NATIONAL REFERENCE CENTER FOR ANTIBIOTIC-RESISTANT GRAM-NEGATIVE BACILLI *(ENTEROBACTERALES, PSEUDOMONAS, ACINETOBACTER)*

Surveillance report 2024

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List of Abbreviations

AST: Antimicrobial susceptibility testing
BLBLI: Beta-lactam/beta-lactamase inhibitors
EUCAST: European Committee on Antimicrobial Susceptibility Testing
MLST: Multilocus Sequence Typing
WGS: Whole Genome Sequencing
MIC: Minimum Inhibitory Concentration
CPAc: Carbapenemase-producing *Acinetobacter*
CPAB: Carbapenemase-producing *Acinetobacter baumannii*
CPPs: Carbapenemase-producing *Pseudomonas*
CPPA: Carbepenemase-producing *Pseudomonas aeruginosa*
CPE: Carbapenemase-producing Enterobacterales
ESBL: Extended-spectrum beta-lactamase
HPAMPC: hyperproduced AmpC cephalosporinase
MBL: Metallo-beta-lactamase
MDR: Multidrug-resistant
NRC: National Reference Center

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1. Introduction

This report summarizes the data analysis up to 2024 of the national microbiological surveillance based on voluntary referral by clinical laboratories of MDR isolates (of any species belonging to Enterobacterales*, Pseudomonas or Acinetobacter*) cultured from clinical or screening samples collected from human patients.

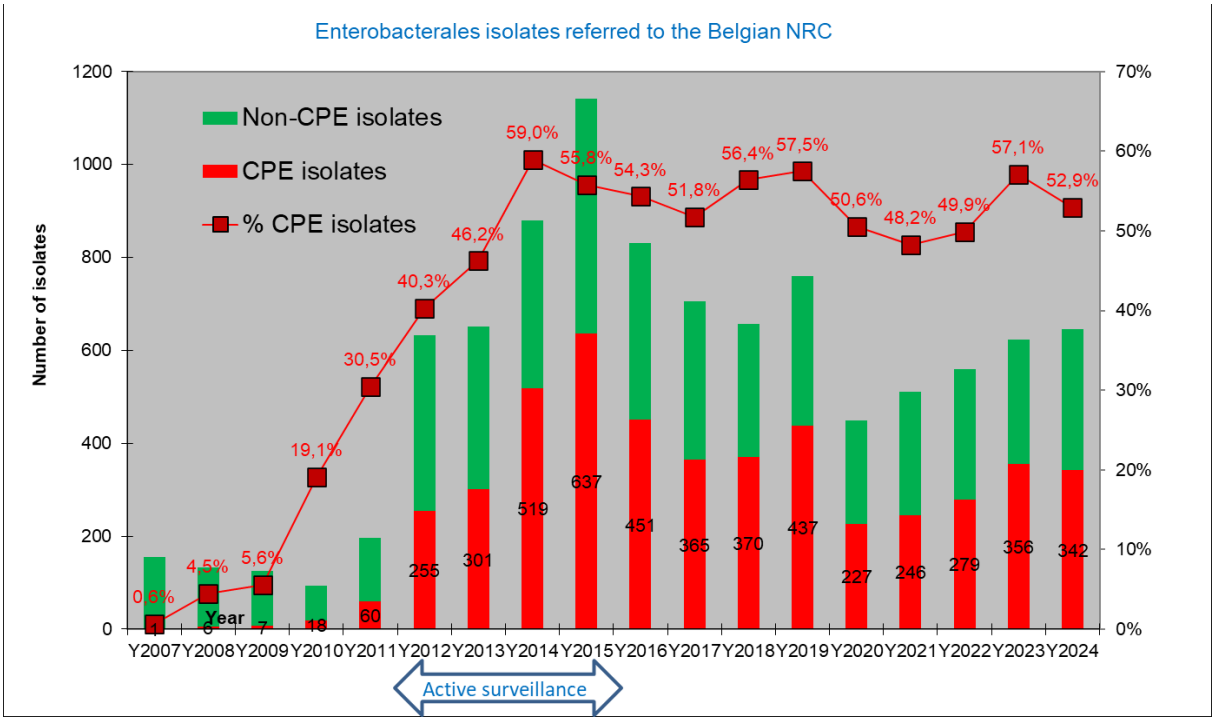
The strains received by the NRC are sent mainly for confirmation of acquired carbapenemase production and/or susceptibility testing for last-line antibiotics active against MDR isolates. Less common requests are confirmation of colistin resistance, identification of hypervirulent *Klebsiella pneumoniae* and genotyping for outbreak investigation.

All carbapenemase-producing isolates were submitted for whole genome sequencing using Illumina and/or Nanopore technologies. For genomic surveillance, resistance determinants encoding genes (including characterization of carbapenemase variants) and clonal determination of MLST were performed for epidemiological description.

2. Multidrug-resistant Enterobacterales

2.1. Characteristics of samples and patients related to isolates

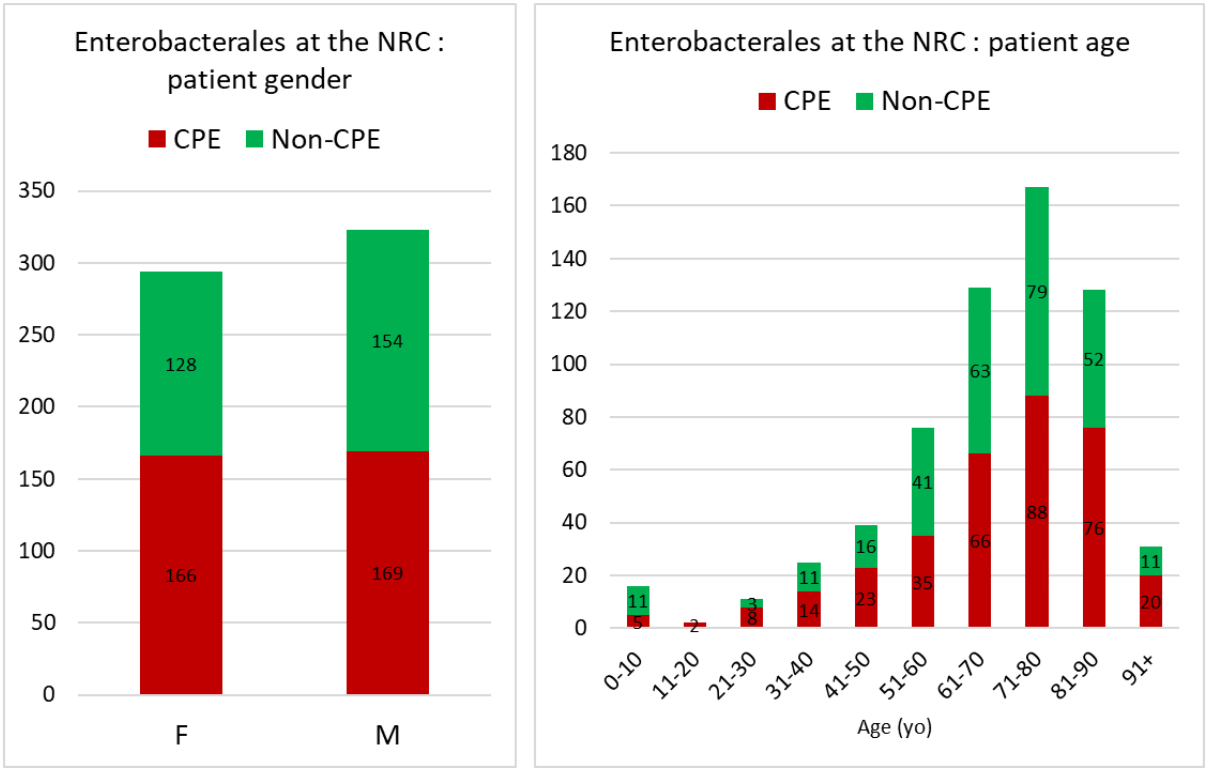
Figure 1 Number of MDR Enterobacterales isolates received per year by the NRC and the proportion (%) of those confirmed as carbapenemase producers (CPE)



From the first report of CPE in 2007, the number of confirmed CPE isolates increased significantly over time. A notable rise was observed during the active surveillance (mandatory epidemiological and microbiological reporting/referral) starting in 2012 and peaking in 2015. Following the end of the active surveillance, the number of isolates and the proportion of confirmed CPE remained stable before dropping down significantly during the COVID-19 pandemics (years 2020-2022). The decline could be due to a lower prevalence of CPE because of control measures, associated with the difficulty of laboratories sending strains during the pandemic. In 2024, the numbers continue to increase back to those observed before the pandemics, reaching 644 isolates analyzed with 53% confirmed as CPE.

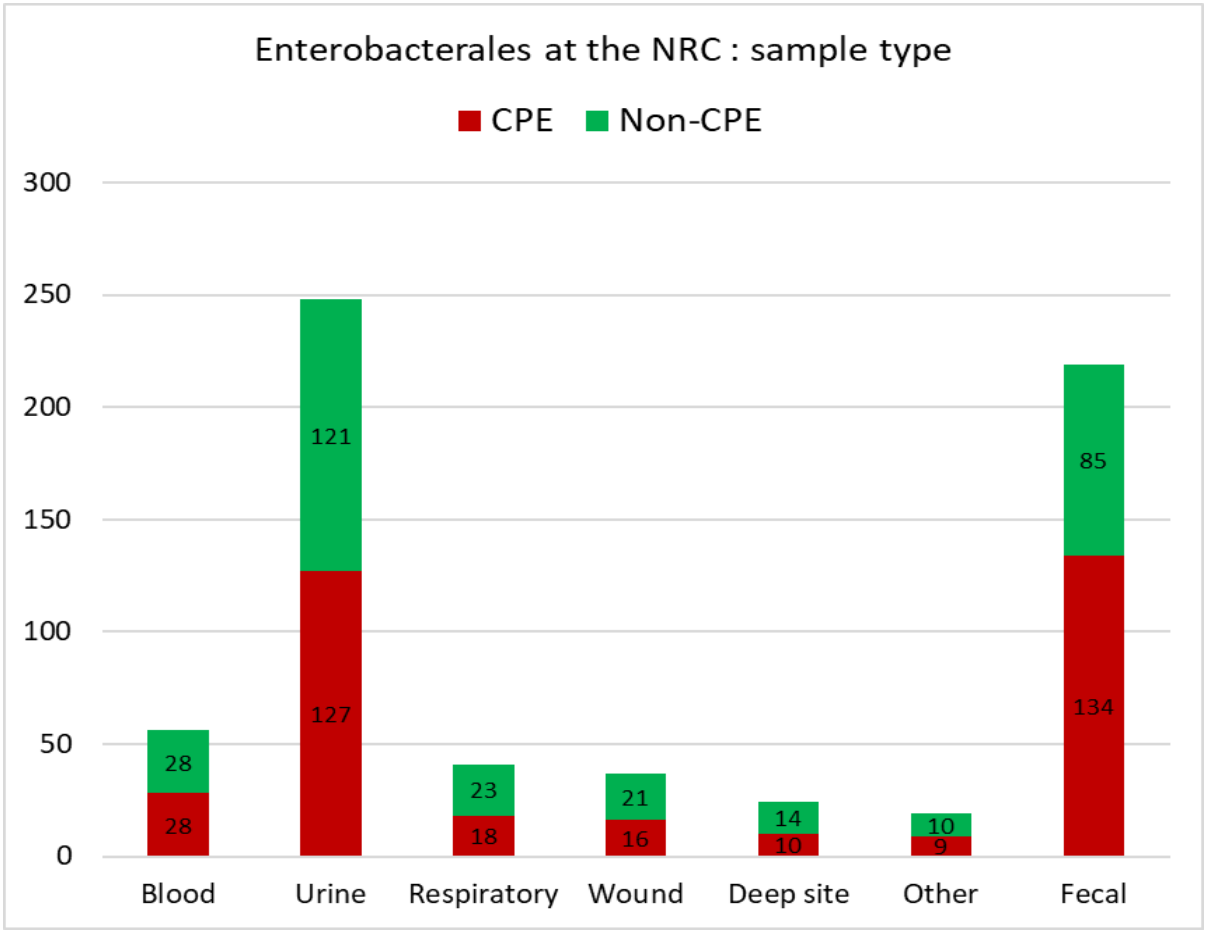
In 2024, 67 (76%) of the 88 clinical laboratories that referred isolates to the NRC (including 55 hospital-based and 12 serving general practitioners in the community) had at least one isolate confirmed as CPE.

Figure 2 Number of Enterobacterales isolates per gender and per age group in 2024.



In 2024, the NRC received more isolates (52%) collected from male patients and mainly (73%) from patients above 60 years old.

Figure 3 Sample types from which Enterobacterales were isolated in 2024.



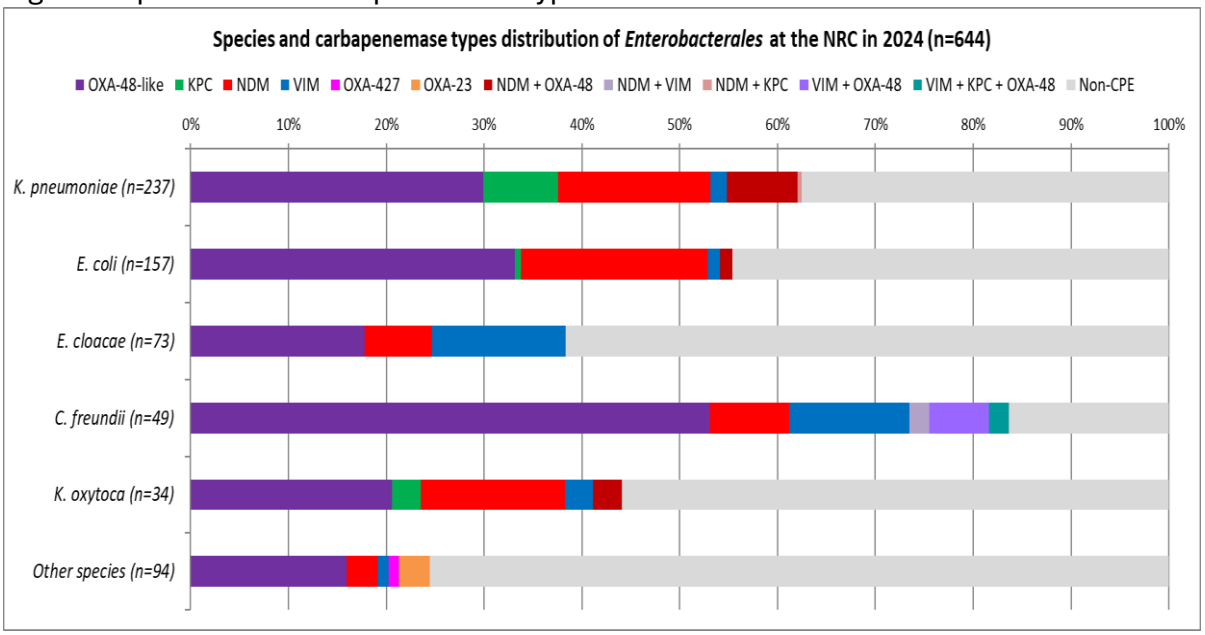
The ‘deep site’ category includes fluid and tissue specimens other than superficial or orifical sample sites. The ‘other’ category includes genital samples, percutaneous catheters and samples of unknown origin.

Of the 644 isolates with indicated sample nature, one third originated from fecal screening samples, while urinary specimens represented the large majority (58%) of sample origins for the 425 clinical isolates. Of the 56 isolates causing bloodstream infections, half were confirmed as carbapenemase producers. The number of blood CPE (n=28) isolates doubled in 2024 compared to 2023 (n=13).

2.2. Carbapenemase-producing Enterobacterales

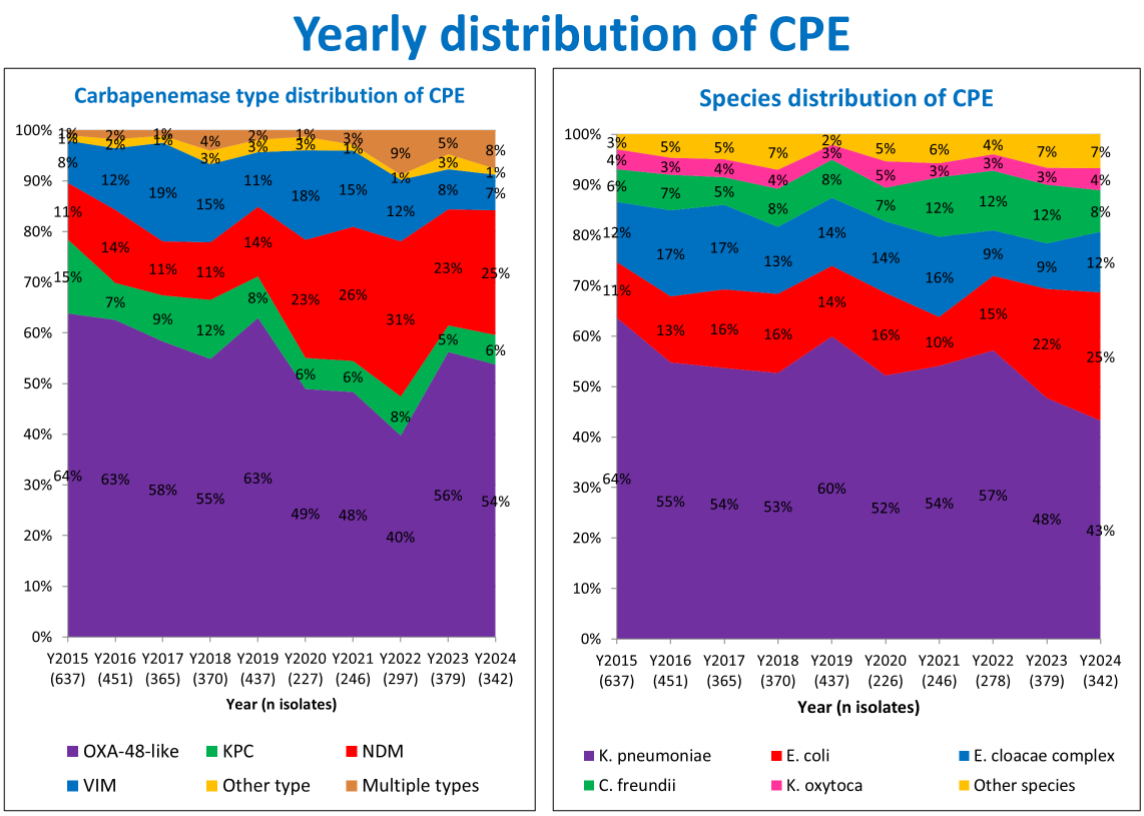
2.2.1. Bacterial species and resistance mechanisms

Figure 4 Species and carbapenemase types distribution of Enterobacterales isolates



In 2024, *K. pneumoniae, E. coli, E. cloacae* complex, *C. freundii* and *K. oxytoca* remained the top 5 species among the total 644 MDR Enterobacterales isolates analyzed for carbapenemase production at the NRC. Among *K. pneumoniae, E. coli or C. freundii*, most of the isolates were confirmed as carbapenemase producers, while for *E. cloacae* complex and *K. oxytoca,* less than 50% were CPE.

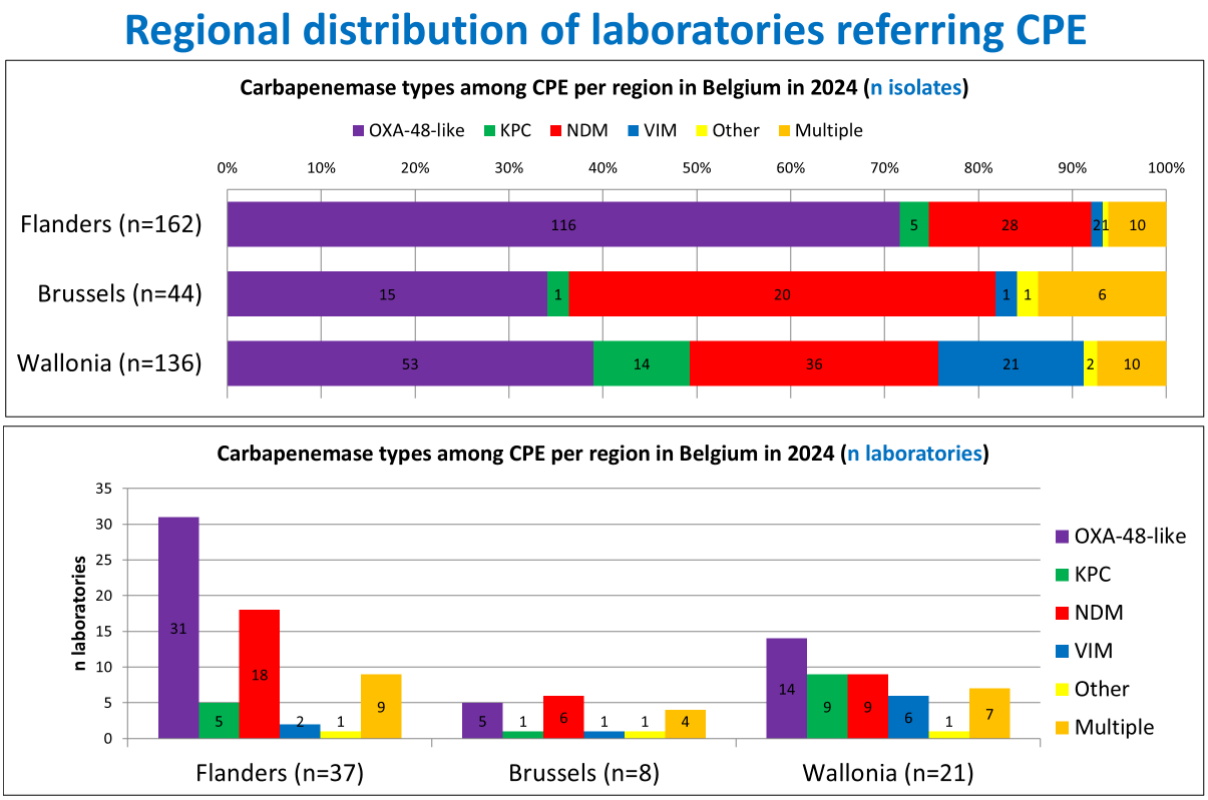
Figure 5 Yearly distribution of species and carbapenemase types among CPE



Among the 342 confirmed CPE isolates in 2024, OXA-48-like enzymes remain the predominant common carbapenemase (54%), followed by NDM (25%), while KPC (6%) and VIM (7%) were less prevalent. The other carbapenemase types include OXA-23-producing *Proteus mirabilis* (n=3) and OXA-427-producing *E. cloacae* complex (n=3). A trend towards diversification of carbapenemase types is evident, with isolates coproducing multiple enzymes being increasingly reported (18 in 2023 and 26 in 2024). 75% of these 44 CPE coproduced the association of NDM and OXA-48-like carbapenemases. The other combination of carbapenemases were sporadically detected in 2023 and included isolates producing VIM + OXA-48 (n=3), IMP + OXA-48 (n=2), NDM + VIM (n=1) enzymes and one isolate co-harbored three carbapenemases (KPC + VIM + OXA-48).

While OXA-48-like and NDM carbapenemases are detected in all species, KPC are mainly detected in *K. pneumoniae* and VIM more frequently associated with *E. cloacae* complex and *C. freundii*.

Figure 6 Regional distribution of CPE



The geographical distribution of CPE showed some regional variations. There is a national North-South gradient with large predominance of OXA-48-like, while a wider heterogeneity of carbapenemase types is observed in Brussels and in Wallonia. In Wallonia, all 4 major carbapenemase types are detected in at least 6 laboratories each.

Figure 7 Yearly numbers of laboratories according to carbapenemase type among CPE

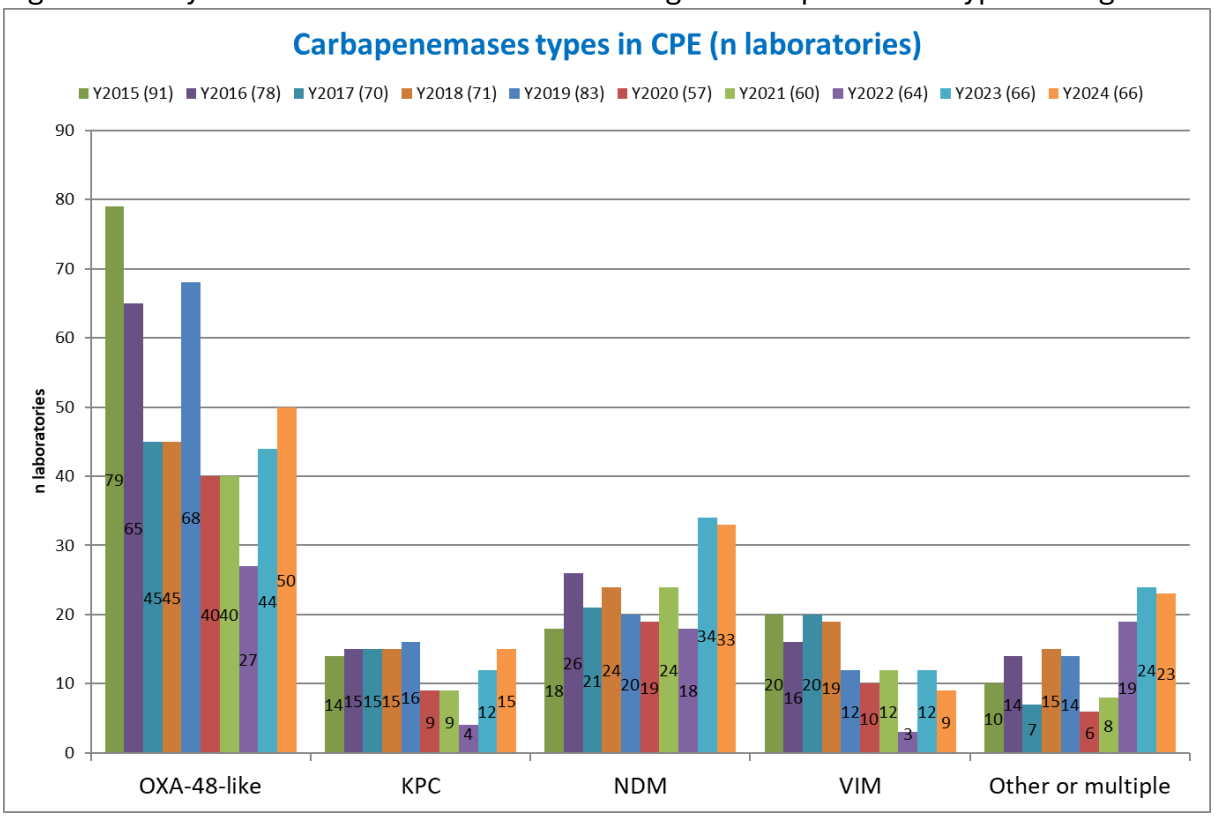


Figure 8 Yearly proportion (%) of CPE-reporting laboratories for each carbapenemase type among CPE

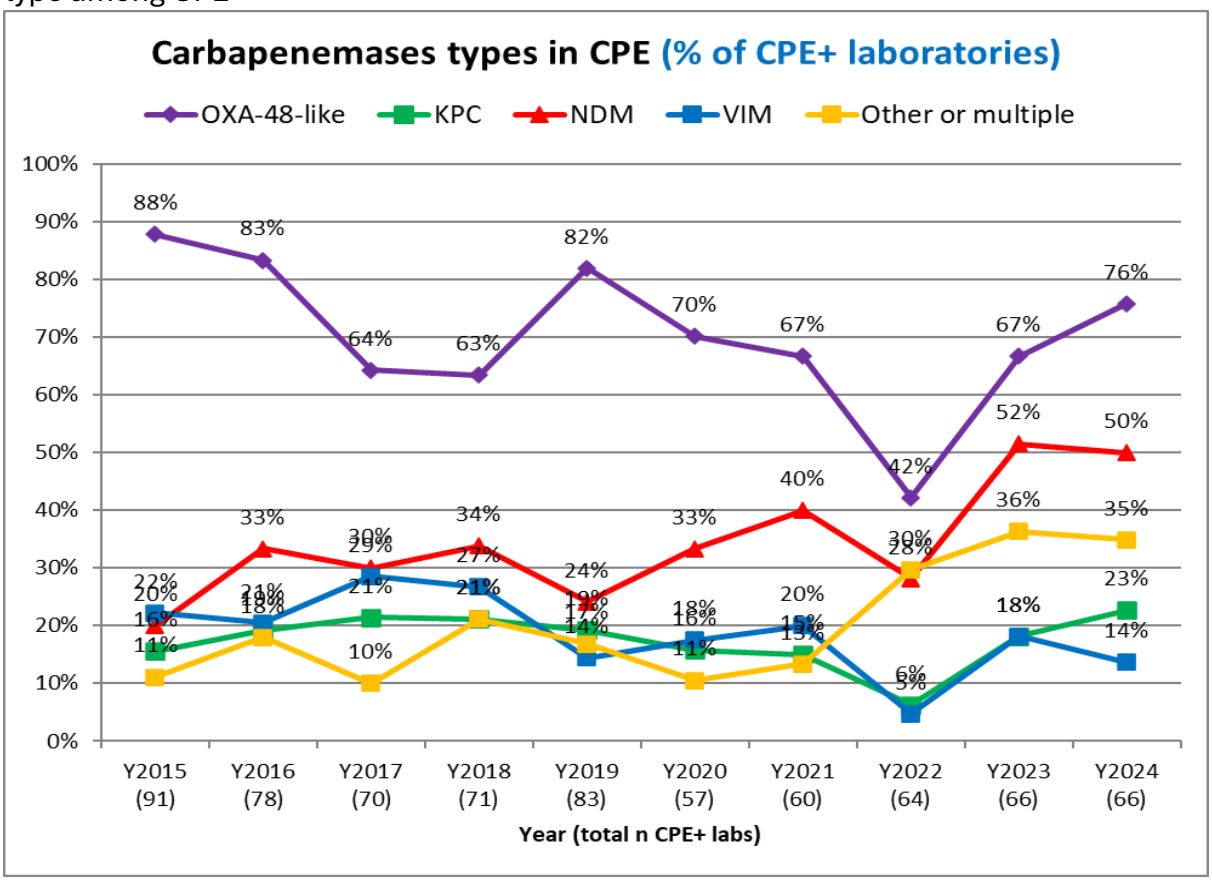
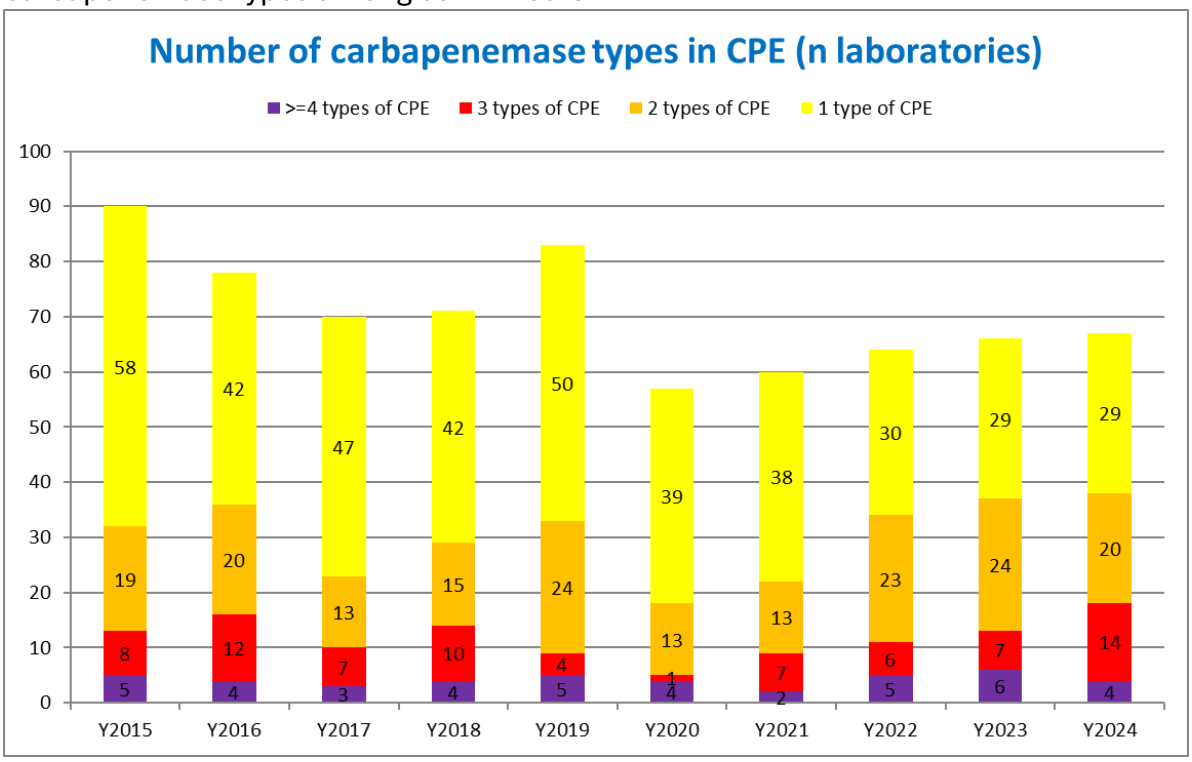


Figure 9 Yearly number/proportion (%) of laboratories reporting one or more carbapenemase types among confirmed CPE



OXA-48-like remains in 2024 the main carbapenemase type reported by the highest number (50) and proportion (76%) of the laboratories. When comparing 2024 to 2019, while there is a lower number of laboratories reporting KPC (15 vs 16) and VIM (9 vs 12) CPE, we observe a steady increase in the number (33 vs 19) and proportion (50% vs 24%) of laboratories reporting NDM type CPE reaching a peak in 2023.

In addition, the number (38 vs 33) and proportion (58% vs 40%) of laboratories reporting more than one types of carbapenemase among CPE also increased comparing 2024 to 2019. These data highly suggest a diversification with spread of multiple carbapenemase types of CPE across Belgian laboratories during the past years.

2.2.2. Genomic surveillance

Figure 10 Distribution of carbapenemase enzyme variants based on bla gene sequence determined by WGS

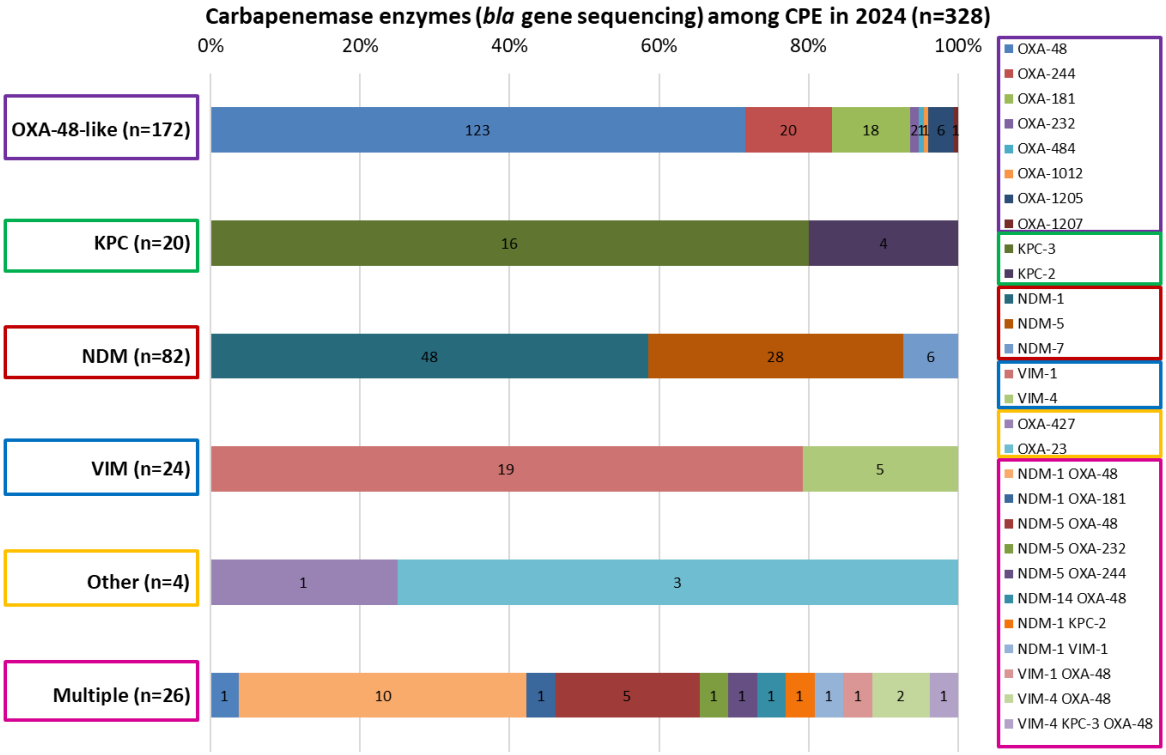
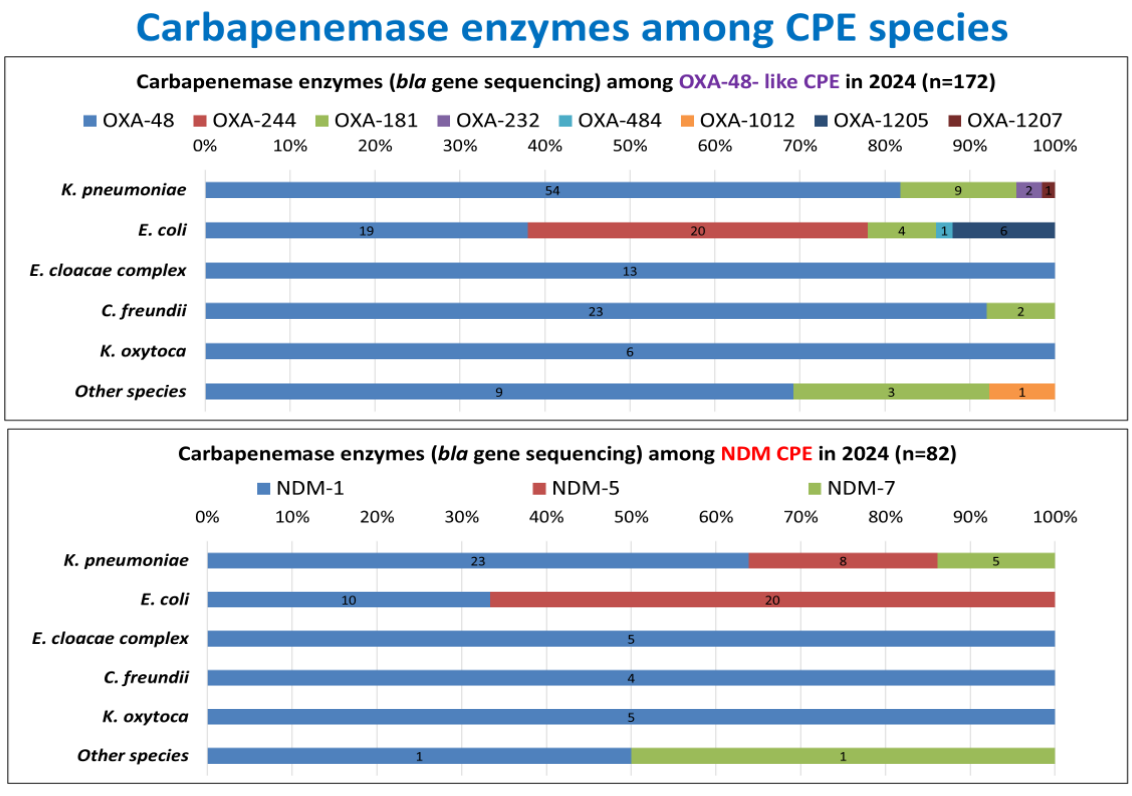


Figure 11 Carbapenemase enzymes based on bla gene sequencing among CPE species



Among OXA-48-like CPE, OXA-48 represented the predominant (76%) enzyme, followed by OXA-244 variant (12%), which were all detected only in *E. coli*. First detected in Belgium in 2016, OXA-244 carbapenemase represented in 2024 the main OXA-48-like enzyme (20/50) CPE among *E. coli.* More than half of these (11/20) OXA-244-producing *E. coli* were reported from 5 private (out of 13) laboratories suggesting also their ongoing spread in the community.

Among NDM CPE, while NDM-1 represented the main (58%) enzyme present among Enterobacterales, NDM-5 represented in 2024 the predominant variant (66%, 20/30) among NDM-producing *E. coli*.

KPC-3 and VIM-1 were largely the predominant enzymes among the other two CPE families. VIM-4 variant was only detected among *C. freundii* (n=4) and *E. cloacae* complex (n=1) CPE.

Figure 12 MLST clonal distribution of CPE among *K. pneumoniae* and *E. coli*

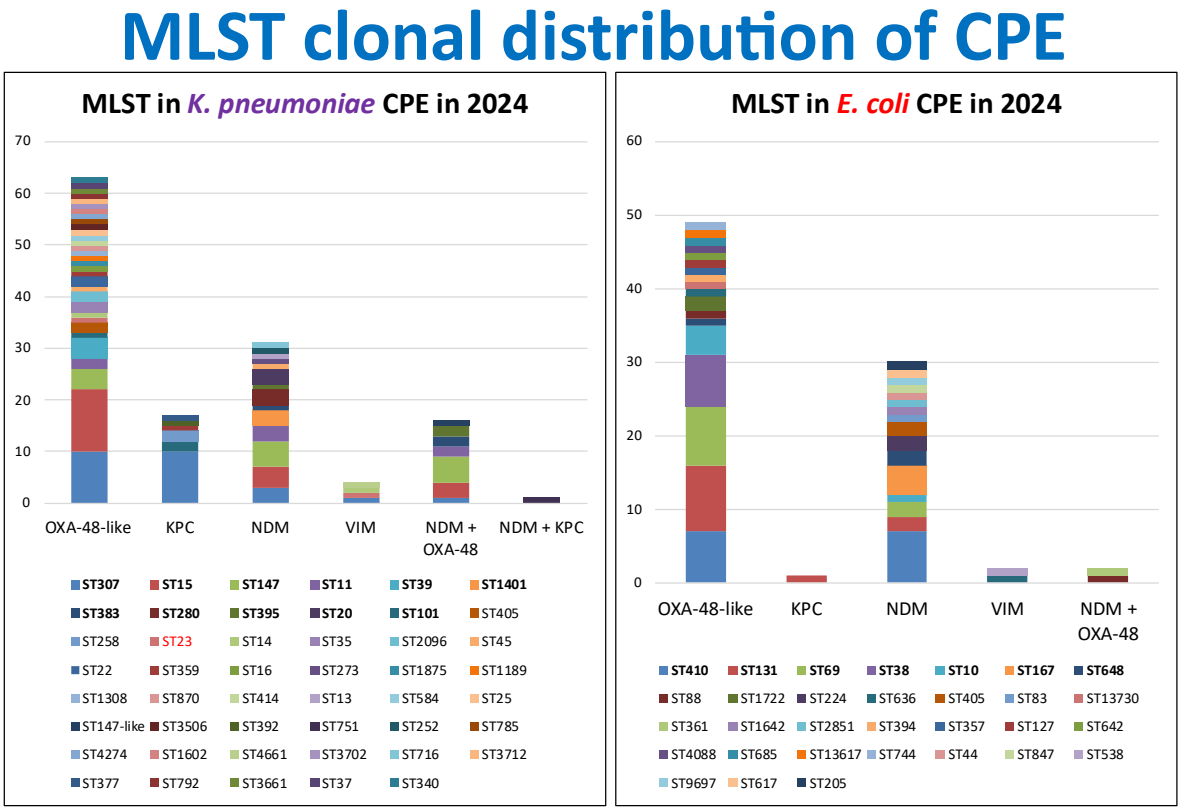


Figure 12 detailed the distribution of sequence types (ST) based on the multilocus sequence typing (MLST) determined by WGS on *K. pneumoniae* and *E. coli* CPE isolates in 2024.

The 132 *K. pneumoniae* CPE isolates belonged to 47 different ST, including notably ST307 (n=25), ST147 (n=14), ST15 (n=19), ST11 (n=7), ST20 (n=3), ST280 (n=3), ST383

(n=3), ST395 (n=3), ST1401 (n=3), which were the top 11 ST clones with at least 3 isolates reported. The predominance of ST307 and ST147 supports the dissemination of these well-recognized high-risk clones (HRC) in Belgium. Of note, 5/15 *K. pneumoniae* CPE co-producing OXA-48-like and NDM-type carbapenemases belonged to ST147. Finally, a hypervirulent ST23 *K. pneumoniae* CPE strain producing VIM-1 carbapenemase was detected for the first time at the NRC in Belgium. The isolate was cultured from a urinary sample of a French female patient who was also carrying a VIM-1 *E. cloacae* isolate.

The 84 *E. coli* CPE isolates showed also high clonal diversity and belonged to 31 different ST, including ST410 (n=14), ST131 (n=12), ST69 (n=10), ST38 (n=7), ST10 (n=5), ST167 (n=4), ST648 (n=3), which were the top 6 ST clones with at least 3 isolates reported. Notably, 79% (15/19) of the *E. coli* producing OXA-244 (OXA-48-like), belonged to one of the top 5 STs (ST69 (n=5), ST131 (n=4), ST38 (n=4) ST10 (n=2)), suggesting the potential multiclonal spread associated with this emerging OXA-48-like variant, known to be usually integrated in the chromosome and phenotypically difficult to detect with very low carbapenem hydrolysis activity. Finally, we observe certain associations of two increasingly detected carbapenemase variants, namely OXA-181, OXA-1205 (both OXA-48-like) and NDM-5 enzymes, with ST410 (n=13) and ST167 (n=4), which are two recently recognized HRC in *E. coli*.

Figure 13 Geographical distribution of carbapenemases among CPE in 2024

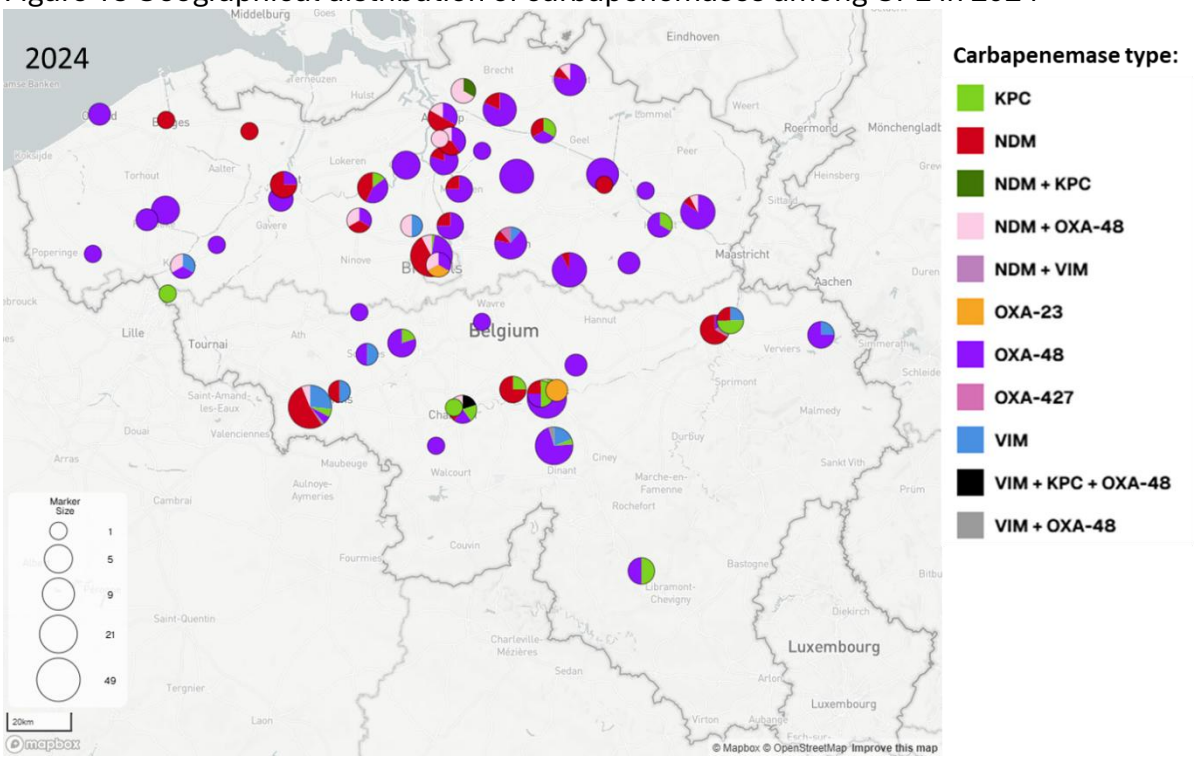


Figure 14 Geographical distribution of top 8 MLST clones among *K. pneumoniae* CPE in 2024

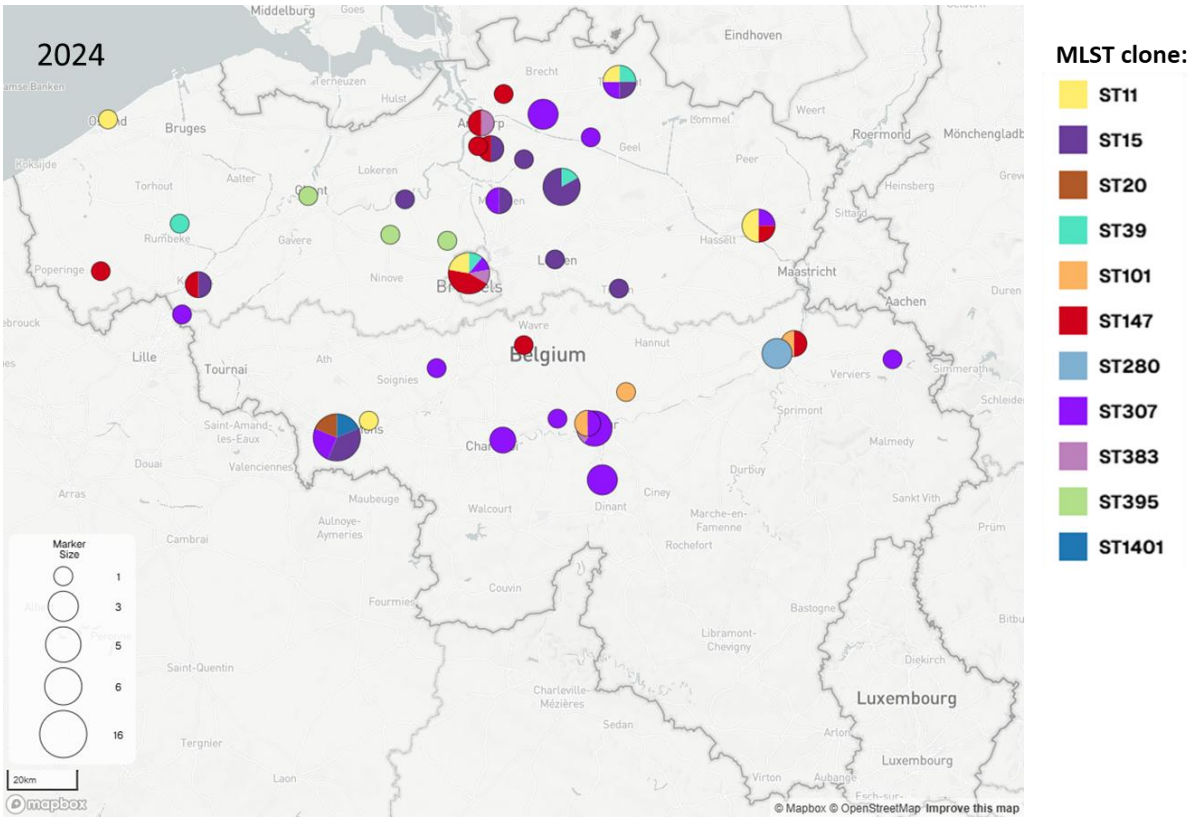
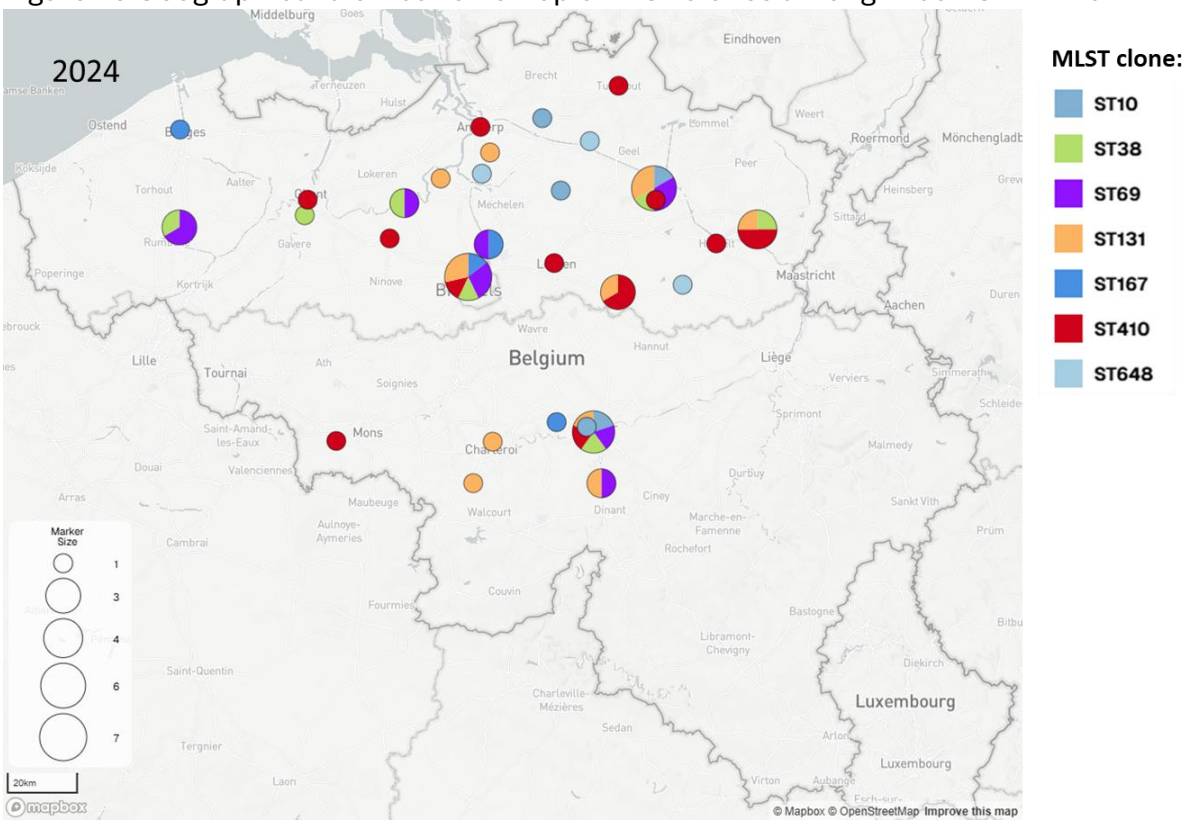


Figure 15 Geographical distribution of top 6 MLST clones among *E. coli* CPE in 2024



2.2.3. Antimicrobial susceptibility

MIC determination was performed by broth microdilution method (BMD) for all confirmed CPE isolates and for part of the non-carbapenemase producers.

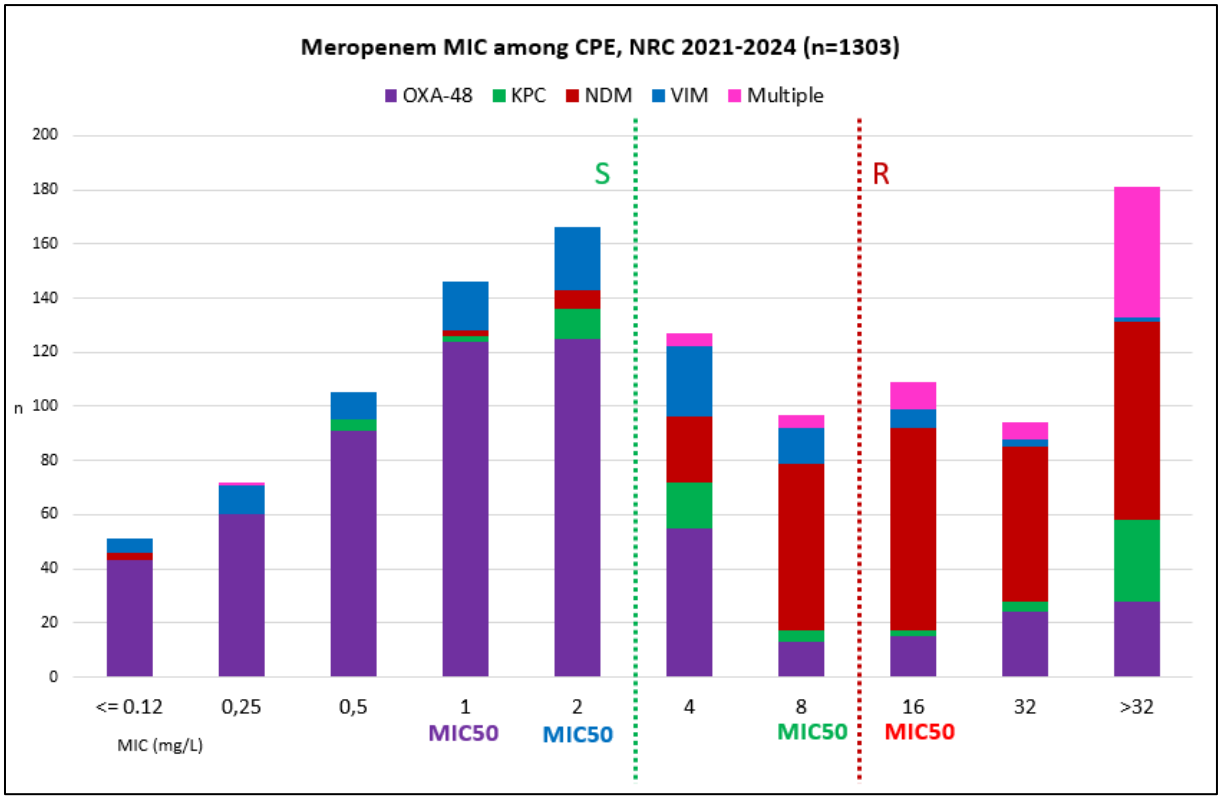
Table 1 Antibiotic susceptibility profile (proportion of susceptible results (S/I) in % according to EUCAST) by broth microdilution method (BMD) according to carbapenemase types for putative CPE at the NRC for 2021-2024 (n=1307)

Enterobacterales (total n=1307)	OXA-48-like	KPC	NDM	VIM	Multiple	non-CPE
Antibiotic \ n isolates	**579**	**74**	**304**	**119**	**76**	**144**
Temocillin	2%	17%	7%	1%	2%	61%
Piperacillin/tazobactam	1%	0%	1%	0%	1%	40%
Aztreonam	48%	0%	12%	49%	14%	45%
Aztreonam/avibactam	99%	96%	97%	100%	95%	25%
Cefotaxime	36%	4%	0%	0%	2%	40%
Ceftazidime	46%	1%	0%	3%	1%	45%
Ceftazidime/avibactam	100%	100%	1%	17%	7%	95%
Cefepime	56%	4%	1%	23%	5%	60%
Ceftolozane/tazobactam	NA	1%	1%	0%	0%	55%
Cefiderocol	97%	82%	75%	88%	75%	91%
Imipenem	87%	21%	25%	58%	12%	96%
Imipenem/relebactam	NA	100%	5%	17%	0%	93%
Meropenem	88%	51%	32%	90%	15%	90%
Meropenem/vaborbactam	NA	98%	NA	NA	15%	89%
Cotrimoxazole	46%	50%	41%	32%	36%	55%
Ciprofloxacin	31%	8%	10%	44%	5%	39%
Gentamicin	67%	63%	46%	71%	29%	65%
Amikacin	91%	58%	54%	93%	34%	87%
Tigecycline	73%	62%	69%	73%	57%	71%
Eravacycline	82%	80%	88%	76%	100%	69%
Colistin	93%	85%	89%	93%	83%	43%
Fosfomycin IV	73%	62%	83%	85%	56%	66%
Chloramphenicol	70%	70%	72%	59%	50%	57%
Mecillinam	19%	0%	22%	0%	0%	31%

*Multiple: isolates producing multiple carbapenemases

Table 1 shows the antibiotic susceptibility testing (AST) profile according to carbapenemase types for CPE isolates analyzed by BMD at the NRC with aggregated data for the 2021-2024 period.

Figure 16 Meropenem MIC distribution by carbapenemase type among CPE



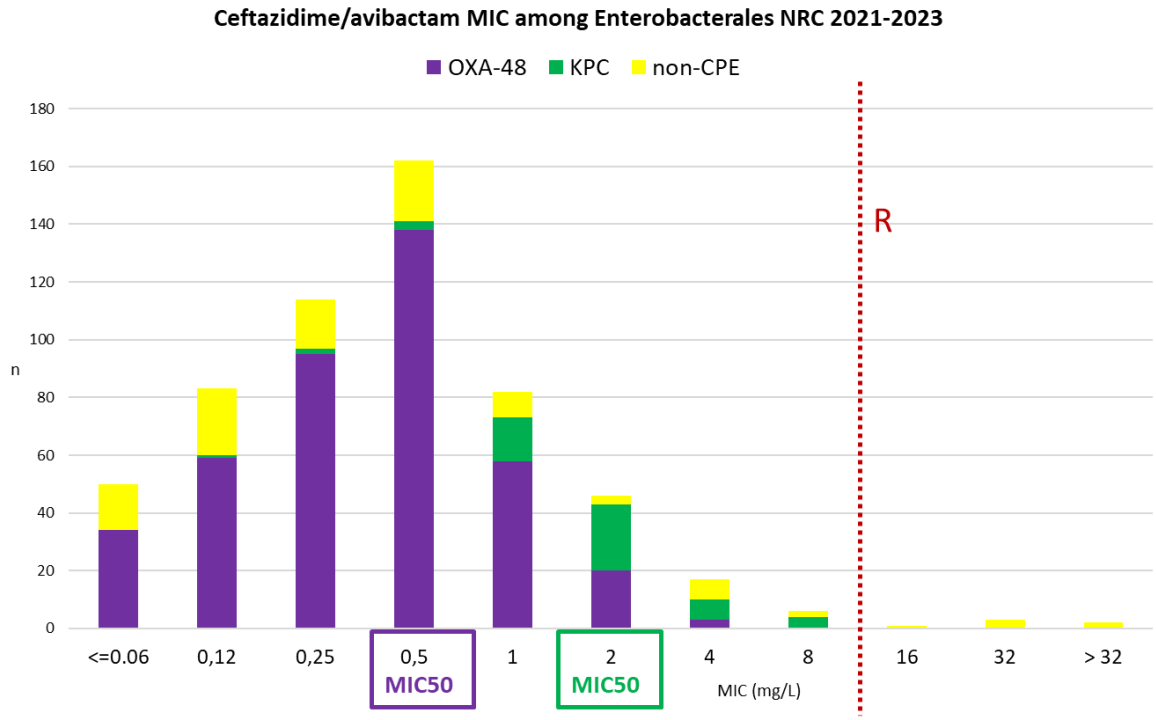
The resistance levels to carbapenems are highly variable depending on the type of carbapenemase produced (and also modulated by other non-enzymatic resistance mechanisms such as porin deficiency not characterized genotypically). While the majority of the KPC and NDM are resistant to meropenem, 88% of OXA-48 and 90% of VIM CPE are susceptible (S/I) to meropenem using EUCAST clinical breakpoint. These data continue to support the use of EUCAST screening breakpoints for the suspicion of carbapenemase production, showing sensitivity of 98% and 96% for ertapenem and meropenem, respectively for the detection of CPE (data not shown).

Nearly all CPE strains were resistant to temocillin (95%) and piperacillin/tazobactam (98%), which can serve as excellent additional phenotypical resistance markers for the suspicion of CPE.

While most NDM, VIM and KPC isolates are resistant to third (>97%) and fourth (>77%) generation cephalosporins (3GC and 4GC), OXA-48-like CPE can retain susceptibility (36%-56%) to 3GC and 4GC when the isolates do not express other large-spectrum beta-lactamases such as extended-spectrum beta-lactamases (ESBLs) or hyperproduced AmpC cephalosporinases (hpAmpC). Aztreonam retains activity against half (49%) of the VIM CPE, but against only 12% the NDM CPE strains which frequently coproduce additional ESBLs and/or hpAmpC.

Susceptibility of CPE against new anti-CPE agents including recent beta-lactam/beta-lactamase inhibitor (BLBLI) combinations are summarized in Table 1 and detailed in the following figures.

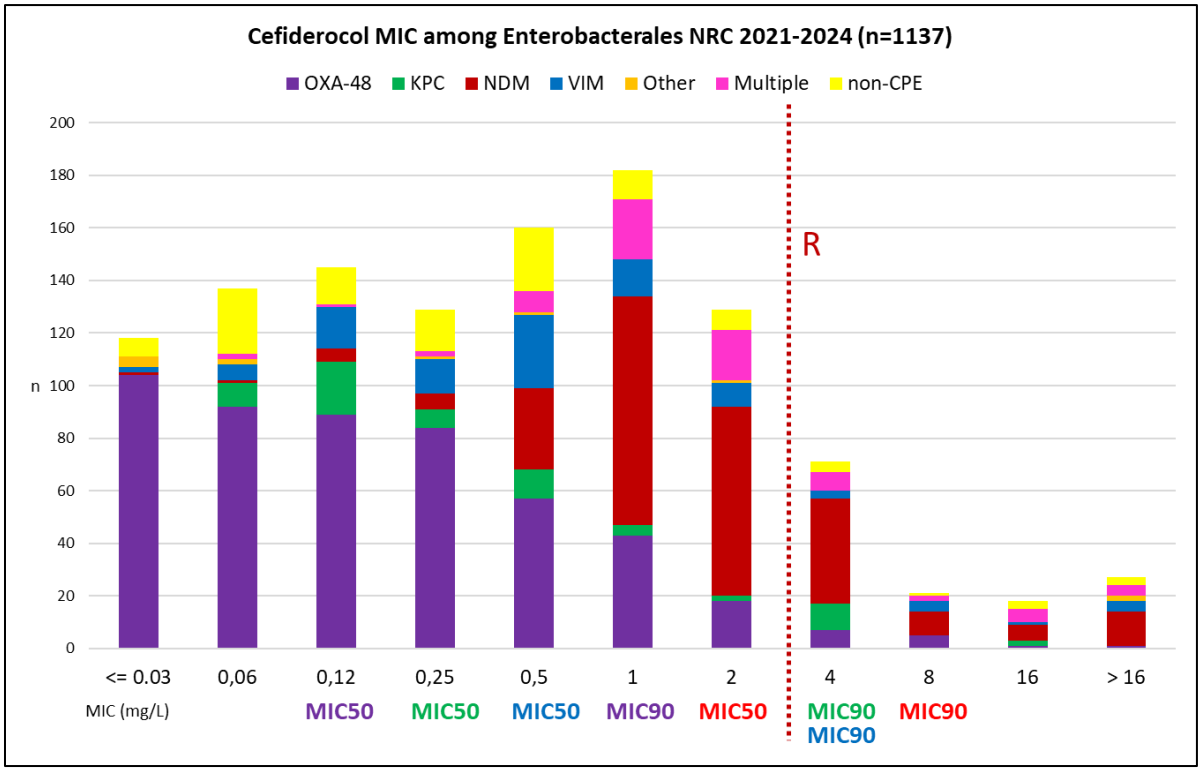
Figure 17 Ceftazidime/avibactam MIC distribution among CPE



Ceftazidime/avibactam maintains 100% activity against all OXA-48 and KPC CPE isolates. The MIC distribution shows higher mean MIC for KPC compared to OXA-48 with 4 KPC strains having ceftazidime/avibactam MIC close to the R breakpoint of 8 mg/l.

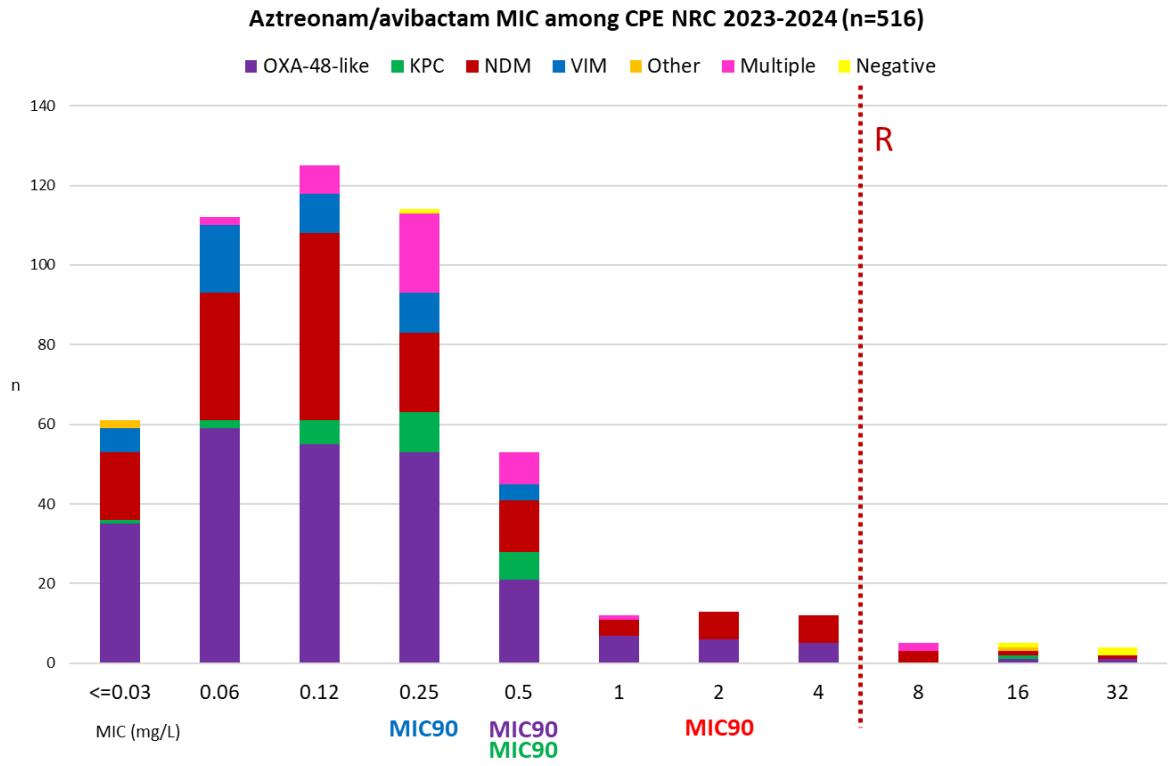
Imipenem/relecbactam and meropenem/vaborbactam were equally active (98-100%) with lower MIC50 of <=0.25 mg/l (data not shown) compared to the MIC50 of 2 mg/l for ceftazidime/avibactam against KPC CPE.

Figure 18 Cefiderocol MIC distribution among CPE



Cefiderocol is a novel siderophore cephalosporin with very wide spectrum of activity against MDR Gram-negatives including strains producing carbapenemase of all three Ambler classes (A, B and D). While cefiderocol displays excellent overall activity of 97% and 88% against OXA-48 and VIM CPE, respectively, it has lower activity rates against KPC (82%) and NDM (75%) strains, including 24 isolates (mainly NDM producers) showing high MIC >16 mg/l. These findings are particularly concerning, as resistance to cefiderocol is already frequently observed among CPE isolates, despite the drug not being available on the Belgian market. This is especially troubling for Ambler class B enzymes (e.g., NDM), where cefiderocol may represent one of the last remaining treatment options.

Figure 19 Aztreonam/avibactam MIC distribution among CPE

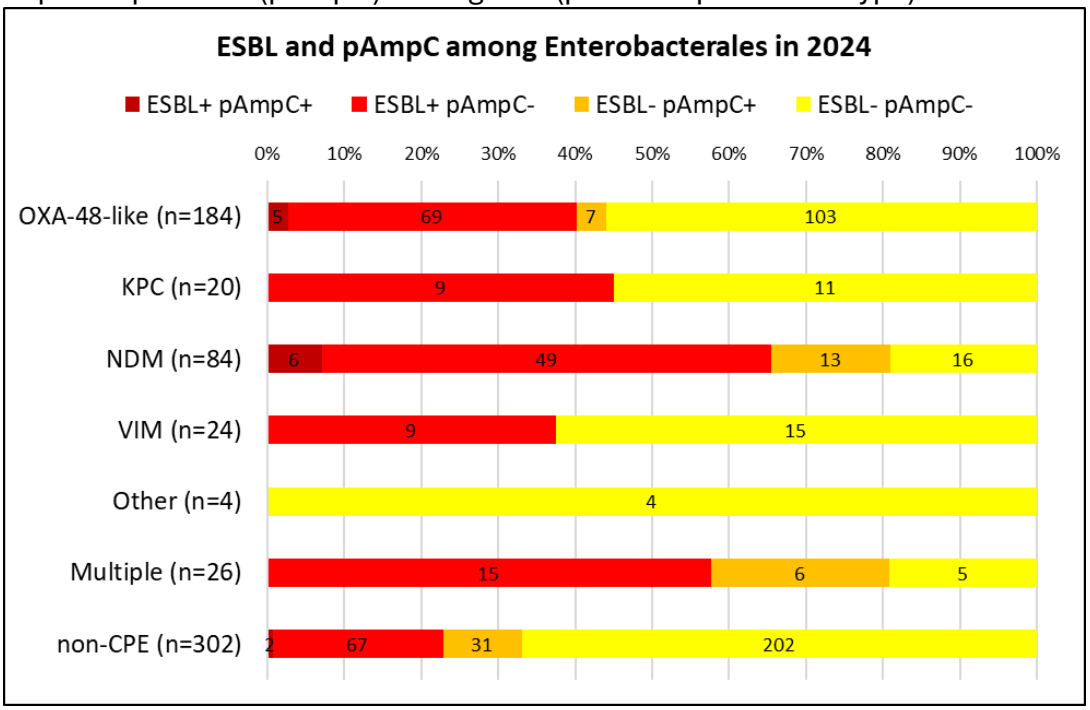


Aztreonam/avibactam is another novel BLBLI with very wide spectrum of activity against MDR Enterobacterales including CPE strains producing KPC and OXA-48-like, but also those producing Ambler class B enzymes. While aztreonam/avibactam shows the highest overall activity (95%-100%) against all four major families of CPE (including strains co-producing multiple carbapenemases), few isolates display higher MIC >=1 mg/l or even above the resistance breakpoint of 4 mg/l. Nearly all of these aztreonam/avibactam resistant isolates are detected in *E. coli* and most produce NDM-5 carbapenemase, variants of CMY-2-like cephalosporinases and/or present modification in the PBP3 gene sequence (result not shown).

Acquired colistin resistance (MIC >2 mg/l) was observed in 45 (out of 358 colistin-tested) Enterobacterales isolates. Plasmid-mediated mobile colistin resistance (MCR) was detected in 16/24 colistin-R *E. coli* and 1/13 colistin-R *K. pneumoniae* isolates. All isolates carried *mcr-1-like* gene and none showed MDR phenotype.

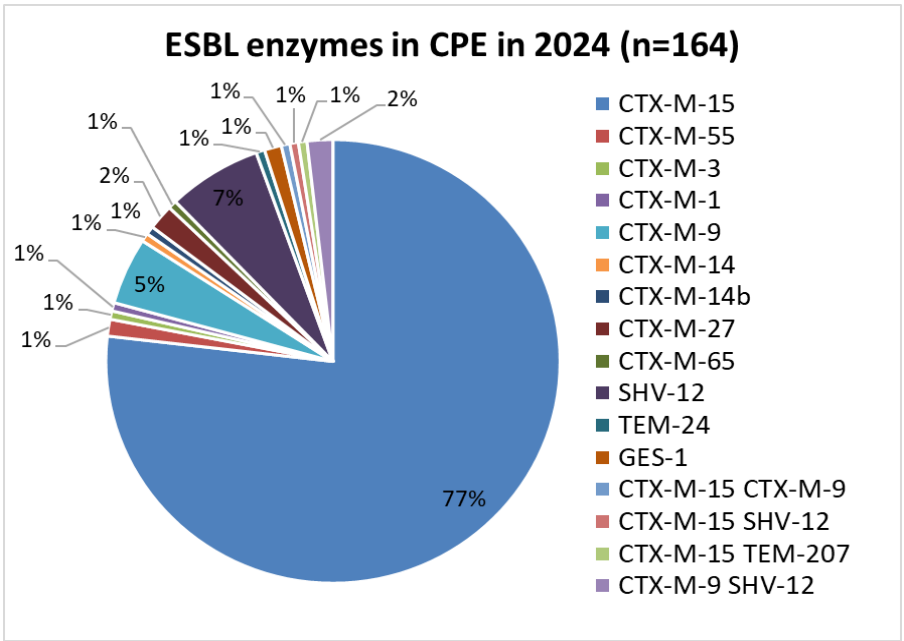
2.3. Extended-spectrum beta-lactamases (ESBL) and plasmidic AmpC cephalosporinases (pAmpC)

Figure 20 Distribution of the presence of ESBL and/or of plasmidic AmpC cephalosporinase (pAmpC) among CPE (per carbapenemase type) and non-CPE



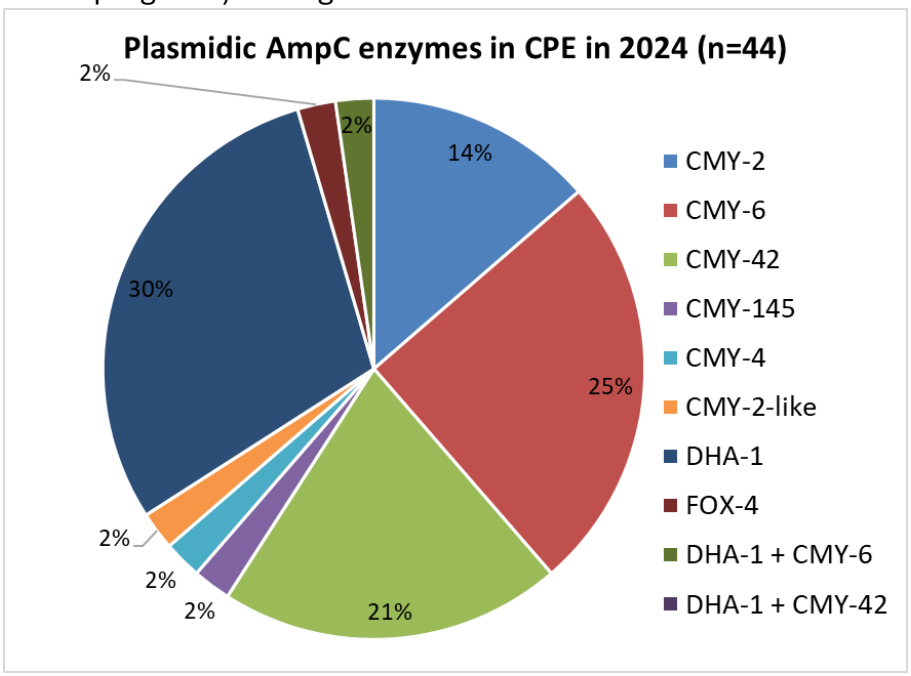
Among CPE, most (81%) of the NDM CPE produce also an ESBL and/or pAmpC, while KPC, OXA-48-like and VIM types CPE, coproduce less frequently (45%, 44% and 38%, respectively) an ESBL and/or pAmpC.

Figure 21 Distribution of ESBL enzymes (based on sequenced blaESBL genes) among CPE



Among CPE, CTX-M-15 represented by far the most frequent (74%) ESBL enzymes among CPE, followed by SHV-12 (7%) and CTX-M-9 (5%) ESBLs.

Figure 22 Distribution of plasmidic AmpC cephalosporinases (based on sequenced blaAmpC genes) among CPE

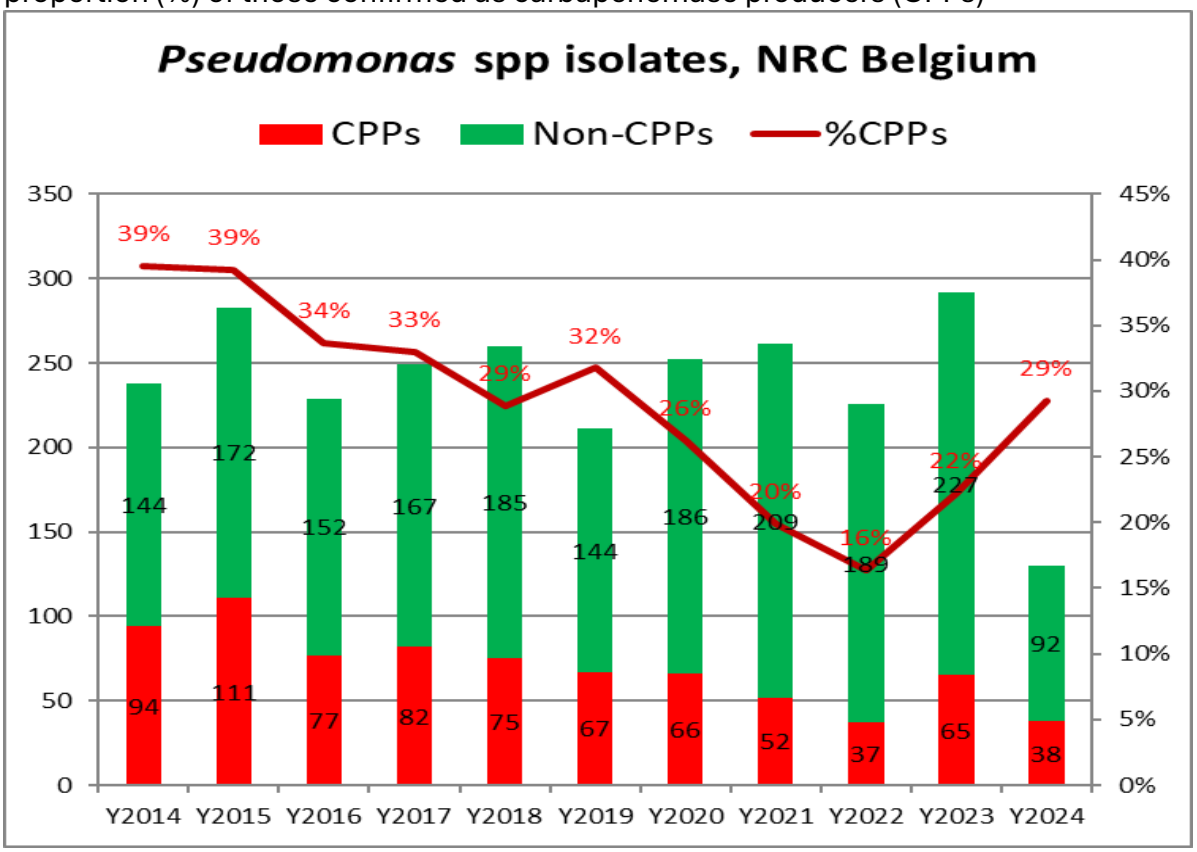


DHA-1 (30%) and/or CMY-2-like (66% including mainly CMY-2, CMY-6, CMY-42) enzymes were the predominant pAmpC cephalosporinases detected among CPE.

3. Multidrug-resistant *Pseudomonas*

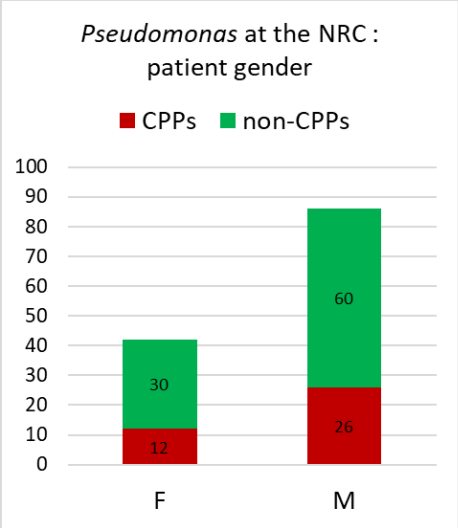
3.1. Characteristics of samples and patients related to isolates

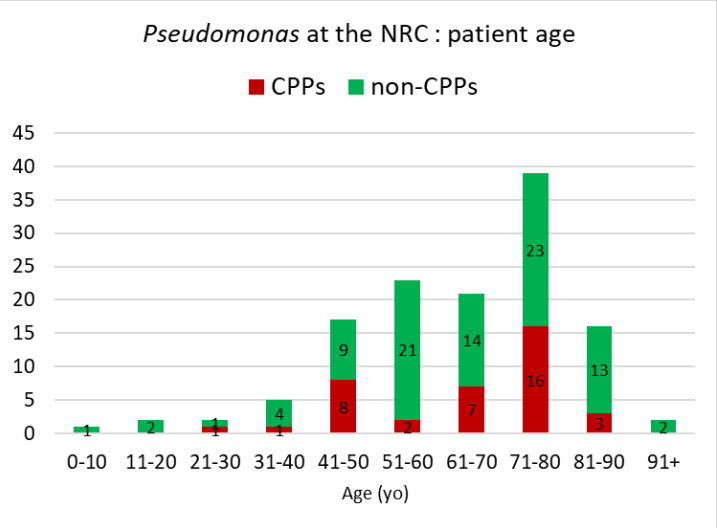
Figure 23 Number of MDR *Pseudomonas* isolates received per year by the NRC and the proportion (%) of those confirmed as carbapenemase producers (CPPs)



The NRC receives yearly a mean number of 240 MDR *Pseudomonas* isolates. The number remained stable over the past 10 years, although reaching the highest peak of 292 isolates in 2023. The number (mean n=70) and the proportion (mean of 29%) of confirmed carbapenemase producers (CPPs) have tended to decrease significantly for the past decade. The increasing ability of local laboratories to detect and identify CPPs might explain the decrease of carbapenemase confirmation by the NRC.

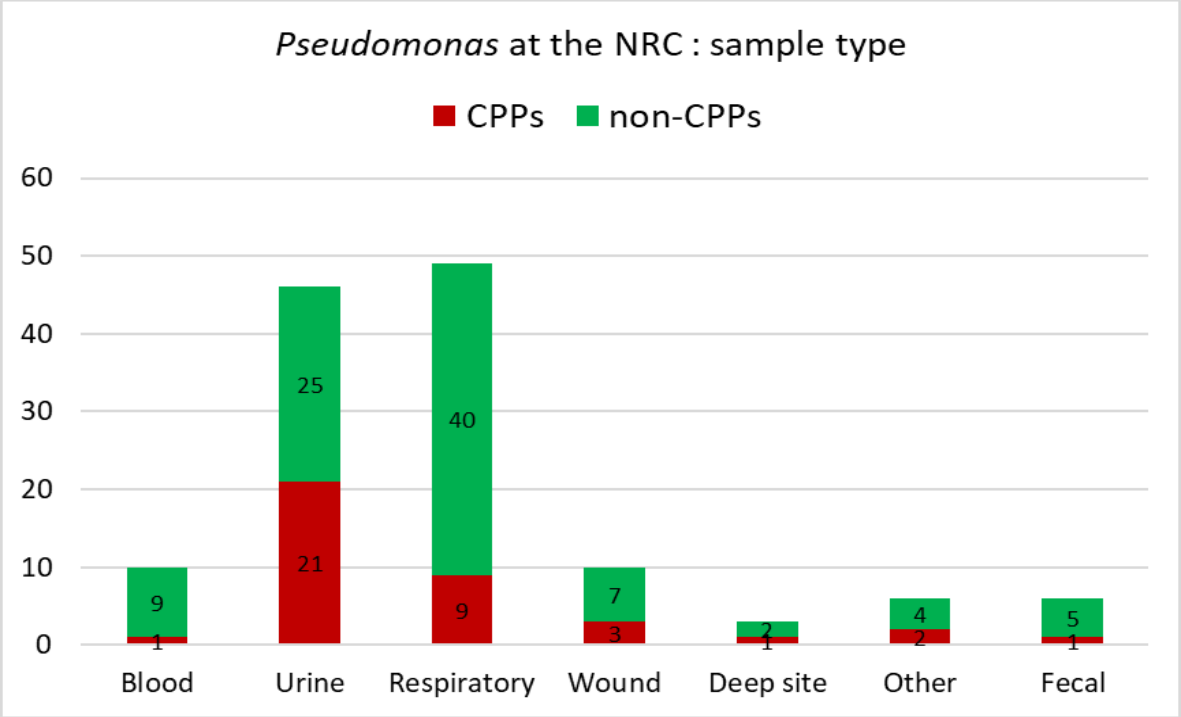
Figure 24 Number of *Pseudomonas* isolates per gender and per age group in 2024





In 2024, the NRC received twice more isolates collected from male (67%) than female patients and mainly (79%) from patients above 50 years old.

Figure 25 Sample types from which Pseudomonas were isolated in 2024



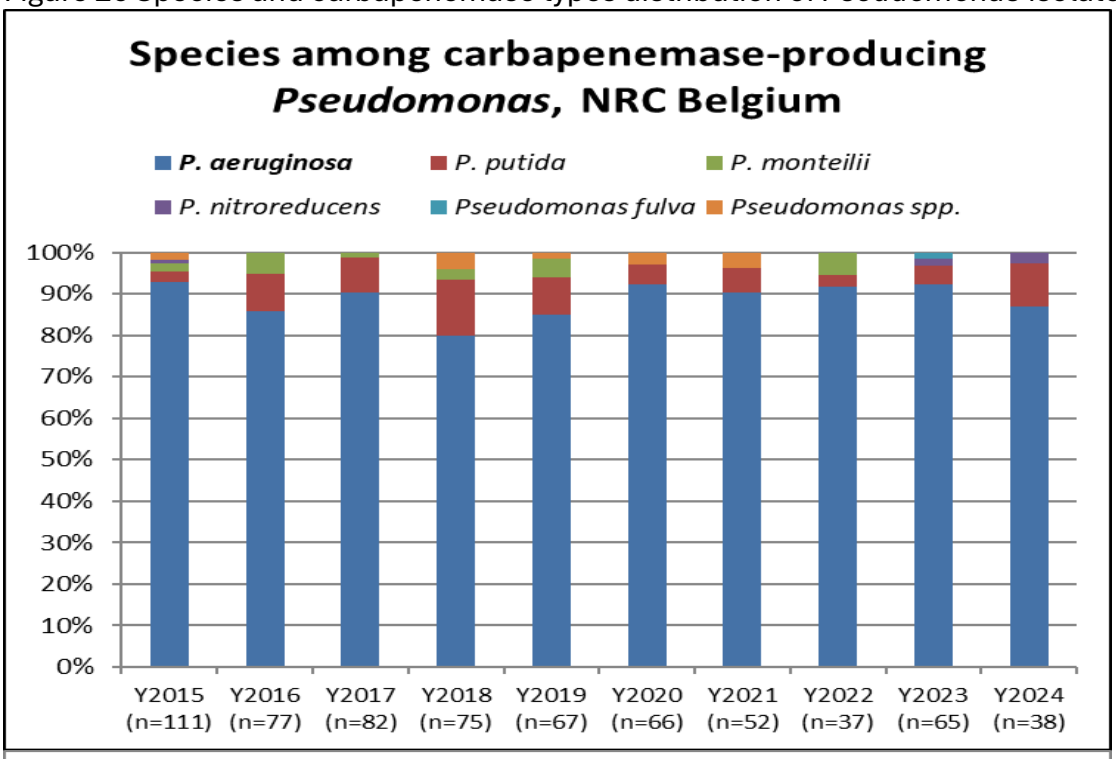
The 'deep site' category includes fluid and tissue specimens other than superficial or orifical sample sites. The 'other' category includes genital samples, percutaneous catheters and samples of unknown origin.

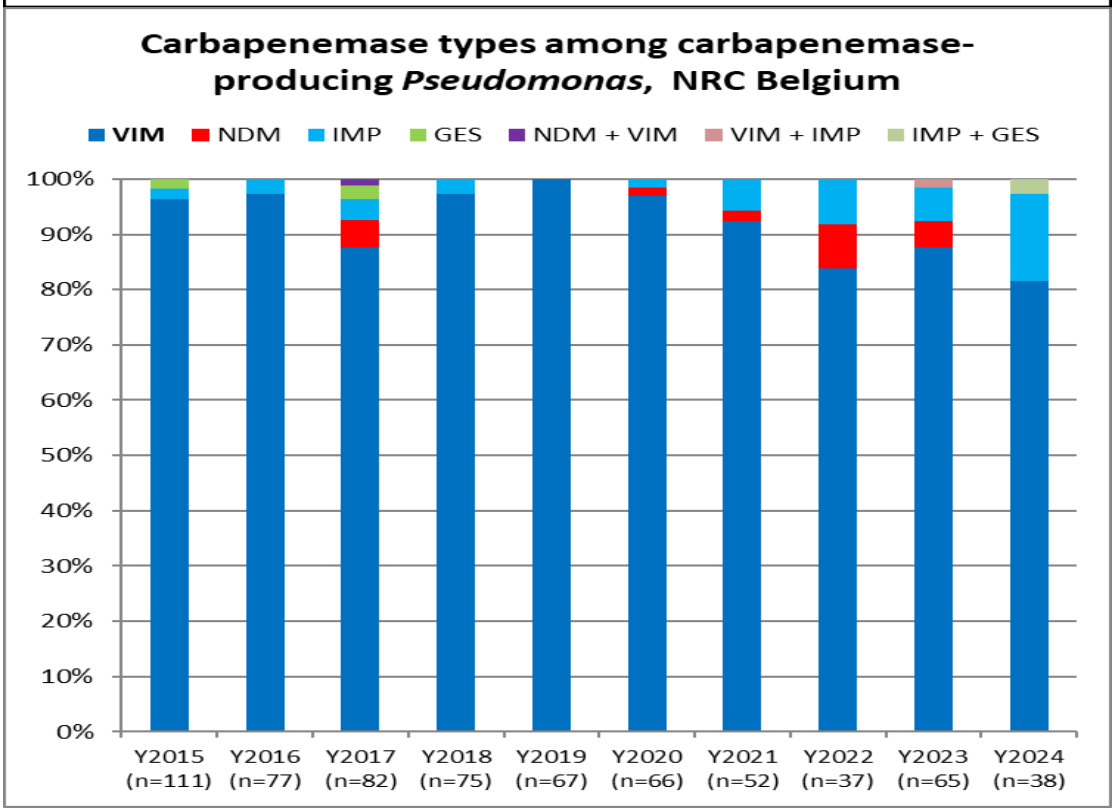
Of the 130 isolates with indicated sample nature, the large majority were cultured from respiratory (39%) and urinary (35%) specimens. Of the 10 isolates causing bloodstream infections, only one was confirmed as carbapenemase producer.

3.2. Carbapenemase-producing *Pseudomonas*

3.2.1. Bacterial species and resistance mechanisms

Figure 26 Species and carbapenemase types distribution of *Pseudomonas* isolates

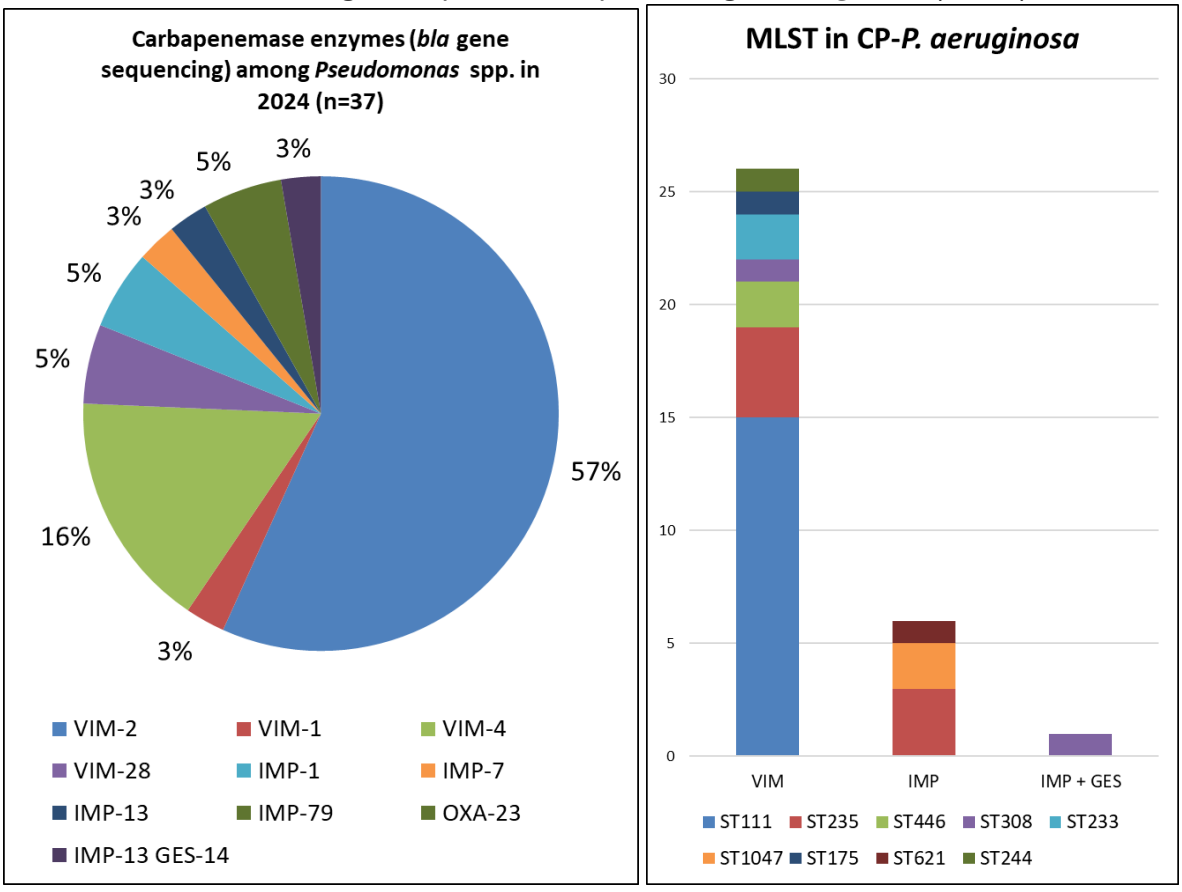




Among the 38 carbapenemase-producing *Pseudomonas* (CPPs) confirmed in 2024, *P. aeruginosa* represented by far the predominant species (88%), with few sporadic isolates of *P. putida* as the other CPPs species (like the data of the previous years). VIM-type carbapenemase remained by far the predominant carbapenemase family (82% in 2024) over the years, although IMP and NDM producers have been sporadically reported over the past years (n=4 and n=3 in 2023, respectively). Of note, NDM CPPs have been detected almost each year since 2020, while they were reported previously only once as a single cluster of 4 isolates in 2017.

3.2.2. Genomic surveillance

Figure 27 Carbapenemase enzymes (sequenced bla gene) among CPPs and the MLST clonal distribution among carbapenemase-producing *P. aeruginosa* (CPPA) in 2024



Among the 37 genome-sequenced CPPs, VIM-2 (57%) and VIM-4 (VIM-1-like;16%) were the two predominant carbapenemase enzymes, while VIM-28 (VIM-1-like) and VIM-1 were produced by two and one isolates each. IMP CPPs had more diversified carbapenemase variants with IMP-1 (n=2), IMP-79 (n=2), IMP-7 (n=1) and IMP-13 (n=1). One ST308 *P. aeruginosa* coproduced IMP-13 and GES-14 carbapenemases.

The 33 carbapenemase-producing *P. aeruginosa* (CPPA) belonged to 9 different MLST and ST111 were by far the predominant clone (45%). Seven of the 9 MLST among CPPA

(ST111, ST175, ST233, ST235, ST244, ST308, ST446) were part of the top 10 international high-risk clones (HRC) defined in a recent review publication (Oliver A et al. CMI, 2024) demonstrating their similar spread in Belgium.

Of note, following the detection in 2023 of NDM-1-producing ST773 CPPA from different patients who had travelled to Ukraine, two IMP-79-producing ST1047 CPPA was recovered from 2 patients originated from Ukraine, suggesting travel abroad still serve as a potential importation source, especially from conflict areas.

Figure 28 Geographical distribution of carbapenemases among CPPA

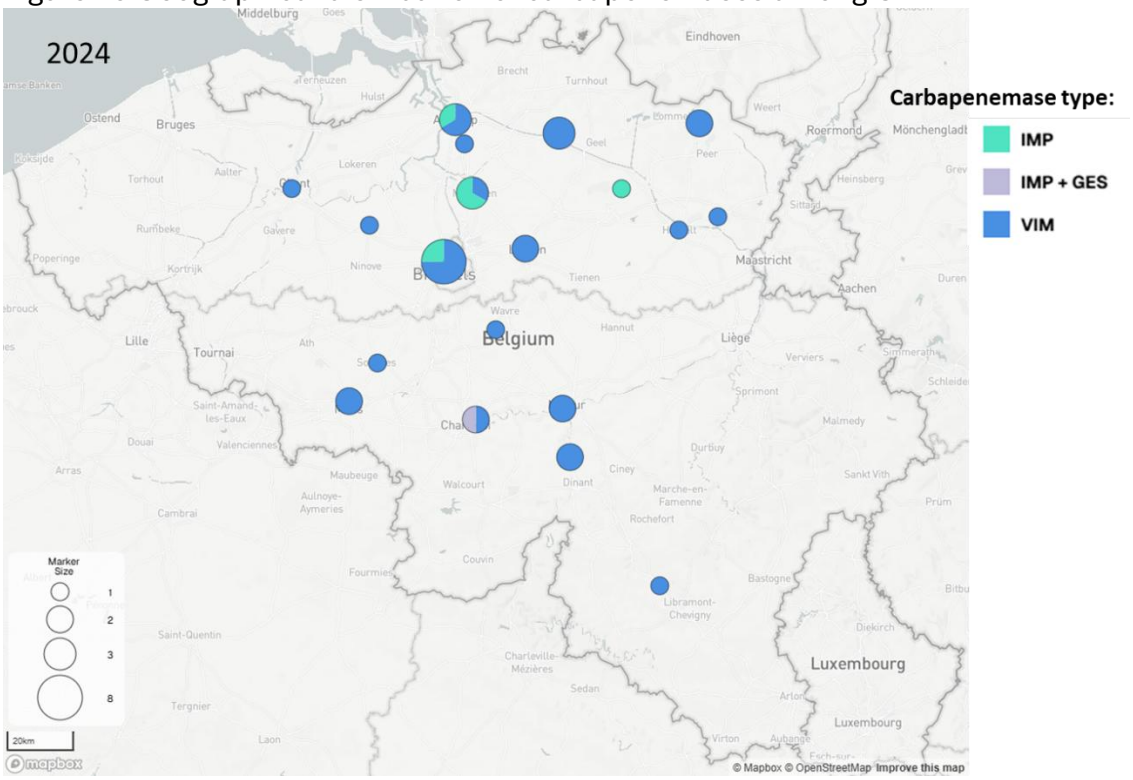
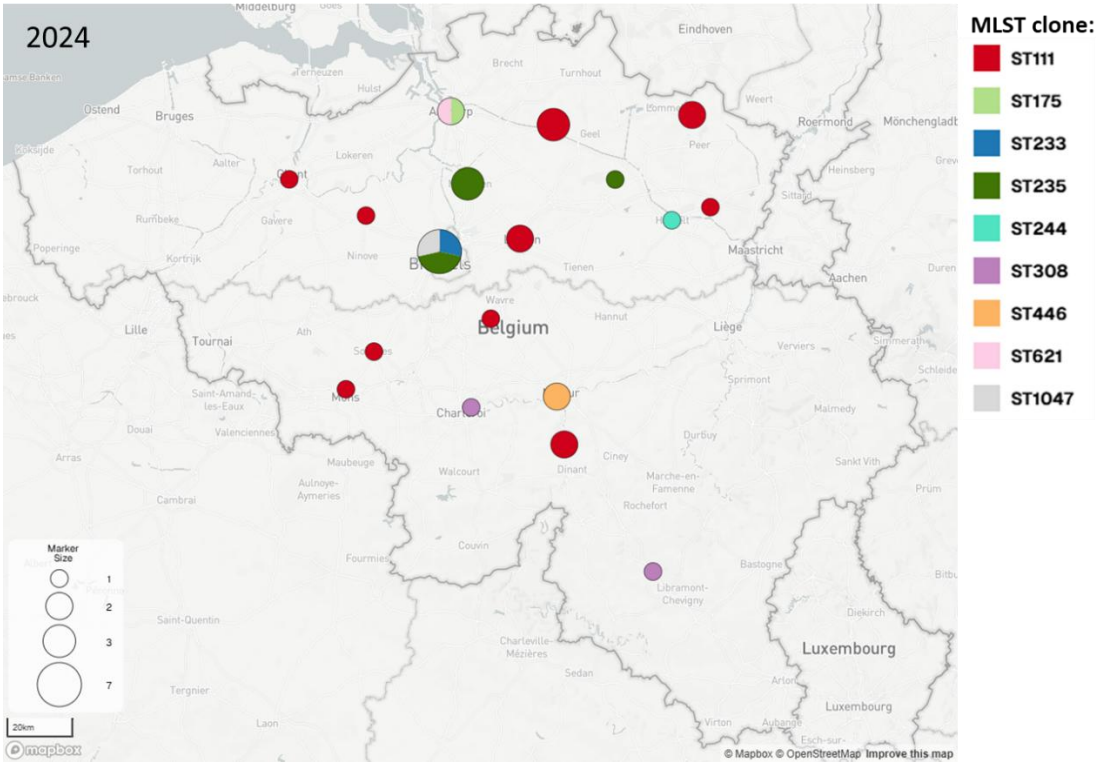


Figure 29 Geographical distribution of MLST clones among CPPA



3.2.3. Antimicrobial susceptibility

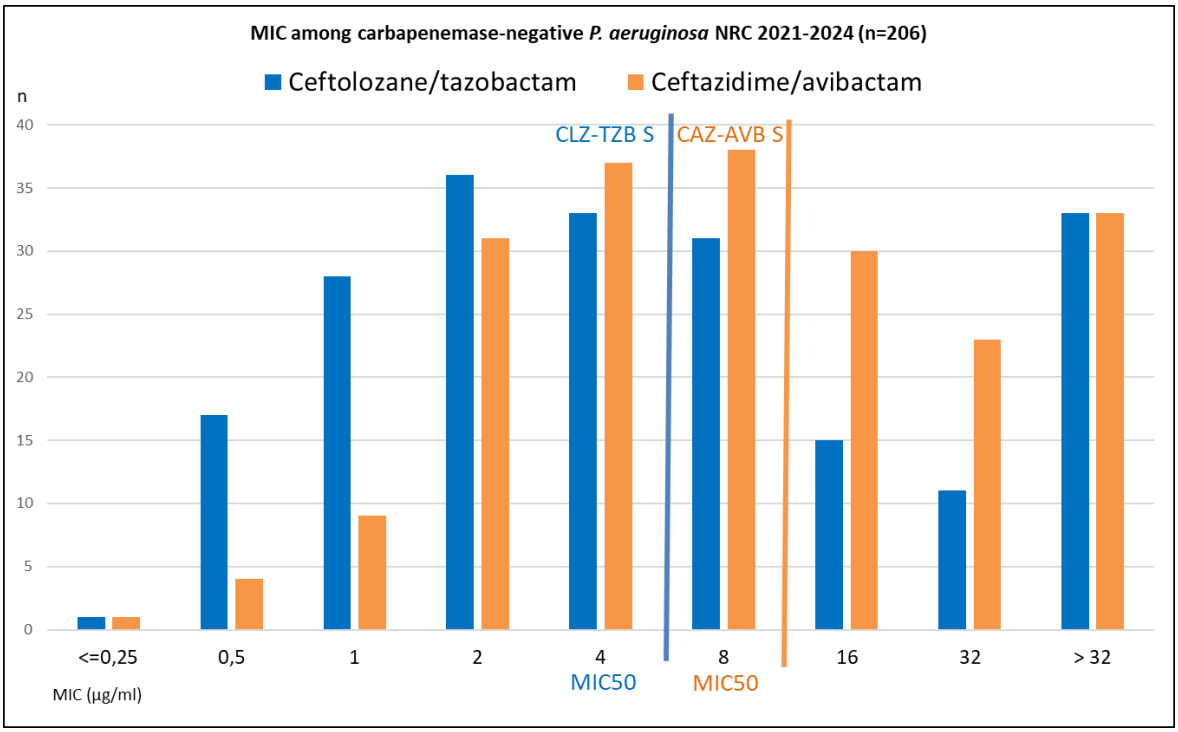
Table 2 Antibiotic susceptibility profile (proportion of susceptible results (S/I) in % according to EUCAST) by broth microdilution method (BMD) according to carbapenemase types for *P. aeruginosa* at the NRC for 2021-2024 (n=376)

***Pseudomonas* spp.** (n=376) **Antibiotic \ n isolates**	**NDM 7**	**VIM 134**	**IMP 13**	**non-CPPA 221**
Piperacillin/tazobactam	0%	3%	15%	27%
Aztreonam	71%	73%	23%	34%
Aztreonam/avibactam	50%	18%	0%	25%
Ceftazidime	0%	0%	0%	26%
Ceftazidime/avibactam	0%	1%	0%	58%
Cefepime	0%	5%	0%	26%
Ceftolozane/tazobactam	0%	1%	0%	56%
Imipenem	0%	0%	17%	16%
Imipenem/relebactam	0%	0%	0%	48%
Meropenem	0%	4%	0%	53%
Meropenem/vaborbactam	0%	7%	0%	64%
Cefiderocol	17%	98%	100%	93%
Ciprofloxacin	0%	5%	0%	31%
Amikacin	0%	22%	38%	83%
Fosfomycin (ECOFF)	57%	91%	100%	83%
Colistin	100%	99%	100%	97%

MIC determination was performed by broth microdilution method (BMD) for all confirmed CPPs isolates and for part of the non-carbapenemase producers.

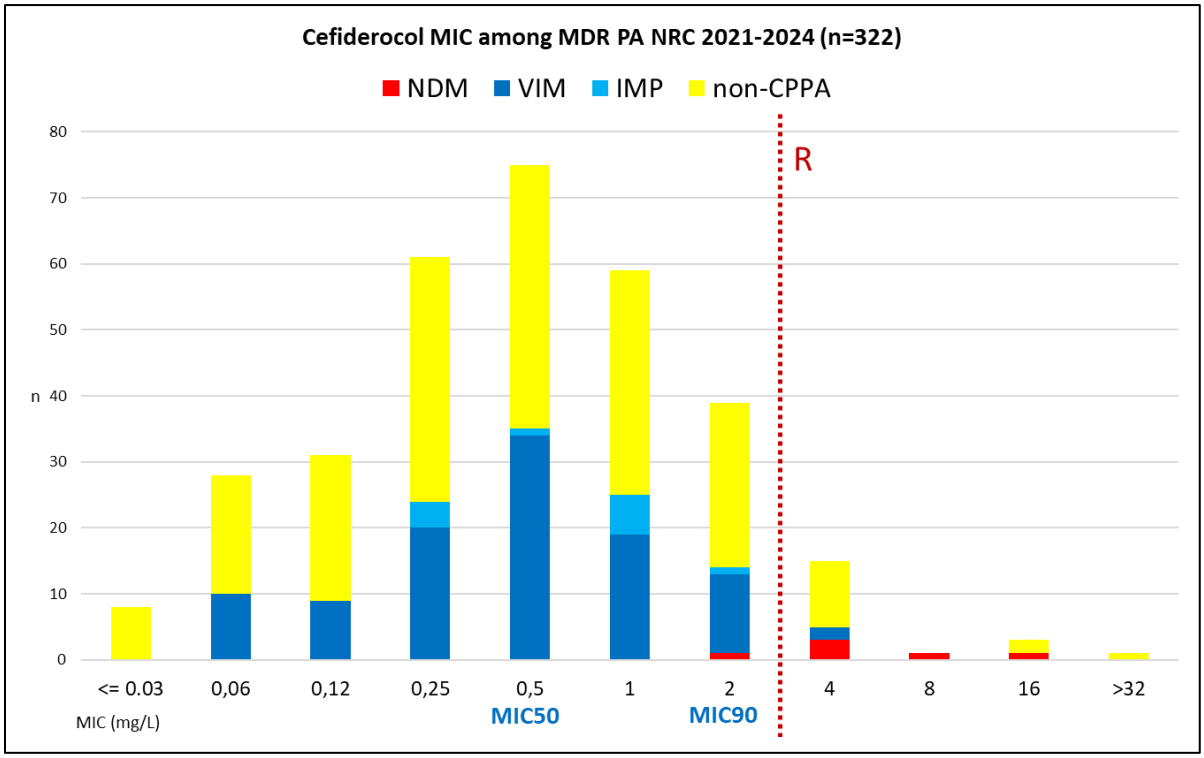
As all CPPA analyzed produced class B enzymes, the large majority of the CPPA isolates are resistant to standard anti-pseudomonal beta-lactams (piperacillin-tazobactam, ceftazidime, cefepime) except aztreonam which remained active in 73% of VIM CPPA that do not express other resistance mechanisms to aztreonam. Recent beta-lactam/beta-lactam inhibitors (avibactam, vaborbactam, relebactam) are not active against these MBL CPPA. Ciprofloxacin and amikacin also displayed poor sensitivity rates as most CPPA carried genes coding for resistance mechanisms to other classes including aminoglycosides-modifying enzymes or 16S RNA methylases, that are frequently associated with carbapenemase genes located within the same mobile genetic elements.

Figure 30 MIC distribution of non-CPPA isolates for ceftolozane/tazobactam and for ceftazidime/avibactam



Multidrug-resistant *P. aeruginosa* (MDRPA) that are not carbapenemase producers (non-CPPA) had a more heterogenous susceptibility profile. Ceftolozane-tazobactam and ceftazidime-avibactam retained substantial activity (57-59%) against non-CPPA.

Figure 31 MIC distribution of CPPA and non-CPPA isolates for cefiderocol



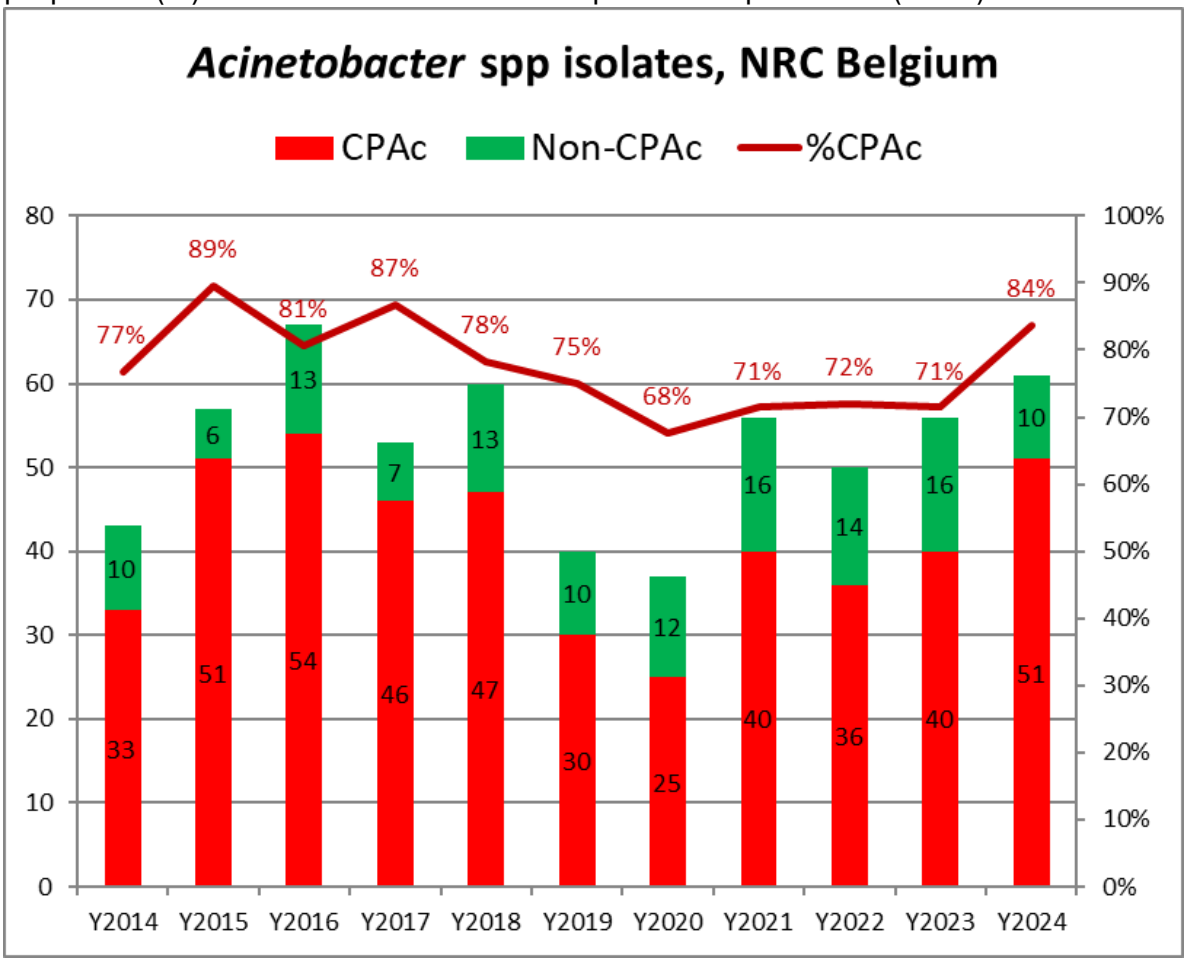
Cefiderocol, the recently developed siderophore cephalosporin, retained high activity (>93%) against CPPA and non-CPPA, except for NDM producers (17%) which showed much higher MIC (2-16 mg/l). Aztreonam/avibactam had only limited activity (16% on CPPA and 25% on non-CPPA) as avibactam cannot restore the activity of aztreonam if the strain is aztreonam resistant by other non-enzymatic resistance mechanisms such as efflux pumps, which are frequently overexpressed in *P. aeruginosa*.

Colistin maintained the highest activity rate overall against CPPA and non-CPPA with resistance detected only among few (3%) sporadic and mainly non-CPPA isolates. For fosfomycin, 91% of the VIM CPPA and 83% of non-CPPA showed MIC below the epidemiological cut-off (ECOFF at 128 mg/l), although it should be reminded that fosfomycin *in vitro* testing for *Pseudomonas* is discouraged by international recommendations.

4. Multidrug-resistant *Acinetobacter*

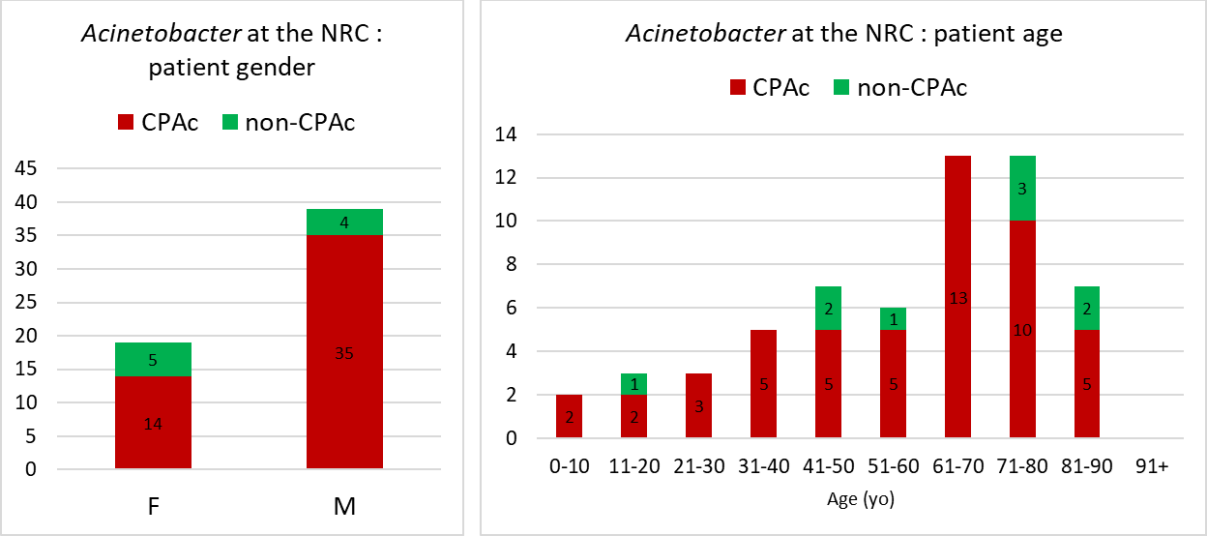
4.1. Characteristics of samples and patients related to isolates

Figure 32 Number of MDR *Acinetobacter* isolates received per year by the NRC and the proportion (%) of those confirmed as carbapenemase producers (CPAc)



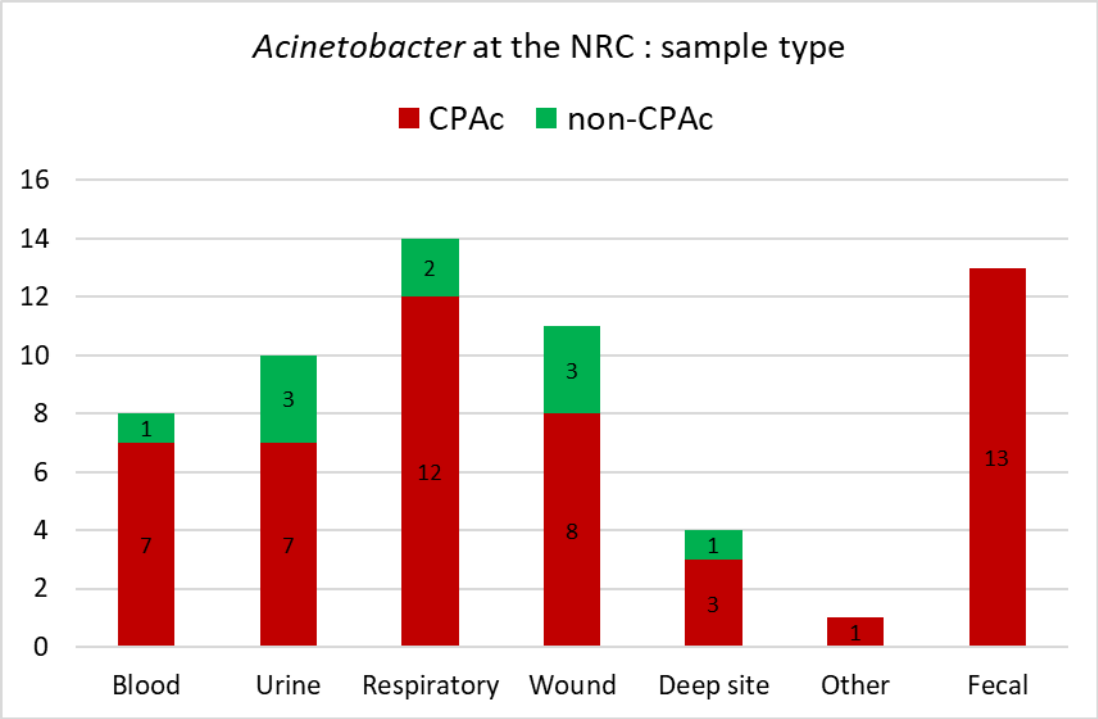
The NRC receives yearly a mean number of 50 MDR (mainly for carbapenem resistance) *Acinetobacter* isolates, this number slightly fluctuating between 37 to 67 over the past 10 years. In 2024, the NRC received 61 *Acinetobacter* isolates for carbapenem resistance and the large majority (84%) were confirmed as carbapenemase producers (CPAc).

Figure 33 Number of *Acinetobacter* isolates per gender and per age group in 2023



In 2024, the NRC received twice more isolates collected from male (67%) than female patients and mainly (60%) from patients above 60 years old.

Figure 34 Sample types from which Acinetobacter were isolated in 2024

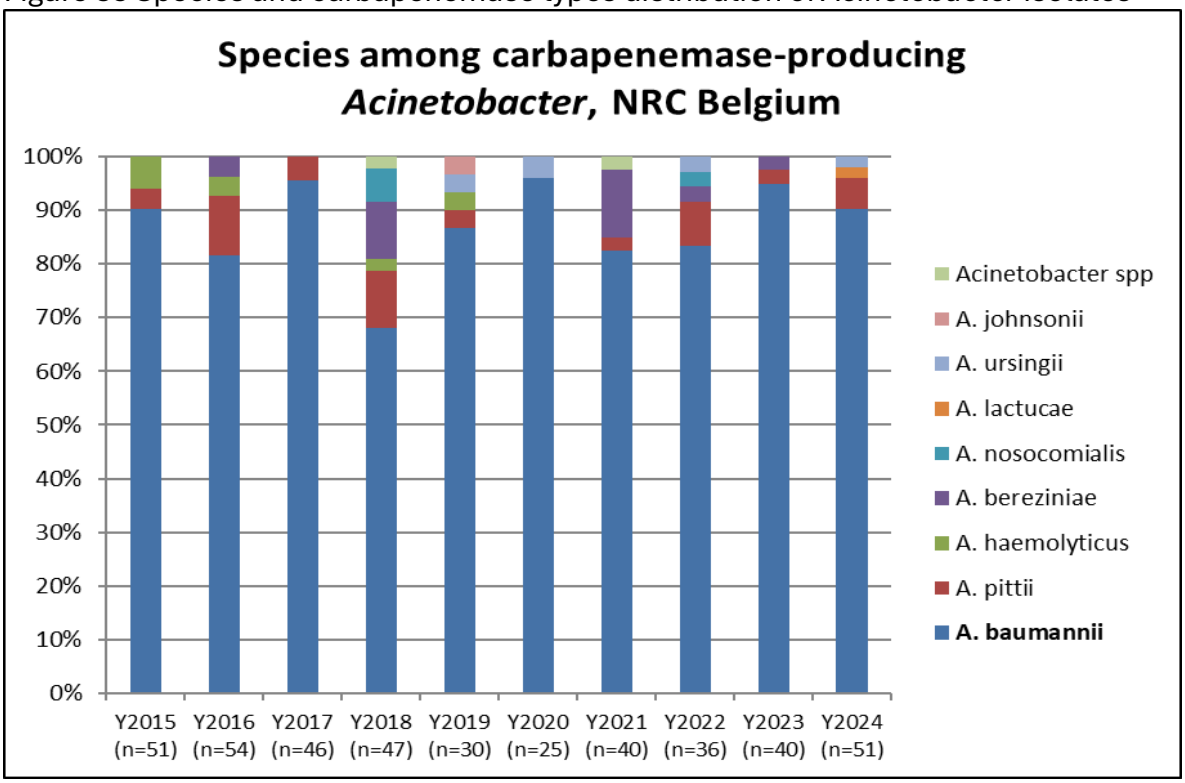


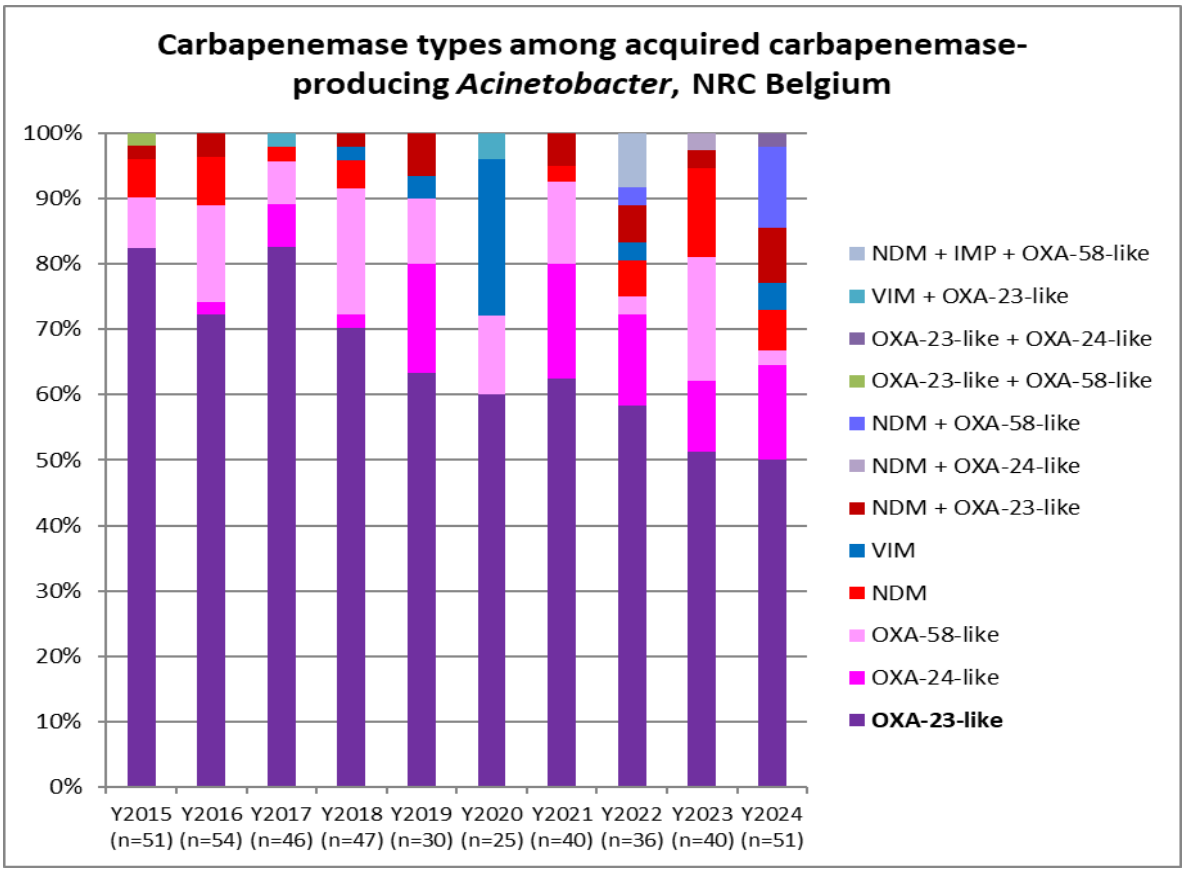
The 'deep site' category includes fluid and tissue specimens other than superficial or orifical sample sites. The 'other' category includes genital samples, percutaneous catheters and samples of unknown origin.

4.2. Carbapenemase-producing *Acinetobacter*

4.2.1. Bacterial species and resistance mechanisms

Figure 35 Species and carbapenemase types distribution of *Acinetobacter* isolates





Among the 51 carbapenemase-producing *Acinetobacter* (CPAc) confirmed in 2024, *A. baumannii* represented by far the predominant species (90%). Three (2 VIM and 1 OXA-58-like) carbapenemase-producing *A. pittii*, one OXA-24-like-producing *A. lactucae* and one NDM-producing *A. ursingii* were the isolates of other CPAc species detected in 2024. OXA-23-like carbapenemase remained the predominant acquired carbapenemase family (52% in 2024) over the years, while OXA-58-like, OXA-24-like and NDM producers have been reported almost each year over the past ten years (n=7, n=1 and n=3 in 2024, respectively).

CPAc coproducing NDM and OXA-23 have been reported nearly each year for the past decade, while the first CPAc isolates associating NDM with OXA-58-like or OXA-24-like were detected in 2022 and 2023, respectively. The number of CPAc carrying multiple carbapenemases reached a peak of 11 isolates in 2024 (6 NDM + OXA-58-like, 4 NDM + OXA-23-like and 1 OXA-23-like + OXA-24-like).

VIM CPAc were only sporadically detected, except for a cluster of 5 VIM-producing *A. baumannii* which was identified in a hospital during the first year of COVID-19 pandemics in 2020. No class A carbapenemase in *Acinetobacter* has ever been confirmed for the past 10 years at the NRC.

4.2.2. Genomic surveillance

Figure 36 Carbapenemase enzymes (sequenced bla gene) among CPAc and the MLST clonal distribution of carbapenemase-producing *A. baumannii* (CPPA) in 2024

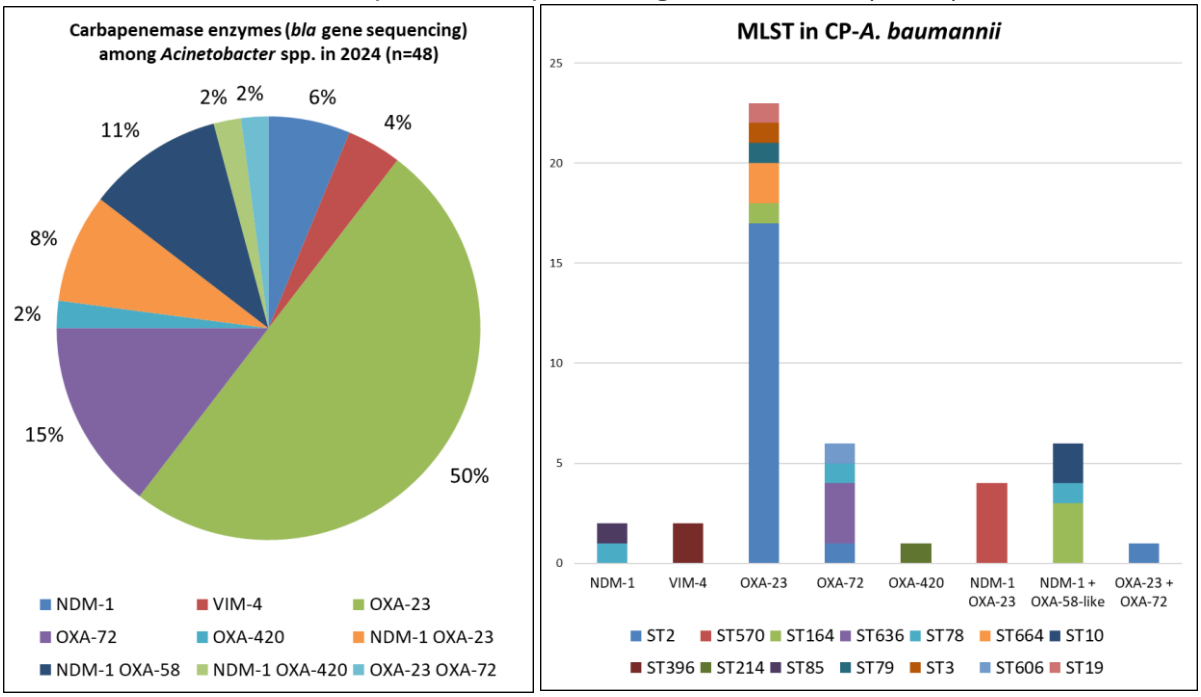
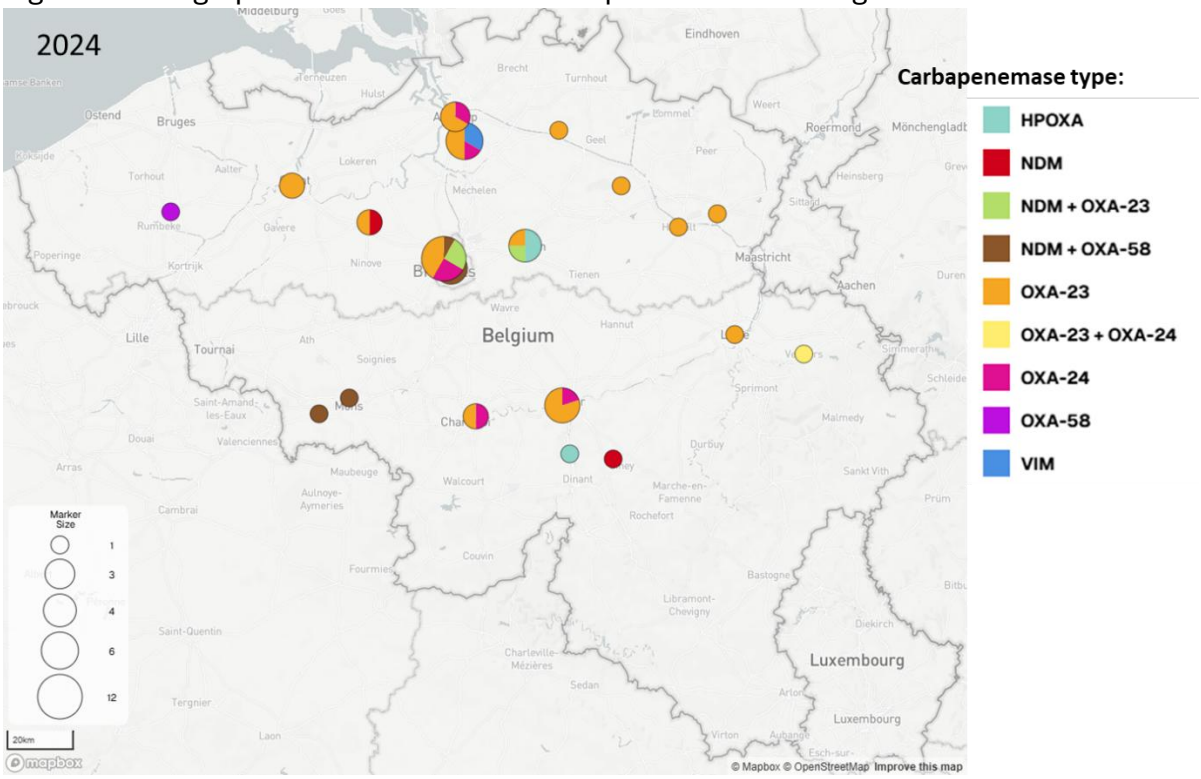
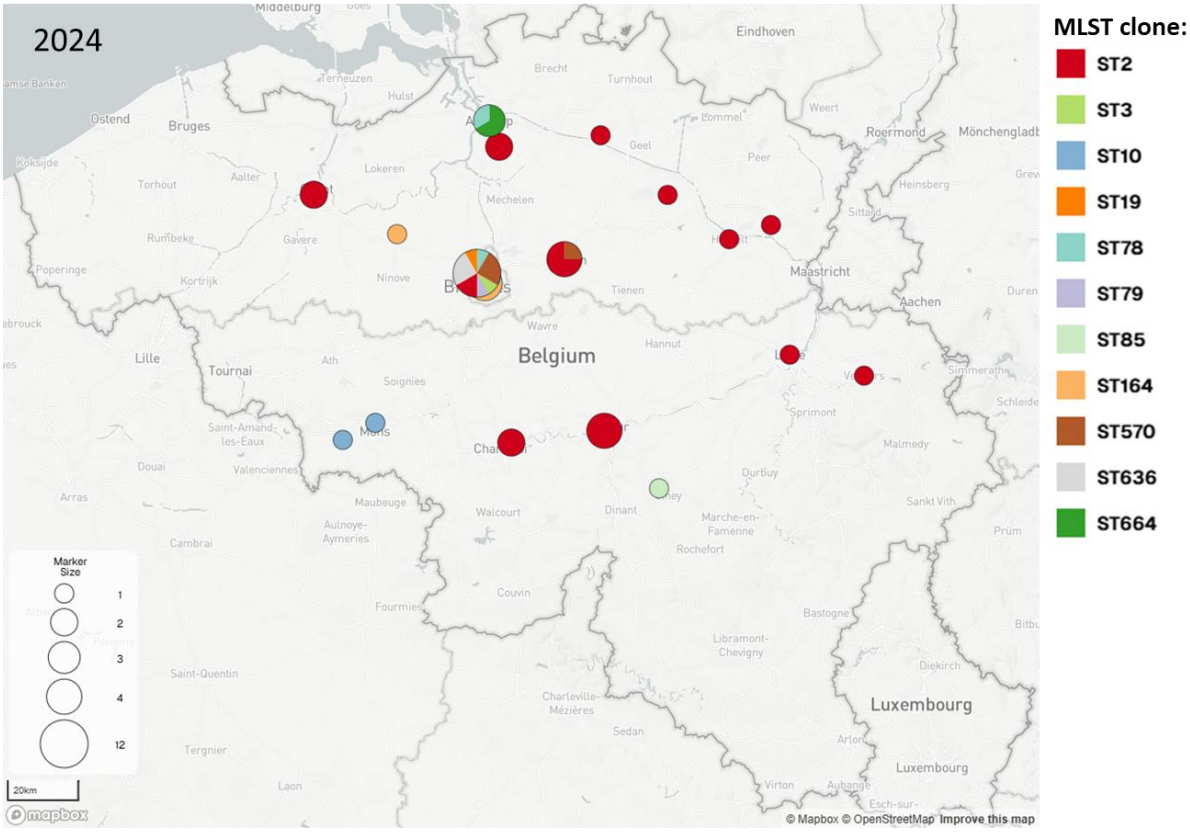


Figure 37 Geographical distribution of carbapenemases among CPAB in 2024



* HPOXA: Overexpression of chromosomal *bla*(OXA-51-like) genes

Figure 38 Geographical distribution of MLST clones among CPAB in 2024



Among the 48 CPAc genome-sequenced in 2024, there was no OXA-23-derived variant other than OXA-23, while all OXA-24-like, VIM and NDM carbapenemases were OXA-72, VIM-4 and NDM-1 enzymes, respectively. OXA-23 (50%), OXA-72 (OXA-24-like, 15%), NDM-1 (6%) were the main single acquired carbapenemase enzymes identified, while the associations NDM-1 + OXA-23 and NDM-1 + OXA-58 were produced by 4 and 5 isolates, respectively. Finally, OXA-420, a variant of OXA-58, was found in two CPAc, including one co-producing NDM-1 and OXA-420. Of note, in 3 *A. baumannii* isolates were detected the presence of the ISAba1 promoter sequence upstream of *bla*OXA-51-like genes, suggesting the contribution to carbapenem resistance through the overexpression of the intrinsic OXA-51-like carbapenemase.

Among the 48 carbapenemase-producing *A. baumannii* (CPPA) determined for their MLST genotype (Pasteur nomenclature), 45 isolates belonged to 14 different MLST (3 isolates were not typeable (NT)) and ST2 were by far the predominant clone (42%), well-recognized as the most prevalent international high-risk clone (HRC) in Europe. Among CPAB coproducing multiple carbapenemases, 3 NDM-1 + OXA-58 coproducing CPAB isolates belonging to a same clone (ST164) were recovered from a cluster of 3 patients hospitalized in the same unit of a hospital. In addition, all 4 CPAB isolates coproducing NDM-1 + OXA-23 referred by 2 hospital laboratories, belonged to the same clone (ST570) and were carried by 4 different patients coming from Romania, suggesting travel-imported cases.

4.2.3. Antimicrobial susceptibility

Table 3 Antibiotic susceptibility profile (proportion of susceptible results (S/I) in % according to EUCAST) by broth microdilution method (BMD) according to carbapenemase types for *Acinetobacter* spp at the NRC for 2019-2024 (n=172)

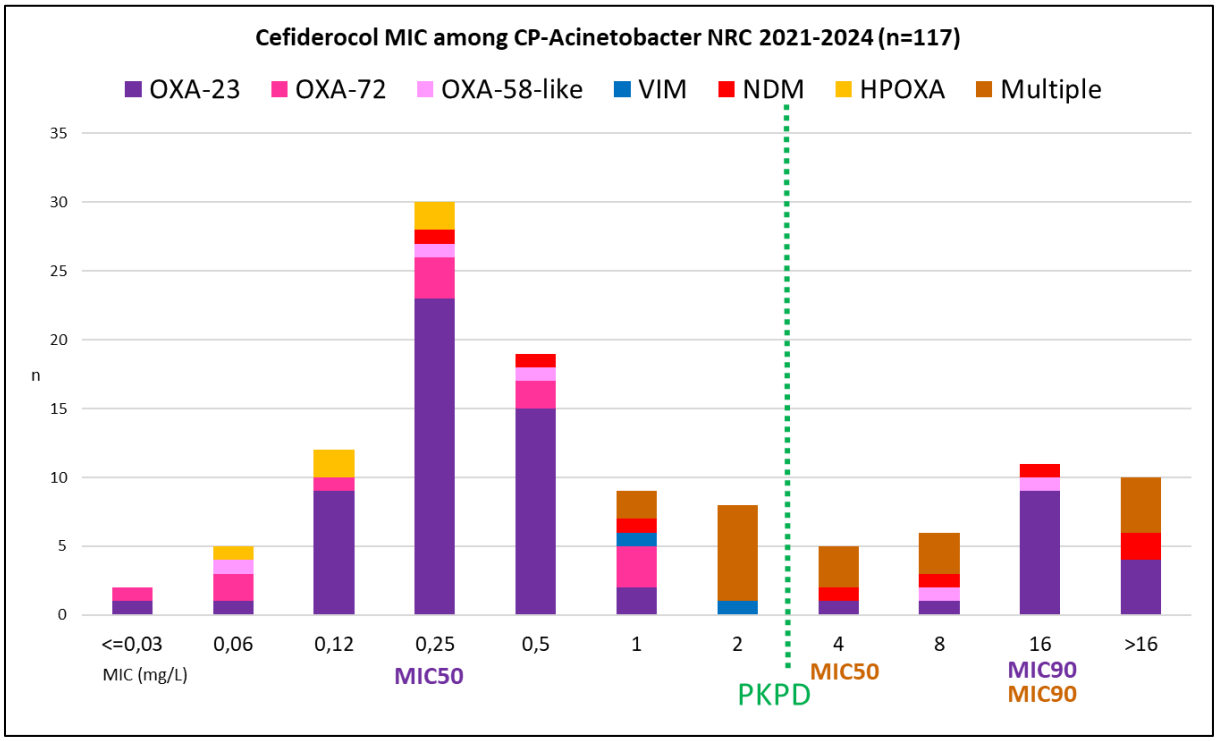
***Acinetobacter* spp** (n=172) **Antibiotic \ n isolates**	**OXA-23** **103**	**OXA-72** **17**	**OXA-58-like** **10**	**VIM** **7**	**NDM** **8**	**Multiple*** **22**
Meropenem	0%	6%	50%	0%	0%	0%
Ampicillin-sulbactam	0%	33%	100%	0%	0%	0%
Sulbactam-durlobactam	83%	100%	100%	0%	0%	0%
Cefiderocol PK/PD	77%	100%	60%	100%	38%	47%
Ciprofloxacin	1%	18%	50%	86%	25%	14%
Cotrimoxazole	17%	33%	0%	50%	0%	10%
Gentamicin	12%	29%	44%	0%	20%	0%
Amikacin	8%	29%	70%	0%	88%	9%
Minocycline	61%	100%	100%	100%	100%	100%
Tigecycline PK/PD	23%	47%	50%	100%	75%	64%
Eravacycline PK/PD	53%	50%	100%	NA	100%	NA
Colistin	85%	100%	80%	71%	88%	100%

* Includes NDM+OXA-23 (n=10), NDM+OXA-72 (n=1), NDM+OXA-58-like (n=7), VIM+OXA-23 (n=1), NDM+IMP+OXA-23 (n=3)

MIC determination was performed by broth microdilution method (BMD) for all confirmed CPAc isolates and for part of the non-carbapenemase producers.

Nearly all CPAc were highly resistant to meropenem, except 50% of OXA-58-like producers with lower carbapenem hydrolysis activity and meropenem MIC. Similar high resistance rates (>80%) were observed for ciprofloxacin, aminoglycosides and tigecycline (using PK/PD breakpoint of 0.5 mg/l) against OXA-23 CPAc, while these classes showed more variable activity against the other carbapenemase families (Table 3).

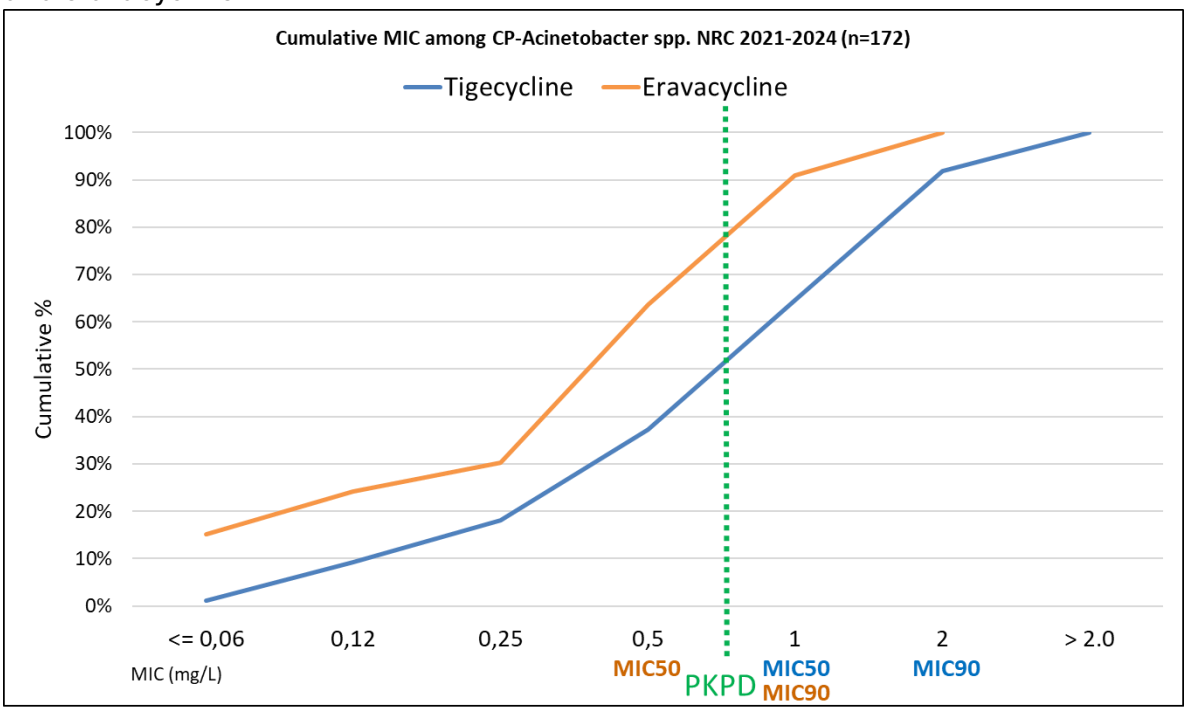
Figure 39 MIC distribution of carbapenemase-producing *Acinetobacter* for cefiderocol



For cefiderocol (using PK/PD breakpoint of 2 mg/l), the *in vitro* activity reached 79-100% against OXA-23 and OXA-72 CPAc, while it appeared more limited (40-67%) against OXA-58-like, NDM or multiple carbapenemase producers, although the low number of isolates tested is a limitation to the interpretation.

Sulbactam–durlobactam is a new antimicrobial combination of two β-lactamase inhibitors specifically designed to overcome MDR in *A. baumannii* complex. Using a preliminary breakpoint of S ≤4 mg/l, sulbactam–durlobactam achieved a very high activity of 86% against class D (OXA-23, OXA-72, OXA-58-like) carbapenemases of CPAc showing a MIC50 of 0.5 mg/l (8 times lower compared to MIC50 of ampicillin-sulbactam). However, the combination has no activity against class B carbapenemases (VIM, NDM).

Figure 40 MIC distribution of carbapenemase-producing *Acinetobacter* for tigecycline and eravacycline



Tetracyclines derivatives were tested against CPAc using French CA-SFM interpretative guidelines for minocycline (R >4 mg/l), and PK/PD breakpoint (0.5 mg/l) for tigecycline and eravacycline, a new synthetic tetracycline with wide-spectrum activity against MDR organisms. While minocycline achieved overall high *in vitro* activity of 79% against CPAc, eravacycline and tetracycline had lower activity of 64% and 37% respectively. On the other hand, eravacycline had the lowest MIC50 of 0.5 mg/l, which was only one dilution lower than those (MIC50 =1 mg/l) of tigecycline and of minocycline.

Colistin retained a globally high activity rate (88%) against CPAc without notable differences between carbapenemase types produced.

5. Summary

The NRC analyzed 644 Enterobacterales, 292 *Pseudomonas*, and 61 *Acinetobacter* isolates, mostly referred for the confirmation of carbapenemase production and susceptibility testing. Isolates characterizations were performed using phenotypical and molecular tools, including whole-genome sequencing (WGS).

In 2024, 53% of Enterobacterales were confirmed as carbapenemase producers (CPE), predominantly *K. pneumoniae* and *E. coli*. OXA-48-like (54%) and NDM (25%) remained the leading carbapenemase families. WGS revealed dissemination of high-risk clones (e.g., ST307 and ST147 in *K. pneumoniae*, ST410 and ST131 in *E. coli*), especially those co-producing NDM and OXA-48-like enzymes. CPE isolates demonstrated high resistance to most beta-lactams, with aztreonam/avibactam and cefiderocol retaining good activity, except against some NDM producers.

Carbapenemase-producing *P. aeruginosa* (CPPA) represented 29% of analyzed strains, with VIM-2 and VIM-4 as dominant enzymes. MLST showed ST111, followed by ST235, as the most prevalent high-risk clones. Susceptibility was generally poor, except for cefiderocol (93%) and colistin (97%), with limited efficacy of newer BLBLIs due to class B enzyme dominance.

Among the 61 *Acinetobacter* isolates, 84% were carbapenemase producers (CPAc), mainly *A. baumannii* with OXA-23 as the main enzyme. Genomic data identified ST2 as the predominant clone. Multi-carbapenemase-producing strains, including NDM + OXA combinations, increased in 2024. New combination sulbactam-durlobactam showed high in vitro activity against OXA-producing strains but was ineffective against class B (NDM/VIM) enzymes. Colistin and cefiderocol remained valuable, although resistance is emerging.

The report emphasizes a shift towards broader carbapenemase diversity, increasing detection of coproducers, and dissemination of high-risk clones across Belgium. It underlines the importance and the need for continuous genomic surveillance and updated treatment strategies for MDR infections.

Acknowledgement

We thank all clinical microbiology laboratories for isolates referral and participation to the national microbiological surveillance.

We thank Professor Bogdan Iorga (Research Director of CNRS, Université Paris-Saclay) for his dedication to the technical assistance of the genomic surveillance.

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