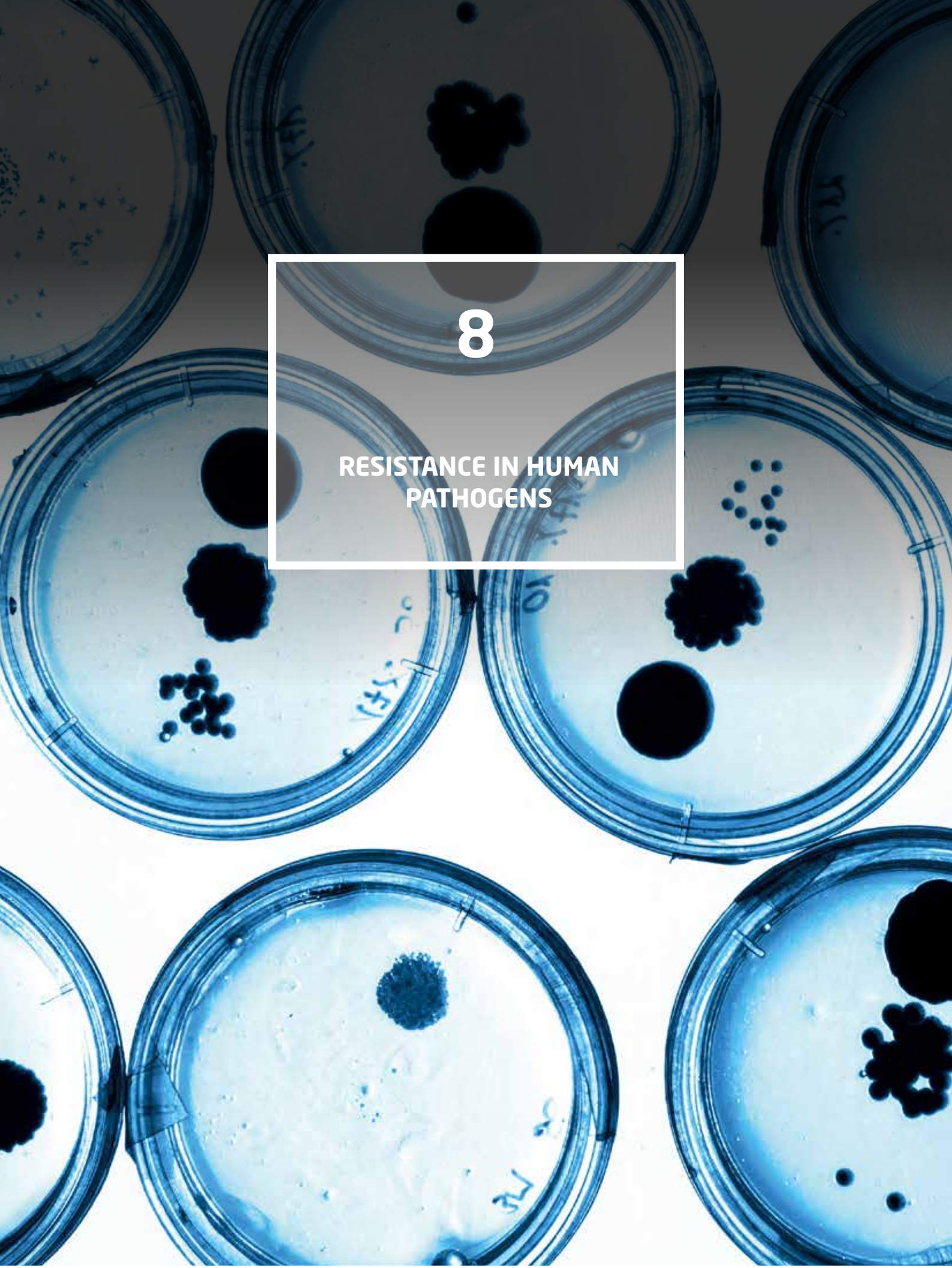


8

RESISTANCE IN HUMAN PATHOGENS

8. Resistance in human pathogens

Highlights

Invasive bacterial infections. The number of invasive infections reached a plateau at 12,373 for the monitored species after increasing steadily for the past ten years (8,954 cases in 2013, a 38% increase). The notable exception are invasive infections with *Streptococcus pneumoniae*, showing an overall decrease over the last decade (789 cases in 2013, 538 cases in 2022), and two significant drops in 2020 and 2021 (363 and 333 total cases, respectively). *Escherichia coli* remained the most common cause responsible for about half of all cases, followed by *Staphylococcus aureus* and *Klebsiella pneumoniae*.

Following declining rates of resistance against most antibiotics in almost all monitored bacterial species during the COVID-19 pandemic, resistance rates are now either stable or increasing but generally remain low.

Escherichia coli. After a substantial decline in the number of ESBL- and pAmpC-producing *E. coli* in bloodstream infections from 2020 to 2021 (352 and 254 cases, respectively), the number of cases resurged to 336 in 2022, a 32% increase compared to the previous year. CTX-M-15 remained the most common ESBL enzyme present in 52% of cases. Resistance rates to gentamicin were declining over the decade (6.5% in 2013, 4.5% in 2022), and resistance to carbapenems remained below 1%.

Klebsiella pneumoniae. Specific note should be taken for piperacillin-tazobactam resistance rates in *K. pneumoniae* as these have been increasing over the past ten years, nearing 10% in both invasive infections (6.2% in 2013, 9.2% in 2022) and urinary tract infections (7.2% in 2013 and 9.9% in 2022) in hospitalized patients.

Combined resistance to 3rd generation cephalosporins, ciprofloxacin and gentamicin remained low in both *E. coli* and *K. pneumoniae* from invasive infections (1.3% and 1.0%, respectively).

Carbapenemase-Producing Organisms/Enterobacterales (CPO/CPE). The number of CPO continued to increase throughout 2022 with 355 affected patients compared to 242 in 2021. This is, in part, due to an influx of patients from Ukraine, but also due to an outbreak caused by a contaminated pharmaceutical product. The number of outbreaks at Danish hospitals remained stable, but for some of the known outbreaks the number of cases continued to grow.

Enterococci. While there has been a decrease in the number of invasive infections with enterococci, the number of vancomycin-resistant enterococci (VRE) continues to increase. In 2022, 845 VRE/vancomycin variable enterococci (VVE) were identified compared to 742 in 2021. This calls for intensified efforts in infection prevention and control.

Staphylococcus aureus. Following a decrease in the number of methicillin-resistant *S. aureus* (MRSA) cases during the COVID-19 pandemic, the number increased by 10% to 2,996 cases from 2021 to 2022. MRSA-screening accounted for 49% of cases, whereas infections accounted for 51%. Thirty-nine MRSA outbreaks were registered at hospitals, nursing homes and other institutions for a total of 143 cases, of which 70 were infections. Fifty bacteraemias were caused by MRSA with seven being livestock-associated MRSA.

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, Denmark, 2022 DANMAP 2022

Routine diagnostics from all 10 DCMs in Denmark. All data are directly identified and extracted in MiBa	
Species	**Inclusion criteria**
Escherichia coli *Klebsiella pneumoniae*	First isolate per patient per year from blood, cerebrospinal fluid or urine (hospitals and 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 exceptional 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 several isolates per patient are received, but for the statistics each patient is counted only once

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 DCMs. MiBa thus simultaneously delivers real time patient data to the DCMs 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 DCMs, beginning with data from just two DCMs in 1995, but quickly expanding to include more than half of the DCMs. Since 2015, all DCMs have contributed to the program resulting in a 100% coverage of hospitalised patients and – since the DCMs 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://miba.ssi.dk/Service/English.aspx] 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 results from 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 invasive *Streptococcus pneumoniae* and *Haemophilus influenzae* serotype b (Hib), methicillin-resistant *S. aureus* (MRSA) and carbapenemase-producing organisms (CPO) from clinical and screening samples, and *Neisseria gonorrhoeae* from all clinical samples (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.

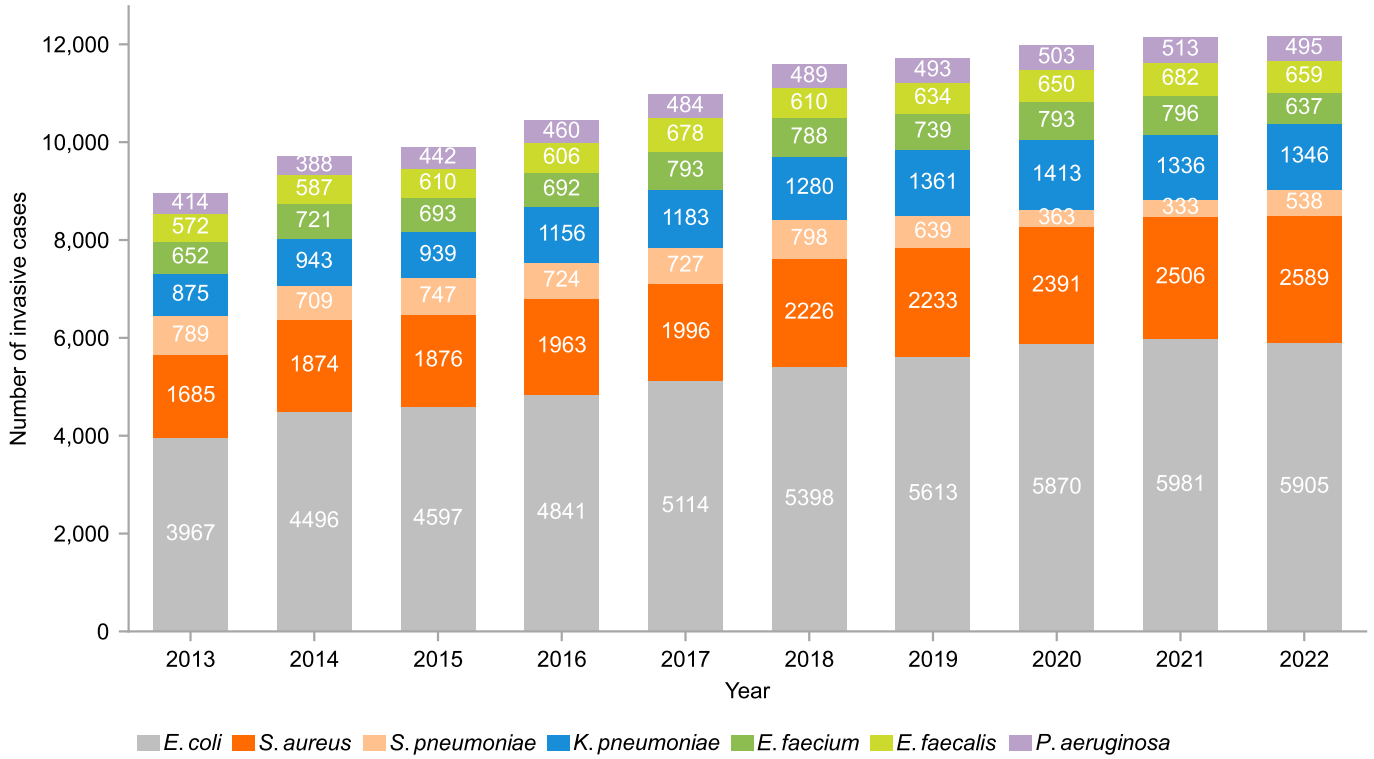
Since 2015/2016, characterisation of antimicrobial resistance mechanisms in bacteria has been performed primarily by whole genome sequencing (WGS).

8.1.3 Number of invasive cases

The total numbers of invasive cases in Denmark bacterial species included in the surveillance programmes for both DANMAP and EARS-Net from 2013 to 2022 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 2013 and 2022).

Between 2013 and 2022, the number of registered individual invasive cases increased by 38% from 8,954 to 12,372 cases in Denmark: *E. coli* 3,967 to 5,905 cases (49% increase), *S. aureus* 1,685 to 2,589 cases (54%) and *K. pneumoniae* 875 to 1,346 cases (54%).

Figure 8.1 Number of invasive cases for bacterial species under surveillance, Denmark, 2013-2022 DANMAP 2022



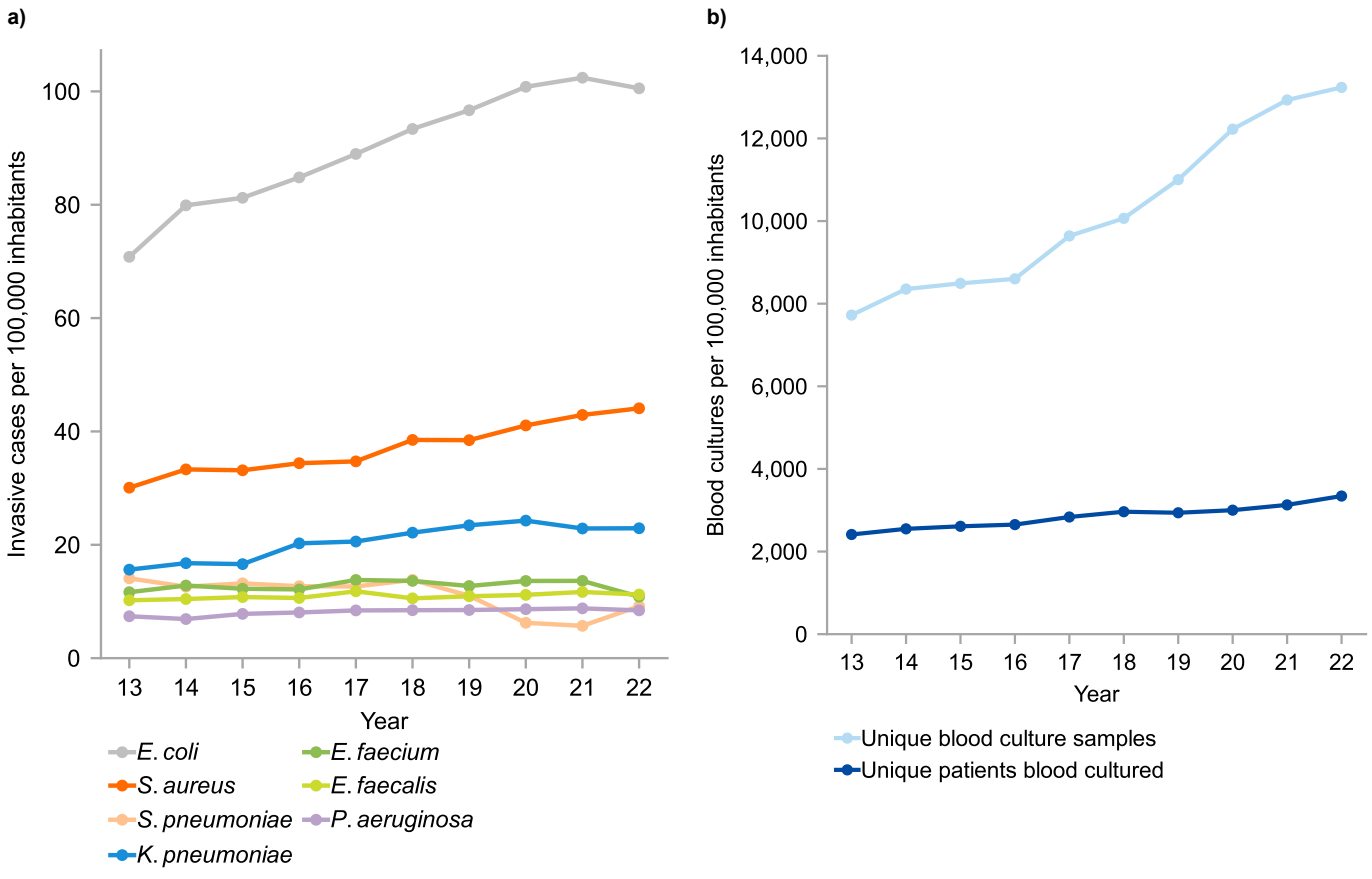
The only species with an overall decreasing number of cases during the past 10 years was *S. pneumoniae*. During the pandemic years 2020 and 2021 an unusual low number of annual invasive cases with *S. pneumoniae* was observed *(363 and 333 cases, respectively)*, but has since increased and the number of cases in 2022 was 538, a 62% increase compared to 2021. This is likely due to a normalization of society post-COVID-19.

Figure 8.2a shows the incidence of invasive cases per 100,000 inhabitants in Denmark per year from 2013 to 2022. During this period, the Danish population increased by 4.8% (from 5,602,628 inhabitants in 2013 to 5,873,420 inhabitants in 2022). For comparison, Figure 8.2b shows the total number of blood cultures taken per 100,000 inhabitants per year for 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 2,413 patients per 100,000 inhabitants in 2013 to 3,343 patients per 100,000 inhabitants in 2022 (an increase of 39%). The total number of blood cultures taken per 100,000 inhabitants increased even more (71%). Thus, on average a higher number of patients have an even higher number of blood cultures taken each year.

Changes in hospital workflow (reduced number of bed-days), healthcare-related infections, improved diagnostics and increased numbers of elderly at risk of infections have probably affected the increased number of total infections.

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

Figure 8.2 a) Invasive cases and b) blood cultures taken per 100,000 inhabitants, Denmark, 2013-2022 DANMAP 2022



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 well as in urine samples from hospitals and primary health care (see details in later paragraphs). Figures 8.3a and 8.3b show the total annual number of invasive isolates and proportion of resistant isolates, respectively, between 2013 and 2022. The percentages of isolates resistant to key antimicrobials are presented in Figure 8.3c.

Invasive cases from hospital patients

In 2022, a total of 5,905 individual patients with invasive *E. coli* isolates were identified in MiBa. Antimicrobial susceptibility test results for key antimicrobials were also extracted for these isolates (See Chapter 10 'Material and methods').

As mentioned in the introduction, an increase in the number of invasive *E. coli* cases was observed over the past decade corresponding to 70.8 cases and 100.5 cases per 100,000 inhabitants respectively (a 42% increase, data not shown).

In 2022, 9.9% of invasive *E. coli* isolates were resistant to cefuroxime. This level has been stable (range 8.6-10%) over the last decade. A minor EUCAST breakpoint change in 2017 influenced data from 2017 compared to data from 2016. For 3rd generation cephalosporins, the percentage of resistant isolates was 6.2% with an increase compared to 2021.

The ciprofloxacin resistance for invasive *E. coli* was 10.8% in 2022, and ranged from 10.3% to 13% in the period 2013-2022. The increased ciprofloxacin resistance rate in 2017 compared to 2016 mainly reflected a relatively large EUCAST breakpoints change in 2017. Ciprofloxacin breakpoints were changed (lowered) once again combined with the introduction of the Area of Technical Uncertainty (ATU) in the EUCAST clinical breakpoint table v. 9.0 in 2019. Therefore, trends for ciprofloxacin resistance should be interpreted with caution.

Piperacillin-tazobactam resistance has gradually increased from 3.8% of invasive *E. coli* isolates being reported as resistant in 2018 to 6.3% in 2022. Gentamicin resistance was 4.5% in 2022 and resistance rates have declined over the last decade, however it increased from 2021 to 2022 (Figure 8.3b and 8.3c). The sharp increase for resistance towards amoxicillin-clavulanic acid can be attributed to removal of data from 2021 to 2022: a major DCM did not meet the inclusion criteria of having tested at least 75% of the isolates, which is clearly mirrored in the number of isolates tested (2021: 4226 isolates tested, 2022: 3076 isolates tested).

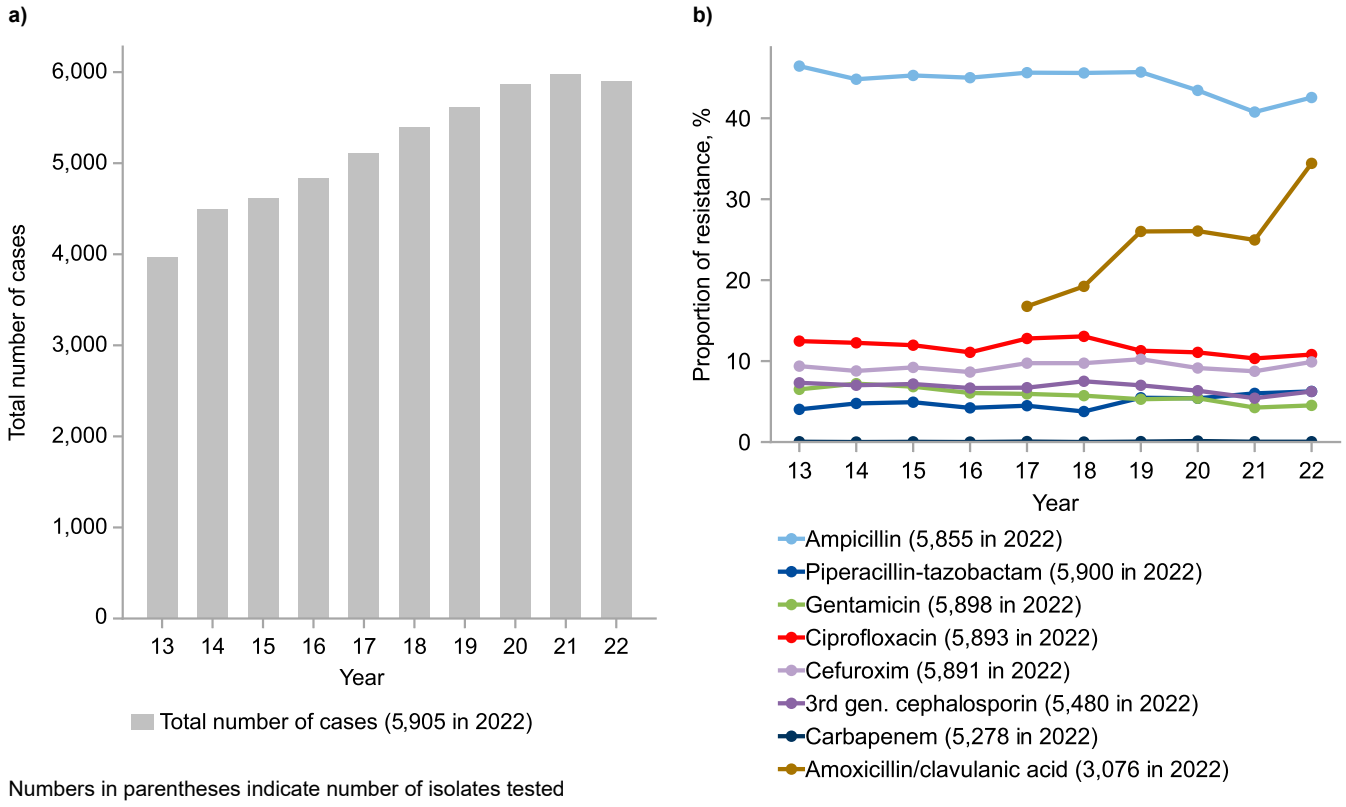
The number of carbapenem-resistant invasive *E. coli* isolates remained low in 2022 with two isolates categorised carbapenem-resistant and six isolates categorised "susceptible, increased exposure" (as per new EUCAST definition of previous intermediate category applicable from January 2019 [www.eucast.org/newsiandr]). The occurrence of multidrug-resistant (combined resistance to 3rd generation cephalosporins, ciprofloxacin and gentamicin) invasive *E. coli* remained low at around 1.3% (Table 8.3).

Table 8.2 *Escherichia coli*. Resistance (%) in isolates from humans, Denmark, 2022 DANMAP 2022

	Invasive isolates, hospitals %	Urine isolates, hospitals %	Urine isolates, primary health care %
Ampicillin	43	40	35
Mecillinam	8.8	6.8	4.3
Piperacillin/tazobactam	6.3	5.4	3.7 (2)
Amoxicillin/clavulanic acid	34.4 (6)	12	6.6 (6)
Sulfonamide		28 (5)	25
Trimethoprim		21	20
Nitrofuratoin		1.0 (5)	0.8
Gentamicin	4.5	4.2	3.2 (3)
Ciprofloxacin	11	9.3	6.9
Cefuroxime	9.9	7.5	5.3 (4)
3rd generation cephalosporins	6.2	6.2	4.8
Carbapenem	0.0	0.0	0.0 (2)
Max. number of isolates tested for resistance to the presented antibiotics	5,900	48,469	104,345

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

Figure 8.3 Invasive *Escherichia coli* isolates from humans: a) annual number of isolates from unique cases, b) proportion of resistant isolates and c) table of resistance percentages, Denmark, 2013-2022 DANMAP 2022



Numbers in parentheses indicate number of isolates tested

c)

Substance	Percent resistant invasive *E. coli* isolates									
	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022
Ampicillin	46.4	44.8	45.3	45	45.6	45.5	45.7	43.4	40.7	42.6
Piperacillin-tazobactam	4	4.8	4.9	4.2	4.5	3.8	5.5	5.4	6.0	6.3
Gentamicin	6.5	7.2	6.8	6.1	6	5.7	5.3	5.4	4.3	4.5
Ciprofloxacin	12.5	12.3	12	11.1	12.8	13	11.3	11.1	10.3	10.8
Cefuroxime	9.4	8.8	9.2	8.6	9.7	9.8	10.2	9.2	8.7	9.9
3rd gen.cephalosporins	7.7	7	7.2	6.7	6.7	7.3	6.9	6.2	5.4	6.2
Carbapenem	0	0	0	0	0.1	0	0.1	0.1	0.0	0.0
Total number of isolates	3,967	4,492	4,618	4,841	5,114	5,398	5,613	5,870	5,981	5,905

Table 8.3 *Escherichia coli*. Combined resistance to 3rd generation cephalosporins, ciprofloxacin, and gentamicin (multidrug-resistance) in invasive isolates from humans, Denmark, 2015-2022 DANMAP 2022

	2015 % (N)	2016 % (N)	2017 % (N)	2018 % (N)	2019 % (N)	2020 % (N)	2021 % (N)	2022 % (N)
Resistance	2.3 (93)	1.8 (87)	1.8 (88)	2.0 (100)	1.8 (93)	1.5 (82)	1.1 (60)	1.3 (70)
Percentage (no.) of isolates tested for combined resistance (multidrug-resistance)	88 (4,071)	98 (4,763)	95 (4,883)	93 (4,997)	94 (5,259)	93 (5,470)	93 (5,564)	93 (5,474)
Total number of invasive isolates	4,614	4,841	5,114	5,398	5,613	5,870	5,981	5,905

Urinary cases from hospitals

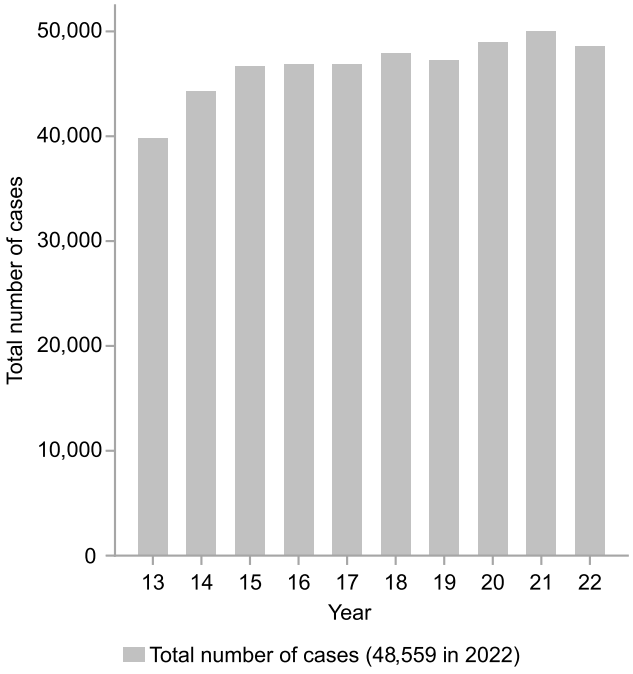
In 2022, 48,559 individual hospital patients had *E. coli* isolated from urine samples, a 22% increase and 2.9% decrease compared to 2013 and 2021, respectively.

Interpretation results from antimicrobial susceptibility test (AST) data on *E. coli* urine isolates were available from all DCMs for ampicillin, mecillinam and ciprofloxacin. In addition, susceptibility test results were reported for: piperacillin-tazobactam (nine DCMs), gentamicin (nine DCMs), cefuroxime (eight DCMs), trimethoprim (eight DCMs), 3rd generation cephalosporins (eight DCMs), amoxicillin/clavulanic acid (eight DCMs), carbapenem (six DCMs), nitrofurantoin (five DCMs) and sulphonamide (four DCMs).

Summarized antimicrobial susceptibility results for *E. coli* isolates (invasive and urine specimen) are shown in Table 8.2. In Figure 8.4, resistance for *E. coli* urine isolates from hospitalised patients are shown for 2013-2022.

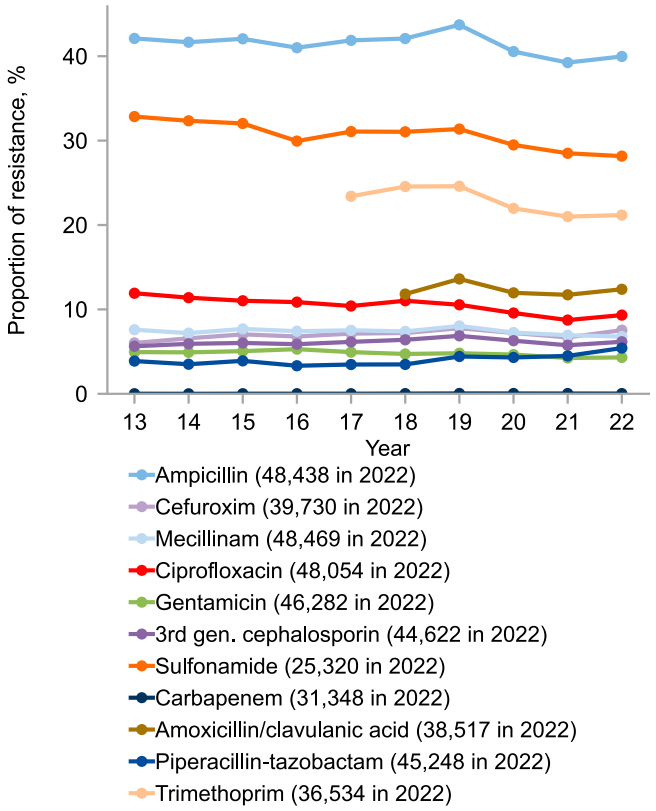
Figure 8.4 Urine *Escherichia coli* isolates from humans (hospitals): a) annual number of isolates from unique cases, b) proportion of resistant isolates and c) table of resistance percentages, Denmark, 2013-2022 DANMAP 2022

a)



Numbers in parentheses indicate number of isolates tested

b)



c)

Substance	Percent resistant *E. coli* isolates from hospital urine									
	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022
Ampicillin	42.1	41.7	42.1	41	41.9	42.1	43.7	40.6	39.2	40.0
Mecillinam	7.6	7.2	7.7	7.4	7.5	7.4	8.1	7.3	6.9	6.8
Piperacillin-tazobactam	3.9	3.5	3.9	3.3	3.7	3.5	4.4	4.3	4.5	5.4
Sulfonamide	32.8*	32.3*	32	34.9	31.1	31*	31.4*	29.4*	28.5*	28.2*
Gentamicin	4.9	4.9	5.1	5.3	4.9	4.7	4.6	4.6	4.2	4.3
Ciprofloxacin	11.9	11.4	11	10.9	10.4	11	10.6	9.6	8.7	9.3
Cefuroxime	6	6.6	7	6.8	7.1	7.2	7.8	7.2	6.7	7.5
3rd gen. cephalosporins	5.7	5.9	5.9	5.9	6.2	6.4	6.9	6.3	5.8	6.2
Carbapenem		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total number of isolates	39,820	44,288	46,723	46,865	46,884	47,914	47,235	48,962	49,986	48,559

* Indicates less than 6 DCMs reported rutine susceptibility testing

As can be seen in Figure 8.4c, a decrease in ciprofloxacin and gentamicin resistance was observed for the past five and ten years. However, for almost all antibiotics in 2022 rising rates of resistance have been observed as compared to 2021.

In 2022, 21 carbapenem-resistant and 21 carbapenem-"susceptible, increased exposure" *E. coli* urine isolates from hospitalised patients were reported, compared to 20 and 10 isolates in 2021, respectively.

Urinary cases from primary health care

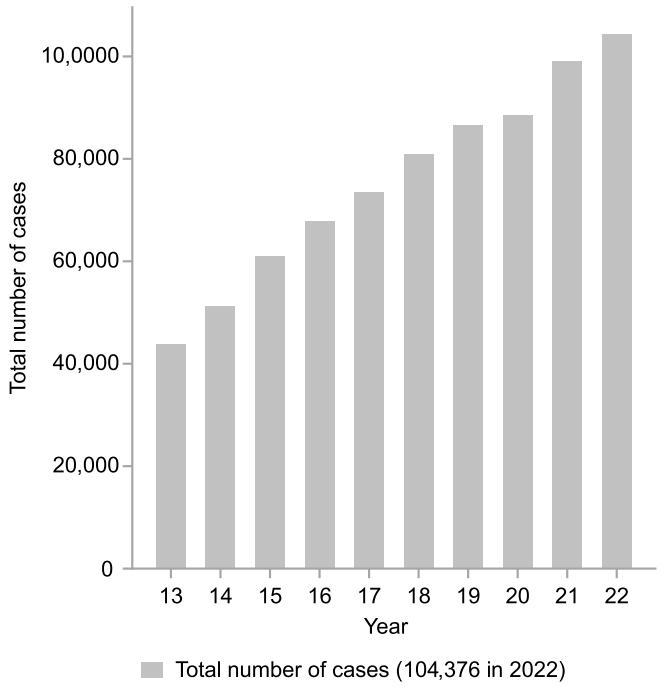
In 2022, 104,376 unique patients in primary health care had *E. coli* isolated from urine samples, a 139% and 5.3% increase compared to 2013 and 2021, respectively.

Susceptibility results for all tested antimicrobials are shown in Table 8.2. In Figure 8.5, resistance for *E. coli* urine isolates from primary health care are shown for the past decade.

As observed with *E. coli* from urinary cases in hospitals, the declining trends in resistance rates observed over the last five years appears to have stagnated or even reversed for most antibiotics and warrants further observation over the coming years.

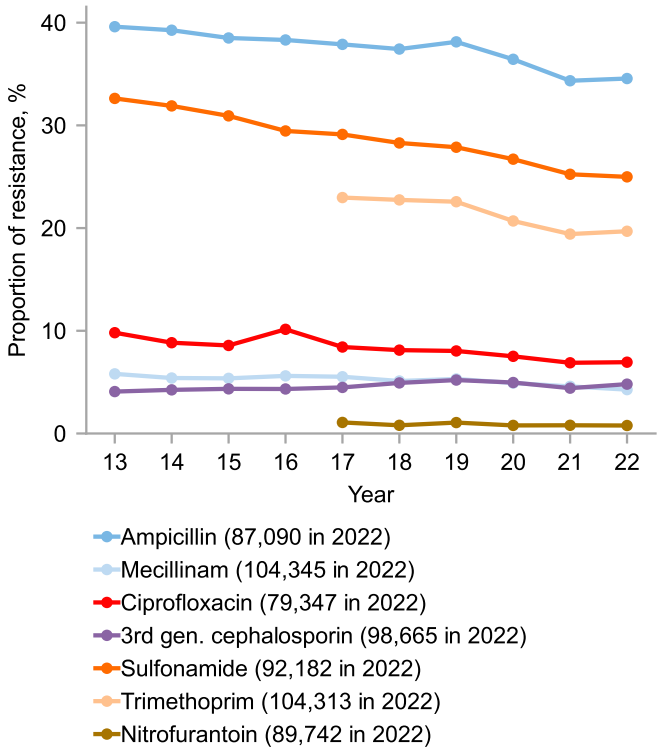
Figure 8.5 Urine *Escherichia coli* isolates from humans (primary health care): a) annual number of isolates from unique cases, b) proportion of resistant isolates and c) table of resistance percentages, Denmark, 2013-2022 DANMAP 2022

a)



Numbers in parentheses indicate number of isolates tested

b)



c)

Substance	Resistance in *E. coli* urine isolates from primary health care									
	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022
Ampicillin	39.6	39.3	38.5	38.3	37.9	37.4	38.1	36.5	34.3	34.6
Mecillinam	5.8	5.2	5.4	5.6	5.5	5.1	5.3	4.9	4.6	4.3
Sulfonamide	32.6	31.9	30.9	29.5	29.1	28.3	27.9	26.7	25.2	25.0
Ciprofloxacin	9.8	8.8	8.6	10.1	8.4	8.1	8	7.5	6.9	6.9
3rd gen. cephalosporins	4.1	4.2	4.3	4.3	4.5	4.9	5.2	5	4.4	4.8
Total number of isolates	43,770	51,272	61,083	67,798	73,497	80,851	86,508	88,462	99,077	104,376

Conclusion

A steady increase in the number of invasive and urinary tract infections (from hospital and primary health care) caused by *E. coli* has been observed since 2013. Around 35-43% of *E. coli* urine isolates are ampicillin-resistant and 7-11% are ciprofloxacin-resistant. Proportion of resistance to cefuroxime and 3rd generation cephalosporins for invasive *E. coli* increased slightly from 8.7% to 9.9% and 5.4% to 6.2% from 2021 to 2022, respectively, reversing the trend from the previous year. Resistance to carbapenems in invasive *E. coli* remains low.

8.2.2 *Klebsiella pneumonia*

Klebsiella pneumoniae is part of the human intestinal tract. The bacteria causes 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 organisms.

The percentage of resistance in invasive *K. pneumoniae* isolates for each key antimicrobial is presented in Table 8.4. Figures 8.6a and 8.6b show total annual numbers of invasive isolates and percentages of resistance in invasive isolates, respectively, between 2013 and 2022. The proportions of isolates resistant to key antimicrobials are presented in Figure 8.6c.

Invasive cases from hospitals

In 2022, a total of 1,346 unique patients were registered in MiBa with invasive *K. pneumoniae* isolates.

As for invasive *E. coli* cases, a continuous increase in the number of invasive *K. pneumoniae* cases was observed over the last decade, from 875 cases in 2013 to 1346 cases in 2022. This corresponds to 15.6 and 22.9 cases per 100,000 inhabitants, respectively (a 46.6% increase).

Resistance in invasive *K. pneumoniae* isolates has decreased over the past 10 years for gentamicin, cefuroxime and 3rd generation cephalosporins, but have noticeably stabilised. However, resistance to ciprofloxacin and piperacillin-tazobactam has increased. For more details, see Figure 8.6b and 8.6c. The increased proportion of ciprofloxacin-resistant isolates in 2017 when compared to 2016 mainly reflects a change in interpretation of S-I-R due to implementation of new EUCAST breakpoints for ciprofloxacin in most Danish DCMs in January 2017. Ciprofloxacin breakpoints changed (lowered) once again in 2019 combined with the introduction of the "Area of Technical Uncertainty" (ATU) in the EUCAST clinical breakpoint table v. 9.0. Therefore, trends for ciprofloxacin resistance should be interpreted with caution. Resistance for piperacillin-tazobactam should also be interpreted with caution. EUCAST breakpoints did not change since 2012 but an ATU for piperacillin-tazobactam was introduced in 2019.

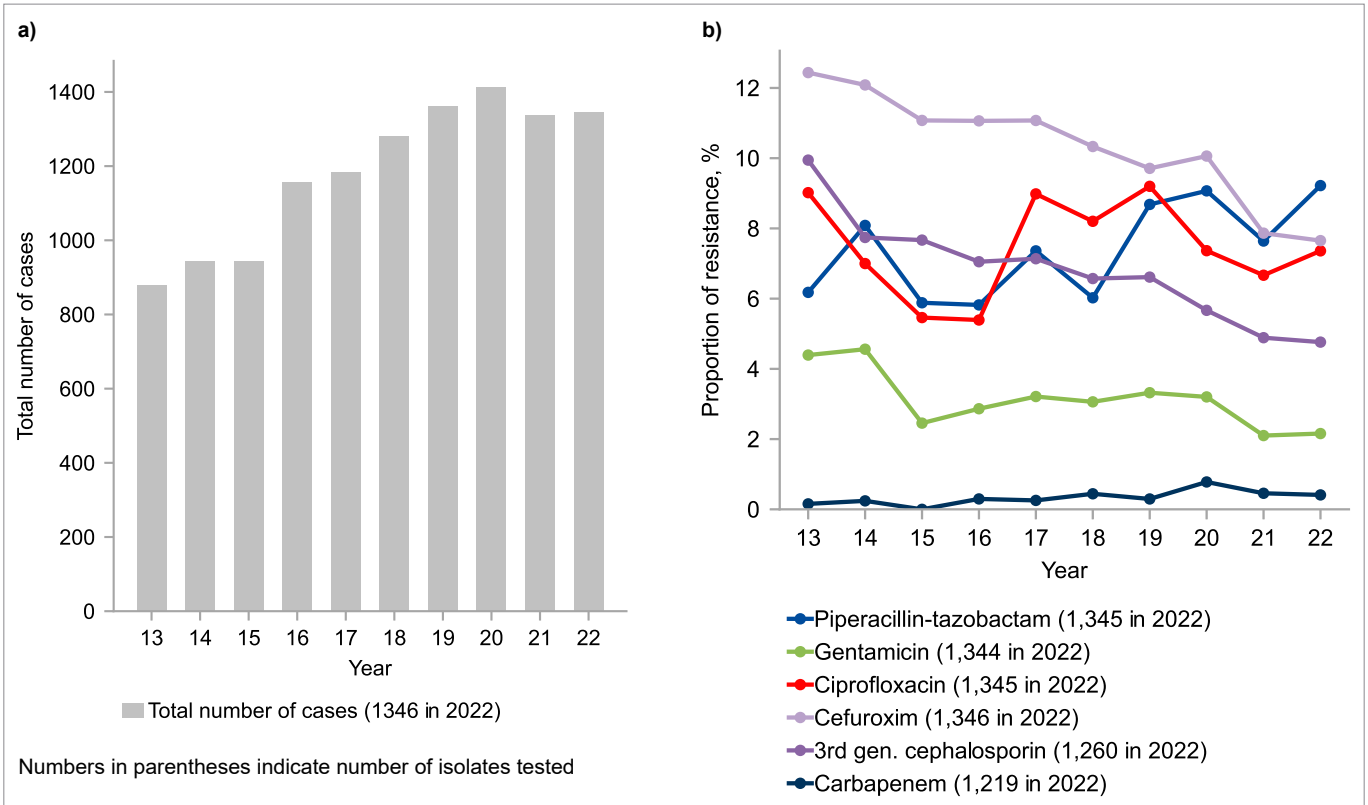
Carbapenem resistance in invasive *K. pneumoniae* is very low (6 resistant isolates out of 1336 tested [0.4%] in 2022). The increase shown in Figure 8.6b and 8.6c in 2020 is mainly due to hospital outbreaks (see Section 8.3.2 Carbapenemase-producing bacteria in Denmark, DANMAP 2020). The level of multidrug-resistant (combined resistance to 3rd generation cephalosporins, ciprofloxacin and gentamicin) invasive *K. pneumoniae* was 1.0% in 2022 (Table 8.5). Two invasive *K. pneumoniae* isolates were registered resistant to colistin. Susceptibility to colistin is not routinely tested.

Table 8.4 *Klebsiella pneumoniae*. Resistance (%) in isolates from humans, Denmark, 2022 DANMAP 2022

Substance	Invasive isolates, hospitals %	Urine isolates, hospitals %	Urine isolates, primary health care %
Mecillinam	8.7	9.4	7.9
Piperacillin/tazobactam	9.2	9.9	8.1 (1)
Amoxicillin/clavulanic acid	14	8.2	4.4 (5)
Sulfonamide		14.7 (4)	11.9
Trimethoprim		14.5	12.3
Nitrofuratoin		34 (5)	29.7
Gentamicin	2.2	2.3	1.5 (2)
Ciprofloxacin	7.4	7.2	5.1
Cefuroxime	7.7	8.1	5.2 (3)
3rd generation cephalosporins	4.8	4.7	3.4
Carbapenem	0.4	0.4	0.1 (1)
Max. number of isolates tested for resistance to the presented antibiotics	1,346	7,892	11,036

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

Figure 8.6 Invasive *Klebsiella pneumoniae* isolates from humans: a) annual number of isolates from unique cases, b) proportion of resistant isolates and c) table of resistance percentages, Denmark, 2013-2022 DANMAP 2022



Numbers in parentheses indicate number of isolates tested

c)

Substance	Percent resistant invasive *K. pneumoniae* isolates									
	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022
Piperacillin/tazobactam	6.2	8.1	5.9	5.8	7.4	6.1	8.7	9.1	7.5	9.2
Gentamicin	4.4	4.6	2.5	2.9	3.2	3.1	3.3	3.2	2.1	2.2
Ciprofloxacin	9.4	7	5.5	5.4	9.0	8.1	9.2	7.4	6.7	7.4
Cefuroxime	12.4	12.1	11.1	11.1	11.1	10.3	9.7	10.1	7.9	7.7
3rd gen. cephalosporins	9.2	7.7	7.7	7.3	7.1	6.1	6.6	5.3	4.9	4.8
Carbapenem	0.2	0.2	0	0.3	0.3	0.5	0.3	0.8	0.5	0.4
Total number of isolates	875	943	939	1,156	1,183	1,280	1,361	1,413	1,336	1,346

Table 8.5 *Klebsiella pneumoniae*. Combined resistance to 3rd generation cephalosporins, ciprofloxacin, and gentamicin (multidrug-resistance) in invasive isolates from humans, Denmark, 2015-2022 DANMAP 2022

	2015 % (N)	2016 % (N)	2017 % (N)	2018 % (N)	2019 % (N)	2020 % (N)	2021 % (N)	2022 % (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)
Percentage (no.) of isolates tested for combined resistance (multidrug-resistance)	89 (840)	98 (1,131)	95 (1,122)	93 (1,188)	94 (1,275)	93 (1,308)	93 (1,248)	94 (1,259)
Total number of invasive isolates	943	1,156	1,183	1,280	1,361	1,413	1,336	1,346

Urinary cases from hospitals

In 2022, 7,911 unique hospital patients had *K. pneumoniae* isolated from urine samples in Denmark. This represents a 34% and 2.7% increase compared to 2013 and 2021, respectively.

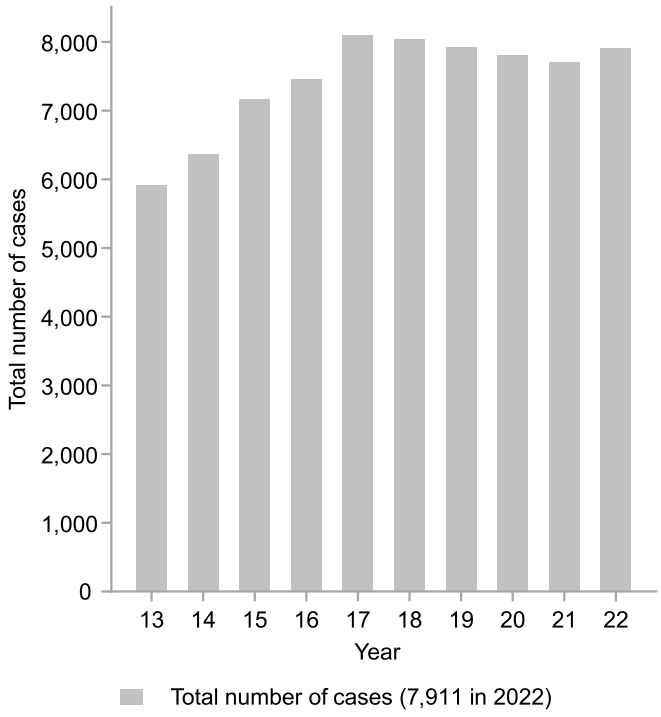
Summarized antibiotic-specific susceptibility results for *K. pneumoniae* isolates (invasive and urine specimen) are shown in Table 8.4. In Figure 8.7, resistance for *K. pneumoniae* urine isolates from hospitals is shown for 2013-2022.

Similar to the changes seen in invasive *K. pneumoniae* isolates, resistance in urine isolates from hospitals has decreased over the past 10 years for gentamicin, ciprofloxacin, cefuroxime and 3rd generation cephalosporins. Meanwhile resistance to piperacillin-tazobactam has been increasing and is now 9.9% The increase in resistance to mecillinam observed in 2017 and 2018 has been followed by a marked decrease to the current 9.4% (Figure 8.7). Amoxicillin/clavulanic acid resistance has only been reported since 2018 in urinary isolates from hospitals, due to less than six DCMs testing isolates previously. The proportion of resistance decreased since then.

In 2022, there were 23 carbapenem-resistant and 13 carbapenem-"susceptible, increased exposure" *K. pneumoniae* urine isolates from hospital patients reported in MiBa, compared to 13 and eight isolates in 2021, respectively.

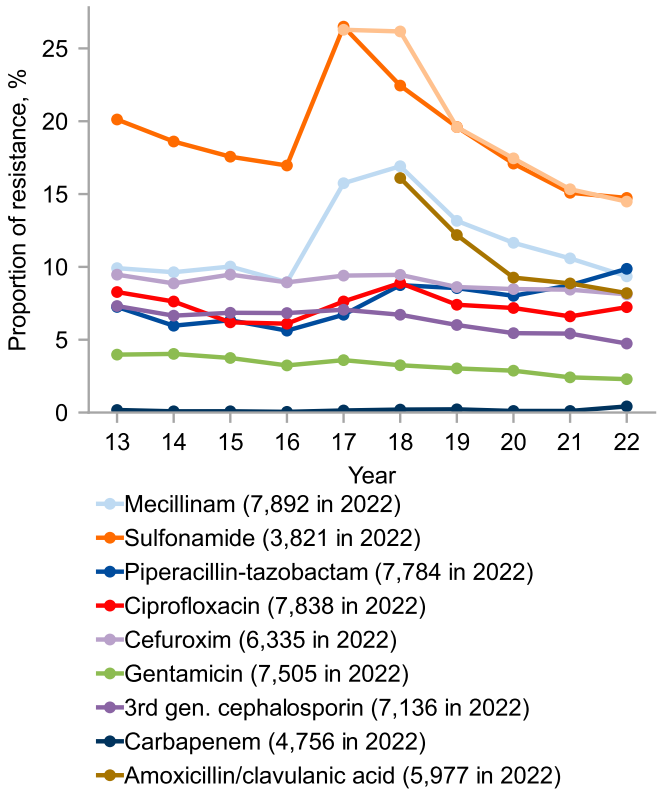
Figure 8.7 Urine *Klebsiella pneumoniae* isolates from humans (hospitals): a) annual number of isolates from unique cases, b) proportion of resistant isolates and c) table of resistance percentages, Denmark, 2013-2022 DANMAP 2022

a)



Numbers in parentheses indicate number of isolates tested

b)



c)

Substance	Resistance in *K. pneumoniae* urine isolates from hospitals									
	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022
Mecillinam	9.9	9.6	10	8.9	15.7	16.9	13.2	11.7	10.6	9.4
Piperacillin/tazobactam	7.2	6	6.3	5.6	6.7	8.7	8.5	8	8.7	9.9
Gentamicin	4	4	3.7	3.2	3.6	3.2	3	2.9	2.4	2.3
Ciprofloxacin	8.3	7.6	6.2	6.1	7.6	8.9	7.4	7.2	7.2	7.2
Cefuroxime	9.5	8.9	9.5	9.1	9.4	9.5	8.6	8.5	8.4	8.1
3rd gen. cephalosporins	7.3	6.6	6.8	6.8	7.1	6.8	6	5.5	5.4	4.7
Total number of isolates	5,919	6,372	7,175	7,467	8,106	8,047	7,926	7,814	7,701	7,911

Urinary cases from primary health care

In 2022, 11,039 unique patients in primary health care had *K. pneumoniae* isolated from urine samples, a 200% and 8.3% increase compared to 2013 and 2021, respectively.

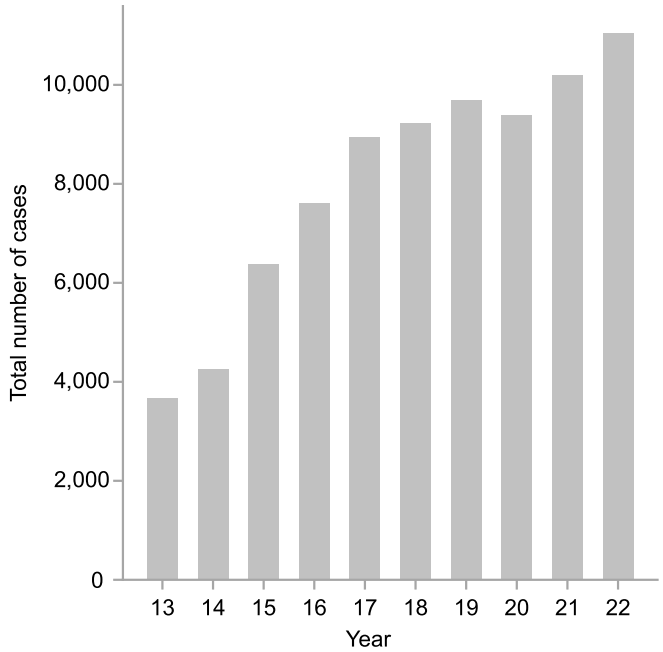
As for the results from invasive isolates and isolates from hospital urine samples, susceptibility results for all tested antimicrobials are shown in Table 8.4. In Figure 8.8, resistance for *K. pneumoniae* urine isolates from primary health care is shown for the past decade.

Following a sharp increase in 2017, resistance to mecillinam and sulfonamides/trimethoprim has since decreased. Additionally, in 2022, the 3rd generation cephalosporins resistance rate has also continued to decline, while resistance towards ciprofloxacin has increased slightly (Figure 8.8).

Five carbapenem-resistant isolates and one carbapenem-"susceptible, increased exposure" isolate were registered in 2022 compared to three and one, respectively, in 2021. However, susceptibility to carbapenems is only routinely reported to MiBa by one DCM.

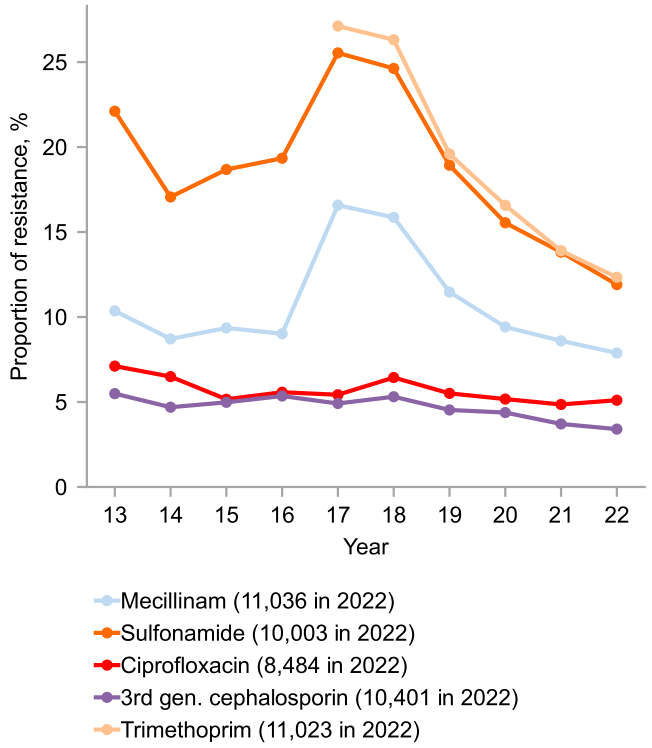
Figure 8.8 Urine *Klebsiella pneumoniae* isolates from humans (primary health care): a) annual number of isolates from unique cases, b) proportion of resistant isolates and c) table of resistance percentages, Denmark, 2013-2022 DANMAP 2022

a)



Numbers in parentheses indicate number of isolates tested

b)



c)

Substance	Percent resistant *K. pneumoniae* isolates from primary health care urine									
	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022
Mecillinam	10.4	8.8	9.4	9	16.6	15.9	11.5	9.4	8.6	7.9
Sulfonamide	22.1	17.1	18.7	19.3	25.5	24.6	18.9	15.5	13.8	11.9
Ciprofloxacin	7.1	6.5	5.2	5.6	5.4	6.4	5.5	5.2	4.9	5.1
3rd gen. cephalosporins	5.5	4.7	4.8	5.4	4.9	5.3	4.5	4.4	3.7	3.4
Total number of isolates	3,669	4,246	6,372	7,615	8,948	9,227	9,696	9,387	10,196	11,039

Conclusion

The general trend for *K. pneumoniae* in all three specimen types (blood/cerebrospinal fluid, urine [hospital/primary health care]) has been a decrease in resistance to important antimicrobials (cephalosporins, gentamicin and ciprofloxacin) over the last ten years. Following a sharp increase in mecillinam and sulfonamide/trimethoprim resistance in urine samples from hospitals and primary care in 2017 and 2018, the proportion of resistant *K. pneumoniae* isolates has since been decreasing. Resistance to piperacillin-tazobactam in *K. pneumoniae* from hospital urinary samples has been increasing and is now 9.9%. The carbapenem resistance in *K. pneumoniae* continues to be very low (<1%).

8.2.3 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is an opportunistic pathogen *that* can colonise the lung, urinary tract, burn wounds, superficial wounds and can 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. The antimicrobial classes which can be used for treatment include: fluoroquinolones, aminoglycosides (tobramycin, gentamicin and amikacin), broad-spectrum beta-lactams (piperacillin-tazobactam, ceftazidime and carbapenems) and colistin. New antibiotic combinations with beta-lactamase inhibitors, such as aztreonam-avibactam and ceftolozane-tazobactam, may be used in serious cases of *Pseudomonas* infections including MBL-producers.

Invasive cases from hospital patients

In 2022, a total of 495 unique patients with invasive *P. aeruginosa* isolates were registered in Denmark. Number of cases over the last decade are presented in Figure 8.9.

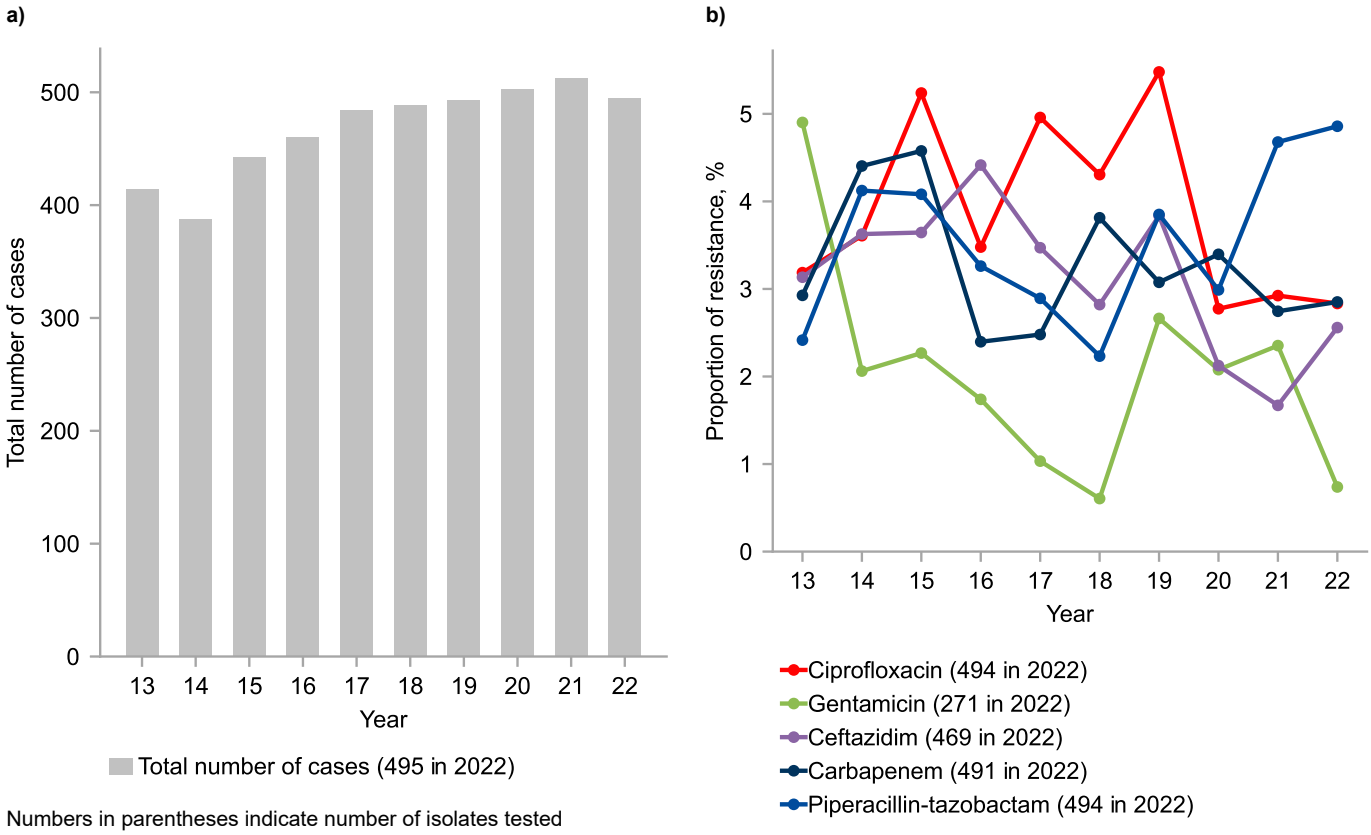
The highest proportion of resistance was reported for ciprofloxacin ranging from 2.8 to 5.4% over the past 10 years. In 2022, meropenem resistance was reported in 2.9% of the cases, but only 0.6% of the cases were resistant to three or more of the five antimicrobials under surveillance.

Conclusion

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

Figure 8.9 Invasive *Pseudomonas aeruginosa* isolates from humans: a) annual number of isolates from unique cases and b) proportion of resistant isolates, Denmark, 2013-2022

DANMAP 2022



Numbers in parentheses indicate number of isolates tested

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. The antimicrobial classes that are recommended for treatment include fluoroquinolones, aminoglycosides, carbapenems and colistin.

Invasive cases from hospitals

In 2022, a total of 93 unique patients with invasive *Acinetobacter* species were reported. Data are presented in Table 8.6 and in Figure 8.10.

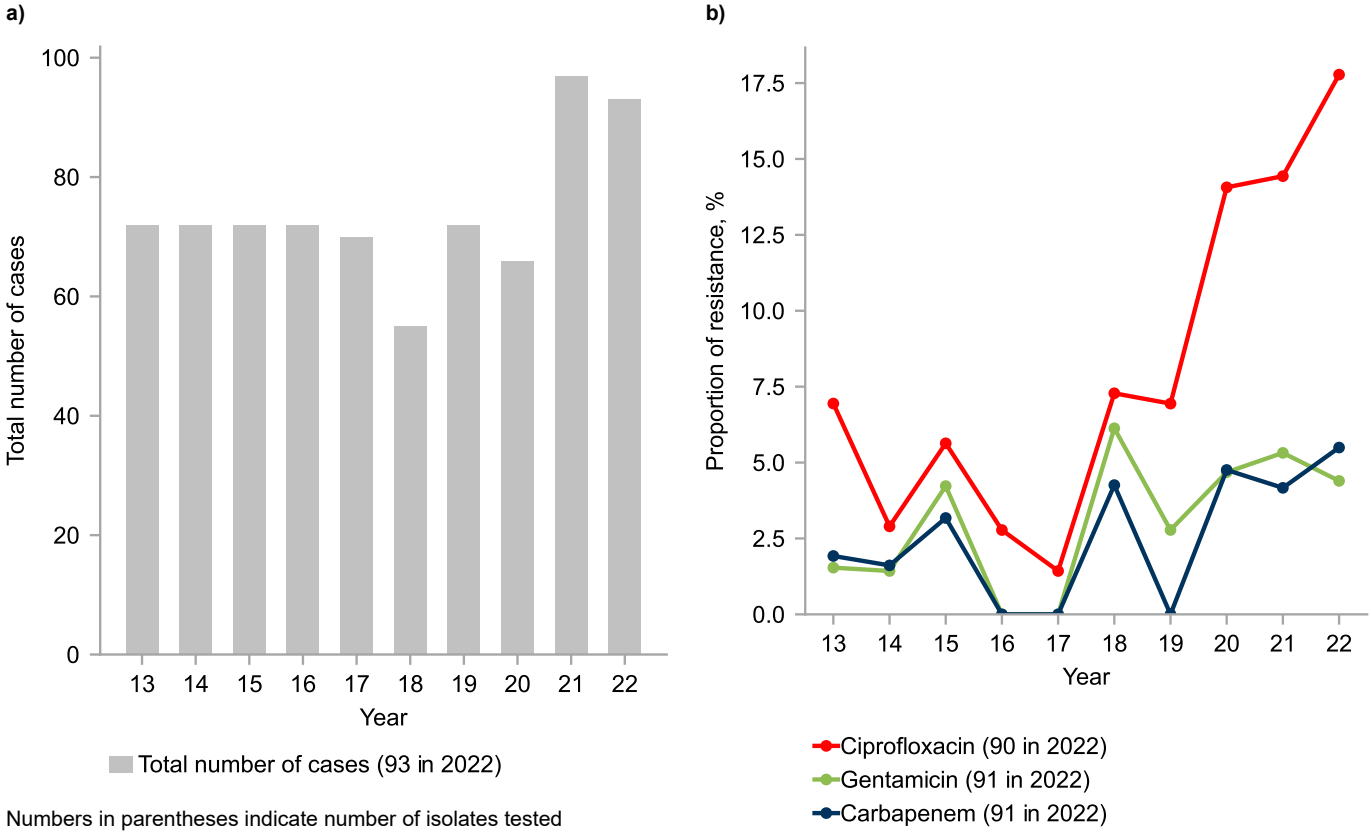
Following the marked increase in the number of invasive *Acinetobacter* spp. cases from 2020 to 2021, the number of cases has stabilized in 2022, but remains high compared to previous years. Five of the 93 isolates identified in 2022 were resistant to meropenem, 16 were resistant to ciprofloxacin and four were resistant to gentamicin. Four isolates had combined resistance to ciprofloxacin and gentamicin and four were reported resistant to colistin, however, susceptibility to colistin, is not routinely tested.

Table 8.6 *Acinetobacter* spp. Tested and resistant invasive isolates, Denmark, 2013-2022 DANMAP 2022

	2013		2014		2015		2016		2017		2018		2019		2020		2021		2022	
	res.	n	res.	n	res.	n	res.	n	res.	n	res.	n	res.	n	res.	n	res.	n	res.	n
Ciprofloxacin	5	72	2	69	4	71	2	72	1	70	4	55	5	72	9	64	14	97	16	92
Gentamicin	1	65	1	70	3	71	0	70	0	70	3	49	2	72	3	64	5	94	4	92
Carbapenem	1	52	1	62	3	68	0	69	0	67	2	47	0	72	3	63	4	96	5	93
Total number of invasive isolates	72		72		71		72		70		55		72		66		97		93	

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

Figure 8.10 Invasive *Acinetobacter* spp. isolates from humans: a) annual number of isolates from unique cases and b) proportion of resistant isolates, Denmark, 2013-2022 DANMAP 2022



Numbers in parentheses indicate number of isolates tested

Conclusion

The number of invasive *Acinetobacter* spp. saw a stabilisation in 2022 compared to the marked increase from 2020 to 2021; however, the proportion of invasive isolates resistant to key antimicrobials remained low in Denmark, but with a marked increase in ciprofloxacin resistance..

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.

Treatment of enterococcal infections may be challenging. Combination therapy based on a synergistic effect of beta-lactam antibiotics (penicillin/ampicillin) and aminoglycoside (most often gentamicin) or glycopeptide (vancomycin) is indicated in cases of complicated infection (e.g. endocarditis). In cases of high-level gentamicin resistance, combination of ampicillin and penicillin V may be used for treatment. The vast majority of *E. faecium* are ampicillin-resistant, and therefore most infections are treated with vancomycin. Antimicrobials such as linezolid and daptomycin can be used for treatment of the multidrug-resistant vancomycin-resistant enterococci (VRE).

Invasive cases from hospitals

In 2022, 659 unique patients with invasive *E. faecalis* isolates and 637 unique patients with invasive *E. faecium* isolates were reported in MiBa.

The proportion of resistant invasive *E. faecalis/faecium* isolates in 2022 are presented for each key antimicrobial in Table 8.7. In Figure 8.11, total numbers of invasive isolates of *E. faecalis* and *E. faecium* and the respective percentages of vancomycin resistance are shown for 2013 to 2022.

The total number of invasive cases caused by *E. faecalis* and *E. faecium* increased by 5.8% from 2013 to 2022. However, a marked decrease of 14% was observed from 2021 to 2022.

A continuous high proportion of ampicillin resistance in invasive *E. faecium* has been observed with proportions of resistant isolates ranging between 92% and 95% since 2010. In 2002, the resistance rate was reported to be 65%. The proportion of invasive vancomycin-resistant *E. faecium* isolates continued to increase to 12.0% in 2022 from 10.2% in 2021 after having stabilised at around 9% in the previous past three years.

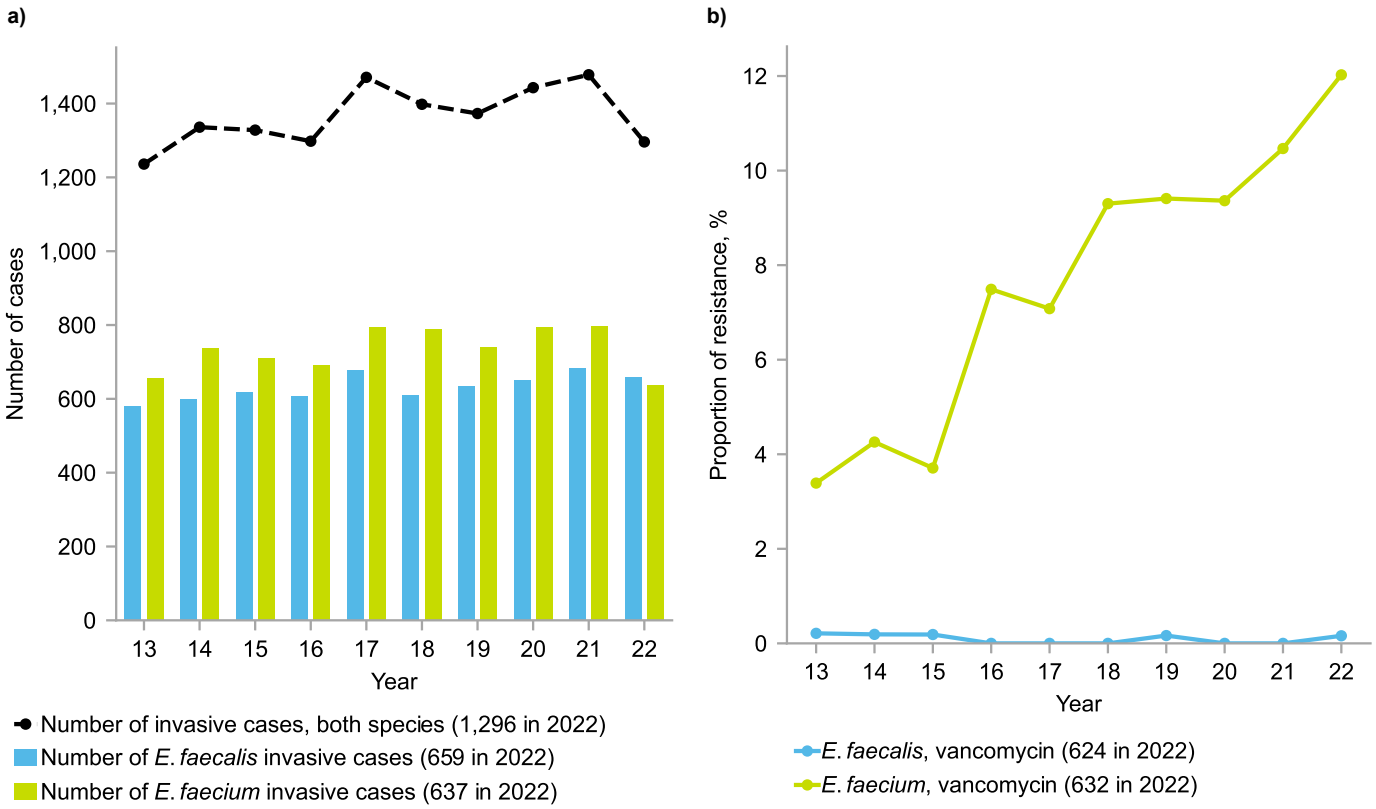
During 2022, five invasive isolates of *E. faecalis* and five invasive isolates of *E. faecium* from ten unique patients were reported linezolid-resistant (Table 8.7). In 2021, the numbers were four *E. faecalis* and two *E. faecium*. Four of the five linezolid-resistant invasive *E. faecium* isolates identified in MiBa in 2022 were also reported resistant towards vancomycin.

Table 8.7 Enterococci. Resistance (%) in invasive isolates from humans, 2022 DANMAP 2022

	E. faecalis	*E. faecium*	Number of included isolates (number of DCM)	
	%	%	*E. faecalis*	*E. faecium*
Ampicillin	0	92.9	658 (10)	607 (9)
Vancomycin	0.2	12.0	624 (9)	632 (10)
Linezolid	1.0	0.8	508 (6)	513 (6)
Teicoplanin	0.4	1.1	233 (2)	177 (2)
Tigecycline	1.0	2.4	104 (1)	82 (1)

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

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, 2013-2022 DANMAP 2022



Numbers in parentheses indicate number of isolates tested

Conclusion

Over the past 20 years, a steady increase in invasive has been observed. However, a noticeable drop occurred from 2021 to 2022. Additionally, a marked increase in invasive *E. faecium* resistant to vancomycin has been observed over the past 10 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 (DCMs) have, on a voluntary basis, submitted third-generation cephalosporin-resistant *E. coli* (3GC-R *Ec*) from bloodstream infections for further characterization at the National Reference Laboratory for Antimicrobial Resistance at Statens Serum Institut (SSI) by adhering to the EUCAST criteria for reduced susceptibility towards cefpodoxime, ceftriaxone, cefotaxime or ceftazidime.

At SSI, the 3GC-R Ec's collected in Denmark through 2022, 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.

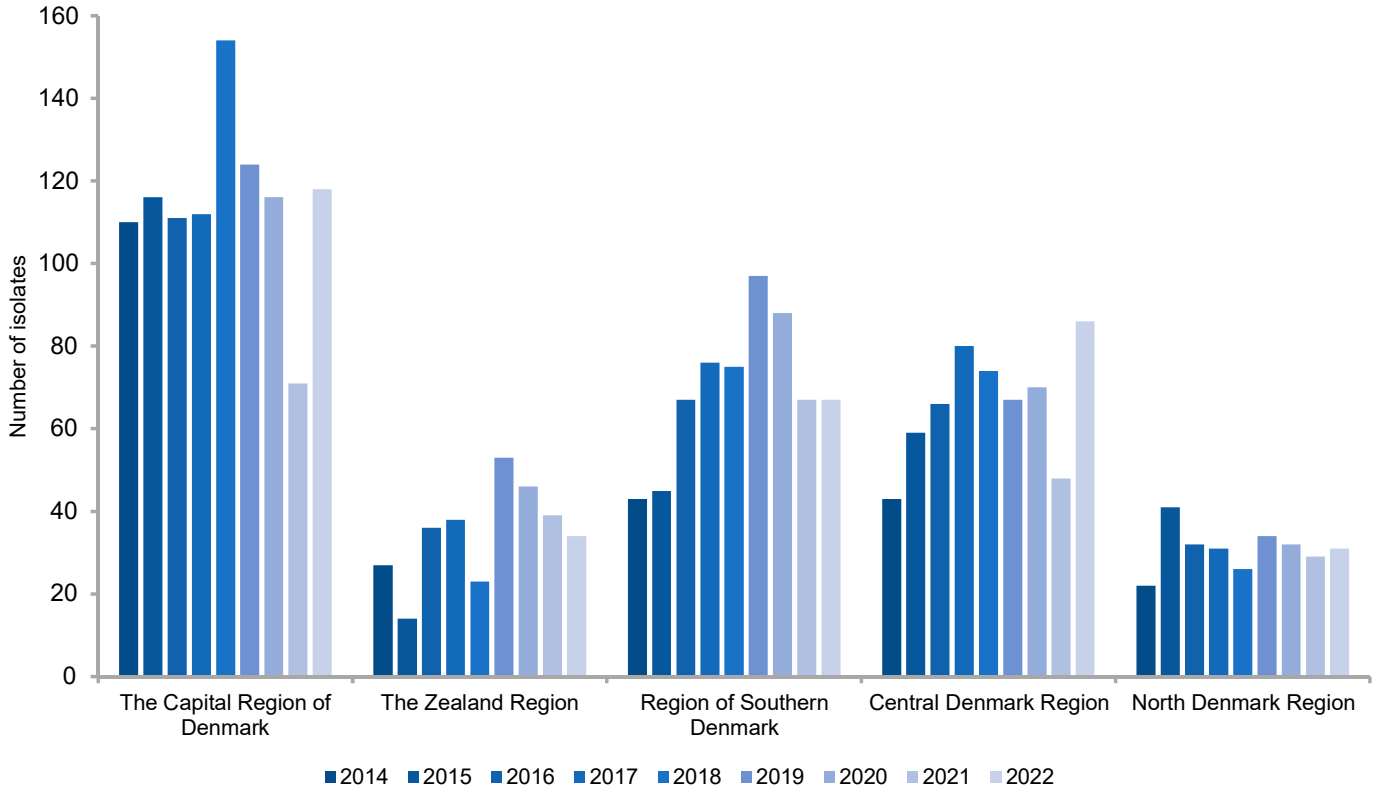
Results

In 2022, a total of 336 *E. coli* isolates from unique patients, were identified with phenotypic test, as ESBL and/or AmpC positive isolates. Demographic data was available for all 336 *E. coli* isolates in 2022; 187 (56%) of the patients were men compared to 147 (58%) in 2021, and 149 (44%) were women compared to 107 (42%) in 2021. The median age at diagnosis was 71 years, ranging from below one year to 96 years. The regional distribution of the 336 isolates with ESBL- and AmpC phenotype was compared to data from previous years (Table 8.8 and Figure 8.12).

Following the overall decreasing trend of reported cases of ESBL/pAmpC *E. coli* in bloodstream infections observed from 2019 to 2021 (32%), the number increased from 2021 to 2022 (32% from 254 to 336 isolates). Increasing numbers were notably observed in The Capital Region and Central Region of Denmark, whereas the numbers in the remaining regions did not change notably.

Whole genome sequencing data were obtained from 181 *E. coli* isolates (as only isolates from every second month and carbapenemase producing *E. coli* were sequenced). Genes encoding ESBL and/or pAmpC were detected in 178 isolates (five having carbapenemase encoding genes detected) and three isolates were AmpC hyperproducers caused by cAmpC mutations. The AmpC hyperproducers will not be analysed further.

Figure 8.12 ESBL/pAmpC producing *E. coli* from bloodstream infections by region, 2014-2022, Denmark DANMAP 2022



In 2022, 20 different genes associated with ESBL-, and pAmpC enzymes were detected among the 178 sequenced isolates encoding ESBL and/or pAmpC genes (Table 8.9). As in previous years, CTX-M-15 was the most prevalent enzyme, remaining relatively stable in occurrence at 52% in 2022, compared to 46% in 2021. In addition, five carbapenemase producers were observed during 2022 among the 181 whole genome sequenced blood infection isolates (3%); two NDM- and three OXA-48-group producing isolates.

Table 8.8 Distribution of ESBL and Carbapenemase producing *E. coli* from bloodstream infections, Denmark, 2015-2022 DANMAP 2022

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

Table 8.9 Most common ESBL enzymes and carbapenemases detected in *E. coli* from bloodstream infections, Denmark 2015-2022 DANMAP 2022

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

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

Table 8.10 Distribution of MLSTs in *E. coli* from bloodstream infections, Denmark, 2015-2022 DANMAP 2022

	DANMAP 2015		DANMAP 2016		DANMAP 2017		DANMAP 2018		DANMAP 2019		DANMAP 2020		DANMAP 2021		DANMAP 2022	
MLST	Number	%	Number	%	Number	%	Number	%	Number*	%	Number*	%	Number*	%	Number*	%
ST131	135	49%	177	57%	175	52%	189	54%	93	47%	89	46%	64	49%	89	50%
ST38	23	8%	21	7%	23	7%	22	6%	13	7%	8	4%	1	1%	11	6%
ST69	10	4%	16	5%	20	6%	27	8%	14	7%	20	10%	7	5%	9	5%
ST648	10	4%	5	2%	8	2%	6	2%	4	2%	0	0%	1	1%	6	3%
ST1193	5	2%	10	3%	7	2%	8	2%	6	3%	9	5%	9	7%	5	3%
ST73	2	1%	4	1%	2	1%	6	2%	4	2%	8	4%	1	1%	5	3%
ST12	9	3%	14	4%	6	2%	5	1%	5	3%	2	1%	5	4%	4	2%
Other STs[1]	51	19%	48	15%	69	20%	69	20%	38	19%	43	22%	34	26%	46	26%

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

In 2022, the 172 of the 178 whole genome sequenced *E. coli* isolates belonged to 44 different known MLSTs, with the remaining 6 isolates typed with novel STs. The most common sequence type (ST) was ST131 (50%), followed by ST38 (6%) and ST69 (5%) (Table 8.10).

Among the 89 *E. coli* isolates belonging to ST131, CTX-M-15 (55%) was the most common enzyme, followed by CTX-M-27 (33%), and CTX-M-14 (6%). The distribution of ESBL and/or pAmpC enzymes observed within ST131 remained stable in 2022.

Conclusion

In 2022, the number of ESBL- and/or AmpC positive isolates increased from 254 to 336 isolates (32% increase). CTX-M-15 remained by far the most prevalent ESBL enzyme in Danish *E. coli* from bloodstream infections in 2022. In isolates belonging to ST131, the relative distribution of ESBL/pAmpC enzymes was stable in 2022 compared to 2020 and 2021.

In 2022, five carbapenemase producers were observed among the 178 whole genome sequenced ESBL- and/or pAmpC blood infection isolates. The relative distribution of sequence types for the whole genome isolates were similar to the distributions in the previous years; the worldwide disseminated ST131 clone was still strongly represented in 2022 (50%).

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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 with 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 (DCMs) 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 confirmed carbapenemase-producing Enterobacterales (CPE), *Pseudomonas* spp. and *Acinetobacter* spp.

During 2022, 392 CPOs were identified from 335 patients compared with 277 CPO isolates from 242 patients in 2021, an increase in isolates of 42%. More than one isolate from the same patient was included if the isolates belonged to different bacterial species and/or if the isolates harboured different carbapenemases. Nineteen out of all CPOs were from bloodstream infections compared to 11 out of all CPOs in 2021.

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 2022, 350 CPE isolates were reported from 304 patients compared to 251 CPE from 221 patients in 2021 resulting in a 39% increase of CPE isolates compared to 2021. In 2022, 22 of the 350 CPE isolates produced both NDM and OXA-48 group enzymes, 208 produced OXA-48-like enzymes alone and 112 were NDM-producers. Furthermore, four KPC-, one VIM-, one IMP-, one KPC-/VIM- as well as one KPC-/NDM-producing CPE isolate(s) were identified (Figure 8.13).

Carbapenemase-producing *Acinetobacter* spp.

In 2022, 23 carbapenemase-producing *Acinetobacter* spp. isolates were reported from 23 patients, compared to 25 isolates from 25 patients in 2021. Of these, 21 patients had been travelling abroad prior to identification of the carbapenemase-producing *Acinetobacter* spp. In 2022, 22 carbapenemase-producing *Acinetobacter baumannii* with the following enzymes were identified: OXA-23 (16), OXA-72 (4), OXA-58 (1) and NDM-5/OXA-23 (1). Furthermore, one OXA-72-producing *Acinetobacter pittii* was identified. All in all, a steady increase in the number of carbapenemase-producing *Acinetobacter* spp. have been observed since the first Danish isolate was identified in 2008 (Figure 8.14).

Figure 8.13 Numbers of carbapenemase-producing Enterobacterales (CPE), Denmark, 2008-2022 DANMAP 2022

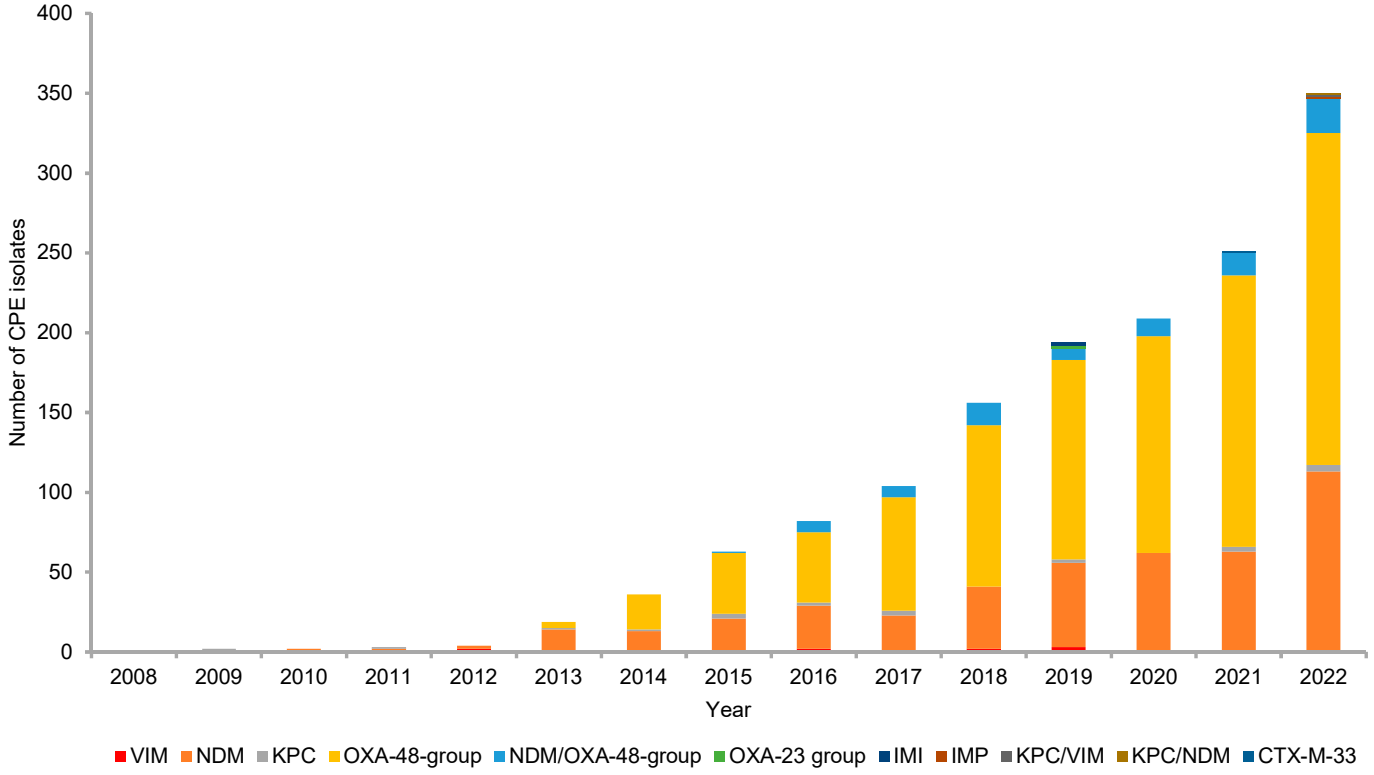
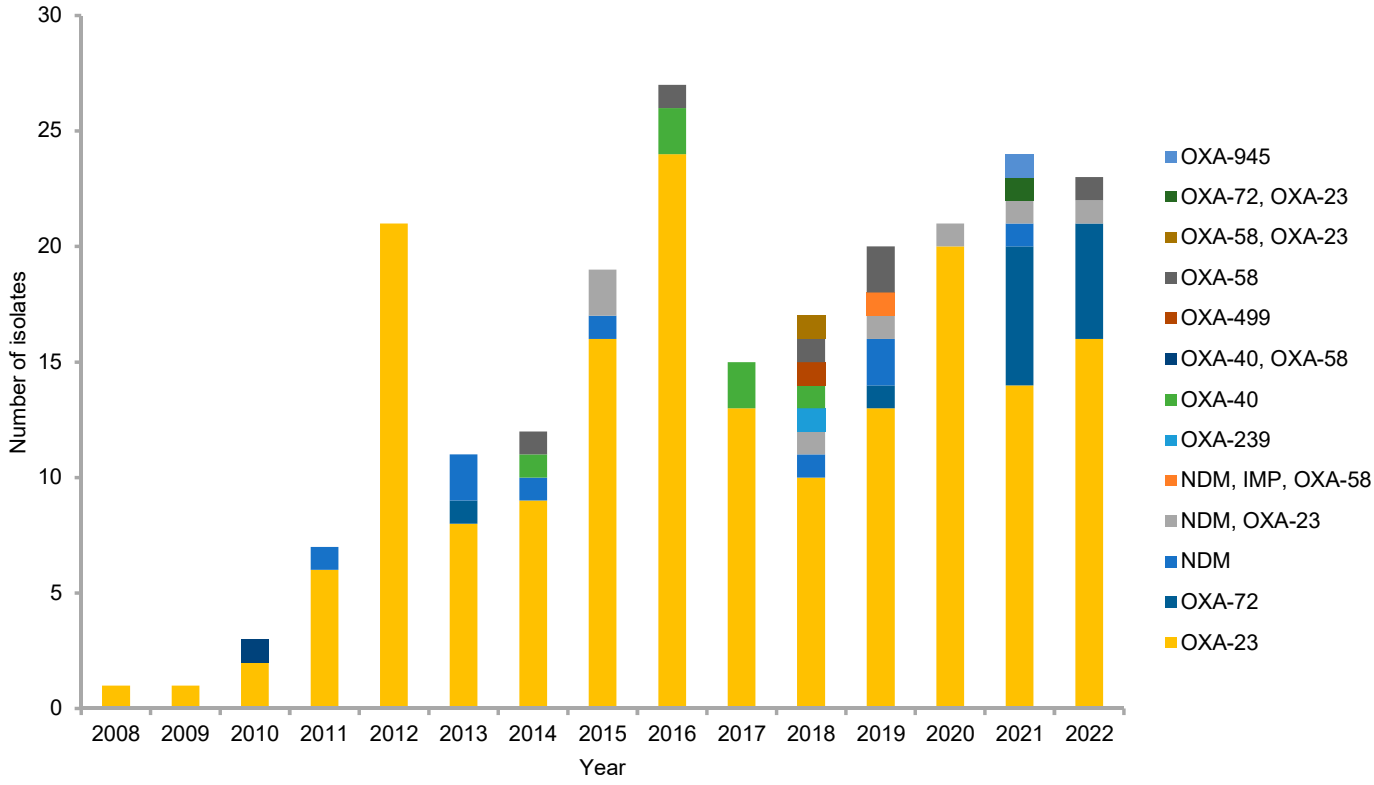


Figure 8.14 Carbapenemase-producing *Acinetobacter* spp. and enzymes identified, Denmark, 2008-2022 DANMAP 2022

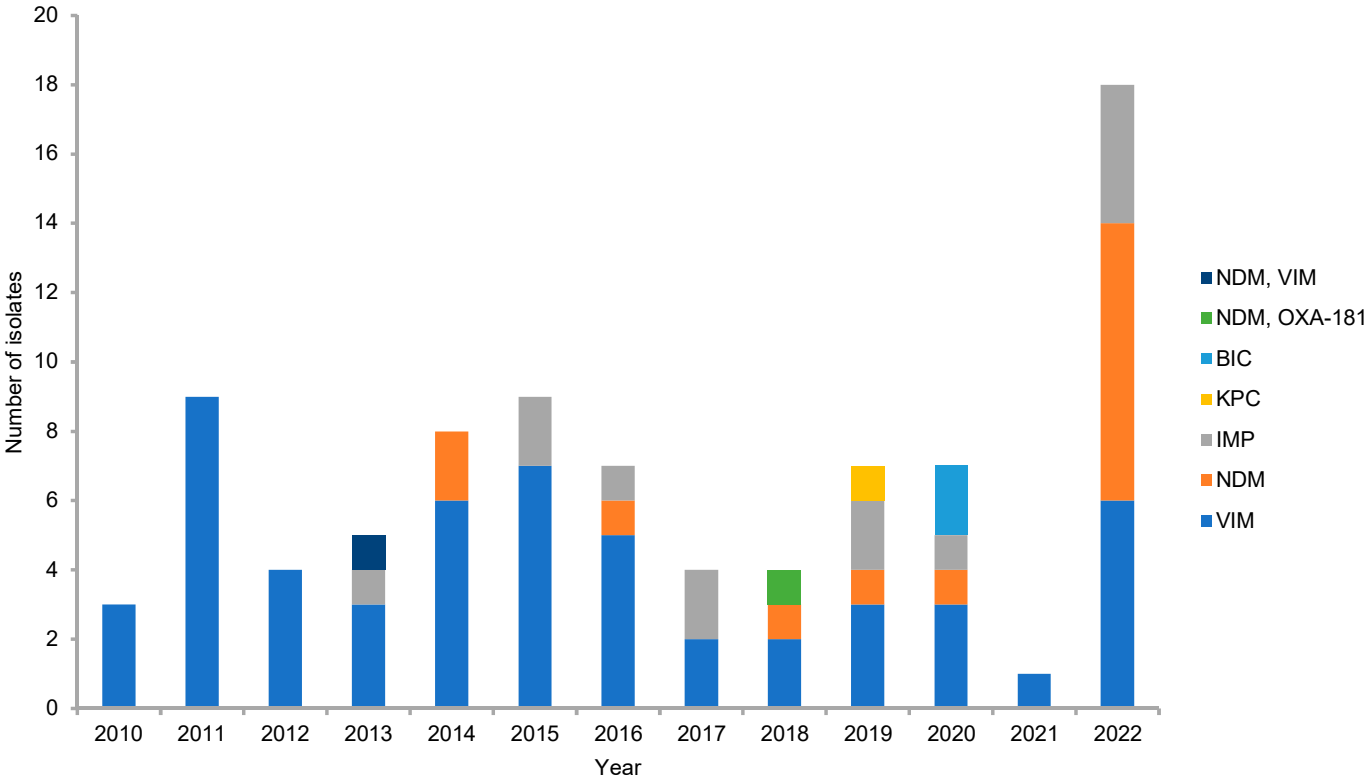


Carbapenemase-producing *Pseudomonas* spp.

In 2022, 19 carbapenemase-producing *Pseudomonas* spp. isolates from 18 patients were reported compared to only one isolate in 2021. In 2022, 18 carbapenemase-producing *Pseudomonas aeruginosa* with the following enzymes were identified: NDM-1 (8), VIM-2 (4), VIM-4 (1), IMP-10 (2), IMP-1 (1), IMP-1/IMP-10 (1) or unknown carbapenemase (1). Furthermore, one VIM-2-producing *Acinetobacter pittii* was identified.

In general, the number of carbapenemase-producing *Pseudomonas* spp. have been relatively stable between 2010 and 2020, with a remarkable decrease in 2021 presumably due to reduced travel activities on account of the COVID-19 pandemic. As opposed to this observation, a large increase in 2022 happened due to the arrival of patients in relation to the current military conflict in Ukraine (Figure 8.15).

Figure 8.15 Carbapenmase-producing *Pseudomonas* spp. and enzymes identified, Denmark, 2010-2022 DANMAP 2022



CPO - Place of origin 2019-2022

It is mandatory for the treating physician to obtain information regarding travel of a CPO-positive patient, if possible, encompassing six months prior to detection. The DCM or a clinical physician can also report a CPO-patient to be colonised in Denmark, implicating that the patient has not been travelling prior to detection of the strain. Depending on this and on the results from WGS performed at the laboratory, the CPO-patient will be classified as belonging to one of five current epidemiological categories: 1) Denmark, sporadic cases 2) Denmark, part of outbreak, 3) travel outside the Nordic countries, 4) patients from Ukraine, and 5) unknown (Figure 8.16).

If a known CPO-patient later is affected by a Danish nosocomial outbreak, the patient will be reclassified as an outbreak-patient. Vice versa, if the index patient (the first patient) in an outbreak was known to be travelling prior to detection of the CPO-strain, the outbreak will be registered according to travel information.

In 2022, the reported travel data showed that 52 (19%) of 279 CPO-positive persons (e.g. patients that were not associated with an outbreak in Denmark) reported travelling outside the Nordic countries, which is is the same proportion as in 2021. In 2019, 43% of the CPO-cases had been travelling outside the Nordic countries. In 2020, supposedly due to the pandemic, the proportion of travel-associated cases had dropped markedly. The most frequent reported travel destinations in 2022 were Asia (11), Africa (10), Middle East (8), and the Mediterranean (7). The most single reported travel destinations were Turkey (10) and Egypt (6).

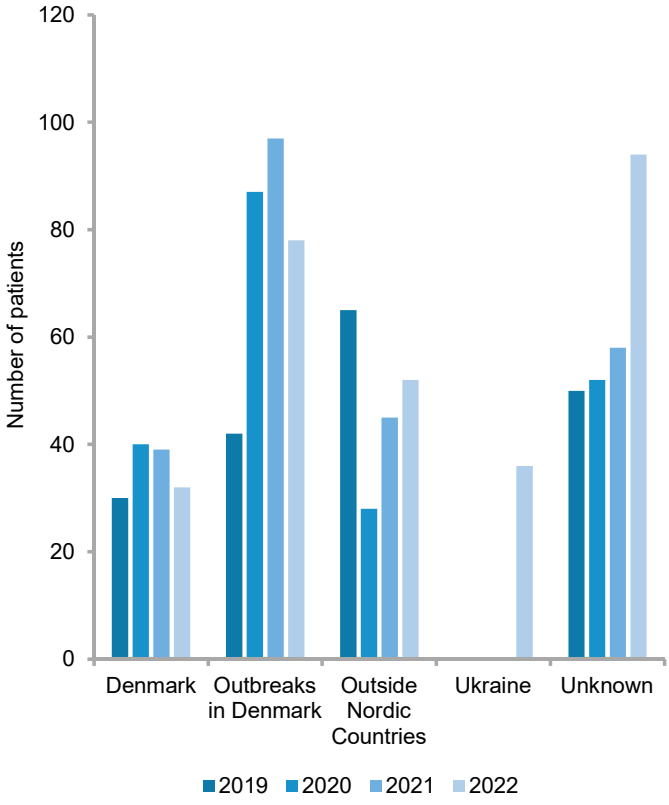
Due to the war in Ukraine, several patients originating from Ukraine have been in contact with the Danish health care system. From February 24th, 2022, through January 23rd, 2023, a total of 371 CPO from 288 patients were obtained as part of the Danish National CPO surveillance. Of the 371 CPO, 77 CPO were collected from 42 patients from Ukraine. The

findings show that the patients originating from Ukraine were colonized and/or infected by many different CPO per patient [Stolberg, et al. 2023, J Glob Antimicrob Resist, 34:15-17].

Outbreaks with CPO during 2022

In Denmark, outbreaks caused by CPO in healthcare settings are registered at SSI in the 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 probable outbreak (Materials and methods, Section 10.12).

Figure 8.16 Place of origin of CPO, 2019-2022 DANMAP 2022



In 2022, a total of 17 CPO-outbreaks were registered compared to 15 CPO-outbreaks in 2021. In seven of the outbreaks, it was possible to establish an epidemiological link between the patients. All epidemiological links were found in healthcare settings, caused by patients sharing the same ward or hospital. Eleven of the seventeen outbreaks had been ongoing for more than two years and two outbreaks more than ten years, meaning that new patients had been identified as belonging to the same cgMLST cluster as found in the previous years.

In 2022, no new outbreak due to *A. baumannii* was detected and no new cases have been detected in the three outbreaks reported in 2021 and were therefore removed from Table 8.11. In total, 78 new patients were affected in 2022 by outbreaks, which is the same level as in 2021. Of the seventeen outbreaks registered in 2022, five new small clusters were identified involving 2-3 patients each, but no epidemiological link could be established in any of the these clusters. Three smaller clusters detected in 2021 had no new patients in 2022 and were removed from the table (Table 8.11).

Outbreaks with CPO of interest

An unusual outbreak with the same unique epitype ST79 *Enterobacter hormaechei* carrying the resistance genes bla_{NDM-5} and bla_{OXA-48} was registered and investigated in Denmark (ID1062) during October 2022 to June 2023. Altogether 15 CPO patients with the epitype and 19 CPO patients with one of the unique resistance genes were detected. Please see Textbox 8.1.

The ST18 NDM-1-producing *Citrobacter freundii* outbreak (ID41), which started in 2012 in the North Denmark Region, continued in 2022 [Hammerum *et al.* 2016, J. Antimicrob. Agents, 71:3117-3124]. Since 2012, the outbreak has spread to four out of five Danish Regions. Until the end of 2022, 93 hospitalized patients have been involved in this outbreak. In all, 20 new patients were identified in 2022 and all had been hospitalized in the North Denmark Region. None of these new outbreak-cases had a prior history of travel.

Since 2015, another large outbreak (ID21) has been ongoing mainly in the Zealand Region and in the Capital Region with spread of ST410 NDM-5/OXA-181 *E. coli*. [Roer *et al.* 2018, mSphere;3(4)]. By the end of 2022, 84 patients in total have been involved in this outbreak. During 2022, twelve new patients were affected by this outbreak. Apart from the first reported patient in 2015, who had been traveling to Egypt [Overballe-Petersen *et al.* 2018, Genome Announc.6(5): e01542-17] none of the patients have a prior history of travel.

Table 8.11 Outbreaks of carbapenemase-producing Enterobacterales (CPE) during 2022, n=17, Denmark DANMAP 2022

Outbreak ID	Year	Patients total	Patients 2022	Carbapenemase	Type of Outbreak	Species (clonal spread)	Regions[1]	Status
41	2012-2022	93	20	NDM-1	Clonal/ plasmid	ST18 *C. freundii*	1 / 2 / 3 / 4	Verified
48	2013-2022	34	4	OXA-436/OXA-48	Clonal/ plasmid	ST90 *E. cloacae*/ ST22 *C. freundii*	1 / 4 / 5	Verified
21	2015-2022	84	12	NDM-5/OXA-181	Clonal	ST410 *E. coli*	1 / 2 / 5	Verified
22	2015-2022	13	3	OXA-181	Clonal	ST440 *E. coli*	1 / 2	Possible
42	2015-2022	13	1	OXA-48	Clonal	ST65 *C. freundii*	1 / 3 / 5	Verified
47	2015-2022	11	3	VIM-2	Clonal	ST111 *P. aeruginosa*	2 / 3	Possible
43	2019-2022	5	2	OXA-48	Clonal	ST323 *C. freundii*	5	Possible
1061	2020-2022	10	4	OXA-181	Clonal	ST22 *C. freundii*	2	Possible
1062	2020-2022	14	11	NDM-5/OXA-48	Clonal/ plasmid	ST79 *E. hormaechei*	1 / 2 / 3 / 4 / 5	Verified
1068	2020-2022	10	1	OXA-48	Clonal	ST18 *C. freundii*	1	Verified
1052	2020-2021	5	1	NDM-1	Clonal	ST18 *C. freundii*	2	Possible
1089	2021-2022	6	4	OXA-244	Clonal	ST131 *E. coli*	2	Verified
10972	2022	3	3	OXA-48/OXA-181	Clonal	ST698 *C. freundii*	1	Possible
10992	2022	2	2	OXA-244	Clonal	ST131 *E. coli*	2 / 4	Possible
11032	2022	3	3	NDM-5	Clonal	ST17 *K. pneumoniae*	1	Possible
11072	2022	2	2	OXA-181	Clonal	ST636 *C. freundii*	5	Possible
11132	2022	2	2	OXA-48	Clonal	ST22 *C. freundii*	5	Possible

[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

Conclusion

The occurrence of carbapenemase-producing bacteria in Denmark continued to increase throughout 2022. The number of patients received from Ukraine contributed to this increase. The level of new nosocomial outbreaks in 2022 was the same as in 2021. The number of patients belonging to the two largest outbreaks in hospital settings continued to increase, highlighting the importance to start early interventions with infection prevention control (IPC) in order to prevent further spread of an outbreak. The spread of CPO 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 detection and control difficult. For the first time in Denmark, an outbreak due to a pharmaceutical product was revealed, emphasizing the importance of national surveillance and the collaboration between national and regional level.

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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. 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 [Kohler, et al, 2018, PLoS One. 2018 Mar 22;13(3)], 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 2015 and 2016, sporadic VVE isolates with different genetic background in relation to concurrent VRE-outbreaks were detected in the Capital Region of Denmark [Hammerum *et al.* Euro Surveill. 2020;25(18)]. In 2016, a new VVE clone belonging to ST1421- CT1134, displaying variable vancomycin susceptibility due to a deletion in the *vanX* gene was detected. [Hansen *et al.*, J. Antimicrob. Agents, 2018, 73: 2936–2940]. 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 (DCMs) 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 isolates characteristics such as species identification, multilocus sequence typing (MLST), detection of *van*-genes and core genome sequence typing (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 2016 were compared to data from clinical VRE reported by the DCMs to MiBa. This comparison showed that the number of submitted VRE/VVE isolates was not complete (Figure 8.17). In 2022, 667 VRE/VVE isolates were submitted to SSI, and were genotyped by WGS. Furthermore, 178 VRE/VVE isolates were identified in MiBa, which had not been submitted to SSI and hence have not been genotyped. This was an increase compared to 2021, were 578 VRE/VVE isolates were sent to SSI and 164 VRE/VVE isolates were identified in MiBa. (Figure 8.17).

Of the 667 clinical VRE/VVE isolates sequenced in 2022, 60 were *vanA E. faecium*, 604 *vanB E. faecium*, 1 *vanA*/*vanB E. faecium*, and 2 *vanB E. faecalis* (Figure 8.17). Until 2020, *vanA E. faecium* were most common, but during the last years this has changed. In 2022, 91% of the *E. faecium* isolates had the *vanB* gene.

WGS-based cgMLST analysis was performed on the 665 E. faecium isolates using SeqSphere+ (Ridom), where a total of 137 unique clonal types (CTs) were observed. When investigating the composition of CTs for *E. faecium*, we observed a clustering tendency between isolates, where CTs were diverging while the allelic differences were minimal within each cluster. To investigate further, Local Single Linkage clustering (SLC) was set up in SeqSphere+, setting the maximal allelic distances to 20. A total of 110 SLCs were detected, of which 70 clusters consisted of 5 or fewer isolates. Each SLC was named according to the ST and CT of the first observed isolate within each cluster. Of these clusters, one SLC (covering several different CTs, but presumably originating from the same clone) was predominant in the Danish surveillance: The ST80-CT2406 *vanB E. faecium* group containing 468 isolates (Table 8.12).

Figure 8.17 Overview and distribution of vancomycin resistance genes in vancomycin-resistant isolates, Denmark, 2015-2022

DANMAP 2022

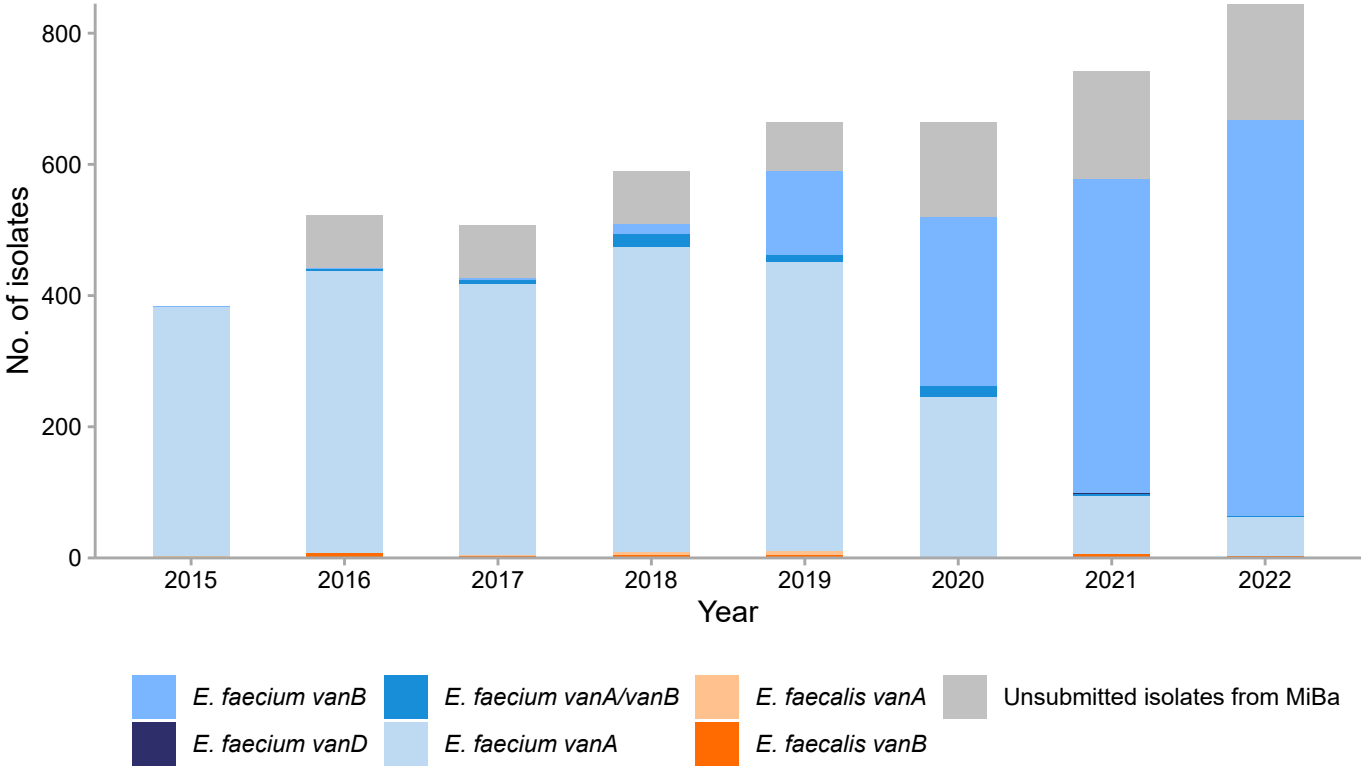


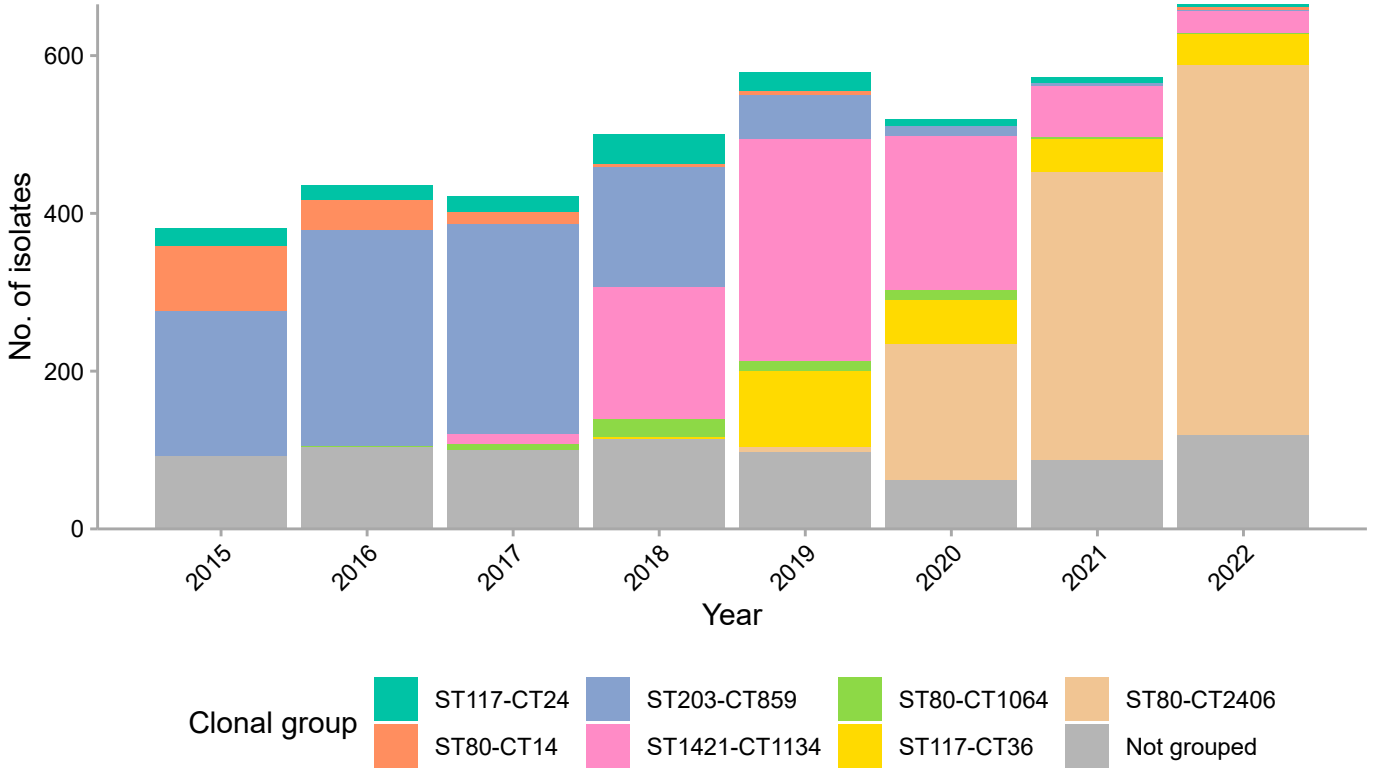
Table 8.12 Description of the most common types of *vanA* and/or *vanB Enterococcus faecium* according to MLST and cgMLST, Denmark, 2016-2022

DANMAP 2022

Types [a]	2016		2017		2018		2019		2020		2021		2022		All years
	(n = 435)		(n = 426)		(n = 518)		(n = 584)		(n = 519)		(n = 565)		(n = 609)		Total
ST117-CT24 group [b]	19	4%	20	5%	38	7%	26	4%	8	2%	7	1%	4	1%	147
ST80-CT14 group [c]	39	9%	16	4%	3	1%	3	1%	1	0%	0	0%	3	0%	147
ST203-CT859 group [d]	273	63%	265	62%	156	30%	57	10%	12	2%	3	1%	2	0%	952
ST1421-CT1134 group [e]	1	0%	12	3%	167	32%	285	49%	197	38%	63	11%	27	4%	752
ST80-CT1064 group [f]	2	0%	7	2%	23	4%	12	2%	13	3%	3	1%	2	0%	62
ST117-CT36 groupG	0	0%	0	0%	3	1%	95	16%	56	11%	43	8%	40	6%	237
ST80-CT2406 group [h]	0	0%	0	0%	0	0%	7	1%	174	34%	356	63%	468	68%	1,005
ST117-CT1686	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	15	2%	15
ST80-CT6438	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	10	1%	10
Other types	101	23%	106	25%	128	25%	99	17%	58	11%	90	16%	120	17%	927

a) ST, sequence type (MLST); CT, cluster type (cgMLST)
b) **CT24**,CT875,CT1180,CT1487,CT1834,CT2456,CT6018
c) **CT14**,CT869,CT1530,CT1797,CT2019
d) **CT859**,CT1051,CT1507,CT1688,CT2257,CT2758,CT5973
e) **CT1134**,CT1749,CT1854,CT2545,CT2911,CT3379,CT5936,CT6048
f) **CT1064**,CT2496,CT6123,CT6520
g) **CT36**,CT991,CT1526,CT2531,CT2659,CT2979
h) **CT2406**,CT2946,CT2949,CT3024,CT3234,CT4189,CT4835,CT5120,CT5143,CT5166,CT5211,CT5215,CT5928,CT5972,CT5974, CT5999,CT6117,CT6132,CT6253,CT6254,CT6417,CT6435,CT6435,CT6436,CT6494,CT6507,CT6531,CT6547,CT6598,CT6610

Figure 8.18 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, 2015-2022 DANMAP 2022



Retrospectively, three clonal groups were conspicuous in the sense of being predominant for a limited time period (see Table 8.12 and Figure 8.18). During 2015-2017, the ST203-CT859 *vanA E. faecium* clonal group was the most prevalent clone. It has since decreased in prevalence and in 2022 less than 1% of the VRE/VVE *E. faecium* isolates belonged to ST203-CT859. In 2017, testing for the presence of *vanA*/*vanB* genes by use of PCR in phenotypically vancomycin-susceptible *E. faecium* isolates from blood cultures was introduced in the DCMs in the Capital Region as a mean of detecting possible VVE [Hammerum *et al.* Euro Surveill. 2020;25(18)]. At that time, the ST1421-CT1134 *vanA E. faecium* clonal group only constituted 3% of the total clinical VRE/VVE *E. faecium* isolates. This clone was initially only detected from clinical samples from the Capital Region, yet in 2018 and 2019 its prevalence increased to 32% and 49%, respectively, and it was now observed in the Capital Region and Zealand Region [Hammerum *et al.* Euro Surveill. 2020;25(18)]. It has since been found in all five regions of Denmark. While the ST1421-CT1134 *vanA E. faecium* VVE clone became the predominant clone throughout 2019 and 2020, it was overtaken by the ST80-CT2406 *vanB E. faecium* clone in 2021, where it decreased to 11%. The earliest detection of the ST80-CT2406 *vanB E. faecium* clone in Denmark was in 2019, where it was present in 1% of the isolates. From 2020 to 2022, the ST80-CT2406 *vanB E. faecium* clone increased from 34% to 68%. Since its introduction, the clone has been detected in all of the five regions, where 74% of cases has been observed on Zealand and in the Capital Region. It is the most frequently occurring clone of the ST80-CT2406 clonal group, and the group itself is the most diversified clonal group, spanning 20 different CT clones. Detailed in Figure 8.18.

Conclusion

The number of VRE/VVE cases increased again in 2022 compared to 2021. Thus it remains clear that more prevention strategies are required to break the annual trend of increasing occurrence of VRE in the Danish health care system.

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8.3.4 Detection of linezolid-resistant enterococci and 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. 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-resistant enterococci (LRE)

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

In 2022, four linezolid-resistant *E. faecalis* isolates and one linezolid-resistant *E. faecium* were sent to SSI. WGS data from the LRE isolates were investigated for 23S rRNA mutations (G2576T and G2505A), and the *optrA*, *cfr*, *cfr(B)* and poxtA genes using the LRE-Finder (https://cge.cbs.dtu.dk/services/LRE-Finder/).

The four *E. faecalis* isolates and the *E. faecium* were all positive for *optrA* (Table 8.13). During the period 2015-2021, 15 linezolid-resistant *E. faecalis* were detected, whereas eight linezolid-resistant *E. faecium* were identified.

Surveillance of linezolid-vancomycin-resistant Enterococci (LVRE)

In order to detect linezolid-resistant VRE isolates (LVRE), the LRE-Finder was also implemented in the automated surveillance at SSI for detection of LRE mutations and LRE genes in genomes from VRE isolates submitted by the DCMs directly for the national VRE Surveillance. During the period 2015-2021, no linezolid-vancomycin-resistant *E. faecalis* were detected, whereas 36 linezolid-vancomycin-resistant *E. faecium* were identified.

In 2022, 15 linezolid-vancomycin-resistant *E. faecium* were identified. All 15 linezolid-resistant *E. faecium* isolates had the G2576T mutation, five of these were positive for the *vanA* gene and 10 were positive *vanB* (Table 8.13).

Conclusion

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

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Table 8.13 Characterization of the 5 linezolid-resistant enterococci (LRE) and the 15 linezolid-vancomycin-resistant enterococci (LVRE), 2022, Denmark DANMAP 2022

	No. of isolates	Species	Linezolid resistance mechanism	Vancomycin resistance gene
LRE	4	*E. faecalis*	*optrA*	none
	1	*E. faecium*	*optrA*	none
LVRE	5	*E. faecium*	G2576T	*vanA*
	10	*E. faecium*	G2576T	*vanB*

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 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 2,578 in 2022 corresponding to 44 cases per 100,000 inhabitants. This is almost the same number as in 2021 (2,511). Fifty (1.9%) of the bacteraemia cases were caused by MRSA. During the last decade this proportion has been between 1.3% (2012) and 2.9% (2014) and remains below most other European countries participating in EARS-Net [EARS-Net 2021]. Livestock-associated (LA) - MRSA CC398 caused seven of the 50 MRSA bacteraemia cases. Within 30 days from the bacteraemia onset, 627 (24%) patients died (all-cause mortality). The mortality for the MRSA bacteraemia cases was 18%.

Table 8.14 Resistance (%) in isolates from *Staphylococcus aureus* bacteraemia cases 2013-2022, Denmark DANMAP 2022

	2013	2014	2015	2016	2017	2018	2019	2020*	2021	2022
Antimicrobial agent	%	%	%	%	%	%	%	%	%	%
Methicillin	1.7	2.9	1.5	2.1	2.2	1.6	2.1	1.6	1.6	1.9
Penicillin	76	77	71	71	72	72	72	72	69	68
Erythromycin	7	8	7	7	6	5	9	7	7	9
Clindamycin	6	8	7	6	5	4	8	7	7	8
Tetracycline	3	5	4	3	3	3	2	3	2	3
Fusidic acid	15	15	16	12	14	17	14	14	13	13
Rifampicin	0	<1	<1	<1	<1	<1	<1	<1	<1	<1
Moxifloxacin#	5	6	6	4	4	4	5	6	4	4
Kanamycin	2	2	3	1	1	2	<1	nt	nt	nt
Linezolid	0	0	0	0	0	0	0	0	0	0
Mupirocin	<1	<1	<1	0	<1	0	<1	<1	<1	<1
Trimethoprim-sulfamethoxazole	1	1	<1	<1	<1	0	<1	<1	<1	<1

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 8.15 The ten most prevalent *spa* types demonstrated in SAB and in non-LA-CC398 MRSA cases, Denmark 2022 DANMAP 2022

SAB			MRSA			
spa type	CC group	No. of cases	*spa* type	CC group	No. of cases	No. causing infections (%)
t127	CC1	120	t304	CC8	229	114 (50)
t084	CC15	115	t127	CC1	168	79 (47)
t091	CC7	101	t223	CC22	122	55 (45)
t002	CC5	96	t002	CC5	121	71 (59)
t230	CC45	78	t008	CC8	110	74 (67)
t008	CC8	73	t4549	CC8	85	69 (81)
t012	CC30	69	t005	CC22	56	33 (59)
t701	CC8	55	t021	CC30	49	31 (63)
t015	CC45	51	t688	CC5	42	29 (69)
t1451	CC398	48	t1476	CC8	37	23 (62)

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

The antimicrobial susceptibility remained at the same level as the previous years for most agents (Table 8.16). Resistance to penicillin in 2022 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 (13%), erythromycin (9%) and clindamycin (8%).

Typing revealed a high diversity with 727 different *spa* types distributed in 27 different clonal complexes (CCs). The ten most prevalent *spa* types, representing 31% of the total number, are presented in Table 8.17 together with *spa* types from the most prominent types of MRSA. The distribution of the most prevalent *spa* types has been very stable over the last decade with only minor changes in the relative order. The Panton-Valentine Leukocidin (PVL) toxin was present in 21 (1%) cases of which six were MRSA. The 21 isolates with the PVL gene were distributed among 15 different *spa* types.

Surveillance of methicillin-resistant *S. aureus*

In 2022, 2,996 MRSA cases were detected (51 per 100,000 inhabitants), a 10% increase compared to 2021 (2,712; Figure 8.21a). The number of new cases was still below the pre-covid levels (3,657 cases registered in 2019). 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 51% of the cases. The proportion of infections in the years 2013 to 2022 has varied between 38% to 51% (Figure 8.21b).

CC398 cases constituted 28% (n = 853) of new MRSA cases, of which 828 belonged to the livestock-associated clone (LA-MRSA CC398) and the remaining 25 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 (41%), which likely reflects the active screening of patients with contact to livestock at admission to healthcare.

MRSA isolates carrying *mecC* were detected in 55 cases (1.8%). Thirty-nine of the cases (71%) 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 (see Textbox 3.3, Chapter 3, DANMAP 2021). 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 357 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 2022 are listed in Table 8.17. They constituted 28% of the total number of non-LA-CC398 MRSA isolates. Table 8.17 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. No change in *spa* type distribution has so far been registered following the war in Ukraine.

Figure 8.19a Number of new MRSA cases 1994-2022, Denmark, with a three years moving average DANMAP 2022

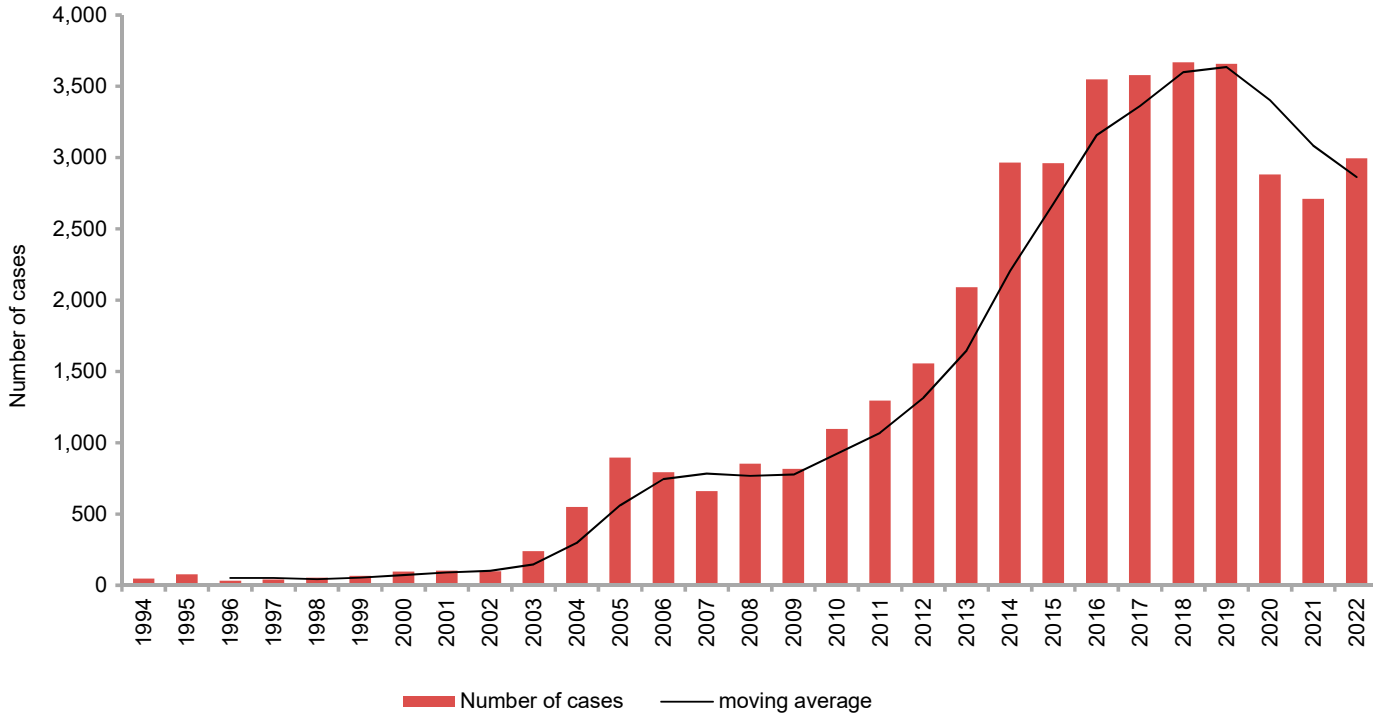

Figure 8.19b Number of new MRSA cases 2013-2022, Denmark, divided in infection and screening samples DANMAP 2022

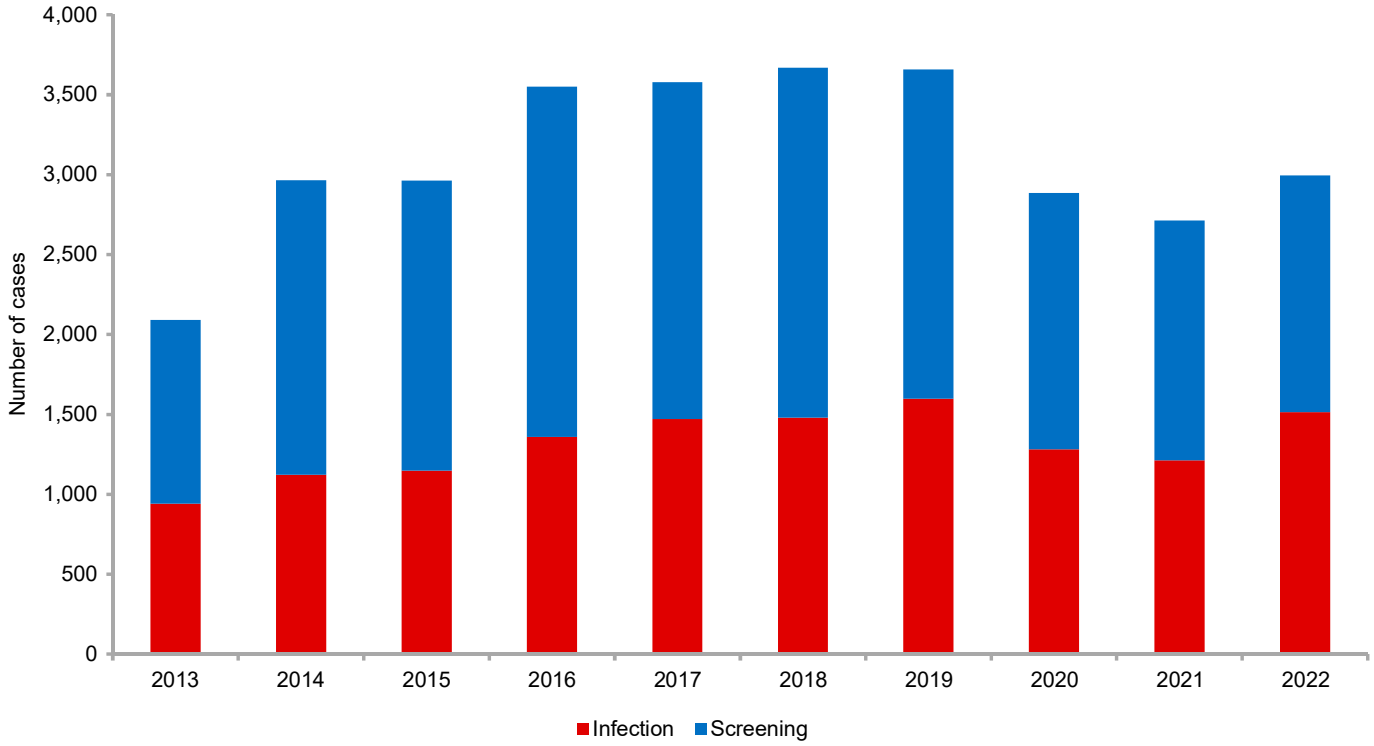

Table 8.16 Epidemiological classification of new MRSA cases, Denmark, 2022 DANMAP 2022

Epidemiologic classification	Exposure	No. of cases (% of total)	No. (%) of cases with infections (% of cases in same epi. class)
Imported (IMP)		**423 (14)**	279 (66)
Hospital-acquired (HA)		**55**	30 (55)
"Health-care associated, community onset (HACO)"		**232 (8)**	
	with known exposure	14	6 (43)
	without known	218	184 (84)
Health care worker		**28 (1)**	20 (71)
Community-acquired (CA)		**1,430 (48)**	
	with known exposure	653	99 (15)
	without known	777	666 (86)
LA-MRSA CC398		**828 (28)**	
	with known exposure	697	138 (20)
	without known	131	92 (70)
Total		**2,996**	**1,514 (51)**

Numbers shown in bold are totals

The PVL encoding gene was detected in 24% of the infections and in 13% of the asymptomatic carriers and most often in relation to isolates with *spa* types t008 (n = 67), t005 (n = 39), t021 (n = 37), t002 (n = 35) and t127 (n = 23).

Thirty-nine MRSA outbreaks were registered at hospitals, nursing homes and other institutions. These outbreaks comprised a total of 143 cases of which 70 had an infection. Seven of the outbreaks occurred in neonatal departments, comprising a total of 46 cases. Additionally, eight outbreaks were registered in other hospital departments, comprising 17 patients and thirteen outbreaks were observed in nursing homes (counting a total of 30 residents).

The total number of cases and the number of cases presenting with infection according to epidemiological classification are shown in Table 8.16. Most of the cases (86%) were acquired in Denmark.

Figure 8.20 Number of MRSA infections according to epidemiological classification, 2013-2022 Denmark DANMAP 2022

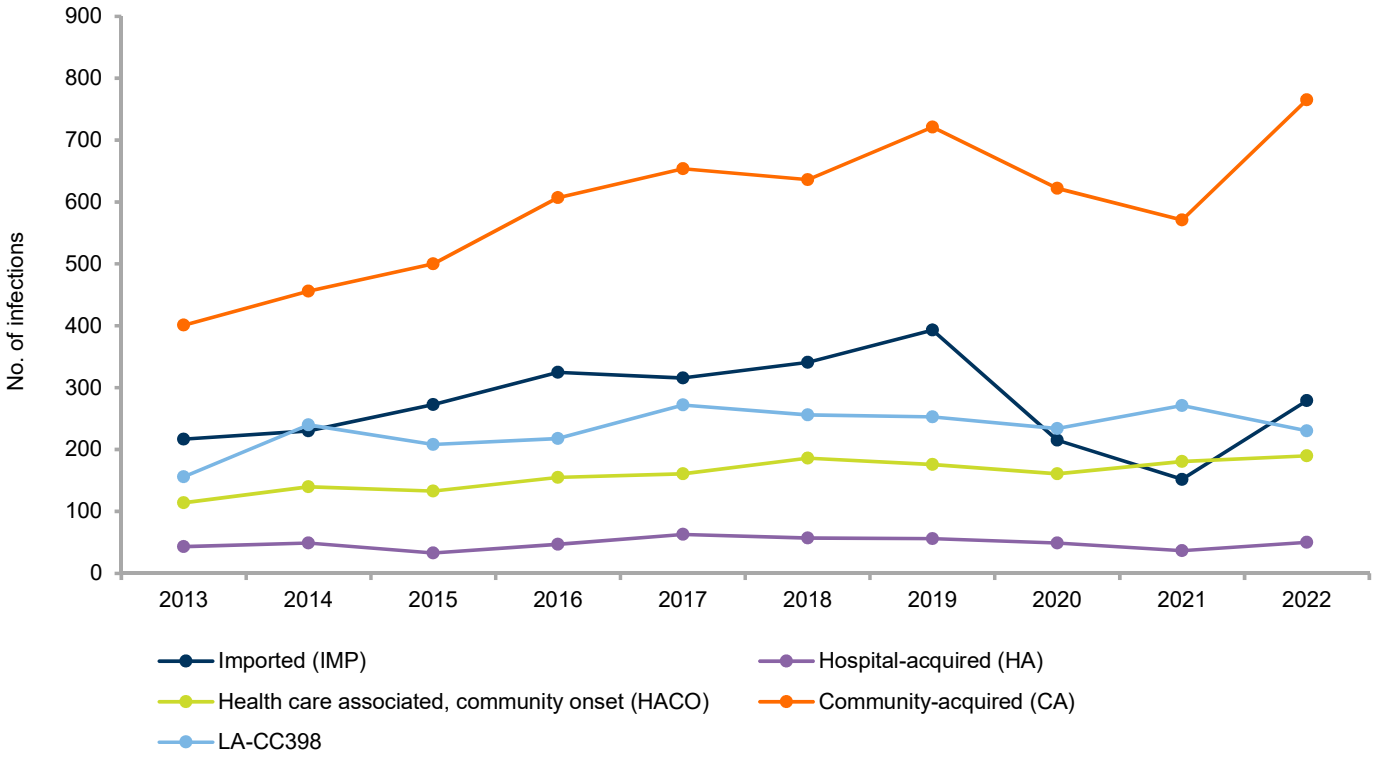

Table 8.17 Resistance (%) in non LA-CC398 MRSA isolates, 2013-2022, Denmark DANMAP 2022

	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022
Erythromycin	32	33	37	34	34	33	33	30	29	34
Clindamycin	24	23	29	25	27	28	23	22	19	21
Tetracycline	20	21	24	26	24	26	22	22	21	23
Fusidic acid	17	17	19	18	16	18	23	22	21	22
Rifampicin	1	<1	<1	1	1	1	<1	<1	<1	<1
Moxifloxacin#	23	27	21	19	20	21	21	17	19	23
Linezolid	<1	<1	0	<1	0	<1	0	<1	<1	<1
Mupirocin	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Trimethoprim-sulfamethoxazole	3	3	4	2	3	3	4	2	<1	1
Number of tested isolates	1,451	1,616	1,242	1,184	1,193	1,233	1,025	1,920*	1,520*	2,043*

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

Resistance among MRSA isolates

Resistance data for non-LA-CC398 isolates are presented in Table 8.17. Resistance prevalences were similar to previous years, with relatively high resistance to erythromycin (34%), fusidic acid (22%), clindamycin (21%), tetracycline (23%) and moxifloxacin (23%), and low resistance (<1%-1) to trimethroprim-sulfamethoxazole, linezolid, mupirocin and rifampicin.

Conclusion

The number of *S. aureus* bacteraemia cases (N=2,578) was almost the same in 2022 as in 2021, with a still low proportion of MRSA <2%. The number of MRSA increased in 2022 after two years of lower numbers due to COVID-19 related restrictions. This was also reflected in an increased number of imported and community-acquired cases, while number of LA-MRSA CC398 decreased.

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The Danish Infection prevention and control guidelines for MRSA, CPO, VRE and other MDRO

The growing numbers of multidrug-resistant bacteria globally demand increased and continued focus on the use of infection prevention and control measures at all levels in the health care system. Although Denmark is regarded a low-prevalent country, it takes efforts to keep the number of outbreaks low and, despite the efforts some of these outbreaks continue to develop. Historically, the emergence of MRSA at Danish hospitals in the 1980´ies paved the way for both surveillance of *S. aureus* infections and infection prevention and control programmes at hospitals, but only within the recent ten to fifteen years, IPC programmes have become more formalised and nationwide. Currently, the following specific programmes exist:

The Guidance on Preventing the Spread of MRSA by the Danish Health Authority was issued in 2006, the year MRSA became notifiable. The applicable 3rd edition of the guideline is from 2016 (Guidance on Preventing the Spread of MRSA - The Danish Health Authority (sst.dk)). The guideline represents the national recommendations and strategic framework for preventing the spread of MRSA at hospitals and in other healthcare settings and nursing homes. Upon referral to a hospital the following high-risk situations are screened for in a patient: being previously diagnosed with MRSA, household-like contact with persons tested positive for MRSA, travelling outside the Nordic countries with admission to a hospital or clinic, and frequent contact with live pigs (for the particular risk of being a carrier of livestock-associated MRSA). Individual risk factors for acquiring colonization or infection with MRSA are wounds, chronic skin diseases and invasive devices etc. As strategy the guideline applies a `search and destroy´ approach: MRSA-carriers can be treated and be declared MRSA-free (in contrast to persons carrying resistant bacteria in the gut, e.g. CPE). Being MRSA-free demands testing negative thrice within a minimum period of six months after completion of treatment. In the case of an outbreak at hospitals, nursing homes and home care, it is necessary to supplement the general precautions with transmission based (isolation) precautions [https://hygiejne.ssi.dk/retningslinjer/infektionshygiejniske-retningslinjer-for-mrsa]. All patients with MRSA are isolated in single rooms or cohort isolation in case of outbreak at hospitals (with contact isolation regime). This is not the case at nursing homes.

The first **national guideline on preventing the spread of CPO by** the Danish Health Authority was issued in 2018 (www.sst.dk/da/udgivelser/2018). The guideline provides a national strategic framework for detection and management of CPO at hospitals. The main purpose of the guideline is to maintain a low prevalence of disease caused by CPO associated with certain high-risk situations. Upon admission to a hospital a patient is screened for: previous diagnosis of CPO, household-like contact with a CPO positive person, travelling outside the Nordic countries with admission to a hospital or clinic and/or receiving antibiotic treatment during the stay.

Individual risk factors for acquiring colonization or infection with CPO are older age (above 80 years), immunosuppression caused by medication or certain chronic and cancer diseases, antibiotic treatment and invasive devices. It is noteworthy that patients are known to be carriers of CPE for several years (in some of the Danish outbreaks more than five years) with no treatment for CPE carrier state available. As a result, compliance with IPC guidelines is extremely important in order to prevent further spread of CPE, and correct hand hygiene and use of personal protective equipment (PPE) are among the most important control measures.

In hospitals, nursing homes and home care, it is necessary to supplement the general precautions with transmission based (isolation) precautions in the case of an outbreak [https://hygiejne.ssi.dk/retningslinjer/infektionshygiejniske-retningslinjer-for-cpo]. All patients with CPO are isolated in single rooms or cohort isolation in case of outbreak at hospitals (with contact isolation regime). This is not the case at nursing homes.

In relation to hospital outbreaks, environmental spread of CPO has occurred through bath drains and toilets. After intensive cleaning and disinfection of these parts it has in some cases been possible to stop an outbreak. This emphasizes the importance of more frequent cleaning and disinfection in an outbreak situation and the importance of well-functioning drains in general.

Infection prevention and control guidelines for VRE and other multidrug-resistant microorganisms (MDRO)
All healthcare professionals are expected to be familiar with and act in compliance with the national guidelines for infection prevention and control (published by the National Centre for Infection Control at SSI); [https://hygiejne. ssi.dk/NIRgenerelle]. The supplemental national guideline includes specific guidance on VRE/VVE and other multidrug-resistant microorganisms and should be followed when being in contact with a patient, for which either clinical infection or carriage of MDRO is suspected or known [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]. As for MRSA and CPO screening of a patient for either VRE or other MDRO is recommended on admission to hospital for defined risk situations, such as travel and hospitalisation outside the Nordic countries or known exposure to a patient with VRE or other MDRO at a Danish ward. Isolation is recommended in the national supplemental IPC-guideline upon verification of VRE in the patient.

All IPC guidelines emphasise the importance of all health care staff, irrespective of profession, to contribute to the management and prevention of infections and all prescribers to prescribe antibiotics with care.

8.3.6 *Streptococcus pneumoniae*

Streptococcus pneumoniae is known to cause various diseases that can be classified into two main groups: non-invasive and invasive. 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 pneumococcal disease worldwide, affecting both children and adults. This is followed by the severe cases of invasive bacteraemia and meningitis. Pneumococci often cause 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, peritonitis, and septic arthritis.

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

Table 8.18 Number of invasive pneumococcal isolates (IPD) observed in Denmark, 2022 DANMAP 2022

Serotype	Included in pneumococcal vaccines	N 2022	PEN-S	PEN-NON-S	Unknown	% Suscep
Unknown		46	0	0	46	0
4	PCV13, PCV15, PCV20, and PPV23	4	4	0	0	100%
6B	PCV13, PCV15, PCV20, and PPV23	0	0	0	0	0%
9V	PCV13, PCV15, PCV20, and PPV23	0	0	0	0	0%
14	PCV13, PCV15, PCV20, and PPV23	0	0	0	0	0%
18C	PCV13, PCV15, PCV20, and PPV23	1	1	0	0	100%
19F	PCV13, PCV15, PCV20, and PPV23	10	10	0	0	100%
23F	PCV13, PCV15, PCV20, and PPV23	1	0	1	0	0%
1	PCV13, PCV15, PCV20, and PPV23	0	0	0	0	0%
3	PCV13, PCV15, PCV20, and PPV23	107	106	1	0	99%
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	1	1	0	0	100%
19A	PCV13, PCV15, PCV20, and PPV23	14	13	1	0	93%
2	PPV23	0	0	0	0	0%
8	PCV20 and PPV23	81	81	0	0	100%
9N	PPV23	16	14	2	0	88%
10A	PCV20 and PPV23	14	14	0	0	100%
11A	PCV20 and PPV23	24	23	1	0	96%
12F	PCV20 and PPV23	4	4	0	0	100%
15B	PCV20 and PPV23	7	7	0	0	100%
17F	PPV23	9	6	3	0	67%
20	PPV23	4	4	0	0	100%
22F	PCV15, PCV20, and PPV23	39	39	0	0	100%
33F	PCV15, PCV20, and PPV23	14	14	0	0	100%
15A		18	16	2	0	89%
7C		18	18	0	0	100%
23B		17	7	10	0	41%
24F		15	15	0	0	100%
35F		15	15	0	0	100%
16F		15	15	0	0	100%
Other		64	58	6	0	91%
Sum		560	486	28	46	95%*

N = number of isolates, PEN-S = Penicillin-susceptible, PEN-NON-S = Penicillin non-susceptible, % Suscep = percentage of IPD isolates susceptible to penicillin. For serotypes not covered by vaccines, isolates were grouped in other if there were fewer than 15
* Calculation = 486 / (560-46).

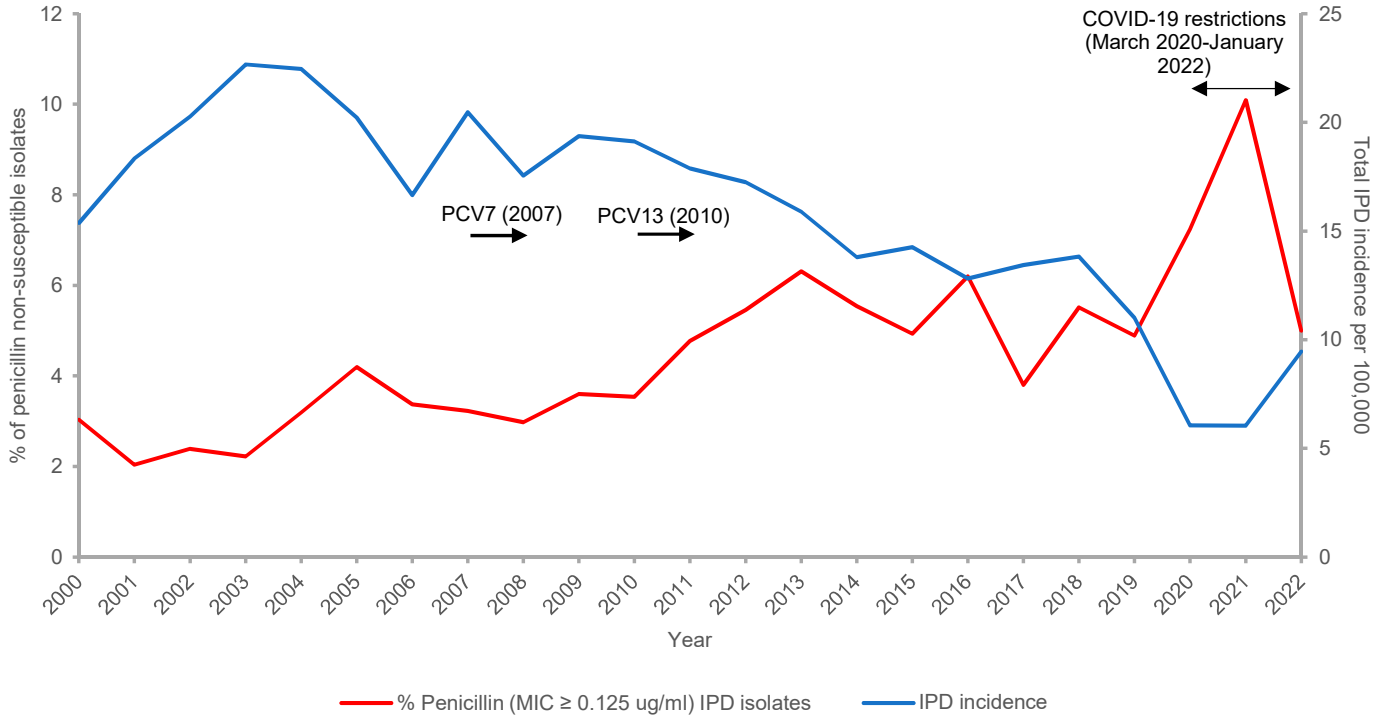
In Denmark, 560 cases of invasive pneumococcal disease (IPD) were registered in 2022 (Table 8.18). The cases were mainly from pneumococci found in either blood (479) or cerebrospinal fluid (70). For 11 cases, pneumococci had been found in other, normally sterile sites. Traditionally, the IPD cases found in other sterile sites than blood and cerebrospinal fluid, have not been included in the DANMAP report, however in 2022 these cases are included in the report. Of the 560 IPD cases identified in MiBa, 514 isolates were received at the reference laboratory. Data for the 46 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 514 cases.

The IPD isolates belonged to 49 different serotypes. For the 514 cases with available penicillin susceptibility data, 486 isolates were susceptible to penicillin (94.6%), and 28 isolates (5.4%) were classified as non-susceptible to penicillin.

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, as it is clear that IPD caused by the seven serotypes included in the first vaccine (PCV7 vaccine from 2007) and later 13 serotypes included in the second vaccine (PCV13 vaccine from 2010) has decreased. The predominant serotype in 2022 was serotype 3 (20.8%) (Table 8.18). One serotype 3 isolate was classified as non-susceptible, while the remaining 106 isolates were susceptible to penicillin. The second and third most frequently isolated serotypes are serotype 8 (81 isolates in 2022) and serotype 22F (39 isolates in 2022), and these isolates were all fully sensitive to penicillin.

Figure 8.21 The incidence of invasive pneumococcal infection (IPD) and the percentage of penicillin non-susceptible IPD isolates in Denmark, 2000–2022. PCV-7 was introduced into the child vaccination program in 2007, and replaced with PCV13 in 2010

DANMAP 2022



Conclusion

The level of penicillin non-susceptible IPD isolates returned to pre covid-19 level in 2022 (5%) compared to the two previous years (10.1% (2021) and 7.2% (2020)). The incidence of invasive pneumococcal disease has also increased to pre-pandemic level, compared to the years 2021 and 2020. 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 seen in other countries as well [Shaw, et al., Lancet Digit Health. 2023 Sep;5(9):e582-e593].

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8.3.7 Beta-haemolytic streptococci

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.

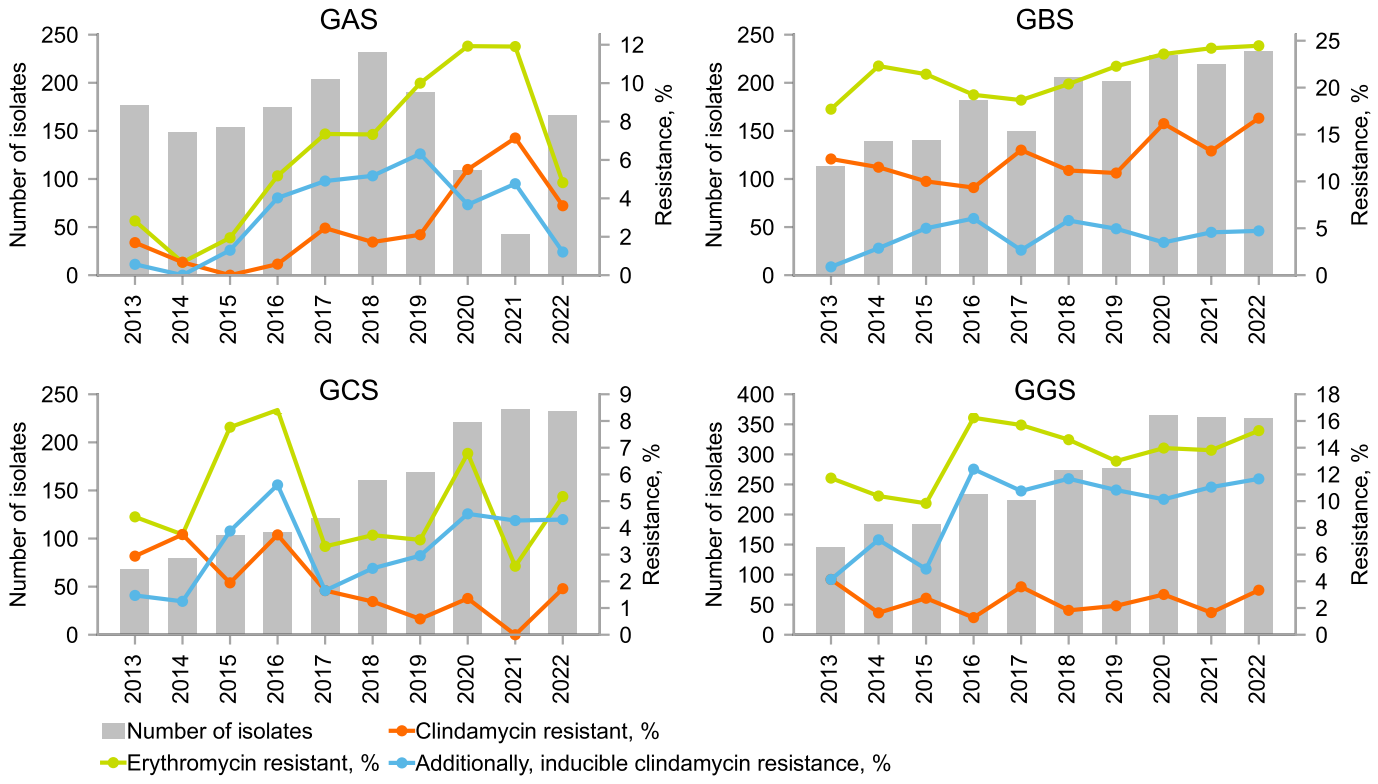
This report presents data on antimicrobial resistance in invasive isolates (i.e. from blood, cerebrospinal fluid or other normally sterile anatomical sites) submitted from the departments of clinical microbiology (DCMs) in 2022 to the Neisseria and Streptococcus Reference laboratory (NSR). This report includes only non-duplicate isolates, i.e. isolates obtained with an interval of more than 30 days. Isolates are received from all DCMs 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 (https://www.eucast.org/) (version 12.0). For all isolates of GAS the *emm* type was determined by whole genome sequencing of the portion of the *emm* gene that dictates the M protein serotype.

In 2022, a total of 1,046 isolates were received. The number of isolates from unique cases was 991, an increase of 16% compared to 2021 (857). Corresponding changes for individual serogroups were: GAS, + 295%; GBS, + 6%; GCS, - 0,9%; and GGS, -0,6%.

Figure 8.22 shows the resistance findings for the years 2013 through 2022. All isolates were fully susceptible to penicillin. Comparing 2022 to 2021, erythromycin resistance as well as clindamycin resistance declined somewhat for GAS and increased for GCS. The percentage of strains with inducible clindamycin resistance was virtually unchanged for all serogroups: GAS, 1.2%; GBS, 4.7%, GCS 4.3%, and GGS, 12%. The percentage of fully susceptible isolates was unchanged compared to 2021 for all four serogroups.

Figure 8.22 Beta-haemolytic streptococci: Antimicrobial resistance testing results, Denmark, 2013-2022 DANMAP 2022



Results of genotyping of *Streptococcus pyogenes*

The GAS isolates belonged to 55 different multi-locus sequence types (MLSTs) and 40 different *emm* types. As shown in Table 8.19 nearly two-thirds (110) of the isolates belonged to eight different combinations of MLSTs and *emm* types. None of these were resistant to erythromycin or clindamycin. The variants 28 / *emm* 1.0 and 36 / *emm* 12.0 accounted for 41% of all isolates. The remaining 56 isolates belonged to 41 different combinations of MLSTs and *emm* types

Table 8.19 *Streptococcus pyogenes*: Combinations of MLSTs and *emm* types DANMAP 2022

MLST / *emm* type	Number
36 / 12.0	36
28 / 1.0	32
101 / 89.0	10
52 / 28.0	10
62 / 87.0	8
44 / 66.0	6
1146 / 1.0	4
28 / 1.25	4

Nine isolates contained genes encoding erythromycin resistance. Seven of these isolates were phenotypically resistant as well as one without detected resistance genes.

Comments and conclusions

The substantial increase from 2021 to 2022 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 GAS isolates is probably a consequence of discontinued COVID-19 related restrictions on upper respiratory tract colonization and droplet 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 2022 to 2021, erythromycin resistance as well as clindamycin resistance declined somewhat for GAS and increased for GCS.

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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 rectal specimens is usually due to contamination with vaginal secretion, while in men who have sex with men (MSM) it is due to unprotected anal sex leading to infection. In both genders, the condition is generally asymptomatic. Pharyngeal gonorrhoea is virtually always asymptomatic.

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

Most of the isolates received in 2022 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 2,145 isolates from 1,841 unique cases of gonorrhoea diagnosed in 2022 (1,131 males, 708 females, and 2 of unknown gender). Only one isolate from each unique case is counted in this report.

Results and discussion

The annual number of received isolates increased considerably from 2011 through 2017 (Figure 8.23). This is 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 (2003-2022). 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 2022. It should be recalled, however, that many cases diagnosed by NAATs are not followed-up by culture.

The ciprofloxacin resistance rate was 40% in 2022 (48% in 2021 and 43% in 2020), thus still considerably lower than the peak of 75% in 2009 (Figure 8.23). Only 0.7% of the isolates were classified as ciprofloxacin intermediate (MIC 0.047-0.064 mg/L).

The percentage of strains producing penicillinase was 14% (23% in 2021 and 17% in 2020). 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 2.9% of the tested isolates (2.8% in 2021 and 2.0% in 2020). 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 8.24, 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 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 many clinicians and has been replaced by ceftriaxone 1 g i.m.

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

In a subset of 134 isolates tested as part of the Danish participation in Euro-GASP, resistance against cefixime (MIC >0.125 mg/L) was 0% in 2022 (Table 8.20), like in 2021. Cefixime is an oral cephalosporin that has never been used in Denmark. Resistance to spectinomycin was also 0%, like in all previous years where data for Euro-GASP were produced. The gentamicin MIC values were in the range 0.5 to 4 mg/L, but clinical breakpoints for this agent have not been established.

Figure 8.23 Number of submitted gonococci isolates from males and females, and the percentage of isolates with ciprofloxacin resistance, azithromycin resistance, and penicillinase production, Denmark, 2003-2022 DANMAP 2022

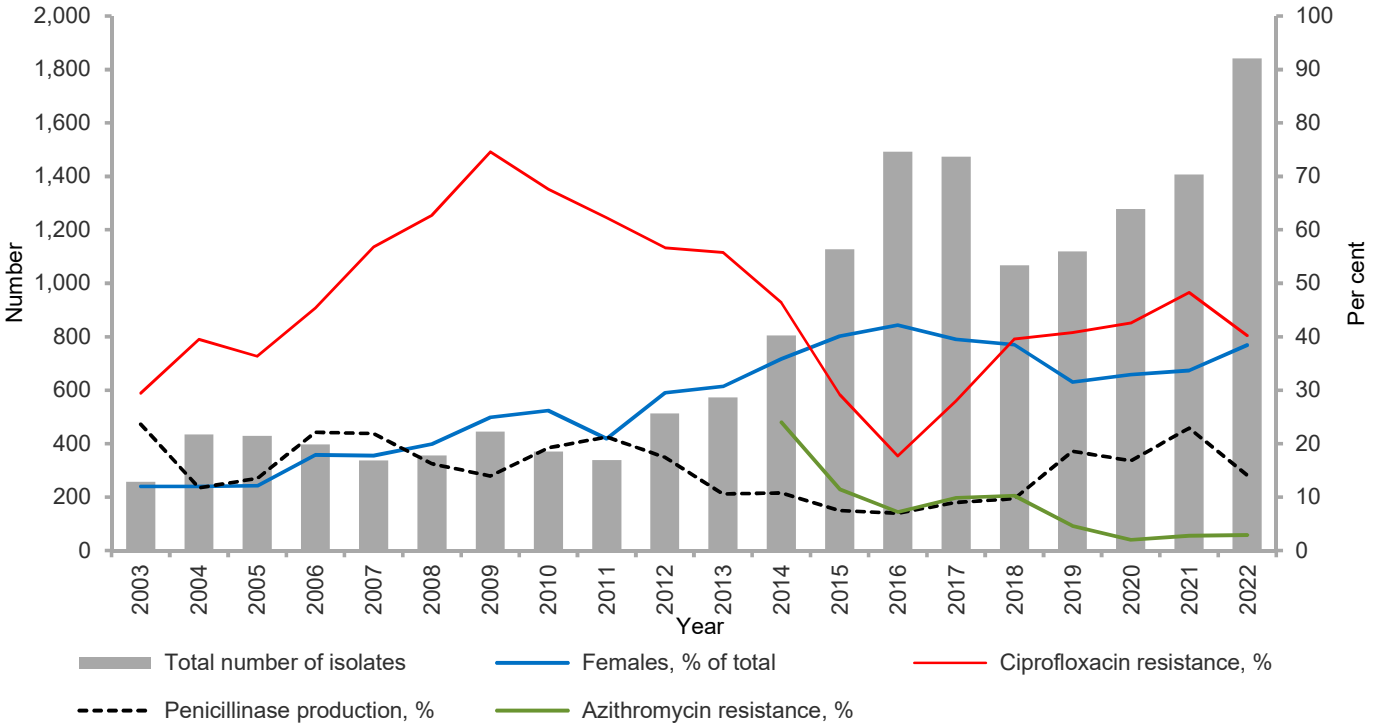

Figure 8.24 Distribution of ceftriaxone MIC (mg/L) values in gonococci, Denmark, 2003–2022 DANMAP 2022

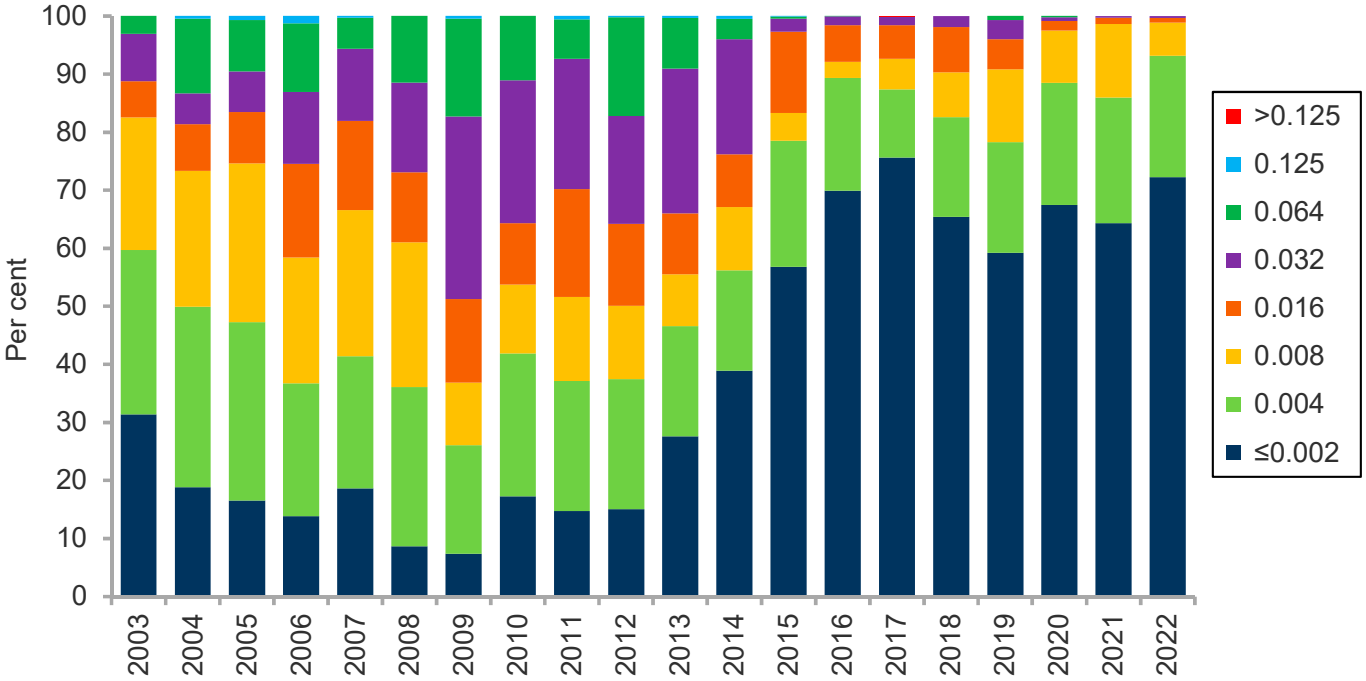

Table 8.20 Distribution of 134 gonococcal isolates according to MIC values for cefixime, gentamicin, and spectinomycin; number of isolates DANMAP 2022

	MIC values (mg/L)							
	≤0.016	0.032	0.064	0.5	1	2	4	8
Cefixime	131	1	2					
Gentamicin				1	35	95	3	
Spectinomycin						2	65	67

In both genders, the ciprofloxacin resistance rate was higher in anorectal isolates than in urogenital and pharyngeal isolates (Table 8.21).

In both genders, the azithromycin resistance rates was higher in pharyngeal isolates than in anorectal and urogenital isolates (Table 8.22).

Penicillinase production was demonstrated at a higher rate among anorectal isolates than among urogenital and pharyngeal isolates (Table 8.23).

Table 8.21 Ciprofloxacin resistance rates by gender and anatomical origin of the isolates DANMAP 2022

	Males		Females		Total	
	No. resistant / total no.	% resistant	No. resistant / total no.	% resistant	No. resistant / total no.	% resistant
Urogenital	622 / 867	72	391 / 616	64	1,013 / 1,483	68
Anorectal	111 / 135	82	11 / 13	85	122 / 148	82
Pharynx	51 / 69	74	34 / 53	64	86 / 122	70
Blood	1 / 1	100	0 / 0	-	1 / 1	100
Eye	4 / 4	100	0 / 0	-	4 / 4	100
Unknown	41 / 55	75	16 / 26	62	57 / 81	70
Total	830 / 1,131	73	452 / 708	64	1,282 / 1,839	70

Table 8.22 Azithromycin resistance rates by gender and anatomical origin of the isolates DANMAP 2022

	Males		Females		Total	
	No. resistant / total no.	% resistant	No. resistant / total no.	% resistant	No. resistant / total no.	% resistant
Urogenital	25 / 867	2.9	14 / 616	2.3	39 / 1,483	2.6
Anorectal	6 / 135	4.4	0 / 13	0	6 / 148	4.1
Pharynx	6 / 69	8.7	4 / 53	7.5	10 / 122	8.2
Blood	0 / 1	0	0 / 0	-	0 / 1	0
Eye	0 / 4	0	0 / 0	-	0 / 4	0
Unknown	0 / 55	0	0 / 26	0	0 / 81	0
Total	37 / 1,131	3.3	18 / 708	2.5	55 / 1,839	3.0

Table 8.23 Penicillinase production by gender and anatomical origin of the isolate DANMAP 2022

	Males		Females		Total	
	No. resistant / total no.	% resistant	No. resistant / total no.	% resistant	No. resistant / total no.	% resistant
Urogenital	119 / 867	14	69 / 616	11	188 / 1,483	13
Anorectal	34 / 135	25	4 / 13	31	38 / 148	26
Pharynx	14 / 69	20	8 / 53	15	22 / 122	18
Blood	0 / 1	0	0 / 0	-	0 / 1	0
Eye	0 / 4	0	0 / 0	-	0 / 4	0
Unknown	7 / 55	13	4 / 26	15	11 / 81	14
Total	1,74 / 1,131	15	85 / 708	12	55 / 1,839	3.0

Conclusions

The ciprofloxacin resistance rate was somewhat lower in 2022 than in 2021 and the ceftriaxone MIC distribution was virtually unchanged. Although resistance problems among gonococci are still not present in Denmark, the frequent emergence globally of gonococcal resistance to antibiotics underlines the importance of maintaining the centralised national surveillance of antimicrobial resistance in gonococci.

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8.3.9 *Haemophilus influenzae*

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.

Invasive *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 now (2022) 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.

In 2022, a total of 118 cases of invasive *H. influenzae* were registered. The majority of cases were in blood (103) or cerebrospinal fluid (12), with some found in other normally sterile sites (3). Out of the 118 *H. influenzae* cases identified in MiBa, 98 isolates were received at the reference laboratory. The age and serotype distribution of the submitted isolates can be seen in Figure 8.25. Invasive *H. influenzae* infections are most commonly observed in the elderly.

Non-capsular *H. influenzae* is still the most commonly tested type (55%), with Hif being the most common serotype (18%), followed by Hib (8%), Hie (2%), and Hic (1%).

Sequence type (ST) and clonal complex (CC) were linked to the serotype of *H. influenzae* (Table 8.24). 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 39 different STs, including three novel STs.

Figure 8.25 The distribution of age and serotype of *H. influenzae* in 2022 DANMAP 2022

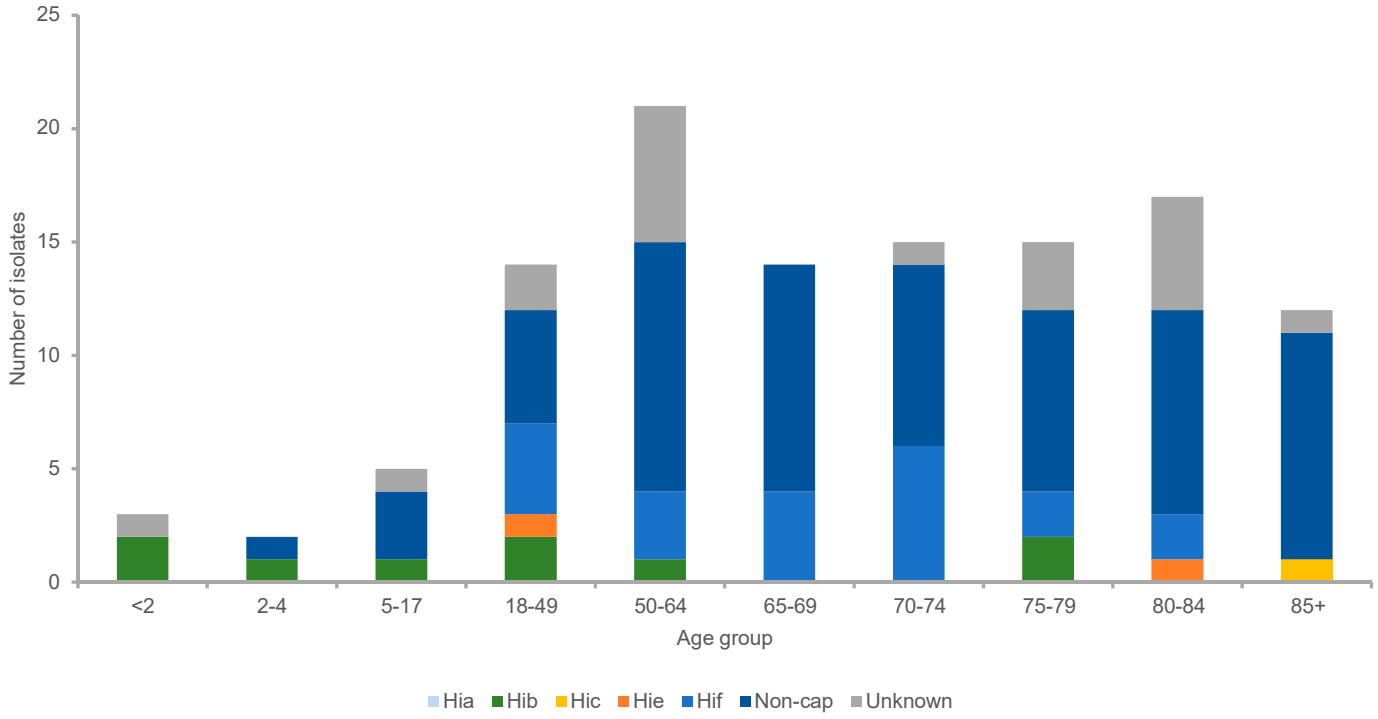

Table 8.24 Sequence type and clonal complex found among the *H. influenzae* isolates with a capsule DANMAP 2022

ST	Clonal complex	A	B	C	D	E	F	Noncap
6	ST-6 complex	0	1	0	0	0	0	0
92	ST-6 complex	0	1	0	0	0	0	0
95	ST-6 complex	0	1	0	0	0	0	0
190	ST-6 complex	0	6	0	0	0	0	0
103	ST-11 complex	0	0	1	0	0	0	0
Novel	ST-18 complex	0	0	0	0	1	0	0
18	ST-18 complex	0	0	0	0	1	0	0
124	ST-124 complex	0	0	0	0	0	19	0
598	ST-124 complex	0	0	0	0	0	1	0
1739	ST-124 complex	0	0	0	0	0	1	0

Table 8.25 Phenotypic resistance against penicillin/ampicillin DANMAP 2022

Sensitivity	Number (%)
Resistant	29 (30)
Susceptible	67 (70)
Total	96

Table 8.26 Phenotypic resistance against penicillin/ampicillin vs. beta-lactam resistance mechanism DANMAP 2022

Beta-lactam resistance mechanism	Resistant	Susceptible	Total (%)
BLNAS	0	67	67 (70)
BLPAR	9	0	9 (9)
BLNAR	18	0	18 (19)
BLPACR	2	0	2 (2)
Total	29	67	96

Data on both molecular and phenotypic antimicrobial susceptibility were available for 96 *H. influenzae* isolates. Of *these*, 30% were penicillin/ampicillin-resistant (Table 8.25). Eleven isolates tested positive for TEM beta-lactamase genes (ten were TEM-1 and one was TEM-234). BLNAR-defining mutations in the *ftsI* gene were found in 20 isolates (19 had the N526K mutation and one had the R517H mutation). The most common *ftsI* types were *IIb* (11), followed by *IIa* (3), *IId* (3), I (2), and *IIc* (1).

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 8.26. A 100% concordance was observed between phenotypic susceptibility testing and molecular resistance gene detection.

Conclusions

The number of invasive *H. influenzae* cases in 2022 were 118 comparable to 101 cases in 2021 and an increase from 59 cases in 2020. The majority of isolates are still of the non-capsular type (55%) while Hif was the most common serotype (18%). Resistance towards penicillin and ampicillin was 30% with 19% BLNAR, 9% BLPAR and 2% BLPACR.

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8.3.10 Meningococci

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. 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 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 (DCMs) during 2012-2022 to the Neisseria and Streptococcus Reference laboratory (NSR). Isolates are received from all DCMs in Denmark. It is voluntary to submit isolates of meningococci, but the coverage rate is estimated to be 100% when compared to the clinical notification system. The two surveillance systems continuously supplement each other.

Surveillance of meningococci and resistance

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 8.26 shows the number of isolates of groups B, C, W, and Y received during 2012-2022. Because of low numbers the following has been omitted: One isolate of group 29E (2017), two isolates of group X (2016 and 2019), and one isolate which was non-groupable (2019). 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 8.27).

Figure 8.26 Number and serogroup of meningococcal isolates received during 2012-2022 DANMAP 2022

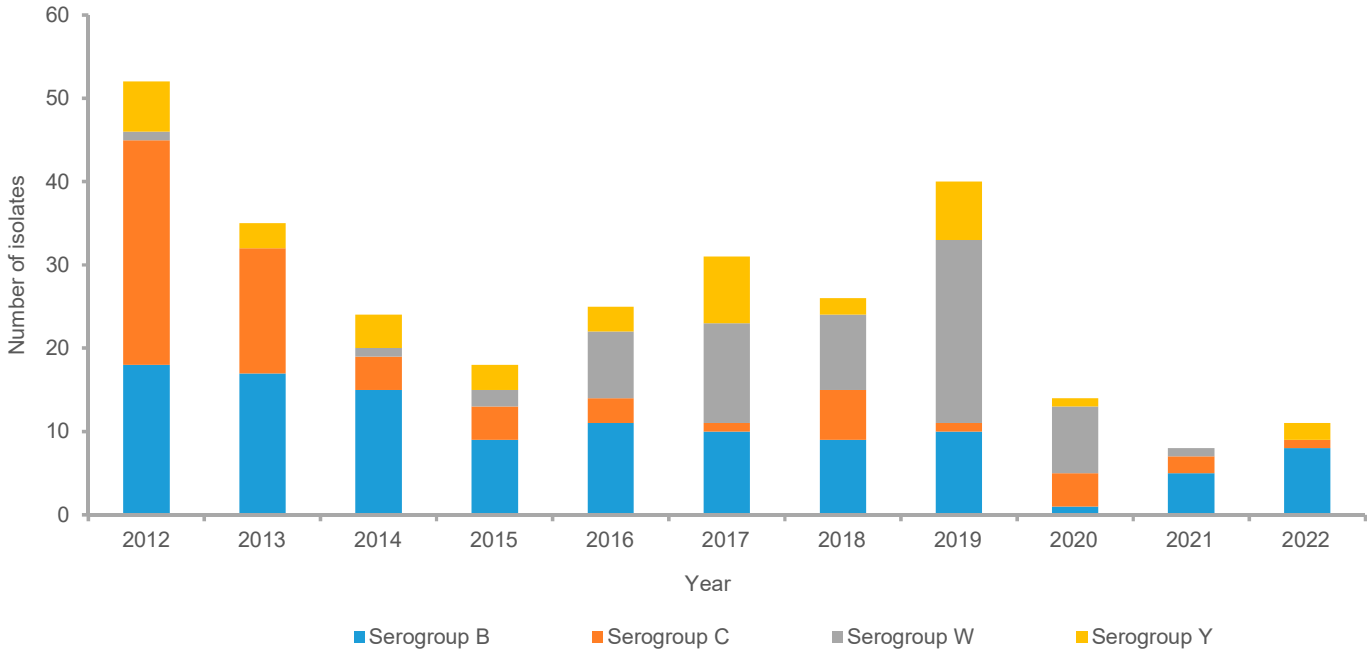


Figure 8.27 Distribution of ceftriaxone MIC values (mg/L) by serogroup, 2012-2022 DANMAP 2022

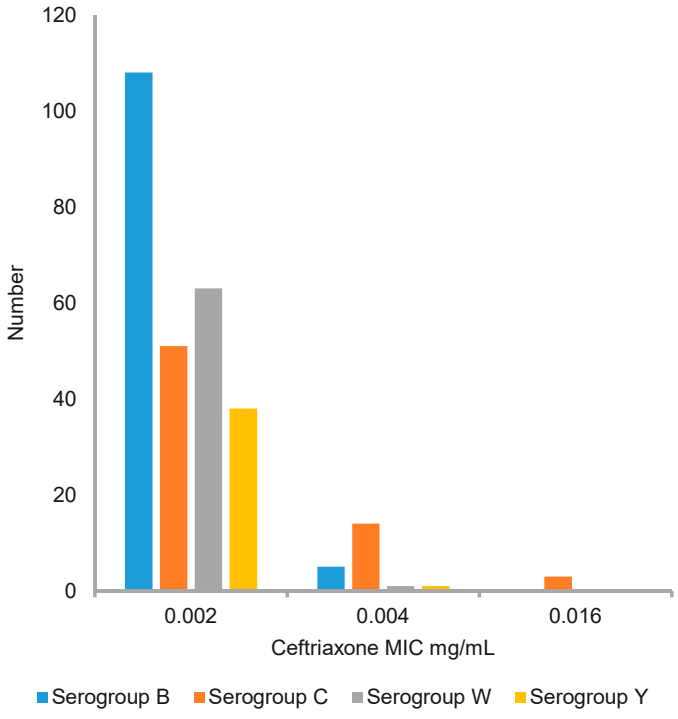
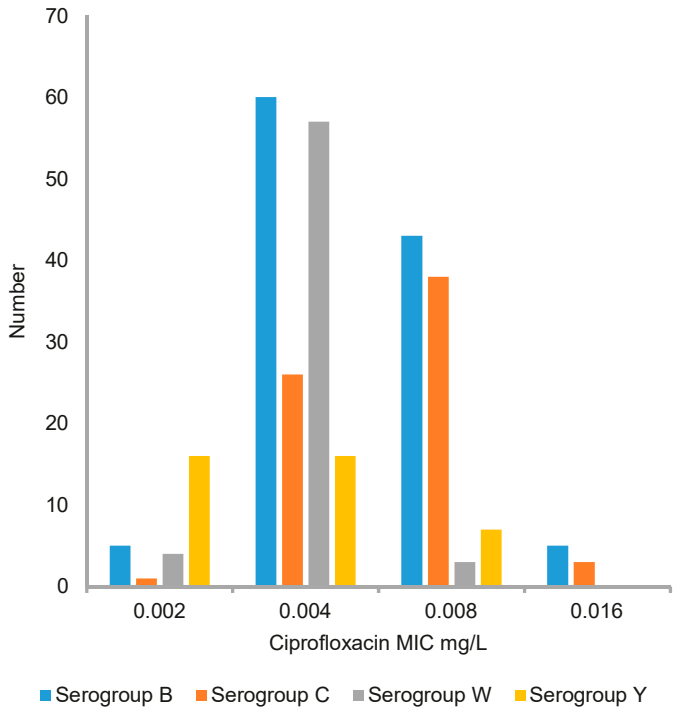


Figure 8.28 Distribution of ciprofloxacin MIC values (mg/L) by serogroup, 2012-2022 DANMAP 2022



All isolates were susceptible to ciprofloxacin (MIC ≤0.016 mg/L) (Figure 8.28). Isolates of serogroup W and Y 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 8.29). Seven (6%) isolates of serogroup B and six (9%) isolates of serogroup C were penicillin-resistant (MIC >0,25 mg/L) (Table 8.27).

Nearly all isolates during the study period were susceptible to rifampicin (MIC ≤ 0.25 mg/L) (Figure 8.30). Only one (from 2017) was resistant (MIC = 32 mg/L).

Figure 8.29 Distribution of penicillin MIC values (mg/L) by serogroup, 2012-2022 DANMAP 2022

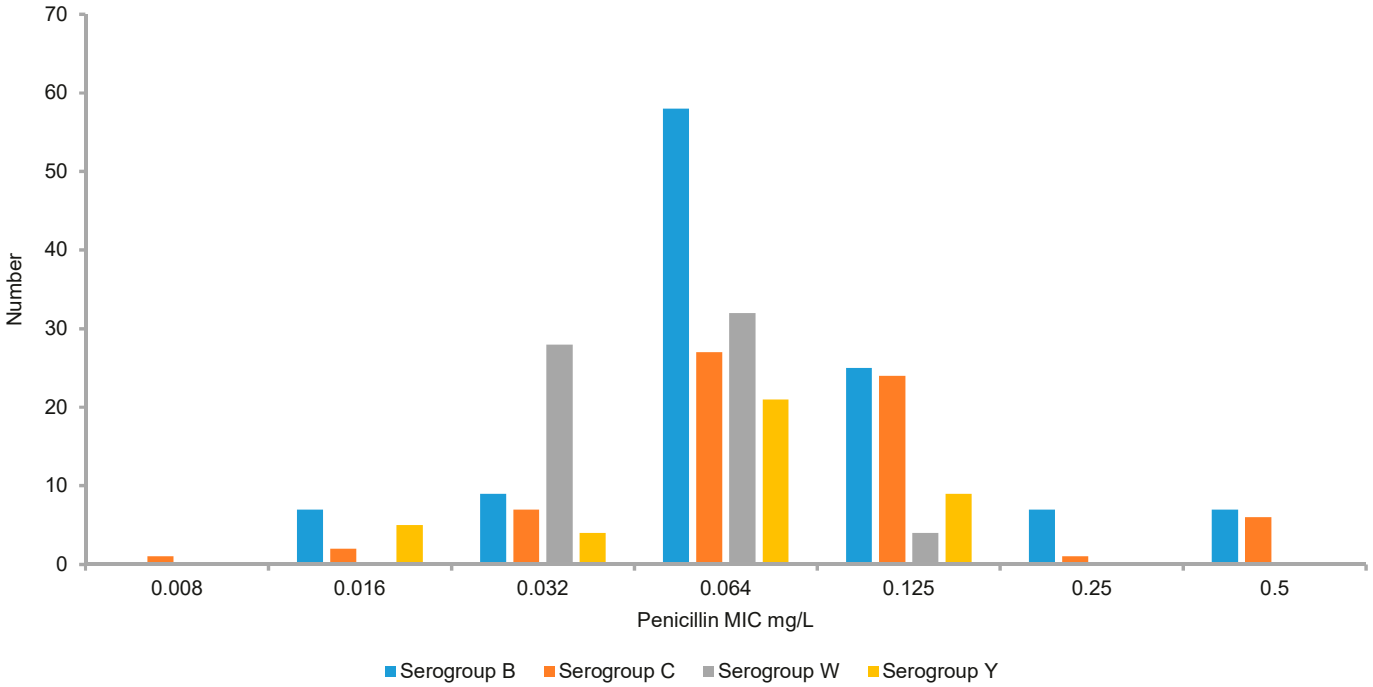
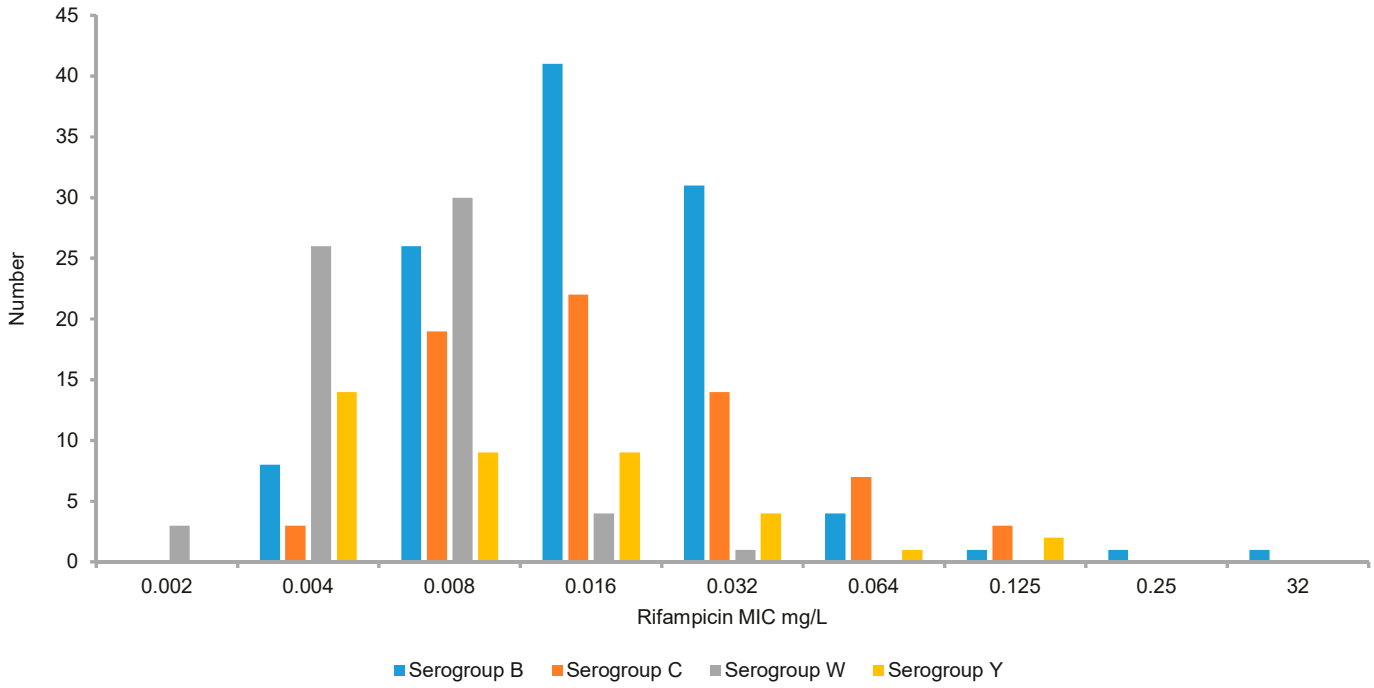


Table 8.27 Number of penicillin-resistant meningococci (MIC = 0.5 mg/L), serogroups B and C, 2013-2022 DANMAP 2022

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

Figure 8.30 Distribution of rifampicin MIC values (mg/L) by serogroup, 2012-2022 DANMAP 2022



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

During the first six months of 2023 nearly 20 cases of IMD have been diagnosed (not described in this report).

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Textbox 8.1

Dicloxacillin capsules contaminated with CPE in Denmark and Iceland

The prevalence of carbapenemase producing Enterobacterales (CPE) has been increasing in Denmark (Figure 8.13), as well as in many other countries, for the last decade. The source for CPE in Danish patients was initially mostly attributed to travel and hospitalization in countries with high(er) prevalence of CPE than Denmark, but in recent years nosocomial spread within Denmark account for more and more cases as part of local or regional outbreaks (Table 8.11). Community onset of CPE may also occur, but here investigations are often difficult to conduct due to sparse epidemiological data as well as lack of national and international surveillance programs in the community.

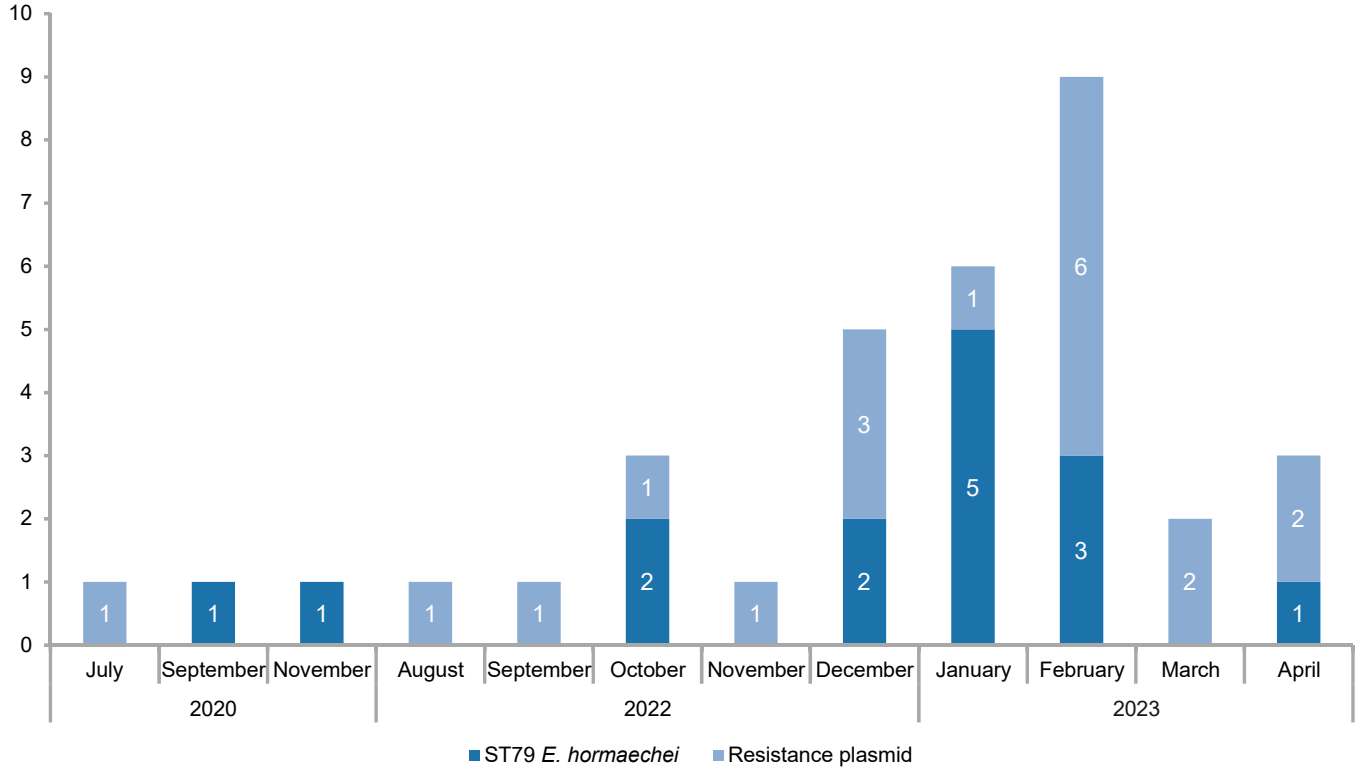
During the winter of 2022/2023, a sudden increase in NDM-5 + OXA-48 producing *Enterobacter hormaechei* ST79 was observed in the national surveillance data. Whole genome sequencing (WGS) analysis showed a strong clonal relationship between the 13 isolates as well as to two highly similar isolates from the autumn 2020 thus suggesting these 15 patients were part of the same outbreak. The isolates were submitted from four of five Danish regions, they originated in most cases from uncomplicated urinary tract infections and the patients had not been hospitalized neither in Denmark nor abroad, indicative of a community source for the outbreak. In the Region of Southern Denmark, three patients submitted samples a few days apart and therefore these three patients were interviewed by the staff of the Department of Clinical Microbiology at Odense University Hospital (OUH). Here, investigation of the interview data identified consumption of the same brand of dicloxacillin product (Dicillin®) prior to the urinary tract infection as a common denominator. This finding was immediately reported to the national outbreak team at Statens Serum Institut (SSI), who contacted the remaining three regions who confirmed that the remaining patients had also been administered Dicillin®. [1]

In parallel with the epidemiological investigations, OUH retracted unused Dicillin® capsules from two of the three patients in the Region of Southern Denmark and was able to culture a CPE *E. hormaechei* ST79 from the surface of one of the capsules. Subsequent WGS analysis confirmed that this capsule isolate clustered with the outbreak isolates, thereby confirming a direct link between the Dicillin capsules and infected patients. Dicillin® was only marketed in Denmark and Iceland (under a different brand name, though) and contact with the Icelandic authorities led to the identification of one identical CPE *E. hormaechei* ST79 isolated from a patient there. This led to retraction of all Dicillin products from the Danish and Icelandic market by February 6th 2023, while the possible source of contamination was being investigated by testing available batches at the company producing Dicillin®. Based on this investigation, at least 12 contaminated batches could be identified between February 2022 and February 2023. The most likely explanation for the contamination was a set of brushes on the packing line even though no viable bacteria were extracted from these. In total, approximately 79 thousand Danish citizens may have ingested the capsules between February 2022 and February 2023.

The two carbapenemase genes ($bla_{\text{NDM-5}}$ and $bla_{\text{OXA-48}}$) were located on a 45,048 bp IncX3 plasmid and a 79,966 bp IncL plasmid, respectively. Both of these two plasmid types were conjugative, and transfer of either one or both plasmids to other Enterobacterales species such as *E. coli*, *Klebsiella* spp. and *R. ornithinolytica* were identified for at least three of the patients carrying the outbreak strain. As Denmark has had an extensive monitoring program of CPE since 2014, where all CPE isolates are submitted to SSI for WGS analysis, a retrospective search within the existing data identified an additional 19 patients between 2020 and 2023, who were carrying other bacterial species than *E. hormaechei*, but with identical plasmids (one or both) as the outbreak strain. As these two plasmids seem to be extremely rare or even unique compared to the data in the international databases such as NCBI and PATRIC, it was most likely that these 19 patients also had received Dicillin®, which was later confirmed through interviews with the patients or their general practitioner (Figure 1).

A decrease in CPE-positive patients with the outbreak strain as well as other bacterial species with the outbreak plasmids has been observed after the contaminated Dicillin® capsules were removed from the market. Therefore, there is a good agreement between the availability of contaminated capsules and the number of new cases. Patients who have ingested the contaminated capsules may therefore to some extent have eliminated the CPE from their digestive system, but it is known from similar cases that patients may be healthy carriers for a longer time [2]. It is therefore important that the national surveillance system is designed to detect both the presence of the outbreak strain and its plasmids in the future.

Figure 1 Epicurve of patients carrying either the outbreak *E. hormaechei* ST79 strain (dark blue) or other bacterial species with either one or both outbreak plasmids (light blue)
DANMAP 2022



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Textbox 8.2

Fungaemia epidemiology, resistance rates and human antifungal consumption: a 2021-2022 update

Candidemia is the most common form of fungemia. It mainly occurs in hospitalized patients with risk factors such as abdominal surgery, presence of intravenous lines and immunosuppression [1] [2]. The 30-day mortality rate is approximately 40% in Denmark and higher in ICU [2].

A Danish nationwide surveillance has existed since 2004. All fungal blood culture isolates from Danish Departments of Microbiology are referred to Statens Serum Institut for confirmatory species identification, susceptibility testing and sequencing (when relevant).

Denmark is a high incidence country compared to our Nordic neighbours. After a peak at 10.1/100.000 inhabitants in 2011, the incidence stabilised at around 8/100.000 but appears to have risen again to 9.1/100.000 (2020-2021) and 9.6 (2022) [3] [4]. The age specific peak incidence in men has risen from 70 to 80-90 years age (Figure 1). The overall incidence changes could be influenced by an ageing population, the COVID-19 epidemic, changed treatment regimens or other factors including change in blood culture system use [5].

The first line treatment for candidaemia is an echinocandin (iv administration only). Acquired echinocandin resistance emerged after the introduction of the echinocandins [6]. However, despite a continued increase in echinocandin usage (+21% since 2013) the acquired echinocandin resistance rate 2021-22 in the five most common species remained low (1.0%). For infection with susceptible isolates, de-escalation to fluconazole is possible and allows outpatient oral treatment. A marked shift from the susceptible species *C. albicans* to the less azole-susceptible species *C. glabrata* has been evident and coupled to a high azole (mainly fluconazole) use [4] [6]. Despite a 34% decline in use 2013-2022, *C. albicans* accounted for only 38% in 2021-2022 (Figure 2). An increase in *C. glabrata* to 34% of the isolates is the main cause for only ~57% of all isolates being fully susceptible to fluconazole.

Figure 1 Incidence by age and gender on 3 or 4-years intervals 2004-2022. M: Males; F: Females; MF: Both genders DANMAP 2022

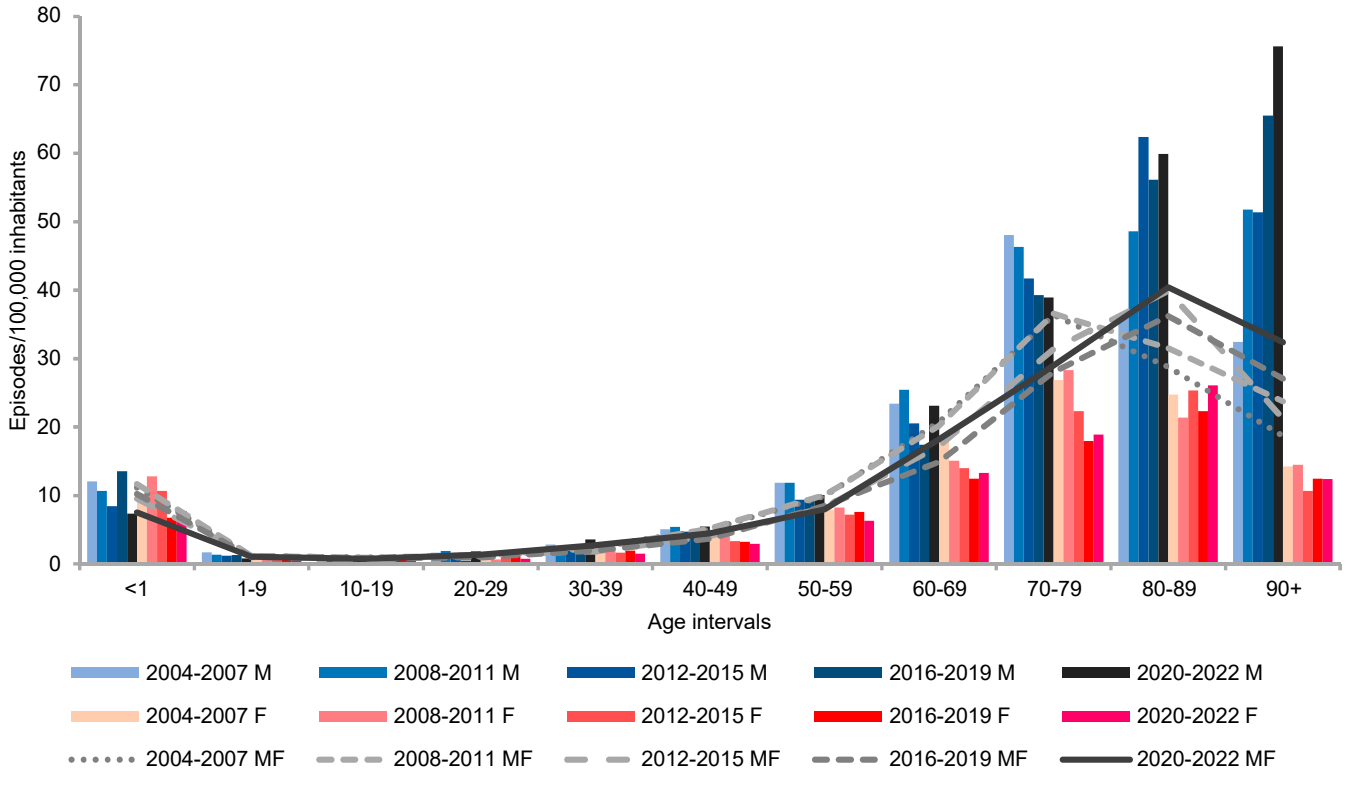
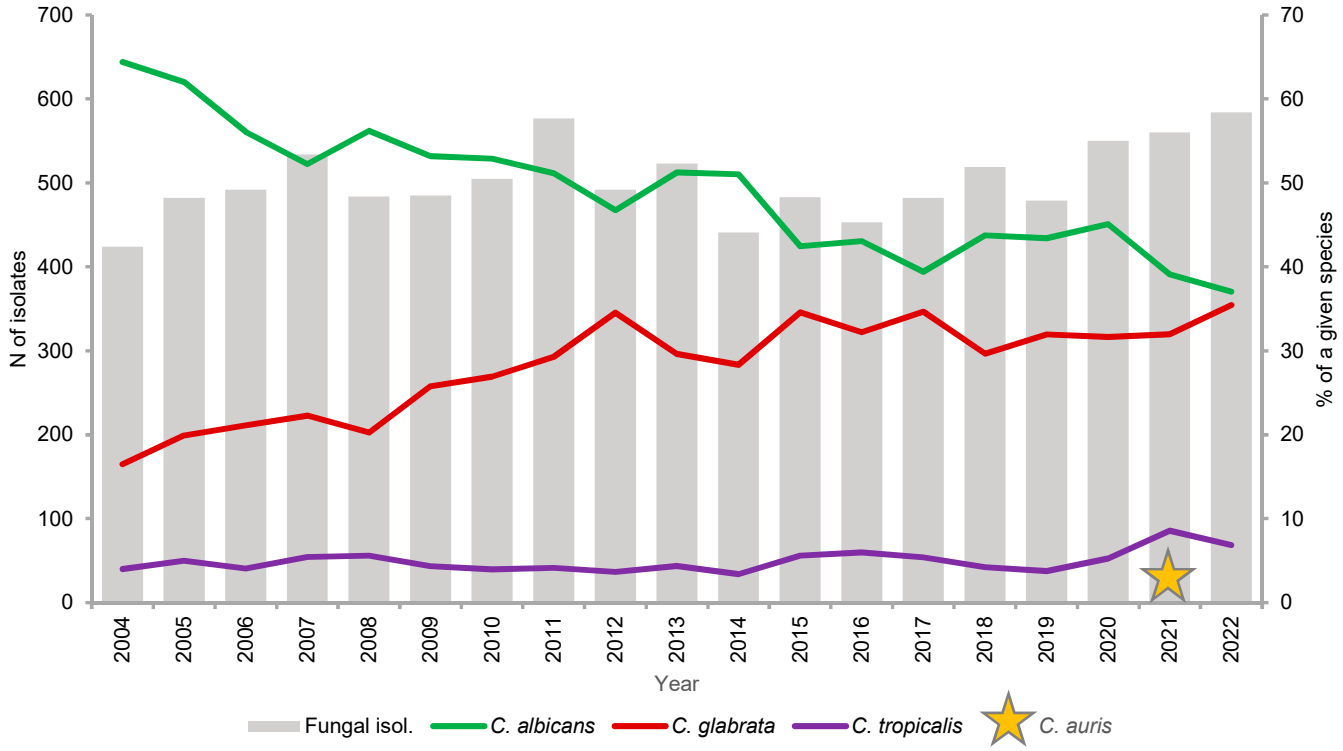


Figure 2 Number of annual isolates and selected trends in species distribution DANMAP 2022



In an international context, emergence of less susceptible species or clones causes concern and poses a threat to standard treatment regimens. *C. auris* is renowned for its near-universal fluconazole resistance and ability to rapidly acquire echinocandin resistance. It has caused hospital outbreaks in multiple countries in Asia, Africa, South and North America and also within Europe [7]. The first and so far only Danish *C. auris* candidemia case occurred in 2021 and involved acquisition of echinocandin resistance [8]. Screening for colonization has been recommended for patients previously admitted in foreign hospitals/countries with known transmission [9] [10]. Moreover, fluconazole-resistant *C. parapsilosis* infections involving clonal outbreaks (and occasionally also echinocandin resistance) have emerged in Southern Europe, Asia, Brazil, South Africa and North America [11].

In conclusion, in Denmark the overall incidence and high proportion of *C. glabrata* remained stable despite a reduction in azole use. Resistance levels remain overall stable but *C. auris* has for the first time been detected in the fungemia surveillance and the global emergence and spreading of resistance in *Candida* is concerning. Consequently, continued surveillance of the shifting epidemiology is important.

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continued ... Textbox 8.2

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Textbox 8.3

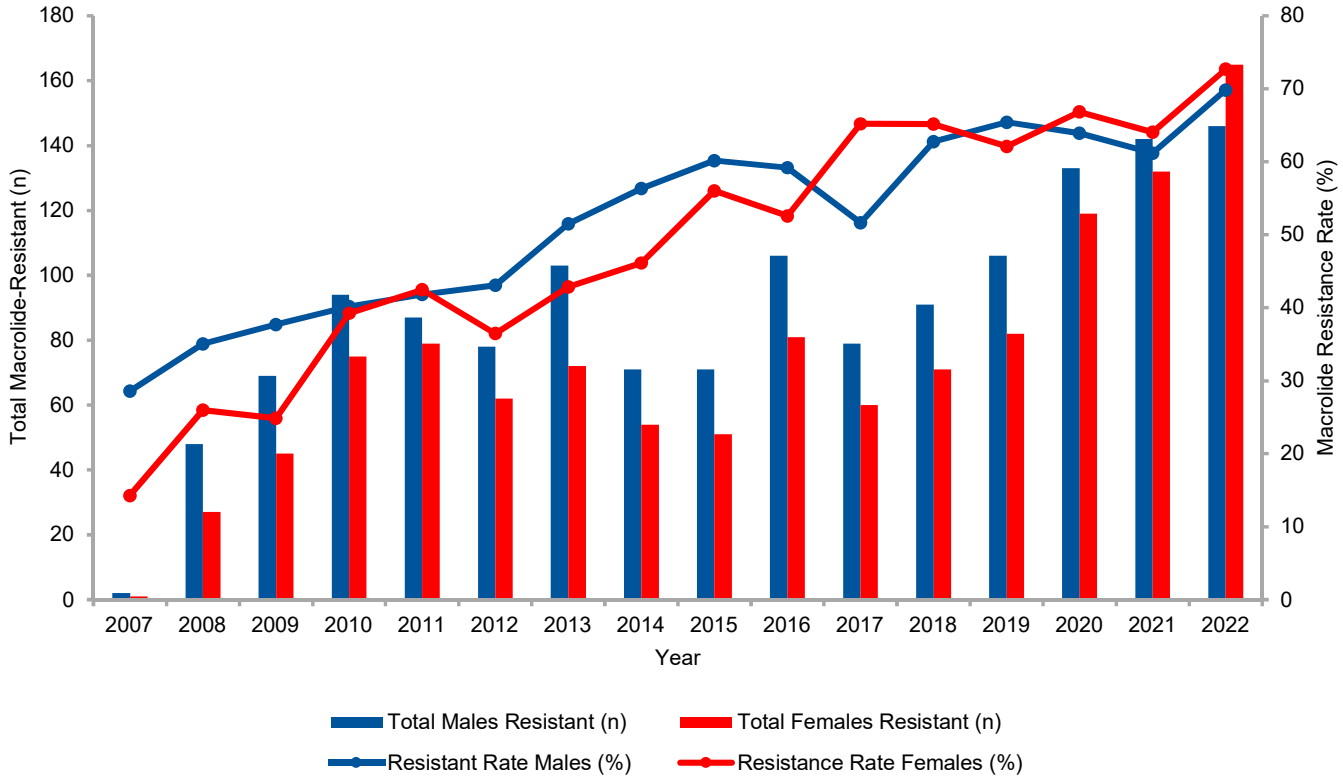
Mycoplasma genitalium

Mycoplasma genitalium (MG) is a sexually transmitted bacterium that was first discovered in 1980. The bacterium causes urethritis, cervicitis, pelvic inflammatory disease and is suspected to be a cause of infertility. The symptoms of an active infection include genital discharge and pain on micturition, however, a large number of infected individuals are asymptomatic. Data show that MG is quite common in the general population, second only to *Chlamydia trachomatis*, with a positive rate of 11.5% of individuals tested in 2022. The prevalence of MG in men with non-gonococcal, non-chlamydial urethritis approaches 25-35%.

Treatment and antimicrobial resistance in *Mycoplasma genitalium*

Treatment for MG in Denmark is based on the European guidelines described by the International Union against Sexually Transmitted Infections (IUSTI), backed by the WHO and ECDC. Broadly, the guidelines recommend that testing for MG is accompanied with molecular detection of resistance to macrolides prior to commencing treatment. For susceptible infections, the first-line treatment is 500 mg azithromycin on Day 1 followed by 250 mg for 2-5 days. Where macrolide resistance mutations (MRM) are detected, the recommended second-line treatment is 400 mg moxifloxacin once daily (fluoroquinolone) for seven days. In cases where moxifloxacin treatment fails, detection of quinolone resistance associated mutations (QRAM) is recommended, in order to discriminate between reinfection and resistance. Third-line treatment requires antimicrobials not registered in Denmark such as pristinamycin or minocycline. Unfortunately, both options are expensive and have an efficacy of only 60-75%. Denmark, like several other countries across the world, has seen an increase in the rate of macrolide resistance. In 2022, 71.3% of all MG positive samples tested at Statens Serum Insitut (SSI) had MRM detected, compared to 21.4% in 2007 when MRM testing was first implemented in Denmark (see Figure 1). The increase has been attributed to selective pressure from azithromycin prescribed for chlamydia.

Figure 1 Number (n) of patients with macrolide resistance mutations (bars) and rate of resistance (%) to macrolides for *Mycoplasma genitalium* infections in Denmark for samples from males and females tested at Statens Serum Institut from 2007-2022 DANMAP 2022



continued ... Textbox 8.3

Prior to 2018, the recommended treatment for uncomplicated chlamydia in Denmark was 1 g single dose azithromycin. Due to an increased awareness of the correlation between the treatment of *Chlamydia trachomatis* with azithromycin and resistance to macrolides in MG infections as well as a lower treatment efficacy in rectal infection, IUSTI and SSI guidelines changed from azithromycin single dose to 100 mg doxycycline twice a day for seven days.

Compared to 2019, 2020 saw a significant decline in the number of azithromycin prescriptions for *C. trachomatis* infections in Denmark. 1.47 prescriptions (per 1000 inhabitants) were issued in 2020 compared to 5 prescriptions (per 1000 inhabitants). At the same time, the number of doxycycline prescriptions issued per 1000 inhabitants saw an increase from 0.26 to 3.43. However, 2021 and 2022 have seen a gradual increase in the number of azithromycin prescriptions issued for *C. trachomatis* infections to 2.88 and 3.14 scripts per 1000 inhabitants, respectively. Consequently, it may be too soon to conclude that medical practitioners in Denmark have changed the treatment regimen for uncomplicated chlamydial infections. Of note, the dramatic decline in the number of azithromycin prescriptions was observed over the COVID-19 period when prescription of macrolides was prohibited.

Testing for resistance to quinolones is not standard practice and a test for detection of QRAM using DNA sequencing methods is currently only offered at the SSI. A retrospective evaluation of MG positive samples submitted to the SSI since 2003 is currently underway. Preliminary results indicate a QRAM rate of 3.7% in 2022 (108 samples tested for both, MRM and QRAM). Of these, two (1.9%) carried mutations for both macrolides and quinolones (dual class resistance). Given that global trends for dual resistance rates exceed 70% in some reports from China, it is of considerable interest to monitor resistance rates for quinolones in Denmark.

Surveillance and treatment strategy for MG

Unlike chlamydia and gonorrhoea, MG is currently not a notifiable disease in Denmark. SSI undertakes testing for MG, MRM and QRAM, however, since 2014 Region Hovedstaden (Capital Region of Denmark) and since 2017 Region Midtjylland (Central Denmark Region) have performed MG and MRM detection. Data from the SSI are, consequently, incomplete since 2014 but suggest that there has been a noteworthy increase in the macrolide resistance rate since 2007. Surveillance of QRAM rates and, preferably, a reduction in the prescription of moxifloxacin would be of significant public health importance considering the absence of an effective third-line treatment. To some extent, this can be obtained by diagnostic stewardship limiting testing for MG to symptomatic patients only, but new, safe, effective, and affordable antimicrobials are also urgently needed.