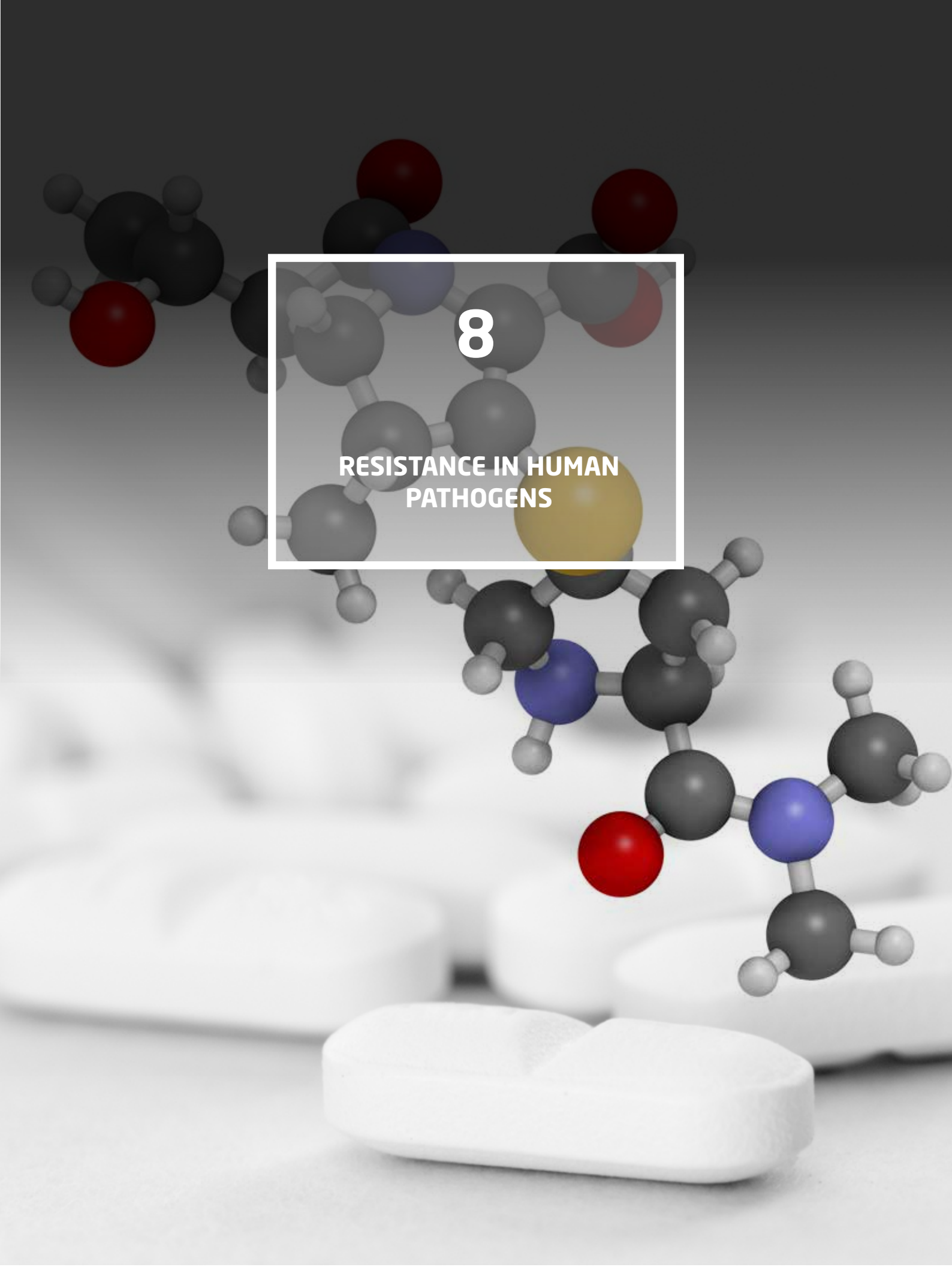


8

RESISTANCE IN HUMAN PATHOGENS

8. Resistance in human pathogens

Highlights: In the observed decade the number of invasive cases has increased considerably for most species. Concomitantly the number of blood cultures taken each year has increased as well (section 8.1).

Blood infections with ***Staphylococcus aureus*** (subsection 8.3.8) have increased gradually by almost 60% from 1,400 cases in 2010 to 2,233 cases in 2019. In 2019, 2.1% of these were methicillin resistant (MRSA). The number of community acquired (CA-) MRSA continued to increase to 1,536 cases, whereas livestock-associated (LA-) MRSA CC398 cases decreased to 1,122, of which 253 cases presented with infection.

Resistance rates to cephalosporins in ***Escherichia coli*** (subsection 8.2.1) have increased gradually in the past decade primarily caused by the production of extended-spectrum beta-lactamases **(ESBLs)** (subsection 8.3.1). In 2019, the prevalence of the ESBL enzyme CTX-M-15 significantly decreased, whereas the prevalence of CTX-M-14 and CTX-M-55 increased in invasive *E. coli*, compared to 2018. In 2019, a new *E. coli* ST23 CTX-M-14 -producing clone was reported by seven of the ten DCMs, primarily from emergency departments.

In 2019 EUCAST introduced the Area of Technical Uncertainty (ATU) for areas/values in which there is uncertainty of susceptibility categorisation for specific combinations of species and agents. Piperacillin-tazobactam in Enterobacterales is an example and trends should be interpretated with caution. Nevertheless increases in piperacillin-tazobactam resistant *E. coli* and ***Klebsiella pneumoniae*** (subsection 8.2.2) in 2019 were observed.

The number of all types of clinical vancomycin resistant and vancomycin variable ***Enterococcus faecium*** **(VRE/VVE)** (subsection 8.3.3) has increased by 11% in Denmark between 2018 and 2019. For **invasive *E. faecium*** cases (subsection 8.2.5), the increase in the proportion of vancomycin-resistance in 2018 (12.1% compared to 7.1% in 2017) was followed by a minor decrease in 2019 to a proportion of 11%.

During 2019, 221 **carbapenemase-producing organisms (CPO)** (subsection 8.3.2) were detected compared with 177 CPO in 2018. Based on whole genome typing data, 16 carbapenemase-producing Enterobacterales outbreaks were registered with new patients in 2019. Seven of these outbreaks were trans-regional, covering patients from at least two different regions. Epidemiological investigations based on patient hospitalisation data and place of residence identified epidemiological links in nine of the 16 outbreaks. All links were identified in healthcare settings: Patients sharing the same ward or hospital.

The level of non-susceptibility to invasive ***Streptococcus pneumoniae*** (subsection 8.3.5) varies in line with the serotypes that are most prevalent in a given year. In total for penicillin in 2019, 95.1% were susceptible, 4.2% were classified as susceptible increased exposure and 0.7% were resistant. For erythromycin, 96.6% were susceptible and 3.4% were resistant.

A nationwide surveillance of azole resistance in ***Aspergillus fumigatus*** (Textbox 8.3) was initiated in October 2018. Data from the first 18 months showed an azole resistance rate of 6.1% with an overweight of resistance mechanisms associated with azole fungicide use in the environment.

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 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 isolates for national resistance surveillance in humans, 2019

DANMAP 2019

Routine diagnostics from all 10 DCMs in Denmark. All data are directly identified and extracted in MiBa	
Species	**Sampling**
Escherichia coli *Klebsiella pneumoniae*	First isolate per patient per year from blood or cerebrospinal fluid, from urine in hospitalised patients and from urine from primary healthcare
Pseudomonas aeruginosa *Acinetobacter species* *Enterococcus faecalis* *Enterococcus faecium*	First isolate per patient per year from blood or cerebrospinal fluid
Voluntary submissions of isolates to the refererance laboratories at SSI	
Species or type	**Sampling**
Staphylococcus areus	One isolate per patient per episode from blood or cerebrospinal fluid
Beta-haemolytic streptococci	One isolate per patient per episode from normally sterile sites
Neisseria gonorrhoeae	One isolate per patient per episode from all sample sites
3rd generation cephalosporin resistant *Escherichia coli*	First isolate per patient within 12 months from blood
Vancomycin-resistant enterococci	First isolate per patient within 12 months irrespective of sample site (excluding screening samples)
Enterococci with exceptional phenotype (e.g. linezolid, daptomycin and tigecycline R)	First isolate per patient within 12 months irrespective of sample site (clinical and screening samples)
All bacterial species with other exceptional phenotypes (e.g. acquired colistin resistance)	One isolate per patient per episode irrespective of sample site
Mandatory submissions of isolates to the reference laboratories at SSI	
Species or type	**Sampling**
Carbapenemase-producing organisms	First isolate per patient within 12 months irrespective of sample site (clinical and screening samples)
Methicillin-resistant *Staphylococcus aureus*	First isolate from all new cases of MRSA postive patients irrespective of sample site (clinical and screening samples)
Streptococcus pneumoniae	One isolate per patient per episode from blood or cerebrospinal fluid
Haemophilus influenzae serotype b, Hib	One isolate per patient per episode from normally sterile sites

Regarding submissions of isolates to the reference laboratories normally more isolates are received, but for the statistics one patient only counts once
For further specification of episodes/cases please see the specific subsections in chapter 9, materials and methods

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 2010 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. Since 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 (DANMAP 2018, Textbox 8.1). Due to the high quality of data in MiBa, this register-based surveillance provides the most accurate prevalence estimates in Denmark. Materials and methods are described in chapter 9.

8.1.2 Surveillance based on data from the reference laboratories

Another surveillance component is based on submission of specified isolates to the reference laboratories at SSI. Isolate-based surveillance gives the opportunity to further characterise isolates 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 isolates has existed since 1957; beginning with the

submission of all isolates of *Staphylococcus aureus* from bloodstream infections. The submission of invasive beta-haemolytic streptococci is also voluntary, while invasive *Streptococcus pneumoniae* and *Haemophilus influenzae* serotype b (Hib) are mandatory to submit. The detection of methicillin-resistant *S. aureus* (MRSA) and *Neisseria gonorrhoeae* from all clinical sites is notifiable but the submission of MRSA isolates is mandatory, while the submission of *N. gonorrhoeae* isolates is voluntary. In addition, the DCMs voluntarily submit isolates of ESBL-producing *E. coli* from blood and vancomycin-resistant enterococci (VRE) from all clinical sites, based on a mutual agreement to survey the development and spread of these often multi-resistant 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 the total numbers of invasive cases in Denmark from 2010 to 2019 for the bacterial species included in the surveillance programs for both DANMAP and EARS-Net. Excluded from the figure is *Acinetobacter* species - these have been registered since 2012 in DANMAP and the number of cases are low (55 to 72 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 are included. Duplicates, within the year of observation, from the same patient are excluded.

Since 2010, the total number of registered invasive cases increased by 46% (from 8,021 to 11,712 cases). The largest increase observed was for *K. pneumoniae* (70%). The only species with an overall decreasing number of cases was *S. pneumoniae* (-33%).

Figure 8.2a shows the number of invasive cases per 100,000 inhabitants in Denmark per year for 2010 to 2019. During this period, the Danish population increased by 4.9% (from 5,534,738 inhabitants in 2010 to 5,806,081 inhabitants in 2019). Figure 8.2b shows the total number of blood cultures taken per 100,000 inhabitants per year for the same period. In addition, the number of unique patients being subjected to blood culture per 100,000 inhabitants per year is shown. In the ten-year period the number of unique patients with at least one blood culture taken per year has increased from 2,059 patients per 100,000 inhabitants in 2010 to 2,914 patients per 100,000 inhabitants in 2019 (an increase of 42%). The total number of blood cultures taken (as registered with a unique sample ID in MiBa) per 100,000 inhabitants has increased even more (63%). Thus, on average more patients have more blood cultures taken each year. 2019 was the first year in the observed decade where less unique patients were blood cultured compared to the previous year but the total number of blood cultures taken increased as previously.

Figure 8.1 Number of submitted invasive isolates for each species under surveillance DANMAP 2019

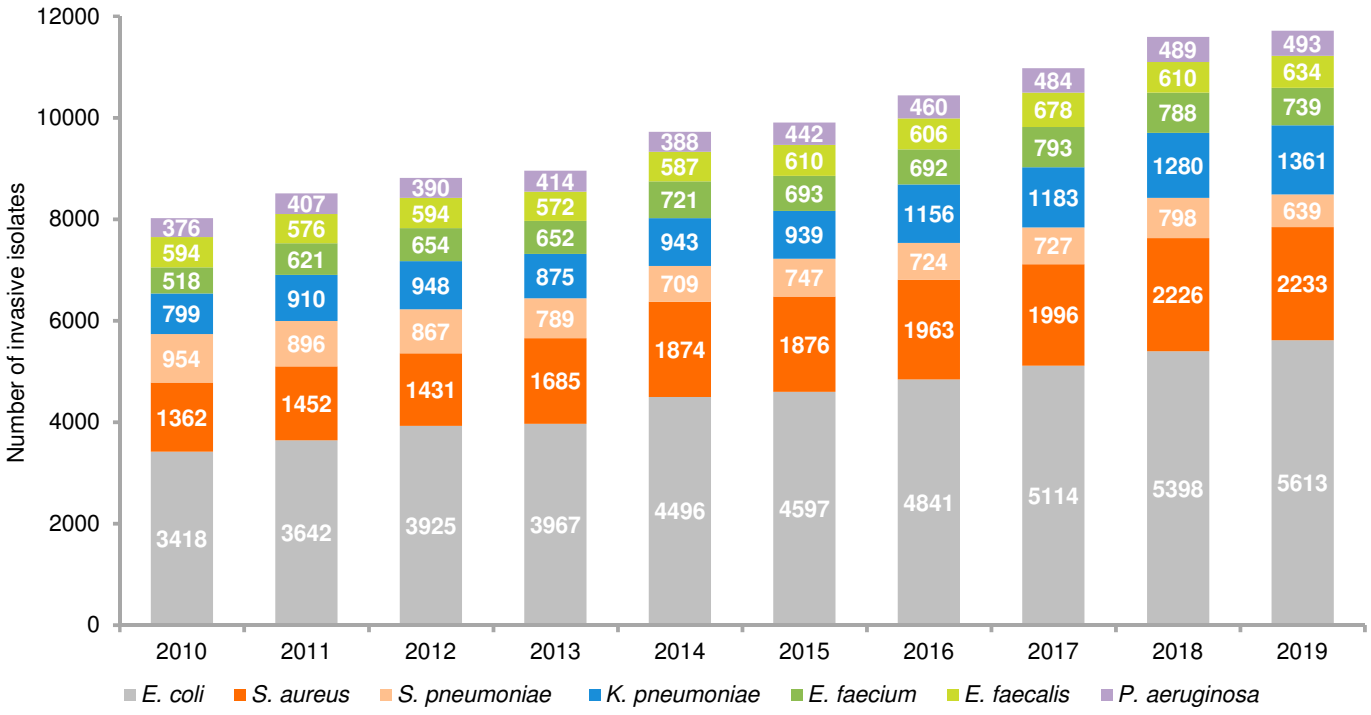
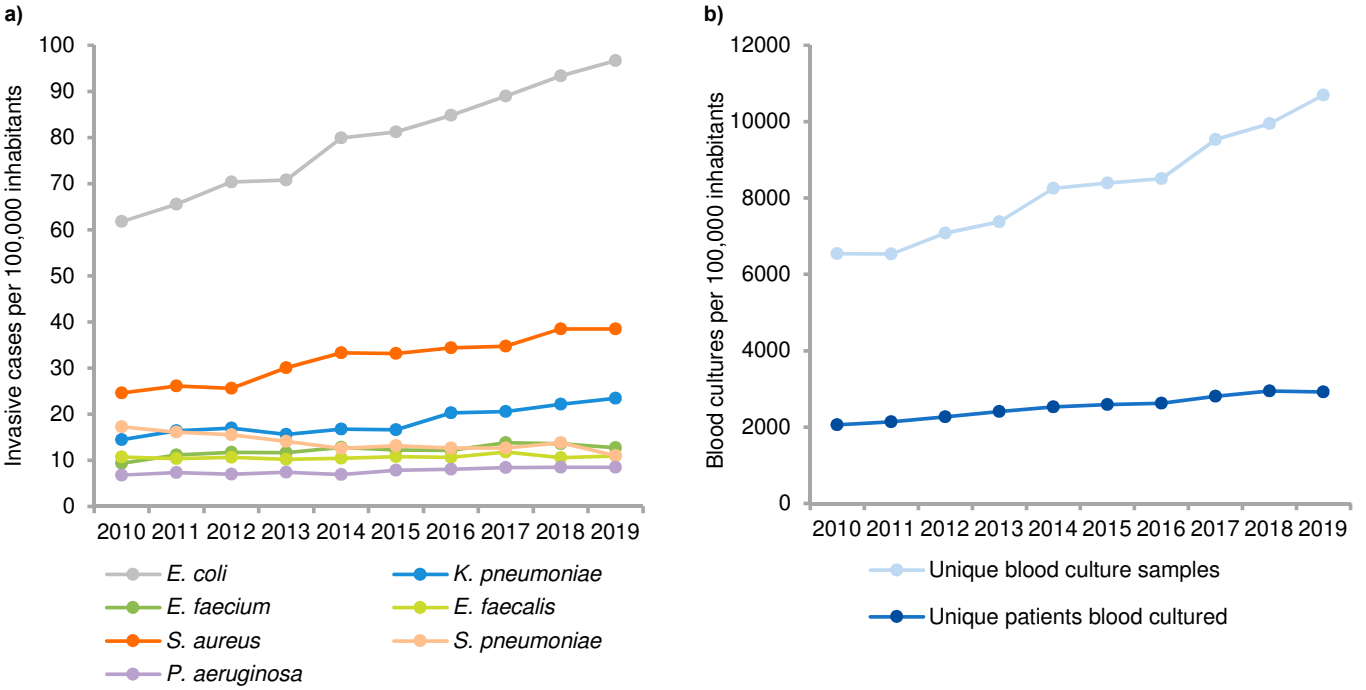


Figure 8.2 Invasive cases and blood cultures taken per 100,000 inhabitants DANMAP 2019



Changes in hospital workflow, improved culturing methods and demographic changes with a growing population of elderly and of chronically ill or immunocompromised patients may explain some of the observed changes. The increasing number of invasive infections is of concern. It demands fast and effective antimicrobial 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 antimicrobials, reserving the most broad-spectrum antimicrobial classes to the patients with multi-resistant infections is underlined as well.

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

8.2 Surveillance based on MiBa data

8.2.1 *Escherichia coli*

Escherichia coli is by far the most frequent cause of community- and hospital acquired urinary tract infections and of bacteraemia in Denmark accounting for approximately 55%, 45% and 21%, respectively, of all registered positive cultures in MiBa (2018 data). It is part of the normal intestinal flora in both animals and humans, where it comes into close proximity to many other bacterial species. Transferable resistance mechanisms are frequently seen in between these gut bacteria as well as 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 biggest drivers of antimicrobial use.

Invasive cases from hospital patients

For 2019, a total of 5,613 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 for ampicillin, ciprofloxacin, piperacillin-tazobactam, gentamicin, cefuroxime, mecillinam and carbapenem in MiBa. In addition, nine DCMs routinely registered antimicrobial susceptibility for 3rd generation cephalosporins and seven routinely registered antimicrobial susceptibility for amoxicillin-clavulanic acid. Tested 3rd generation cephalosporins were ceftazidime, except for two DCMs where it was ceftriaxone and cefpodoxime. The tested carbapenem was meropenem for all DCMs in 2019. Antimicrobial susceptibility 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 upon in specific cases.

Resistance rates for all tested antimicrobials, presented as a national mean for each antimicrobial class, are summarized in Table 8.2. In Figure 8.3 total numbers of invasive isolates and rates of resistance are shown for the past decade. Time trends and significance levels, 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 have varied over time, making comparison of the results difficult and time trends unreliable. Also amoxicillin-clavulanic acid test results are excluded from Figure 8.3 for which reason resistance rates are commented on later in this section.

Table 8.2 *Escherichia coli*. Resistance (%) in isolates from humans, 2019 DANMAP 2019

	Invasive isolates, hospitals %	Urine isolates, hospitals %	Urine isolates, primary health care %
Ampicillin	46	44	38
Mecillinam	14	8.1	5.3
Piperacillin-tazobactam	5.5	4.4	6.5*
Amoxicillin-clavulanic acid	26	14	6.5*
Sulfonamide		31*	28
Trimethoprim		25	23
Nitrofuratoin		1.2*	1.1
Gentamicin	5.3	4.8	3.9*
Ciprofloxacin	11	11	8.0
Cefuroxime	10	7.8	5.7*
3rd generation cephalosporins	6.9	6.9	5.2
Carbapenem	0.1	0.0	0.0*

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 * marks where less than 6 (out of totally 10 DCM) tested a sufficient percentage of their samples. For carbapenems, piperacillin/tazobactam and gentamicin in urines from primary health care, only one DCM tested >75% of their isolates

A continuous increase in the number of invasive *E. coli* cases was observed throughout the years, from 3,426 cases in 2010 to 5,613 cases in 2019. This corresponds to 61.8 cases and 96.7 cases per 100,000 inhabitants respectively (a 57% increase). Simultaneously, the total number of blood cultures performed also increased steeply with 63% per 100.000 inhabitants (commented on in subsection 8.1.3).

For cefuroxime in invasive *E. coli* a significant increase in resistance was observed for the past decade as well as for the past five years. A minor EUCAST breakpoint change in 2017 influenced 2017 data compared to 2016, but there has not been any breakpoint change since then. The same increase was not observed for 3rd generation cephalosporins. The increased ciprofloxacin resistance rate in 2017 compared to 2016 mainly reflected a relatively large change in EUCAST breakpoints in 2017. Ciprofloxacin breakpoints changed (lowered) once again combined with the introduction of the Area of Technical Uncertainty (ATU) in the EUCAST clinical breakpoint Table v. 9.0 in 2019. Therefore curves for ciprofloxacin resistance should be interpreted with caution and time trend calculations are not conducted. For piperacillin-tazobactam the interpretation should be taken with caution as well, even though there have been no EUCAST breakpoint changes since 2012. Several local rules regarding downgrading piperacillin-tazobactam from I to R or S to R has existed during the years and also technical problems with the testing has been a recurring issue. Furthermore, in 2019 an ATU, for piperacillin-tazobactam was introduced. With a calculation based only on zone diameters 2.5% of the isolates were resistant (<17 mm) in 2018 compared to 3.1% in 2019, indicating a "true" increase in resistance towards piperacillin-tazobactam (Figure A8.1 in web annex). Gentamicin resistance rates have declined in the decade. For more details see Figure 8.3.

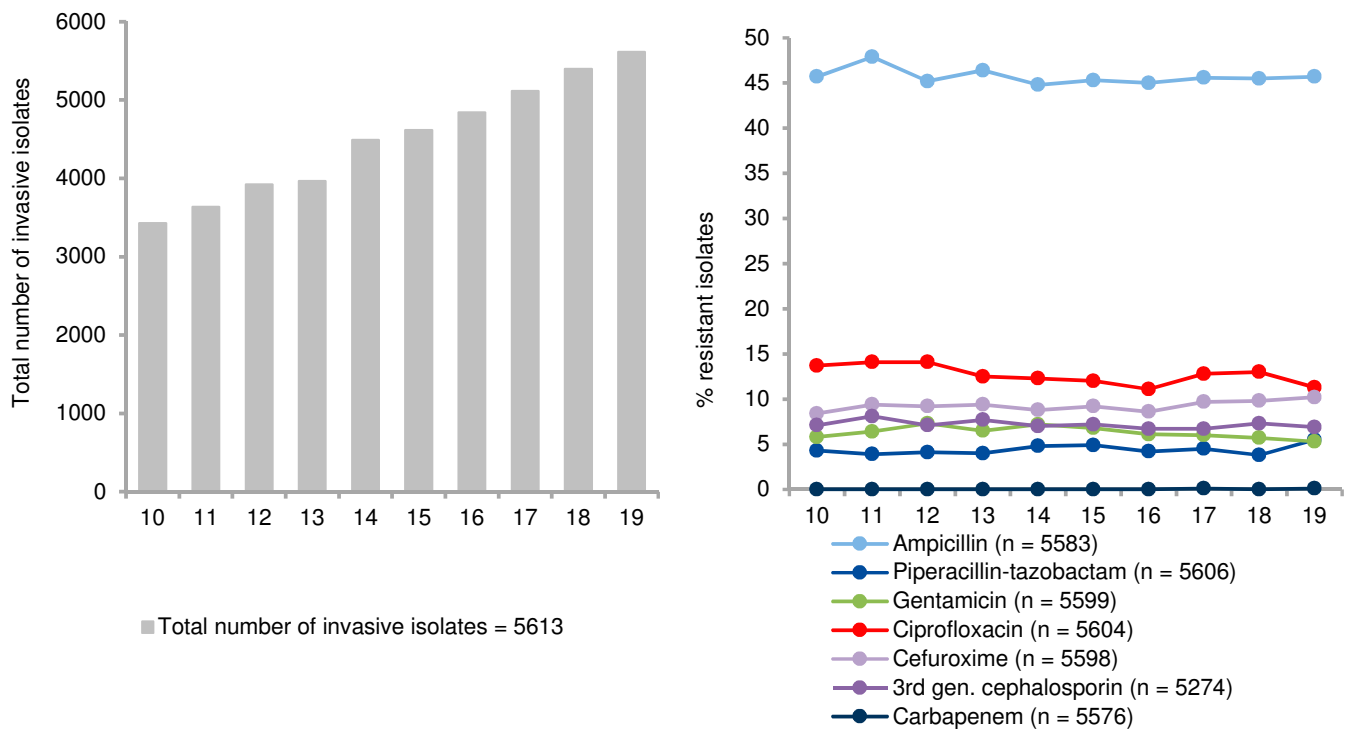
Reporting of amoxicillin-clavulanic acid resistance rates needs special concerns. There are two different breakpoints defined; one more restrictive breakpoint and one less restrictive only to be used in uncomplicated UTI, and there is an ATU that was introduced by EUCAST in 2019. According to the reported S-I-R interpretations, an increase from 19% to 26% resistant cases from 2018 to 2019 was noticed. Looking into the reported zone diameters though, no such change was observed (Figure A8.2 in web annex).

The defined clinical breakpoint by EUCAST for amoxicillin-clavulanic acid is S≥19 mm, R<19 mm, with an ATU of 19-20 mm, and the breakpoint to be used only in uncomplicated UTI is S≥16 mm, R<16 mm (EUCAST v.9.0). Evaluation of the zone distributions reveals changes from 2018 to 2019 only above 18 mm with less isolates in the ATU (19-20 mm) in 2019 compared to 2018, and instead more isolates with reported zones of 21 mm. The isolates for which the results fall in the ATU range, EUCAST suggests the following alternatives depending on the situation: Repeat the test, use an alternative test, downgrade the susceptibility category or include the uncertainty as part of the report. Some DCMs simply do not report any result on amoxicillin-clavulanic acid to the clinicians and only handle it if needed. If all reported zones are interpreted with the same breakpoint of 19 mm, the resistance rates in 2018 and 2019 are 31% and 30% respectively. Here we report interpretations as reported to MiBa by the DCMs in Table 8.2.

The number of carbapenem resistant *E. coli* isolates remained continuously very low with three carbapenem resistant isolates and two additional isolates which were susceptible increased exposure in the invasive cases in 2019. The level of multiresistant (combined resistance to 3rd generation cephalosporins, ciprofloxacin and gentamicin) invasive *E. coli* remained

at around 2%, Table 8.3. For colistin, four of the invasive *E. coli* were registered resistant, however colistin resistance is not tested routinely and in 2019 colistin susceptibility testing was reported for 521 of the isolates, constituting 9.3% of the invasive cases.

Figure 8.3 *Escherichia coli*. Resistance (%) in invasive isolates from humans DANMAP 2019



c)

Substance	Percent resistant invasive *E. coli* isolates										Time trends (Cochran-Armitage test)	
	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	10 years (2010-2019)	5 years (2015-2019)
Ampicillin	45.7	47.9	45.2	46.4	44.8	45.3	45	45.6	45.5	45.7	↓ p = 0.114	↑ p = 0.277
Piperacillin-tazobactam	4.3	3.9	4.1	4	4.8	4.9	4.2	4.5	3.8	5.5	NA	NA
Gentamicin	5.8	6.4	7.3	6.5	7.2	6.8	6.1	6	5.7	5.3	↓ p = 0.001	↓ p = 0.001
Ciprofloxacin	13.7	14.1	14.1	12.5	12.3	12	11.1	12.8	13	11.3	NA	NA
Cefuroxime	8.4	9.4	9.2	9.4	8.8	9.2	8.6	9.7	9.8	10.2	↑ p = 0.005	↑ p = 0.007
3rd gen.cephalosporins	7.1	8.1	7.1	7.7	7	7.2	6.7	6.7	7.3	6.9	↓ p = 0.01	↑ p = 0.459
Carbapenem	0	0	0	0	0	0	0	0.1	0	0.1	↑ p = 0.046	↑ p = 0.303
Total number of isolates	3426	3636	3922	3967	4492	4618	4841	5114	5398	5613		

a) Total numbers of invasive isolates per year. b) Resistance rates among tested isolates c) Resistance rates and 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 2019. 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 2019

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

Urinary cases from hospitals

For 2019, a total of 47,235 unique patients with *E. coli* isolates, cultured in urine samples from hospital patients from all DCMs in Denmark, were identified in MiBa. A total of 2,090 unique patients with *E. coli* isolates, cultured in urine samples were excluded because it was not possible to categorise them as hospital or primary care urine samples.

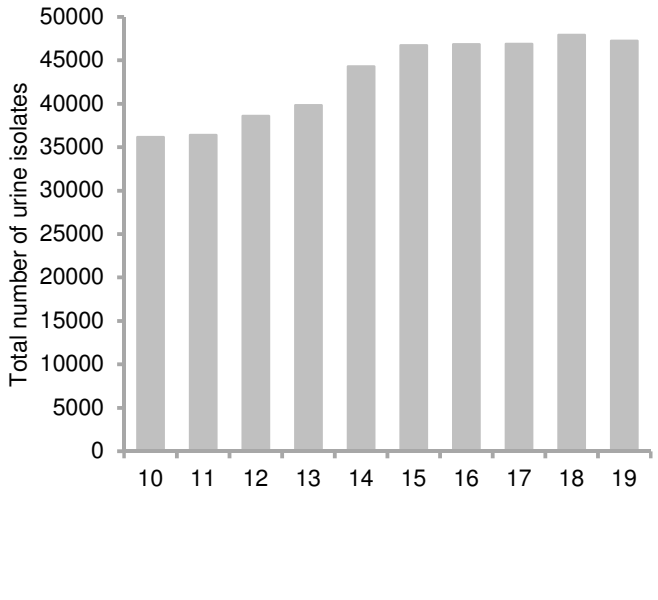
All 10 DCMs routinely (>75% of isolates) registered antimicrobial susceptibility interpretations for ampicillin, mecillinam, piperacillin/tazobactam and ciprofloxacin in MiBa. In addition nine DCMs routinely registered antimicrobial susceptibilities for gentamicin and eight DCMs for cefuroxime and trimethoprim.

Six DCMs routinely registered antimicrobial susceptibilities for amoxicillin-clavulanic acid, 3rd generation cephalosporins and carbapenem and five DCMs for nitrofurantoin. Three DCMs routinely registered antimicrobial susceptibilities for sulphonamide.

Susceptibility results for all tested antimicrobials are summarized together with the results from the invasive isolates as a national mean for each antimicrobial class in Table 8.2. In Figure 8.4, rates of resistance are plotted for the past decade. Time trends and significance levels, based on the resistance rates for the past five and 10 years respectively, are presented in Figure 8.4c.

Figure 8.4 *Escherichia coli*. Resistance (%) in urine isolates from humans in hospitals DANMAP 2019

a)



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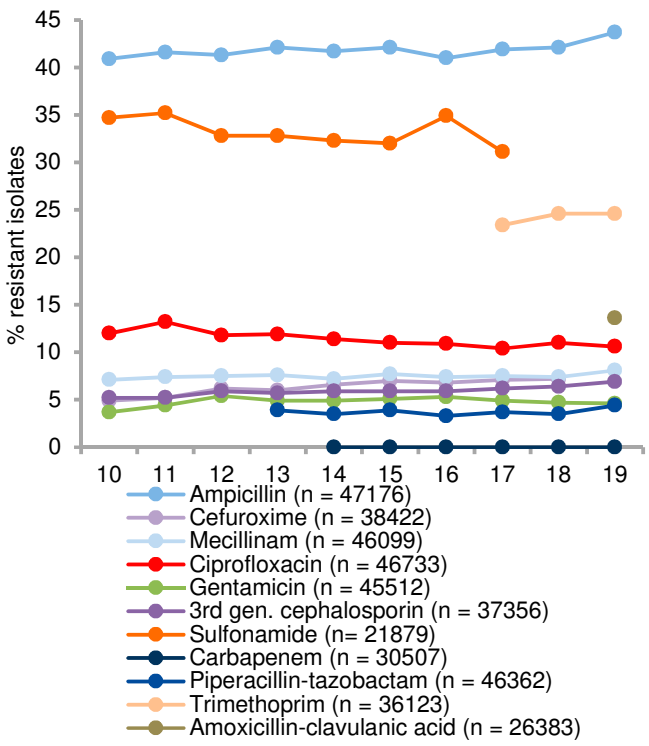


c)

Substance	Percent resistant *E. coli* isolates from hospital urine										Time trends (Cochran-Armitage test)	
	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	10 years (2010-2019)	5 years (2015-2019)
Ampicillin	40.9	41.6	41.3	42.1	41.7	42.1	41	41.9	42.1	43.7	↑ p <0.001	↑ p <0.001
Mecillinam	7.1	7.4	7.5	7.6	7.2	7.7	7.4	7.5	7.4	8.1	↑ p <0.001	↑ p = 0.016
Piperacillin-tazobactam				3.9	3.5	3.9	3.3	3.7	3.5	4.4	NA	NA
Sulfonamide	34.7	35.2	32.8	32.8*	32.3*	32.0	34.9	31.1	31.0*	31.4*	NA	NA
Gentamicin	3.7	4.4	5.4	4.9	4.9	5.1	5.3	4.9	4.7	4.6	↓ p <0.001	↓ p = 0.004
Ciprofloxacin	12	13.2	11.8	11.9	11.4	11	10.9	10.4	11	10.6	NA	NA
Cefuroxime	4.9	5.2	6.2	6	6.6	7	6.8	7.1	7.2	7.8	↑ p <0.001	↑ p <0.001
3rd gen. cephalosporins	5.2	5.2	5.9	5.7	5.9	5.9	5.9	6.2	6.4	6.9	↑ p <0.001	↑ p <0.001
Carbapenem					0	0	0	0	0	0	NA	↑ p = 0.004
Total number of isolates	36149	36389	38610	39820	44288	46723	46865	46884	47914	47235		

a) Total numbers of urine isolates per year, b) Resistance rates among tested isolates, c) Resistance rates and 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 2019. NA = Not applicable
*Indicates that less than 6 DCMs reported rutine testing

For ampicillin, mecillinam, cefuroxime and 3rd generation cephalosporins there are slow but significant increases in resistance for the past decade as well as for the past five years. For more details see Figure 8.4.

In 2019 there was not noted any increase in carbapenem resistance with 19 carbapenem resistant and 16 susceptible increased exposure *E. coli* urine isolates from hospital patients registered, compared to 24 and 20 in 2018.

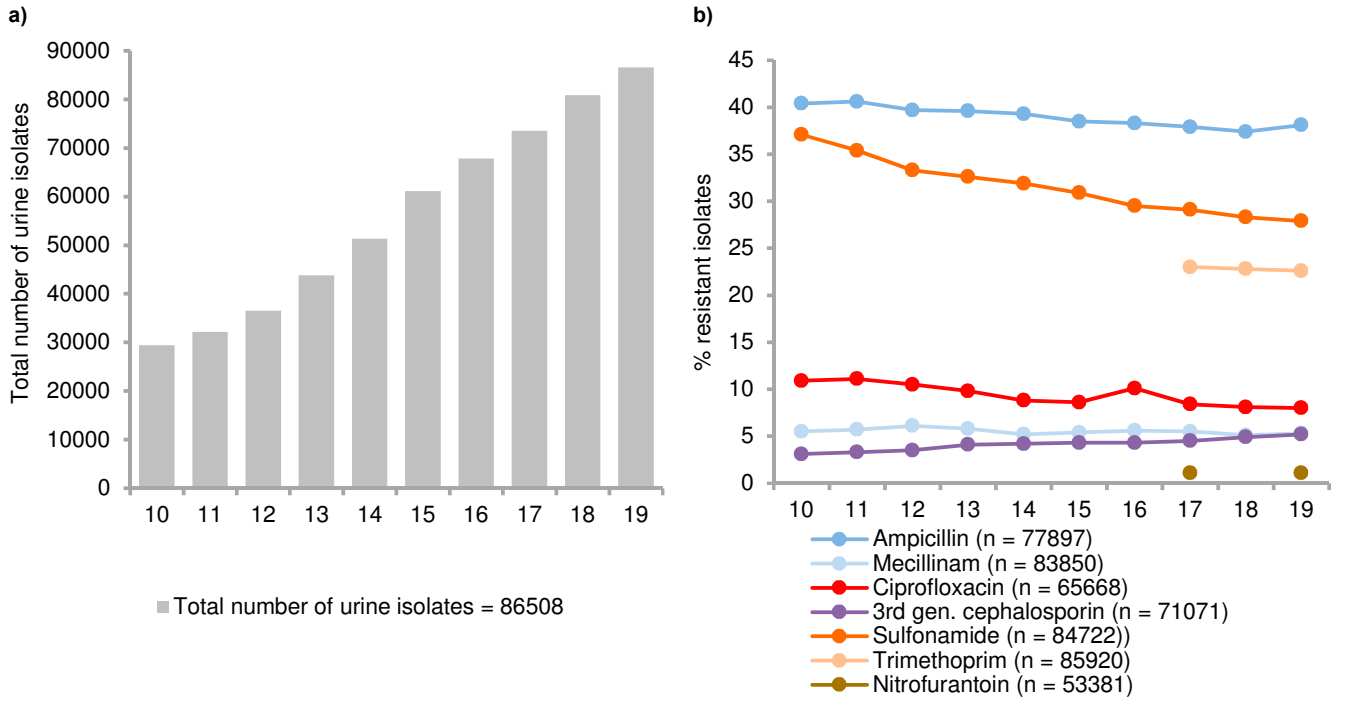
Urinary cases from primary health care

For 2019, a total of 86,508 unique patients with *E. coli* isolates, cultured in urine samples from primary health care (PHC), from nine DCMs in Denmark, were identified in MiBa. The general practitioners (GPs) 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 for mecillinam, sulphonamide and trimethoprim in MiBa. In addition eight DCMs routinely registered antimicrobial susceptibilities for ampicillin, six DCMs for 3rd generation cephalosporins, ciprofloxacin and nitrofurantoin and four DCMs for amoxicillin-clavulanic acid. Three DCMs routinely registered antimicrobial susceptibilities for cefuroxime and just one DCM routinely registered antimicrobial susceptibilities for piperacillin-tazobactam, gentamicin and carbapenem.

As for the results from invasive isolates and isolates from hospital urines, susceptibility results for all tested antimicrobials are shown as national means in Table 8.2. In Figure 8.5, rates of resistance are plotted for the past decade. Time trends and significance levels, based on the resistance rates for the past five and 10 years respectively, are presented in Figure 8.5c.

Figure 8.5 *Escherichia coli*. Resistance (%) in urine isolates from humans in primary health care DANMAP 2019



c)

Substance	Percent resistant *E. coli* isolates from PHC urine										Time trends (Cochran-Armitage test)	
	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	10 years (2010-2019)	5 years (2015-2019)
Ampicillin	40.4	40.6	39.7	39.6	39.3	38.5	38.3	37.9	37.4	38.1	↓ p <0.001	↓ p = 0.007
Mecillinam	5.5	5.7	6.1	5.8	5.2	5.4	5.6	5.5	5.1	5.3	↓ p = 0.001	↓ p = 0.01
Sulfonamide	37.1	35.4	33.3	32.6	31.9	30.9	29.5	29.1	28.3	27.9	↓ p <0.001	↓ p <0.001
Ciprofloxacin	10.9	11.1	10.5	9.8	8.8	8.6	10.1	8.4	8.1	8	NA	NA
3rd gen. cephalosporins	3.1	3.3	3.5	4.1	4.2	4.3	4.3	4.5	4.9	5.2	↑ p <0.001	↑ p <0.001
Total number of isolates	29421	32117	36475	43770	51272	61083	67798	73497	80851	86508		

a) Total numbers of urine isolates per year, b) Resistance rates among tested isolates, c) Resistance rates and 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 2019. NA = Not applicable

A continuing slow increase in resistance to 3rd generation cephalosporins, a slow decline in mecillinam resistance and a more pronounced decline in sulphonamide resistance were observed. All changes are significant calculating both 10 and 5 years back. For more details see Figure 8.5.

In 2019, six carbapenem resistant and nine susceptible increased exposure *E. coli* isolates from PHC urinary cases were registered. As noted, registration of carbapenem susceptibility results in urine samples from PHC is only routinely done in one 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. Throughout 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 worrisome trend for all three categories of *E. coli* isolates were an increase in cephalosporin (cefuroxime and/or 3rd generation cephalosporins) resistance rates both for the past decade, and for the past five years. One exception was the resistance to 3rd generation cephalosporins in invasive *E. coli*, which showed no increase in resistance rates. In Europe an increase in resistance to 3rd generation cephalosporins in invasive *E. coli* was observed (EU/EEA population-weighted mean 15.1%) [EARS-Net annual report, 2018]. Resistance to 3rd generation cephalosporins often occur through production of extended-spectrum beta-lactamases (ESBLs) (for more information see section 8.3). When the population of ESBL-positive *E. coli* isolates increases, this might lead to increased use of broader spectrum antimicrobials and thereby a vicious circle selecting for even more resistance can run. Whenever possible, a downscaling to narrow spectrum antimicrobials is recommended.

Resistance to carbapenem in invasive and urinary *E. coli* isolates is still contained at an acceptable level. Besides antimicrobial stewardship many initiatives, including active surveillance and infection control programs are effectuated each day to keep it this way. In 2019 a new national surveillance database with registration of all verified and possible outbreaks with carbapenemase-producing Enterobacterales (CPE) in health care settings was established (for further information, please see section 8.3.2).

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8.2.2 *Klebsiella pneumoniae*

Klebsiella 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* frequently displays plasmid-mediated resistance mechanisms which are transferrable and may be transferred to other organisms.

Invasive cases from hospitals

For 2019, a total of 1,361 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 for ciprofloxacin, piperacillin-tazobactam, gentamicin, cefuroxime and meropenem in MiBa. In addition, nine DCMs routinely registered antimicrobial susceptibilities for mecillinam and 3rd generation cephalosporins and seven routinely registered antimicrobial susceptibilities for amoxicillin-clavulanic acid. Tested 3rd generation cephalosporins were ceftazidime, except for two DCMs where it was ceftriaxone and cefpodoxime. The tested carbapenem was meropenem for all DCM in 2019. Antimicrobial susceptibility 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 upon in specific cases.

Resistance rates for 2019 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. Time trends and significance levels, based on the resistance rates for the past five and ten years respectively, are presented in Figure 8.6c. 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 differ and/or have varied over time, making comparison of the results difficult and time trends unreliable.

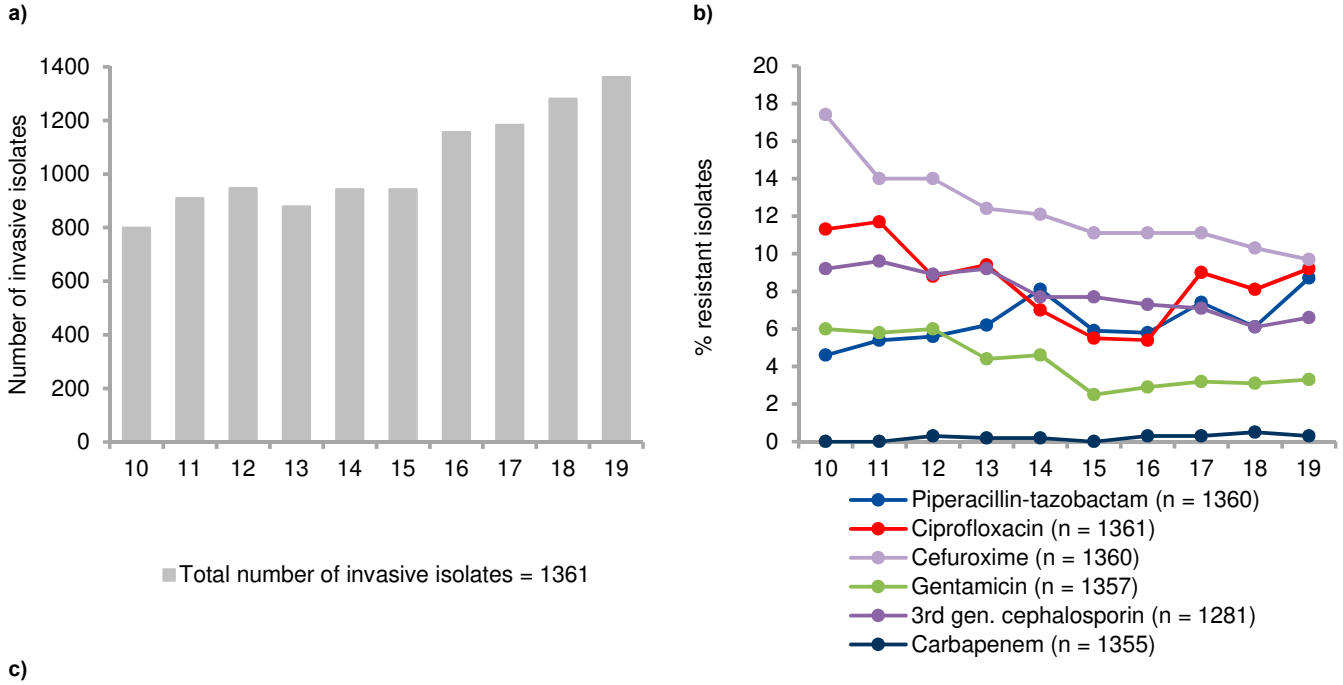
As for invasive *E. coli* cases, a continuous increase in the number of invasive *K. pneumoniae* cases was observed throughout the years, from 799 cases in 2010 to 1,361 cases in 2019. This corresponds to 14.4 cases and 23.4 cases per 100,000 inhabitants respectively (a 62% increase). Simultaneously, the total number of blood cultures taken also increased steeply with 63% per 100.000 inhabitants (commented on in subsection 8.1.3).

Table 8.4 *Klebsiella pneumoniae*. Resistance (%) in isolates from humans, 2019 DANMAP 2019

Substance	Invasive isolates, hospitals %	Urine isolates, hospitals %	Urine isolates, primary healthcare %
Mecillinam	14	13	11
Piperacillin/tazobactam	8.7	8.5	9.4*
Amoxicillin/clavulanic acid	17	12	7.7*
Sulfonamide		20*	19
Trimethoprim		20	20
Nitrofuratoin		33*	33*
Gentamicin	3.3	3.0	2.0*
Ciprofloxacin	9.2	7.4	5.5
Cefuroxime	9.7	8.6	4.5*
3rd generation cephalosporins	6.6	6.0	4.5
Carbapenem	0.3	0.2	0.1*

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

Figure 8.6 *Klebsiella pneumoniae*. Resistance (%) in invasive isolates from humans DANMAP 2019



c)

Substance	Percent resistant invasive *K. pneumoniae* isolates										Time trends (Cochran-Armitage test)	
	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	10 years (2010-2019)	5 years (2015-2019)
Piperacillin/tazobactam	4.6	5.4	5.6	6.2	8.1	5.9	5.8	7.4	6.1	8.7	NA	NA
Gentamicin	6.0	5.8	6.0	4.4	4.6	2.5	2.9	3.2	3.1	3.3	↓ p <0.001	↑ p = 0.125
Ciprofloxacin	11.3	11.7	8.8	9.4	7	5.5	5.4	9.0	8.1	9.2	NA	NA
Cefuroxime	17.4	14.0	14.0	12.4	12.1	11.1	11.1	11.1	10.3	9.7	↓ p <0.001	↓ p = 0.097
3rd gen. cephalosporins	9.2	9.6	8.9	9.2	7.7	7.7	7.3	7.1	6.1	6.6	↓ p <0.001	↓ p = 0.1
Carbapenem	0.0	0.0	0.3	0.2	0.2	0	0.3	0.3	0.5	0.3	↑ p = 0.033	↑ p = 0.083
Total number of isolates	799	910	947	879	943	943	1156	1183	1280	1361		

a) Total numbers of invasive isolates per year. b) Resistance rates among tested isolates
c) Resistance rates and 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 2019. NA = Not applicable

In invasive *K. pneumoniae* resistance rates have decreased over the past 10 years for gentamicin, ciprofloxacin, cefuroxime and 3rd generation cephalosporins, but with insignificant or no decrease in the past five years. For more details see Figure 8.6. The increased ciprofloxacin resistance rate in 2017 compared to 2016 mainly reflected a relatively large change in EUCAST breakpoints in 2017. Ciprofloxacin breakpoints changed (lowered) once again combined with the introduction of the Area of Technical Uncertainty (ATU) in the EUCAST clinical breakpoint Table v. 9.0 in 2019. Therefore curves for ciprofloxacin resistance should be interpreted with caution and time trend calculations are not conducted. For piperacillin-tazobactam, the interpretation should be taken with caution as well, even though there have been no EUCAST breakpoint changes since 2012. Several local rules regarding downgrading piperacillin-tazobactam from I to R or S to R have existed during the years and also technical problems with the testing has been a recurring issue. Furthermore, in 2019 an ATU, for piperacillin-tazobactam was introduced. With a calculation based only on zone diameters 4.3% of the isolates were resistant (<17 mm) in 2018 compared to 5.7% in 2019, indicating a "true" increase in resistance towards piperacillin-tazobactam (Figure A8.3 in web annex).

For carbapenem resistance in invasive *K. pneumoniae* a very small but significant increase was observed over the past 10 years. However total numbers of meropenem resistant invasive isolates are low, and no increase was observed in 2019 with four resistant and two susceptible increased exposure isolates compared to seven resistant and one susceptible increased exposure invasive *K. pneumoniae* isolates in 2018. 2018 was the year with most meropenem resistant invasive *K. pneumoniae* isolates until now. 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, though susceptibility to colistin is not routinely tested.

Urinary cases from hospitals

For 2019, a total of 7,926 unique patients with *K. pneumoniae* isolates, cultured in urine samples from hospitalised patients from all DCMs in Denmark, were identified in MiBa. 225 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 urine samples.

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

Resistance rates for all tested antimicrobials are summarized together with the results from the invasive isolates as a national mean for each antimicrobial class in Table 8.4. In Figure 8.7 rates of resistance are shown for the past decade. 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 invasive *K. pneumoniae* resistance rates in urine isolates from hospitals have decreased over the past 10 years for gentamicin, ciprofloxacin, cefuroxime and 3rd generation cephalosporins, but with less or no decrease in the past five years. For 2019 a decrease in resistance to mecillinam was observed. Thereby the very steep increase observed in 2017 and confirmed in 2018 seems to be on reverse. For more details see Figure 8.7.

In 2019, in total 15 carbapenem resistant and 11 susceptible increased exposure *K. pneumoniae* isolates from hospital urinary cases were registered compared to 19 and 6 in 2018.

Urinary cases from primary health care

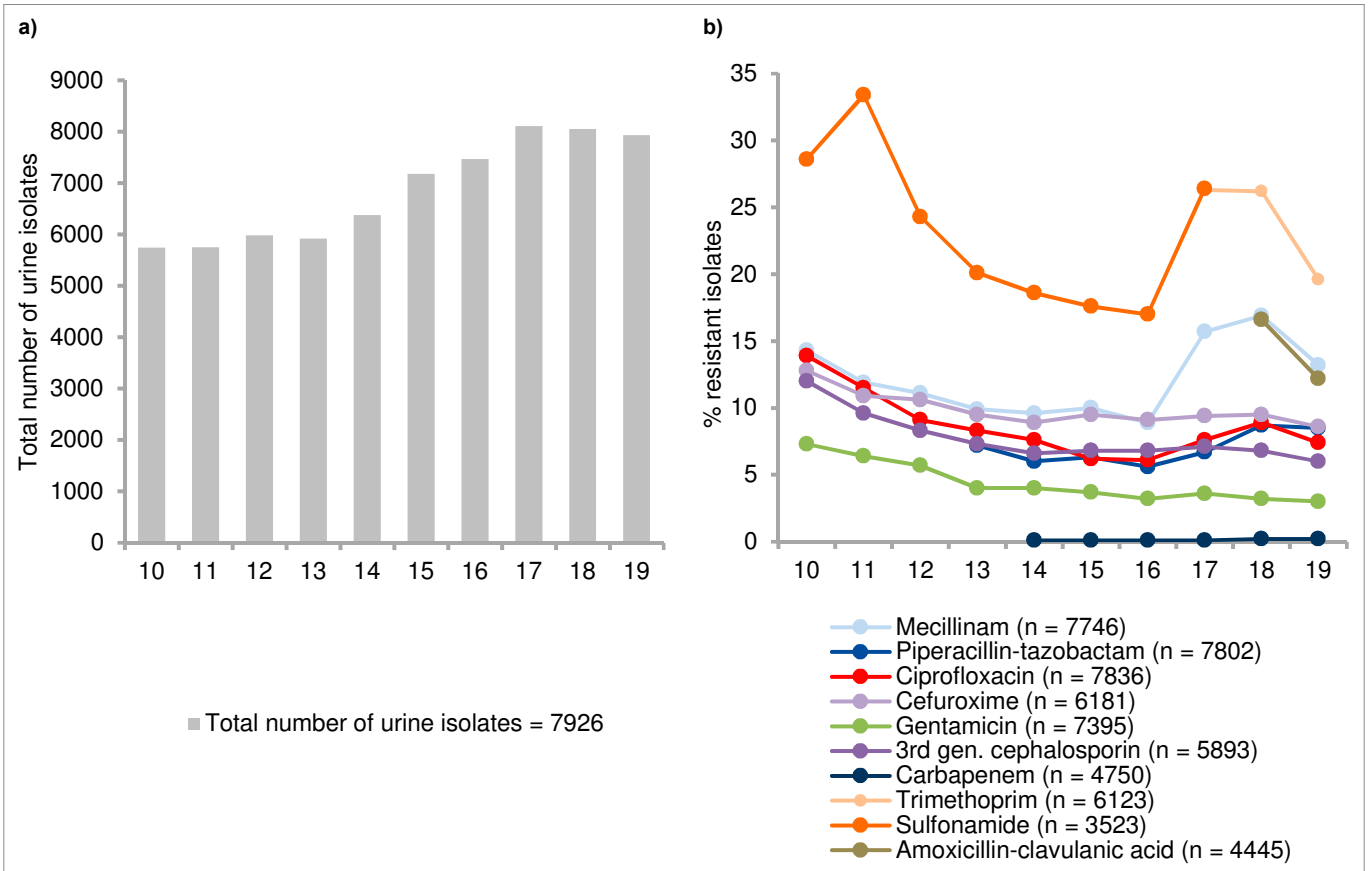
For 2019, a total of 9,696 unique patients with *K. pneumoniae* isolates, cultured in urine samples from primary health care (PHC), from nine DCMs in Denmark, were identified in MiBa. The general practitioners (GPs) in Denmark are all serviced by those nine DCMs except for a few GPs in the Capital Region of Denmark whom a serviced by one private laboratory.

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

DANMAP 2019

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

Figure 8.7 *Klebsiella pneumoniae*. Resistance (%) in urine isolates from humans in hospitals DANMAP 2019



c)

Substance	Percent resistant *K. pneumoniae* isolates from hospital urine										Time trends (Cochran-Armitage test)	
	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	10 years (2010-2019)	5 years (2015-2019)
Mecillinam	14.3	11.9	11.1	9.9	9.6	10	8.9	15.7	16.9	13.2	↑ p <0.001	↑ p <0.001
Piperacillin/tazobactam				7.2	6	6.3	5.6	6.7	8.7	8.5	NA	NA
Gentamicin	7.3	6.4	5.7	4	4	3.7	3.2	3.6	3.2	3	↓ p <0.001	↓ p = 0.024
Ciprofloxacin	13.9	11.5	9.1	8.3	7.6	6.2	6.1	7.6	8.9	7.4	NA	NA
Cefuroxime	12.8	10.9	10.6	9.5	8.9	9.5	9.1	9.4	9.5	8.6	↓ p <0.001	↓ p = 0.126
3rd gen. cephalosporins	12	9.6	8.3	7.3	6.6	6.8	6.8	7.1	6.8	6	↓ p <0.001	↓ p = 0.048
Total number of isolates	5740	5746	5982	5919	6372	7175	7467	8106	8047	7926		

a) Total numbers of invasive isolates per year, b) Resistance rates among tested isolates, c) Resistance rates and 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 2019. NA = Not applicable

All nine DCMs routinely (>75% of isolates) registered antimicrobial susceptibility interpretations for mecillinam and trimethoprim in MiBa. In addition eight DCMs routinely registered antimicrobial susceptibilities for sulphonamide, six DCMs for 3rd generation cephalosporins and ciprofloxacin, five DCMs for nitrofurantoin, four DCMs for amoxicillin-clavulanic acid, three DCMs for cefuroxime and one DCM for piperacillin-tazobactam, carbapenem and gentamicin.

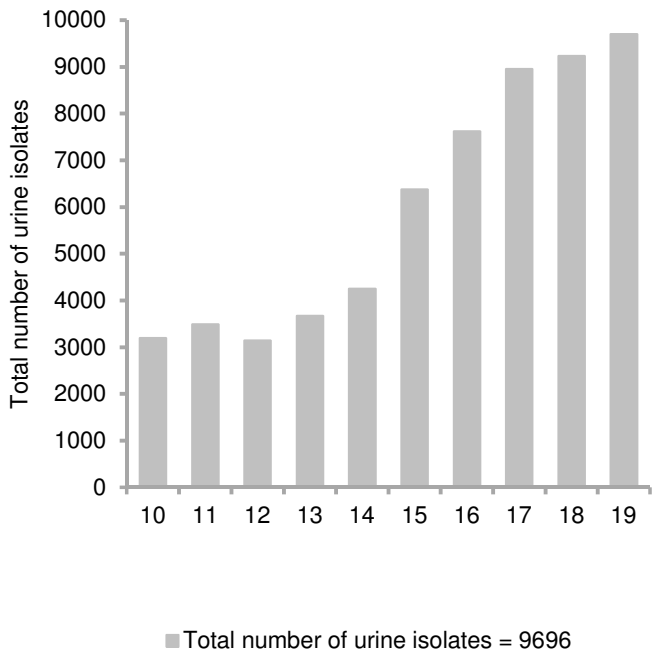
As for the results from invasive isolates and isolates from hospital urines resistance results for all tested antimicrobials are shown as national means in Table 8.4. In Figure 8.8, rates of resistance are shown for the past decade. Time trends and significance levels, based on the resistance rates for the past five and ten years respectively, are presented in Figure 8.8c.

Also in urine isolates from PHC a decrease in resistance to mecillinam was observed in 2019 and thereby the very steep increase observed in 2017 and confirmed in 2018 seems to be on reverse. For more details see Figure 8.8.

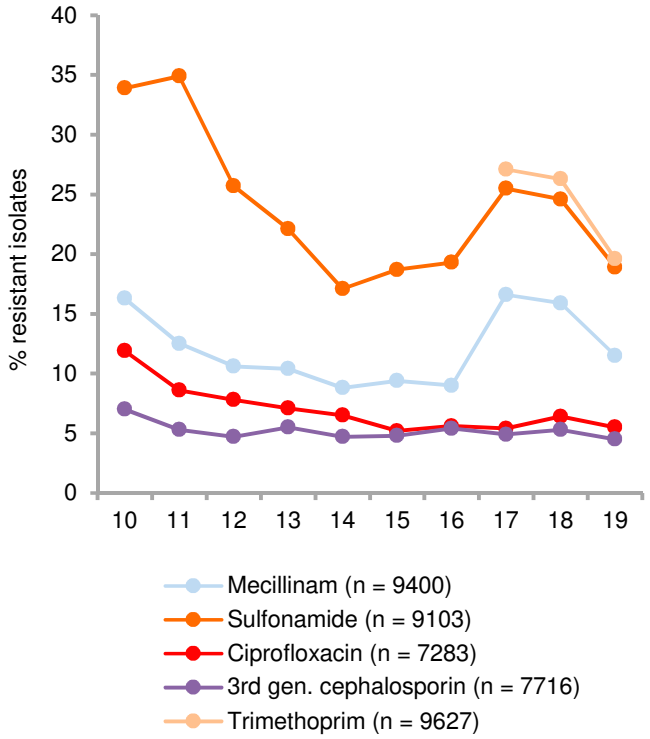
In total numbers three carbapenem resistant and two susceptible increased exposure isolates were registered in 2019 compared to four and two in 2018. Susceptibility results for carbapenem though, is only routinely reported in MiBa from one DCM.

Figure 8.8 *Klebsiella pneumoniae*. Resistance (%) in urine isolates from humans in primary health care DANMAP 2019

a)



b)



c)

Substance	Percent resistant *K. pneumoniae* isolates from PHC urine										Time trends (Cochran-Armitage test)	
	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	10 years (2010-2019)	5 years (2015-2019)
Mecillinam	16.3	12.5	10.6	10.4	8.8	9.4	9	16.6	15.9	11.5	↑ p <0.001	↑ p <0.001
Sulfonamide	33.9	34.9	25.7	22.1	17.1	18.7	19.3	25.5	24.6	18.9	↓ p <0.001	↑ p = 0.074
Ciprofloxacin	11.9	8.6	7.8	7.1	6.5	5.2	5.6	5.4	6.4	5.5	NA	NA
3rd gen. cephalosporins	7	5.3	4.7	5.5	4.7	4.8	5.4	4.9	5.3	4.5	↓ p = 0.001	↓ p <0.001
Total number of isolates	3197	3489	3142	3669	4246	6372	7615	8948	9227	9696		

a) Total numbers of invasive isolates per year, b) Resistance rates among tested isolates, c) Resistance rates and 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 2019. NA = Not applicable

Conclusion

The general trend for *K. pneumoniae* in all three specimen categories have been declines in resistance rates to important antimicrobials as cephalosporins, gentamicin and ciprofloxacin over a ten years period. These declines have slowed down or stagnated during the past five years. For mecillinam a worrisome increase in resistance in 2017 and 2018 has reversed in 2019. Amoxicillin-clavulanic acid has only been observed since 2017, and in the urinary isolates resistance rates has declined in the period. Even though caution should be taken when interpreting reported susceptibility results towards piperacillin-tazobactam a worrisome change in the zone distributions for invasive isolates of *K. pneumoniae* with more isolates being less than 17mm (resistant) was observed.

The small, but increasing levels of carbapenem resistance in *K. pneumoniae* is worrisome. Often those isolates are also resistant to other important antimicrobials. In 2019 though, no increase in numbers of carbapenem resistant cases was observed. In the southern and south-eastern part of Europe high levels of carbapenem resistance, with Greece peaking at 63.9%, as well as combined resistance to 3rd generation cephalosporins, ciprofloxacin and gentamicin is truly problematic [EARS-Net annual report, 2018].

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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 hospital patients

In 2019, a total of 493 unique patients with invasive *P. aeruginosa* isolates from all 10 departments of clinical microbiology (DCMc) in Denmark, were identified in MiBa. All 10 DCMs routinely (>75% of isolates) registered antimicrobial susceptibility interpretations for ciprofloxacin, piperacillin-tazobactam, gentamicin and meropenem in MiBa and nine DCMs routinely registered antimicrobial susceptibilities for ceftazidime. Antimicrobial susceptibility 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.

The highest level of resistance was reported for ciprofloxacin with 5.5%. Meropenem resistance was reported in 3.1% of the cases, and only in 1.7% of the cases resistance to three or more of the five antimicrobials under surveillance were reported. None of the invasive *P. aeruginosa* isolates identified in MiBa for 2019 were registered as colistin resistant.

Conclusion

Regarding invasive *P. aeruginosa* the situation in Denmark is quite stable both in number of cases and resistance profiles among those, with relatively low overall prevalence of resistance. EARS-Net 2018 reported a small decreasing trend in resistance in the EU/EEA population-weighted mean for all antimicrobial groups under surveillance during the period 2015-2018. In 2018 EU/EEA population-weighted means for prevalence of resistance were: ceftazidime (14.1%), fluoroquinolones (19.7%), aminoglycosides (11.8%), piperacillin-tazobactam (18.3%), carbapenems (17.2%) and for combined resistance (resistance to three or more of the five monitored antimicrobials) (12.8%). Large inter-country variations are reported between South East Europe and North Europe [EARS-Net annual report, 2018].

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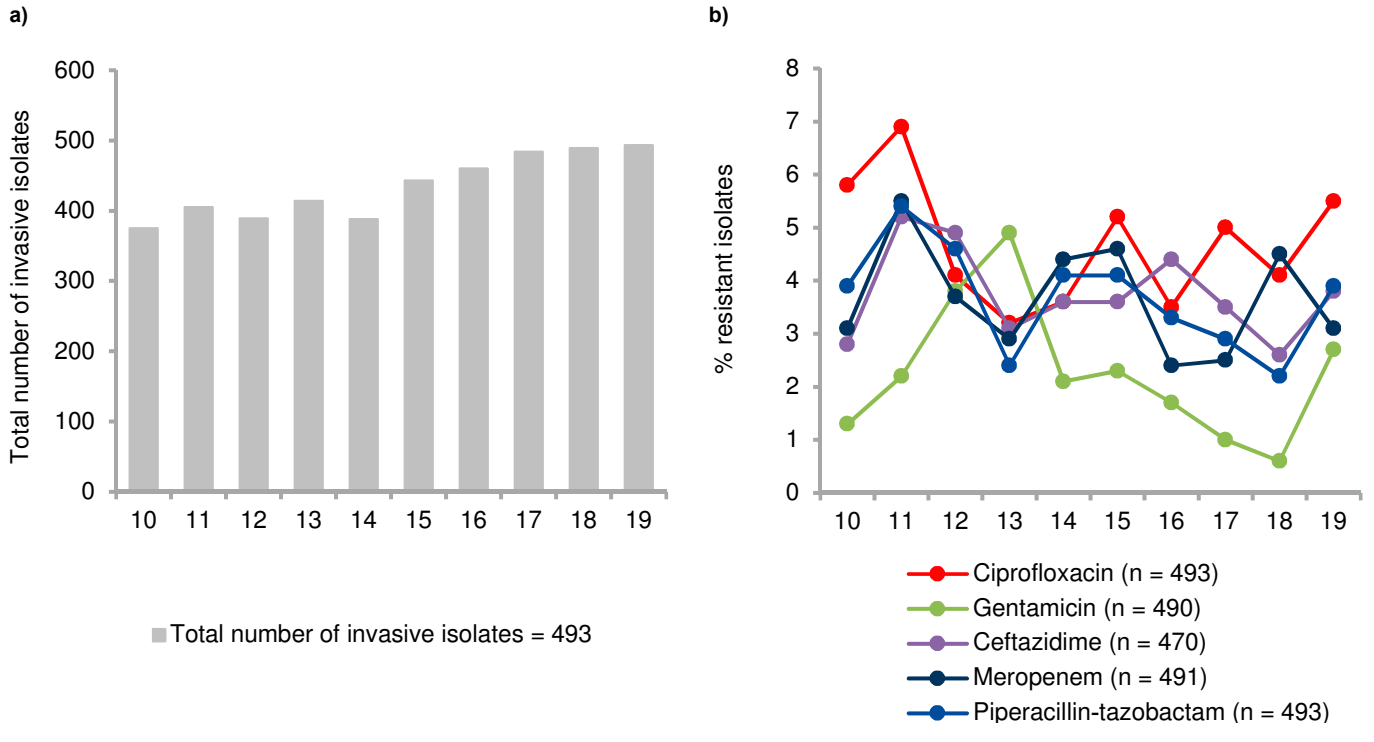
Figure 8.9 *Pseudomonas aeruginosa*. Resistance (%) in invasive isolates from humans DANMAP 2019



a) Total numbers of invasive isolates per year
b) Resistance rates among tested isolates. The number (n) in parentheses represents the numbers of isolates tested in 2019

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* is intrinsically resistant to a broad range of antimicrobials mediated by a low membrane permeability and constitutive expression of various efflux systems. The antimicrobial classes, which can be used for treatment include some fluoroquinolones, aminoglycosides, carbapenems and colistin. Particularly for *A. baumannii*, multiresistant clones are widespread in the hospital environment in many South- and East European countries, where they cause nosocomial 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*.

Invasive cases from hospitals

In 2019, a total of 72 unique patients with invasive *Acinetobacter* species were identified in MiBa from nine departments of clinical microbiology (DCMs) in Denmark. All nine DCMs routinely (>75% of isolates) registered antimicrobial susceptibility interpretations for ciprofloxacin, meropenem and gentamicin. Antimicrobial susceptibility 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.

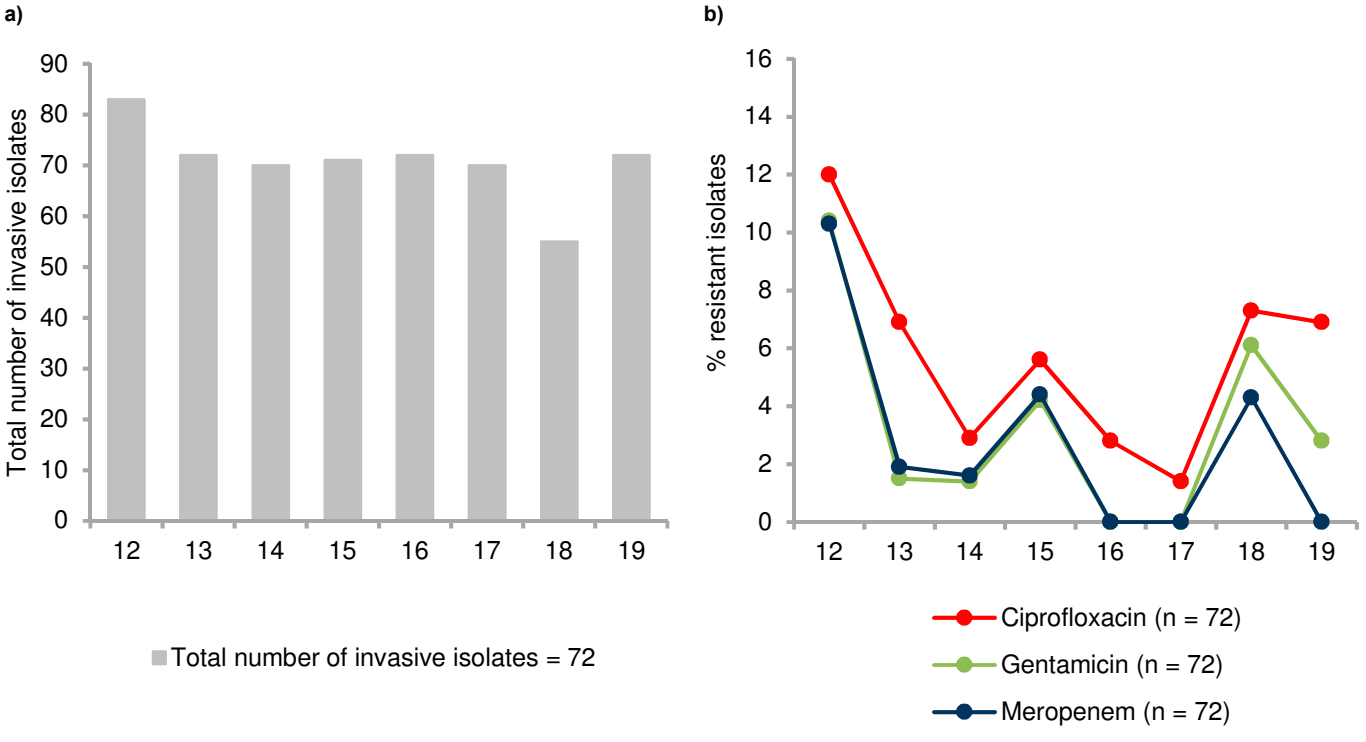
Data are presented in Table 8.6 and in Figure 8.10.

Tabel 8.6 *Acinetobacter* spp. Tested and resistant invasive isolates DANMAP 2019

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

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

Figure 8.10 *Acinetobacter* species. Resistance (%) in invasive isolates from humans DANMAP 2019



a) Total numbers of invasive isolates per year
b) Resistance rates among tested isolates. The number (n) in parentheses represents the numbers of isolates tested in 2019

The number of invasive *Acinetobacter* cases were similar to the years 2013 – 2017. None of the 72 isolates were resistant to meropenem, 5 isolates were resistant to ciprofloxacin and 2 were resistant to gentamicin. None had combined resistance to ciprofloxacin and gentamicin. None of the invasive *Acinetobacter* species were reported resistant to colistin, however, susceptibility to colistin, is not routinely tested.

Conclusion

In general, low total numbers of invasive *Acinetobacter* species are 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. Particularly the Baltic and Southern and South-Eastern countries of Europe reported on problems with high resistance levels and the most common have been combined resistance to fluoroquinolones, aminoglycosides and carbapenems with up to 91% of isolates with combined resistance in Croatia in 2018 as the highest and a EU/EAA population-weighted mean of 29%. The northern countries reported in between 0% and 4.3% combined resistance in 2018 [EARS-Net annual report, 2018].

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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 intrinsically 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 colonization 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.

Treatment 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 antibiotics (penicillin/ampicillin) with an aminoglycoside (most often gentamicin) or glycopeptide (vancomycin) is required in cases of endocarditis.

Invasive cases from hospitals

For 2019, a total of 634 unique patients with invasive *E. faecalis* isolates and 739 unique patients with invasive *E. faecium* isolates from all 10 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 for ampicillin in MiBa. In addition, nine DCMs routinely reported antimicrobial susceptibilities for vancomycin, six DCMs for linezolid, two DCMs for teicoplanin and one DCM for tigecycline. For *E. faecium*, all 10 DCMs routinely (>75% of isolates) reported antimicrobial susceptibility interpretations for ampicillin and vancomycin in MiBa. In addition, six DCMs routinely reported antimicrobial susceptibilities for linezolid, two DCMs for teicoplanin and one DCM for tigecycline. Antimicrobial susceptibility 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. For vancomycin all isolates reported as VRE in MiBa (based on PCR results for *vanA/B* genes) were calculated as vancomycin resistant independently from the actual zone/MIC result and there was not distinguished between VRE and VVE (vancomycin-variable enterococci). High-level resistance to gentamicin was calculated with EUCAST break points (MIC >128 mg/L and/or zone diameters <8 mm) on MIC and/or zone diameters as registered in MiBa. Gentamicin MIC and/or zone diameters were routinely reported from three DCMs.

Resistance to all tested antimicrobials 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.

The total number of invasive cases of *E. faecalis* and *E. faecium* have been stable in 2018 and 2019.

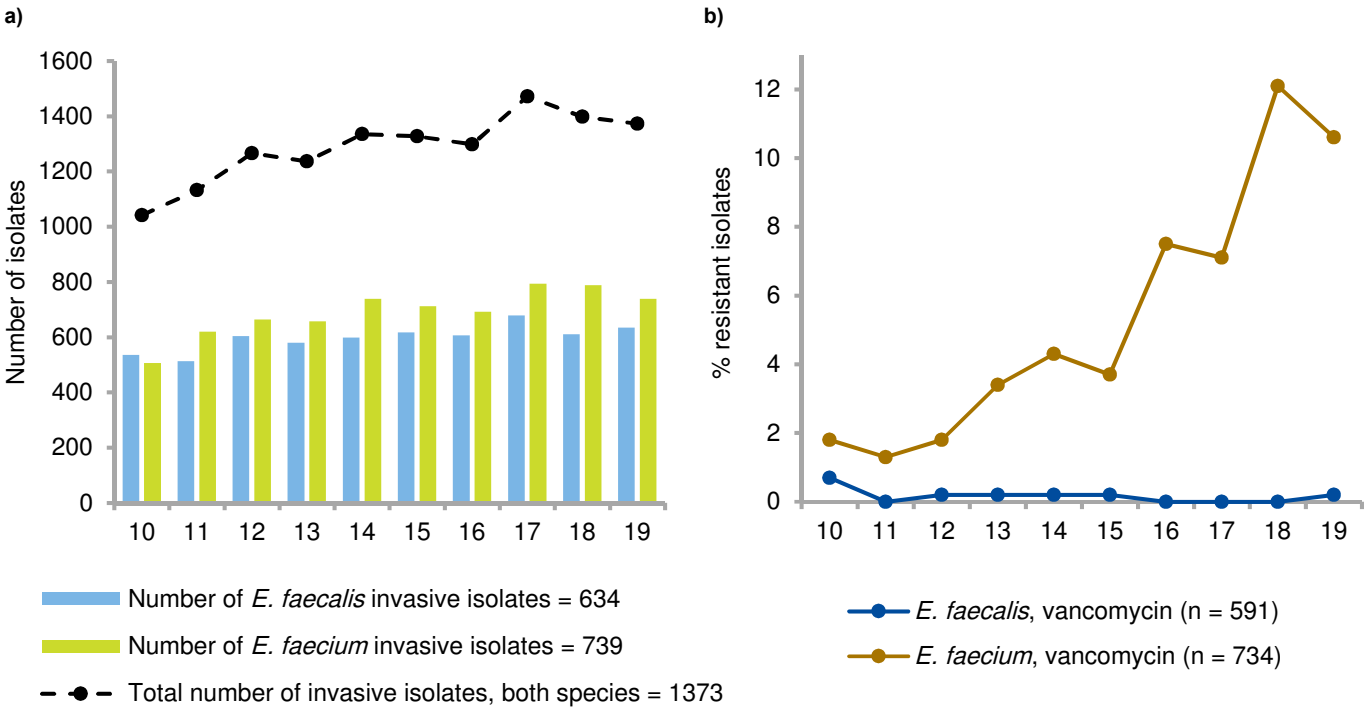
Table 8.7 Enterococci. Resistance (%) in invasive isolates from humans, 2019 DANMAP 2019

	E. faecalis	*E. faecium*	Number of included isolates (number of DCM)	
	%	%	*E. faecalis*	*E. faecium*
Ampicillin	0.2	93	632 (10)	735 (10)
Vancomycin	0.2	11	591 (9)	734 (10)
Linezolid	1.7	0.2	470 (6)	535 (6)
High-level gentamicin	9.4	33	278 (3)	296 (3)
Teicoplanin	1.0	6	198 (2)	218 (2)
Tigecycline	0.0	0.0	95 (1)	91 (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

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

DANMAP 2019



a) Total numbers of invasive isolates per year, b) Resistance rates among tested isolates. The number (n) in parentheses represents the numbers of isolates tested in 2019

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

The steep increase in the proportion of vancomycin resistant invasive *E. faecium* (12% compared to 7.1% in 2017) in 2018 was followed by a minor decrease to 11% in 2019. One vancomycin resistant invasive *E. faecalis* were reported in 2019. In total numbers 79 unique patients had a vancomycin resistant *E. faecium*/*E. faecalis* as the first invasive *Enterococcus* isolate in 2019. This number was 97 patients in 2018 and 56 in 2017.

The proportion of high-level gentamicin resistance is based on reporting from one DCM in 2010-2017, four DMCs in 2018 and from three DCMs in 2019. These rather sparse data show a decreasing trend in high-level gentamicin resistance in invasive *E. faecalis* over the decade, from 36% in 2010 to 20% in 2016, 7.1% in 2017 and a levelling with 9.4% in 2019. In *E. faecium* the level has been oscillating between 55% and 75% in the same time period, but a decreasing trend has been observed since 2017 with 43%, and further in 2019 with 33%.

During 2019, eight invasive isolates of *E. faecalis* and one invasive isolate of *E. faecium* from nine unique patients were reported linezolid resistant by the six DCMs routinely reporting interpretations to linezolid in MiBa (Table 8.7). In 2018 the numbers were five *E. faecalis* (five reporting DCMs) and three *E. faecium* (six reporting DCMs). All linezolid resistant invasive isolates identified in MiBa in 2019, were found susceptible to vancomycin.

Conclusion

An increase of invasive enterococci, mainly caused by an increase in invasive *E. faecium*, has been observed since 2002 (Figure 8.1.1. DANMAP 2015). The increase was combined with, an increase in the proportion of ampicillin resistant *E. faecium* (65% in 2002 and more than 90% since 2010) in the beginning, and since 2013 combined with an increase in vancomycin resistant *E. faecium*. In 2018, yet a steep increase in the percentage of vancomycin resistant invasive *E. faecium* was observed while the total number of invasive enterococci did not increase further compared to 2017. In 2019, the percentage of vancomycin resistant invasive *E. faecium* as well as the total number of invasive enterococci did not increase any further. The proportion of invasive vancomycin resistant *E. faecium* is relatively high in Denmark, 11% in 2019, especially when compared to the other Nordic countries with a range of 0-2.3%. But also southern European countries like France and Spain have lower percentages of vancomycin resistant invasive *E. faecium* than Denmark. However, EARS-Net also reported a worrisome increase in vancomycin resistant *E. faecium* in several countries from 2015 to 2018 [EARS-Net annual report, 2018].

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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, 2019 Denmark

Background

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

Since 2014, the Danish Departments of Clinical Microbiology (DCMs) have, on a voluntary basis, submitted 3rd generation cephalosporin-resistant *E. coli* (3GC-R Ec) from bloodstream infections for further characterization at the National Reference Laboratory for Antimicrobial Resistance at Statens Serum Institut (SSI) by adhering to the EUCAST criteria for reduced susceptibility towards cefpodoxime, ceftriaxone, cefotaxime or ceftazidime.

At SSI, the 3GC-R Ec's collected in Denmark through 2019, were phenotypically tested for ESBL-production. ESBL- and/or pAmpC-positive isolates from January, March, May, July, September, and November were subjected to whole-genome sequencing and characterized according to Multi locus 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 production.

Results

In 2019, a total of 375 *E. coli* isolates from unique patients, were identified with phenotypic test, as ESBL, AmpC or carbapenemase-producing isolates. Demographic data was available for all 375 *E. coli* isolates in 2019; 206 (55%) of the patients were men compared to 185 (53%) in 2018, and 169 (45%) were women compared to 167 (47%) in 2018. The average age at diagnosis was 72 years, ranging from below one year to 99 years.

The regional distribution of the 375 isolates with ESBL, AmpC or carbapenemase pheonotype was compared to data from previous years (Table 8.8 and Figure 8.12).

From 2014 to 2019, the reported cases of *E. coli* ESBL/AmpC or carbapenemase-producing isolates from bloodstream infections increased with 53% from 245 to 375, compared to the overall number of *E. coli* bloodstream infections which increased with 25% in the same period.

From 2018 to 2019, the number increased from 352 to 375 isolates, a 7% increase, compared to the overall number that increased with 4%.

In The Capital Region, the number of reported cases decreased from 154 cases in 2018 to 124 cases in 2019 (p = 0.003), whereas the reported number of cases in The Zealand Region increased significantly from 23 cases in 2018 to 53 cases in 2019 (p = 0.0008). For the remaining three regions, the reported number of cases were stable in 2019 compared with 2018.

Whole genome sequencing data were obtained from 197 *E. coli* isolates (as only isolates from every second months were sequenced). Genes encoding ESBL and/or pAmpC were detected in 192 (97%) of the isolates while 5 isolates were cAmpC hyper producers only; these 5 isolates were not investigated further.

In 2019, 15 different ESBL-, and pAmpC-enzymes were detected among the 192 sequenced isolates (Table 8.9). As in previous years, CTX-M-15 was the most prevalent enzyme, decreasing from 57% in 2018 to 43% in 2019 (p=0.0006). The presence of CTX-M-14 increased from 9% in 2018 to 17% in 2019 (p=0.005), and the presence of CTX-M-55 increased from 1% in 2018 to 4% in 2019 (p=0.02). No new ESBL- or pAmpC-enzymes were detected among the isolates in 2019.

No carbapenemase producers were observed among the 197 whole genome sequenced ESBL- and/or pAmpC *E. coli* isolates from blood infection (from every second months were sequenced), but two carbapenemase producing *E. coli* isolates from bloodstream infections were detected in June and October.

Table 8.8 Distribution of ESBL/pAmpC -producing *E. coli* from bloodstream infections, Denmark DANMAP 2019

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

Figure 8.12 Regionwide distribution of ESBL/pAmpC-producing *E. coli* from bloodstream infections, Denmark DANMAP 2019

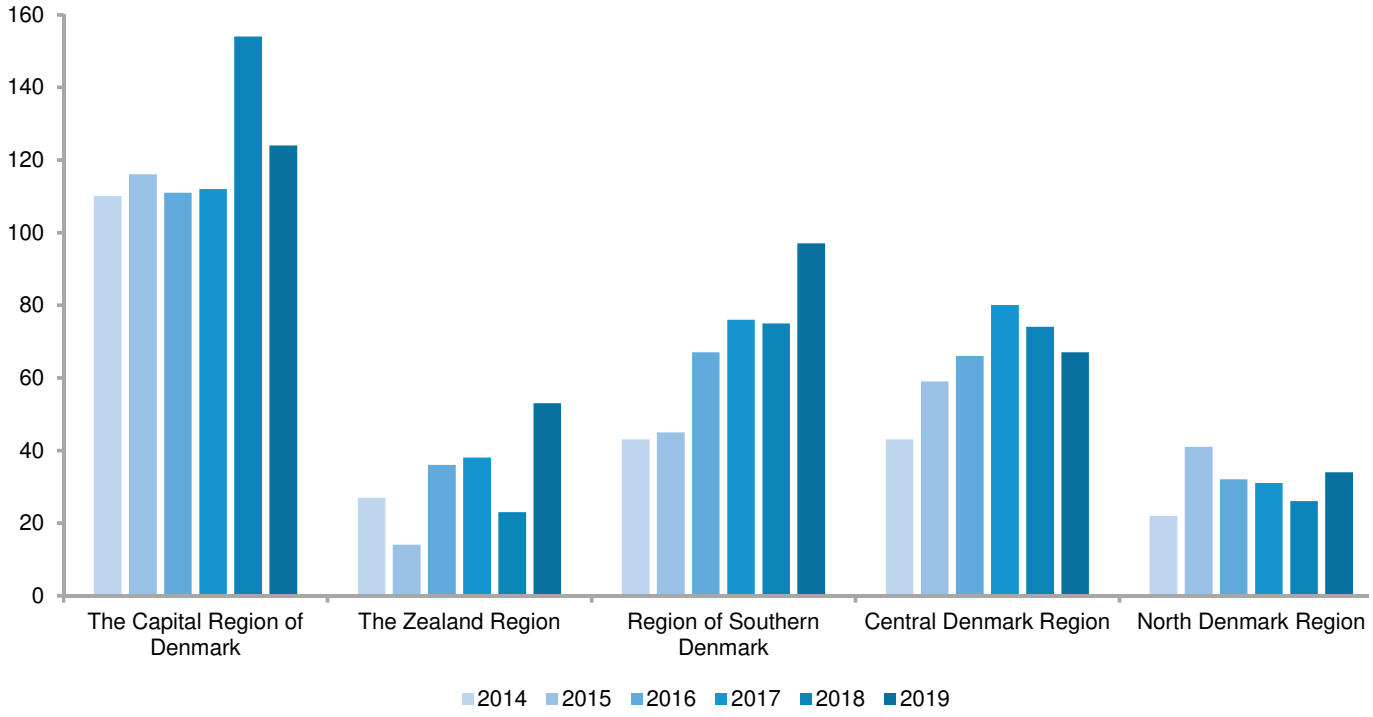

Table 8.9 Most common ESBL enzymes, pAmpC enzymes and carbapenemases detected in *E. coli* from bloodstream infections, Denmark DANMAP 2019

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

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

In 2019, the 197 whole genome sequenced *E. coli* isolates belonged to 39 different MLSTs, with the most common sequence type (ST) being ST131 (47%), followed by ST69 (7%) and ST38 (7%) (Table 8.10).

The proportion of ST23 isolates increased from below 1% in 2018 to 6% in 2019 (p=0.00005). Between 2014 and 2018, ST23 was only observed seven times. The 11 isolates from 2019 all produced CTX-M-14, and cgMLST analysis of the clones showed highly identical strains with maximum two allele differences between the isolates. The isolates were reported by seven of the ten DCMs, and primarily from emergency departments.

Among the 93 *E. coli* isolates belonging to ST131, CTX-M-15 (52%) was the most common enzyme, followed by CTX-M-27 (32%), and CTX-M-14 (8%). The presence of CTX-M-15 decreased from 67% in 2018 to 52% in 2019 (p=0.01), whereas the presence of CTX-M-27 increased from 20% in 2018 to 32% in 2019 (p=0.03).

Table 8.10 Distribution of MLSTs in ESBL/pAmpC-producing *E. coli* from bloodstream infections, Denmark DANMAP 2019

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

[1] Each ST found in less than 2% of the isolates in 2019
*Numbers based on sequenced data from odd months

The whole genome sequencing data from the 192 human bloodstream infections, were compared with the collection of ESBL/AmpC- and carbapenemase-producing isolates of animal origin collected in 2018 and 2019. No clonal relationship were identified by single-nucleotide polymorphism (SNP) analysis for isolates sharing the same combination of ST and ESBL-pAmpC-genes. Horizontal gene transfer of ESBL/pAmpC or carbapenemase genes were not investigated.

Conclusion

In 2019, the number of ESBL- and/or AmpC positive isolates increased from 352 to 375 isolates (7% increase). Changes of ESBL enzymes produced by the isolates were observed in 2019, where CTX-M-15 decreased, and CTX-M-14 and CTX-M-55 increased. In isolates belonging to ST131, the relative abundance of CTX-M-15 decreased while it increased for CTX-M-27.

The relative distribution of sequence types for the 197 whole genome sequences isolates were similar to the distributions in the previous years; the worldwide disseminated ST131 clone was still strongly represented in 2019 (47%), however, a new ST23 CTX-M-14 clone was observed in 2019.

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8.3.2 Carbapenemase producing bacteria in Denmark, 2019

Background

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

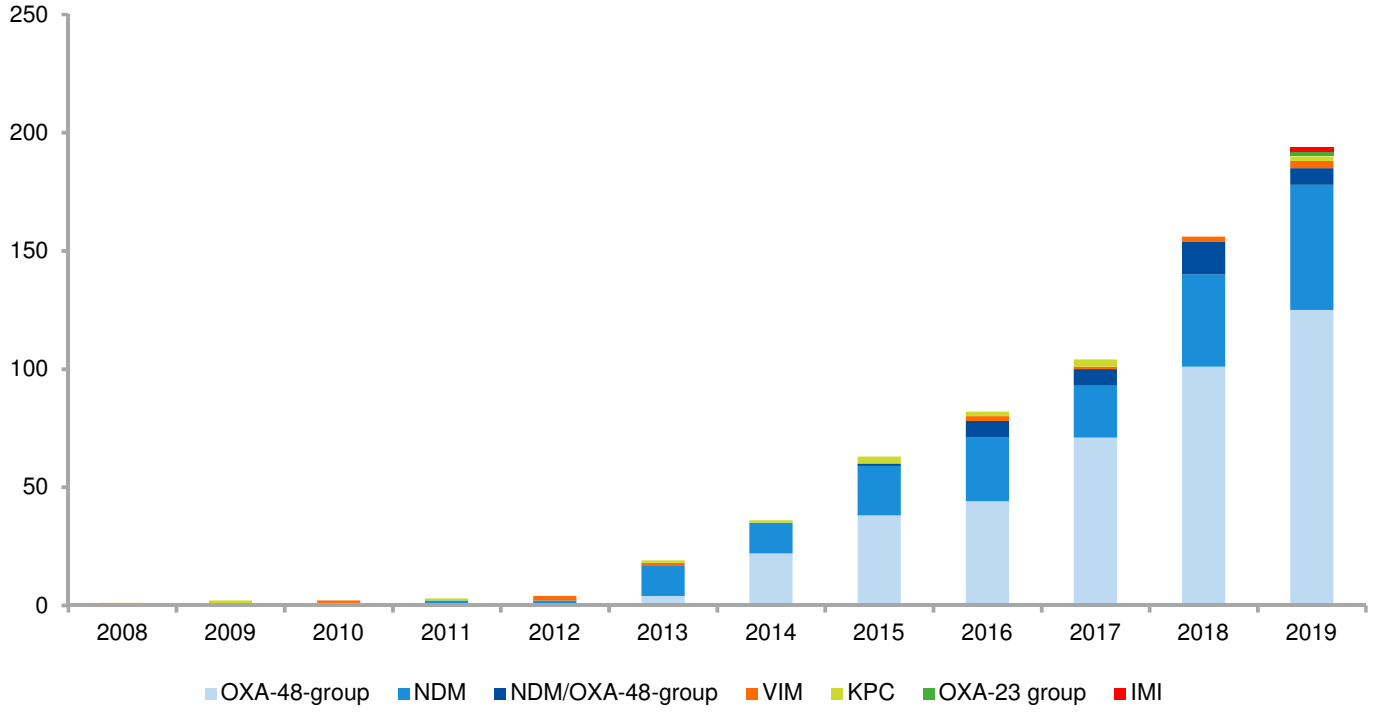
In recent years, Danish departments of clinical microbiology (DCMs) have on a voluntary basis submitted carbapenem-resistant isolates for verification and genotyping at the National Reference Laboratory for Antimicrobial Resistance at Statens Serum Institut (SSI). The Danish Health Authority made CPO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

During 2019, 221 carbapenemase-producing organisms (CPO) were detected from 187 patients compared with 177 CPO from 160 patients in 2018. 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. Ten of the CPO (nine Enterobacterales and one *Acinetobacter* spp.) were from bloodstream infections compared with 18 of the CPO in 2018.

Enterobacterales

In 2019, 194 CPE isolates were detected from 168 patients compared to 156 CPE from 141 patients in 2018 leading to a 23% increase of submitted CPE isolates compared to 2018. In 2019, seven of the 194 CPE isolates produced both NDM and OXA-48 group enzymes, 125 produced OXA-48-like enzymes and 53 were NDM-producing. Furthermore, three VIM-producing isolates and two KPC-producing isolates were detected. For the first time, two OXA-23 group CPE isolates were detected (Figure 8.13).

Figure 8.13 Numbers of carbapenemase-producing Enterobacterales (CPE), 2008-2019, Denmark DANMAP 2019



OXA-244 belong to the OXA-48-enzyme group. In recent years, an increase in OXA-244-producing *E. coli* isolates has been observed in the national surveillance of CPO in Denmark. Molecular characterisation of 24 OXA-244-producing *E. coli* isolates from January 2016 to August 2019 was initiated and epidemiological investigation of cases, including telephone interviews, was conducted. In conclusion, import of OXA-244 *E. coli* isolates from travelling abroad seemed likely for the majority of cases. Community sources were also possible, as many of the patients had no history of hospitalisation and many of the *E. coli* isolates belonged to STs that are present in the community. It was not possible to point at a single country or a community source as risk factor for acquiring OXA-244-producing *E. coli* [Hammerum et al. 2020, Euro Surveill.;25(18)].

Outbreaks with CPE during 2019

In Denmark, outbreaks with CPE in healthcare settings are registered at SSI in a newly established national database (KURS) for CPE-outbreaks. At SSI, CPE isolates are routinely characterised by whole genome sequencing (WGS). Cluster analysis is conducted to detect possible clustering between the CPE isolates. Isolates from two or more persons sharing the same unique genotype are defined as an outbreak. When epidemiological investigations can establish a link between at least two of the patients in an outbreak cluster, the outbreak is classified as a verified outbreak. When no epidemiological link can be established between the patients, the outbreak is classified as a possible outbreak (Materials and methods, section 9.12)

In total, 16 ongoing CPE-outbreaks were registered with new patients in 2019. In nine of these outbreaks, it was possible to establish an epidemiological link between the patients. All epidemiological links were found in healthcare settings: Patients sharing the same ward or hospital. Of the 16 outbreaks, five of them were new outbreak clusters, each including two patients, identified in the CPE surveillance in 2019. Three of the newly identified outbreak clusters had index patients before 2019 (Table 8.11).

In 2019, an investigation of possible clonal transmission of carbapenemase-producing *K. pneumoniae* complex member isolates in Denmark was carried out using core genome MLST and National Patient Registry Data. Thirteen clusters, including 103 isolates of carbapenemase-producing *K. pneumoniae* from 2014 – 2018, were investigated. In five of these clusters, the patients had stayed at the same ward at the same time, showing a direct epidemiological link. The study found that cgMLST combined with patient hospital admission data and travel information was a reliable and detailed approach for detecting possible transmission of carbapenemase-producing *K. pneumoniae* complex members [Hammerum et al. 2020, Intl J Antimicrob. Agents, 55:105931].

Larger outbreaks with CPE

The ST18 NDM-1-producing *Citrobacter freundii* outbreak, which started in 2012 in the North Denmark Region, continued in 2019 [Hammerum et al. 2016, J. Antimicrob. Agents, 71:3117-3124]. Until the end of 2019, 39 patients have been involved in the outbreak. None of the patients have a prior history of travel. In 2016, an already known patient from the North Denmark Region was detected positive in the Capital Region. During 2018 and 2019, the ST18 NDM-1-producing *C. freundii* also spread in the Central Denmark Region affecting six patients. The origin of the NDM-1-producing *C. freundii* is

Table 8.11 Outbreaks of carbapenemase-producing Enterobacterales (CPE) during 2019 DANMAP 2019

Outbreak ID	Year	Patients total	Patients 2019	Carbapenemase	Type of Outbreak	Species (clonal spread)	Regions	Status
41	2012-2019	39	12	NDM-1	Clonal/ plasmid	ST18 *C. freundii*	Capital Region/Central Denmark Region/North Denmark Region	Verified
48	2013-2019	19	5	OXA-436	Clonal/ plasmid	ST90 *E. cloacae*/ ST22 *C. freundii*	Capital Region/South Denmark Region/Zealand Region	Verified
24	2014-2019	9	2	OXA-181	Clonal	ST410 *E. coli*	Capital Region	Verified
25	2014-2019	6	1	OXA-48	Clonal	ST38 *E. coli*	Capital Region/Zealand Region	Verified
26	2014-2019	3	1	OXA-48	Clonal	ST448 *E. coli*	Capital Region	Possible
49	2014-2019	5	1	NDM-1	Clonal	ST91 *C. freundii*	Central Denmark Region	Possible
21	2015-2019	41	12	NDM-5/OXA-181	Clonal	ST410 *E. coli*	Capital Region/Zealand Region	Verified
22	2015-2019	5	1	OXA-181	Clonal	ST440 *E. coli*	Capital Region/Central Denmark Region	Possible
42	2015-2019	7	2	OXA-48	Clonal	ST65 *C. freundii*	Capital Region/North Denmark Region/Zealand Region	Verified
1*	2016-2019	2	1	OXA-181	Clonal	ST410 *E. coli*	Capital Region	Verified
15	2016-2019	3	1	OXA-48	Clonal	ST38 *E. coli*	Capital Region/South Denmark Region	Possible
33	2016-2019	7	1	OXA-232	Clonal	ST231 *K. pneumoniae*	Central Denmark Region	Verified
40*	2018-2019	2	1	OXA-48	Clonal	ST15 *K. pneumoniae*	Capital Region	Verified
51*	2018-2019	2	1	OXA-48	Clonal	ST73 *E. coli*	Central Denmark Region	Possible
7*	2019	2	2	NDM-5	Clonal	ST167 *E. coli*	Capital Region	Possible
43*	2019	2	2	OXA-48	Clonal	ST323 *C. freundii*	Zealand Region	Possible

*Outbreak clusters identified in 2019

unknown. The NDM-1 encoding plasmid has primarily been detected in ST18 *C. freundii* (56 isolates), but has also been detected in ST161 *E. coli* (3 isolates), ST8 *C. freundii* (1 isolate), ST17 and ST1890 *K. pneumoniae* (2 isolates respectively) and others, indicating plasmid transfer. During 2019, twelve new patients were part of this outbreak.

Since 2015, another large outbreak has been ongoing in the Zealand Region with spread of ST410 NDM-5/OXA-181 *E. coli*. [Roer et al. 2018, mSphere;3(4).]. Until the end of 2019, 41 patients had been involved in this outbreak. From 2018 spread of ST410 NDM-5/OXA-181 *E. coli* were also detected in the Capital Region. During 2019, twelve new patients were part of this outbreak. Apart from the first outbreak patient in 2015, who had been traveling to Egypt [Overballe-Petersen et al. 2018, Genome Announc.6(5): e01542-17] none of the patients had a prior history of travel.

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]. The outbreak was still ongoing with five new patients in 2019. In total, 19 patients have been detected with OXA-436-producing CPE isolates (Tabel 8.11), where both plasmid and clonal spread have been seen. Clonal spread of ST90 OXA-436-producing *E. cloacae* isolates was detected in the Region of Southern Denmark (Textbox 8.1).

Besides the twenty-nine new patients in the three larger outbreak registered in 2019, only one or two new patients have been detected in the majority of the other outbreaks (Table 8.11). It seems very likely that the increase in OXA-48-producing CPE was due to plasmid transfer, but this was not investigated further.

For all outbreaks, several infection control measures have been implemented, e.g. extensive screening of patients, enforced hand hygiene, implementation of extensive use of Personal Protective Equipment (PPE), isolation precautions, and implementation of extensive cleaning. Patients are known to be carriers of CPE for years and infection hygiene control measures are important tools to prevent further spread of CPE.

Acinetobacter spp.

In 2019, 20 carbapenemase-producing *Acinetobacter* spp. isolates were detected compared to 18 isolates in 2018. All but three patients had been travelling abroad prior to detection of the carbapenemase-producing *Acinetobacter* spp. In 2019, 19 carbapenemase-producing *Acinetobacter baumannii* with the following enzymes were detected: OXA-23 (13), NDM-1 (1), NDM-1/OXA-23 (2), OXA-58 (2) and OXA-72 (1). Furthermore, one NDM-1/OXA-58/IMP-48-like-producing *Acinetobacter pittii* was detected.

***Pseudomonas* spp.**

In 2019, seven carbapenemase-producing *Pseudomonas* spp. isolates were detected compared to three isolates in 2018. All but one patient had been travelling abroad prior to detection of the carbapenemase-producing *Pseudomonas* spp. isolates. In 2019, six carbapenemase-producing *Pseudomonas aeruginosa* with the following enzymes were detected: VIM-2 (2), NDM-1 (1), KPC-2 (1), IMP-34-like (1) and IMP-45-like (1). Furthermore, one VIM-2-producing *Pseudomonas putida* were detected.

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. It is unknown whether part of the observed increase in 2019 could be explained by the new Guidance for prevention of spread of CPO in 2018 resulting in alterations in screening and/or referral procedures.

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Textbox 8.1

Investigation of an *Enterobacter cloacae* OXA-436 carbapenemase-outbreak – Shower drains as reservoirs

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]. During 2017-2019, ST90 OXA-436-producing *E. cloacae* isolates were detected from seven patients in the Region of Southern Denmark [ECCMID 2020; abstract 508]. They had all been hospitalized at the same department at some point during 2017-2019. However, there was no direct epidemiological link between several of the patients. Rectal screening of all patients at the involved department was performed several times during the outbreak, but the results were negative. The infection control team audited staff and procedures, but no source or route of transmission was revealed. Finally, an investigation focusing on the department facilities, including sinks and drains, was performed.

Seven drains, 25 sinks and three bedpan boilers/instrument washers were sampled. ST90 OXA-436-producing *E. cloacae* were detected from two shower drains in the patient bathrooms. When comparing the ST90 OXA-436 *E. cloacae* isolates from the two shower drains with the isolate from the index patient, it seems highly plausible that the drains were reservoirs for the ST90 OXA-436 *E. cloacae* isolates. During sampling, staff reported that the shower drains had been partly clogged from time to time, which meant that the patients were standing in water returning from the drains. After the drains were unclogged, no further ST90 OXA-436 *E. cloacae* isolates have been detected in the Danish national surveillance of CPO.

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8.3.3 Increase in both *vanA* and *vanB E. faecium* in Denmark

Background

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

In recent years, *E. faecium* harboring the *vanA* gene complex, but being phenotypically susceptible to vancomycin, have been described in different countries. These enterococci are referred to as vancomycin-variable enterococci (VVE). VVE have caused nosocomial outbreaks and development of reverting mutants becoming vancomycin resistant *in vitro* and *in vivo* has been described. Thus, VVE are clinically relevant and their detection is thus critical in order to avoid treatment failure with vancomycin. However, VVE cannot be selectively cultured on

vancomycin-containing media and can only be detected by molecular methods. In 2015 and 2016, sporadic VVE isolates with different genetic background in relation to concurrent VRE-outbreaks were detected in the Capital Region of Denmark, [Hammerum et al. Euro Surveill. 2020;25(18)]. In 2016, a new VVE clone belonging to ST1421- CT1134, which displays variable vancomycin susceptibility due to a deletion in the *vanX* gene, was detected. [TA Hansen et al., J. Antimicrob. Agents, 2018, 73: 2936–2940].

Surveillance of VRE/VVE

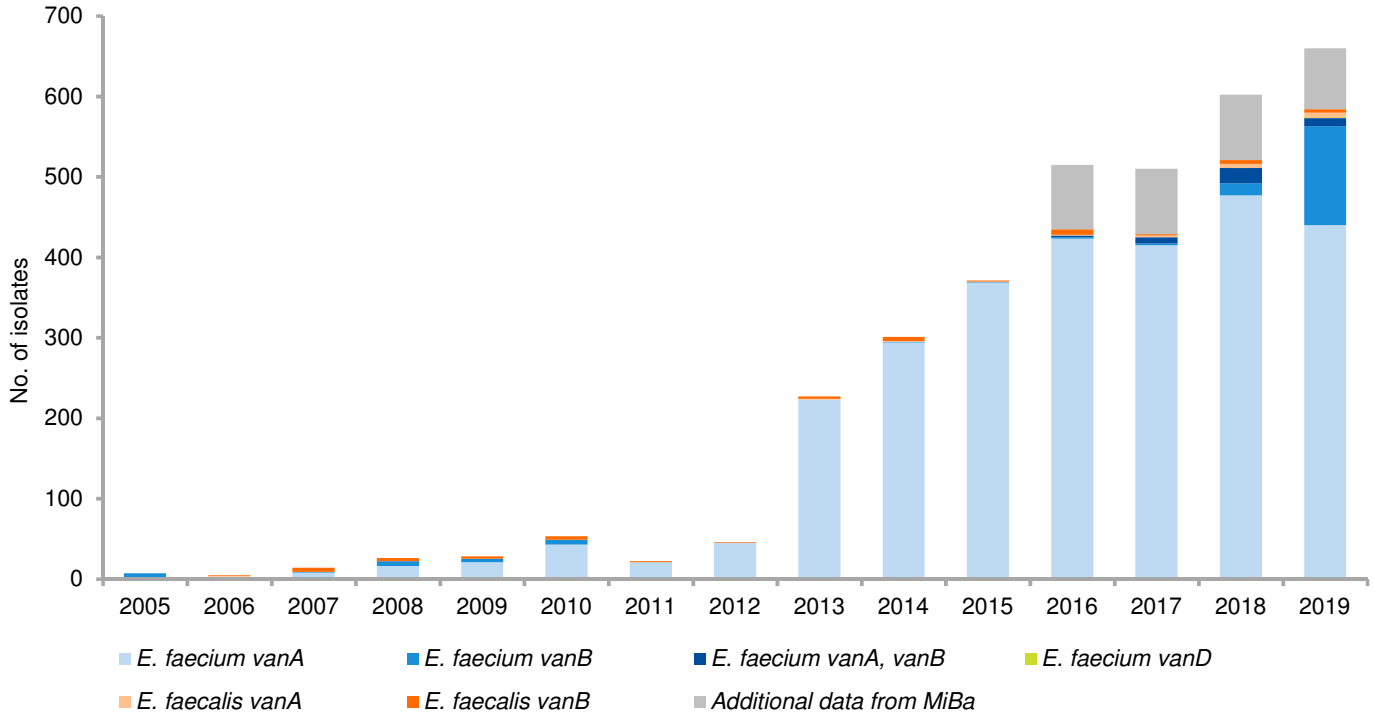
Since 2005, Danish departments of clinical microbiology (DCMs) have voluntarily submitted clinical VRE (one isolate per patient per 12 months) for species identification, genotyping and surveillance to the Antimicrobial Resistance Reference Laboratory at Statens Serum Institut (SSI).

In 2017, testing of phenotypically vancomycin-susceptible *E. faecium* isolates from blood cultures for the presence of *vanA*/*vanB* genes by PCR was introduced in the DCMs in the Capital Region for detection of possible VVE. During 2018, PCR testing was expanded to all clinical *E. faecium* isolates. Similarly in 2018, molecular testing by PCR of *E. faecium* from all clinical samples were implemented in one of the four DCMs in the Region of Southern Denmark. Furthermore, *E. faecium* isolates from blood cultures were tested by PCR for *vanA*/*vanB* genes in a second DCM in the Region of Southern Denmark and in the DCM in the Central Denmark Region in 2018. In 2019, diagnostic algorithms to detect VVE expanded, as most of the DCMs across Denmark as a minimum tested all blood culture *E. faecium* isolates for the presence of *vanA* genes using PCR [Hammerum et al. Euro Surveill. 2020;25(18)].

To determine any underreporting in the submissions, the number of VRE/VVE submitted to SSI in 2016, 2017, 2018 and 2019 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, 78 and 76 patients in surveillance in 2016, 2017, 2018 and 2019, respectively (Figure 8.14). In 2019, 584 VRE/VVE isolates were submitted to SSI. By adding the 76 VRE/VVE isolates extracted from MiBa, this summed up to 660 VRE/VVE isolates from 660 patients in 2019 compared to 603 VRE/VVE isolates from 599 patients in 2018 (Figure 8.14).

From 2013, a sharp increase in clinical VRE isolates has been observed. Until 2018, the increase was mostly seen for *vanA E. faecium*, but during 2019 an increase was detected for *vanB E. faecium* too (Figure 8.14).

Figure 8.14 Numbers of *Enterococcus faecium* and *Enterococcus faecalis* isolates carrying *vanA* and *vanB* genes from clinical samples submitted to SSI 2005-2019 supplemented with data obtained from MiBa from 2016-2019, Denmark DANMAP 2019



From 2015 through 2019, the clinical VRE/VVE isolates received at SSI have been analysed by whole-genome sequencing (WGS). In 2019, 584 VRE/VVE were analysed by WGS. From the WGS data, species identification, multilocus sequence type (MLST), core genome MLST (cgMLST) and van-genes were identified *in silico*.

Of the 584 clinical VRE/VVE isolates, 440 were *vanA E. faecium*, 123 *vanB E. faecium*, 10 *vanA*/*vanB E. faecium*, one *vanD E. faecium*, six *vanA E. faecalis* and four *vanB E. faecalis* (Figure 8.14). WGS-based cgMLST analysis was performed on the ten *E. faecalis* isolates and the 574 *E. faecium* isolates using SeqSphere+ (Ridom). The ten *E. faecalis* isolates were subdivided into nine different complex types (CTs), whereas the 574 *E. faecium* isolates were subdivided into 72 CTs. Three clones were predominant: ST1421-CT1134 *vanA E. faecium*, ST117-CT36/CT991 *vanB E. faecium* and ST203-CT859/CT1051/CT1507 *vanA E. faecium* (Table 8.12).

ST203-CT859 *vanA E. faecium* isolates were first detected during the end of 2014 [Hammerum et al. J Antimicrob Chemother. 2017 Aug 1;72(8):2184-2190]. This type increased rapidly in numbers and became the most prevalent *vanA E. faecium* type (together with its subtypes CT1051 and CT1507) during 2015 to 2017, but decreased in 2018 and 2019 (Table 8.12). In 2019, only 9% of the VRE/VVE *E. faecium* isolates belonged to ST203-CT859.

In 2017, 3% of the *E. faecium* isolates belonged to the VVE clone, ST1421-CT1134 *vanA E. faecium*. This type was only detected from clinical samples from the Capital Region. In 2018, 34% of the *E. faecium* isolates belonged to ST1421-CT1134, they were detected in the Capital Region, the Region Zealand and from one DCM in the Region of Southern Denmark 2019 [Hammerum et al. Euro Surveill. 2020;25(18)]. During 2019, ST1421-CT1134 *vanA E. faecium* was the most prevalent type observed (50%) (Table 8.12). Furthermore, ST1421-CT1134 *vanA E. faecium* has spread to the Faroe Islands during 2018 and 2019 through patient transferring from Denmark [Hammerum et al. Euro Surveill. 2020;25(18)].

During 2019, the ST117-CT36 *vanB E. faecium* and its subtype ST117-CT991 *vanB E. faecium* increased in Denmark, which was both related to several introductions into Denmark from hospitals abroad and spread between hospitals due to patient transfer. Only two isolates belonged to this type in 2018, whereas 90 isolates were detected during 2019 (Table 8.12).

Conclusion

The increasing number of VRE/VVE cases in 2019 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 a difficult task. Infection control should include proper cleaning, focus on 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 because VVE diagnostic is challenging and therefore, the clone is likely to be underdiagnosed. The increase during 2019 of *vanB E. faecium* is also of concern.

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Table 8.12 Description of the most common types of *vanA* and/or *vanB Enterococcus faecium* according to MLST and cgMLST, 2015-2019, Denmark

DANMAP 2019

Types[a]	2015		2016		2017		2018		2019	
	(n = 369)		(n = 427)		(n = 425)		(n = 515)		(n = 574)	
ST80-CT14 *vanA*	81	22%	38	9%	15	4%	1	<1%	1	<1%
ST80-CT24 *vanA*	23	6%	19	5%	11	3%	2	<1%	4	<1%
ST80-CT866 *vanA*	14	4%	10	2%	7	2%	N.D.	N.D.	N.D.	N.D.
ST80-CT1064 *vanA/vanB*	N.D.	N.D.	2	<1%	8	2%	23	4%	11b	2%
ST80-CT1729 *vanA*	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	22	4%	2	<1%
ST117-CT1180 *vanA*	N.D.	N.D.	N.D.	N.D.	9	2%	30	6%	14	2%
ST117-CT36/CT991 *vanB*	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	2	<1%	90	16%
ST203-CT859/CT1051/CT1507 *vanA*	188	51%	271	64%	265	63%	161	31%	54	9%
ST1421-CT1134 *vanA*	N.D.	N.D.	2	<1%	13	3%	176	34%	285	50%
Other types	62	17%	85	20%	97	23%	98	19%	113	20%

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

8.3.4 Detection of linezolid resistant enterococci and linezolid-vancomycin resistant enterococci

Background

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

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

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

In DANMAP 2018, LRE isolates from 2015-2018 were investigated. During this period, eight linezolid-resistant *E. faecium* isolates and eight linezolid-resistant *E. faecalis* isolates were send to SSI (only one isolate per patient were included). In 2019, two linezolid-resistant *E. faecalis* isolates were send to SSI.

WGS data from the LRE isolates were investigated for 23S rRNA mutations (G2576T and G2505A), and the *optrA*, *cfr*, *cfr*(B) and *poxtA* genes using the LRE-Finder [https://cge.cbs.dtu.dk/services/ LRE-Finder/].

Among the LRE isolates from 2015-2019, LRE-Finder detected seven *E. faecium* with the G2576T mutation, one *E. faecium* with the G2505A mutation and 10 *E. faecalis* isolates with *optrA* (Table 8.13).

Surveillance of linezolid-vancomycin-resistant Enterococci (LVRE)

In order to detect linezolid-resistant VRE isolates (LVRE), the LRE-Finder was also implemented in the automated surveillance at SSI for detection of LRE mutations and LRE genes in genomes from VRE isolates submitted for the national VRE Surveillance and LRE/LVRE submitted from DCMs directly. During the period 2015-2019, no linezolid vancomycin resistant *E. faecalis* were detected, whereas, 11 linezolid-vancomycin resistant *E. faecium* were identified. Seven linezolid resistant *E. faecium* isolates had the G2576T mutation and were positive for the *vanA* gene encoding vancomycin resistance, three *E. faecium* isolates were positive for *optrA* and *vanA* and one *E. faecium* isolate were positive for *cfr*(B) and *vanB* (Table 8.13).

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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Table 8.13 Characterization of the 18 linezolid resistant enterococci (LRE) and the 11 linezolid vancomycin resistant enterococci (LVRE), 2015-2019, Denmark DANMAP 2019

	No. of isolates	Species	Linezolid resistance mechanism	Vancomycin resistant gene
LRE	1	*E. faecium*	G2505A	none
	7	*E. faecium*	G2576T	none
	10	*E. faecalis*	*optrA*	none
LVRE	7	*E. faecium*	G2576T	*vanA*
	3	*E. faecium*	*optrA*	*vanA*
	1	*E. faecium*	*cfr*(B)	*vanB*

8.3.5 *Streptococcus pneumoniae*

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

In Denmark, 639 cases of invasive pneumococcal disease (IPD) were registered in 2019. The cases were mainly from pneumococci found in either blood (582) or cerebrospinal fluid (41). For 16 cases, pneumococci had been found in other, normally sterile sites (ascites, joint, pleura, brain), but data from these are by tradition not included in this report. In total 606 isolates were received at the reference laboratory out of the 623 cases of bacteraemia and meningitis identified in MiBa. Four of the 606 received isolates were not viable for susceptibility testing, but serotyping was nevertheless possible. Data for the seventeen remaining cases where isolates were not provided were retrieved from MiBa, and the failure of submitting an isolate for serotyping was mainly found to be due to non-viable isolates or diagnosis through PCR. Antimicrobial susceptibility data for those cases were retrieved, when available, through MiBa. In total, serotypes were available for 606 cases and antimicrobial susceptibility data for both penicillin and erythromycin was available for 612 cases (two additional cases had data for only one of the antimicrobials each registered in MiBa).

The 606 isolates from blood or cerebrospinal fluid belonged to 41 different serotypes. For the 612 cases with fully available susceptibility data, 569 were susceptible to both penicillin and erythromycin (93.0%). For penicillin, 583 out of 613 were susceptible (95.1%), 26 (4.2%) were classified as susceptible increased exposure and four isolates of different serotypes (0.7%) were classified as resistant. For erythromycin, 592 of 613 isolates were susceptible (96.6%) and 21 isolates (3.4%) were resistant.

Table 8.14 Number of invasive isolates and distribution of resistance in the most common sero-types of pneumococci, Denmark

DANMAP 2019

Serotype	N 2019	PEN-S_ ERY-S	PEN-S_ ERY-R	PEN-I_ ERY-S	PEN-I_ ERY-R	PEN-R_ ERY-S	PEN-R_ ERY-R	Unk	% S-S	N (% S-S) 2018	N (% S-S) 2017
8	162	162							100.0%	194 (100%)	192 (99%)
3	69	68						1	100.0%	70 (97%)	57 (100%)
22F	49	47	1	1					95.9%	69 (100%)	58 (100%)
12F	44	42	1	1					95.5%	55 (100%)	69 (99%)
9N	28	27		1					96.4%	62 (98%)	56 (98%)
23B	18	9		8	1				50.0%	14 (7%)	11 (27%)
16F	17	16	1						94.1%	19 (84%)	21 (95%)
11A	17	15	1	1					88.2%	19 (95%)	19 (89%)
24F	15	10	5						66.7%	17 (76%)	19 (79%)
15A	15	13			1			1	92.9%	25 (76%)	16 (63%)
23A	14	14							100.0%	13 (100%)	18 (100%)
35B	13	11		1	1				84.6%	15 (100%)	20 (90%)
33F	13	10	3						76.9%	17 (88%)	13 (92%)
20	13	13							100.0%	24 (100%)	26 (100%)
15C	11	9		1	1				81.8%	4 (100%)	3 (100%)
35F	9	8			1				88.9%	14 (100%)	13 (100%)
10A	9	9							100.0%	15 (100%)	9 (100%)
10B	9	9							100.0%	9 (100%)	9 (100%)
19A	8	4	1	1	1		1		50.0%	11 (82%)	5 (100%)
17F	8	5		3					62.5%	12 (50%)	10 (80%)
15B	8	8							100.0%	9 (100%)	14 (93%)
7C	8	8							100.0%	6 (83%)	3 (100%)
31	6	6							100.0%	15 (100%)	8 (88%)
19F	6	6							100.0%	7 (71%)	13 (69%)
6C	5	4		1					80.0%	5 (40%)	13 (92%)
Other	49	36		1		2	1	9	90.0%	42 (88%)	39 (87%)
Sum	623	569	13	20	6	2	2	11	93.0%	762 (93%)	734 (94%)

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

Figure 8.15 Non-wild-type (%) in *Streptococcus pneumoniae* blood and spinal fluid isolates from humans, Denmark DANMAP 2019

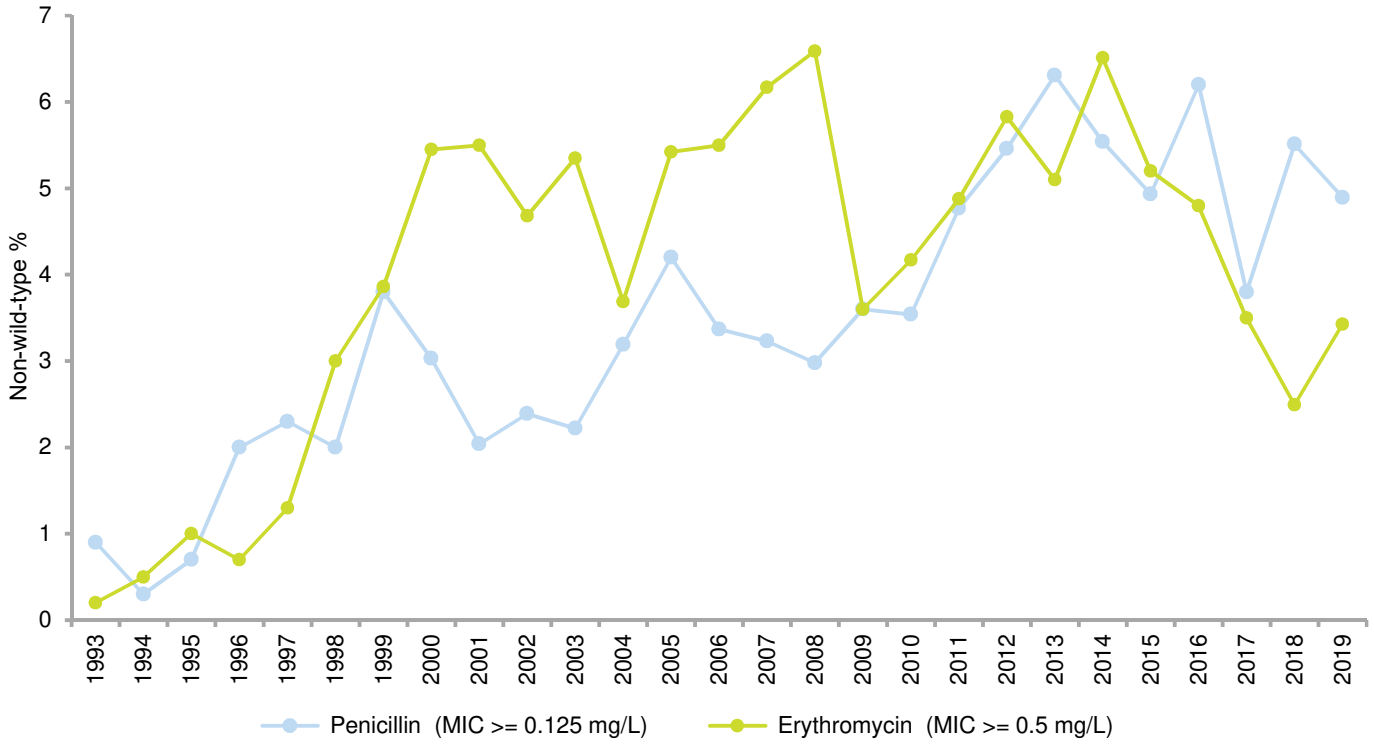
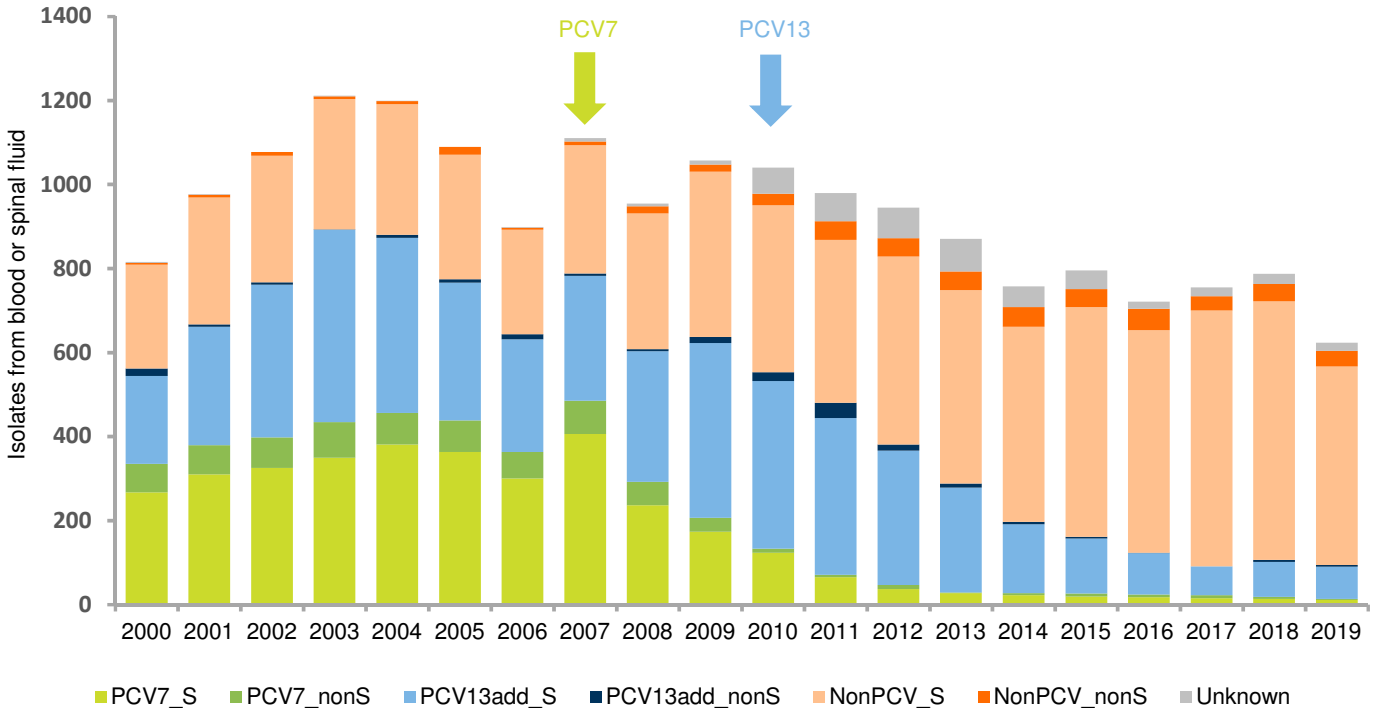


Figure 8.16 *Streptococcus pneumoniae* blood and spinal fluid isolates from humans. Vaccine serotypes and non-vaccine serotypes in combination with susceptibility to penicillin and erythromycin, Denmark DANMAP 2019



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

Antimicrobial susceptibility in pneumococci is highly correlated to serotypes (Table 8.14), and variations in susceptibility patterns through the years often reflects changes in circulating serotypes. The pneumococcal vaccine introduced in the childhood vaccination programme in 2007 has had a profound effect on the serotypes that are causing invasive pneumococcal disease in Denmark (Figure 8.16), as it is clear that IPD caused by the seven serotypes included in the vaccine (PCV7 vaccine from 2007) and later 13 serotypes (PCV13 vaccine from 2010) has decreased markedly. The current predominant serotype is serotype 8 (26% in 2019), of which all invasive isolates isolated in 2019 and 2018 were fully sensitive to both penicillin and erythromycin.

Comparing the obtained results of antimicrobial susceptibility of IPD in Denmark in 2019 to the data reported in 2018 from our neighbouring countries, the levels of penicillin non-wild-type reported by EARS-Net were: Sweden (5.2%), Norway (5.0%) and Germany (5.3%). The levels of erythromycin resistance were: Sweden (4.5%), Norway (7.6%) and Germany (7.2%). Thus, the results of non-wild-type (I+R) for invasive pneumococci from Denmark in 2019 were similar to the reported values from 2018 from neighbouring countries with respect to penicillin, but markedly lower with respect to erythromycin resistance.

Conclusion

For penicillin, the level of non-wild-type in 2019 was lower than in 2018 (4.9% compared to 5.5%). For erythromycin, the level of resistance in 2019 was slightly higher than the level in 2018 (3.4% compared to 2.5%), but was nevertheless lower than for any of the years from 1999 to 2017, Figure 8.15.

There has been a trend of decreasing resistance to erythromycin since 2013, while the non-wild-type levels to penicillin are more variable. More information on the surveillance of invasive pneumococcal disease in Denmark can be found on the SSI homepage (EPI-NEWS, No 10-2020, https://en.ssi.dk/news/epi-news/2020/no-10---2020).

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8.3.6 Beta-haemolytic streptococci

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

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

Streptococcus agalactiae (group B streptococci; GBS) may be present in the vaginal flora of 20-25% of women in the childbearing age. If these bacteria are transferred to the child during labour, meningitis and septicaemia may develop in the newborn. Such infections also occur in elderly or immunocompromised patients.

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

This report presents data on antimicrobial resistance in non-duplicate invasive isolates (i.e. from normally sterile sites) of BHS submitted in 2019 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.

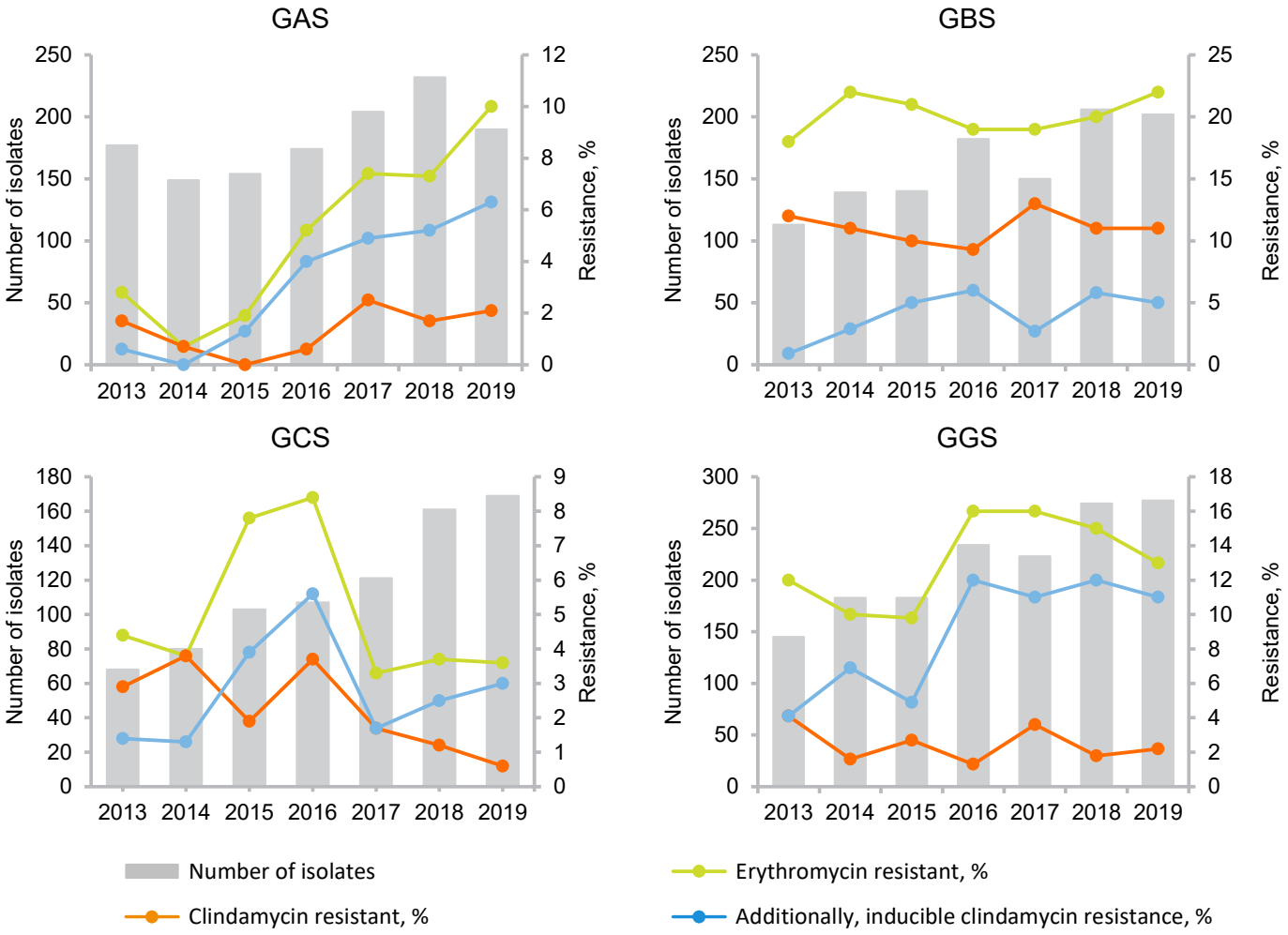
Infections with BHS are usually treated with penicillins or macrolides. All submitted isolates of BHS group A, B, C and G were therefore tested for susceptibility to penicillin, erythromycin, and clindamycin as well as inducible clindamycin resistance. Susceptibility testing was performed with disk diffusion methods. If resistance was observed, MIC was determined with Etest. All results were interpreted according to MIC breakpoints issued by EUCAST. For all isolates of GAS the *emm* type was determined by whole genome sequencing of the portion of the *emm* gene that dictates the M protein serotype.

Figure 8.17 shows the resistance findings for the years 2013 through 2019. In 2019, the number of submitted isolates from unique cases was 838, a slight decrease of 4% compared to 2018 (873). Corresponding changes for individual serogroups were: GAS, -18%; GBS, -2%; GCS, +5%; and GGS, +0.3%.

All isolates were susceptible to penicillin. The erythromycin resistance rate as well as the clindamycin resistance rate remained virtually unchanged compared to 2018 for all four serogroups. The percentage of isolates with inducible clindamycin resistance was: GAS, 6.3%; GBS, 5.0%, GCS 3.0%, and GGS, 11%. These percentages represented a slight increase for GAS and GCS, and a slight decrease for GBS and GGS. The percentage of isolates susceptible to all three antimicrobials was unchanged for all four serogroups.

The GAS isolates belonged to 32 different *emm* types. The majority of the received isolates (124; 65%) belonged to six *emm* types, each of which were represented by at least nine isolates (Table 8.15). The remaining 66 isolates (35%) belonged to 26 different *emm* types.

Figure 8.17 Beta-haemolytic streptococci: Antimicrobial resistance testing results. Numbers of isolates and resistance in percent
DANMAP 2019



GAS = group A streptococci, GBS = group B streptococci, GCS = group C streptococci, GGS = group G streptococci

Table 8.15 Group A streptococci 2019: *emm* types, clindamycin resistance and erythromycin resistance. Number of isolates
DANMAP 2019

	CLI-R		CLI-S		
emm type	ERY-R	ERY-S	ERY-R	ERY-S	Total
1.0	0	0	0	33	33
28.0	1	0	0	28	29
89.0	2	0	0	25	27
12.0	0	0	0	16	16
4.0	0	0	0	10	10
75.0	1	0	0	8	9
Subtotal	4	0	0	120	124
Other	12	0	3	51	66
Total	16	0	3	171	190

Abbreviations: CLI = clindamycin, ERY = erythromycin, R = resistant, S = sensitive, *emm* = the M protein gene
Numbers of isolates are shown for individual *emm* types if ≥9. Otherwise, if <9, the numbers are summarized in the "Other" category

Conclusions

The number of submitted isolates of group A was considerably lower in 2019 than in 2018, but virtually unchanged for the three other serogroups. All isolates were susceptible to penicillin. The erythromycin resistance rate as well as the clindamycin resistance rate remained virtually unchanged compared to 2018 for all four serogroups.

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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 cause infections, with otitis media and bacterial sinusitis being the most common clinical manifestations. Invasive infections with *H. influenzae* happen relatively rarely and occur predominantly in the very young or elderly patients but may also afflict individuals with underlying conditions, such as chronic obstructive pulmonary disease or cancer. *H. influenzae* is classified into six capsular serotypes (a, b, c, d, e and f, also known as Hia, Hib, Hic, Hid, Hie and Hif), as well as noncapsular (non-typeable, NTHi). Introduction of the polysaccharide type b vaccine in 1993 significantly reduced the number of cases with systemic infection caused by *H. influenzae* type b (Hib) isolates. Before the vaccine was introduced, there were around 80 cases of Hib meningitis annually among infants in Denmark. This has been reduced to 0-2 cases per year. NTHi for which no vaccine yet exists is now the predominant type found among invasive *H. influenzae* infections.

Invasive *Haemophilus influenzae*

Surveillance of invasive Hib is mandatory by submission of the isolate to Statens Serum Institut (SSI). By tradition, most departments of clinical microbiology are voluntarily submitting all isolates of invasive *H. influenzae*. The received isolates are then serotyped and biotyped by the reference laboratory at SSI. Thus, isolates are submitted for the majority of cases, and the remaining cases can be identified through the Danish Microbiological Database (MiBa). Thereby, all invasive infections with *H. influenzae* are registered in the surveillance database, and for the majority of cases serotypes are available. In 2019, all isolates were tested at SSI for antimicrobial susceptibility with disc diffusion assays and the betalactamase test. Whole genome sequencing was also performed on the received isolates, and the data were analysed for the presence of the plasmid-borne beta-lactamase genes TEM-1 and ROB-1. For cases where isolates were not received, antimicrobial susceptibilities were retrieved through MiBa, when available.

The present report includes all episodes of invasive *H. influenzae* as identified through MiBa, where the date of sampling was in 2019. A total of 114 cases were identified, of which isolates from 100 (88%) were received at the reference laboratory. *H. influenzae* were isolated from cerebrospinal fluid in nine of the 114 cases, from blood in 102, from pleural fluid in two and from joint fluid in one of the cases. The serotypes of the received isolates were: six Hib (6%), one Hie (1%), fifteen Hif (15%) and 78 NTHi (78%). The age-distribution of the cases is presented in Figure 8.18.

Figure 8.18 Different serotypes in invasive *H. influenzae* cases according to age, 2019, Denmark DANMAP 2019

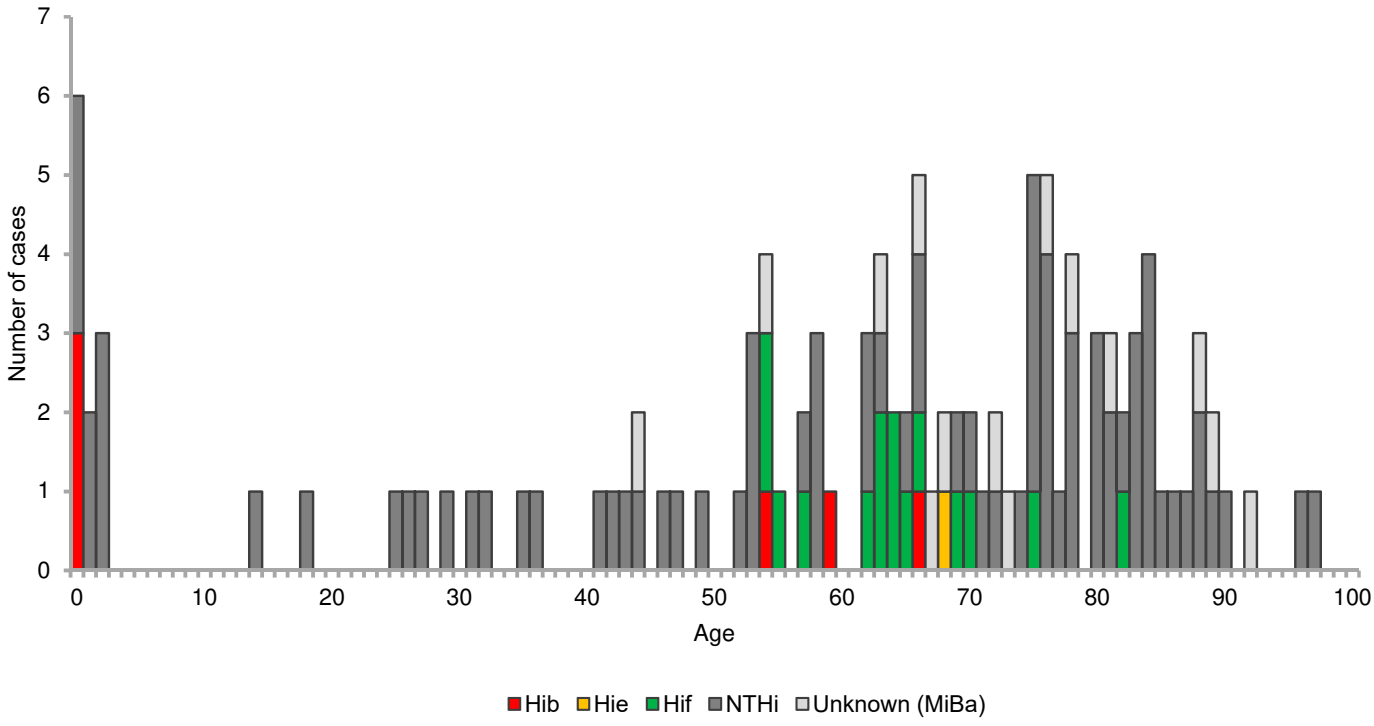

Table 8.16 Distribution of antimicrobial susceptibilities in invasive *H. influenzae* according to serotypes, 2019, Denmark

DANMAP 2019

	Hia	Hib	Hie	Hif	NTHi	Unknown*	All (2019)	All(2018)
Penicillin: no result registered	-	-	-	-	-	1	1	1
Penicillin: I and S	-	5 (83%)	1 (100%)	15 (100%)	57 (73%)	6 (46%)	84 (74%)	89 (74%)
Penicillin: R	-	1 (17%)	-	-	21 (27%)	7 (54%)	29 (26%)	31 (26%)
Ampicillin: no result registered	-	-	-	1	1	3	5	4
Ampicillin: I and S	-	5 (83%)	1 (100%)	14 (100%)	57 (74%)	8 (73%)	85 (78%)	94 (80%)
Ampicillin: R	-	1 (17%)	-		20 (26%)	3 (27%)	24 (22%)	23 (20%)
Cefuroxime: no result registered	-	-	-	-	-	1	1	18
Cefuroxime: I and S	-	6 (100%)	1 (100%)	15 (100%)	69 (88%)	8 (62%)	99 (88%)	87 (84%)
Cefuroxime: R	-	-	-	-	9 (12%)	5 (38%)	14 (12%)	16 (16%)
Amoxi-clav: no result registered	-	-	-	-	-	3	3	29
Amoxi-clav: I and S	-	6 (100%)	1 (100%)	15 (100%)	70 (90%)	8 (73%)	100 (90%)	83 (90%)
Amoxi-clav: R	-	-	-	-	8 (10%)	3 (27%)	11 (10%)	9 (10%)

*The group "unknown" represent the 14 cases that were registered in MiBa, but where an isolate was not received at the reference laboratory at SSI, and therefore not serotyped
Amoxi-clav = amoxicillin-clavulanic acid

Nineteen of the received isolates harboured the TEM-1 gene (one Hib and 18 NTHi) which represented 20% of 93 analysed isolates. All TEM-1 gene positive isolates had corresponding phenotypical betalactamase activity. None of the isolates harboured the ROB-1 gene. Susceptibility results, divided in to serotypes, for penicillin, ampicillin, amoxicillin-clavulanic acid and cefuroxime, are presented in Table 8.16. Due to differences across the departments of clinical microbiology in the reporting of non-resistant isolates, the "S" and "I" interpretations are combined in the table.

The results from antimicrobial susceptibility testings showed that in total there was 26% resistance to penicillin, 22% to ampicillin, 12% to cefuroxime and 10% to amoxicillin-clavulanic acid. Some variation across serotypes was observed. These figures are very similar to what was observed in 2018 (where data were exclusively extracted from MiBa).

In summary, the majority of isolates from invasive infections with *H. influenzae* are of the non-capsular type. This is similar to previous years and also similar to what is observed generally in Europe [R. Whittaker, Emerg Infect Dis, 2017, Mar;23(3):396-404]. The majority of the invasive *H. influenzae* isolates in Denmark in 2019 were found to be non-resistant to the antimicrobials tested, with the non-capsular isolates showing the highest degree of resistance.

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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 reported cases and bacterial isolates submitted to SSI has been almost complete (94-97%).

Laboratory and clinical notification of all cases of methicillin-resistant *S. aureus* (MRSA) has existed since November 2006. At SSI, all referred isolates are initially tested using a multiplex PCR detecting: the *spa*, *mecA*, *hsd*, *scn* and *lukF-PV* (PVL) genes. *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 necrotising 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 2019, altogether 2,233 *S. aureus* bacteraemia cases corresponding to 38.5 cases per 100,000 inhabitants were reported from the departments of clinical microbiology (DCMs) in Denmark. This is almost the same number as in 2018 (2,276). Forty-six (2.1%) of the bacteraemia cases were caused by MRSA. During the last decade the proportion has been between 1.3% (2010 and 2012) and 2.9% (2014) and remains below most other European countries participating in EARS-Net [EARS-Net 2018]. LA-MRSA CC398 caused eight of the 46 MRSA bacteraemia cases. The number of LA-MRSA CC398 bacteraemia cases in 2018 was eight as well. Within 30 days from the bacteraemia onset, 518 (23%) patients died (all cause mortality). The mortality for the MRSA bacteraemia cases was 17% (n = 8, of which 1 was due to CC398 MRSA).

A total of 549 representative isolates were tested for antimicrobial resistance. Results from antimicrobial susceptibility testing in *S. aureus* bacteraemia isolates from 2010–2019 are presented in Table 8.17. Resistance to penicillin in 2019 was 72%. At the beginning of the 1990s resistance to penicillin was around 86% and in 2010 75%, thereby a continuing decreasing trend was observed. The highest frequency of resistance to other antimicrobials than penicillin was observed for fusidic acid (14%), erythromycin (9%), clindamycin (8%) and norfloxacin (5%). For most antimicrobial agents, the susceptibility remained at the same level as the previous years.

Table 8.17 Resistance (%) in isolates from *Staphylococcus aureus bacteraemia* cases 2010-2019, Denmark DANMAP 2019

Antimicrobial agent	2010 %	2011 %	2012 %	2013 %	2014 %	2015 %	2016 %	2017 %	2018 %	2019 %
Methicillin	1.4	1.4	1.2	1.7	2.9	1.5	2.1	2.2	1.6	2.1
Penicillin	75	77	74	76	77	71	71	72	72	72
Erythromycin	5	7	6	7	8	7	7	6	5	9
Clindamycin	4	6	6	6	8	7	6	5	4	8
Tetracycline	3	2	2	3	5	4	3	3	3	2
Fusidic acid	13	13	14	15	15	16	12	14	17	14
Rifampicin	<1	<1	<1	0	<1	<1	<1	<1	<1	<1
Norfloxacin	3	4	4	5	6	6	4	4	4	5
Kanamycin	1	<1	1	2	2	3	1	1	2	<1
Linezolid	0	0	0	0	0	0	0	0	0	0
Mupirocin	<1	<1	<1	<1	<1	<1	0	<1	0	<1
Trimethoprim-sulfamethoxazole	nt	<1	1	1	1	<1	<1	<1	0	<1
Numbers tested	1416	1515	1523	962	381	502	560	551	504	549

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

Table 8.18 The ten most prevalent *spa* types demonstrated in SAB and in non-LA-CC398 MRSA cases, Denmark 2019 DANMAP 2019

SAB			MRSA			
spa type	CC group	No. of cases	*spa* type	CC group	No. of cases	No. causing infections (%)
t127	CC1	116	t304	CC8	230	99 (43)
t091	CC7	95	t223	CC22	181	80 (44)
t084	CC15	91	t008	CC8	153	95 (62)
t230	CC45	73	t127	CC1	151	74 (49)
t002	CC5	72	t002	CC5	134	58 (43)
t012	CC30	65	t4549	CC8	109	89 (82)
t021	CC30	65	t044	CC80	69	31 (45)
t008	CC8	59	t005	CC22	54	32 (59)
t701	CC8	56	t019	CC30	48	34 (71)
t015	CC45	36	t021	CC30	43	23 (53)

CC = Clonal complex, SAB = *S. aureus* bacteraemia, spa = *S. aureus*-specific staphylococcal protein A, MRSA = Methicillin-resistant *Staphylococcus aureus*

Typing revealed 632 different *spa* types distributed in 27 different clonal complexes (CCs). The ten most prevalent *spa* types, representing 33% 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 41 (1.8%) cases of which six were MRSA. The 41 PVL presenting isolates were distributed among 22 different *spa* types and 11 different CCs.

Surveillance of methicillin-resistant *S. aureus*

In 2019, 3,657 MRSA cases were detected (63.0 per 100,000 inhabitants). This was almost the same number as in 2018 (3,669; Figure 8.19). For the last four years, the numbers of new MRSA cases seem to be levelling off. A case was defined when a person for the first time tested positive for a specific MRSA strain regardless of clinical situation (infection or colonisation).

CC398 cases constituted 32% (n = 1,163) of new MRSA cases, of which 1,122 belonged to the LA-MRSA CC398 and the remaining 41 to a human adapted variant harbouring the PVL encoding genes. The number of LA-MRSA CC398 is lower than the previous five years. The decrease 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 35 cases (1.0%). Twenty-seven of the cases (77%) had infections at the time of diagnosis. Two patients reported contact to horses, which previously have been shown to be reservoirs for *mecC* MRSA. The remaining 33 patients reported no known contact to any livestock.

In the course of 2019, 23 MRSA outbreaks were registered at hospitals, nursing homes and other institutions. These outbreaks comprised a total of 157 cases. Seven of the outbreaks occurred in neonatal departments, comprising a total of 85 cases. Additionally, eight outbreaks were observed in nursing homes (counting a total of 38 residents) and in two residential schools (11 pupils).

The total number of cases and the number of cases presenting with infection according to epidemiological classification are shown in Table 8.19. Most of the cases (82%) were acquired in Denmark. At the time of diagnosis, 44% (n = 1,599) of cases had infection, which was similar to 2018 (40%).

Figure 8.19 Number of new MRSA cases with a three years moving average, Denmark DANMAP 2019

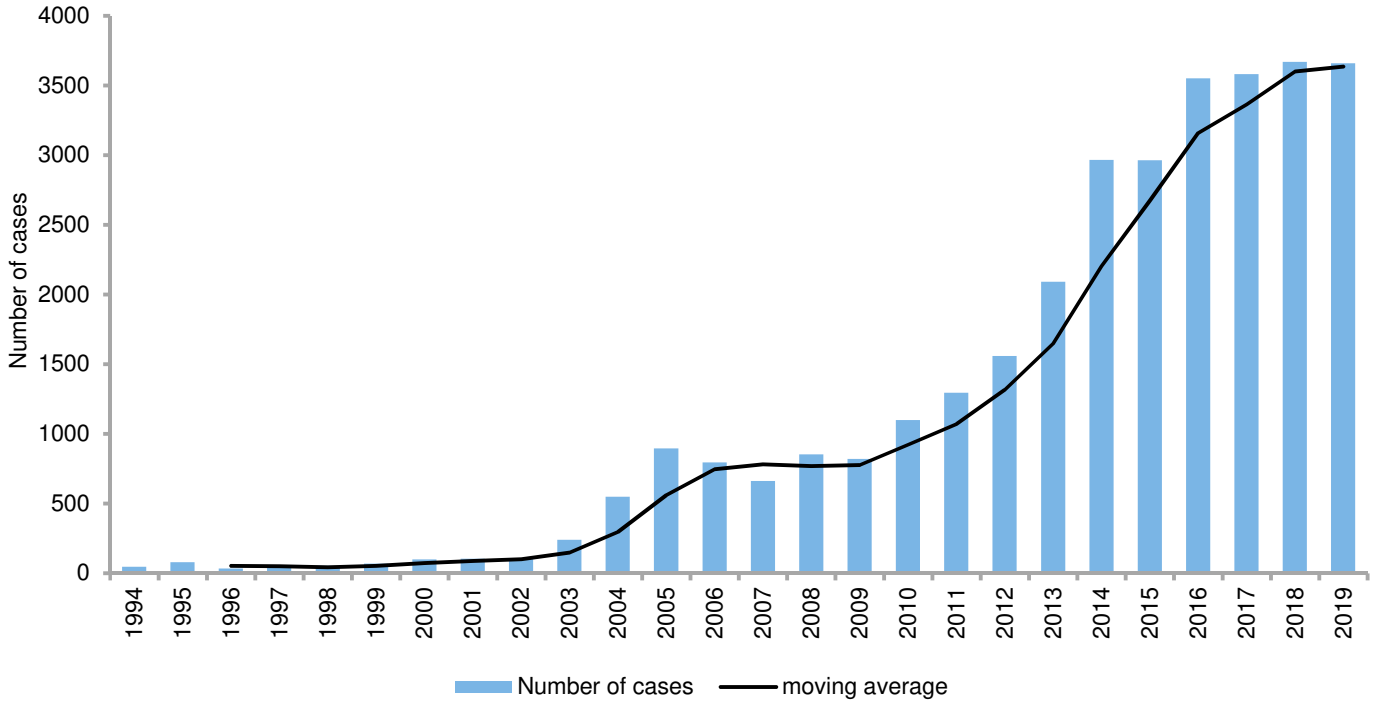
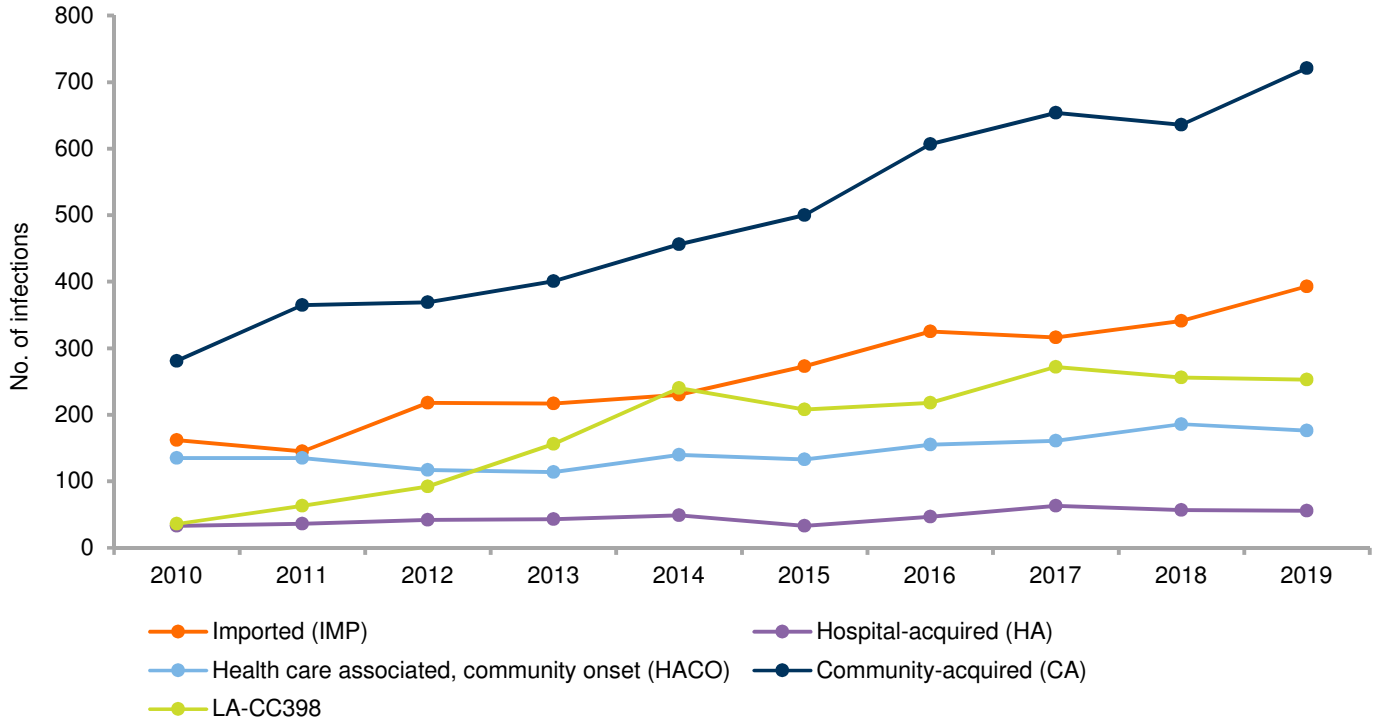


Table 8.19 Epidemiological classification of new MRSA cases, Denmark 2019 DANMAP 2019

Epidemiologic classification	Exposure	No. of cases (% of total)	No. (%) of cases with infections
Imported (IMP)		**651 (18)**	393 (60)
Hospital-acquired (HA)		**75 (2)**	36 (48)
Healthcare associated, community onset (HACO)		**229 (6)**	
	with known exposure	26	12 (46)
	without known exposure	203	162 (80)
Healthcare worker		**44 (1)**	22 (50)
Community-acquired (CA)		**1536 (42)**	
	with known exposure	844	143 (17)
	without known exposure	692	578 (84)
LA-MRSA CC398		**1122 (31)**	
	with known exposure	993	167 (17)
	without known exposure	129	86 (67)

Numbers shown in bold are totals

Figure 8.20 Number of MRSA infections according to epidemiological classification, Denmark DANMAP 2019



The trend of MRSA infections for 2010-2019 based on their epidemiological classification is shown in Figure 8.20. Community-acquired infections increased to 721 cases (corresponding to that 47% of the total number of CA MRSA cases presented with infections in 2019, Table 8.19). Imported cases presented with infections in 60% of the cases and the number of infections in this category has been increasing from less than 100 cases in 2007 to 393 cases in 2019. The number of HA, HACO and LA cases with infection were at a similar level as 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 some HA-MRSA cases may not be recognized before patients are discharged.

Molecular typing of the MRSA strains

In total, *spa* typing revealed 365 different strain types, not including isolates belonging to LA-CC398. Among the infections, 285 *spa* types were demonstrated. The 10 dominating non-LA-CC398 *spa* types isolated in 2019 are listed in Table 8.18. They constituted 47% 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. Table 8.17 does not list *spa* types in CC398, which for many years has been the dominating CC.

The PVL encoding gene was detected in 29% of the infections and in 13% of the asymptomatic carriers and most often in

Table 8.20 Resistance (%) in non LA-CC398 MRSA isolates, 2010-2019, Denmark DANMAP 2019

	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019
Erythromycin	44	37	38	32	33	37	34	34	33	33
Clindamycin	34	27	29	24	23	29	25	27	28	23
Tetracycline	20	17	18	20	21	24	26	24	26	22
Fusidic acid	16	15	17	17	17	19	18	16	18	23
Rifampicin	3	2	1	1	<1	<1	1	1	1	<1
Norfloxacin	32	27	25	23	27	21	19	20	21	21
Kanamycin	33	31	26	29	30	32	28	26	28	31
Linezolid	0	0	0	<1	<1	0	<1	0	<1	0
Mupirocin	4	<1	<1	<1	<1	<1	<1	<1	<1	<1
Trimethoprim-sulfamethoxazole	ND	1	2	3	3	4	2	3	3	4
Number of tested isolates	984	1125	1324	1451	1616	1242	1184	1193	1233	1025

relation to isolates with *spa* types t008 (n = 109), t044 (n = 65), t019 (n = 45), t005 (n = 38) and t021 (n = 37).

Resistance among MRSA isolates

Resistance data for non-LA-CC398 isolates are presented in Table 8.20. Forty percent of non-LA-CC398 isolates received in 2019 was tested (n = 1,025). Resistance prevalences were similar to previous years.

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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 also sometimes be demonstrated in specimens from the pharynx or rectum in either gender. In females, the finding of gonococci in rectal specimens is usually due to contamination with vaginal secretion, while in men who have sex with men (MSM) it may be due to unprotected anal sex leading to infection. In both genders, the condition is generally asymptomatic. Pharyngeal gonorrhoea is virtually always asymptomatic.

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

Surveillance

Methods: 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 were determined using the Etest® on chocolate agar incubated at 35°C in 5% CO_2. The breakpoints used were those defined by EUCAST. The breakpoints for azithromycin were changed as of January 1, 2019 (EUCAST version 9.0) where only an epidemiological cut-off value (ECOFF), MIC >1 mg/L was defined. This is because azithromycin should only be used in conjunction with another efficient agent. Until January 1, 2019, S was defined by MIC ≤ 0.25 mg/L and R by MIC >0.5 mg/L. These breakpoints were used in this report in order to enable comparison of azithromycin data for 2019 with those from previous years.

Both resistant and intermediate susceptible isolates have through the years been categorised as resistant in the DANMAP reports. However, isolates with intermediate susceptibility/susceptible increased exposure are non-existing according to EUCAST breakpoints for ceftriaxone and azithromycin and they are exceedingly rare regarding ciprofloxacin. Penicillinase production was tested for using the Nitrocephin assay.

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

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

The NSR Laboratory received isolates from 1,119 unique cases of gonorrhoea diagnosed in 2019. The annual number increased considerably from 2011 through 2016 (Figure 8.21). This was partly because of the widespread use of combined nucleic acid amplifications tests (NAATs) for *Chlamydia trachomatis* and *N. gonorrhoeae* which have identified unexpected cases of gonorrhoea (sometimes followed by culture), and partly due to an increasing incidence of gonorrhoea, especially among young heterosexual persons, among whom an increas-

ing proportion are women, at least until 2016. A slight decrease in the annual number of isolates from unique cases was observed in 2017 and a more pronounced decrease in 2018 continuing in 2019. It should be recalled, however, that many cases diagnosed by NAATs are not followed-up by culture.

Figure 8.21 Number of submitted gonocccus isolates from males and females, and the percentage of isolates with ciprofloxacin resistance, azithromycin resistance, and penicillinase production DANMAP 2019

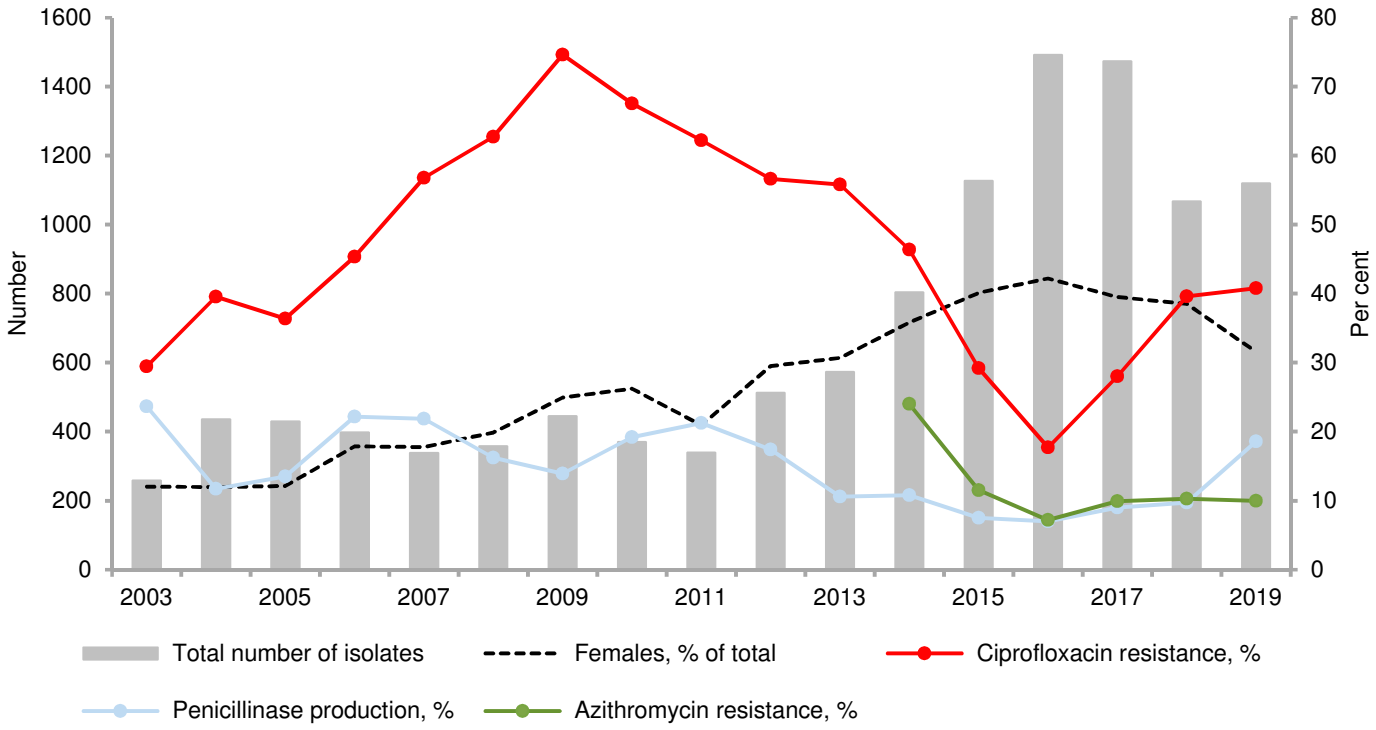
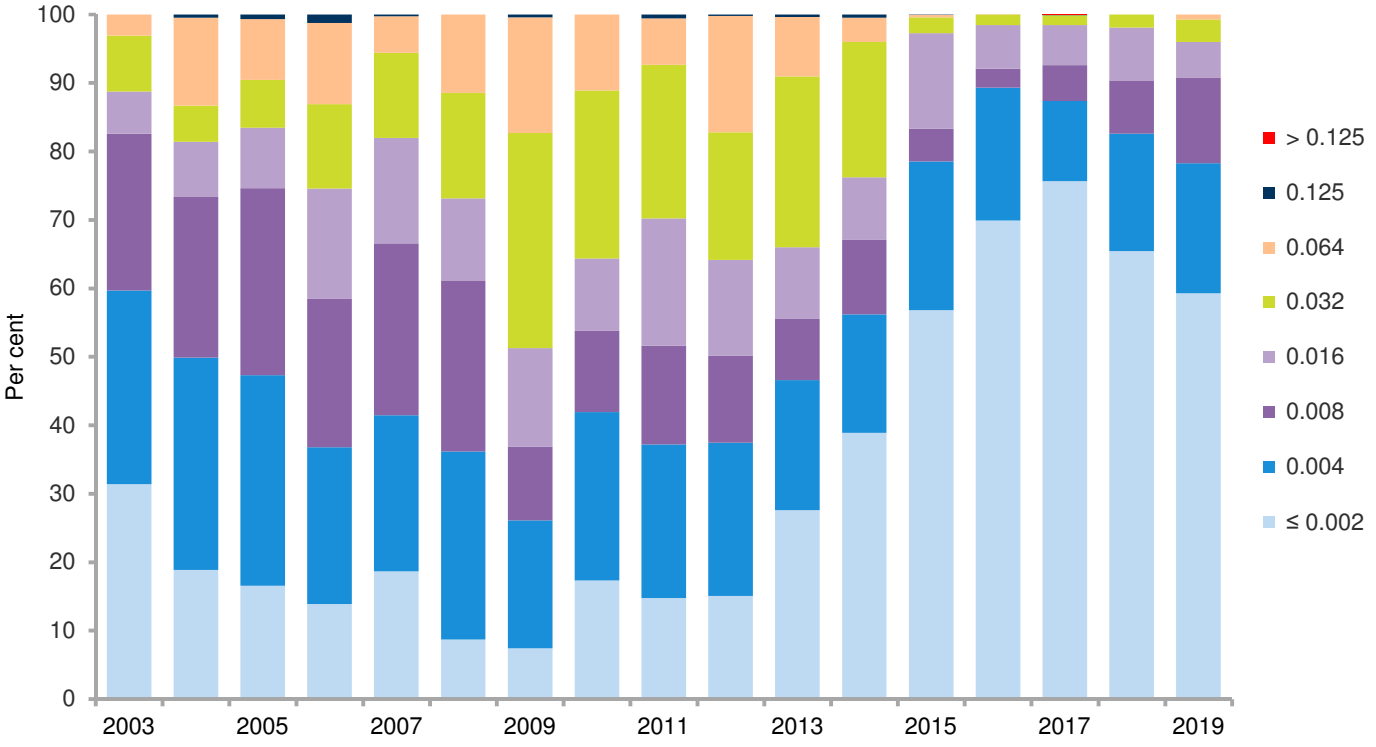


Figure 8.22 Distribution of ceftriaxone MIC (mg/L) values in gonococci DANMAP 2019



The ciprofloxacin resistance rate was 41% in 2019 (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 19%, i.e. an increase since 2018 (10%). It has fluctuated between 22% in 2005 and 7% in 2016. Azithromycin non-wild-type (MIC above the present ECOFF >1 mg/L) was found in 4.6% of the tested isolates. Using the old resistance breakpoint on 2019 data (MIC >0.5 mg/L) 10% of the isolates was found resistant, the same as in 2018 (Figure 8.21).

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, although slightly increasing to 22% in 2019.

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. The use of this combination therapeutic regimen has been gradually abandoned during 2019.

In a subset of 119 isolates, resistance against cefixime (MIC >0.125 mg/L) was 0% in 2019, like in 2018. Cefixime is an oral cephalosporin that has never been used in Denmark. Resistance against spectinomycin was 0% in 2019 as well as in the years 2015 through 2018. MIC values for gentamicin were 1 to 4 mg/L, but no breakpoints are defined for this agent against gonococci.

Conclusions

The ciprofloxacin and azithromycin resistance rates and the ceftriaxone MIC distribution were virtually unchanged in 2019 compared to 2018. Although resistance problems among gonococci are still not present in Denmark, the frequent emergence globally of gonococcal resistance to antibiotics underlines the importance of maintaining the centralised national surveillance of antimicrobial resistance in gonococci.

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Textbox 8.2

Incidence of multiresistant bacteria in Greenland

Background: Greenland has a population of 55,992 inhabitants (January 2019) and Nuuk is the capital with around 18,000 inhabitants. Greenland is an autonomous administrative country of Denmark; it has its own Ministry of Health and the country is divided into five health regions. Although sparsely populated, due to its big geographic dispersion, there are five smaller hospitals, one national hospital and 11 health care centres in the five health regions. The national and largest hospital, Dronning Ingrids Hospital (182 beds), is situated in Nuuk. Around 15-16,000 persons are admitted to hospital at least once a year. Patients with specific or serious diseases that cannot be treated at Dronning Ingrids Hospital are transferred to Den-mark or Iceland for further treatment e.g. haemodialysis, cancer treatment, brain surgery etc.

Resistant bacteria: From 2000 to 2019, 54 patients were diagnosed with methicillin-resistant *Staphylococcus aureus* (MRSA), 116 patients with extended spectrum beta-lactamase (ESBL)-producing Enterobacterales, four patients with vancomycin-resistant enterococci (VRE), and 177 patients with *Clostridioides difficile* (formerly known as *Clostridium difficile*) infection among whom 55 had the 027 type.

MRSA: Since 2015, a nearly 4-fold increase in incidence of MRSA has been observed. The largest increase was seen during 2017, and the main reason for this was an outbreak involving 12 persons in Tasiilaq at the East coast of Greenland (described in details in DANMAP 2017). In 2018, only four new persons were reported with MRSA including one premature child whom carried MRSA in nose and throat. The child was colonised with MRSA while hospitalised in Denmark due to an MRSA-outbreak (MRSA t223, CC22) at the neonatal ward. The mother of the child was MRSA-negative at time of the detection in 2018, but in February 2019 she was also tested positive from the nose with the same MRSA-type as her child. In 2019, there were also four new persons with MRSA: three adults (including the above mentioned mother) and one child. The adults were all MRSA-carriers – two of them in the nose and one in the throat. Two adults were husband and wife and the wife was colonised with MRSA while hospitalised in Denmark; one month later the husband was positive with the same MRSA-type (MRSA t902, CC22). The child (3½ years old) had a wound in the nose without any history of hospitalisation or traveling. In all four cases there was no further transmission in Greenland among family/household or at the hospital.

VRE: In spite of ongoing VRE outbreaks in Denmark, only four patients have been diagnosed with VRE in Greenland. Two patients were colonised with VRE in the rectum, one patient had pleurisy and one patient had VRE in the urine. In all four cases VRE occurred after hospitalisation in Denmark. No transmission was observed in the wards.

CPO: In recent years, an increase in incidence of carbapenemase-producing organisms (CPO) in Denmark has been observed but until now, no CPO has been reported in Greenland.

Other resistant bacteria: Most of the other resistant bacteria observed were imported from Denmark or abroad, but in some cases, especially in patients with ESBL-producing Enterobacterales, treatment with broad-spectrum antimicrobial agents in Greenland probably selected for these bacteria. From 2012 to 2013, there were outbreaks with *C. difficile* type 027 in several hospitals, and transmission within the country occurred. But due to a great effort in infection prevention and control from the hospital staff, these outbreaks were quickly stopped. Of the 12 new patients with *C. difficile* infection diagnosed in 2019, one patient was infected with the 027 type.

Conclusion: Continued focus on surveillance of multiresistant bacteria, compliance to screening procedures, the use of broad-spectrum antimicrobial agents, and on compliance to guidelines for infection prevention and control remain necessary in order to combat multiresistant bacteria in Greenland also in the future.

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Textbox 8.3

Azole resistance in *Aspergillus fumigatus* – Nationwide surveillance data from the first 18 months

Azole resistance in *Aspergillus fumigatus* complicates patient management with a negative impact on survival (1,2). Resistance is driven by either target gene mutations in *cyp51A*, other gene mutations or efflux (3). The dominating environmentally driven resistance mechanisms are TR_{34}/L98H and TR_{46}/Y121F/T289A (4). Both resistance mechanisms have been detected in *A. fumigatus* from patients and in the environment (5-8).

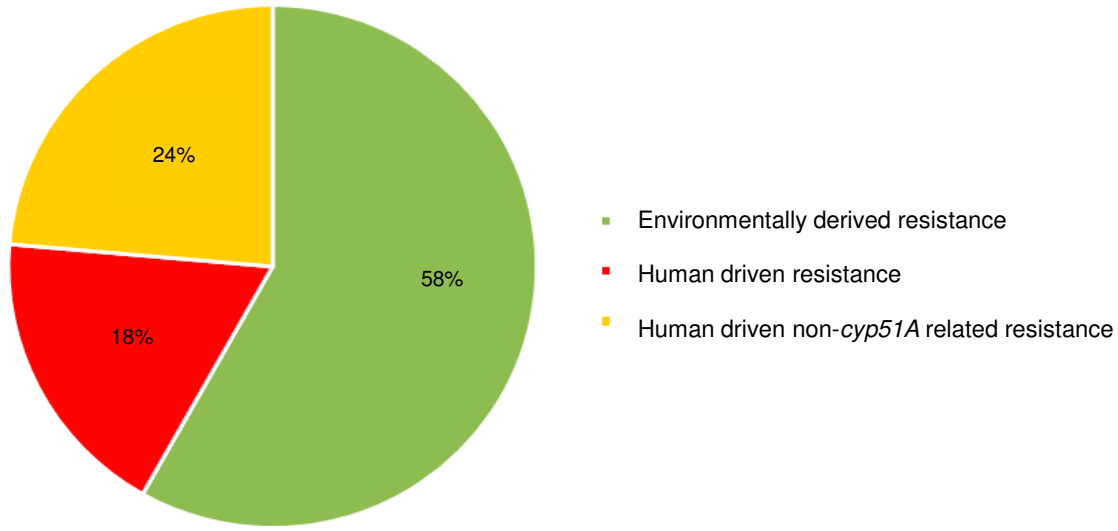
The nationwide surveillance was initiated in October 2018 with participation from all 10 Danish departments of clinical microbiology. The azole resistance rate is based on the following isolates: Clinically relevant *A. fumigatus* isolates and *A. fumigatus* isolates found on a Monday to represent background prevalence, the so-called Monday samples. The EUCAST E.Def 10.1 method using VIPcheck azole agar plates (Mediaproducts BV, Groningen NL) is used for screening and EUCAST E.Def 9.3.2 for susceptibility testing. Susceptibility classification was determined according to the revised EUCAST antifungal clinical breakpoints v 10.0, released in February 2020 (9). All non-susceptible isolates underwent *cyp51A* target gene sequencing. If more than one isolate was received from the same patient within 30 days, only the initial isolate was included in the data analysis unless differential susceptibility and/or resistance mechanism were documented. The azole resistance rate was determined at patient level.

From the first 18 months 1422 *A. fumigatus* isolates from 901 patients were included. Among those, 1419 isolates from 898 patients were susceptibility tested of which 78 isolates (5.5%) were specifically marked as Monday samples. At the patient level, 55 out of 898 patients had one or more resistant *A. fumigatus* isolates resulting in an azole resistance rate of 6.1% nationally. Thirty-two patients (3.6% of all patients and 58% of patients with resistance) had one or more isolates with an environmentally driven mechanism (Figure 1). These patients derived from various regions of Denmark and including both Zealand, Funen and Jutland and with no clear difference over time (Figure 2-3).

We report a nationwide azole resistance rate of 6.1% in *A. fumigatus*, with an overweight of resistance mechanisms associated with azole fungicide use in the environment. This may have an impact on human health.

Figure 1 Classification of the resistant *A. fumigatus* isolates at the patient level according to underlying type and origin of the resistance mechanism

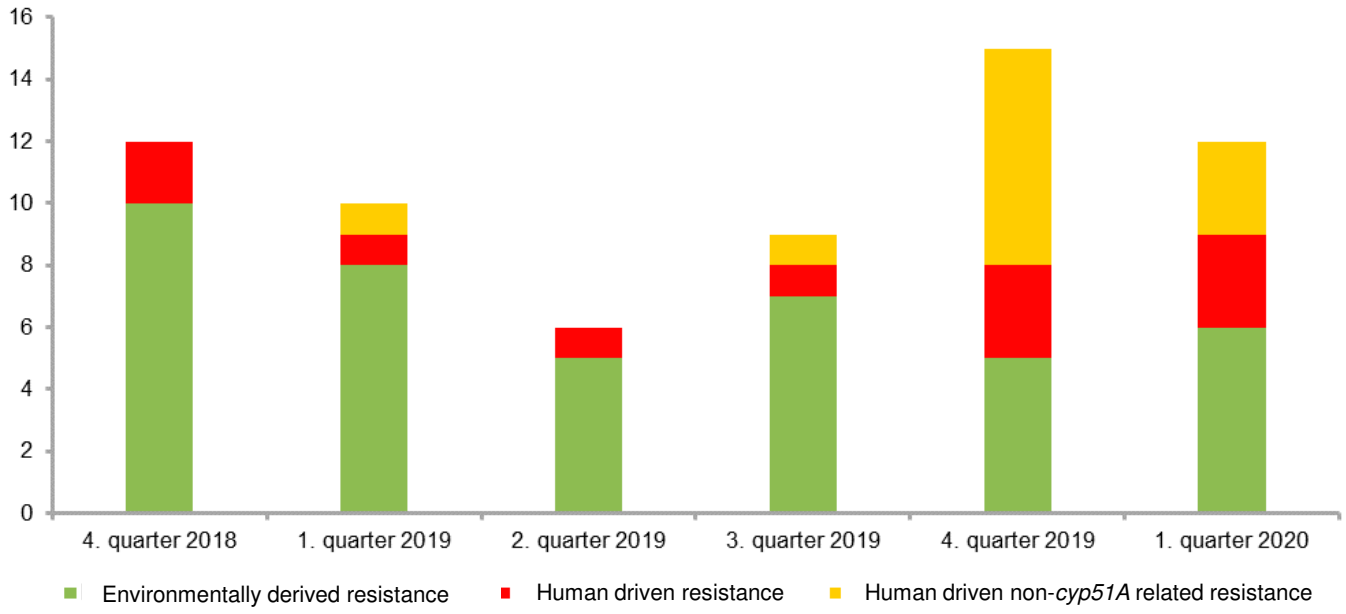
DANMAP 2019



The identified environmental resistance mechanisms consisted of TR_{34}/L98H, TR_{34}/L98H and TR_{34}/L98H/S297T/F495I. The human driven resistance mechanisms were G54R, G54W, M220K, M220R, M220I, P216S and G432S. The non-*cyp51A* related resistance consisted of isolates with *cyp51A* wildtype but elevated voriconazole MICs (≥2 mg/L). Three patients harboured resistant isolates with target gene mutations (TR_{34}/L98H, M220R and P216S, respectively) and also a resistant isolate with *cyp51A* wildtype

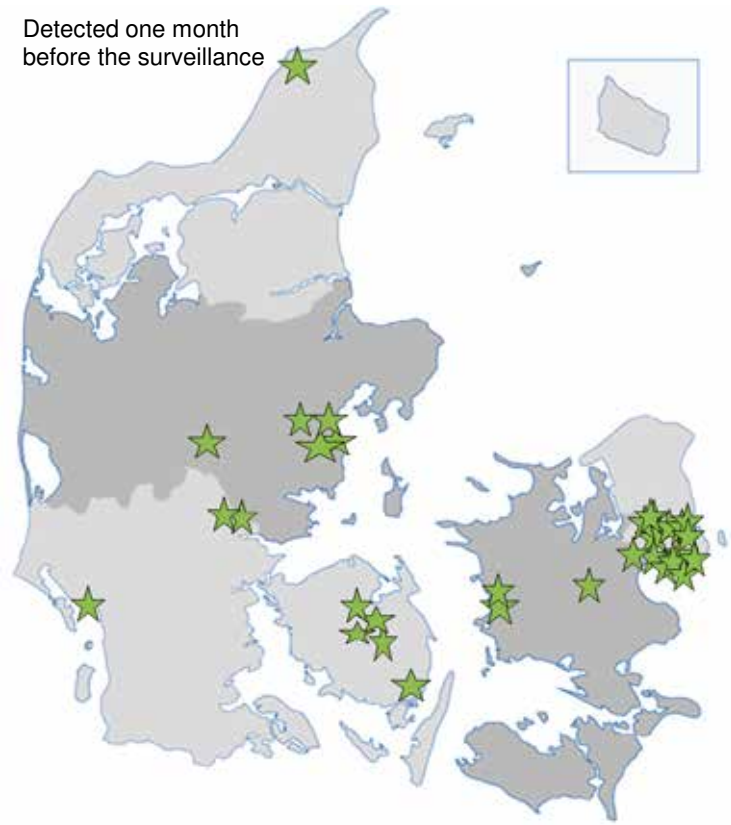
continued ... Textbox 8.3

Figure 2 Number of patients with resistant isolates shown in quarters DANMAP 2019



One patient might be repeated in another quarter. One patient with a human driven target gene mutation in 4. quarter also had a resistant isolate with *cyp51A* wildtype, which is also shown

Figure 3 Geographical areas where resistant *A. fumigatus* isolates from patients with an environmental resistance mechanism have been found DANMAP 2019



The stars mark the locations of hospitals or general practitioners from where the patient samples have been referred

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