Addendum to DANMAP 2024 report, chapter 8.3.2
Supplementary data on carbapenemase-producing Enterobacterales (CPE)

In the DANMAP 2024 report, data on carbapenemase-producing Enterobacterales is shown based on isolates referred to the Antimicrobial Resistance (AMR) Reference Laboratory at Statens Serum Institut (SSI). This surveillance includes one isolate per patient per bacterial species and carbapenemase-type within 12 months.

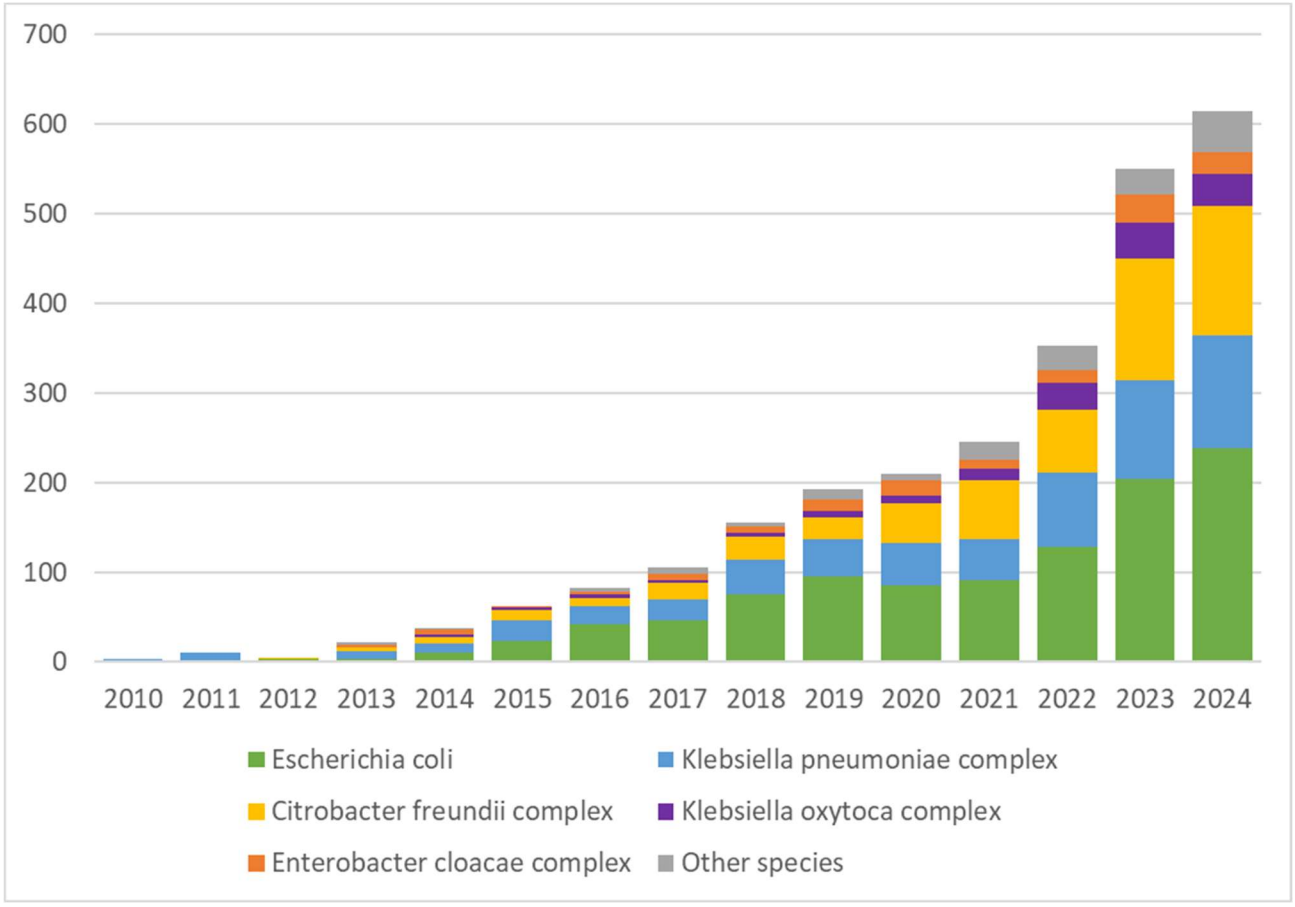
In this addendum, supplementary data from the AMR Reference Laboratory isolate collection is shown. On a representative subset of the CPE-isolates received in 2024, phenotypic susceptibility testing was performed for a broad range of antibiotics.
In addition, data was extracted from MiBa (the Danish Microbiology Database) to examine the distribution of samples types and specimen and quantify the burden of CPE in Denmark.

1. Supplementary data from the AMR Reference Laboratory

1.1. Species distribution

The species distribution of the 5 most common species is shown in Figure 1. From 2020 to 2024, the species distribution has been stable with an average of 38% *Escherichia coli*, 21% *Klebsiella pneumoniae* complex and 23% *Citrobacter freundii* complex.

Figure 1: Species distribution of CPE-isolates received at the AMR Reference Laboratory from 2010-2024.



1.2. Phenotypic susceptibility pattern

From the 616 CPE isolates received during 2024 at the AMR Reference Laboratory, selected isolates from outbreaks and all available non-outbreak isolates (total n=463) underwent phenotypic testing.

Susceptibility testing for amoxicillin-clavulanic acid, piperacillin-tazobactam, cefotaxime, ceftazidime, ceftazidime-avibactam, meropenem, ertapenem, imipenem, aztreonam, aztreonam-avibactam, colistin, gentamicin, tobramycin, amikacin, ciprofloxacin, trimethoprim-sulfamethoxazole and tigecycline was performed with broth microdilution. Susceptibility testing for mecillinam, cefiderocol and fosfomycin was performed using the European Committee on Antimicrobial Susceptibility Testing (EUCAST) disk diffusion methodology (www.eucast.org). Susceptibility testing results were interpreted according to EUCAST breakpoint tables, version 15.0.

Table 1 shows an overview over species and carbapenemase types of the 463 isolates which underwent phenotypic susceptibility testing. The carbapenemase-type distribution differed for the different species. Of the 196 *E. coli* isolates tested, 69% harboured OXA-48-group, 27% NDM and 3% both NDM and OXA-48-group. Of the 120 *K. pneumoniae* complex isolates, 46% carried OXA-48-group, 23% NDM and 18% both NDM and OXA-48-group. In the 63 *C. freundii* complex isolates, 83% had OXA-48-group, 11% NDM and 3% both enzymes.

Table 2 shows an overview over resistance rates to the tested agents, stratified by carbapenemase-type and Table 3 stratified by species.

Novel β-lactam antibiotics

The vast majority of OXA-48-group and KPC-positive isolates were ceftazidime-avibactam susceptible (99.4% and 100%, respectively). Two OXA-48-group isolates were resistant, one *E. coli* and one 1 *K. pneumoniae* complex isolate, both were retested and high resistant (MIC >16 mg/L).

Most of the 463 tested isolates were susceptible for aztreonam-avibactam. Of the 26 isolates (5.6%) which tested aztreonam-avibactam resistant, one was an OXA-48-group *C. freundii* complex isolate and the remaining isolates were *E. coli* (8 OXA-48-group, 14 NDM and 3 NDM+OXA-48-group).
Five of the *E. coli* isolates (all carrying NDM) were also resistant to cefiderocol, all tested aminoglycosides, ciprofloxacin and trimethoprim-sulfamethoxazole. Thus, the only available treatment options were colistin, fosfomycin and tigecycline, none of which is a first line treatment option for monotherapy of invasive infections.

Testing of cefiderocol was performed by disk diffusion according to EUCAST recommendations. Resistance rates for cefiderocol differed for the different carbapenemase-types. Of the 309 CPE-isolates harbouring OXA-48-group enzymes (and no other carbapenemases), 11% were cefiderocol resistant and 11% were in the area of technical uncertainty (ATU). These rates were considerably higher in the 132 isolates carrying NDM (incl. in combination with other carbapenemases) with 78% being cefiderocol resistant and 33% in the ATU.

Colistin

Colistin resistance in total was low (3%). However, 8% of the 120 *K. pneumoniae* complex isolates were colistin resistant. One of the 3 phenotypically colistin resistant *E. coli* isolates harboured the resistance gene *mcr*-1.1 and had a colistin MIC 4 mg/L. Another *E. coli* isolate was *mcr*-1.1 positive, but was found phenotypically susceptible (MIC 2 mg/L).

Other agents

Resistance rates for aminoglycosides were 38% for gentamicin, 46% for tobramycin, 18% for amikacin and 16% for all three aminoglycosides. Of the 463 tested CPE isolates, 63 % were ciprofloxacin resistant and 47% trimethoprim-sulfamethoxazole resistant. For tigecycline, breakpoints have only been established for *E. coli* (3% resistance) and *C. koseri* (no isolate resistant) and for fosfomycin only for *E. coli* (4% resistant).

Multiresistance

Resistance rates to non-β-lactam agents were unevenly distributed among carbapenemase-types and species. Resistance to all aminoglycosides (gentamicin, tobramycin, amikacin), ciprofloxacin and trimethoprim-sulfamethoxazole occurred only in 2% of the 309 CPE-isolates harbouring OXA-48-group enzymes (and no other carbapenemases). In contrast, 33% of the 132 isolates carrying NDM (incl. in combination with other carbapenemases) were resistant against all of these agents.

Resistance rates in *K. pneumoniae* complex isolates were higher than in *E. coli* isolates (Table 3). While only 5% of *E. coli* isolates were resistant to all aminoglycosides (gentamicin, tobramycin, amikacin), ciprofloxacin and trimethoprim-sulfamethoxazole, 27% of *K. pneumoniae* complex isolates displayed this multiresistant phenotype.

Table 1. Bacterial species and carbapenemase types of the 463 CPE-isolates from 2024 which underwent phenotypic susceptibility testing at the AMR Reference Laboratory.

	OXA-48-group	NDM	NDM + OXA-48-group	KPC	KPC + OXA-48-group	NDM + KPC	NDM + KPC + OXA-48-group	VIM	VIM + KPC	IMI	IMP	*Total*
Escherichia coli	135	52	5	2	1						1	*196*
***Klebsiella pneumoniae* complex**	55	28	21	12		1	1	1	1			*120*
Citrobacter freundii complex	52	7	2	1				1				*63*
Enterobacter cloacae complex	15	6	1							1		*23*
Klebsiella oxytoca complex	21	1										*22*
Citrobacter amalonaticus complex	10	1										*11*
Citrobacter koseri	8											*8*
Morganella morganii	2	1		1								*4*
***Raoultella* species**	4											*4*
***Providencia* species**		3										*3*
***Serratia* species**	2	1										*3*
Klebsiella aerogenes	2											*2*
Atlantibacter subterranea	1											*1*
Hafnia alvei	1											*1*
***Kluyvera* species**	1											*1*
Proteus mirabilis		1										*1*
Total	*309*	*101*	*29*	*16*	*1*	*1*	*1*	*2*	*1*	*1*	*1*	*463*

Table 2. Resistance rates [%] in 463 CPE-isolates, stratified by carbapenemase-type. R: resistant; ATU: area of technical uncertainty; NA: not applicable (mecillinam, tigecycline, fosfomycin: no clinical breakpoints for the respective species; colistin: expected resistant phenotype for the respective species). NT: not tested.

	Amoxicillin-clavulanic acid % R	Piperacillin-tazobactam % R	Mecillinam % R	Cefotaxime % R	Ceftazidime % R	Ceftazidime-avibactam % R	Cefiderocol % R	Cefiderocol % in ATU	Meropenem % R	Ertapenem % R	Imipenem % R	Aztreonam % R	Aztreonam-avibactam % R	Colistin % R	Gentamicin % R	Tobramycin % R	Amikacin % R	Ciprofloxacin % R	Trimethoprim-sulfamethoxazole % R	Tigecycline % R	Fosfomycin % R
OXA-48-group	100	100	20	44	35	1	11	11	6	81	7	39	3	1	28	30	5	50	35	2	4
NDM	100	100	35	100	100	100	75	30	91	100	90	76	14	2	51	69	42	88	76	0	4
NDM + OXA-48-group	100	100	90	100	100	100	86	48	100	100	97	86	10	14	86	97	72	97	72	0	20
KPC	100	100	100	100	100	0	25	6	56	94	75	100	0	0	50	81	13	88	38	50	0
KPC + OXA-48-group	100	100	100	100	100	0	100	100	0	100	0	100	0	0	100	100	100	100	0	0	0
NDM + KPC	100	100	100	100	100	100	100	0	100	100	100	100	0	0	100	100	100	0	100	NA	NA
NDM + KPC + OXA-48-group	100	100	100	100	100	100	100	0	100	100	100	100	0	100	100	100	100	100	100	NA	NA
VIM	100	100	100	100	100	100	100	50	100	100	100	50	0	50	50	100	0	100	50	NA	NA
VIM + KPC	100	100	100	100	100	100	100	0	100	100	100	100	0	100	0	100	100	100	100	NA	NA
IMI	100	0	0	0	0	0	0	0	100	100	100	0	0	0	0	0	0	0	0	NA	NA
IMP	100	100	0	100	100	NT	0	0	NT	NT	NT	100	0	0	0	100	0	100	100	100	0
Total	*100*	*100*	*32*	*63*	*56*	*30*	*32*	*17*	*33*	*87*	*34*	*53*	*6*	*3*	*38*	*46*	*18*	*63*	*47*	*2*	*4*

Table 3. Resistance rates [%] in 463 CPE-isolates, stratified by species. R: resistant; ATU: area of technical uncertainty; NA: not applicable (mecillinam, tigecycline, fosfomycin: no clinical breakpoints for the respective species; colistin: expected resistant phenotype for the respective species).

	Amoxicillin-clavulanic acid % R	Piperacillin-Tazobactam % R	Mecillinam % R	Cefotaxime % R	Ceftazidime % R	Ceftazidime -avibactam % R	Cefiderocol % R	Cefiderocol % in ATU	Meropenem % R	Ertapenem % R	Imipenem % R	Aztreonam % R	Aztreonam-avibactam % R	Colistin % R	Gentamicin % R	Tobramycin % R	Amikacin % R	Ciprofloxacin % R	Trimethoprim-Sulfamethoxazole % R	Tigecycline % R	Fosfomycin % R
Escherichia coli	100	100	21	58	52	30	33	10	27	78	27	50	13	2	20	26	9	49	51	3	4
***Klebsiella pneumoniae* complex**	100	100	52	74	70	45	44	27	63	97	64	64	0	8	53	69	43	88	50	NA	NA
***Citrobacter freundii* complex**	100	100	38	79	75	16	25	29	17	95	11	67	2	0	73	76	11	76	59	NA	NA
***Enterobacter cloacae* complex**	100	96	17	61	48	30	35	4	26	100	30	39	0	0	30	39	26	57	35	NA	NA
***Klebsiella oxytoca* complex**	100	100	36	27	14	5	0	14	5	86	5	45	0	0	32	32	5	55	14	NA	NA
***Citrobacter amalonaticus* complex**	100	100	18	45	45	9	9	0	9	91	9	36	0	0	36	45	0	55	18	NA	NA
Citrobacter koseri	100	100	0	0	0	0	13	25	0	88	0	0	0	0	13	13	13	25	0	0	NA
Morganella morganii	100	100	NA	75	50	25	25	25	0	25	75	25	0	NA	50	50	0	50	50	NA	NA
***Raoultella* species**	100	100	25	0	0	0	0	0	0	100	0	0	0	0	25	25	0	25	0	NA	NA
***Providencia* species**	100	100	NA	100	100	100	33	67	100	100	100	33	0	NA	33	67	33	100	100	NA	NA
***Serratia* species**	100	100	NA	100	33	33	33	33	33	100	100	67	0	NA	33	33	33	67	0	NA	NA
Klebsiella aerogenes	100	100	50	50	0	0	0	0	50	100	50	0	0	0	0	0	0	50	0	NA	NA
Atlantibacter subterranea	100	100	NA	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	NA	NA
Hafnia alvei	100	100	NA	100	100	0	0	0	100	100	100	100	0	NA	0	0	0	0	0	NA	NA
***Kluyvera* species**	100	100	NA	0	0	0	0	0	0	100	0	0	0	0	0	100	0	100	100	NA	NA
Proteus mirabilis	100	100	0	100	100	100	0	100	100	100	100	0	0	NA	100	100	0	100	100	NA	NA
Total	*100*	*100*	*32*	*63*	*56*	*30*	*32*	*17*	*33*	*87*	*34*	*53*	*6*	*3*	*38*	*46*	*18*	*63*	*47*	*2*	*4*

2. Data from MiBa (the Danish Microbiology Database)

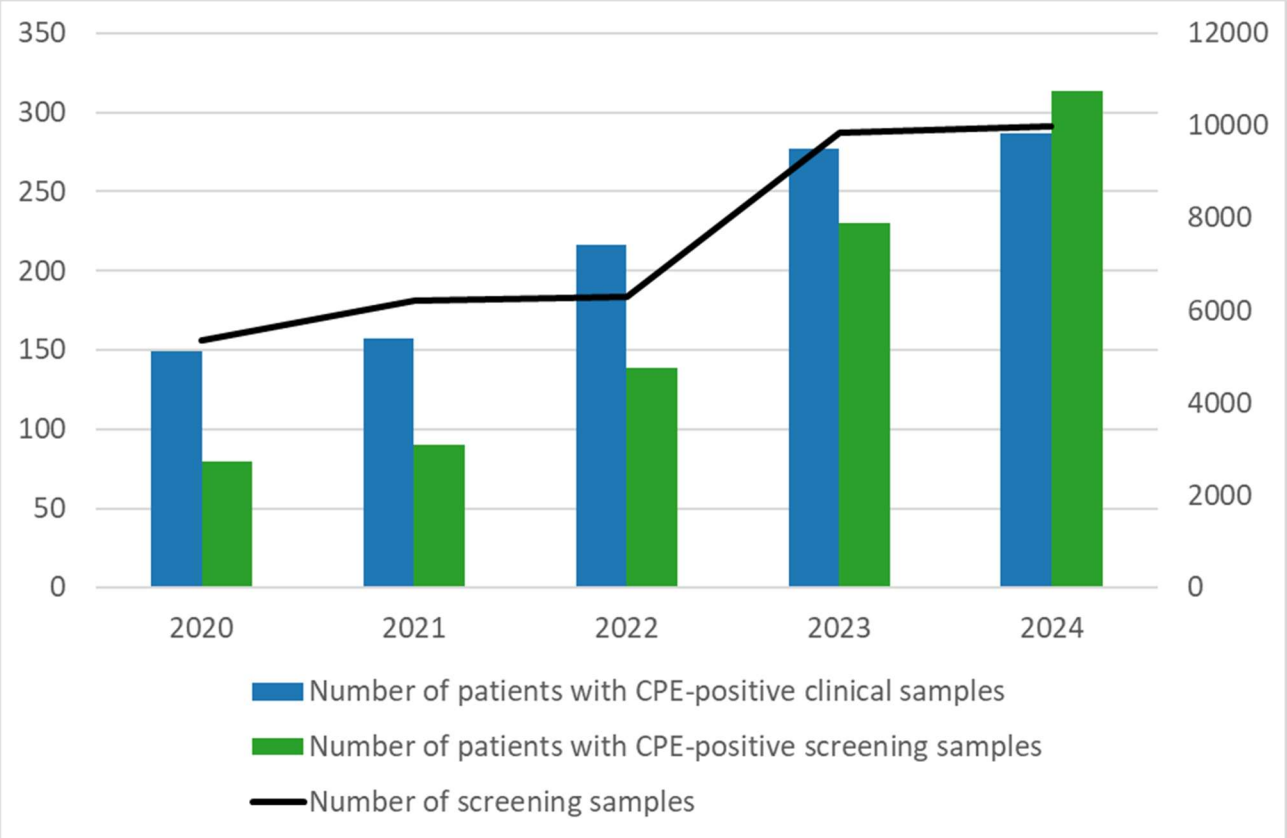
The isolates referred to the AMR Reference Laboratory are based on the first isolate per patient and subsequently one isolate per patient per bacterial species and carbapenemase-type after >12 months. Therefore, this data cannot be used with regards to burden within specimen types, e.g. how many patients presented with CPE-bacteremia. Therefore, CPE-surveillance was supplemented with data from MiBa, which includes all microbiology reports from all ten Danish Departments of Clinical Microbiology.

Data was extracted from MiBa for 2020-2024 and validated by comparison with both data from CPE-isolates received at the AMR Reference Laboratory and with data extractions from Departments of Clinical Microbiology. In MiBa, 3195 CPE-positive samples with a total of 3650 CPE-isolates were identified from 1583 patients.

2.1. CPE in clinical versus screening samples and screening activity

An increase in both CPE-positive screening and clinical samples was seen during the 5-years' period. However, while the number of patients, where CPE was found in a clinical sample stagnated from 2023 to 2024, the number of patients where CPE was found in screening samples continued to increase. The number of screenings samples increased markedly from 2022 with 6295 screening samples to 9848 in 2023 with only a slight increase in 2024.

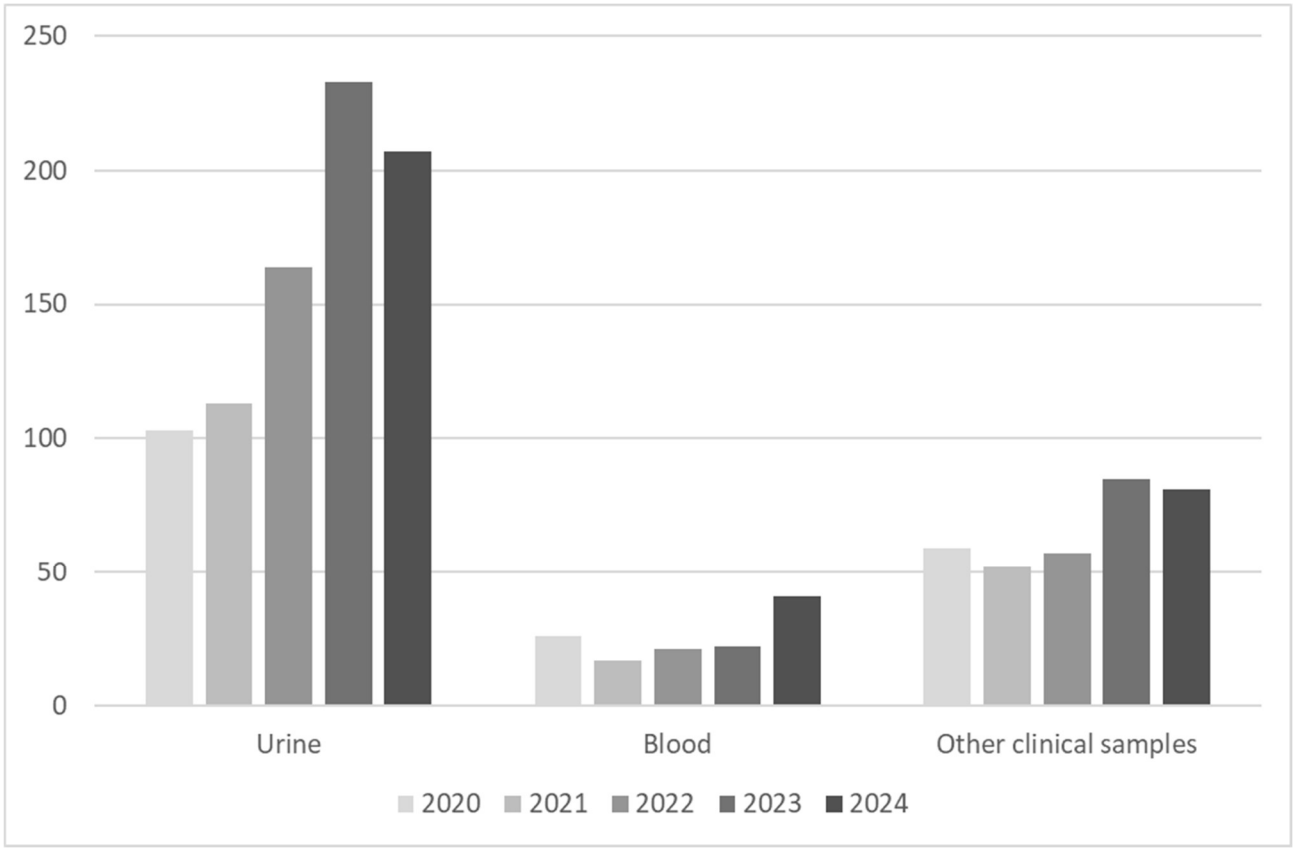
Figure 2: Number of patients with CPE-positive samples in the Danish Microbiology Database, 2020-2024. Numbers of patients with CPE-positive clinical (blue columns) and screening (green columns) samples, 2020-2024. Patients are included in both categories if they had both a positive screening and clinical sample in the same year. The black line (secondary y-axis) represents the total number of screening samples.



2.2 CPE in clinical samples: specimen types

Urine was the specimen, where CPE was most frequently found. The number of patients with CPE -positive urine samples increased from 2020 to 2023, but decreased slightly from 2023 to 2024. In the period 2020-2024, 124 patients were diagnosed with CPE-bacteremia, with the highest number (n=41 patients) in 2024, corresponding to an incidence of 0.69 /100 000 inhabitants.

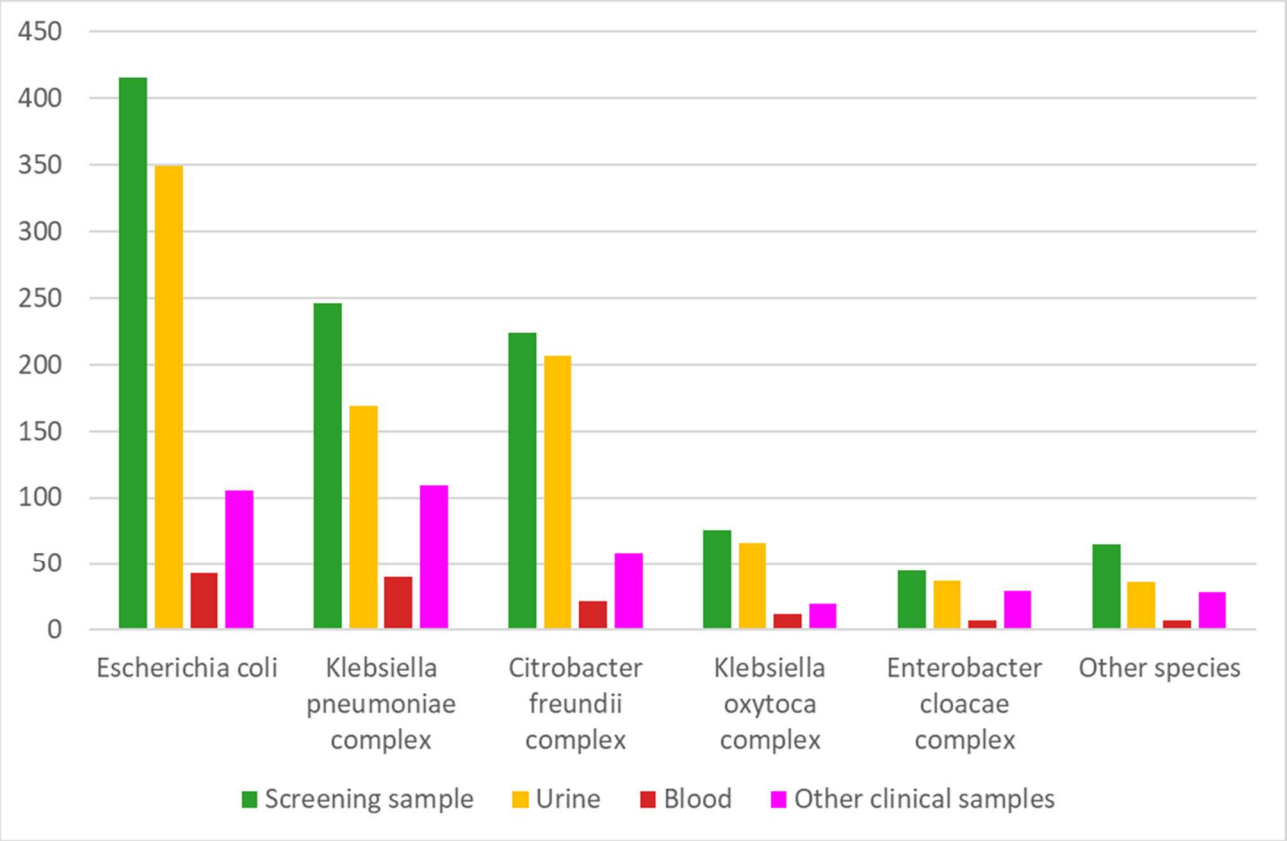
Figure 3: Numbers of patients with CPE-positive clinical samples, 2020-2024, stratified by specimen type. Patients can be included in >1 category and in >1 year.



2.3. Species distribution in sample and specimen types

Figure 4 shows the distribution of species in different specimen. The different species showed similar patterns. All of the five most frequent species were found in blood cultures.

Figure 4: Numbers of CPE-isolates (one isolate per patient per species per year), 2020-2024, stratified by bacterial species and specimen type.
Patients can be included with >1 species and >1 specimen type.



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