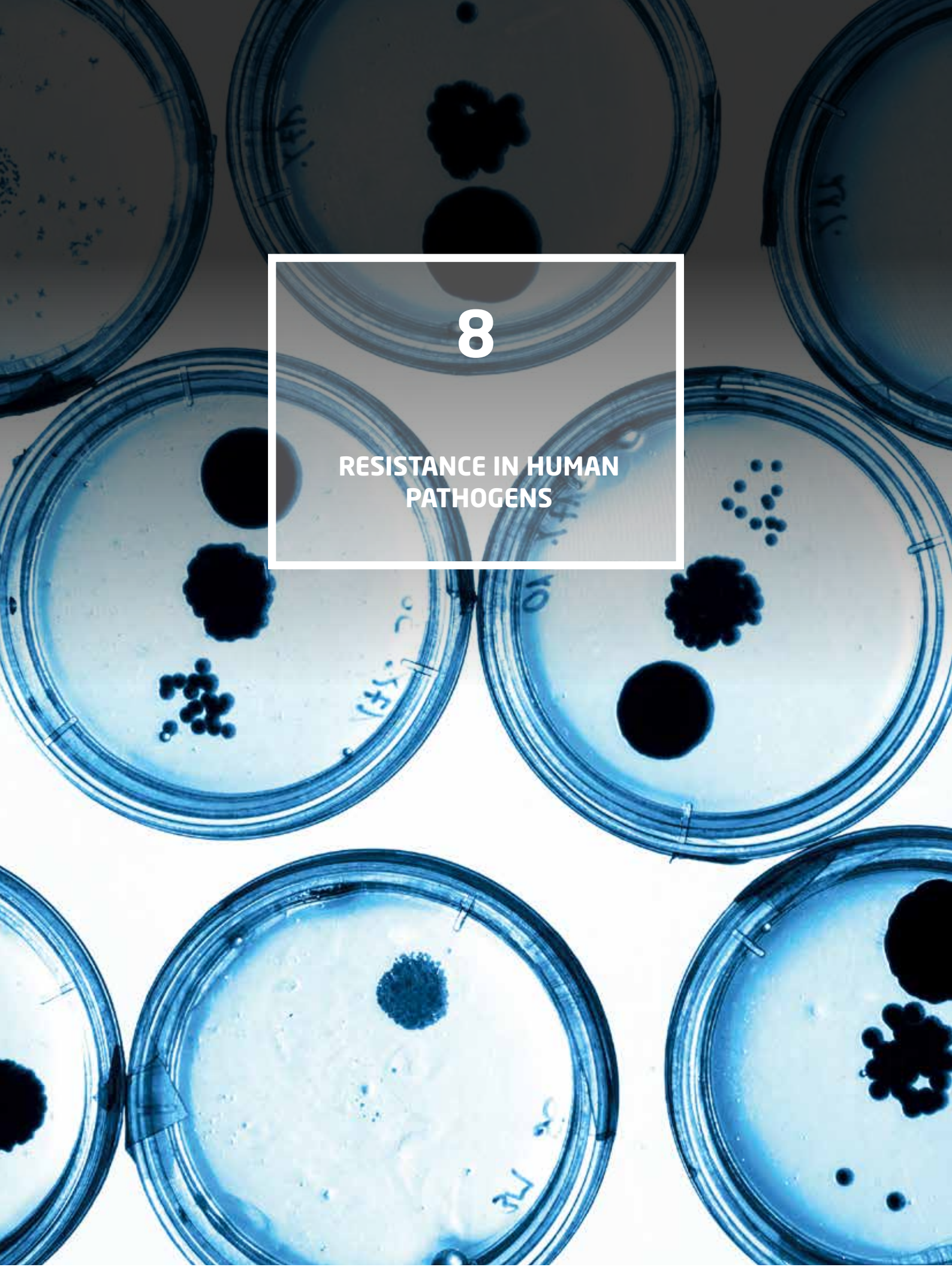

8

RESISTANCE IN HUMAN PATHOGENS

8. Resistance in human pathogens

Highlights

Invasive infections. After years of increasing invasive infections, the numbers stagnated with a total of 12,205 cases. However, invasive infections with *Klebsiella pneumoniae* (1,399 cases) and *Staphylococcus aureus* (2,559 cases) continued to rise. Invasive infections with *Streptococcus pneumoniae* (602 cases) saw a steep decline after the introduction of vaccinations for young children and during the COVID-19 pandemic, but have since risen to near pre-pandemic levels.

Escherichia coli. Resistance levels have generally been stable over the past decade. However, resistance to piperacillin/tazobactam in invasive infections climbed from 4.8% in 2014 to 6.3% in 2023 for Denmark as a whole. These increases were driven by rising trends in the Capital Region, Region Zealand and the Central Denmark Region, whereas the North Denmark Region had a decreasing trend. Combined resistance to 3rd generation cephalosporins, ciprofloxacin and gentamicin remained low at 1.2%. Combined resistance to ampicillin and gentamicin was at 4.1% in 2023, decreasing from 6.9% in 2014. Carbapenem-resistance remained below 1% with no noticeable trend.

Klebsiella pneumoniae. While resistance levels have been decreasing for the past decade, piperacillin/tazobactam-resistance in invasive infection in 2023 surpassed 10% for Denmark as a whole and in four out of five regions. Combined resistance to 3rd generation cephalosporins, ciprofloxacin and gentamicin increased from 1.1% in 2015 to 1.9% in 2023. Additionally, cefuroxime-resistance increased from 7.7% to 9.8% from 2022 to 2023. The rising resistance coupled with an increase in invasive infections with *K. pneumoniae* warrants increased efforts to curb the development.

Carbapenemase-producing organisms/Enterobacterales (CPO/CPE). CPE were increasingly spreading in Danish hospitals, with cases rising 43% from 2022 to 2023, reaching 436 individual cases. CPE, historically linked to travel, are now increasingly found domestically. CPE outbreaks increased in hospitals despite extensive screening and cleaning efforts. 138 new CPO patients were associated with outbreaks in Denmark in 2023. Although bloodstream infections remained rare, the rise in hospital-acquired CPE indicates the emergence of domestic reservoirs. For non-outbreak related cases, 86 patients reported travelling outside Nordic countries, however, the number of cases with no travel information reported was high (130 cases). Additionally, 36 cases were patients from Ukraine.

Staphylococcus aureus. The number of S. aureus bacteraemia cases was 2,571 in 2023 and at the same level as in 2022. Of these, 39 cases (1.5%) were caused by methicillin-resistant *S. aureus* (MRSA) with nine being livestock-associated-MRSA (LA-MRSA). Resistance to penicillin continued to decrease and was 68% in 2023. There were 3,649 cases of MRSA from both screening (45% of cases) and infections (55%), which was a 20% increase compared to 2022. Forty-one MRSA outbreaks were registered at hospitals, nursing homes and other institutions for a total of 199 cases with 80 being infections.

Neisseria gonorrhoeae. Over the decade the number of received isolates and of reported cases increased significantly. In 2023, the reference laboratory at SSI received 2,653 isolates from 2,152 unique cases. Ciprofloxacin resistance was at 45%. Azithromycin-resistance was found in 6% of tested isolates in 2023 compared to 2.9% of tested isolates in 2022.

8.1 Introduction

The Danish national surveillance programme of antimicrobial resistance in human clinical bacteria collects data on routine diagnostics from all of Denmark. It is an active catchment system collecting results from all clinical and screening samples from patients. Data coverage is high; microbiology data from all hospitals and the majority of general practitioners feed into the system, hereby covering a close to complete proportion of microbiological analyses performed in Denmark.

In addition, the programme includes phenotypic resistance and typing results from the reference laboratories at SSI. Isolates are submitted to SSI either as part of the programme on mandatory notifiable diseases or based on voluntary submission of species carrying resistance mechanisms of concern (Table 8.1).

Table 8.1 Summary of species/types, sampling and sources of isolates for national resistance surveillance in humans, 2023

DANMAP 2023

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

Regarding submissions of isolates to the reference laboratories often more isolates per patient are received, but for the statistics only one isolate per patient is counted

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

An important part of the national surveillance programme consists of systematically extracted data from a register (EpiMiBa) linking to the Danish Microbiology Database (MiBa). MiBa was established in 2010 with the aim to build a cross-national database that included and made available all microbiology analyses performed by the individual DCM. MiBa thus simultaneously delivers real time patient data to the DCM for clinical use and data to SSI for national surveillance. Until the establishment of MiBa, surveillance was based on voluntary manual reporting from the individual DCM, beginning with data from just two DCM in 1995, but quickly expanding to include more than half of the DCM. Since 2015, all DCM have contributed to the program resulting in a 100% coverage of hospitalised patients and – since the DCM perform microbiology analyses for GPs and private specialists as well – also a very high coverage of results from the primary sector.

A description of MiBa and the usage and validation of data from the associated database EpiMiBa can be found on the following homepage [https://mibaen.ssi.dk/] and in DANMAP 2018, Textbox 8.1.

The MiBa-based surveillance includes all invasive isolates of *Escherichia coli, Klebsiella pneumoniae, Enterococcus faecalis, Enterococcus faecium, Pseudomonas aeruginosa* and *Acinetobacter* species and all urine isolates of *E. coli* and *K. pneumoniae*. For inclusion, the following case definition applies: The first sample of each given bacterial species per individual patient per year, by date of sample collection, an approach identical to the surveillance performed by the European Antimicrobial Resistance Surveillance Network (EARS-Net). All duplicates, patient-related or sample-related within the year of observation, are excluded.

Materials and methods are further described in paragraph 10.10 in Chapter 10.

8.1.2 Surveillance based on data from the reference laboratories

Antimicrobial resistance is also monitored in isolates submitted to the reference laboratories at SSI.

Submission of isolates began in 1957 with the voluntary referral of *S. aureus* from bloodstream infections. Still today, many isolates are sent as part of voluntary programmes; however, the majority of isolates are referred as part of surveillance programmes for notifiable diseases.

The mandatory programme includes methicillin-resistant *S. aureus* (MRSA) and carbapenemase-producing organisms (CPO) from clinical and screening samples, *S. pneumoniae* and *Haemophilus influenzae* serotype b (Hib) from invasive infections and *Nesseria gonorrhoeae* from all clinical samples.

The latter three irrespective of antimicrobial resistance, but resistance mechanisms are investigated both locally and at SSI (Table 8.1).

The voluntary programme includes day-to-day referral of vancomycin-resistant enterococci and monthly or periodically reporting of ESBL-positive invasive *E. coli*, invasive beta-haemolytic streptococci and invasive *S. aureus*.

In addition, the reference laboratory for antibiotic resistance at SSI receives highly resistant strains or strains carrying atypical antimicrobial resistance mechanisms of specific concern irrespective of bacterial species.

Since 2015/2016, characterisation of antimicrobial resistance mechanisms in bacteria has been performed primarily by whole genome sequencing (WGS).

8.1.3 Number of invasive cases

The total numbers of invasive cases of the monitored bacterial species included in the surveillance programmes for both DANMAP and EARS-Net from 2014 to 2023 are presented in Figure 8.1. Invasive cases caused by *Acinetobacter* species are not shown in the figure due to the small numbers of isolates (ranging from 55 to 97 cases/year between 2014 and 2023).

Between 2014 and 2023, the number of registered individual invasive cases increased by 25% from 9,790 to 12,205 cases in Denmark: *E. coli* 4,496 to 5,835 cases (30% increase), *S. aureus* 1,874 to 2,559 cases (37%) and *K. pneumoniae* 943 to 1,399 cases (48%).

Figure 8.1 Number of invasive cases for bacterial species under surveillance, Denmark, 2014-2023 DANMAP 2023

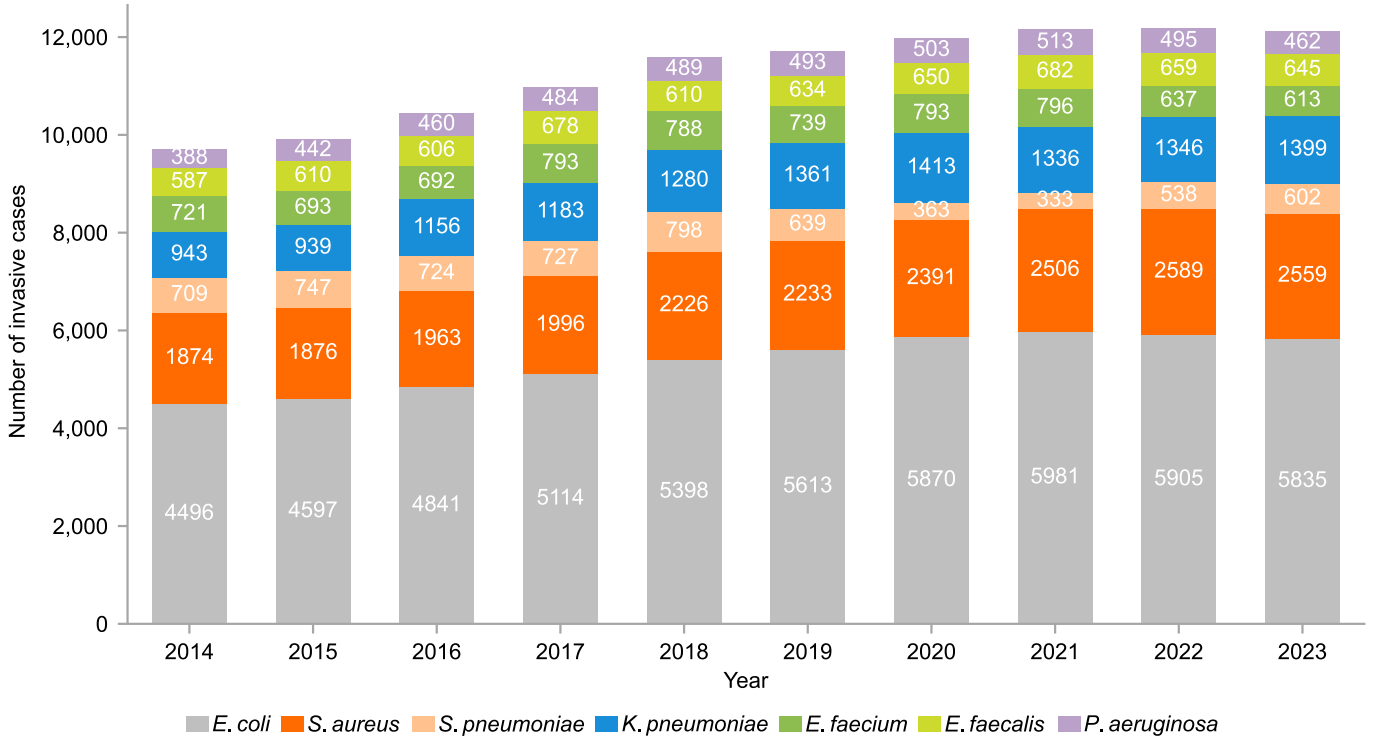

Figure 8.2a shows the incidence of invasive cases of the seven monitored species per 100,000 inhabitants in Denmark per year from 2014 to 2023. During this period, the Danish population increased by 5.4% (from 5,627,235 inhabitants in 2014 to 5,932,654 inhabitants in 2023). For comparison, Figure 8.2b shows the total number of blood cultures taken per 100,000 inhabitants per year in the same period. Additionally, the number of individual patients with minimum one blood culture taken per 100,000 inhabitants per year is shown. In the ten-year period the number of individual patients with at least one blood culture taken per year increased from 2,549 patients per 100,000 inhabitants in 2014 to 3,313 patients per 100,000 inhabitants in 2023 (an increase of 30%). The total number of blood samples (unique samples in MiBa) taken per 100,000 inhabitants increased even more (54%). The incidence of positive blood cultures with pathogenic species increased from 490 per 100,000 inhabitants in 2014 to 628 per 100,000 inhabitants in 2023.

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

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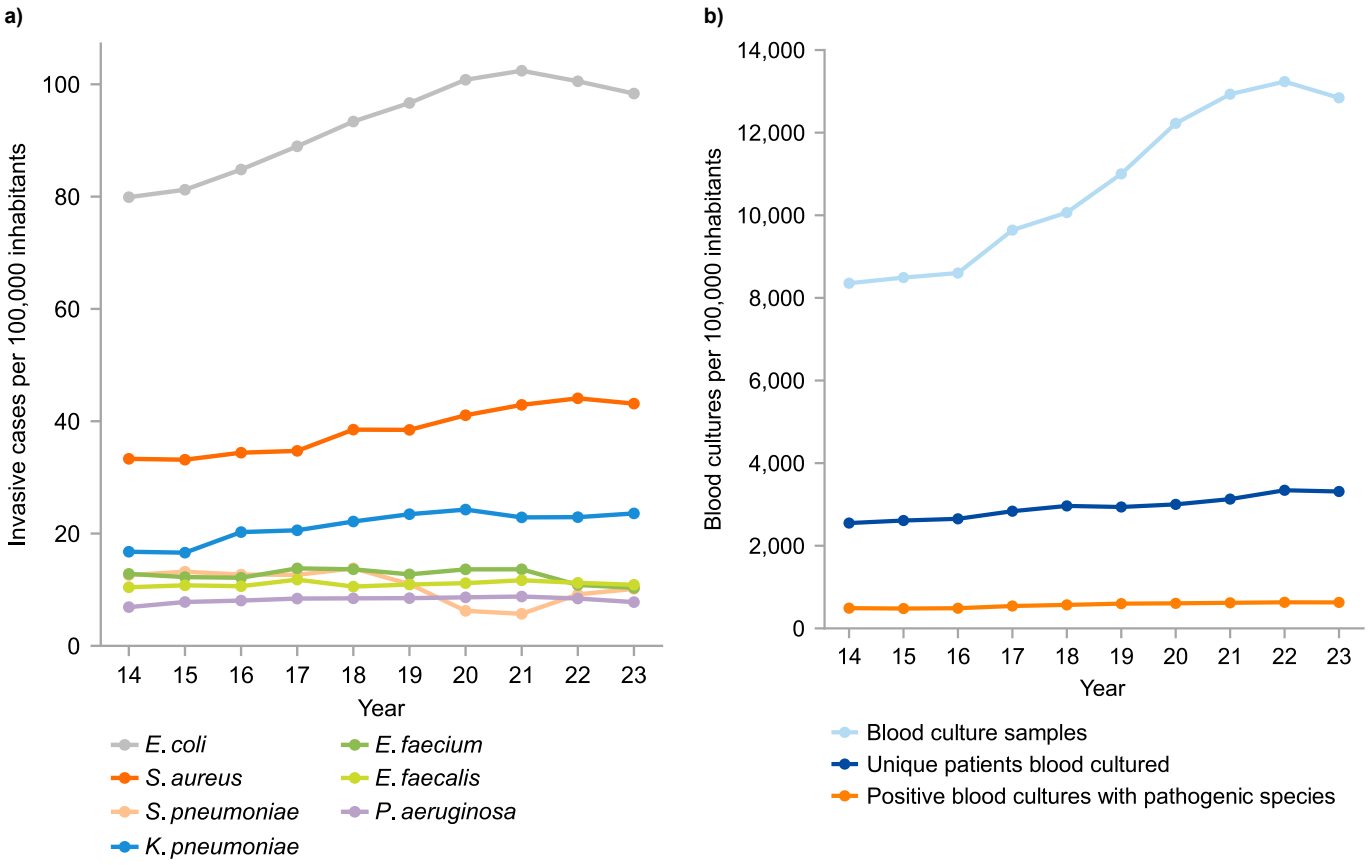
Figure 8.2 a) Invasive cases and b) blood cultures taken per 100,000 inhabitants, Denmark, 2014-2023 DANMAP 2023



8.2 Results from MiBa data surveillance

8.2.1 *Escherichia coli*

Escherichia coli is by far the most frequent cause of community and hospital acquired bacteraemia and urinary tract infections (UTI) in Denmark. The pathogen is part of the normal intestinal flora in both animals and humans, where it comes into close proximity to many other bacterial species. Transferable resistance mechanisms are frequently seen in these gut bacteria as well as resistance conferred by mutations. Since *E. coli* is the most frequent cause of UTI and bacteraemia, it is also responsible for a large proportion of the antimicrobials used to treat these conditions.

The percentages of resistance in invasive *E. coli* isolates for each key antimicrobial are presented in Table 8.2, as are urine samples from hospitals and primary health care (see details in later paragraphs).

Invasive cases from hospital patients

In 2023, a total of 5,835 individual patients with invasive *E. coli* isolates were identified in EpiMiBa. Antimicrobial susceptibility test results for key antimicrobials were also extracted for these isolates (See Chapter 10 'Material and methods').

Figure 8.3 and Table 8.3 shows the total annual number of invasive isolates and proportion of resistant isolates by region between 2014 and 2023. Most resistance levels decreased or stagnated over the last decade, apart from resistance to piperacillin-tazobactam, which increased in three of the five healthcare regions, leading to an overall increase from 4.5% in 2014 to 6.3% in 2023.

Resistance to carbapenems remained below 1%. The percentages of multidrug resistant invasive isolates are presented in Table 8.4. Combined resistance to ciprofloxacin, cephalosporins and gentamicin decreased from 2.3% in 2015 to 1.2% in 2023 and combined resistance to ampicillin and gentamicin from 6.3% to 4.1%.

Urinary cases from hospitals

In 2023, *E. coli* was isolated from urine samples of 44,389 individual hospital patients. In Table 8.2 summarized antimicrobial susceptibility results for *E. coli* isolates (invasive and urine specimen) are shown. In Figure 8.4 and Table 8.5, resistance for *E. coli* urine isolates from hospitalised patients are shown for 2014-2023.

Urinary cases from primary health care

In 2023, *E. coli* were isolated from urine samples from 106,236 unique patients in primary health care. Susceptibility results for all tested antimicrobials are shown in Table 8.2. In Figure 8.5 and Table 8.6, resistance for *E. coli* urine isolates from primary health care are shown for the past decade.

Conclusion

The number of invasive cases of *E. coli* appears to have reached a plateau. Resistance levels are generally stable for all specimen types, however, the slow rise in piperacillin-tazobactam resistance in *E. coli* from invasive infections and hospital urines are cause for concern.

Table 8.2 *Escherichia coli*. Resistance (%) in isolates from humans, Denmark, 2023 DANMAP 2023

Substance	Invasive isolates, hospitals %	Urine isolates, hospitals %	Urine isolates, primary health care %
Ampicillin	41	39	34
Mecillinam	6.5	6.5	4.0
Piperacillin/tazobactam	6.3	5.4	4.3 (1)
Amoxicillin/clavulanic acid	28.8 (4)	10.1	6.7
Sulfonamide		31 (4)	26
Trimethoprim		21	20
Nitrofuratoin		0.8	0.6
Gentamicin	4.4	4.5	3.7 (1)
Ciprofloxacin	10.6	9.9	7.5
Cefuroxime	9.6	7.7	6.1 (3)
3rd generation cephalosporins	5.8	6.5	5.3
Carbapenem	0.0	0.1	0.0 (2)
Max. number of isolates tested for resistance to the presented antibiotics	5,830	44,263	106,203

Note: Included are results from all DCM where >75% of the isolates in each antibiotic/sample group were susceptibility tested
Numbers in parentheses indicate the number of DCM included if less than six

Figure 8.3 Antimicrobial resistance in invasive *Escherichia coli* isolates from humans by region, Denmark, 2014-2023 DANMAP 2023

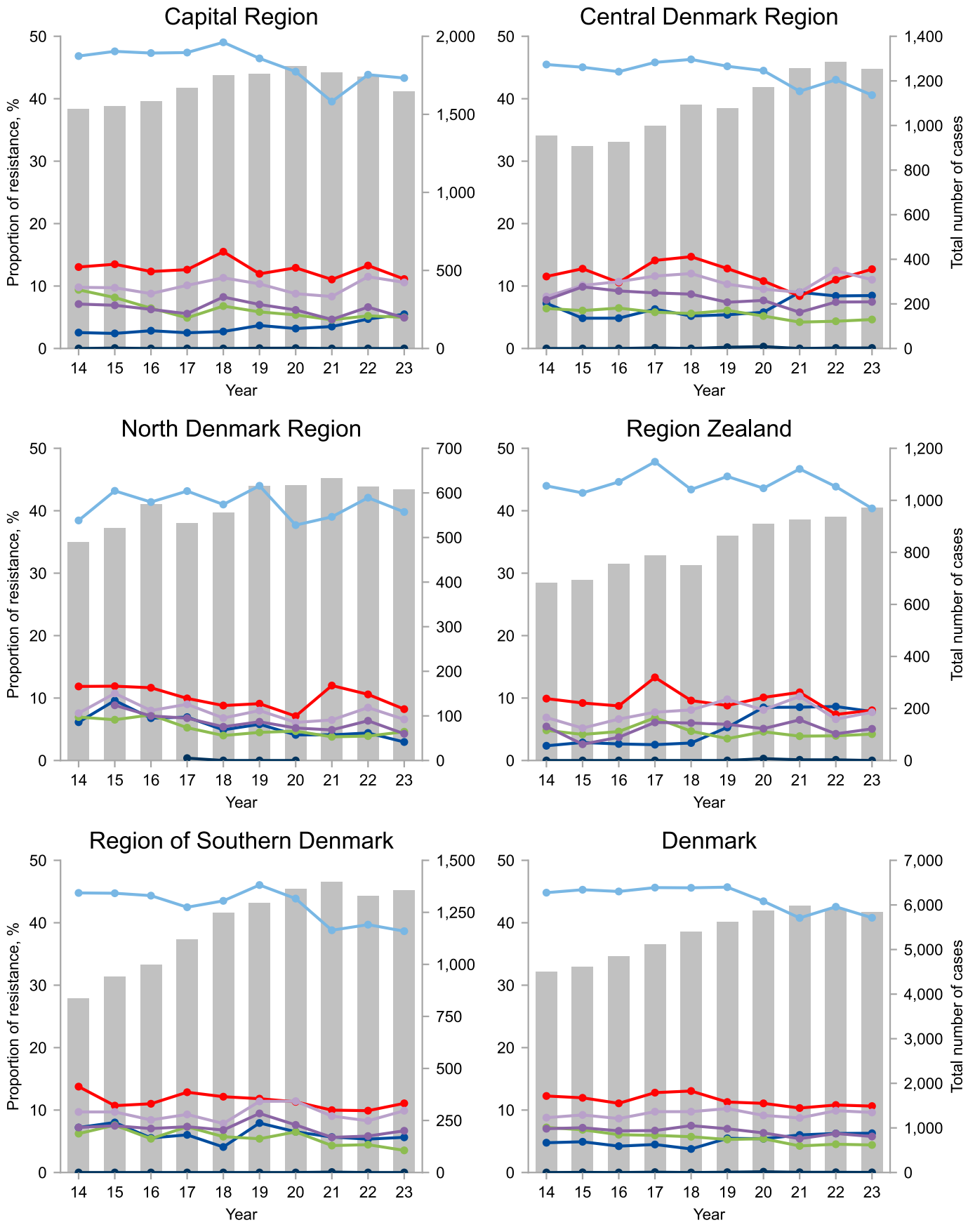


Table 8.3 *Escherichia coli* from invasive infections. Table of resistance percentages, Denmark, 2014-2023 DANMAP 2023

Substance	Percent resistant invasive *E. coli* isolates									
	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023
Ampicillin	44.8	45.3	45	45.6	45.5	45.7	43.4	40.7	42.6	40.8
Piperacillin-tazobactam	4.8	4.9	4.2	4.5	3.8	5.5	5.4	6.0	6.3	6.3
Gentamicin	7.2	6.8	6.1	6.0	5.7	5.3	5.4	4.3	4.5	4.4
Ciprofloxacin	12.3	12.0	11.1	12.8	13.0	11.3	11.1	10.3	10.8	10.6
Cefuroxime	8.8	9.2	8.6	9.7	9.8	10.2	9.2	8.7	9.9	9.6
3rd gen.cephalosporins	7.0	7.2	6.7	6.7	7.3	6.9	6.2	5.4	6.2	5.8
Carbapenem	0.0	0.0	0.0	0.1	0.0	0.1	0.1	0.0	0.0	0.0
Total number of isolates	4,492	4,618	4,841	5,114	5,398	5,613	5,870	5,981	5,905	5,835

Table 8.4 *Escherichia coli*. Combined resistance to 1) ampicillin and gentamicin and 2) 3rd generation cephalosporins, ciprofloxacin, and gentamicin (multiresistance) in invasive isolates from humans, Denmark, 2014-2023 DANMAP 2023

Combination		2014 % (N)	2015 % (N)	2016 % (N)	2017 % (N)	2018 % (N)	2019 % (N)	2020 % (N)	2021 % (N)	2022 % (N)	2023 % (N)
AMP/GEN	Resistance	6.9 (284)	6.3 (254)	5.8 (278)	5.8 (289)	5.5 (284)	5.2 (275)	5.1 (287)	4.0 (229)	4.1 (233)	4.1 (231)
	Percentage (no.) of isolates tested	92 (4,138)	87 (4,009)	99 (4,816)	98 (5,015)	96 (5,170)	95 (5,308)	95 (5,583)	96 (5,745)	96 (5,679)	96 (5,602)
3GC/CIP/ GEN	Resistance	1.8 (72)	2.3 (93)	1.8 (87)	1.8 (88)	2.0 (100)	1.8 (93)	1.5 (82)	1.1 (60)	1.3 (70)	1.2 (63)
	Percentage (no.) of isolates tested	90 (4,039)	88 (4,071)	98 (4,763)	95 (4,883)	93 (4,997)	94 (5,259)	93 (5,470)	93 (5,564)	93 (5,474)	93 (5,417)
	Total number of invasive isolates	4,495	4,614	4,841	5,114	5,398	5,613	5,870	5,981	5,905	5,835

Table 8.5 *Escherichia coli* from hospital urines. Table of resistance percentages, Denmark, 2014-2023 DANMAP 2023

Substance	Percent resistant *E. coli* isolates from hospital urine									
	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023
Ampicillin	41.7	42.1	41.0	41.9	42.1	43.7	40.6	39.2	40.0	39.4
Mecillinam	7.2	7.7	7.4	7.5	7.4	8.1	7.3	6.9	6.8	6.5
Piperacillin-tazobactam	3.5	3.9	3.3	3.7	3.5	4.4	4.3	4.5	5.4	5.4
Sulfonamide	32.3*	32.0	34.9	31.1	31.0*	31.4*	29.4*	28.5*	28.2*	30.9*
Gentamicin	4.9	5.1	5.3	4.9	4.7	4.6	4.6	4.2	4.3	4.5
Ciprofloxacin	11.4	11.0	10.9	10.4	11.0	10.6	9.6	8.7	9.3	9.9
Cefuroxime	6.6	7.0	6.8	7.1	7.2	7.8	7.2	6.7	7.5	7.7
3rd gen. cephalosporins	5.9	5.9	5.9	6.2	6.4	6.9	6.3	5.8	6.2	6.5
Carbapenem	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
Total number of isolates	44,288	46,723	46,865	46,884	47,914	47,235	48,962	49,986	48,559	44,389

* Indicates less than 6 DCM reported rutine susceptibility testing

Table 8.6 *Escherichia coli* from urines from primary health care. Table of resistance percentages, Denmark, 2014-2023 DANMAP 2023

Substance	Resistance in *E. coli* urine isolates from primary health care									
	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023
Ampicillin	39.3	38.5	38.3	37.9	37.4	38.1	36.5	34.3	34.6	34.2
Mecillinam	5.2	5.4	5.6	5.5	5.1	5.3	4.9	4.6	4.3	4.0
Sulfonamide	31.9	30.9	29.5	29.1	28.3	27.9	26.7	25.2	25.0	25.5
Ciprofloxacin	8.8	8.6	10.1	8.4	8.1	8.0	7.5	6.9	6.9	7.5
3rd gen. cephalosporins	4.2	4.3	4.3	4.5	4.9	5.2	5.0	4.4	4.8	5.3
Total number of isolates	51,272	61,083	67,798	73,497	80,851	86,08	88,462	99,077	104,376	106,236

Figure 8.4 Antimicrobial resistance in *Escherichia coli* isolates from urine in humans (hospitals) by region, Denmark, 2014-2023

DANMAP 2023

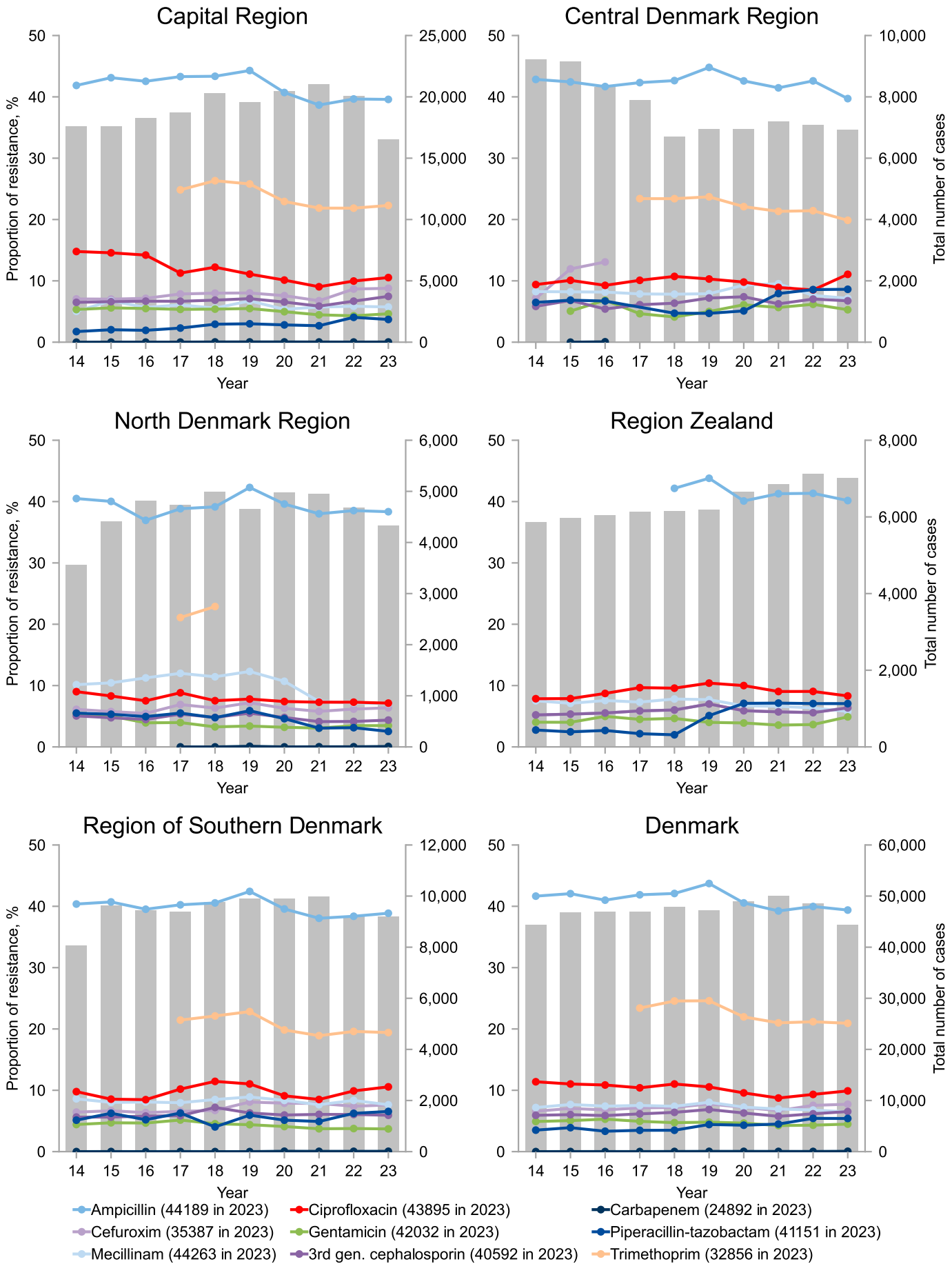
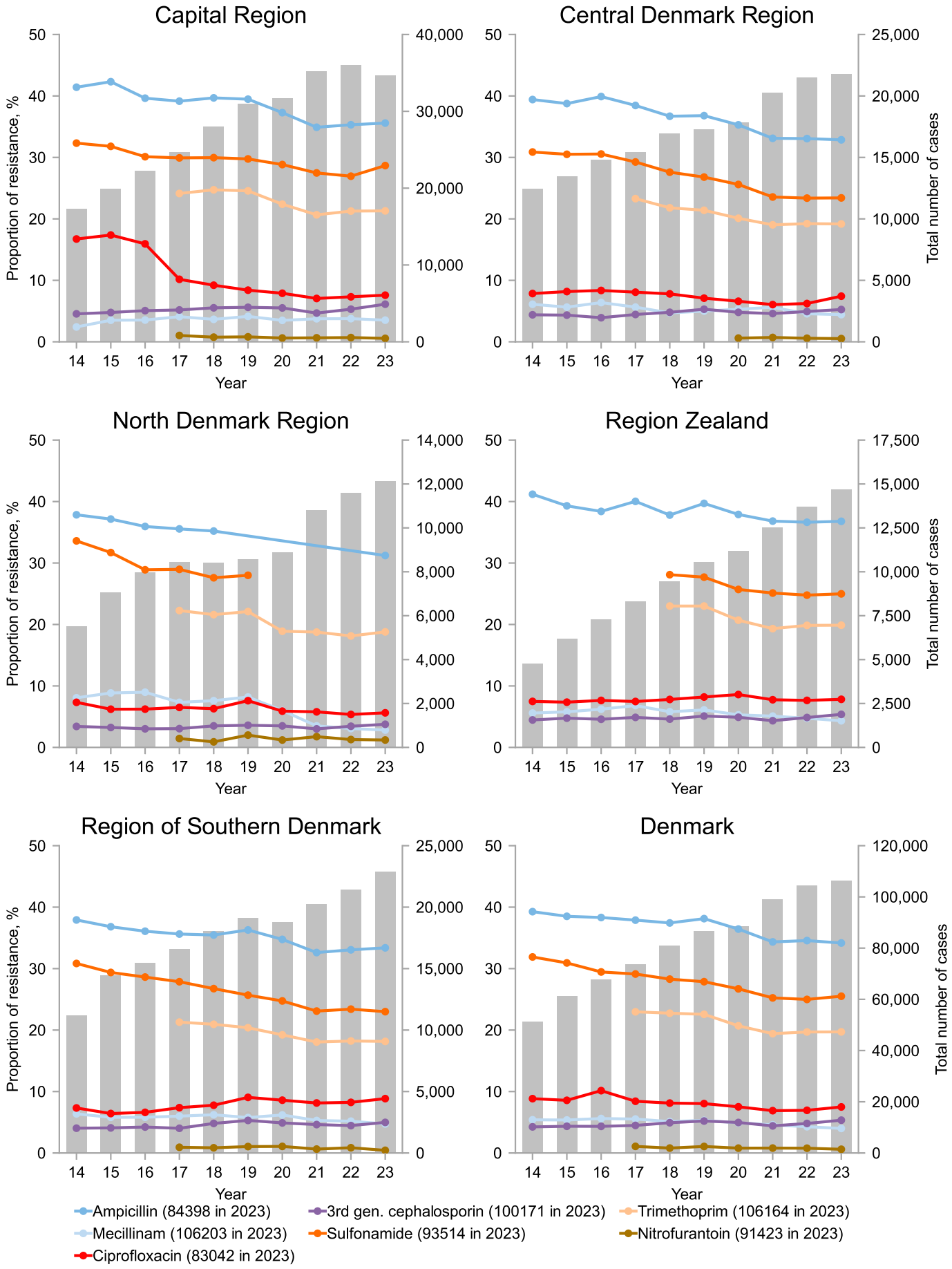


Figure 8.5 Antimicrobial resistance in *Escherichia coli* isolates from urine in humans (primary health care) by region, Denmark, 2014-2023

DANMAP 2023



8.2.2 *Klebsiella pneumonia*

Klebsiella pneumoniae is part of the human intestinal tract. The bacteria cause urinary tract infections, intra-abdominal infections, bacteraemia and ventilator-associated pneumonia (VAP). *K. pneumoniae* may cause nosocomial outbreaks. *K. pneumoniae* frequently displays plasmid-mediated resistance mechanisms, which are transferrable to other bacterial species.

The percentage of resistance in *K. pneumoniae* isolates for key antimicrobials in isolates from invasive infections, urines from hospitals and urines from general praxis, respectively, is presented in Table 8.7.

Invasive cases from hospitals

In 2023, a total of 1,399 unique patients were registered in MiBa with invasive *K. pneumoniae* isolates. Figure 8.6 shows total annual numbers and numbers per region of invasive isolates and percentages of resistance in invasive isolates between 2014 and 2023. From 2014 to 2021, particularly for cephalosporins decreasing trends in resistance were observed. From 2022 to 2023, resistance levels reverted and showed increases for most of the monitored antibiotics. Of particular interest is the almost continous increase in resistance levels to piperacillin-tazobactam, which increased from 8.1% in 2014 to 10.5% in 2023 in total, an increase that was observed for all regions. Resistance levels to carbapenems showed sligth increases over the decade (0.2% to 0.5%) as did combined resistance to ciprofloxacin, cephalosporins and carbapenems (1.1% to 1.9%).

The proportions of isolates resistant to key antimicrobials for the decade are presented in Table 8.8. The percentages of multidrug resistant invasive isolates are presented in Table 8.9.

Table 8.7 *Klebsiella pneumoniae*. Resistance (%) in isolates from humans, Denmark, 2023 DANMAP 2023

Substance	Invasive isolates, hospitals %	Urine isolates, hospitals %	Urine isolates, primary health care %
Mecillinam	8.5	10.0	7.2
Piperacillin/tazobactam	10.5	10.5	9.0 (1)
Amoxicillin/clavulanic acid	16 (5)	7.5	4.7 (5)
Sulfonamide		19.6 (3)	13.4
Trimethoprim		13.9	12.3
Nitrofuratoin		36 (5)	30
Gentamicin	2.8	2.3	1.6 (1)
Ciprofloxacin	7.4	7.5	5.2
Cefuroxime	9.8	9.4	5.9 (3)
3rd generation cephalosporins	5.9	5.7	4.6
Carbapenem	0.5	0.4 (5)	0.2 (2)
Max. number of isolates tested for resistance to the presented antibiotics	1,398	7,459	11,502

Note: Included are results from all DCM where >75% of the isolates in each antibiotic/sample group were susceptibility tested
Numbers in parantheses indicate the number of DCM included if less than six

Figure 8.6 Invasive *Klebsiella pneumoniae* isolates from humans: proportion of resistant isolates and annual number of isolates from unique cases, Denmark, 2014-2023

DANMAP 2023

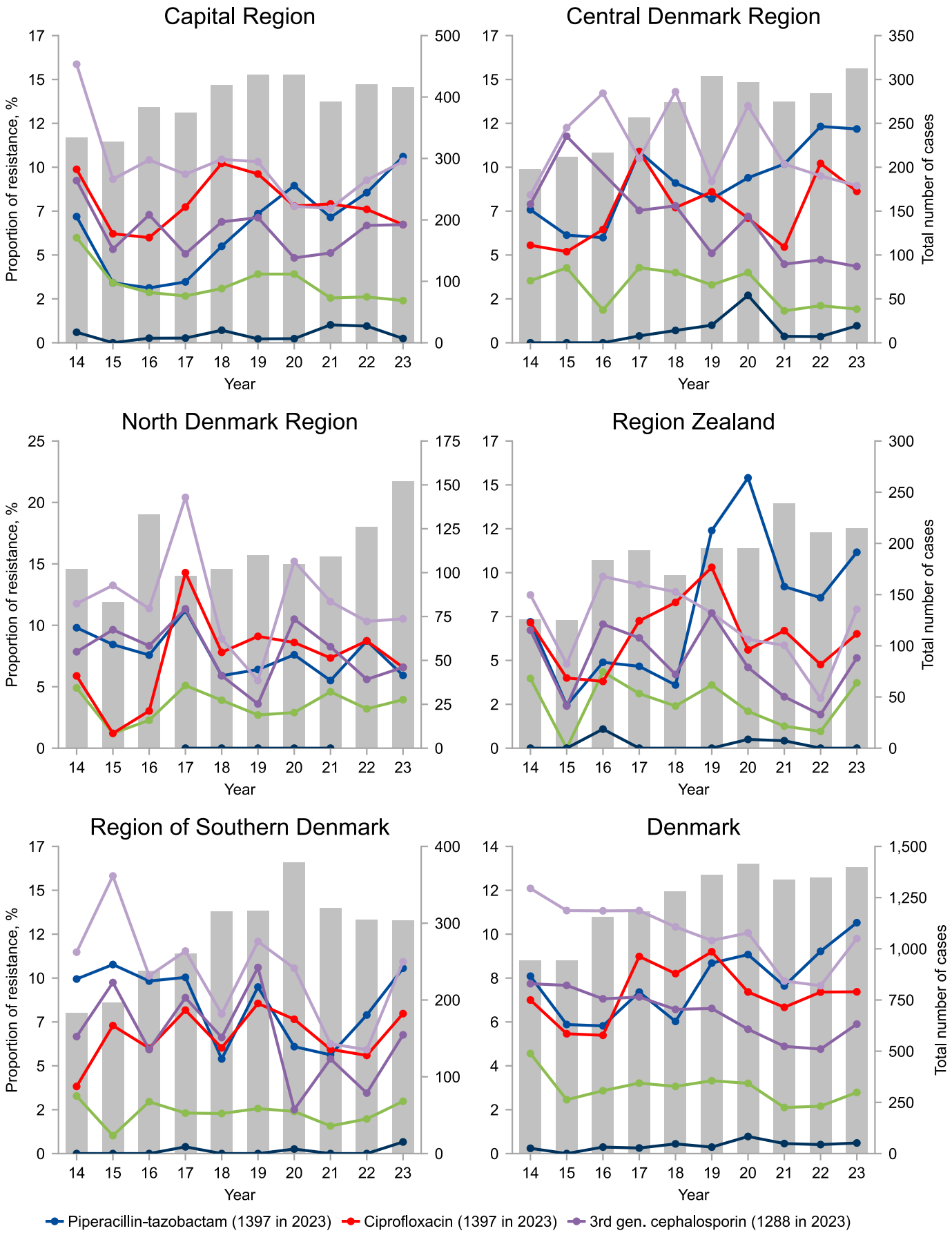


Table 8.8 Invasive *Klebsiella pneumoniae*. Table of resistance percentages, 2014-2023 DANMAP 2023

Substance	Percent resistant invasive *K. pneumoniae* isolates									
	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023
Piperacillin/tazobactam	8.1	5.9	5.8	7.4	6.1	8.7	9.1	7.5	9.2	10.5
Gentamicin	4.6	2.5	2.9	3.2	3.1	3.3	3.2	2.1	2.2	2.8
Ciprofloxacin	7.0	5.5	5.4	9.0	8.1	9.2	7.4	6.7	7.4	7.4
Cefuroxime	12.1	11.1	11.1	11.1	10.3	9.7	10.1	7.9	7.7	9.8
3rd gen. cephalosporins	7.7	7.7	7.3	7.1	6.1	6.6	5.3	4.9	4.8	5.9
Carbapenem	0.2	0.0	0.3	0.3	0.5	0.3	0.8	0.5	0.4	0.5
Total number of isolates	943	939	1,156	1,183	1,280	1,361	1,413	1,336	1,346	1,399

Table 8.9 Invasive *Klebsiella pneumoniae*. Combined resistance to 3rd generation cephalosporins, ciprofloxacin, and gentamicin (multidrug-resistance) in invasive isolates from humans, Denmark, 2015-2023 DANMAP 2023

	2015 % (N)	2016 % (N)	2017 % (N)	2018 % (N)	2019 % (N)	2020 % (N)	2021 % (N)	2022 % (N)	2023 % (N)
Resistance	1.1 (9)	1.6 (18)	2.4 (27)	1.7 (20)	2.4 (30)	1.5 (19)	1.0 (13)	1.0 (13)	1.9 (24)
Percentage (no.) of isolates tested for combined resistance (multiresistance)	89 (840)	98 (1,131)	95 (1,122)	93 (1,188)	94 (1,275)	93 (1,308)	93 (1,248)	94 (1,259)	92 (1,287)
Total number of invasive isolates	943	1,156	1,183	1,280	1,361	1,413	1,336	1,346	1,399

Table 8.10 *Klebsiella pneumoniae* from hospital urines. Table of resistance percentages, Denmark, 2014-2023 DANMAP 2023

Substance	Resistance in *K. pneumoniae* urine isolates from hospitals									
	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023
Mecillinam	9.6	10.0	8.9	15.7	16.9	13.2	11.7	10.6	9.4	10.0
Piperacillin/tazobactam	6.0	6.3	5.6	6.7	8.7	8.5	8.0	8.7	9.9	10.5
Gentamicin	4.0	3.7	3.2	3.6	3.2	3.0	2.9	2.4	2.3	2.3
Ciprofloxacin	7.6	6.2	6.1	7.6	8.9	7.4	7.2	7.2	7.2	7.5
Cefuroxime	8.9	9.5	9.1	9.4	9.5	8.6	8.5	8.4	8.1	9.4
3rd gen. cephalosporins	6.6	6.8	6.8	7.1	6.8	6.0	5.5	5.4	4.7	5.7
Total number of isolates	6,372	7,175	7,467	8,106	8,047	7,926	7,814	7,701	7,911	7,492

Table 8.11 *Klebsiella pneumoniae* from urines from primary health care. Table of resistance percentages, Denmark, 2014-2023 DANMAP 2023

Substance	Resistance in *K. pneumoniae* urine isolates from primary health care									
	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023
Mecillinam	8.8	9.4	9.0	16.6	15.9	11.5	9.4	8.6	7.9	7.2
Sulfonamide	17.1	18.7	19.3	25.5	24.6	18.9	15.5	13.8	11.9	13.4
Ciprofloxacin	6.5	5.2	5.6	5.4	6.4	5.5	5.2	4.9	5.1	5.2
3rd gen. cephalosporins	4.7	4.8	5.4	4.9	5.3	4.5	4.4	3.7	3.4	4.6
Total number of isolates	4,246	6,372	7,615	8,948	9,227	9,696	9,387	10,196	11,039	4,246

Figure 8.7 *Klebsiella pneumoniae* isolates from urines in humans (hospitals): proportion of resistant isolates and annual number of isolates from unique cases, Denmark, 2014-2023

DANMAP 2023

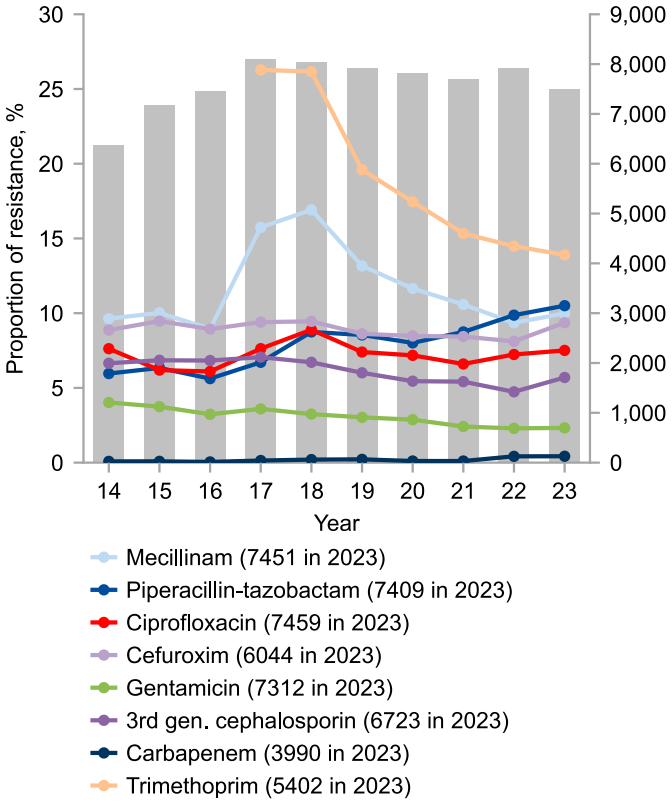
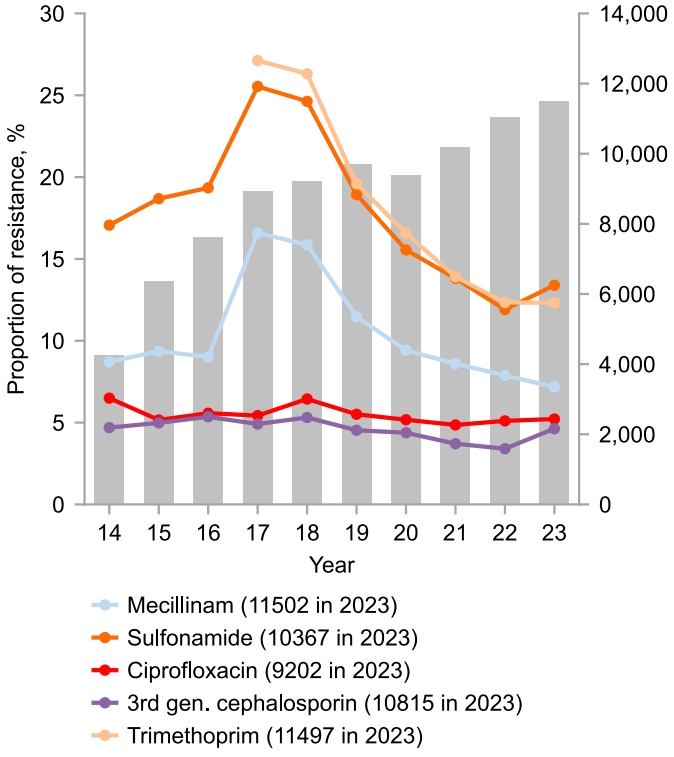


Figure 8.8 *Klebsiella pneumoniae* isolates from urines in humans (primary health care): proportion of resistant isolates and annual number of isolates from unique cases, Denmark, 2014-2023

DANMAP 2023



Urinary cases from hospitals

In 2023, *K. pneumoniae* from urine samples were isolated from 7,492 unique hospital patients in Denmark.

Summarized antibiotic-specific susceptibility results for *K. pneumoniae* isolates (invasive and urine specimen) for 2023 are shown in Table 8.7. In Figure 8.7 and Table 8.10, resistance for *K. pneumoniae* urine isolates from hospitals is shown for 2014-2023.

Urinary cases from primary health care

In 2023, *K. pneumoniae* was isolated from urine samples of 11,039 unique patients in primary health care. As for the results from invasive isolates and isolates from hospital urine samples susceptibility results for all tested antimicrobials are shown in Table 8.7. In Figure 8.8 and Table 8.11, resistance for *K. pneumoniae* urine isolates from primary health care is shown for the past decade.

Conclusion

The number of invasive cases of *K. pneumoniae* have increased by 38% since 2014. Resistance levels to cephalosporins have decreased steadily over the decade but reverted from 2022 to 2023, a change that should be followed closely. As for *E. coli*, a concerning trend is seen with regards to piperacillin-tazobactam for which resistance levels have now surpassed 10% for both invasive infections and hospital urines.

8.2.3 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is an opportunistic pathogen that can colonise the lung, urinary tract, burn wounds, superficial wounds and cause invasive infections. The pathogen is capable of biofilm formation and has the ability to colonise medical devices (e.g. indwelling catheters and implants) which in many cases causes complicated infections requiring prolonged and combination treatment. *P. aeruginosa* infections are more prevalent in certain at-risk groups such as immunocompromised, diabetes and cystic fibrosis patients. *P. aeruginosa* exhibits intrinsic resistance to various antimicrobial agents through chromosomal gene mutations and has the ability to acquire β-lactamases (extended-spectrum β-lactamases (ESBLs) and carbapenemases (especially class B carbapenemases or metallo-β-lactamases [MBLs]) by horizontal transmission.

Invasive cases from hospital patients

In 2023, a total of 495 unique patients with invasive P. *aeruginosa* isolates were registered in Denmark. Figure 8.9 shows the total annual number of invasive isolates and proportion of resistant isolates by region between 2014 and 2023.

Conclusion

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

8.2.4 *Acinetobacter* species

The genus *Acinetobacter* includes several species and is found widespread in nature (soil and water) as well as in animals and humans. In humans, *Acinetobacter* species (spp.) can colonise the skin and wounds but may also cause hospital-acquired infections such as central line-associated bloodstream infections, nosocomial pneumonia, UTIs and wound infections, most often in severely ill patients. Many of the different subspecies are phenotypically alike, and thus identification at species level can be difficult. Species belonging to the *A. baumannii* group are considered as the most clinically important. *Acinetobacter* spp. is intrinsically resistant to a broad range of antimicrobials mediated by a low membrane permeability and constitutive expression of various efflux systems.

Invasive cases from hospitals

In 2023, a total of 90 unique patients with invasive *Acinetobacter* species were reported. Data are presented in Table 8.12 and in Figure 8.10.

Conclusion

The number of invasive *Acinetobacter* spp. appears to have stabilized at a new level compared to the marked increase from 2020 to 2021; however, the proportion of invasive isolates resistant to key antimicrobials remains low in Denmark.

Figure 8.9 Invasive *Pseudomonas aeruginosa* isolates from humans: proportion of resistant isolates and annual number of isolates from unique cases, Denmark, 2014-2023 DANMAP 2023

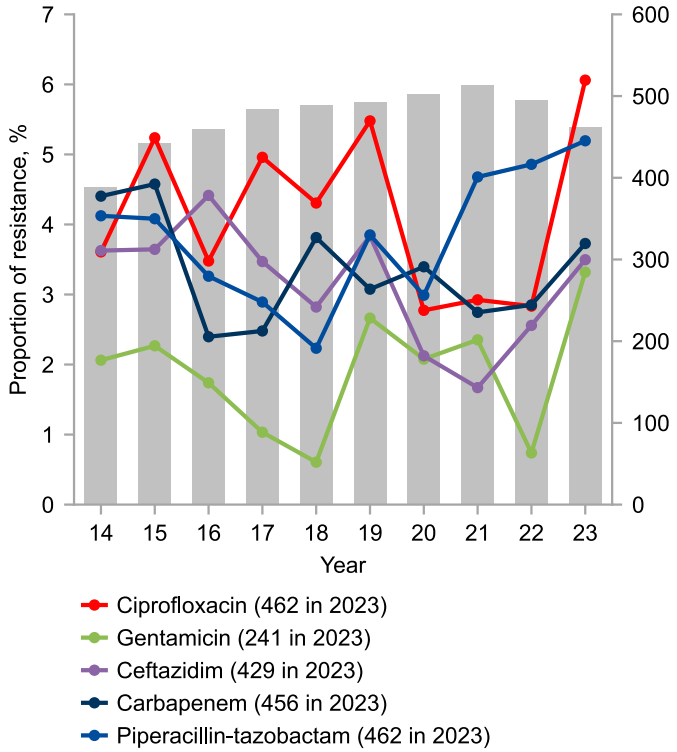


Figure 8.10 Invasive *Acinetobacter* spp. isolates from humans: a) annual number of isolates from unique cases and b) proportion of resistant isolates, Denmark, 2014-2023 DANMAP 2023

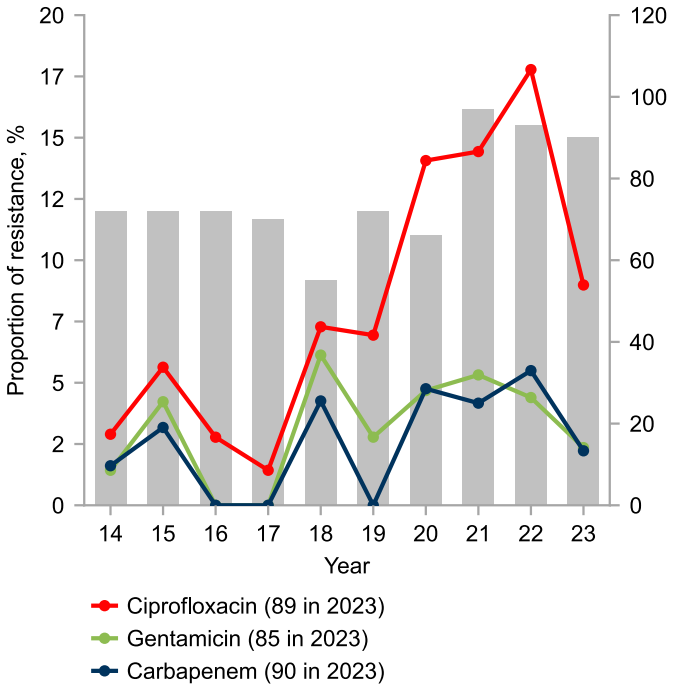


Table 8.12 *Acinetobacter* spp. tested and resistant invasive isolates, Denmark, 2014-2023 DANMAP 2023

	2014		2015		2016		2017		2018		2019		2020		2021		2022		2023	
	res.	n	res.	n	res.	n	res.	n	res.	n	res.	n	res.	n	res.	n	res.	n	res.	n
Ciprofloxacin	2	69	4	71	2	72	1	70	4	55	5	72	9	64	14	97	16	92	8	89
Gentamicin	1	70	3	71	0	70	0	70	3	49	2	72	3	64	5	94	4	92	2	85
Carbapenem	1	62	3	68	0	69	0	67	2	47	0	72	3	63	4	96	5	93	2	90
Total number of invasive isolates	72		71		72		70		55		72		66		97		93		90	

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

8.2.5 Enterococci

Enterococci are part of the normal intestinal microbiota in humans and animals. More than 54 species belonging to the genus *Enterococcus* have been described. The majority of human infections are caused by *E. faecalis* and *E. faecium* and in rare cases by *E. casseliflavus*, *E. gallinarum* and *E. raffinosus*. Most common clinical infections include urinary tract infections, intra-abdominal infections, bacteraemia and infective endocarditis.

Enterococci are intrinsically resistant to many groups of antimicrobials which gives them a selective advantage in e.g. hospitalised patients under antibiotic treatment, leading to colonization or infection. The source of hospital infection is often associated with invasive medical devices.

Invasive cases from hospitals

In 2023, *E. faecalis* isolated from 645 unique patients and *E. faecium* isolated from 613 unique patients were reported in MiBa. Table 8.13 shows resistance percentages towards the most important antibiotics for both species for 2023 and Figure 8.11 shows the annual numbers of invasive isolates and resistance to vancomycin.

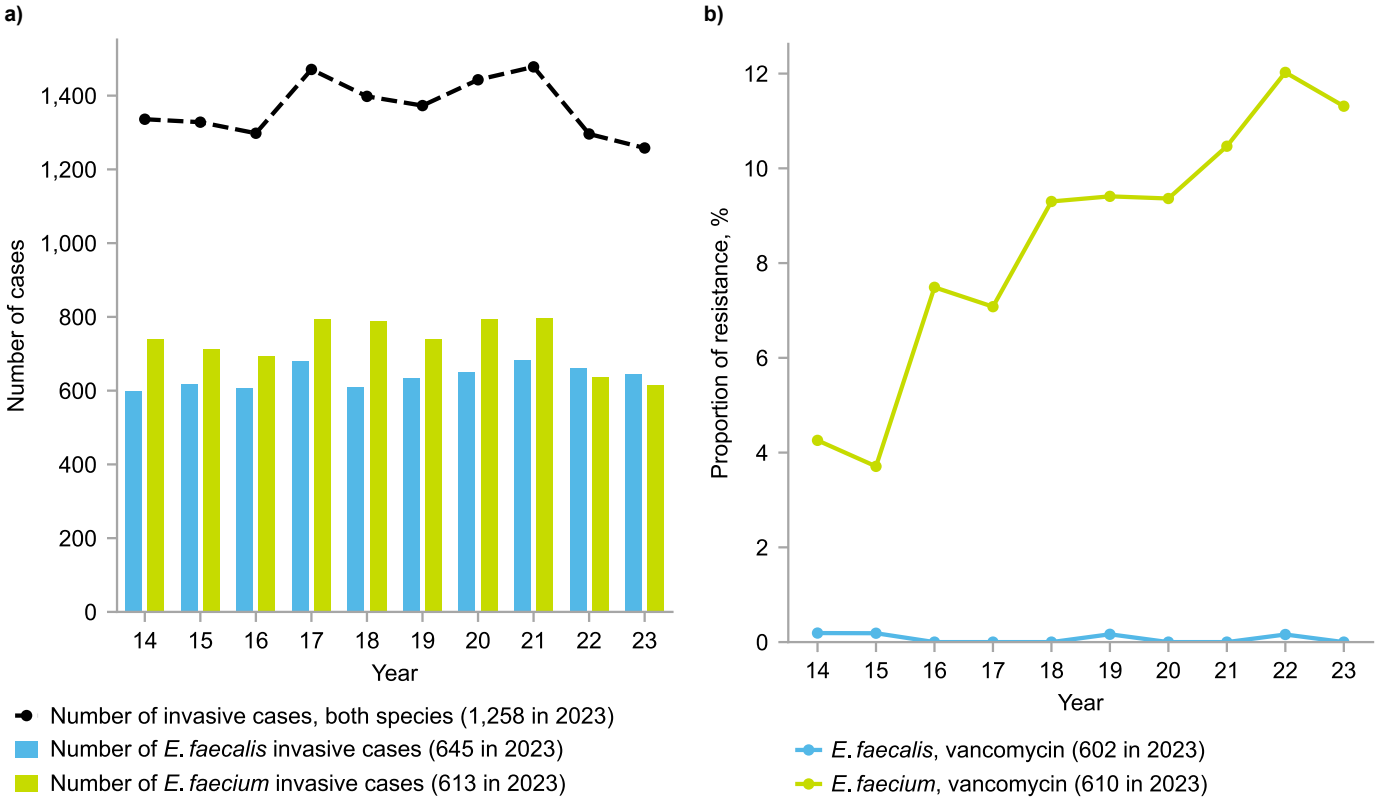
Table 8.13 Enterococci. Resistance (%) in invasive isolates from humans, 2023 DANMAP 2023

	E. faecalis	*E. faecium*	*E. faecalis*	*E. faecium*
	%	%	Number of included isolates (number of DCM)	
Ampicillin	0.2	93.1	645 (10)	592 (9)
Vancomycin	0.0	11.3	602 (9)	610 (10)
Linezolid	0.8	0.0	504 (7)	483 (6)
Teicoplanin	0.4	0.5	227 (2)	196 (2)
Tigecycline	0.0	0.0	110 (1)	104 (1)

All proportions of resistance are presented for each antibiotic as a mean of the resistance rates reported by the DCM. Included are all DCM that report routine testing (>75% of the isolates)

Figure 8.11 Invasive Enterococci faecalis/faecium isolates from humans:
a) annual number of isolates from unique cases and b) proportion of vancomycin resistant isolates, Denmark, 2014-2023

DANMAP 2023



Conclusion

The number of invasive infections with *E. faecium* seems to be decreasing while the number of infections with *E. faecalis* remains stable and resistance to vancomycin is non-existent in *E. faecalis,* however for *E. faecium* is has been increasing for the last decade and has surpassed 10% for the past three years.

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8.3 Results from the reference laboratories

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

Background

Resistance to third-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 (DCM) have, on a voluntary basis, submitted third-generation cephalosporin-resistant *E. coli* (3GC 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 EC's collected in Denmark through 2023, 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 Multilocus Sequence Types (MLSTs) and the encoding ESBL-, pAmpC- and carbapenemase genes.

Results

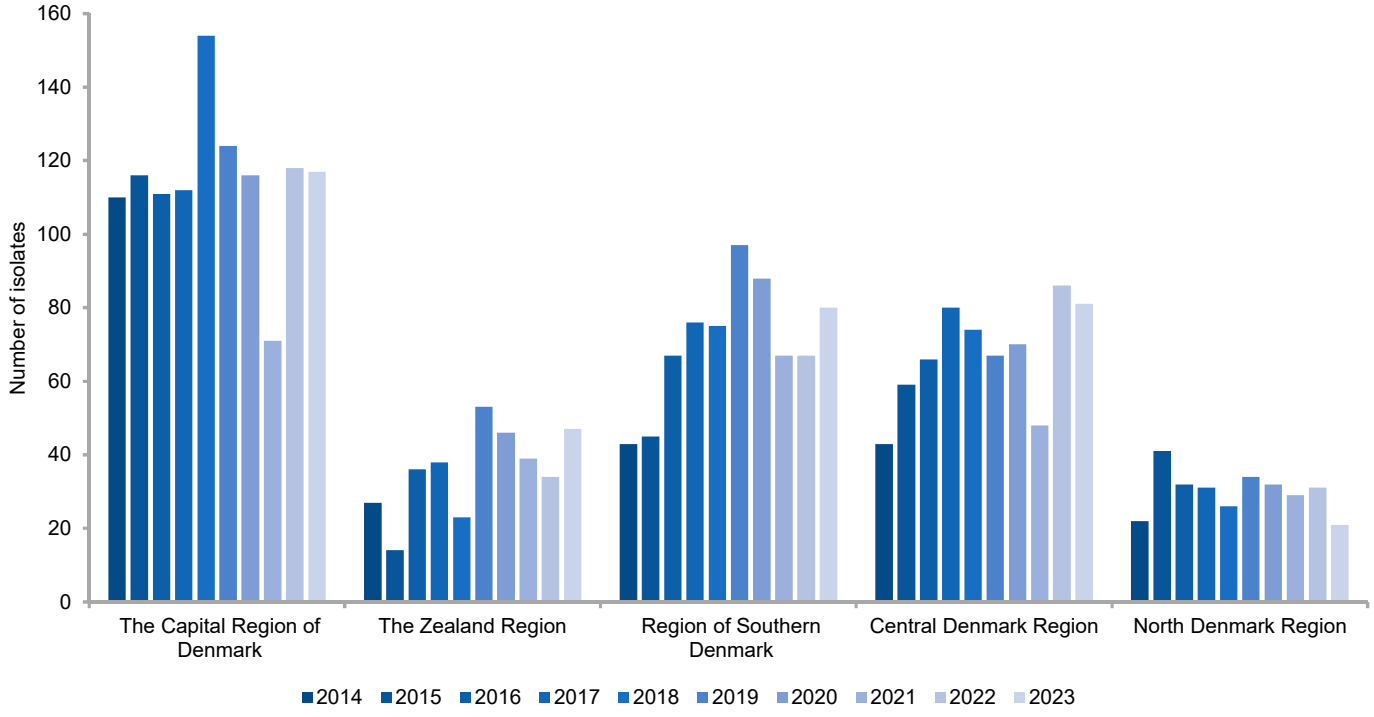
In 2023, a total of 346 *E. coli* blood isolates from unique patients, were identified with phenotypic test, as ESBL and/or AmpC positive isolates. Demographic data was available for all 346 *E. coli* isolates; the median age at diagnosis was 71 years, ranging from below one year to 99 years. In 2023, 164 (47%) of the patients were men compared to 187 (56%) in 2022. The change in men/women distribution observed in 2023 was statistically significant, compared to 2022. Further, it was the first time during surveillance of 3GC EC, that women outnumbered men.

The regional distribution of the 346 isolates with ESBL- and AmpC phenotype was compared to data from previous years (Figure 8.12 and Table 8.14). Following the notable increase in numbers of reported cases of ESBL/pAmpC *E. coli* in bloodstream infections observed from 2021 to 2022 (32% from 254 to 336 isolates), the number reported in 2023 remained stable compared to the previous year, with no significant changes within regions.

Whole genome sequencing data were obtained from 188 *E. coli* isolates (as only isolates from every second month and/or carbapenemase producing *E. coli* were sequenced). Genes encoding ESBL, pAmpC and/or carbapenemase were detected in 179 isolates, and nine isolates were AmpC hyperproducers caused by cAmpC mutations. The AmpC hyperproducers were not included in the further analysis.

In 2023, 16 different genes associated with ESBL-, and pAmpC enzymes were detected among the 179 sequenced isolates encoding ESBL and/or pAmpC genes, (Table 8.15). As in previous years, CTX-M-15 was the most prevalent enzyme, remaining relatively stable in occurrence at 58% in 2023, compared to 52% in 2022. In addition, the carbapenemase enzyme OXA-48 was observed only once during 2023 among the 179 whole genome sequenced bloodstream isolates.

Figure 8.12. ESBL/pAmpC producing *E. coli* from bloodstream infections by region, 2014-2023, Denmark DANMAP 2023



In 2023, the 179 analyzed whole genome sequenced *E. coli* isolates belonged to 45 different known MLSTs. In 2023, the most common sequence type (ST) was still ST131, however a decrease was observed for this type as only 68 (38%) of the isolates belonged to this type in 2023 compared to 89 (50%) in 2022. Additionally, an increase in isolates belonging to ST69 was observed from 9 (5%) in 2022 to 27 (15%) in 2023 (Table 8.16).

Among the 68 *E. coli* isolates belonging to ST131, CTX-M-15 (59%) was the most common enzyme, followed by CTX-M-27 (32%). Furthermore, among the 27 *E. coli* isolates belonging to ST69, CTX-M-15 (48%) was also the most common enzyme, followed by CTX-M-14 (15%), DHA-1 (15%) and CTX-M-27 (11%).

Table 8.14 Distribution of ESBL and carbapenemase producing *E. coli* from bloodstream infections, Denmark, 2015-2023 DANMAP 2023

Region	DANMAP 2015 Numbers	DANMAP 2016 Numbers	DANMAP 2017 Numbers	DANMAP 2018 Numbers	DANMAP 2019 Numbers	DANMAP 2020 Numbers	DANMAP 2021 Numbers	DANMAP 2022 Numbers	DANMAP 2023 Numbers
The Capital Region of Denmark	116	111	112	154	124	116	71	118	117
The Zealand Region	14	36	38	23	53	46	39	34	47
Region of Southern Denmark	45	67	76	75	97	88	67	67	80
Central Denmark Region	59	66	80	74	67	70	48	86	81
North Denmark Region	41	32	31	26	34	32	29	31	21
Total Numbers	275	312	337	352	375	352	254	336	346

Table 8.15 Most common ESBL enzymes and carbapenemases detected in *E. coli* from bloodstream infections, Denmark, 2015-2023 DANMAP 2023

Enzyme	DANMAP 2015 Number	%	DANMAP 2016 Number	%	DANMAP 2017 Number	%	DANMAP 2018 Number	%	DANMAP 2019 Number	%	DANMAP 2020 Number*	%	DANMAP 2021 Number*	%	DANMAP 2022 Number*	%	DANMAP 2023 Number*	%
blaCTX-M-1	7	3%	8	3%	17	5%	25	7%	8	4%	7	4%	6	4%	1	<1%	3	2%
blaCTX-M-14	33	12%	40	13%	48	14%	31	9%	33	17%	15	8%	12	9%	17	9%	17	9%
blaCTX-M-14b	5	2%	9	3%	3	1%	10	3%	3	2%	4	2%	0	0%	3	2%	2	1%
blaCTX-M-15	139	51%	157	50%	164	49%	200	57%	82	43%	100	52%	63	46%	94	52%	103	57%
blaCTX-M-27	33	12%	44	14%	52	15%	53	15%	37	19%	36	19%	29	21%	34	19%	32	18%
blaCTX-M-3	4	1%	7	2%	8	2%	5	1%	4	2%	1	1%	3	2%	1	<1%	0	0%
blaCTX-M-55	14	5%	6	2%	13	4%	4	1%	8	4%	4	2%	5	4%	3	2%	1	<1%
blaCMY-2	6	2%	10	3%	7	2%	6	2%	5	3%	5	3%	2	1%	2	1%	3	2%
blaDHA-1	3	1%	5	2%	6	2%	10	3%	4	2%	7	4%	3	2%	11	6%	9	5%
blaSHV-12	5	2%	5	2%	3	1%	4	1%	2	1%	5	3%	3	2%	3	2%	3	2%
Other CMY variants	10	4%	3	1%	3	1%	3	1%	5	3%	0	0%	1	1%	1	<1%	1	<1%
Other ESBL enzymes	23	8%	17	5%	10	3%	10	3%	3	2%	8	4%	6	4%	7	4%	2	1%
Carbapenemase enzymes	3	1%	1	<1%	1	<1%	5	1%	0	0%	7	4%	4	3%	5	3%	1	<1%

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

Table 8.16. Distribution of MLSTs in *E. coli* from bloodstream infections, Denmark, 2015-2023 DANMAP 2023

MLST	DANMAP 2015 Number	%	DANMAP 2016 Number	%	DANMAP 2017 Number	%	DANMAP 2018 Number	%	DANMAP 2019 Number*	%	DANMAP 2020 Number*	%	DANMAP 2021 Number*	%	DANMAP 2022 Number*	%	DANMAP 2023	
ST131	135	49%	177	57%	175	52%	189	54%	93	47%	89	46%	64	49%	89	50%	68	38%
ST69	10	4%	16	5%	20	6%	27	8%	14	7%	20	10%	7	5%	9	5%	27	15%
ST12	9	3%	14	4%	6	2%	5	1%	5	3%	2	1%	5	4%	4	2%	8	4%
ST38	23	8%	21	7%	23	7%	22	6%	13	7%	8	4%	1	1%	11	6%	7	4%
ST1193	5	2%	10	3%	7	2%	8	2%	6	3%	9	5%	9	7%	5	3%	7	4%
ST95	ND	ND	5	2%	4	1%	4	1%	3	2%	4	2%	3	2%	2	1%	5	3%
ST2279	ND	ND	0	0%	0	0%	2	1%	0	0%	3	2%	1	1%	2	1%	5	3%
ST73	2	1%	4	1%	2	1%	6	2%	4	2%	8	4%	1	1%	5	3%	5	3%
Other STs[1]	91	33%	65	21%	100	30%	89	25%	59	30%	50	26%	39	30%	51	29%	47	26%

[1] Found in less than 2% in 2022
* Numbers based on sequenced data from odd months

Conclusion
In 2023, the number of ESBL- and/or AmpC positive isolates remained stable compared to 2022, even though a significant shift in the gender distribution was observed. CTX-M-15 remained by far the most prevalent ESBL enzyme in Danish 3GC EC in 2023 (58%). Isolates belonging to ST131 decreased, whereas an increase was observed for isolates belonging to ST69.

In 2023, only one carbapenemase producer was observed among the 179 sequenced ESBL- and/or pAmpC blood infection isolates. Even though, the relative distribution of sequence types for the whole genome sequenced isolates changed compared to previous years; the worldwide disseminated ST131 clone was still strongly represented in 2023 (38%).

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8.3.2 Carbapenemase-producing organisms (CPO)

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

Carbapenemase-producing organisms (CPO) comprise two main groups: 1) Intestinal bacteria: Carbapenemase-producing Enterobacterales (CPE), e.g. *E. coli* and *K. pneumoniae*, and 2) environmental bacteria: *P. aeruginosa* and *A. baumannii*. The term CPE is used to designate intestinal bacteria. Thus, CPE are a subset of CPO.

CPO have been notifiable in Denmark since 5th September 2018 [https://www.retsinformation.dk/eli/lta/2018/1091]. Before this date, Danish departments of clinical microbiology (DCM) have submitted carbapenem-resistant isolates for verification and genotyping at the National Reference Laboratory for Antimicrobial Resistance at Statens Serum Institut on a voluntary basis. This section describes findings for carbapenemase-producing Enterobacterales (CPE), *Pseudomonas* spp. and *Acinetobacter* spp.

During 2023, 589 CPOs were identified from 458 patients compared with 392 CPO isolates from 335 patients in 2022, an increase in isolates of 50%. More than one isolate from the same patient was included if the isolates belonged to different bacterial species and/or if the isolates harbored different carbapenemases.

Collection of travel information is part of the CPO surveillance in Denmark. Thus, information of travelling abroad in the period of 6 months prior to testing positive for CPO is requested and reported to SSI, together with the clinical sample information.

Carbapenemase-producing Enterobacterales
In 2023, a total of 552 CPE isolates were reported from 436 patients compared with 350 CPE from 304 patients in 2022, resulting in a 58% increase of CPE isolates and a 43% increase in numbers of patients compared to 2022. In 2023, 37 of the 552 CPE isolates produced both NDM and OXA-48 group enzymes, 385 produced OXA-48-like enzymes alone and 109 were only NDM-producing. Furthermore, 12 KPC-, three VIM-, three KPC-/NDM- two VIM/OXA-48- group as well as one VIM/NDM-producing CPE isolate(s) were identified (Figure 8.13).

The large increase in CPE in 2023 was caused by a combination of an increase in the number of both clinical and screening isolates in most of the five regions (Figure 8.14). However, especially in one region (Region Zeeland), an intensified screening regime was implemented in 2023 in order to adhere to the national guidelines issued by the Danish Health Authorities to handle hospital outbreaks caused by CPO. This intensified screening is therefore an important contributing factor in explaining the increase in CPE in this particular region, together with an increase in clinical isolates in the region.

Among the species most frequently detected in CPE outbreaks the dominating bacterial species were *C. freundii* and *E. coli*, (table 8.17), while in travel-related findings the most dominating species were *K. pneumoniae* and *E. coli* (not shown).

No information is available as to the mortality in these cases.

Figure 8.13 Numbers of carbapenemase-producing Enterobacterales (CPE), Denmark, 2008-2023 DANMAP 2023

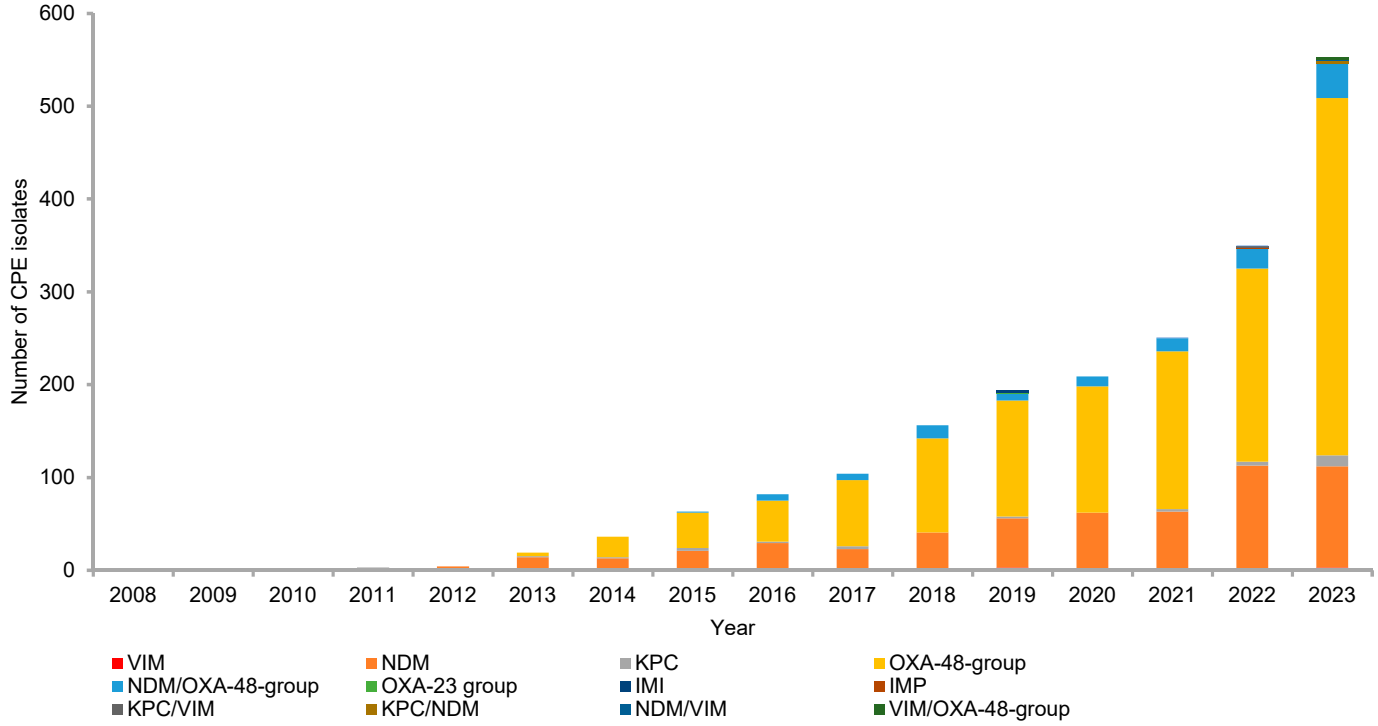
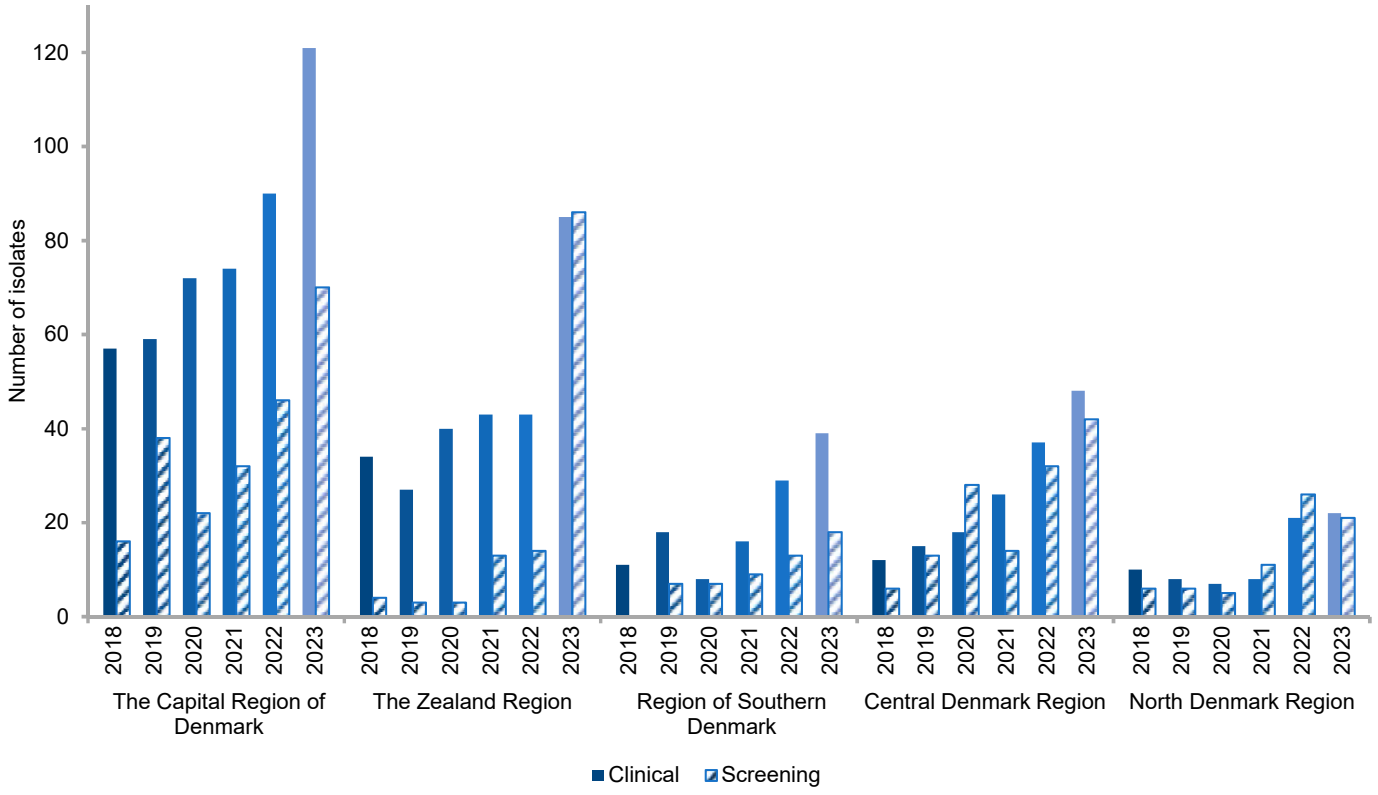


Figure 8.14 Number of clinical and screening CPE isolates per region, Denmark, 2018-2023 DANMAP 2023



Carbapenemase-producing *Acinetobacter* spp.

In 2023, 21 carbapenemase-producing *Acinetobacter* spp. isolates were reported from 21 patients, compared with 23 isolates from 23 patients in 2022. Of these 21 patients, eight patients had been travelling abroad and seven patients had relation to Ukraine prior to identification of the carbapenemase-producing *Acinetobacter* spp. No travel information was reported for six of the patients. In 2023, 18 carbapenemase-producing *Acinetobacter baumannii* with the following enzymes were identified: OXA-23 (12), OXA-72 (2), OXA-23/OXA-72 (3) and NDM-5/OXA-23 (1). Furthermore, one OXA-253- producing *Acinetobacter bereziniae*, one VIM-producing *Acinetobacter monteilii*, one NDM/OXA-58 *A. bereziniae* and one NDM-1-producing *A. lwoffii* were identified. (Figure 8.15).

Carbapenemase-producing *Pseudomonas* spp.

In 2023, 16 carbapenemase-producing *Pseudomonas* spp. isolates from 16 patients were reported compared to 19 isolates in 2022. Of these 16 patients, one patient had not been travelling, four patients had been travelling abroad, eight patients had relation to Ukraine and no information was given for three patients prior to identification of the carbapenemase-producing *Pseudomonas* spp. In 2023, 15 carbapenemase-producing *Pseudomonas aeruginosa* with the following enzymes were identified: NDM-1 (3), VIM (6) and IMP (6). In general, the number of carbapenemase-producing *Pseudomonas* spp. seems to be relatively stable over the years until the onset of Covid-19, which led to a large decrease in the number of isolates and then the onset of the war in Ukraine, which again has led to a large increase (Figure 8.16).

Figure 8.15 Carbapenemase-producing *Acinetobacter* spp. and enzymes identified, Denmark, 2008-2023 DANMAP 2023

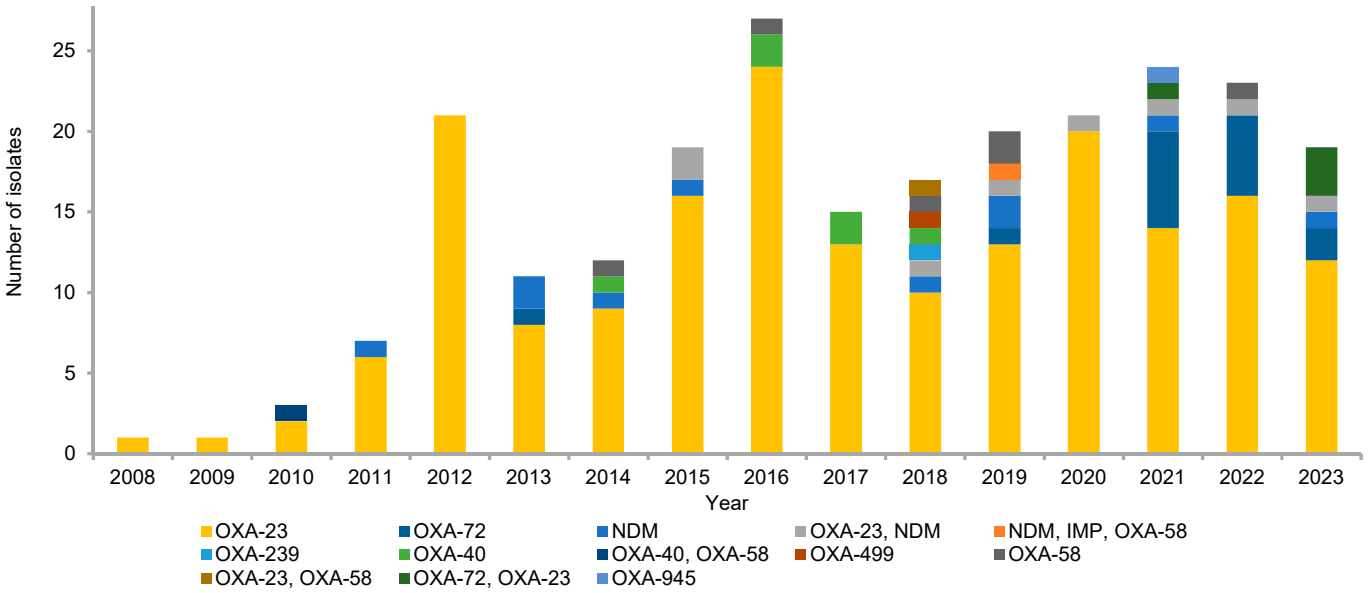
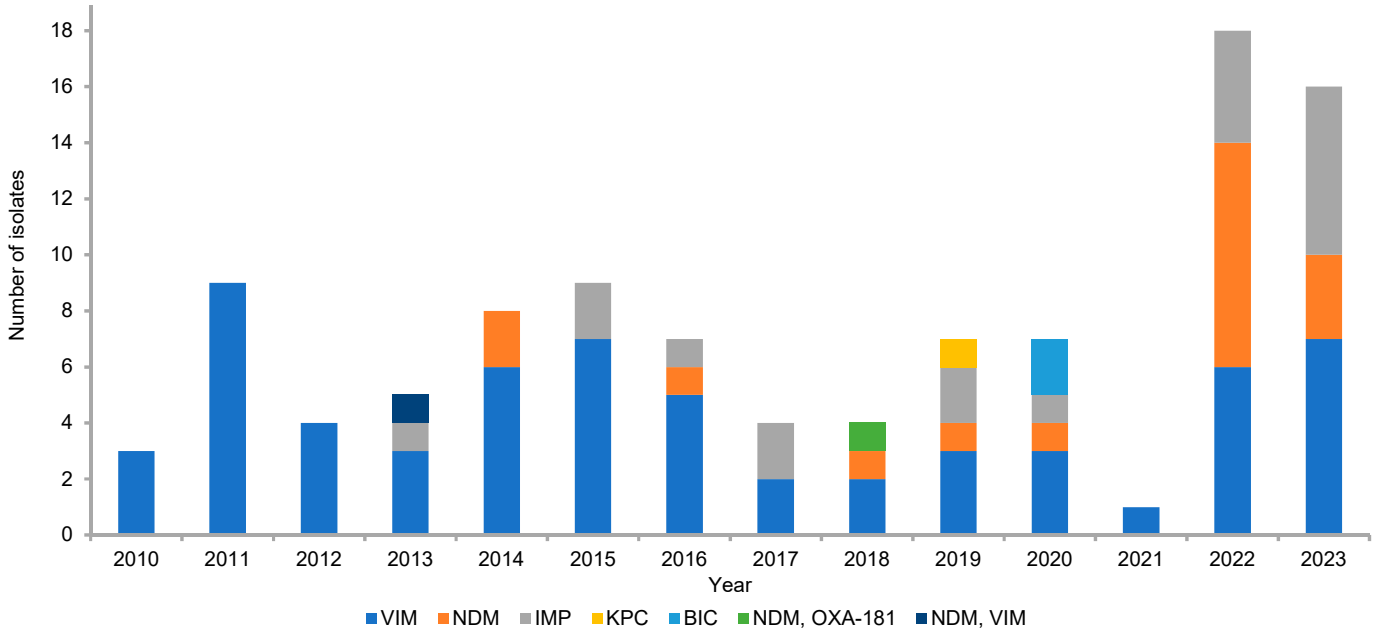


Figure 8.16 Carbapenemase-producing *Pseudomonas* spp. and enzymes identified, Denmark, 2010-2023 DANMAP 2023



CPO - Place of origin 2019-2023

If a patient is detected positive with CPO, the Clinical Departments or a clinical physician must report travel in the period of six months before day of sample. The CPO-patient will then be classified as a travel-associated CPO-patient. The Clinical Departments or a clinical physician can also report a CPO-patient to be colonized or infected in Denmark implicating that the patient has not been travelling prior to positive sample. In order to qualify the information regarding the origin of a colonization with CPO in a Danish patient, the reported CPO-data from 2019 to 2023 has been evaluated and categorized into four categories: 1) colonized or infected in Denmark, 2) part of Danish outbreak, 3) travel outside the Nordic countries, and 4) unknown (Figure 8.16).

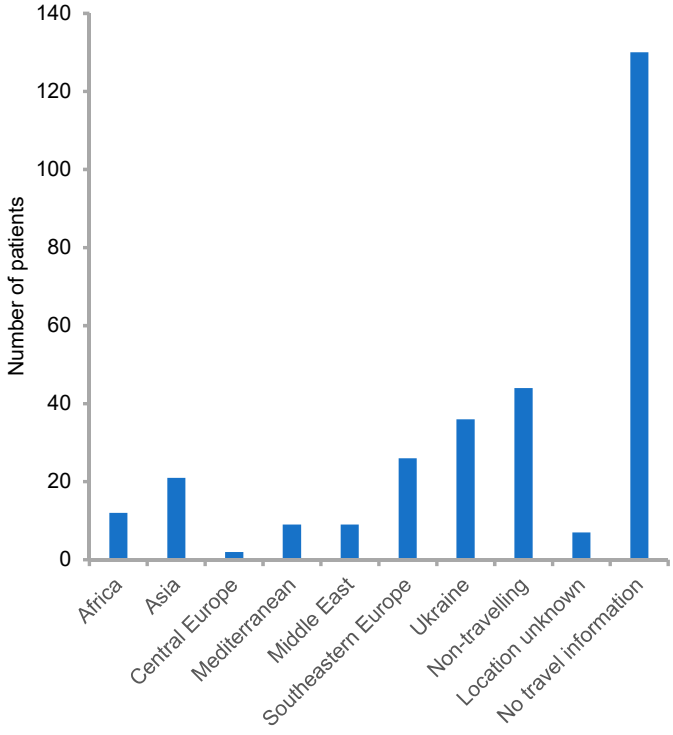
A CPO-patient can be affected by a Danish nosocomial outbreak and will be classified as an outbreak-patient. The presumed index patient (the first patient identified) in an outbreak will be registered according to possible travel information.

In 2023, 86 (29%) of 296 CPO cases with no relation to Danish domestic outbreaks reported travelling outside the Nordic countries, representing an increase compared to 2022, where 52 (19%) of 279 CPO positive cases had been travelling. The number of cases where no travel information is provided was high, 130 cases (44%).

The most frequent reported travel destinations in 2023 were Southeastern Europe (26), Asia (21), Middle East and Mediterranean (9 respectively) (Figure 8.17). The most reported single country travel destinations were Turkey (22), India (8), Pakistan and Thailand (5 respectively).

Due to the still ongoing war in Ukraine, a number of patients from Ukraine are still receiving care in the Danish healthcare system. In 2023, a total of 58 CPO isolates were collected from 36 patients from Ukraine. According to the Danish Health Authority, Denmark has received a total of 101 patients from Ukraine as part of medical evacuations in 2023. More than one isolate was included from individual patients, if the isolates belonged to different bacterial species and/or had different carbapenemases. Among the 58 CPO, 43 were CPE isolates, seven were *Acinetobacter* ssp. and eight were *Pseudomonas* spp. The findings show that the patients originating from Ukraine were colonized and/or infected by many CPO per patient [Stolberg et al. 2023, J Glob Antimicrob Resist. 34:15-17].

Figure 8.17 Place of origin of CPO, 2019-2023 DANMAP 2023



Outbreaks with CPO during 2023

In Denmark, outbreaks caused by CPO in healthcare settings are registered at SSI in a national database (KURS). At SSI, CPO isolates are routinely characterized by whole genome sequencing (WGS). Cluster analysis is conducted on all isolates using cgMLST to detect possible clustering between the CPO isolates. A genomic cluster is defined as at least two isolates (from different patients) belonging to the same cgMLST cluster (or having less than 15 alleles difference between the isolates). Due to the risk of long-term colonization of the patients, cluster analysis is carried out regardless of the time of isolation. When epidemiological investigations can establish a link (e.g., the patients had been at the same hospital ward at the same time) between at least two patients in a genomic cluster, the patients are classified as belonging to a "verified outbreak". When no epidemiological link can be established, the patients related to the genomic cluster are classified as part of a possible outbreak (Materials and methods, section 10.12).

In 2023, a total of 26 CPO outbreaks were registered compared to 17 CPO-outbreaks in 2022 (Table 8.17). In 18 of the outbreaks, it was possible to establish an epidemiological link between the majority of the patients. All, except for two of the outbreaks, epidemiological links were found in healthcare settings, caused by patients sharing the same ward or staying at the same hospital at the same time. Sixteen of the outbreaks have been ongoing for more than two years. Two outbreaks for more than ten years.

In total, 138 new patients were associated with outbreaks in Denmark in 2023. This is an increase compared to 2022 where 78 new patients were affected. Ten patients were part of more than one outbreak. Of the 26 outbreaks registered in 2023, eight new small clusters were identified involving two to five patients each. Five of the new clusters was identified in Region Zeealand, where the intensified screening regime for CPO also identified more CPO outbreaks than previous years.

Table 8.17 Outbreaks of carbapenemase-producing Enterobacterales (CPE) during 2023, n = 26, Denmark DANMAP 2023

Outbreak ID	Year	Patients total	Patients 2023	Carbapenemase	Type of Outbreak	Species (clonal spread)	Regions[1]	Status
Outbreaks of carbapenemase-producing Enterobacterales (CPE)								
41	2012 - 2023	108	15	NDM-1	Clonal/ plasmid	ST18 *C. freundii*	1 / 2 / 3 / 4 / 5	Verified
48	2013 - 2023	38	4	OXA-436/OXA-48	Clonal/ plasmid	ST90 *E. cloacae*/ ST22 *C. freundii*	1 / 4 / 5	Verified
21	2015 - 2023	96	12	NDM-5/OXA-181	Clonal	ST410 *E. coli*	1 /2 / 5	Verified
22	2015 - 2023	15	2	OXA-181	Clonal	ST440 *E. coli*	1 / 2	Verified
42	2015 - 2023	16	3	OXA-48	Clonal	ST65 *C. freundii*	1 / 3 / 5	Verified
47	2015 - 2023	12	1	VIM-2	Clonal	ST111 *P. aeruginosa*	2 / 3	Possible
1066	2017 - 2023	30	12	OXA-48	Clonal	ST91 *C. freundii*	1 / 5	Possible
1070	2017 - 2023	9	3	OXA-48	Clonal	*C. farmeri*	5	Verified
1081	2017 - 2023	6	1	NDM-5/OXA-48	Clonal	ST22 *C. freundii*	1 / 5	Possible
43	2019 - 2023	22	17	OXA-48	Clonal	ST323 *C. freundii*	1 / 5	Verified
1061	2020 - 2023	14	4	OXA-181	Clonal	ST22 *C. freundii*	2	Possible
1062	2020 - 2023	38	24	NDM-5	Clonal/ plasmid	ST79 *E. hormaechei*	National	Verified
1110	2020 - 2023	4	2	OXA-181	Clonal	ST116 *C. freundii*	1	Possible
1089	2021 - 2023	7	1	OXA-244	Clonal	ST131 *E. coli*	2	Verified
1132	2021 - 2023	10	2	NDM-1	Plasmid	*C. freundii*	1	Possible
1107	2022 - 2023	5	3	OXA-181	Clonal	ST636 *C. freundii*	5	Verified
1113	2022 - 2023	6	4	OXA-48	Clonal	ST22 *C. freundii*	5	Verified
1115	2022 - 2023	4	1	NDM-1	Clonal	ST2 *K. oxytoca*	3	Verified
1112	2023	5	5	OXA-244	Clonal	ST10 *E. coli*	1	Verified
1146	2023	5	4	OXA244	Clonal	ST13730 *E. coli*	1 / 5	Possible
1127	2023	3	3	NDM-1/OXA-181	Clonal	ST781 *E. ludwigii*	5	Verified
1133	2023	2	2	OXA-48	Clonal	ST124 *C. freundii*	5	Possible
1131	2023	4	4	OXA-181	Clonal	ST2 *K. oxytoca*	5	Verified
1135	2023	3	3	OXA-181	Clonal	ST36 *K. oxytoca*	5	Verified
1139	2023	4	4	OXA-181	Clonal	ST65 *C. freundii*	5	Verified
Outbreaks of carbapenemase-producing organisms (CPO)								
1129	2023	2	2	OXA-23	Clonal	ST2069 *A. baumannii*	1 / 5	Verified

[1] Regions will be listed as: 1) Capital Region, 2) Central Denmark Region, 3) North Denmark Region, 4) Southern Denmark Region and 5) Zealand Region

Outbreaks with CPO of special interest

The single outbreak affecting most new cases in 2023 was the unusual outbreak with the same unique epitype ST79 *Enterobacter hormaechei* (outbreak ID1062), caused by medical treatment with contaminated dicloxacillin capsules from the same producer. The outbreak was described in the DANMAP report 2022 (Textbox 8.1). Since September 2023, no new cases have been identified in this outbreak.

In 2019, two cases were identified as part of the same cluster of ST323 *Citrobacter freundii* (outbreak ID43). In 2021 and 2022, three more cases were identified as part of the cluster. In 2023, the intensified screening in Region Zealand has identified several new cases, which explains the increase in the number of cases in this outbreak. The screening strategy recommended in the CPO guideline is an important tool, together with the infection control measures (IPC) to control and stop an outbreak due to antimicrobial resistance (AMR).

The ST18 NDM-1-producing *Citrobacter freundii* outbreak (ID41), which started in 2012 in the North Denmark Region, continued in 2023 [Hammerum et al. 2016, J. Antimicrob. Agents, 71:3117-3124]. Since 2012, the outbreak has now spread to all five Danish regions. The main reason for the spread between regions is the movement of infected patients between regions. By the end of 2023, a total of 108 cases were involved in this outbreak. In 2023, 15 new cases were identified, a decrease from the previous year when 20 new cases were identified. None of the cases had a travel history.

Since 2015, another large outbreak (ID21) has been ongoing, primarily in two regions of the Zealand Region and in the Capital Region. The outbreak is associated with the spread of ST410 NDM-5/OXA-181 *E. coli*. [Roer et al. 2018, mSphere;3(4)]. By the end of 2023, a total of 96 cases have been identified as part of this outbreak. During 2023, 12 new cases were detected to be part of this outbreak. With the exception of the initial cases reported in 2015, who had been traveling to Egypt [Overballe-Petersen et al. 2018, Genome Announc.6(5): e01542-17] none of the cases had a history of travel.

Conclusion

During 2023 the numbers of CPO in Denmark continued to increase. The number of patients received from Ukraine are contributing to this increase, but the main contributing factor is a general increase in CPO at Danish hospitals and the increase in numbers of CPE in nosocomial outbreaks. Furthermore, intensified screening, particularly in one region, also contributed to the increase in CPE cases in 2023.

The national outbreak caused by medical treatment with Dicloxacillin capsules from the same producer also contributed to the number of new outbreak-patients in 2023.

The number of new nosocomial detected outbreaks in 2023 has increased since 2022. For the first time 10 patients were involved in more than one outbreak. The number of patients belonging to the largest outbreaks in hospital settings continued to increase. This highlight the importance to start early interventions with infection prevention control (IPC) in order to prevent further spread of CPO in hospitals and between hospitals.

Travel outside the Nordic countries is a contributing factor to the number of CPO isolates detected in Denmark. The most frequently reported travel destinations in 2023 were South Eastern Europe and Asia. The number of cases where no travel information is provided is high.

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 infection, which makes outbreak control difficult and have consequences for medical treatment.

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8.3.3 Vancomycin-resistant/vancomycin-variable enterococci

Background

Enterococci are intrinsically resistant to a number of first-line antimicrobial agents, including cephalosporins, which increases the risk of enterococci being selected for in-hospital environments and emerging in patients that receive cephalosporins for other conditions. Most hospital-acquired *Enterococcus 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 within the last decade, both internationally as well as in Denmark. Newer antibiotics such as linezolid and daptomycin can be used for treatment of VRE, but both antimicrobial agents may lead to adverse events, and the development of resistance, particularly against linezolid, is relatively common and has also been reported against daptomycin. In recent years, isolates of phenotypically vancomycin-susceptible *E. faecium* have been described to harbor a *vanA*-gene complex, in various countries. These isolates are referred to as vancomycin-variable enterococci (VVE). It has been demonstrated that VVE retain the ability to become vancomycin resistant upon exposure to vancomycin [Patel 2018 – PLOS one], and are often associated with nosocomial outbreaks. However, VVE cannot be selectively cultured on vancomycin-containing media and can only be detected by molecular methods. In 2016, a new VVE clone belonging to ST1421- CT1134, displaying variable vancomycin susceptibility due to a deletion in the *vanX* gene, was detected. [TA Hansen *et al.*, J. Antimicrob. Agents, 2018, 73: 2936–2940]. The VVE clone has spread to all five Danish regions [Hammerum *et al.* Euro Surveill. 2024;29(23)].

Conclusively, VVE may be of clinical relevance and timely detection as well as continuous monitoring are critical to avoid treatment failure and further transmission.

Surveillance of VRE/VVE

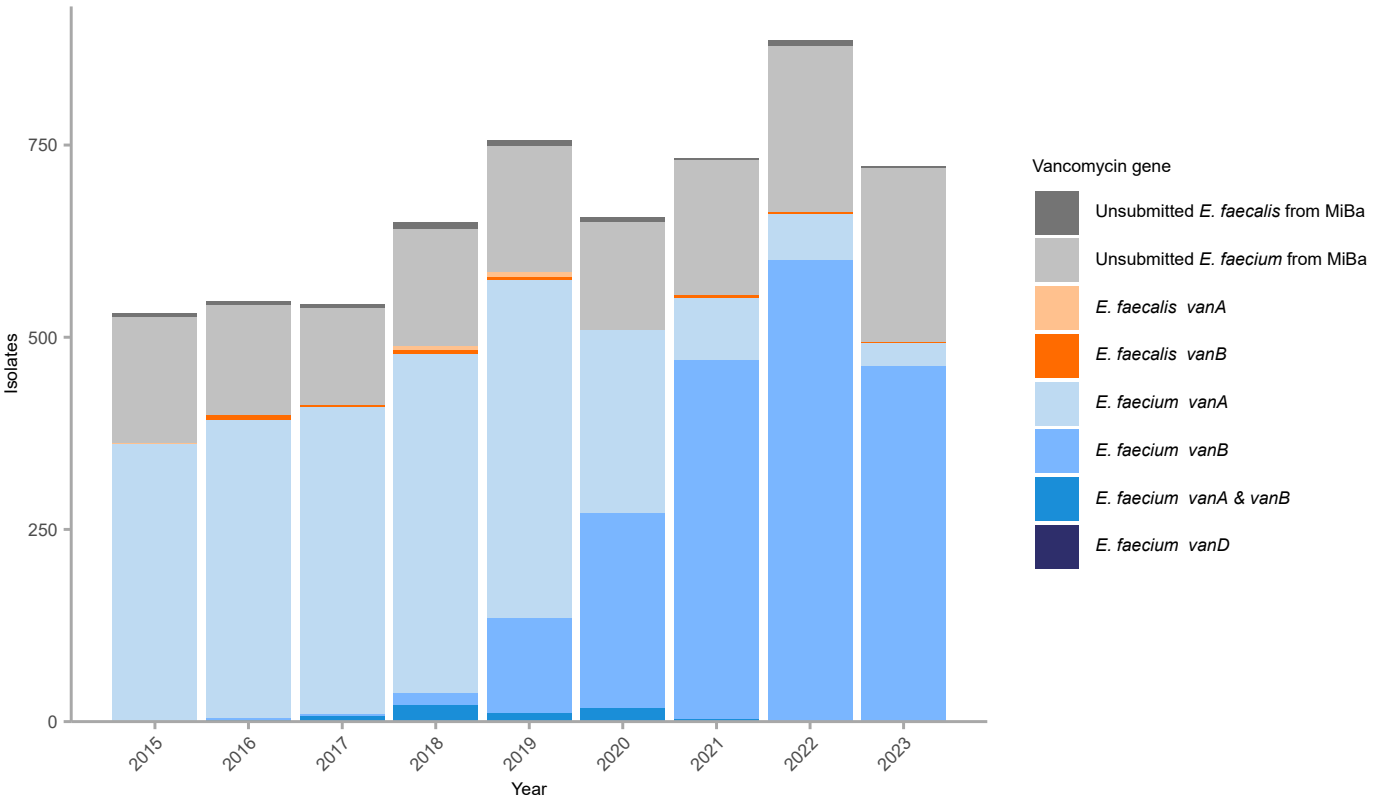
Since 2005, Danish departments of clinical microbiology (DCM) have voluntarily submitted clinical VRE (one VRE/VVE isolate per patient, isolated within 12 months) for species identification, genotyping and surveillance purposes to the Antimicrobial Resistance Reference Laboratory at Statens Serum Institut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

Since 2015, the received clinical VRE/VVE isolates have been analysed using whole-genome sequencing (WGS). The WGS data are used for in silico genotyping of isolate characteristics such as species identification, multilocus sequence typing (MLST), core genome sequence typing (cgMLST) and detection of van-genes and core locus MLST (cgMLST) used for clonal detection (see Section 10.13.3).

To determine any underreporting in the submissions of VRE isolates, the number of VRE/VVE submitted to SSI since 2015 were compared to data from clinical VRE reported by the DCM to MiBa (the Danish Microbiology Database). These isolates were not genotyped, yet they are included in Figure 8.18. This comparison showed that the number of submitted VRE/VVE isolates to SSI was not complete (Figure 8.18). The MiBa isolates were distributed evenly throughout the study period and it did not seem to be a systematic loss. In 2023, 494 VRE/VVE isolates were submitted to SSI, and were genotyped by WGS. Furthermore, 228 VRE/VVE isolates were identified in MiBa, which had not been submitted to SSI and hence have not been genotyped. This was a decrease compared to 2022, were 662 VRE/VVE isolates were sent to SSI, while 224 VRE/VVE isolates were identified in MiBa yet not submitted to SSI. (Figure 8.18).

Figure 8.18 Overview and distribution of vancomycin resistance genes in vancomycin-resistant isolates, Denmark, 2015-2023

DANMAP 2023



Of the 494 clinical VRE/VVE isolates sequenced in 2023, 31 were *vanA E. faecium*, 462 vanB *E. faecium*, and 1 *vanB E. faecalis* (Figure 8.18). Until 2020, *vanA E. faecium* were most common, but during the last years this has changed. In 2023, 93,7% of the *E. faecium* isolates had the *vanB* gene.

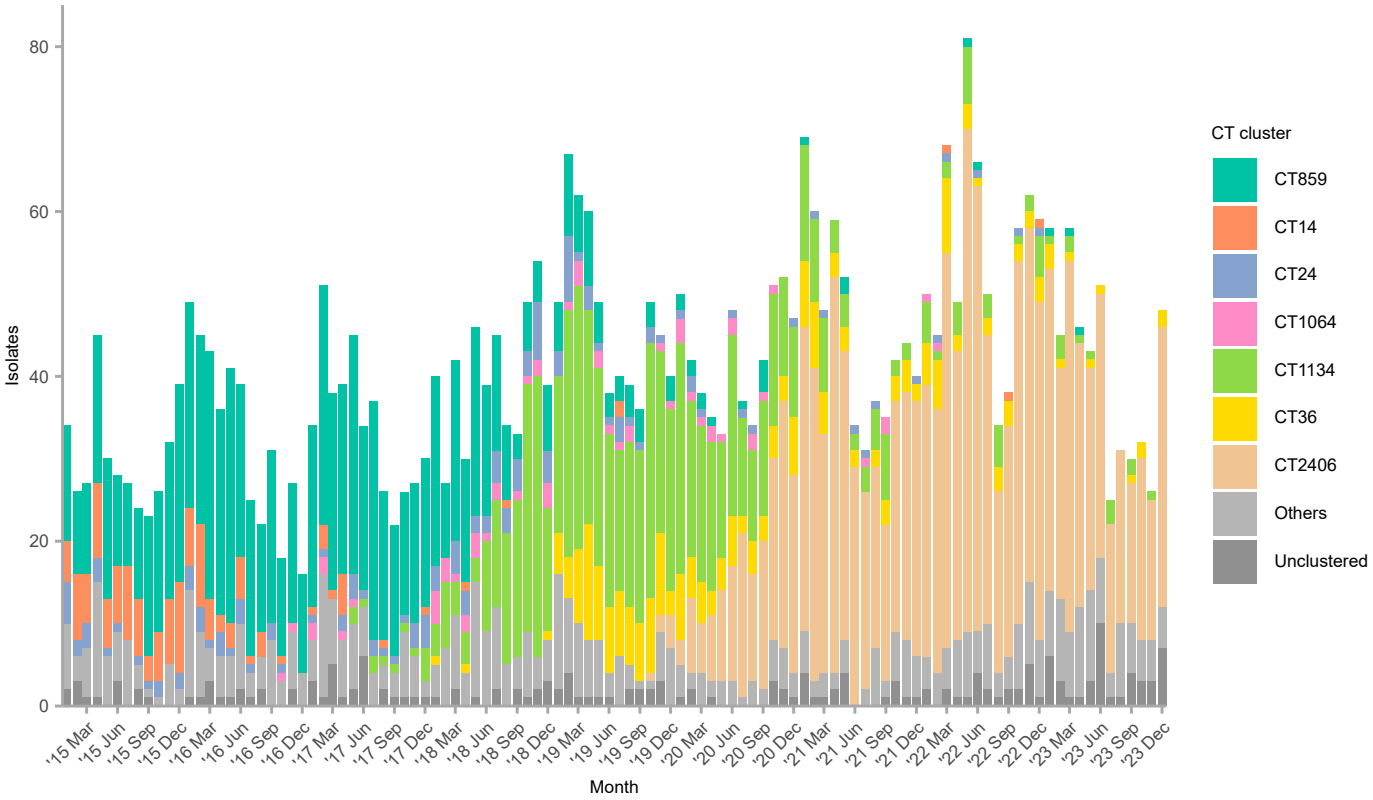
WGS-based cgMLST analysis was performed on the 494 *E. faecium* isolates using SeqSphere+ (Ridom). The 494 *E. faecium* isolates belonged to unique 113 CT clusters. When investigating the composition of sequence types (ST) and clonal types (CT) for *E. faecium*, we observed a clustering tendency between isolates, where ST's and CT's were diverging while the allelic differences were minimal within each cluster. MLST clusters were set up using the MLST algorithm of SeqSphere+, setting the maximal allelic distances to 20. The top seven complex type clusters were devised, based on clustering of the isolates. Each complex type cluster were named according to the ST and CT of the earliest observed isolate within each cluster. One clonal group (covering several different CTs but presumably originating from the same clone) were predominant: The ST80-CT2406 *vanB E. faecium* group containing 332 isolates (Table 8.18).

Table 8.18 Description of the most common types of *vanA* and/or *vanB Enterococcus faecium* according to MLST and cgMLST, Denmark, 2015-2023

DANMAP 2023

Complex type	2015		2016		2017		2018		2019		2020		2021		2022		2023		All years	
Cluster	(n = 361)		(n = 392)		(n = 409)		(n = 478)		(n = 574)		(n = 509)		(n = 551)		(n = 660)		(n = 493)		(n = 427)	
ST203-CT859	179	50%	249	64%	260	64%	153	32%	57	10%	12	2%	3	0,5%	2	0,3%	3	0,6%	918	21%
ST80-CT14	85	24%	37	9%	12	3%	2	0,4%	2	0,3%	0	0,0%	0	0%	3	0,5%	0	0%	141	3%
ST117-CT24	21	6%	18	5%	20	5%	39	8%	25	4%	8	2%	6	1%	5	0,8%	0	0%	142	3%
ST80-CT1064	0	0%	2	0,5%	6	1%	23	5%	12	2%	14	3%	3	0,5%	2	0,3%	0	0%	62	1%
ST1421-CT1134	0	0%	0	0%	13	3%	161	34%	285	50%	196	39%	63	11%	35	5%	14	3%	767	17%
ST117-CT36	0	0%	0	0%	0	0%	3	0,6%	94	16%	54	11%	43	8%	41	6%	12	2%	247	6%
ST80-CT2406	0	0%	0	0%	0	0%	0	0%	7	1%	178	35%	370	67%	476	72%	332	67%	1363	31%
Other clusters	63	17%	72	18%	74	18%	83	17%	73	13%	37	7%	45	8%	73	11%	89	18%	609	14%
Unclustered	13	4%	14	4%	24	6%	14	3%	19	3%	10	2%	18	3%	23	3%	43	9%	178	4%

Figure 8.19 Timeline of the clonal group prevalence in all sequenced VRE isolates. Clonal groups are named according to sequence type and clonal type of the earliest observed member, Denmark, 2015-2023 DANMAP 2023



From 2015 to 2023, seven *E. faecium* clusters dominated: ST80-CT14 *vanA*, ST117-CT24 *vanA*, ST203 -CT859 *vanA*, ST1421-CT1134 *vanA*, ST80-CT1064 *vanA-vanB*, ST117-CT36 *vanB* and ST80-CT2406 *vanB* [Hammerum et al. Euro Surveill. 2024;29(23)]. Figure 8.19 shows the monthly count of these seven clusters alongside the remaining clusters during the period 2015 to 2023.

We detected ST80-CT14 *vanA E. faecium* in all Danish Regions during 2015. On a national level, the numbers of ST80-CT14 *vanA E. faecium* decreased from 2016 to 2018. In 2022, only a few isolates ST80-CT14 *vanA E. faecium* were detected. ST117-CT24 *vanA E. faecium* was first detected in the Capital Region in January 2015. Between 2015 and 2022 it was detected in all five regions. ST203-CT859 *vanA E. faecium* was first detected in Denmark in December 2014. It was one of the dominating clones from 2015 through July 2018 and was detected in all five regions. In 2022, only a few isolates belonging to ST203-CT859 *vanA E. faecium* were detected. ST80-CT1064 *vanA-vanB E. faecium* was first detected in Central Denmark Region in October 2016. It spread to the North Jutland Region during April 2018. During 2019, a single case of ST80-CT1064 *vanA-vanB E. faecium* was detected. This clone disappeared during January 2022. *E. faecium* isolates belonging to ST1421-CT1134 *vanA E. faecium* (VVE cluster) were first detected in clinical samples in 2016. In 2018, 34% of the *E. faecium* isolates belonged to ST1421-CT1134, and they were mostly detected in The Capital Region. During 2019, ST1421-CT1134 *vanA E. faecium* was the most prevalent type (44%) but decreased during 2021 and 2022. ST117-CT36 *vanB E. faecium* was detected in January 2019 in Capital Region of Denmark. During 2019, ST117-CT36 vanB *E. faecium* was detected in all five Danish Regions. In October 2019, the first clinical ST80-CT2406 *vanB E. faecium* sample was detected in a patient hospitalised in the Capital Region. It spread further to other patients in the Capital Region in 2019. During 2020, this cluster was detected in all Danish regions except the Northern Region of Denmark but during 2021, it was also detected there. [Hammerum et al. Euro Surveill. 2020;25(32)]. Since 2022, the ST80-CT2406 have been and continues to be, the main dominant complex type cluster in all Danish regions. [Hammerum et al. Euro Surveill. 2024;29(23)].

Infection prevention and control guidelines for VRE

Acting in compliance with the national guidelines for infection prevention and control (published by National Centre for Infection Control at SSI) is key in preventing the spread of multidrug-resistant microorganisms, with correct hand hygiene and correct use of personal protective equipment (PPE) among the most important control measures [https://hygiejne. ssi.dk/NIRgenerelle]. The supplemental national guideline for infection prevention and control includes specific guidance on VRE/VVE and should be followed when examining and treating patients, residents and citizens with multidrug-resistant microorganisms [https://hygiejne.ssi.dk/NIRsupplerende]. Precautions include the use of PPE such as a disposable gown and gloves

and proper cleaning of the environment [https:// hygiejne.ssi.dk/NIRdesinfektion]. Moreover, the guidelines emphasise the importance of prescribing antibiotics with caution. According to the national supplemental infection prevention control (IPC) guideline, screening of a patient is recommended on admission to hospital if the patient: 1) is transmitted from a hospital outside the Nordic countries, 2) has been hospitalised outside the Nordic countries within the last 6 months, 3) has been hospitalised in wards in Denmark or another Nordic country with outbreaks of VRE within the last 6 months or 4) previously has been diagnosed with a VRE-infection or carrier state. Isolation is recommended in the national supplemental IPC-guideline in case of verification of VRE in the patient.

Conclusion

The number of VRE/VVE cases has decreased from 2022 to 2023 to a level that is comparable to 2021. While the decrease is a desired development, the level of VRE infections is still high. Therefore, more prevention strategies are required to prevent spread of VRE in the Danish health care system.

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8.3.4 Detection of linezolid-vancomycin resistant enterococci

Background

Linezolid is an antimicrobial belonging to the oxazolidinones. Its indication of use are nosocomial pneumonia and complicated skin and soft tissue infections caused by Gram-positive bacteria. It belongs to the defined last line choices and should be used with caution, based on microbiological testing and only if other antibiotics are not available. In Denmark it is primarily used in combination treatments for patients with very complicated Gram-positive infections and as treatment against 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.

Particular interest has been paid to the transferable resistance genes and these are monitored in the Danish surveillance system due to a potential risk of a shared pool of resistance genes with enterococci stemming from animals that have been treated with pleuromutilines, a group of antimicrobials related to the oxazolidinones.

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-vancomycin-resistant Enterococci (LVRE)

In order to detect linezolid-resistant VRE isolates (LVRE), the LRE-Finder was implemented in the automated surveillance at SSI for detection of LRE mutations and LRE genes in genomes from VRE isolates submitted by the DCM directly for the national VRE Surveillance. During the period 2015 to 2023, no linezolid vancomycin resistant *E. faecalis* were detected, whereas 48 linezolid-vancomycin resistant *E. faecium* were identified.

In 2023, nine linezolid-vancomycin resistant *E. faecium* were identified. All nine LVRE *E. faecium* had the G2576T mutation and were all positive for *vanB* (Table 8.19).

Conclusion

As in previous years the numbers of LVRE have been low, however the findings are of concern as linezolid is important for the treatment of VRE. Often, only few antimicrobial agents are available for treatment of infections with LVRE.

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Table 8.19 Characterization of the nine linezolid vancomycin resistant enterococci (LVRE), 2023, Denmark DANMAP 2023

Linezolid genotype	Vancomycin gene	2015 (n = 1)	2016 (n = 2)	2017 (n = 0)	2018 (n = 2)	2019 (n = 5)	2020 (n = 4)	2021 (n = 10)	2022 (n = 15)	2023 (n = 9)	All years (n = 48)
G2576T	*vanA*	1	2	0	2	0	4	0	5	0	14
G2576T	*vanB*	0	0	0	0	1	0	9	10	9	29
G2576T & *optrA*	*vanA*	0	0	0	0	0	0	1	0	0	1
cfrB	*vanB*	0	0	0	0	1	0	0	0	0	1
optrA	*vanA*	0	0	0	0	3	0	0	0	0	3

8.3.5 *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.

Surveillance of *S. aureus* bacteraemia

The number of *S. aureus* bacteraemia cases were 2,571 in 2023 corresponding to 43 cases per 100,000 inhabitants. This is almost the same number as in 2022 (2,578). Thirty-nine (1.5%) of the bacteraemia cases were caused by MRSA. During the last decade this proportion has been between 1.5% and 2.9% (2014) and remains below most other European countries participating in EARS-Net [EARS-Net 2022]. LA-MRSA CC398 caused nine of the 39 MRSA bacteraemia cases. Within 30 days from the bacteraemia onset, 617 (24%) patients died (all-cause mortality). The mortality for the MRSA bacteraemia cases was 28%.

The antimicrobial susceptibility remained at the same level as the previous years for most agents (Table 8.20). Resistance to penicillin in 2023 was 68%, which confirms the decreasing trend since the beginning of the 1990s, where resistance to penicillin was around 86%. The highest frequency of resistance to antimicrobials other than penicillin was observed for fusidic acid (12%), erythromycin (9%) and clindamycin (8%).

Typing revealed a high diversity with 764 different *spa* types distributed in 30 different clonal complexes (CCs). The ten most prevalent *spa* types, representing 31% of the total number, are presented in Table 8.21 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 38 (1.5%) cases of which eight were MRSA. The 38 isolates with the PVL gene were distributed among 24 different *spa* types.

Surveillance of methicillin-resistant *S. aureus*

In 2023, 3,649 MRSA cases were detected (62 per 100,000 inhabitants), a 20% increase compared to 2022 (2,982; Figure 8.20a), reflecting the return to normality after covid-19. 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). Infections constituted 55% of the cases. The proportion of infections in the years 2014 to 2023 has varied between 38% to 55% (Figure 8.20b).

Table 8.20 Resistance (%) in isolates from *Staphylococcus aureus* bacteraemia cases 2014-2023, Denmark DANMAP 2023

	2014	2015	2016	2017	2018	2019	2020*	2021	2022	2023
Antimicrobial agent	%	%	%	%	%	%	%	%	%	%
Methicillin	2.9	1.5	2.1	2.2	1.6	2.1	1.6	1.6	1.9	1.5
Penicillin	77	71	71	72	72	72	72	69	68	68
Erythromycin	8	7	7	6	5	9	7	7	9	9
Clindamycin	8	7	6	5	4	8	7	7	8	8
Tetracycline	5	4	3	3	3	2	3	2	3	2
Fusidic acid	15	16	12	14	17	14	14	13	13	12
Rifampicin	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Moxifloxacin#	6	6	4	4	4	5	6	4	4	4
Kanamycin	2	3	1	1	2	<1	nt	nt	nt	nt
Linezolid	0	0	0	0	0	0	0	0	0	0
Mupirocin	<1	<1	0	<1	0	<1	<1	<1	<1	<1
Trimethoprim-sulfamethoxazole	1	<1	<1	<1	0	<1	<1	<1	<1	<1

Notes: nt = not tested. * From 2020 and onwards, resistance data was extracted from the Danish Microbiology database, MiBa. # Prior to 2021 testing for fluoroquinolone resistance was reported for norfloxacin

Table 8.21 The ten most prevalent *spa* types demonstrated in SAB and in non-LA-CC398 MRSA cases, Denmark 2023 DANMAP 2023

SAB			MRSA			
spa type	CC group	No. of cases	*spa* type	CC group	No. of cases	No. causing infections (%)
t127	CC1	125	t304	CC8	263	58
t084	CC15	116	t127	CC1	188	53
t091	CC7	87	t008	CC8	162	69
t230	CC45	80	t223	CC22	149	54
t002	CC5	76	t002	CC5	136	61
t021	CC30	69	t4549	CC8	129	83
t012	CC30	68	t005	CC22	84	69
t008	CC8	65	t688	CC5	82	70
t1451	CC398	61	t1476	CC8	62	63
t701	CC8	44	t272	CC121	47	77

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

Figure 8.20a Number of new MRSA cases 1994-2023, Denmark, with a three years moving average DANMAP 2023

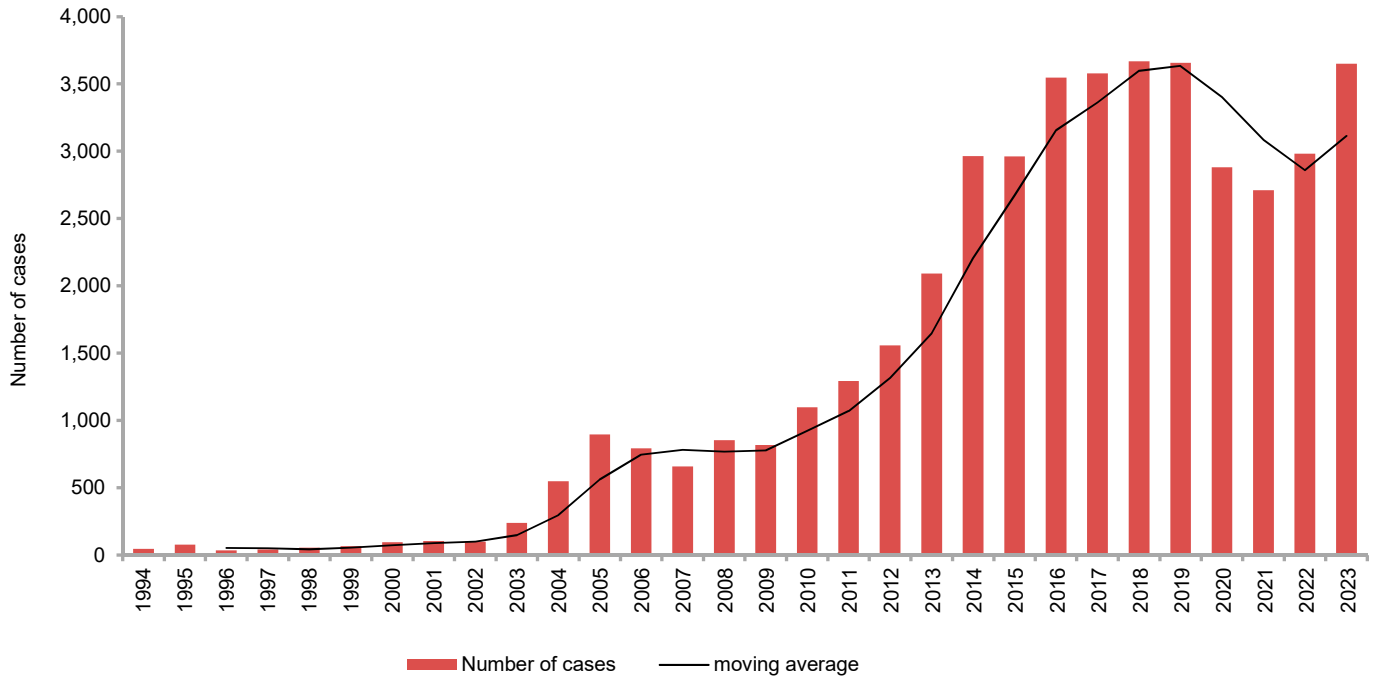
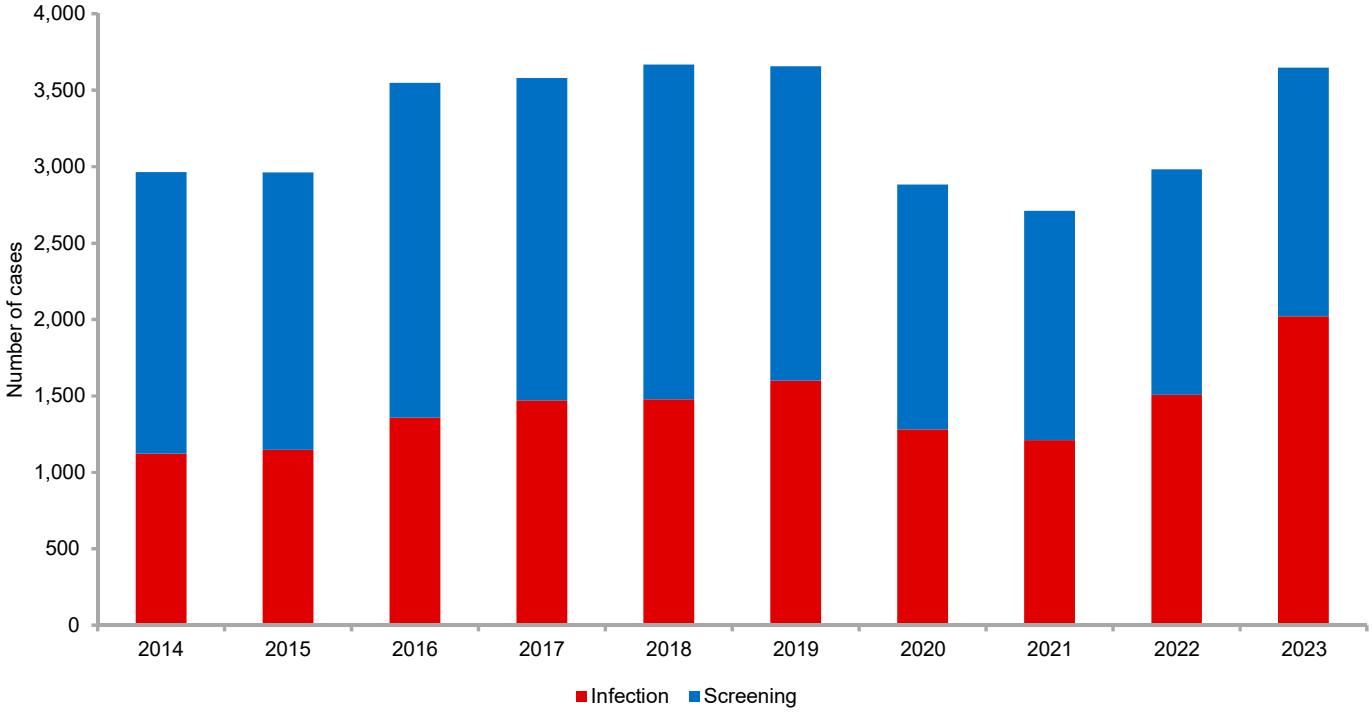


Figure 8.20b Number of new MRSA cases 2014-2023, Denmark, divided in infection and screening samples DANMAP 2023



CC398 cases constituted 25% (n = 897) of new MRSA cases, of which 856 belonged to the livestock-associated clone (LA-MRSA CC398) and the remaining 41 to a human adapted variant harbouring the PVL encoding genes. More LA-MRSA CC398 isolates (72%) were found in healthy carriers compared to MRSA of other types (45%), which likely reflects the active screening of patients with contact to livestock at admission to healthcare.

MRSA isolates carrying *mecC* were detected in 58 cases (1.6%). Forty-five of the cases (78%) had infections at the time of diagnosis. Three patients reported contact to hedgehogs, which recently has been shown to be a major environmental reservoir for *mecC* MRSA. One patient worked with livestock, one patient had a family member working with livestock, while the remaining 50 patients did not report any contact to livestock or other animals.

spa typing revealed 410 different strain types, not including isolates belonging to LA-CC398. Among the infections, 299 *spa* types were identified. The 10 dominating non-LA-CC398 *spa* types isolated in 2023 are listed in Table 8.21. They constituted 47% of the total number of non-LA-CC398 MRSA isolates. Table 8.21 does not list *spa* types in CC398, which for many years has been the dominating CC. *spa* types t304 and t223 have been among the five most prevalent *spa* types since 2016, when their increase was linked to the refugee crisis following the civil war in Syria. This year *spa* type t127 was the second most numerous. This type has been involved in several outbreaks in neonatal units in recent years.

The PVL encoding gene was detected in 26% of the infections and in 12% of the asymptomatic carriers and most often in relation to isolates with *spa* types t008 (n = 90), t005 (n = 73), t355 (n = 45), t034 (n = 32) and t021 (n = 31).

Forty-one MRSA outbreaks were registered at hospitals, nursing homes and other institutions. These outbreaks comprised a total of 199 cases of which 80 had an infection. Seven of the outbreaks occurred in neonatal departments, comprising a total of 48 cases. Additionally, eight outbreaks were registered in other hospital departments, comprising 18 patients and thirteen outbreaks were observed in nursing homes (counting a total of 29 residents).

The total number of cases and the number of cases presenting with infection according to epidemiological classification are shown in Table 8.22. Most of the cases (86%) were acquired in Denmark.

The trend of MRSA infections for 2014-2023 based on their epidemiological classification is shown in Figure 8.21. The number of infections in community-acquired cases increased in 2023 when compared to 2022, and the number of infections among imported cases were also higher. Importantly, number of infections among hospital-acquired MRSA remained low in 2023 (59 cases).

Resistance among MRSA isolates

Resistance data for non-LA-CC398 isolates are presented in Table 8.23. Resistance prevalences were similar to previous years, with relatively high resistance to erythromycin (32%), fusidic acid (26%), clindamycin (21%), tetracycline (21%) and moxifloxacin (24%), and low resistance (<1%-1) to trimethroprim-sulfamethoxazole, linezolid, mupirocin and rifampicin.

Table 8.22 Epidemiological classification of new MRSA cases, Denmark 2023 DANMAP 2023

Epidemiologic classification	Exposure	No. of cases (% of total)	No. (%) of cases with infections (% of cases in same epi. class)
Imported (IMP)		**573 (16)**	433 (76)
Hospital-acquired (HA)		**102 (3)**	44 (43)
Health-care associated, community onset (HACO)		**281 (8)**	
	with known exposure	43	27 (63)
	without known	238	215 (90)
Health care worker		**33 (1)**	15 (45)
Community-acquired (CA)		**1,799 (49)**	
	with known exposure	808	144 (18)
	without known	991	904 (91)
LA-MRSA CC398		**856 (23)**	
	with known exposure	691	119 (17)
	without known	165	117 (71)
Total		**3,649**	**2,020 (55)**

Note: Numbers shown in bold are totals

Figure 8.21 Number of MRSA infections according to epidemiological classification, 2014-2023, Denmark DANMAP 2023

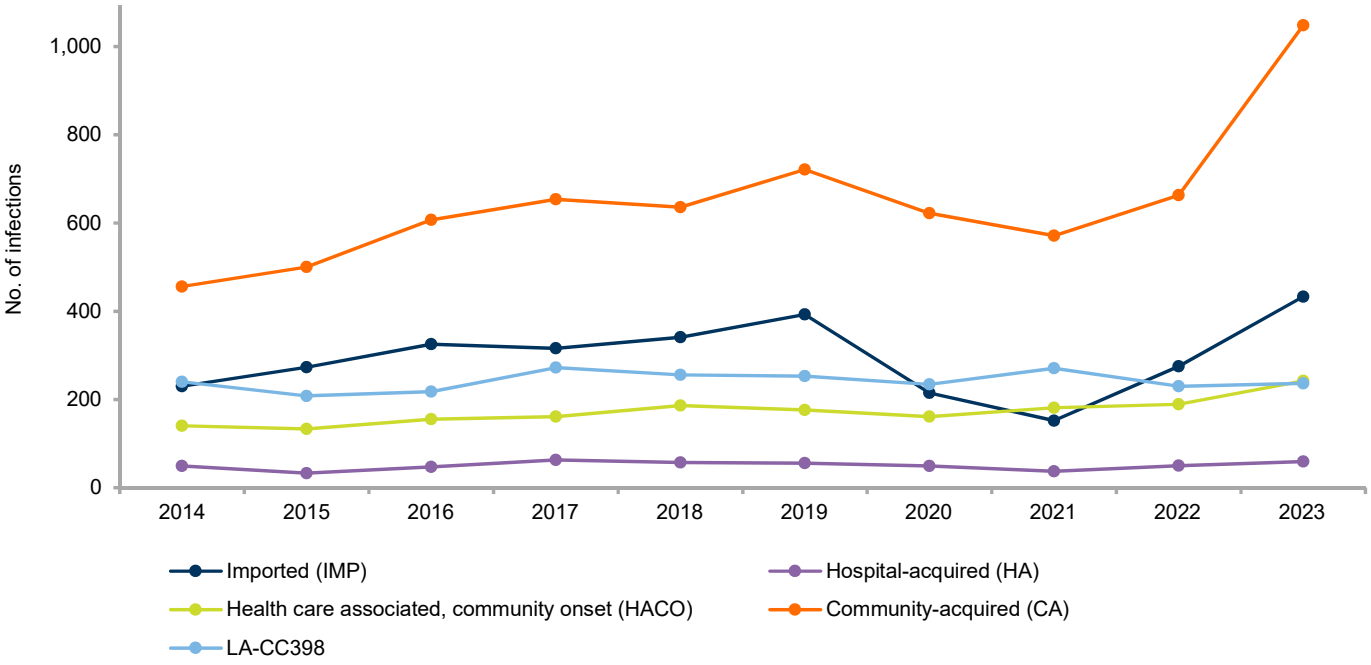


Table 8.23 Resistance (%) in non LA-CC398 MRSA isolates, 2014-2023, Denmark DANMAP 2023

	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023
Erythromycin	33	37	34	34	33	33	30	29	34	32
Clindamycin	23	29	25	27	28	23	22	19	21	21
Tetracycline	21	24	26	24	26	22	22	21	23	21
Fusidic acid	17	19	18	16	18	23	22	21	22	26
Rifampicin	<1	<1	1	1	1	<1	<1	<1	<1	1
Moxifloxacin#	27	21	19	20	21	21	17	19	23	24
Linezolid	<1	0	<1	0	<1	0	<1	<1	<1	<1
Mupirocin	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Trimethoprim-sulfamethoxazole	3	4	2	3	3	4	2	<1	1	1
Number of tested isolates	1,616	1,242	1,184	1,193	1,233	1,025	1,920*	1,520*	2,043*	2,696*

*Not all isolates were tested for all listed antimicrobials
Prior to 2021 testing for fluoroquinolone resistance was reported for norfloxacin

Conclusion

The number of *S. aureus* bacteraemia cases was 2,571 in 2023 and at the same level as in 2022. Of these, 39 cases (1.5%) were caused by methicillin-resistant *S. aureus* (MRSA) with nine being livestock-associated-MRSA (LA-MRSA). Resistance to penicillin continued to decrease and was 68% in 2023. There were 3,649 cases of MRSA from both screening (45% of cases) and infections (55%), which was a 20% increase compared to 2022. Forty-one MRSA outbreaks were registered at hospitals, nursing homes and other institutions for a total of 199 cases with 80 being infections.

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8.3.6 *Streptococcus pneumoniae*

Background

Streptococcus pneumoniae is known to cause various diseases that can be classified into two main groups: non-invasive and invasive diseases. Among these, invasive pneumococcal diseases (IPD) are considered the most severe, while non-invasive pneumococcal infections are more commonly observed. Pneumonia, which can be categorized as non-invasive or invasive, is estimated to be the leading cause of pneumococcal disease worldwide, affecting both children and adults. This is followed by the severe cases of the invasive disease's bacteraemia and meningitis. Pneumococcus often causes the non-invasive acute otitis media (AOM) in children, with more than half of Danish children estimated to experience this condition. AOM is frequently treated with antibiotics, although their effectiveness is uncertain. In addition to these well-known pneumococcal-related diseases, pneumococci are also associated with other common infections such as non-invasive sinusitis and bronchitis, as well as invasive diseases like endocarditis (infection of the heart valves), peritonitis, and septic arthritis.

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

Results

In Denmark, 635 cases of invasive pneumococcal disease (IPD) were registered in 2023 (Table 8.24). Pneumococci were mainly found in either blood (571) or cerebrospinal fluid (48). For 16 cases, pneumococci were found in other, normally sterile sites. Earlier, only IPD cases from blood and cerebrospinal fluid, were included in the DANMAP report. This year, we have included all IPD cases, in the report. Of the 635 IPD cases identified in MiBa, 597 isolates were received at the reference laboratory. Data for the 35 remaining cases, where isolates were not provided, were retrieved from MiBa and serotypes for these cases are unknown. Failure of submitting an isolate for serotyping was mainly due to non-viable isolates or diagnosis through PCR. In total, data for serotypes and antimicrobial susceptibility data for penicillin were available for six cases.

The IPD isolates belonged to 39 different serotypes. For the 616 cases with available penicillin susceptibility data, 580 isolates were susceptible to penicillin (94.0%), 30 isolates (4.9%) were classified as non-susceptible to penicillin. Of note, one IPD isolate obtained from cerebrospinal fluid was penicillin resistant with a MIC of 2 mg/L (serotype 9N).

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 8.22), 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. The predominant serotype in 2023 was serotype 3 (20.9%) (Table 8.24). All 133 serotype 3 isolates were susceptible to penicillin. The second and third most frequently isolated serotypes are serotype 8 (84 isolates in 2023) and serotype 22F (69 isolates in 2023), and these isolates were all fully sensitive to penicillin.

Conclusion

The level of penicillin non-susceptible IPD isolates in 2023 (6%) increased slightly compared to 2022 (5%). Both the great fluctuation in the prevalence of penicillin non-susceptible IPD isolates and the general IPD incidence in the years 2020 and 2021 is assumed to be an effect of the COVID-19 restrictions on the general prevalence of infectious diseases associated with respiratory droplet transmission. Such fluctuations have been observed in other countries as well [Shaw, et al., Lancet Digit Health. 2023 Sep;5(9):e582-e593].

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Table 8.24 Number of invasive pneumococcal isolates (IPD) observed in Denmark, 2023 DANMAP 2023

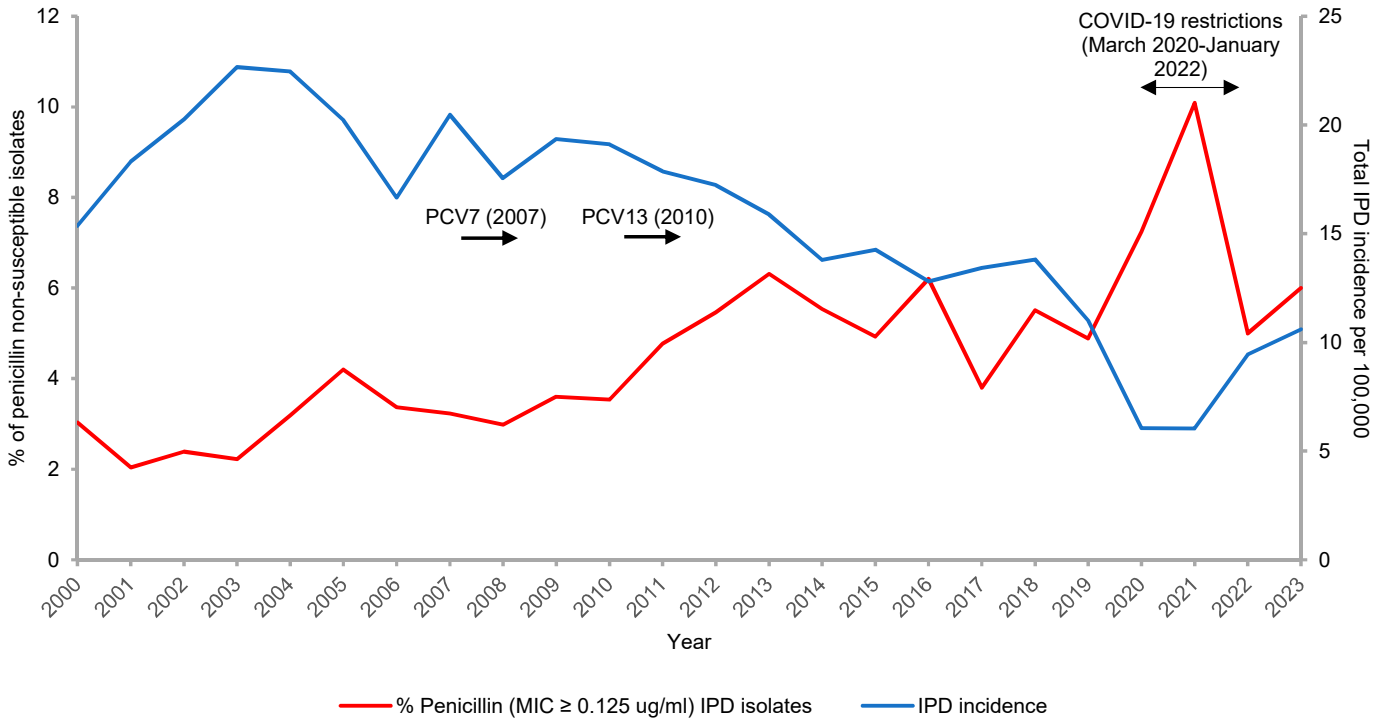
Serotype	Included in pneumococcal vaccines	N 2023	PEN-S	PEN-NON-S	Unknown	% Suscep
Unknown		38	18	1	19	*95%
4	PCV13, PCV15, PCV20, and PPV23	3	2	1	0	67%
6B	PCV13, PCV15, PCV20, and PPV23	1	0	1	0	0%
9V	PCV13, PCV15, PCV20, and PPV23	0	0	0	0	0%
14	PCV13, PCV15, PCV20, and PPV23	1	1	0	0	100%
18C	PCV13, PCV15, PCV20, and PPV23	0	0	0	0	0%
19F	PCV13, PCV15, PCV20, and PPV23	7	1	0	0	14%
23F	PCV13, PCV15, PCV20, and PPV23	1	1	0	0	100%
1	PCV13, PCV15, PCV20, and PPV23	0	0	0	0	0%
3	PCV13, PCV15, PCV20, and PPV23	133	133	0	0	100%
5	PCV13, PCV15, PCV20, and PPV23	0	0	0	0	0%
6A	PCV13, PCV15, and PCV20	2	1	1	0	50%
7F	PCV13, PCV15, PCV20, and PPV23	0	0	0	0	0%
19A	PCV13, PCV15, PCV20, and PPV23	17	15	2	0	88%
2	PPV23	0	0	0	0	0%
8	PCV20 and PPV23	84	84	0	0	100%
9N	PPV23	35	32	3	0	91%
10A	PCV20 and PPV23	17	17	0	0	100%
11A	PCV20 and PPV23	14	14	0	0	100%
12F	PCV20 and PPV23	6	6	0	0	100%
15B	PCV20 and PPV23	10	10	0	0	100%
17F	PPV23	4	1	3	0	25%
20	PPV23	5	5	0	0	100%
22F	PCV15, PCV20, and PPV23	69	69	0	0	100%
33F	PCV15, PCV20, and PPV23	7	7	0	0	100%
24F		29	28	1	0	97%
15A		19	15	4	0	79%
23A		17	16	1	0	94%
38		17	16	1	0	94%
35F		16	16	0	0	100%
7C		12	12	0	0	100%
23B		11	4	7	0	36%
35B		10	10	0	0	100%
15C		10	10	0	0	100%
16F		8	6	2	0	75%
31		8	8	0	0	100%
6C		5	5	0	0	100%
10B		5	5	0	0	100%
17A		3	2	1	0	67%
34		2	1	1	0	50%
21		2	2	0	0	100%
24B		2	2	0	0	100%
35D		2	2	0	0	100%
16A		1	1	0	0	100%
33C		1	1	0	0	100%
9A		1	1	0	0	100%
Sum		635	580	30	19	*94%

N = number of isolates, PEN-S = Penicillin susceptible, PEN-NON-S = Penicillin non-susceptible, % Suscep = percentage of IPD isolates susceptible to penicillin

* Calculations are based on available isolates

Figure 8.22 The incidence of invasive pneumococcal infection (IPD) and the percentage of penicillin non-susceptible IPD isolates in Denmark, 2000–2023. PCV-7 was introduced into the child vaccination program in 2007, and replaced with PCV13 in 2010

DANMAP 2023



8.3.7 Beta-haemolytic streptococci

Background

Beta-haemolytic streptococci (BHS) are classified 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 soft tissue infections, 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 blood, cerebrospinal fluid or other normally sterile anatomical sites) submitted from the departments of clinical microbiology (DCM) in 2022 to the Neisseria and Streptococcus Reference laboratory (NSR). This report includes only non-duplicate isolates, i.e. isolates obtained with an interval of more than 30 days. Isolates are received from all DCM in Denmark. It is voluntary to submit isolates of BHS.

Infections with BHS are usually treated with penicillins or macrolides. All submitted isolates of BHS were therefore tested for susceptibility to penicillin, erythromycin, and clindamycin as well as inducible clindamycin resistance. Susceptibility testing was performed with disk diffusion methods. If resistance was observed, MIC was determined with Etest. All results were interpreted according to MIC breakpoints issued by EUCAST (http://www.eucast.org/) (version 13.0).

Results

In 2023, a total of 1,651 isolates from invasive cases were received. The number of isolates from unique invasive cases was 1,615, i.e. an increase of 39% compared to 2023 (991). The number of GAS isolates increased by a factor 3.7 compared to 2022, while the number in 2022 had increased by a factor 3.9 compared to 2021. For GBS these ratios were 1.2 and 1.1, respectively. For GCS they were 1.1 and 1.0, respectively, and for GGS they were 1.3 and 1.0.

Figure 8.23 shows the resistance findings for the years 2014 through 2023. All isolates were fully susceptible to penicillin. Comparing GAS in 2023 to 2022, the rates of erythromycin resistance, clindamycin resistance and inducible clindamycin resistance all decreased. For GBS, GCS and GGS these rates all remained nearly unchanged. The percentage of fully susceptible isolates compared to 2022 was increasing for GAS and remained virtually unchanged for the three other serogroups.

Comments and conclusions

The substantial increase from 2021 to 2022 and further in 2023 for GAS was part of the national as well as international surge of invasive and non-invasive GAS infections, including a high proportion of cases with a serious course. The increase in the number of submitted invasive GAS isolates is probably a consequence of discontinued COVID-19 related restrictions on upper respiratory tract colonization and airborne transmission of this species.

The increase cannot be explained by changes in the prevalence of antimicrobial resistance. All isolates were fully susceptible to penicillin. Comparing GAS in 2023 to 2022, the rates of erythromycin resistance, clindamycin resistance and inducible clindamycin resistance all decreased. For GBS, GCS and GGS these rates all remained nearly unchanged.

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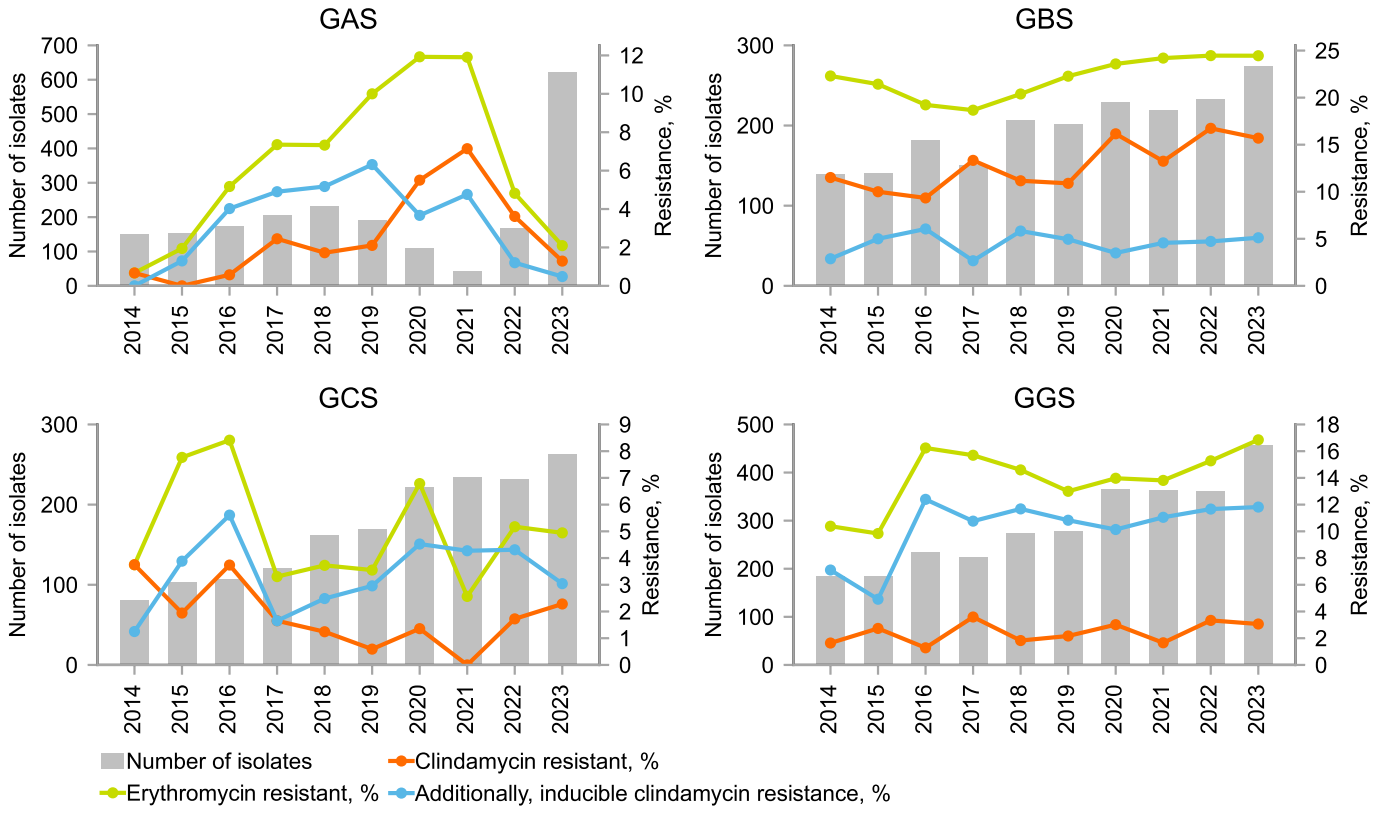
Figure 8.23 Invasive beta-haemolytic streptococci: Antimicrobial resistance testing results, Denmark, 2014-2023 DANMAP 2023



8.3.8 *Neisseria gonorrhoeae*

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

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

Gonococcal Surveillance

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

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

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

Results and discussion

Most of the isolates received in 2023 were from urethra or cervix, while clinicians only rarely obtained specimens from rectum and pharynx. Occasionally, the NSR laboratory receives isolates from other anatomical sites, such as conjunctivae, joint fluid, blood, Bartholin's abscess, etc.

Repetitive detections of gonococci in a patient are considered to represent unique cases of gonorrhoea if separated by more than 21 days. The NSR laboratory received 2,653 isolates from 2,152 unique cases of gonorrhoea diagnosed in 2023 (1,350 males, 802 females). Only one isolate from each unique case is counted in this report.

The annual number of received isolates increased considerably from 2011 through 2017 (Figure 8.24). This was most likely due to 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 also to an increasing incidence of gonorrhoea, especially among young heterosexual persons, among whom an increasing proportion until 2016 were women. The proportion of female cases has decreased slightly since 2017, but it is still substantially higher than in the beginning of the observation period (2003-2016). A decrease in the annual number of isolates from unique cases was seen in 2018 followed by an increase in 2019-2021 and in particular from 2021 to 2023 [Pedersen et al., Euro Surveill. 2024 Feb;29(7):2400059]. It should be recalled, however, that many cases diagnosed by NAATs are not followed-up by culture.

The ciprofloxacin resistance rate was 45% in 2023 (40% in 2022 and 48% in 2021), (Figure 8.24). Only 0.9% of the isolates were classified as ciprofloxacin intermediate (MIC 0.047-0.064 mg/L).

The percentage of strains producing penicillinase was 14% (14% in 2022 and 23% in 2021). Previously, it has fluctuated between 22% in 2005 and 7% in 2016. Azithromycin resistance (MIC above the present ECOFF >1 mg/L) was found in 6% of the tested isolates (2.9% in 2022 and 2.8% in 2021). In 2018, azithromycin resistance was observed in 6% of the isolates and intermediary susceptibility in 4%. However, EUCAST breakpoints were changed as of January 1, 2019 leading to a greater proportion of strains being classified as sensitive, compared to previously, albeit only if used in conjunction with another effective agent.

Ceftriaxone resistant gonococci (MIC >0.125 mg/L) as well as ceftriaxone treatment failure in patients with gonorrhoea have occurred in several countries during recent years. As shown in Figure 8.25, the ceftriaxone MIC distribution changed from 2003 through 2009 with the highly susceptible isolates becoming gradually less frequent. However, since then, this change has completely reversed. In 2017, there was a single case among the submitted isolates with ceftriaxone MIC of 0.25 mg/L (i.e. resistant) and azithromycin MIC of 0.25 mg/L. Secondary cases were not reported and no strains with ceftriaxone resistance have been encountered among the submitted isolates since 2017.

The Danish National Board of Health issued guidelines in 2015 for the diagnosis and treatment of sexually transmitted infections. A combination of high dose ceftriaxone (500 mg i.m.) and azithromycin (2 g p.o.) was recommended for the empiric treatment of gonorrhoea. This therapeutic regimen has gradually been abandoned during 2019 and onwards by most clinicians.

Ciprofloxacin (500 mg p.o. as a single dose) may still be used for treatment if the strain is fully susceptible.

Figure 8.24 Number of submitted gonococcus isolates from unique cases of *gonorrhoea* in males and females, and the percentage of isolates with ciprofloxacin resistance, azithromycin resistance, and penicillinase production, Denmark, 2003-2023 DANMAP 2023

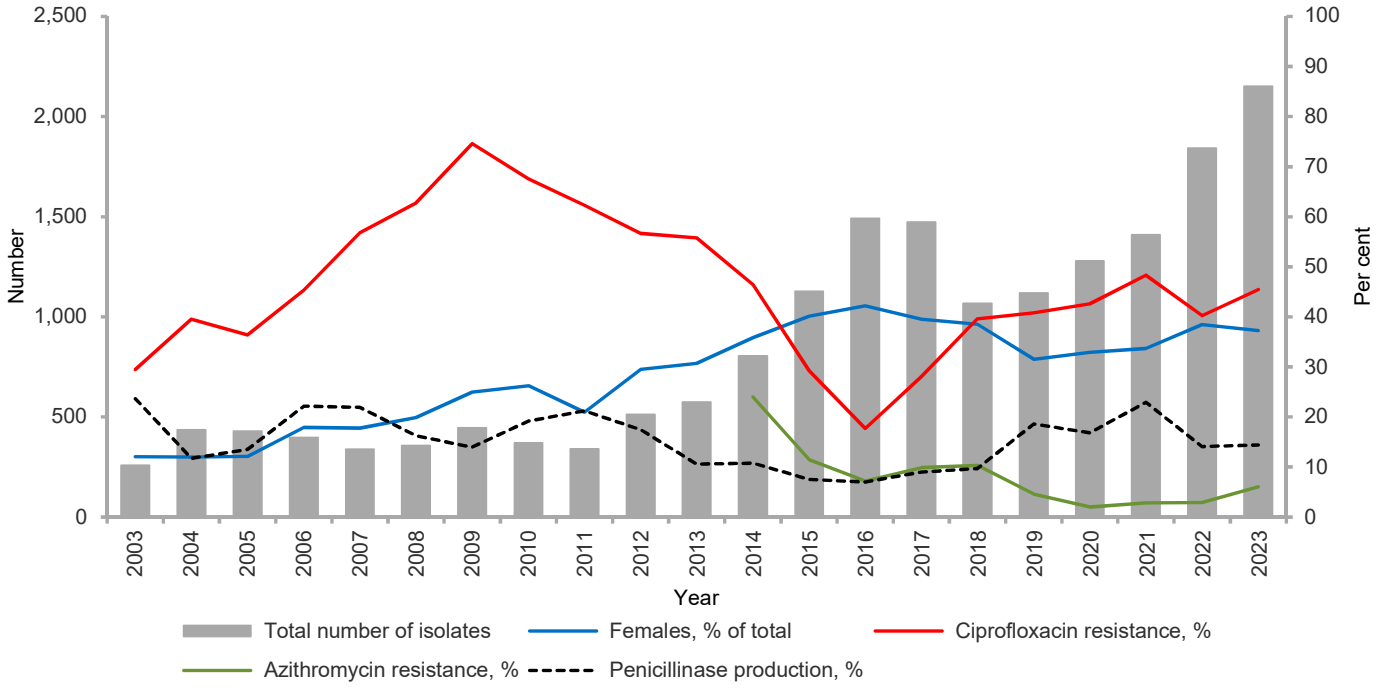
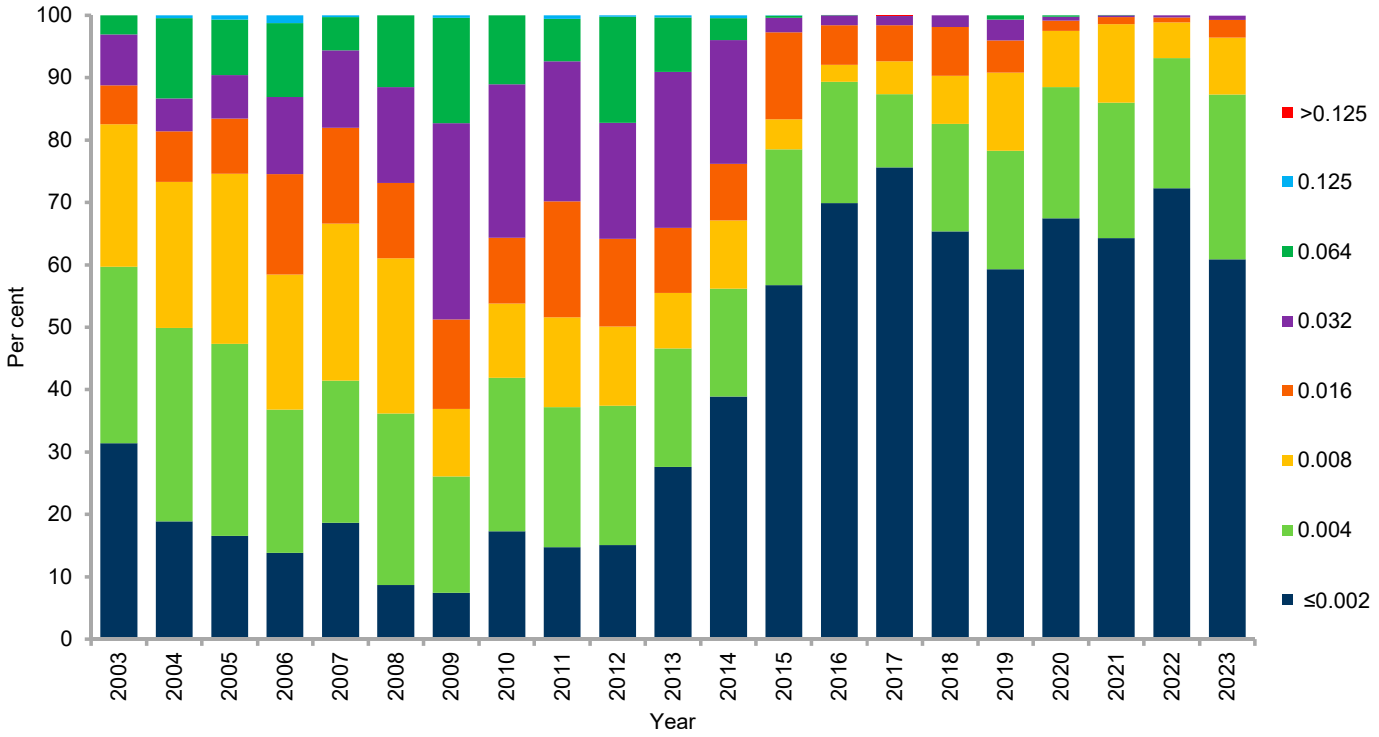


Figure 8.25. Distribution of ceftriaxone MIC (mg/L) values in gonococci, Denmark, 2003–2023 DANMAP 2023



Participation in Euro-GASP

In a subset of 128 isolates tested as part of the Danish participation in Euro-GASP, resistance against cefixime (MIC >0.125 mg/L) was 0% in 2023 (Table 8.25), like in 2022. Cefixime is an oral cephalosporin that has never been used in Denmark.

Resistance against tetracycline (MIC >0.5 mg/L) was 34%. Susceptibility testing against spectinomycin and gentamicin are only performed in selected years and was not carried out in 2023.

Resistance findings by gender and anatomical origin of the isolates

In males, the ciprofloxacin resistance rate was higher in anorectal isolates than in urogenital and pharyngeal isolates (Table 8.26).

The azithromycin resistance rate among males was higher in pharyngeal isolates than in anorectal and urogenital isolates, while in females it was close to the rate in anorectal isolates (Table 8.27).

Penicillinase production was demonstrated at a higher rate among anorectal isolates than among urogenital and pharyngeal isolates in males, while no differences were noted among isolates from females (Table 8.28).

Table 8.25 Distribution of 128 gonococcal isolates according to MIC values for cefixime and tetracycline; number of isolates

DANMAP 2023

	MIC values (mg/L)					
	≤0.016	0.032	0.064	0.125	0,25	0,5
Cefixime	106	16	6			
Tetracycline		1	8	14	12	50

Table 8.26 Ciprofloxacin resistance rates by gender and anatomical origin of the isolates DANMAP 2023

	Males		Females		Total	
	No. resistant / total no.	% resistant	No. resistant / total no.	% resistant	No. resistant / total no.	% resistant
Urogenital	525 / 1,067	49	214 / 683	31	739 / 1,750	42
Anorectal	115 / 151	76	5 / 22	23	120 / 173	69
Pharynx	70 / 120	58	41 / 97	42	111 / 217	51
Blood	1 / 1	100	0 / 0	-	1 / 1	100
Eye	5 / 6	83	0 / 0	-	5 / 6	83
Other or unknown	2 / 5	40	0 / 0	-	2 / 5	40
Total	718 / 1,350	55	260 / 802	32	978 / 2,152	45

Table 8.27 Azithromycin resistance rates by gender and anatomical origin of the isolates DANMAP 2023

	Males		Females		Total	
	No. resistant / total no.	% resistant	No. resistant / total no.	% resistant	No. resistant / total no.	% resistant
Urogenital	57 / 1.067	5.3	38 / 683	5.6	95 / 1750	5.4
Anorectal	6 / 151	4	3 / 22	13.6	9 / 173	5.2
Pharynx	12 / 120	10	12 / 97	12.4	24 / 217	11.1
Blood	1 / 1	0	0 / 0	-	0 / 1	0
Eye	1 / 1	0	0 / 0	-	0 / 6	0
Other or unknown	0 / 5	0	0 / 0	-	0 / 5	0
Total	77 / 1,350	5.7	53 / 802	6.6	128 / 2,152	5.9

Table 8.28 Penicillinase production by gender and anatomical origin of the isolate DANMAP 2023

	Males		Females		Total	
	No. resistant / total no.	% resistant	No. resistant / total no.	% resistant	No. resistant / total no.	% resistant
Urogenital	173 / 1,067	16	62 / 683	9	235 / 1,750	13
Anorectal	45 / 151	30	2 / 22	9	47 / 173	27
Pharynx	15 / 120	13	9 / 97	9	24 / 217	11
Blood	0 / 1	0	0 / 0	-	0 / 1	0
Eye	2 / 6	33	0 / 0	-	2 / 6	33
Unknown	2 / 5	40	0 / 0	-	2 / 5	40
Total	237 / 1,350	18	73 / 802	9	128 / 2,152	6

Conclusions

The ciprofloxacin resistance rate was higher in 2023 (45%) than in 2022 (40%) but lower than in 2021 (48%) and the ceftriaxone MIC distribution remained without notable changes. Although resistance problems among gonococci are still not present in Denmark, the emergence globally of gonococcal resistance to antibiotics underlines the importance of maintaining the centralised national surveillance of antimicrobial resistance in gonococci.

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8.3.9 *Haemophilus influenzae*

Background

Haemophilus influenzae is part of the normal upper respiratory tract flora, with colonization varying by age. *H. influenzae* frequently causes upper and lower respiratory infections, while invasive infections are relatively rare and primarily occur in young children and elderly individuals, particularly those with underlying conditions like chronic obstructive pulmonary disease or cancer. Bacteremia/sepsis is the predominant form (80-90%), while meningitis is observed in approximately 10% of cases. *H. influenzae* is categorized into six capsular serotypes (a, b, c, d, e, and f), also known as Hia, Hib, Hic, Hid, Hie, and Hif, and there are noncapsular (non-typeable, NTHi) strains as well. The introduction of the polysaccharide type b vaccine in 1993 significantly reduced the incidence of systemic infection caused by *H. influenzae* type b (Hib) isolates, as the vaccine specifically protects against Hib. Antimicrobial resistance in *H. influenzae* is an increasing problem globally, especially within beta-lactam antibiotics. The molecular antibiotic resistance in *H. influenzae* is complex, where both beta-lactamase production and alterations in penicillin binding proteins (PBPs), particularly PBP3, encoded by the *ftsI* gene, contribute to resistance to betalactam antibiotics.

Surveillance of *Haemophilus influenzae*

The surveillance of *H. influenzae* causing invasive disease in Denmark involves submitting clinical isolates to Statens Serum Institut (SSI). Cases without a submitted isolate are identified through MiBa. At SSI, the isolates are only subjected to whole-genome sequencing to extract the serotype, sequence type, and beta-lactam resistance genes (beta-lactamase genes and mutations in the *ftsI* gene encoding penicillin-binding protein 3). Phenotypic beta-lactam susceptibility is retrieved through MiBa, if available.

Results

In 2023, a total of 123 cases of invasive *H. influenzae* were registered. The majority of cases were in blood (117) or cerebrospinal fluid (4), and two isolates from other normally sterile sites. The age and serotype distribution of the submitted isolates can be seen in Figure 8.26. Invasive *H. influenzae* infections are most commonly observed in the elderly.

Figure 8.26 The distribution of age and serotype of *H. influenzae* in Denmark, 2023 DANMAP 2023

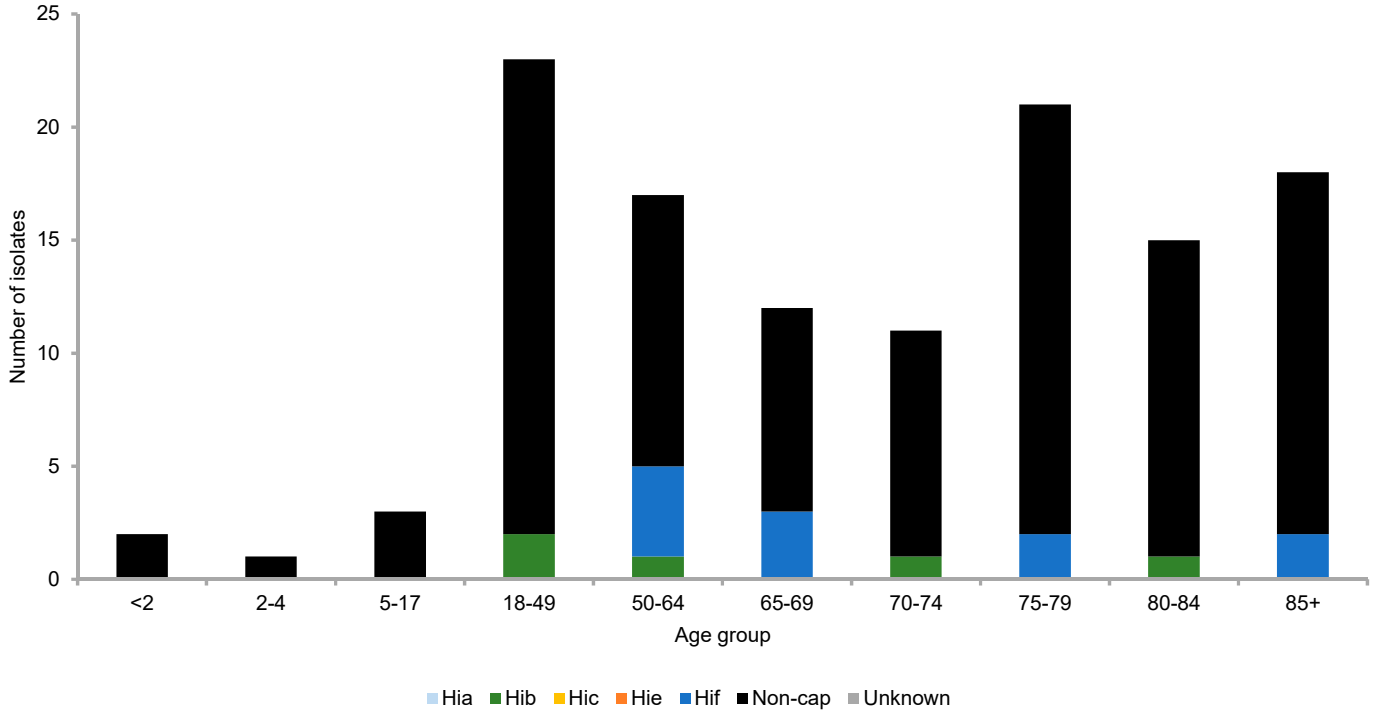

Table 8.29 Sequence type and clonal complex found among the *H. influenzae* isolates with a capsule DANMAP 2023

ST	Clonal complex	A	B	C	D	E	F	Noncap
6	ST-6 complex	0	5	0	0	0	0	1
124	ST-124 complex	0	0	0	0	0	11	0

Table 8.30 Phenotypic resistance against penicillin/ampicillin in *Haemophilus influenzae* DANMAP 2023

Sensitivity	Number (%)
Resistant	40 (33)
Susceptible	80 (67)
Total	120

Table 8.31 Phenotypic resistance against penicillin/ampicillin vs. beta-lactam resistance mechanism in *H. influenzae* DANMAP 2023

Beta-lactam resistance mechanism	Pen/ampi resistant	Pen/ampi susceptible	Total (%)
BLNAS	1	79	80 (67)
BLPAR	20	0	20 (17)
BLNAR	19	1	20 (17)
BLPACR	0	0	0 (0)
Total	40	80	120

Non-capsular *H. influenzae* is still the most commonly tested type (87%), with Hif being the most common serotype (9%), followed by Hib (4%).

Sequence type (ST) and clonal complex (CC) were linked to the serotype of *H. influenzae* (Table 8.29). Thus, the MLST type can indicate the correct identification of the genotype because the capsular isolates appear to be part of only a few clonal complexes. This was not the case for the non-capsular *H. influenzae* isolates which had 53 different STs, including five novel STs.

Data on both molecular and phenotypic antimicrobial susceptibility were available for 120 *H. influenzae* isolates. Of these, 33,3% were penicillin/ampicillin-resistant (Table 8.30). Twenty isolates were tested positive for TEM beta-lactamase genes (all were *TEM-1*). BLNAR-defining mutations in the *ftsI* gene were found in 20 isolates (18 had the N526K mutation and two had the R517H mutation). The most common *ftsI* type were IIb (9), followed by IId (6), IIa (3) and III-like (2).

A comparison between phenotypic beta-lactam susceptibility and beta-lactam resistance mechanisms (BLNAS=beta-lactamase-negative ampicillin-susceptible; BLPAR=beta-lactamase positive ampicillin-resistant; BLNAR=beta-lactamase negative ampicillin-resistant; BLPACR=beta-lactamase-positive amoxicillin-clavulanic acid-resistant) can be found in Table 8.31. A 98% concordance was observed between phenotypic susceptibility testing and molecular resistance gene detection: one isolate with no beta-lactam resistance markers was tested penicillin/ampicillin resistant, and one isolate with a BLNAR resistance mechanism was tested penicillin/ampicillin susceptible.

Only three *H. influenzae* isolates had other resistance genes (aminoglycoside-modifying enzymes).

Conclusions

The number of invasive *H. influenzae* cases in 2023 were 123 comparable to 118 cases in 2022 and 101 cases in 2021. The majority of isolates are still of the noncapsular type (87%) while Hif was the most common serotype (9%). Resistance towards penicillin and ampicillin was 33% with 17% BLNAR and 17% BLPAR (none were observed with a BLPACR resistance mechanism).

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8.3.10 Meningococci

Background

Neisseria meningitidis (meningococci) are capable of causing invasive meningococcal disease (IMD), usually meningitis or septicaemia or both, which may be life threatening and may cause permanent neurological sequelae. Asymptomatic throat carriage is frequent and only rarely leads to invasive disease. IMD is usually treated with intravenous penicillin or ceftriaxone. Single-dose ciprofloxacin or rarely rifampicin may be used for prophylaxis to close contacts to patients with IMD.

Meningococci are classified into serological groups (A, B, C, W, X, Y, Z, and 29E) according to antigenic properties of the polysaccharide capsule. In Denmark, serogroups B and C are the most frequent ones in most years, although serogroup W was quite frequent in 2016 through 2019. Vaccination against meningococci has never been part of the Danish childhood immunization programme but is used for prophylaxis to close contacts to patients with IMD. Specific vaccines exist for group B and combined vaccines for groups A, C, W, and Y.

This report presents data on antimicrobial resistance in non-duplicate invasive isolates (i.e. usually from blood, cerebrospinal fluid) submitted from the departments of clinical microbiology (DCM) during 2012-2023 to the Neisseria and Streptococcus Reference laboratory (NSR). Isolates are received from all DCM in Denmark. Until November 1, 2023

it was voluntary to submit isolates of meningococci, but the coverage rate is estimated to be 100% when compared to the mandatory clinical notification system. The two surveillance systems continuously supplement each other.

Results

During the years 2012 through 2019, 18 to 52 isolates were received annually. In 2020, where restrictions to counteract the spread of COVID-19 were implemented, the number was 14, decreasing to 8 and 11, respectively, in 2021 and 2022. Figure 8.27 shows the number of isolates of groups B, C, W, and Y received during 2012-2023. Because of low numbers the following have been omitted: One isolate of group 29E (2017), three isolates of group X (2016, 2019 and 2023), and two isolates which were non-groupable (2019 and 2023). The susceptibility pattern of these four isolates did not show any specific deviations from the remaining findings and are therefore not further included in the present report.

All isolates were susceptible to ceftriaxone (MIC ≤0.125 mg/L), Figure 8.28.

All isolates were susceptible to ciprofloxacin (MIC ≤0.016 mg/L), Figure 8.29. Isolates of serogroup W (none in 2023) and Y (11 in 2023) tended to have slightly lower levels of ciprofloxacin MIC values than isolates of serogroup B and C.

In total, 95% of the isolates during the study period were susceptible to penicillin (MIC ≤0.25 mg/L), Figure 8.30. Eight isolates of serogroup B including one from 2023 and six isolates of serogroup C were penicillin-resistant (MIC >0,25 mg/L), (Table 8.32).

Nearly all isolates during the study period were susceptible to rifampicin (MIC ≤0.25 mg/L), Figure 8.31. Only one (from 2017) was resistant (MIC = 32 mg/L).

Figure 8.27 Number and serogroup of meningococcal isolates received during 2012-2023 DANMAP 2023

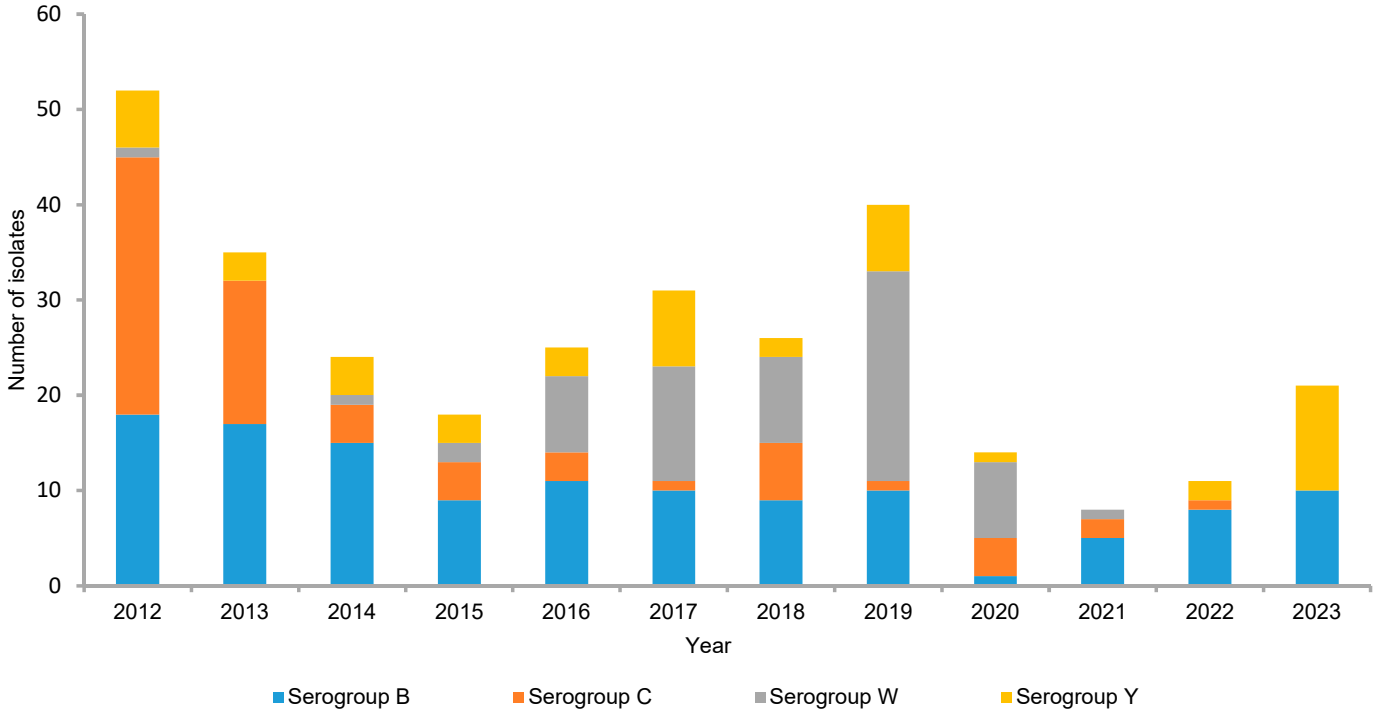


Figure 8.28 Distribution of ceftriaxone MIC values (mg/L) by serogroup, 2012-2023 DANMAP 2023

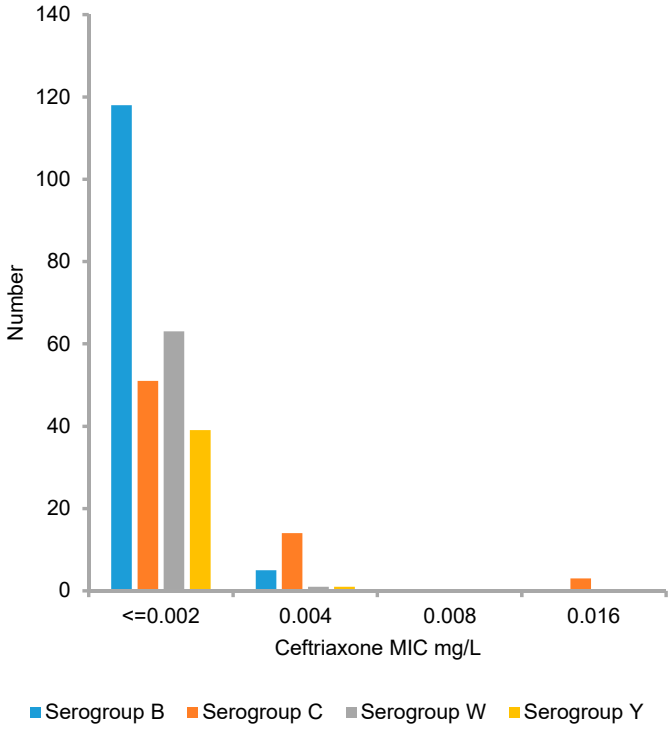


Figure 8.29 Distribution of ciprofloxacin MIC values (mg/L) by serogroup, 2012-2023 DANMAP 2023

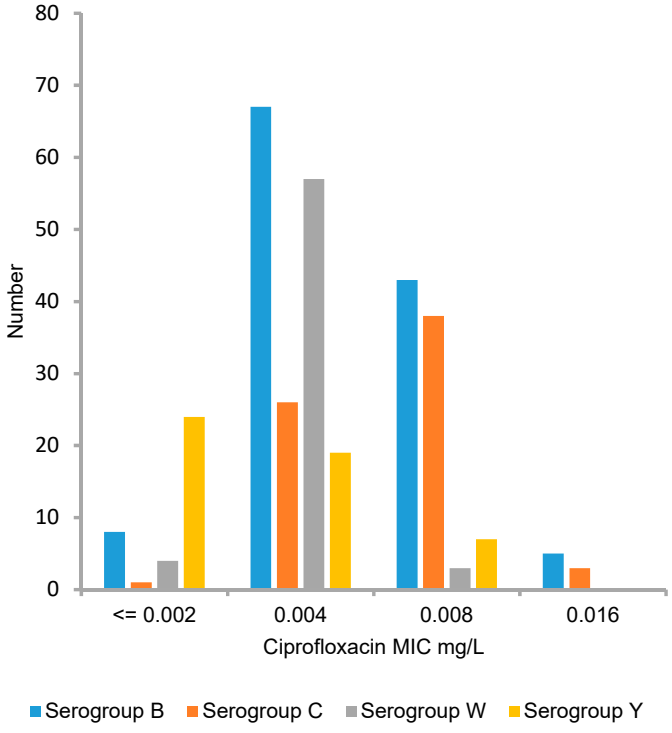


Figure 8.30 Distribution of penicillin MIC values (mg/L) by serogroup, 2012-2023 DANMAP 2023

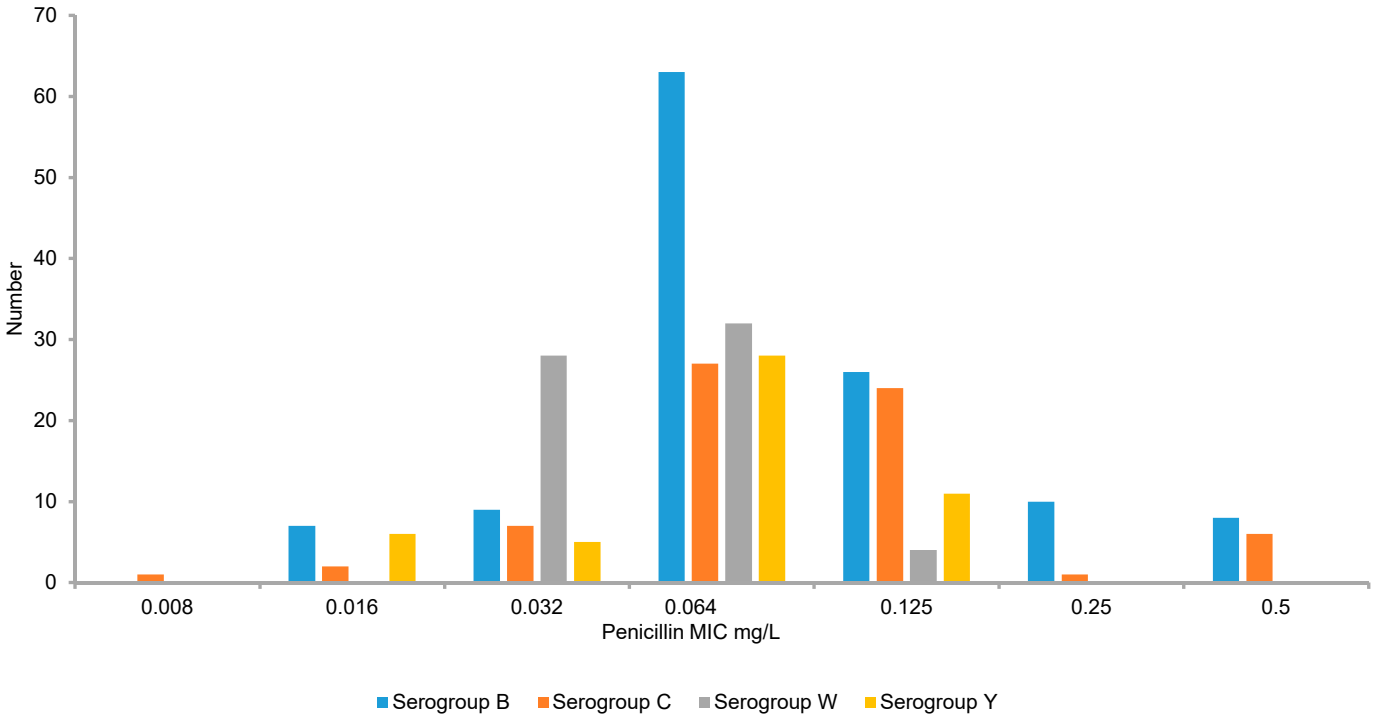
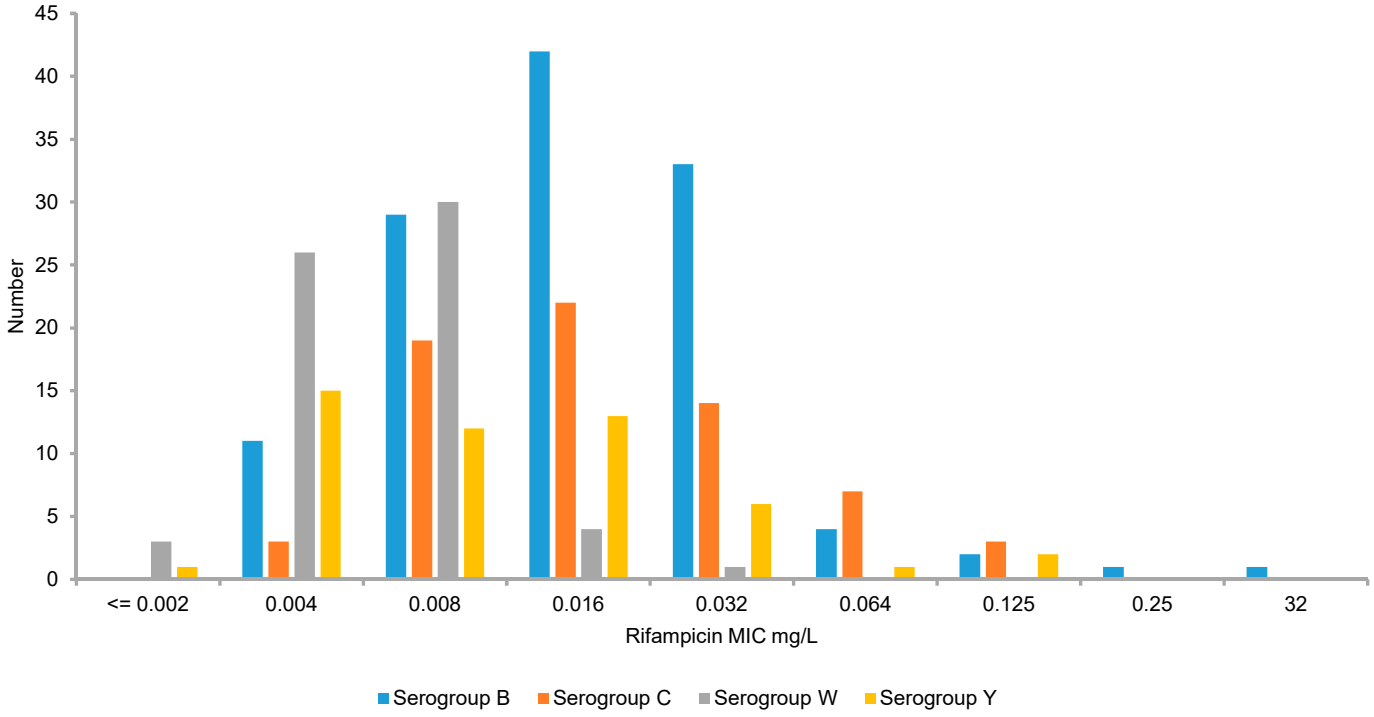


Table 8.32 Number of penicillin-resistant meningococci (MIC = 0.5 mg/L), serogroups B and C, 2012-2023 DANMAP 2023

	2013	2014	2016	2017	2018	2020	2022	2023
Serogroup B	1	2	1	2			1	1
Serogroup C		1			2	3		

Figure 8.31 Distribution of rifampicin MIC values (mg/L) by serogroup, 2012-2023 DANMAP 2023



Comments and conclusions

The substantial decrease of received meningococcal isolates during and following 2020 are most likely due to the social restrictions implemented in April 2020 because of COVID-19. Likewise, the modest increase during 2023 probably represent the influence of the lifting of the restrictions which have enabled more respiratory transmission than during the preceding 2-3 years.

The antibiotic susceptibility patterns of meningococci do not reveal any imposing threats of resistance.

During the first five months of 2024 a total of 13 cases of IMD have been diagnosed in Denmark (not described in this report).

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