based on the S-I-R system. One exception is high-level gentamicin resistance from one DCM, were MIC and/or zone diameters in MiBa were used because interpretations were not reported in MiBa.

Resistance to all tested antibiotics are presented as a national mean of the combined DCMs reporting in Table 8.7. In Figure 8.11, total numbers of invasive isolates of *E. faecalis* and *E. faecium* and the ratio of resistance to vancomycin in both, for the past decade, are shown.

Figure 8.11 Enterococci. Number of isolates and rates of resistance to vancomycin (%) in invasive isolates from humans, Denmark DANMAP 2018



The number (n) in parentheses represents the numbers of isolates tested in 2018. In 2009 the presented data covers 75% of the Danish population. From 2010 to 2014 data covers 95% of the Danish population and from 2015 the total Danish population is covered

Table 8.7 Enterococci. Resistance (%) in invasive isolates from humans, 2018 DANMAP 2018

	E. faecalis	*E. faecium*	Number of tested isolates (number of DCMs)	
	%	%	*E. faecalis*	*E. faecium*
Ampicillin	0.3	94	608 (10)	787 (10)
Vancomycin	0.0	12	576 (9)	785 (10)
Linezolid	1.2	0.5	427 (5)	602 (6)
High-level gentamicin	11	40	326 (4)	396 (4)
Teicoplanin	0.0	16	213 (2)	246 (2)
Tigecycline	0.0	0.0	105 (1)	103 (1)

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

From 2017 to 2018, the total number of invasive cases decreased from 678 to 610 for *E. faecalis* and from 793 to 788 for *E. faecium*.

A continuing high prevalence of ampicillin resistance in invasive *E. faecium* have been observed with rates between 92% and 95% since 2010. In 2002 the resistance rate was 65%.

In 2018, yet a steep increase in the proportion of invasive vancomycin resistant *E. faecium* (12% compared to 7.1% in 2017) was observed. A part of this was due to the spread and detection of the "VVE clone", ST1421-CT1134 vanA *E. faecium*, which even though variable in vancomycin resistance, has the ability to revert to vancomycin resistance, and therefore is interpreted as vancomycin resistant in the clinical setting. For more, go to subsection 8.3.3, VVE and VRE. In this subsection there is no distinction between VRE and VVE. None vancomycin resistant invasive *E. faecalis* were reported in 2018. In total numbers 97 unique patients had invasive VRE/VVE as the first invasive enterococcus isolate in 2018. This number was 56 patients in 2017 and 51 in 2016. Thirty-four out of 90 of those patients (38%) in 2018, died within 30 days of diagnosis. Many of the VRE patients were chronically ill patients and the high mortality might be due to this.

The proportion of high-level gentamicin resistance (MIC > 128 mg/L and/or zone diameters < 8 mm) was reported in MiBa from four DCMs (>75% of isolates tested), Table 8.7. In the previous years, only one DCM reported on the proportion of high-level gentamicin resistance to DANMAP. Based on these rather sparse data, a decreasing trend in high-level gentamicin resistance in invasive *E. faecalis* has been observed over the decade, from 35% in the first years to 20% in 2016, 7.1% in 2017 and 11% in 2018. In *E. faecium* the level has been oscillating between 55% and 75% in the same time period, but decreasing to 43% in 2017 and 40% in 2018.

In 2018, five isolates of *E. faecalis* and three isolates of *E. faecium* from unique patients were reported linezolid resistant from the five and six DCMs routinely reporting interpretations to linezolid (Table 8.7). In 2017 the numbers were six *E. faecalis* (three reporting DCMs) and eight *E. faecium* (six reporting DCMs). Of all linezolid resistant invasive isolates identified in MiBa in 2018, two *E. faecium* were also VVE but still susceptible to tigecycline and daptomycin, the rest were susceptible to vancomycin.

Conclusion

An increase of invasive enterococci, mainly caused by an increase in invasive *E. faecium*, has been observed during the past 17 years (Figure 8.1.1. DANMAP 2015). The increase was combined with firstly, an increase in the proportion of ampicillin resistant *E. faecium* (65% in 2002 and more than 90% since 2010) and since 2013, an increase in vancomycin resistant *E. faecium*. In 2018, yet a steep increase in the percentage of vancomycin resistant invasive *E. faecium* was observed but the total number of invasive enterococci did not increase any further compared to 2017. The proportion of invasive vancomycin resistant *E. faecium* is high in Denmark, especially when compared to the other Nordic countries, where Norway has the next highest percentage (4.5%). But also southern European countries like France and Spain have lower percentages of vancomycin resistant invasive *E. faecium* than Denmark [EARS-Net annual report, 2017]. The increase is worrisome and reflects the sharp increase in Denmark since 2013 in all types of clinical VRE (subsection 8.3.3). A high mortality was observed in patients with invasive VRE/VVE. There is an ongoing focus, on dealing with the VRE/VVE problem in Denmark.

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8.3 Surveillance based on data from the reference laboratories

8.3.1 Characterisation of ESBL- and pAmpC-producing *Escherichia coli* from bloodstream infections, 2018, Denmark

Background

Resistance to 3rd generation cephalosporins in *Escherichia coli* often occur through production of extended-spectrum beta-lactamases (ESBL), carbapenemases, plasmid-mediated AmpC (pAmpC) or through mutations within the promoter/attenuator region of chromosomal AmpC (cAmpC).

Since 2014, the Danish departments of clinical microbiology have, on a voluntary basis, submitted 3rd generation cephalosporin resistant *E. coli* (3GC-R Ec) from bloodstream infections for characterisation at the National Reference Laboratory for Antimicrobial Resistance at Statens Serum Institut. The 3GC-R Ec were defined by the EUCAST criteria to cefpodoxime, ceftriaxone, cefotaxime or ceftazidime.

The 3GC-R Ec collected in Denmark through 2018, were characterized according to Multilocus Sequence Types (MLSTs), and the encoding ESBL-, pAmpC- and carbapenemase genes. For isolates with no ESBL-, pAmpC-, or carbapenemase-encoding genes detected, the sequences were investigated for promotor mutations presumed to up-regulate cAmpC.

Results

In 2018, whole genome sequencing data were obtained from 369 *E. coli* isolates from unique patients. Genes encoding ESBL and/or pAmpC were detected in 352 (95%) of the isolates while 17 isolates were cAmpC hyper producers only; these 17 isolates were not further investigated.

Demographic data was available for all 352 *E. coli* isolates in 2018; 185 (53%) of the patients were men compared to 209 (62%) in 2017, and 167 (47%) were women compared to 128 (38%) in 2017. The average age at diagnosis was 69 years,

ranging from below one to 96 years. Fifty patients (14%; 28 men and 22 women) of the 352 patients died within 30 days of diagnosis (average age at death was 78 years; ranging from 53 to 96 years).

The regional distribution of the 352 isolates with ESBL- and pAmpC encoding genes was compared to data from previous years (Table 8.8 and Figure 8.12).

From 2014 to 2018, the reported cases of ESBL/pAmpC *E. coli* in bloodstream infections have changed from 245 to 352 per year, a 44% increase. In the same time period the total number of unique patients with invasive *E. coli* (irrespective of the resistance profile) only increased by 20%.

In the Capital Region, the number of reported cases increased significantly from 112 cases in 2017 to 154 cases in 2018 (p = 0.005), whereas the reported number of cases in the Zealand Region decreased significantly from 38 cases in 2017 to 23 cases in 2018 (p = 0.029). For the remaining three regions, the reported number of cases were stable in 2018 compared to 2017.

In 2018, 24 different ESBL-, pAmpC- and carbapenemase-enzymes were detected among the 352 isolates (Table 8.9). As in previous years, CTX-M-15 was the most prevalent enzyme, with a significant increase from 164 cases in 2017 to 200 cases in 2018 (p = 0.032), whereas the presence of CTX-M-14 and CTX-M-55 decreased significantly from 48 and 13 cases in 2017 to 31 and four cases in 2018, respectively (p = 0.025 and p = 0.021). In five cases, a carbapenemase-enzyme was detected along with an ESBL-enzyme.

Table 8.8 Distribution of ESBL/pAmpC producing *E. coli* from bloodstream infections, Denmark DANMAP 2018

	2014		2015		2016		2017		2018	
Region	Numbers	%	Numbers	%	Numbers	%	Numbers	%	Numbers	%
The Capital Region of Denmark	110	45	116	42	111	36	112	33	154	44
The Zealand Region	27	11	14	5	36	12	38	11	23	7
Region of Southern Denmark	43	18	45	16	67	21	76	23	75	21
Central Denmark Region	43	18	59	21	66	21	80	24	74	21
North Denmark Region	22	9	41	15	32	10	31	9	26	7
Total Numbers	245		275		312		337		352	

Figure 8.12 Regionwide distribution of ESBL/pAmpC-producing *E. coli* from bloodstream infections, Denmark DANMAP 2018

Table 8.9 Most common ESBL enzymes, pAmpC enzymes and carbapenemases detected in *E. coli* from bloodstream infections, Denmark DANMAP 2018

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

In some isolates more than one enzyme was detected

Table 8.10 Distribution of MLSTs in ESBL/pAmpC-producing *E. coli* from bloodstream infections, Denmark DANMAP 2018

	2014		2015		2016		2017		2018	
MLST	Numbers	%	Numbers	%	Numbers	%	Numbers	%	Numbers	%
ST131	124	51%	135	49%	177	57%	175	52%	189	54%
ST38	18	7%	23	8%	21	7%	23	7%	22	6%
ST405	13	5%	12	4%	7	5%	9	3%	9	3%
ST410	4	2%	11	4%	6	4%	6	2%	3	1%
ST69	10	4%	10	4%	16	3%	20	6%	27	8%
ST648	7	3%	10	4%	5	2%	8	2%	6	2%
ST12	5	2%	9	3%	14	2%	6	2%	5	1%
ST88	2	1%	1	< 1%	0	-	5	1%	2	1%
ST1193	2	1%	5	2%	10	2%	7	2%	8	2%
ST10	0	-	6	2%	2	2%	4	1%	7	2%
ST73	3	1%	2	1%	4	2%	2	1%	6	2%
Other STs[1]	57	24%	51	19%	50	16%	72	21%	70	20%

[1] less than 5 isolates per ST in 2018

In 2018, the 352 *E. coli* isolates belonged to 62 different MLSTs, with the most common sequence type (ST) being ST131 (54%), followed by ST69 (8%) and ST38 (6%) (Table 8.10). No significant changes were observed in the MLSTs in 2018, compared to 2017.

Among the 189 *E. coli* isolates belonging to ST131, CTX-M-15 (67%) was most common, followed by CTX-M-27 (20%), and CTX-M-14 (7%). A significant increase (p = 0.021) of CTX-M-15 in ST131 was observed from 2017 (97; 55%) to 2018 (127; 67%), which explains the increase of CTX-M-15 in the collection of the 352 *E. coli* isolates. The five carbapenemase producers belonged to ST38 (2), ST405 (1), ST648 (1) and ST744 (1).

Conclusion

In 2018, the number of isolates carrying CTX-M-15 increased significantly in ST131, where 67% of the isolates were carrying the CTX-M-15 enzyme. In 2018, five isolates were also carbapenemase producers (OXA-48 group and NDM), and a minor part (3%) of the isolates carried CMY-variants. The distribution of sequence types for the 352 isolates did not change according to previous years; the worldwide disseminated ST131 clone was still strongly represented in 2018 (54%).

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8.3.2 Carbapenemase producing bacteria in Denmark, 2018

Background

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

In recent years, Danish departments of clinical microbiology (DCMs) have on a voluntary basis submitted carbapenem-resistant isolates for verification and genotyping at the National Reference Laboratory for Antimicrobial Resistance at Statens Serum Institut. The Danish Health Authority made CPO notifiable as of 5th September 2018 [https://www.sst.dk/da/udgivelser/2018/~/media/52D5C295BCEA48E6BC596C0083367FF3.ashx]. The present textbox describes carbapenemase-producing Enterobacterales (CPE), *Pseudomonas* spp. and *Acinetobacter* spp.

Carbapenemase-producing organisms

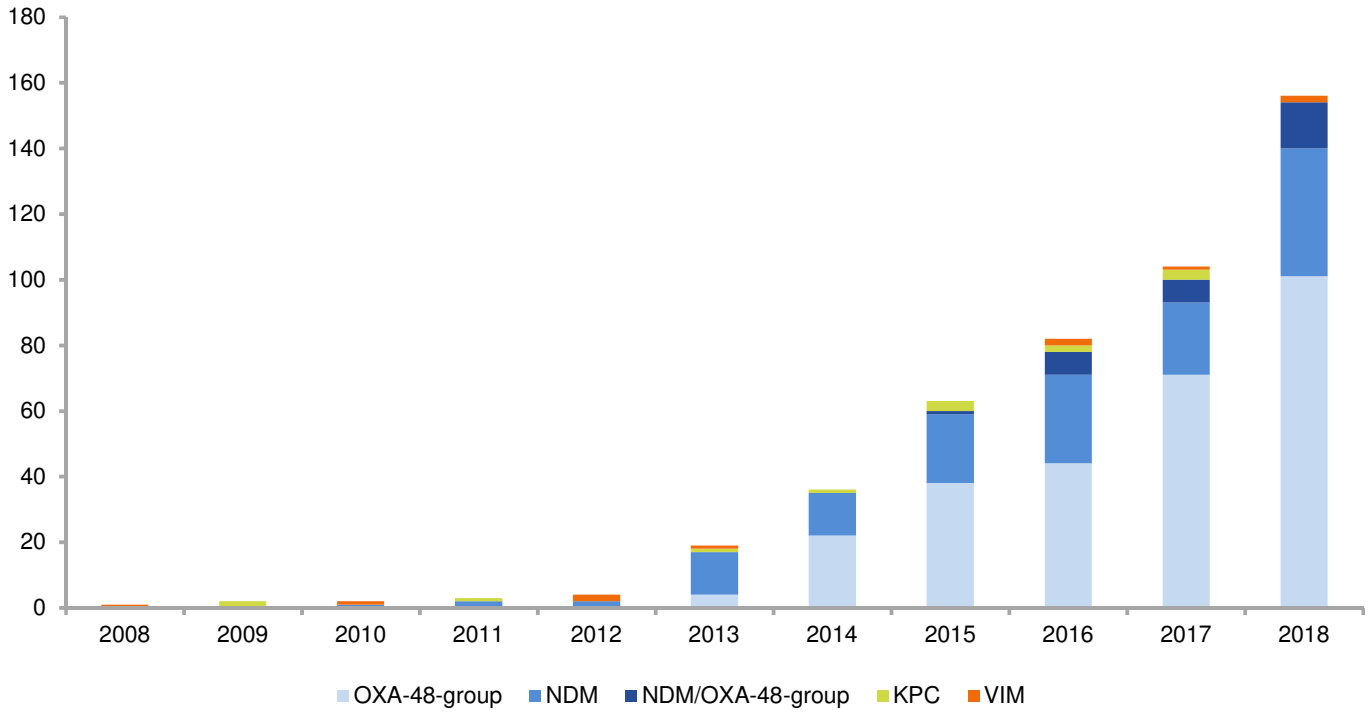
During 2018, 177 carbapenemase-producing organisms (CPO) were detected from 160 patients compared with 123 CPO from 115 patients in 2017 leading to a 44% overall increase of submitted CPO isolates compared to 2017. More than one isolate from the same patient were included, if the isolates belonged to different bacterial species and/or if the isolates harboured different carbapenemases. Eighteen of the CPO (15 Enterobacterales and three *Acinetobacter* spp.) were from bloodstream infections compared with five of the CPO in 2017.

Enterobacterales

In 2018, 156 CPE isolates were detected from 141 patients compared to 104 CPE from 96 patients in 2017 (Figure 8.13) leading to a 50% increase of submitted CPE isolates compared to 2017. In 2018, 35 of the patients had reported travelling abroad prior to detection of the CPE; three of the patients had no reported history of recent travel and for the remaining 103 patients, travel information was unavailable. As many of the patients with unavailable travel information were involved in the detected outbreaks, it seems that the CPE were obtained in Denmark, at least for these patients. Better reporting will be obtained in the future, since CPE has become notifiable.

Fourteen of the 156 CPE isolates produced both NDM and OXA-48 group enzymes, 101 produced OXA-48-like enzymes and 39 were NDM-producing (Figure 8.13). Furthermore, two VIM-producing isolates were detected.

Figure 8.13 Numbers of carbapenemase-producing Enterobacterales (CPE), 2008-2018, Denmark DANMAP 2018



Larger outbreaks with CPE during 2018

The ST18 NDM-1-producing *Citrobacter freundii* outbreak, which started in 2012 in the North Denmark Region, continued in 2018 [Hammerum et al. 2016, J. Antimicrob. Agents, 71:3117-3124]. During 2018, an ST18 NDM-1-producing *C. freundii* was also detected from a patient in the Central Denmark Region, with epidemiologic link to the North Denmark Region. Furthermore, the NDM-1encoding plasmid (pT1) was detected in an ST8 *C. freundii*, indicating possible plasmid transfer from an ST18 NDM-1-producing *C. freundii*. Until the end of 2018, 27 patients had been involved in this outbreak in the period 2012-2018. During 2018, eight new patients were part of 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.

Another large outbreak was detected in Zealand with spread of ST410 NDM-5/OXA-181 *E. coli*. [Roer et al. 2018, mSphere;3(4).]. The first patient with ST410 NDM-5/OXA-181 *E. coli* was hospitalised in the Capital Region in 2015 after hospitalisation in Egypt [Overballe-Petersen et al. 2018, Genome Announc.6(5): e01542-17]. The other patients with ST410 NDM-5/OXA-181 *E. coli* were hospitalised in the Region Zealand in 2016-2018. By the end of 2018, 28 patients had been involved in this outbreak in the period 2015-2018 and 18 of these were detected during 2018.

In 2013, OXA-436, a novel carbapenemase belonging to the OXA-48 enzyme group, was detected in Denmark [Samuelsen et al. 2017, Antimicrob. Agents and Chemother 62(1): e01260-17]. From 2013 to the end of 2018, 14 patients had had OXA-436-producing CPE isolates, both plasmid and clonal spread have been seen. Spread of ST90 OXA-436-producing *E. cloacae* between three patients occurred in the Region of Southern Denmark in 2018.

During 2018, spread of ST231 OXA-232-producing *K. pneumoniae* were detected between five patients in the Central Denmark Region. Furthermore, spread of ST101 OXA-48-produing *K. pneumoniae* was detected between eight patients in the Capital Region during 2018.

Besides these larger outbreaks, possible spread of CPE between two patients were observed several times during 2018. It seems very likely that the increase in OXA-48-producing CPE was due to plasmid transfer, but this was not investigated further.

***Acinetobacter* spp.**

In 2018, 18 carbapenemase-producing *Acinetobacter* spp. isolates were detected compared to 15 isolates in 2017. Ten of the 18 patients with carbapenemase-producing *Acinetobacter* spp. had been travelling abroad prior to detection. In 2018, 16 carbapenemase-producing *Acinetobacter baumannii* with the following enzymes were detected: OXA-23 (12), NDM-1 (1), NDM-1/OXA-23 (1), OXA-239 (1) and OXA-72 (1). Furthermore, one OXA-58 -producing *Acinetobacter bereziniae* and one OXA-499 -producing *Acinetobacter calcoaceticus* were detected.

***Pseudomonas* spp.**

In 2018, one VIM-2-producing *Pseudomonas aeruginosa*, one NDM-1-producing *P. aeruginosa* and one VIM-2-producing *Pseudomonas monteilii* were detected. All three patients had been travelling abroad prior to detection of the carbapenemase-producing *Pseudomonas* spp.

Conclusion

The occurrence of carbapenemase-producing bacteria in Denmark continues to increase, a trend worrisome to patients and clinicians. Especially the spread of CPE among patients in Denmark is of concern, since Enterobacterales can be carried in the intestine for a long time (years) without any symptoms of infections, which makes outbreak control difficult.

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8.3.3 Further increase of *vanA Enterococcus faecium* in Denmark

Background

Enterococci are intrinsically resistant to a number of first-line antimicrobial agents, including cephalosporins. Therefore, antibiotic treatment of enterococcal infections may be challenging. In addition, most hospital-acquired *E. faecium* are resistant to ampicillin, thus limiting the treatment possibilities. Vancomycin is an important drug for the treatment of severe *E. faecium* infections, but an increase in the occurrence of vancomycin-resistant enterococci (VRE) has been observed in Denmark and internationally. Newer antibiotics such as linezolid and daptomycin can be used for treatment of VRE, but both antimicrobial agents have side effects and development of resistance has been reported.

In recent years, *E. faecium* harboring the *vanA* gene complex, but being phenotypically vancomycin susceptible, has been described in different countries. These enterococci are referred to as vancomycin-variable enterococci (VVE). VVE have caused nosocomial outbreaks and development of revertant mutants becoming vancomycin resistant *in vitro* and *in vivo* has been described. This makes the detection of VVE highly clinically relevant in order to avoid treatment failure with vancomycin. VVE can only be detected by molecular methods and cannot be cultured on selective vancomycin-containing media. In 2015 and 2016, sporadic VVE with different genetic background were detected in the Capital Region of Denmark, in relation to concurrent VRE-outbreaks [B Holzknecht, personal communication]. In 2016, a new VVE clone belonging to ST1421-CT1134 was detected, which displays variable vancomycin

susceptibility due to a deletion in the *vanX* gene. [TA Hansen et al., J. Antimicrob. Agents, 2018, 73: 2936-2940].

Surveillance of VRE/VVE

Since 2005, Danish departments of clinical microbiology (DCMs) have voluntarily submitted VRE (first isolate per patient per 12 months) for species identification, genotyping and surveillance to the Antimicrobial Resistance Reference Laboratory at Statens Serum Institut (SSI). In 2017, VVE isolates were included in the VRE surveillance. However, VVE diagnostics differ substantially in the different regions. In 2017, testing of invasive *E. faecium* isolates was introduced in some DCMs in the Capital Region, which was during 2018 expanded to testing of all clinical *E. faecium* isolates for the presence of *vanA/vanB* genes. During 2018, VVE screening was also implemented in one DCM in the Region of Southern Denmark. Furthermore, invasive *E. faecium* isolates were tested by PCR for *vanA/vanB* genes in another DCM in the Region of Southern Denmark and in the DCM in Central Denmark Region.

To determine any underreporting in the submissions, the number of VRE/VVE submitted to SSI in 2016, 2017 and 2018 were compared to data from clinical VRE reported by the DCMs to MiBa (the Danish Microbiology Database). This comparison showed that the number of submitted VRE/VVE isolates were not complete, since VRE/VVE isolates were missing from 80, 81 and 78 patients in surveillance in 2016, 2017 and 2018, respectively (Figure 8.14). In 2018, 525 VRE/VVE isolates were submitted to SSI. One patient had both a vancomycin resistant *E. faecium* and a vancomycin resistant *E. faecalis*. By adding the 81 and 78 VRE/VVE isolates extracted from MiBa, this added up to 603 VRE/VVE isolates from 599 patients in 2018 compared to 510 VRE/VVE isolates from 508 patients in 2017 (Figure 8.14).

From 2013, a sharp increase in clinical VRE isolates has been observed. The increase has mostly been seen for *vanA E. faecium* (Figure 8.14).

From 2015 through 2018, all clinical VRE/VVE isolates have been submitted to whole-genome sequencing (WGS). In 2018, 525 VRE/VVE were submitted to WGS. From the WGS data, multilocus sequence type (MLST), core genome MLST (cgMLST) and *van*-genes were extracted *in silico*.

Of the 525 VRE/VVE isolates, 480 were *vanA E. faecium*, 16 *vanB E. faecium*, 19 *vanA/vanB E. faecium*, five *vanA E. faecalis* and five *vanB E. faecalis* (Figure 8.14). cgMLST analysis was performed on the 10 *E. faecalis* isolates and 515 *E. faecium* isolates using SeqSphere+. The 10 *E. faecalis* isolates were subdivided into nine different complex types (CTs) by cgMLST, whereas the 515 *E. faecium* isolates were subdivided into 65 CTs. Two types dominated: ST203-CT859 *vanA E. faecium* and ST1421-CT1134 *vanA E. faecium* (Table 8.11). The number of *vanA E. faecium* isolates belonging to ST203-CT859 was high during 2015-2017, but the number decreased during 2018.

Figure 8.14 Numbers of *Enterococcus faecium* and *Enterococcus faecalis* isolates carrying *vanA* and *vanB* genes from clinical samples submitted to SSI 2009-2018 supplemented with data obtained from MiBa from 2016-2018, Denmark DANMAP 2018

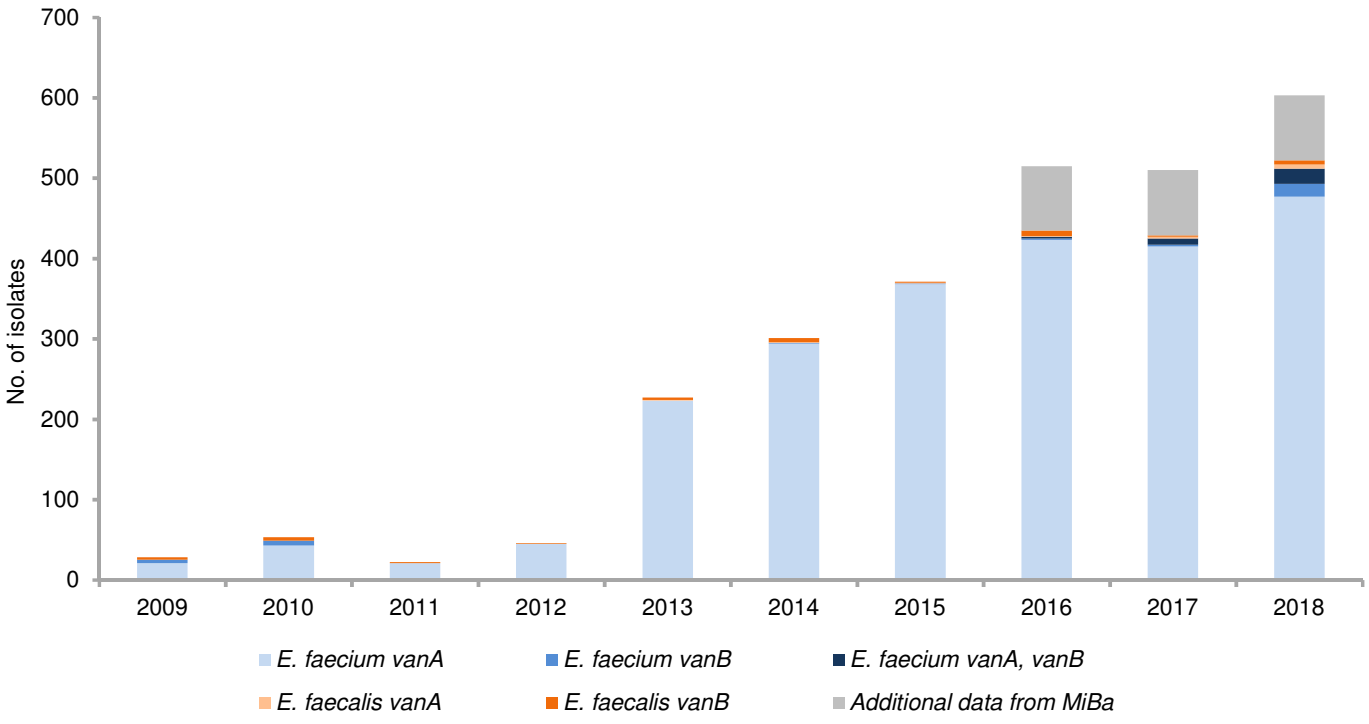

Table 8.11 Distribution of the most common types of *vanA Enterococcus faecium* according to MLST and cgMLST, Denmark DANMAP 2018

	2015		2016		2017		2018	
Types[a]	(n = 369)		(n = 427)		(n = 425)		(n = 515)	
ST80-CT14 *vanA*	81	22%	38	9%	15	4%	1	<1%
ST80-CT24 *vanA*	23	6%	19	5%	11	3%	2	<1%
ST80-CT860 *vanA*	7	2%	11	3%	N.D.	N.D.	N.D.	N.D.
ST80-CT866 *vanA*	14	4%	10	2%	7	2%	N.D.	N.D.
ST80-CT991 *vanA*	N.D.	N.D.	11	3%	9	2%	6	1%
ST80-CT1160 *vanA*	N.D.	N.D.	N.D.	N.D.	7	2%	10	2%
ST80-CT1064 *vanA/vanB*	N.D.	N.D.	2	<1%	8	2%	23	5%
ST80-CT1729 vanA	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	22	4%
ST117-CT873 *vanA*	5	1%	12	3%	N.D.	N.D.	N.D.	N.D.
ST117-CT1180 *vanA*	N.D.	N.D.	N.D.	N.D.	9	2%	30	6%
ST203-CT859 (subtypes CT1051 and CT1507) *vanA*	188	51%	271	64%	265	63%	161	31%
ST1421-CT1134 *vanA*	N.D.	N.D.	2	<1%	13	3%	176	34%
other types	51	14%	51	12%	81	19%	84	16%

[a]ST, sequence type (MLST); CT, cluster type (cgMLST); N.D., not determined

In 2017, the VVE clone ST1421-CT1134 *vanA E. faecium* was only detected in clinical samples from the Capital Region and accounted for 3% of the *E. faecium* isolates. In 2018, 34% (n = 176) of the *vanA E. faecium* isolates belonged to ST1421-CT1134 (Table 8.11) and this clone was detected from all DCMs in the Capital Region, the DCM in Region Zealand and from one DCM in the Region of Southern Denmark. It seems very likely that VVE have been underreported, since not all DCMs in Denmark have implemented systematic molecular testing.

Conclusion

The still increasing number of VRE/VVE cases in 2018 in Denmark is worrying. VRE can be carried in the intestine for a long period without showing any symptoms. Moreover, VRE can persist in the hospital environment, which makes infection control difficult. Infection control should include proper cleaning, good hand hygiene, VRE/VVE screening and subsequent isolation of patients. The spread of the "VVE clone", ST1421-CT1134 *vanA E. faecium*, in Denmark is of concern, especially since VVE diagnostic is challenging and therefore, the clone is likely to be underdiagnosed. Close surveillance of VVE is important in the future.

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8.3.4 Detection of the 23S rRNA mutations encoding linezolid resistance and the *optrA* in enterococci from Denmark

Background

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

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

Surveillance of linezolid-resistant enterococci (LRE)

Danish departments of clinical microbiology (DCMs) have, on voluntarily basis, submitted LRE for surveillance to the National Reference Laboratory for Antimicrobial Resistance at Statens Serum Institut (SSI).

During 2015-2018, eight linezolid-resistant *E. faecium* isolates and eight linezolid-resistant *E. faecalis* isolates were send to SSI (only one isolate per patient were included). WGS data from the 16 LRE isolates were investigated for 23S rRNA mutations (G2576T and G2505A), and the *optrA, cfr, cfr(B)* and *poxtA* genes using the LRE-Finder (https://cge.cbs.dtu.dk/services/LRE-Finder/). Among the LRE isolates, LRE-Finder detected seven *E. faecium* with the G2576T mutation, one *E. faecium* with the G2505A mutation and eight *E. faecalis* isolates with *optrA* (Table 8.12).

Table 8.12 Characterisation of the 16 linezolid resistant enterococci (LRE) and the eight linezolid vancomycin resistant enterococci (LVRE), 2015-2018, Denmark DANMAP 2018

	No. of isolates	Species	Linezolid resistance mechanism	Vancomycin resistant gene
LRE	1	*E. faecium*	G2505A	none
	7	*E. faecium*	G2576T	none
	8	*E. faecalis*	*optrA*	none
LVRE	6	*E. faecium*	G2576T	*vanA*

Surveillance of linezolid-vancomycin-resistant Enterococci (LVRE)

In order to detect linezolid-resistant VRE isolates (LVRE), the LRE-Finder was also used for detection of LRE mutations and LRE genes in genomes from VRE isolates from the VRE Surveillance. In total, 1757 genomes from VRE isolates from 2015-2018 were investigated using the LRE-Finder. No linezolid vancomycin resistant *E. faecalis* were detected, whereas, six linezolid-vancomycin resistant *E. faecium* were detected. The linezolid resistant *E. faecium* isolates had the G2576T mutation and were positive for the *vanA* gene encoding vancomycin resistance (Table 8.12).

Conclusion

The findings of LRE and LVRE are of concern. Linezolid is used for treatment of VRE. Only a limited number of antimicrobial agents are available for treatment of infections with LVRE.

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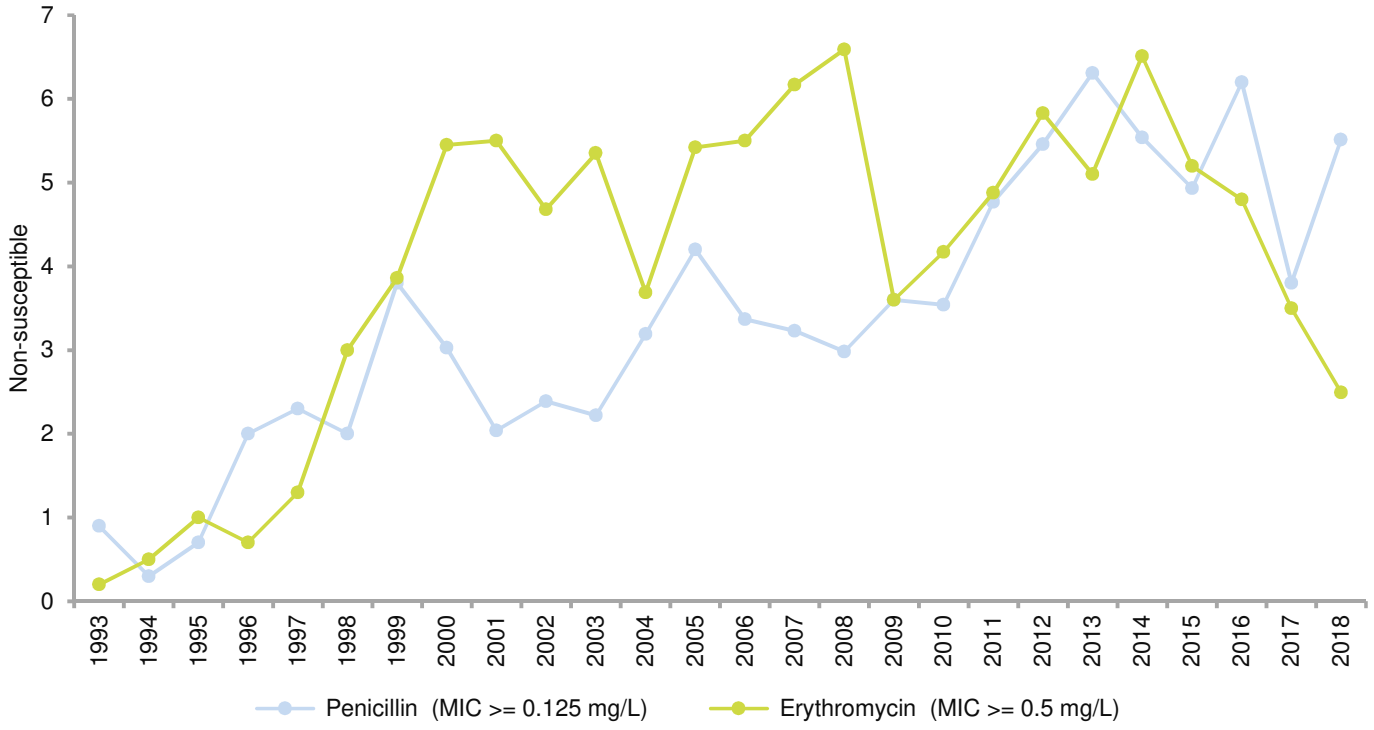
8.3.5 *Streptococcus pneumoniae*

The surveillance of pneumococci (*Streptococcus pneumoniae*) causing invasive disease in Denmark happens through mandatory submission of clinical isolates to Statens Serum Institut (SSI). At SSI, the isolates are serotyped and tested for antimicrobial susceptibility.

In Denmark, 798 cases of invasive pneumococcal disease were registered in 2018. Isolates were received from 768 of these cases. The isolates mainly originated from either blood (713 isolates from bacteraemias, nine of which the patient additionally had a positive identification of pneumococci in the cerebrospinal fluid) or from cerebrospinal fluid alone (51 isolates). Four isolates were moreover received from other, normally sterile sites (ascites, joint), but results from these are by tradition not included in this report. Two isolates could not be serotyped. The remaining 762 isolates from blood or cerebrospinal fluid belonged to 40 different serotypes, and 711 of those were fully susceptible to both penicillin and erythromycin (93.3%). For penicillin, 720 isolates were fully

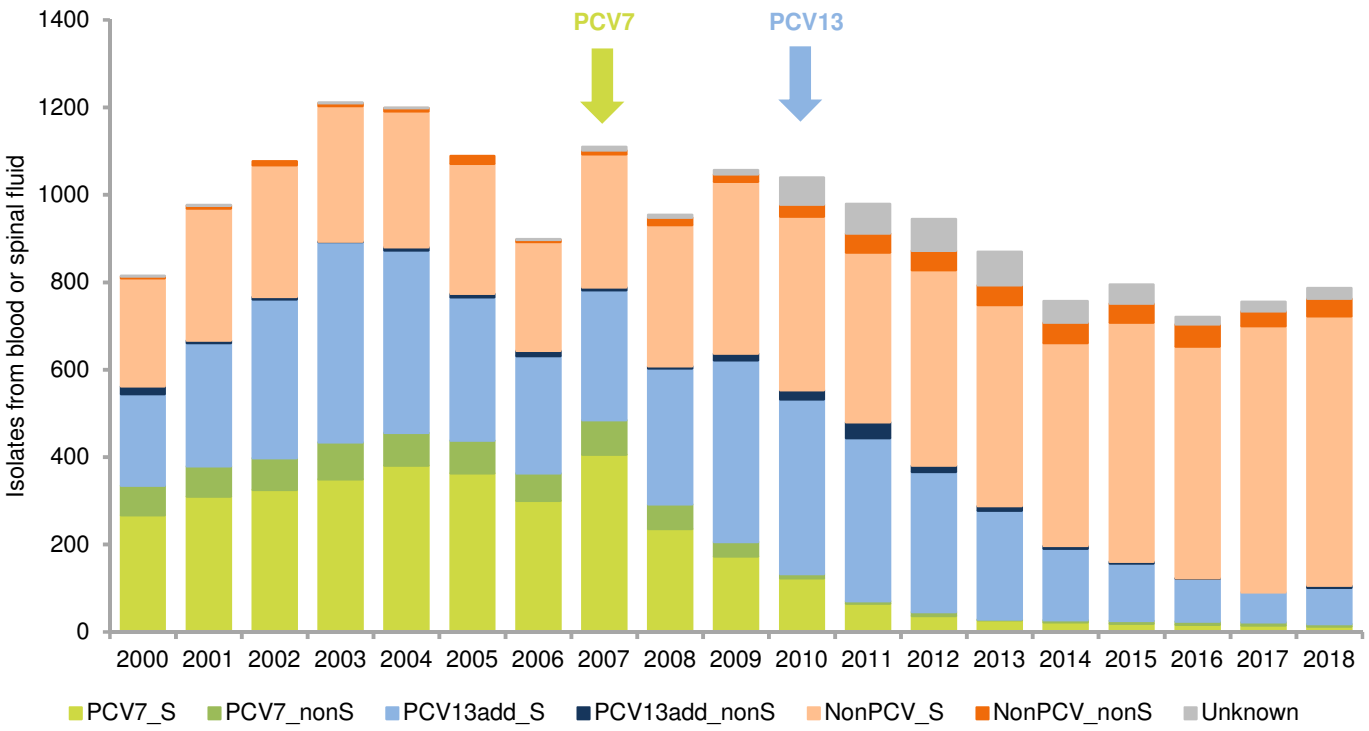
Figure 8.15 Non-susceptibility (%) in *Streptococcus pneumoniae* blood and spinal fluid isolates from humans, Denmark DANMAP 2018



susceptible (94.5%), 41 isolates (5.4%) were intermediate susceptible and one (of serotype 14) was resistant (0.1%). For erythromycin, 743 isolates were fully susceptible (97.5%) and 19 isolates (2.5%) were resistant. For penicillin, the level of non-susceptibility in 2018 was higher than in 2017 (3.8%) but lower than in 2016 (6.2%). For erythromycin, the level of non-susceptibility in 2018 was the lowest since 1998, Figure 8.15.

Comparing these results to the data reported in 2017 from our neighbouring countries, the levels of penicillin non-susceptibility reported to EARS-Net were: Sweden (6.1%), Norway (4.8%) and Germany (4.8%). The levels of erythromycin non-susceptibility were: Sweden (4.8%), Norway (5.9%) and Germany (7.1%). Thus, the results of non-susceptibility for invasive pneumococci from Denmark in 2018 were similar to the reported values from 2017 from neighbouring countries with respect to penicillin, but markedly lower with respect to erythromycin.

Figure 8.16 *Streptococcus pneumoniae* blood and spinal fluid isolates from humans. Vaccine serotypes and non-vaccine serotypes in combination with susceptibility to penicillin and erythromycin, Denmark DANMAP 2018



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

Table 8.13 Number of invasive isolates and distribution of resistance in the most common sero-types of pneumococci, Denmark

DANMAP 2018

Serotype	N 2018	PenS, EryS	PenS, EryR	PenI, EryS	PenI, EryR	PenR, EryS	%PenSEryS	N 2017	%PenSEryS2017
8	194	194					100.0%	192	99.5%
3	70	68	2				97.1%	57	100.0%
22F	69	69					100.0%	58	100.0%
9N	62	61		1			98.4%	56	98.2%
12F	55	55					100.0%	69	98.6%
15A	25	19			6		76.0%	16	62.5%
20	24	24					100.0%	26	100.0%
11A	19	18		1			94.7%	19	89.5%
16F	19	16		3			84.2%	21	95.2%
24F	17	13	4				76.5%	19	78.9%
33F	17	15	2				88.2%	13	92.3%
10A	15	15					100.0%	9	100.0%
31	15	15					100.0%	8	87.5%
35B	15	15					100.0%	20	90.0%
23B	14	1		13			7.1%	11	27.3%
35F	14	14					100.0%	13	100.0%
23A	13	13					100.0%	18	100.0%
17F	12	6		6			50.0%	10	80.0%
19A	11	9		2			81.8%	5	100.0%
15B	9	9					100.0%	14	92.9%
19F	7	5	1		1		71.4%	13	69.2%
6C	5	2		2	1		40.0%	13	92.3%
Other	61	55		3	2	1	90.2%	54	90.7%

N = number of isolates, Pen = penicillin, Ery = erythromycin

Conclusion

There has been a trend of decreasing non-susceptibility to erythromycin since 2013, while the non-susceptibility levels to penicillin are more variable. Antimicrobial susceptibility is highly correlated to serotypes, and therefore fluctuations in susceptibility often reflects changes in the circulating serotypes. More information on the surveillance of invasive pneumococcal disease can be found on the SSI homepage (EPI-NEWS, No 40/41-2018, https://en.ssi.dk/news/epi-news/2018/no-40-41---2018).

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8.3.6 Beta-haemolytic streptococci

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

Streptococcus pyogenes (group A streptococci; GAS) cause pharyngitis, tonsillitis, otitis media, wound infections, and superficial skin infections, but also more severe infections, e.g., bacteraemia, necrotizing myofasciitis, and rarely meningitis. In Denmark, the rate of asymptomatic throat carriage of GAS is approximately 2%. *Streptococcus agalactiae* (group B streptococci; GBS) may be present in the vaginal flora of 20-25% of women in the childbearing age. If these bacteria are transferred to the child during labour, meningitis and septicaemia may develop in the newborn. Such infections also occur in elderly or immuno-compromised patients.

Streptococcus dysgalactiae subsp. *equisimilis* (group C streptococci; GCS, and group G streptococci; GGS) predominantly cause soft-tissue infections and sometimes bacteraemia.

This report presents data on antimicrobial resistance in non-duplicate isolates from normally sterile sites (e.g. blood, cerebrospinal fluid, synovial fluid, pleural fluid, ascites, and tissue obtained during surgery) of BHS submitted in 2018 to the Neisseria and Streptococcus Reference laboratory. Isolates are received from all DCMs in Denmark. It is voluntary to submit isolates of BHS, and not all DCMs submit BHS of all groups.

Infections with BHS are usually treated with penicillins or macrolides. All submitted isolates of BHS group A, B, C and G are therefore tested for susceptibility to penicillin, erythromycin, and clindamycin as well as inducible clindamycin resistance. For all isolates of GAS the *emm* type (the M protein gene that dictates the M serotype) was determined by whole genome sequencing.

Figure 8.17 shows the resistance findings for the years 2014 through 2018. In 2018, the number of submitted isolates from

unique cases was 873, an increase of 25% compared to 2017. Corresponding changes for individual serogroups were: GAS; + 14%, GBS; + 37%, GCS; + 33%, and GGS; + 23%. All isolates were fully susceptible to penicillin.

Figure 8.17 Beta-haemolytic streptococci: Antimicrobial resistance testing results. Numbers of isolates and resistance in percent, Denmark

DANMAP 2018

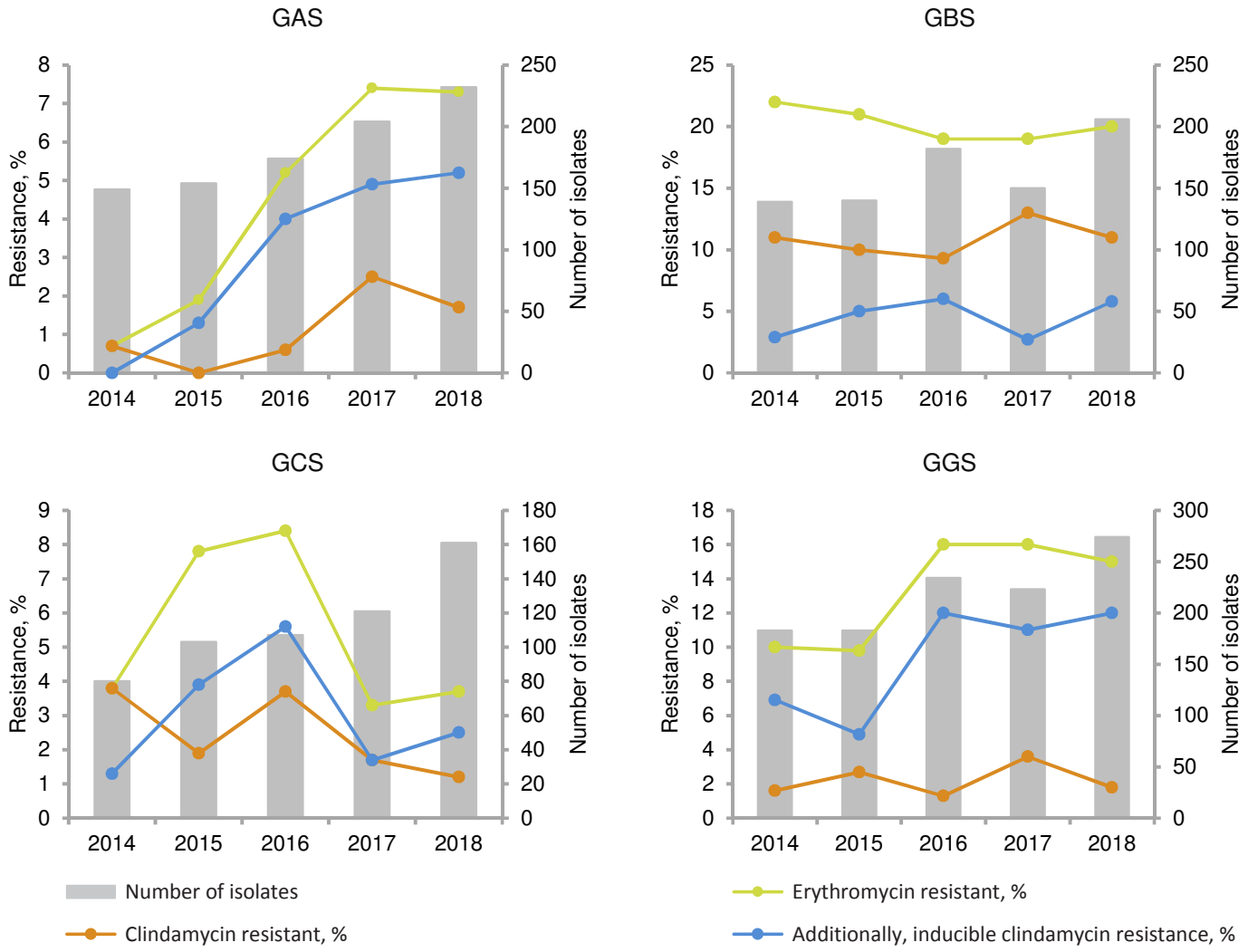

Table 8.14 Group A streptococci 2018: Distribution of *emm* types, clindamycin resistance and erythromycin resistance, Denmark

DANMAP 2018

		CLI-S		
emm type	CLI-R	ERY-R	ERY-S	Total
1.0	0	0	83	83
12.0	0	0	13	13
28.0	0	0	10	10
66.0	0	0	20	20
89.0	0	0	27	27
108.1	0	10	1	11
Subtotal	0	10	154	164
Other	3 (*)	4 (#)	61	68
Total	3 (*)	14 (#)	215	232

Note: Numbers of isolates are shown for individual *emm* types if ≥ 10. Otherwise, if < 10, the numbers are summarized in the "Subtotal" category.
(*) All were ERY-R and all were *emm* type 11.0
(#) One was *emm* type 11.0
CLI = clindamycin, ERY = erythromycin, R = resistant, S = sensitive

The erythromycin resistance rate remained virtually unchanged compared to 2017 for all four serogroups. The clindamycin resistance rate showed a slight decrease for all serogroups. The percentage of strains with inducible clindamycin resistance was virtually unchanged for GAS and GGS, but showed an increase for GBS and GCS. The percentage of fully susceptible isolates was unchanged for all four serogroups.

The GAS isolates belonged to 35 different *emm* types. The majority of the received isolates (164; 71%) belonged to six *emm* types, each of which were represented by at least ten isolates (Table 8.14). The remaining 68 isolates (29%) belonged to 29 different *emm* types. All three clindamycin resistant isolates and one clindamycin sensitive, erythromycin resistant isolate were *emm* type 11.0. There were no erythromycin sensitive isolates of *emm* type 11.0.

Conclusions

The number of submitted isolates in 2018 compared to 2017 increased for all four serogroups. The erythromycin resistance rate was unchanged for all four serogroups. The clindamycin resistance rate, including inducible resistance, decreased slightly for GAS and GGS and increased slightly for GBS and GCS.

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8.3.7 *Haemophilus influenzae*

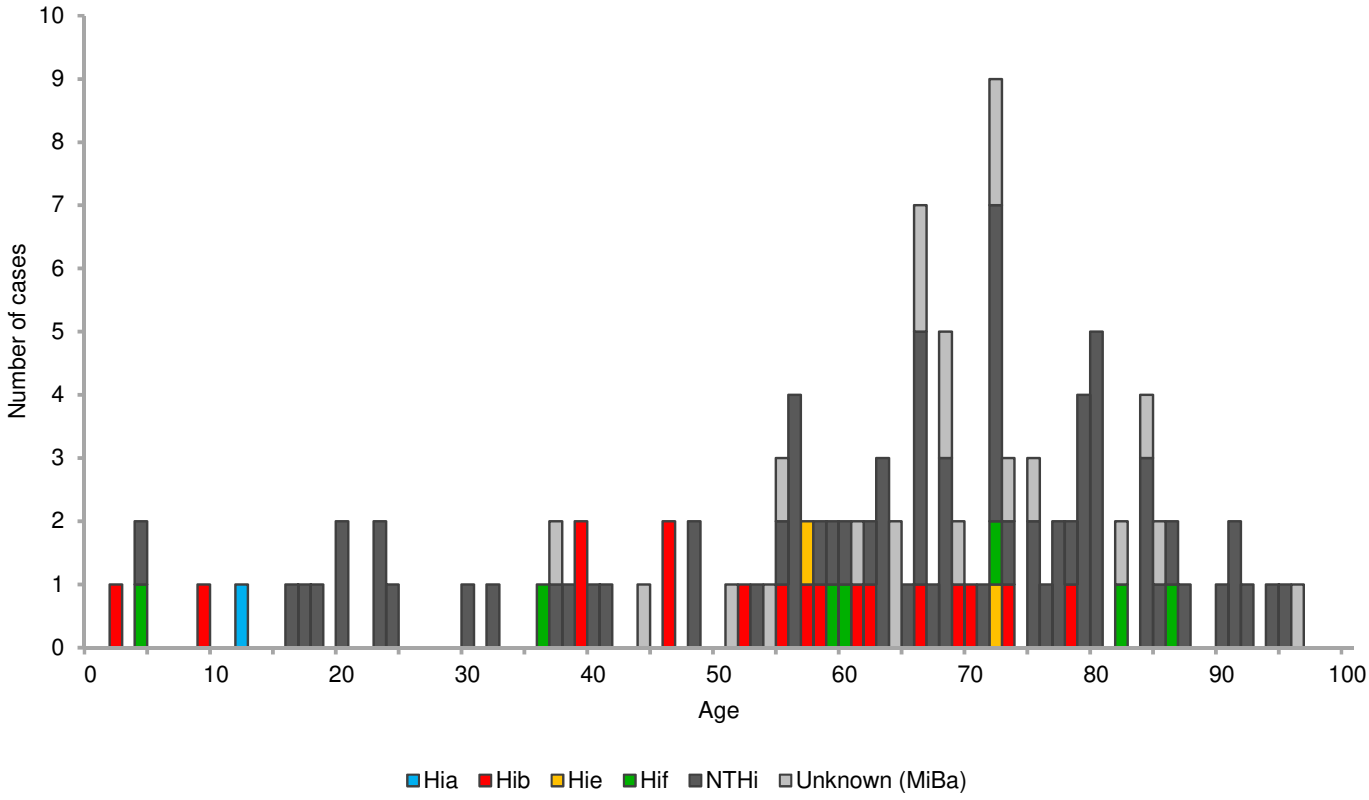
Haemophilus influenzae is part of the normal upper respiratory tract flora, where colonisation varies with age. *H. influenzae* can also be the cause of infections, with otitis media and bacterial sinusitis being the most common clinical manifestations.

Invasive infections with *H. influenzae* happen relatively rarely and occur predominantly in the very young and the elderly but may also afflict individuals with underlying conditions, such as chronic obstructive pulmonary disease or cancer. *H. influenzae* can be divided into six capsular serotypes (a, b, c, d, e and f, also known as Hia, Hib, Hic, Hid, Hie and Hif), as well as non-capsular (non-typeable, NTHi). Introduction of the polysaccharide type B vaccine in 1993 significantly reduced the number of cases with systemic infection caused by *H. influenzae* type b (Hib) isolates. Before the vaccine was introduced, there were around 80 cases of Hib meningitis anually among infants in Denmark, and this is now down to 0-2 per year. NTHi for which no vaccine yet exists now dominates the invasive infections.

Invasive *Haemophilus influenzae*

Surveillance of invasive Hib is mandatory by submission of the isolate to Statens Serum Institut (SSI). By tradition, most departments of clinical microbiology are voluntarily submitting all isolates of invasive *H. influenzae* and not just Hib. The received isolates are then serotyped and biotyped by the reference laboratory at SSI. Isolates are submitted for the

Figure 8.18 Different serotypes in invasive *H. influenzae* cases according to age, 2018, Denmark DANMAP 2018



majority of cases, and the remaining cases can be identified through the Danish Microbiological Database (MiBa). Thus, all invasive infections with *H. influenzae* are registered in the surveillance database, and for the majority of cases serotypes are available. Whole genome sequencing is performed on the received isolates, and the data are analysed for the presence of the plasmid-borne beta-lactamase genes TEM-1 and ROB-1. Antimicrobial susceptibilities of the isolates are found through MiBa.

The present report includes all episodes of invasive *H. influenzae* identified in MiBa, where the date of sampling was in 2018. A total of 121 cases were identified, of which isolates from 100 (83%) were received at the reference laboratory. For five of the 121 cases, *H. influenzae* were isolated from cerebrospinal fluid, two were from pleural fluid, and 114 were from blood. The serotypes of the received isolates were one Hia (1%), 17 Hib (17%), two Hie (2%), seven Hif (7%) and 73 NTHi (73%). The age-distribution of the cases is presented in Figure 8.18.

Seventeen of the received isolates harboured the TEM-1 gene (five Hib and 12 NTHi) (17%), and all of these had corresponding resistance to penicillin as registered in MiBa. Susceptibility results, divided in to serotypes, for the antibiotics that were most frequently registered in MiBa, are presented in Table 8.15.

The results from antimicrobial susceptibility testings registered in MiBa showed that there was 26% resistance to pencillin, 1% to ciprofloxacin, 20% to ampicillin, 16% to cefuroxime and 10% to amoxicillin/clavunulate. Some variation across serotypes was observed.

In summary, the majority of isolates from invasive infections with *H. influenzae* are of the non-capsular type. This is similar to what is observed generally in Europe [R. Whittaker, Emerg Infect Dis, 2017, Mar;23(3):396-404]. Only 48% of the isolates were registered as fully susceptible to penicillin. However, 80% of the isolates were registered as susceptible to ampicillin.

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Table 8.15 Distribution of antimicrobial susceptibilities in invasive *Haemophilus influenzae* according to serotypes, 2018, Denmark

DANMAP 2018

	Hia	Hib	Hie	Hif	NTHi	Unknown*	All
Penicillin_no result registered	-	-	-	-	1	-	1
Penicillin_I	-	3 (18%)	1 (50%)	3 (43%)	17 (24%)	7 (33%)	31 (26%)
Penicillin_R	-	5 (29%)	-	1 (14%)	21 (29%)	4 (19%)	31 (26%)
Penicillin_S	1 (100%)	9 (53%)	1 (50%)	3 (43%)	34 (47%)	10 (48%)	58 (48%)
Ciprofloxacin_no result registered	-	-	-	-	3	-	3
Ciprofloxacin_R	-	-	-	-	1 (1%)	-	1 (1%)
Ciprofloxacin_S	1 (100%)	17 (100%)	2 (100%)	7 (100%)	69 (99%)	21 (100%)	117 (99%)
Ampicillin_no result registered	-	-	-	-	3	1	4
Ampicillin_R	-	5 (29%)	-	-	15 (21%)	3 (15%)	23 (20%)
Ampicillin_S	1 (100%)	12 (71%)	2 (100%)	7 (100%)	55 (79%)	17 (85%)	94 (80%)
Cefuroxime_no result registered	-	1	-	1	13	3	18
Cefuroxime_I	-	-	-	-	-	1 (6%)	1 (1%)
Cefuroxime_R	-	4 (25%)	-	-	12 (20%)	-	16 (16%)
Cefuroxime_S	1 (100%)	12 (75%)	2 (100%)	6 (100%)	48 (80%)	17 (94%)	86 (83%)
Amoxi/Clav_no result registered	-	3	-	1	23	2	29
Amoxi/Clav_R	-	3 (21%)	-	-	6 (12%)	-	9 (10%)
Amoxi/Clav_S	1 (100%)	11 (79%)	2 (100%)	6 (100%)	44 (88%)	19 (100%)	83 (90%)

* The group "unknown" represent the 21 cases that were registered in MiBa, but where an isolate was not received at the reference laboratory at SSI, and therefore not serotyped.

8.3.8 *Staphylococcus aureus*

Staphylococcus aureus is part of the normal flora of the skin and mucosa. Approximately 50% of humans will be colonized with *S. aureus* in their nose or throat at any given time, some of these carrying the strain only intermittently, others for longer periods of time. *S. aureus* causes infections ranging from superficial skin infections e.g. impetigo and boils to deeper and more complicated infections. These may be health care associated such as post-operative wound infections and infections related to intravenous catheters and prosthetic devices but they can also be caused by endogenous spread of bacteria causing septic arthritis, osteomyelitis, endocarditis and bacteraemia. Most of these may have a fulminant progress and are associated with high mortality.

In Denmark, a voluntary surveillance program of all *S. aureus* bacteraemia cases was established in 1957. By comparison with the numbers of bacteraemia cases registered in the Danish Microbiology Database (MiBa) since 2010, the number of cases reported to SSI has been almost complete (94-97%).

Laboratory and clinical notification of all cases of methicillin-resistant *S. aureus* (MRSA) has existed since November 2006. At SSI, all referred isolates are initially tested using a multiplex PCR detecting: the *spa, mecA, hsd, scn* and *pvl* gene (lukF-PV). *Spa* is used as *S. aureus* specific marker and for subsequent typing by Sanger sequencing, *mecA* to determine MRSA status, and *scn* and *hsd* as markers for human adaptation and relation to the clonal complex (CC) 398, respectively. PVL has been closely linked to skin abscesses and the very rare condition of severe necrotizing pneumonia. PVL is rarely found in methicillin-susceptible *S. aureus* (MSSA) causing bacteraemia but has been associated with certain community acquired (CA) MRSA strains. Isolates positive for *mecA* and the CC398 specific *hsd* fragment but negative for *scn* (human adaptive factor) and *pvl* genes are considered typical livestock associated MRSA (LA-MRSA) and are not *spa* typed. All others, including human adapted CC398 isolates, are spa typed. In addition, all bacteraemia cases and *mecA* negative presumptive MRSA are tested for presence of the *mecC* gene.

A representative selection of bacteraemia isolates is tested for antimicrobial susceptibility against 17 antimicrobials (see chapter 9 for more information). For MRSA cases, 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 (LA) MRSA. For CA, HACO and LA, classification was separated into known and not known exposure.

Surveillance of bacteraemia

In 2018, altogether 2,276 *S. aureus* bacteraemia cases corresponding to 39.4 cases per 100,000 inhabitants were reported from the departments of clinical microbiology (DCMs) in Denmark. This is an increase after approximately 2,000 reported cases in 2014-2016 and 2,104 cases in 2017. Thirty-seven (1.6%) of the bacteraemia cases were caused by MRSA. This proportion is almost identical to the previous years, and remains below most other European countries participating in EARS-Net [EARS-Net 2017]. Eight of the 37 MRSA cases were caused by LA-MRSA CC398 (4 LA-MRSA CC398 in 2017).

Within 30 days from the bacteraemia onset, 510 (22%) patients died (all cause mortality). The mortality for the MRSA bacteraemia cases was 19% (n = 7, of which 2 were due to CC398 MRSA).

A total of 504 representative isolates was susceptibility tested. Results from antimicrobial resistance testing in *S. aureus* bacteraemia isolates from 2009-2018 is presented in Table 8.16. Resistance to penicillin was 72%, showing a decreasing trend from 77% in 2009. The highest frequency of resistance to other antimicrobials than penicillin was observed for fusidic acid (17%), erythromycin (5%), clindamycin (4%) and norfloxacin (4%). For most antimicrobial agents, the susceptibility remained at the same level as the previous years. However, resistance to fusidic acid increased for the second consecutive year (14% in 2017 and 12% in 2016).

Typing revealed 630 different *spa* types distributed in 27 different clonal complexes (CCs). The ten most prevalent *spa* types, representing 34% of the total number, are presented in Table 8.17 together with *spa* types from the most prominent types of MRSA. The distribution of the most prevalent *spa* types has been very stable over the last decade with only minor changes in the relative order. The PVL toxin was present in 29 (1.3%) cases of which five were MRSA. The 29 PVL presenting isolates were distributed among 25 different *spa* types and 11 different CCs.

Table 8.16 Resistance (%) in isolates from *Staphylococcus aureus* bacteraemia cases, Denmark DANMAP 2018

Antimicrobial agent	2009 %	2010 %	2011 %	2012 %	2013 %	2014 %	2015 %	2016 %	2017 %	2018 %
Methicillin	1.6	1.4	1.4	1.2	1.7	2.9	1.5	2.1	2.2	1.6
Penicillin	77	75	77	74	76	77	71	71	72	72
Erythromycin	7	5	7	6	7	8	7	7	6	5
Clindamycin	6	4	6	6	6	8	7	6	5	4
Tetracycline	2	3	2	2	3	5	4	3	3	3
Fusidic acid	9	13	13	14	15	15	16	12	14	17
Rifampicin	<1	<1	<1	<1	0	<1	<1	<1	<1	<1
Norfloxacin	2	3	4	4	5	6	6	4	4	4
Kanamycin	1	1	<1	1	2	2	3	1	1	2
Linezolid	0	0	0	0	0	0	0	0	0	0
Mupirocin	<1	<1	<1	<1	<1	<1	<1	0	<1	0
Trimethoprim-sulfamethoxazole	nt	nt	<1	1	1	1	<1	<1	<1	0
Numbers tested	1479	1416	1515	1523	962	381	502	560	551	504

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

Table 8.17 The ten most prevalent *spa* types demonstrated in SAB and in non-LA-CC398 MRSA cases, Denmark 2018 DANMAP 2018

SAB			MRSA			
spa type	CC group	No. of cases	spa type	CC group	No. of cases	No. causing infections (%)
t127	CC1	137	t304	CC8	234	78 (33)
t002	CC5	101	t223	CC22	213	70 (33)
t084	CC15	105	t002	CC5	177	76 (43)
t230	CC45	81	t127	CC1	142	72 (51)
t091	CC7	74	t008	CC8	97	60 (62)
t012	CC30	70	t044	CC80	62	29 (47)
t021	CC30	61	t019	CC30	61	40 (66)
t008	CC8	53	t4549	CC8	58	43 (74)
t015	CC45	49	t005	CC22	50	27 (54)
t701	CC8	40	t437	CC59	48	33 (69)

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

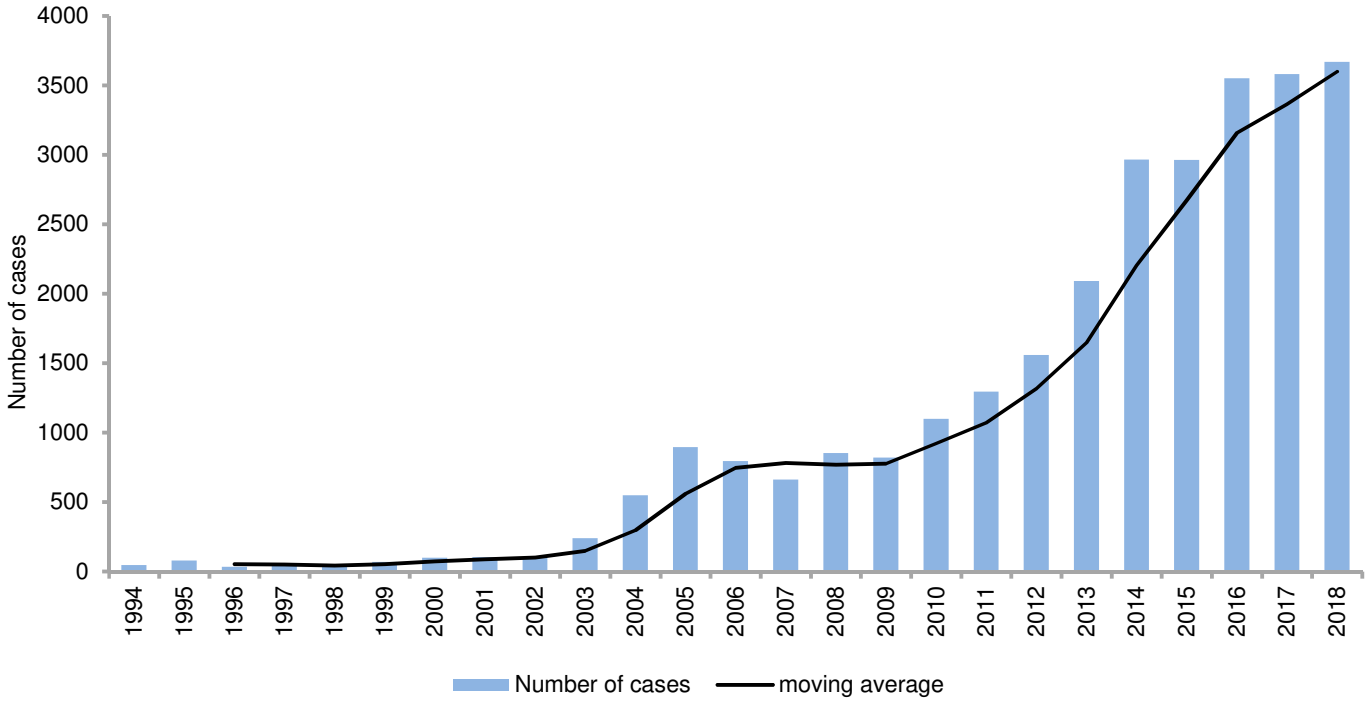
Surveillance of methicillin-resistant *S. aureus*

In 2018, 3,669 MRSA cases were detected (63.5 per 100,000 inhabitants). This was a slight increase compared to 2017 (3,579; Figure 8.19) and followed the increasing trend registered since 2009. A case was defined when a person for the first time tested positive for a specific MRSA strain regardless of clinical situation (infection or colonisation only).

CC398 cases constituted 34% (n = 1,250) of new MRSA cases, of which 1,215 belonged to the LA-MRSA CC398 and the remaining 35 to a human adapted variant harbouring the PVL encoding genes. The number of LA-MRSA CC398 is at the same level as the previous four years. The levelling in number of cases may be influenced by the fact that only new cases are registered in the surveillance program. Many people in contact with livestock have already been examined and tested positive at an earlier stage and also cases where the clinical situation changes from colonisation to infection will thus not be registered as new cases.

MRSA isolates carrying *mecC* were detected in 52 cases (1.4%). Twenty-two of the cases (42%) had infections at the time of diagnosis. Three patients reported contact to horses, which previously have been shown to be reservoirs for *mecC* MRSA. The remaining 27 patients reported no known contact to any livestock.

Figure 8.19 Number of new MRSA cases with a three years moving average, Denmark DANMAP 2018



The total number of cases and the number of cases presenting with infection according to epidemiological classification are shown in Table 8.18. Most of the cases (84%) were acquired in Denmark. At the time of diagnosis, 40% (n = 1,478) of cases had infection, which was similar to 2017.

The trend of MRSA infections for 2009-2018 based on their epidemiological classification is shown in Figure 8.20. For the first time in almost a decade the number of CA infections did not increase in 2018 and infections caused by LA-MRSA CC398 seemed to level off, whereas imported and HACO infections continued to increase. Imported cases presented with infections in 58% of the cases and the number of infections in this category has been increasing from less than 100 cases in 2007 to 341 cases in 2018. HA-MRSA infections remains at a low level with only 100 new cases registered in 2018. It should be noted that the average time patients are hospitalised has decreased over the years to approximately three days, which means that HA-MRSA cases may not be recognized before patients are discharged.

Table 8.18 Epidemiological classification of new MRSA cases, 2018, Denmark DANMAP 2018

Epidemiologic classification	Exposure	No. of cases (% of total)	No. (%) of cases with infections
Imported (IMP)		**588 (16)**	341 (58)
Hospital-acquired (HA)		**100 (3)**	39 (39)
Health-care associated, community onset (HACO)		**244 (7)**	
	with known exposure	18	8 (44)
	without known exposure	226	178 (79)
Health care worker		**42 (1)**	20 (48)
Community-acquired (CA)		**1457 (40)**	
	with known exposure	797	109 (14)
	without known exposure	660	527(80)
LA-MRSA CC398		**1215 (33)**	
	with known exposure	1057	169 (16)
	without known exposure	158	87 (55)
Unknown/missing		**23**	

Numbers shown in bold are totals

Figure 8.20 Number of MRSA infections according to epidemiological classification, Denmark DANMAP 2018

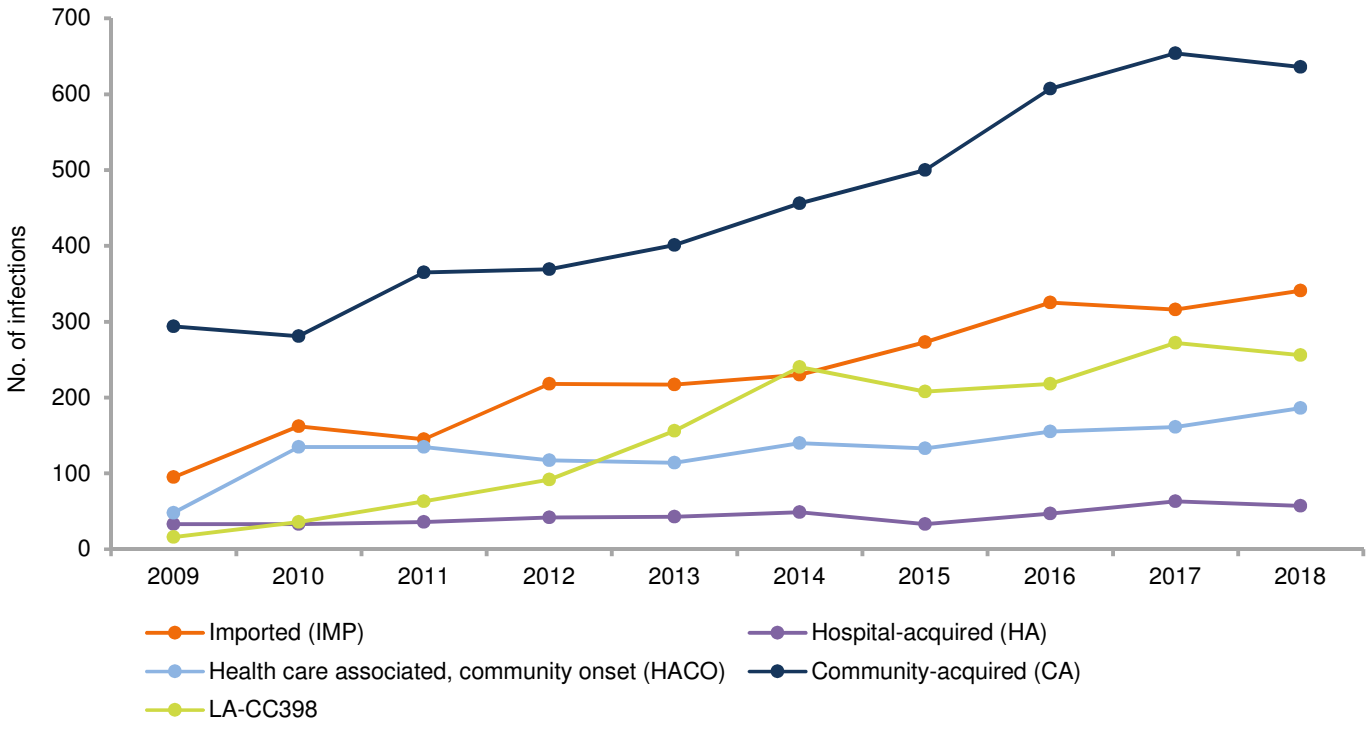


Table 8.19 Resistance (%) in non LA-CC398 MRSA isolates, 2018, Denmark DANMAP 2018

	% non-CC398
Erythromycin	33
Clindamycin	28
Tetracycline	26
Fusidic acid	18
Rifampicin	1
Norfloxacin	21
Kanamycin	28
Linezolid	<1
Mupirocin	<1
Trimethoprim-sulfamethoxazole	3
Number of tested isolates	1233

Molecular typing of the MRSA strains.
In total, *spa* typing revealed 363 different strain types, not including isolates belonging to LA-CC398. Among the infections, 278 *spa* types were demonstrated. The 10 dominating non-LA-CC398 *spa* types isolated in 2018 are listed in Table 8.17. They constituted 43% of the total number of non-LA-CC398 MRSA isolates. *spa* types t304 and t223 have been among the five most prevalent *spa* types since 2016 and can be linked to the refugee crisis following the civil war in Syria. Ten years ago, t024/CC8 was among the five most prevalent *spa* types, but has since decreased in prevalence. Table 8.17 does not list *spa* types in CC398, which for many years has been the dominating CC.

The *pvl* gene was detected in 29% of the infections and in 12% of the asymptomatic carriers and most often in relation to isolates with *spa* types t008 (n = 66), t019 (n = 57), t044 (n = 56), t437 (n = 37) and t002 (n = 36).

Resistance among MRSA isolates
Resistance data for non-LA-CC398 isolates are presented in Table 8.19. Every other non-LA-CC398 isolate received in 2018 was tested (n = 1,233). Resistance prevalences were similar to previous years.

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Textbox 8.2

LA-MRSA CC398 in animals and humans

LA-MRSA CC398 infections in humans

The annual number of registered LA-MRSA CC398 infections in people with livestock contact has been relatively constant since 2014, with 121-174 new cases per year (Figure 1). Similarly, the number of LA-MRSA CC398 infections in people with no livestock contact, i.e. the general population, seems to have reached a plateau, with 87-98 cases during 2016-2018 (Figure 1). It should be noted that these numbers do not include people who have been found positive for LA-MRSA CC398 in previous years. This is particularly relevant for people who have been working in the sector for some time, as many of them have already been screened and found positive for LA-MRSA CC398.

Figure 1 LA-MRSA CC398 in conventional pig production herds and as a cause of human infections DANMAP 2018

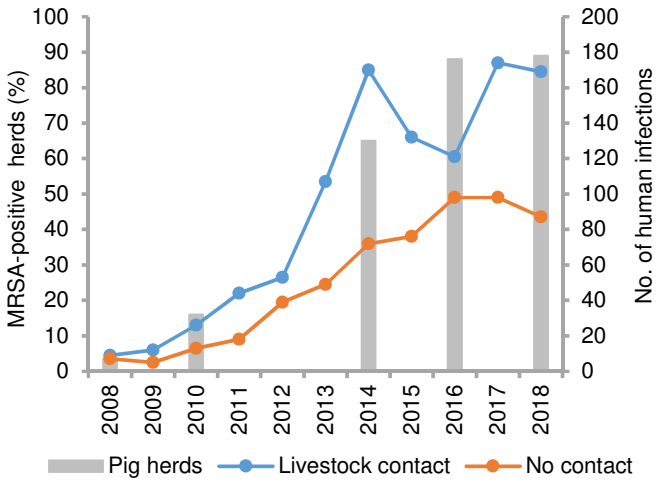

Table 1 Occurrence of MRSA in various animal populations, 2018, Denmark DANMAP 2018

Farm/herd type	MRSA-positive (%)
Conventional production pigs	89% (116/130)
Conventional breeding pigs	83% (34/41)
Organic/free-range pigs	20% (21/104)
Mink	25% (31/122)
Horses	8% (10/123)
Dairy cows	6% (8/132)
Layer hens	3% (4/124)
Turkeys	0% (0/19)

MRSA surveys in animals

In 2018, the Danish Veterinary and Food Administration (DVFA) conducted MRSA surveys in conventional pig herds (breeding and production), free-range pig herds (both organic and non-organic), mink farms, horse herds, dairy cattle herds, layer hen and turkey flocks. For pigs, mink, horses, and dairy cattle, 25 individual animals were tested per herd/farm and the samples analysed as pools of five samples, whereas sampling of layer hen and turkey flocks was performed by collecting five sock samples per flock. Thus, the results described below represent prevalence estimates at the farm level (between-farm prevalence) rather than at the animal level (within-farm prevalence). The results of the MRSA surveys are summarised in Table 1.

MRSA in conventional pig herds

A total of 130 randomly selected production herds and 41 breeding herds were tested; The overall prevalence of LA-MRSA was 89%, which is similar to the results obtained in 2016 (88%) but higher than in previous years (Figure 1). Among the breeding herds, 83% tested MRSA-positive, which is an increase compared to 2016 (66%). All 150 isolates had *spa*-types associated to CC398, with *spa*-types t034 (n = 109) and t011 (n = 28) being the most prevalent, while 10 other *spa*-types were found in one to three isolates.

MRSA in organic and free-range pigs

Testing of 104 organic and non-organic free-range pig herds in 2018 revealed that 20% were positive for LA-MRSA, compared with 6% in 2015. It should be noted, however, that the 2015 survey only included organic herds and also differed in size and sample selection procedure, which makes it difficult to compare the results. It has been speculated that the lower frequency of MRSA in organic and non-organic free-range pigs compared to conventionally raised pigs is due to differences in animal density, access to open air, and antimicrobial use. A study will be conducted in 2020-2021 to determine the impact of these factors. All 21 MRSA isolates had *spa*-types associated to CC398, most of which belonged to t034 (n = 15) and t011 (n = 4).

continued ... Textbox 8.2

MRSA in mink

Previous studies have revealed that LA-MRSA in mink is most often located on the paws and in the pharynx, probably reflecting that mink acquire MRSA from contaminated food, which contains slaughter offal including by-products from the Danish pig production. Testing of paws from mink from 122 mink farms revealed that 25% were positive for MRSA. This is similar to the prevalence of 29% found in a 2015 survey [Hansen et al., Vet. Microbiol. 2017; 207:44-9]. Of the 31 MRSA isolates, 30 had CC398-associated *spa*-types, with t034 (n = 19) and t011 (n = 6) being the predominant *spa*-types, while a single isolate had *spa*-type t13790 associated with CC1. In a recent study, it was shown that most of the LA-MRSA CC398 isolates obtained from mink resembled isolates found in the Danish pig production, supporting the hypothesis that mink become colonized through contaminated mink feed [Fertner et. al, Vet. Microbiol. 2019; 231: 80-86].

MRSA in horses

Of the 123 horse herds tested, 8% were found positive for MRSA, which is similar to the prevalence of 9% found in a previous study from 2015 [Islam et al., Front. Microbiol. 2015; 8:543]. Of the 10 MRSA isolates, nine had *spa*-types t011 or t034 associated with CC398, whereas a single isolate had *spa*-type t843 associated with CC130 and carried the methicillin resistance gene *mecC* rather than the typical *mecA* gene found in LA-MRSA CC398 and CC1.

MRSA in dairy cows

In 2018, 132 dairy herds were tested for LA-MRSA, of which 6% were positive. Previous surveys of bulk milk showed a lower percentage of MRSA positive samples, which may indicate that sampling using nasal swabs is more sensitive. All MRSA isolates had *spa*-type t034 associated with CC398, except from a single isolate with *spa*-type t267 associated with CC97. MRSA with *spa*-type t267 has previously been described in bovine samples.

MRSA in layer hens

In 2018, a total of 124 layer hen flocks were examined, of which 3% tested positive. The four MRSA isolates had *spa*-types t011 or t034 associated with CC398. All 19 tested turkey farms were MRSA-negative.

MRSA in turkeys

In 2015, a single turkey flock tested positive, whereas all broiler and layer hen flocks were negative.

Conclusions

The results presented above show that conventionally raised pigs are still the primary reservoir for LA-MRSA CC398, and that LA-MRSA CC398 is capable of spreading to other animal species, such as cattle, mink, horses, and poultry, and to people in contact with those animals. The prevalence of LA-MRSA CC398-positive pig farms and the number of LA-MRSA CC398 infections in the general population seem to have reached a maximum in the last couple of years (Figure 1), which suggests that the occurrence of LA-MRSA CC398 in humans is due to a constant spillover from pig farms. Nonetheless, the continued spread of LA-MRSA CC398 to people with no livestock contact is worrisome because they include a higher proportion of elderly and immunocompromised people with an elevated risk of developing serious and life-threatening infections. In addition, there are international concerns about the possibility that LA-MRSA CC398 can adapt to humans, which would result in increased spread in the human population and an increasing number of infections in people with no livestock contact. It is therefore important to continue the ongoing surveillance and efforts to prevent spillover of LA-MRSA CC398 from the farm through people or via the environment.

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8.3.9 *Neisseria gonorrhoeae*

Neisseria gonorrhoeae is the causative agent of the sexually transmitted infection gonorrhoea, which is usually located in the urethra in males and cervix in females. *N. gonorrhoeae* (gonococci) may sometimes be demonstrated in specimens from the pharynx or rectum in either gender. In females, the finding of gonococci in rectal specimens is usually due to contamination with vaginal secretion, while in men who have sex with men (MSM) it is due to unprotected anal sex leading to infection. In both genders, the condition is generally asymptomatic. Pharyngeal gonorrhoea is virtually always asymptomatic.

Complications of gonorrhoea include e.g. salpingitis, epididymitis, orchitis, and prostatitis. Furthermore, conjunctivitis may occur in newborns after transmission from an infected mother during labour and rarely in adults following direct inoculation.

Surveillance

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

At the NSR laboratory ceftriaxone, ciprofloxacin and azithromycin MICs are determined. Both resistant and intermediate susceptible isolates are categorized as resistant in this report.

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

Results and discussion

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

The NSR laboratory received isolates from 1067 unique cases of gonorrhoea diagnosed in 2018. The annual number increased considerably from 2011 through 2016 (Figure 8.21), partly because the widespread use of combined nucleic acid amplifications tests for *Chlamydia trachomatis* and *N. gonorrhoeae* has identified unexpected cases of gonorrhoea (sometimes followed by culture), and partly due to an increasing incidence of gonorrhoea, especially among young heterosexual persons, among whom an increasing proportion are women. A decrease in the annual number of unique cases has been observed in 2017, continuing in 2018.

Figure 8.21 The number of submitted gonocccus isolates from males and females, and the percentage of isolates with ciprofloxacin resistance, azithromycin resistance, and penicillinase production, Denmark

DANMAP 2018

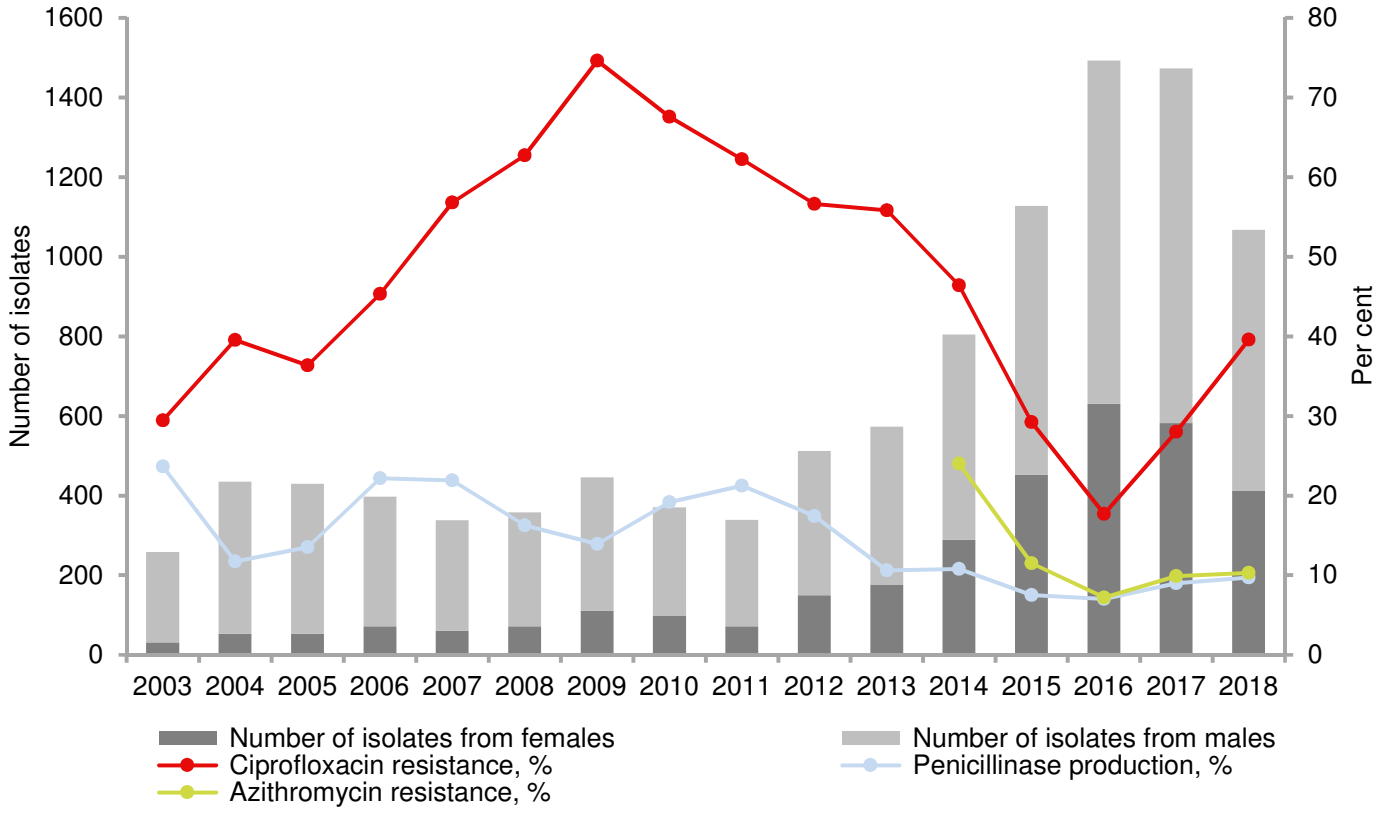
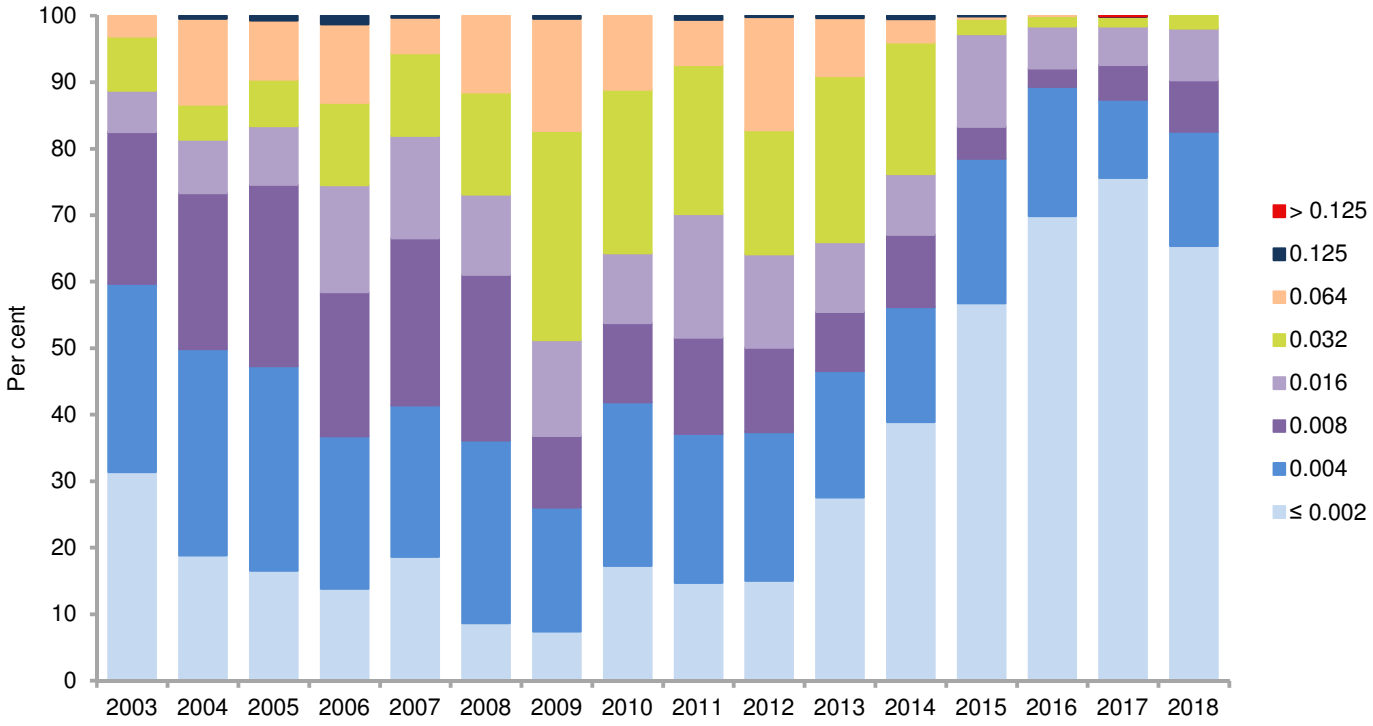


Figure 8.22 Distribution of ceftriaxone MIC (mg/L) values in gonococci, Denmark DANMAP 2018



The ciprofloxacin resistance rate was 40% in 2018 (28% in 2017 and 18% in 2016), thus still considerably lower than the peak of 75% in 2009 (Figure 8.21). The percentage of strains producing penicillinase was 10%. It has fluctuated between 22% in 2005 and 7% in 2016. Azithromycin resistance was found in 6% (10% in 2017) and intermediate susceptibility in 4% of the isolates (4% in 2017).

Ceftriaxone resistant gonococci (MIC > 0.125 mg/L) as well as ceftriaxone treatment failure in patients with gonorrhoea have occurred in several countries during recent years. During 2003 through 2009, the proportion of isolates with ceftriaxone MIC ≥ 0.008 mg/L gradually increased from 40% to nearly 75% (Figure 8.22). During recent years this trend has nearly reversed, the proportion being 44% in 2014, 11% in 2016 and 17% in 2018

The Danish National Board of Health issued guidelines in 2015 for the diagnosis and treatment of sexually transmitted infections. A combination of high dose ceftriaxone (500 mg i.m.) and azithromycin (2 g) is recommended for the treatment of gonorrhoea. Ciprofloxacin (500 mg p.o.) may be used for treatment if the strain is fully susceptible. In 2017, there was a single case among the submitted isolates with ceftriaxone MIC of 0.25 mg/L and azithromycin MIC of 0.25 mg/L.

In a subset of 120 isolates, resistance against cefixime (MIC > 0.125 mg/L) was 0% in 2018 (0.8% in 2017 and 0% in 2016 and 2015). Cefixime is an oral cephalosporin that has never been used in Denmark. Resistance against spectinomycin was 0% in 2018 as well as in the years 2015 through 2017. MIC values for gentamicin were 1 to 4 mg/L, but no break-points are defined for this agent against gonococci.

Conclusions

The ciprofloxacin resistance rate among gonococci increased in 2018. Although resistance problems are still not present in Denmark, the emerging ceftriaxone treatment failures in other countries underlines the importance of maintaining the centralised national surveillance of antimicrobial resistance in gonococci.

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