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

Surveillance report 2023

CHU UCL NAMUR
Yvoir, Belgium

Medical staff

Te-Din Daniel Huang, MD, PhD
Olivier Denis, MD, PhD
Carlota Montesinos, MD, PhD

Scientific staff

Pierre Bogaerts, Ir. PhD
Stéphanie Evrard, MSc
Nicolas Gilliard, Msc

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List of Abbreviations

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

CPP: Carbapenemase-producing Pseudomonas

CPE: Carbapenemase-producing Enterobacterales

MDR: Multidrug-resistant

NRC: National Reference Center

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1. Introduction

This report summarizes the data analysis up to 2023 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.

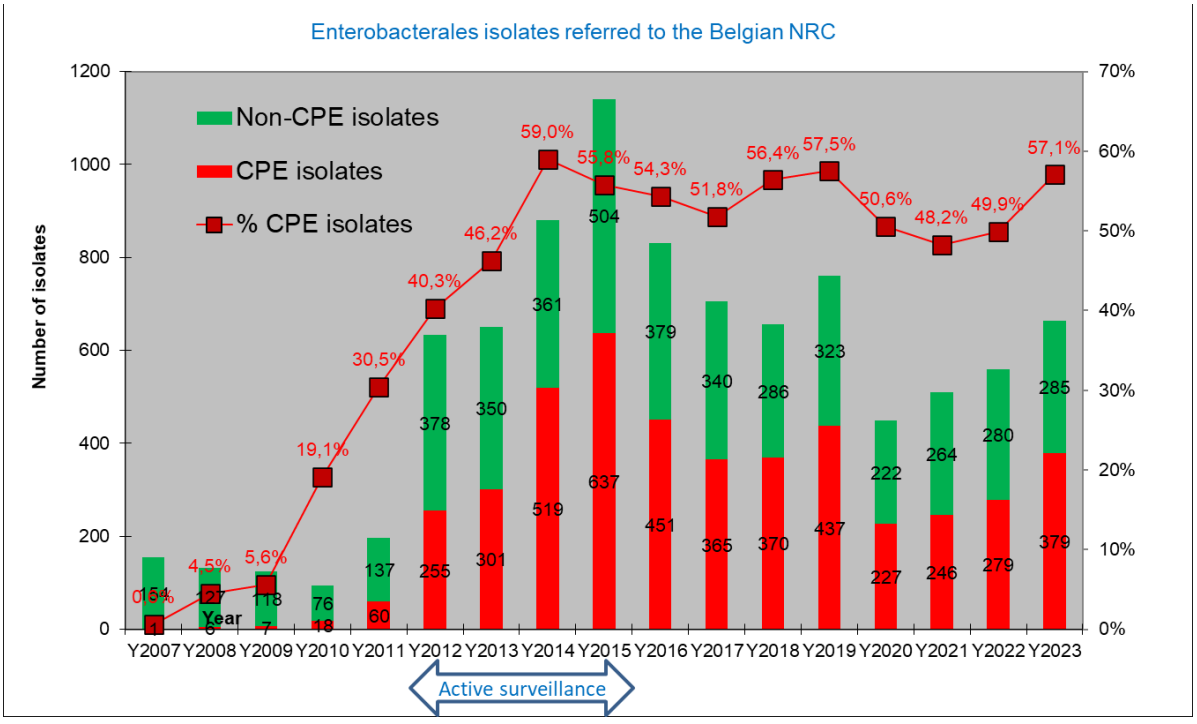
The strains received by the NRC are sent mainly for confirmation of acquired carbapenemase production and/or susceptibility testing for last-line antibiotics active against MDR isolates. Less common requests are confirmation of colistin resistance, identification of hypervirulent *Klebsiella pneumoniae*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

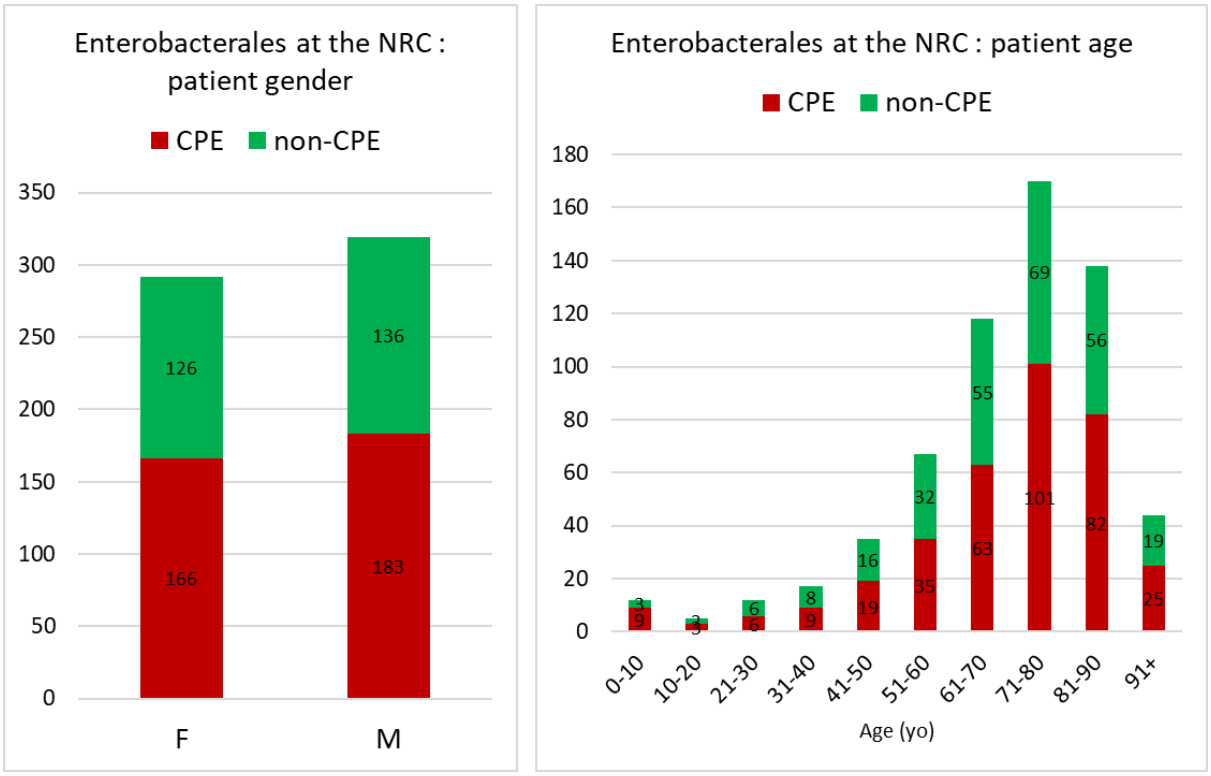
Figure 1. Number of Enterobacterales isolates received per year by the NRC and the proportion (%) of those confirmed as carbapenemase producers (CPE).



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 significantly during the COVID-19 pandemics (years 2020-2022). The decline could be due to both a lower prevalence of CPE as a consequence of control measures, associated to the inability of laboratories to send strains during the pandemic. In 2023, the numbers continue to increase back to those observed before the pandemics, reaching 664 isolates analyzed with 57% confirmed as CPE.

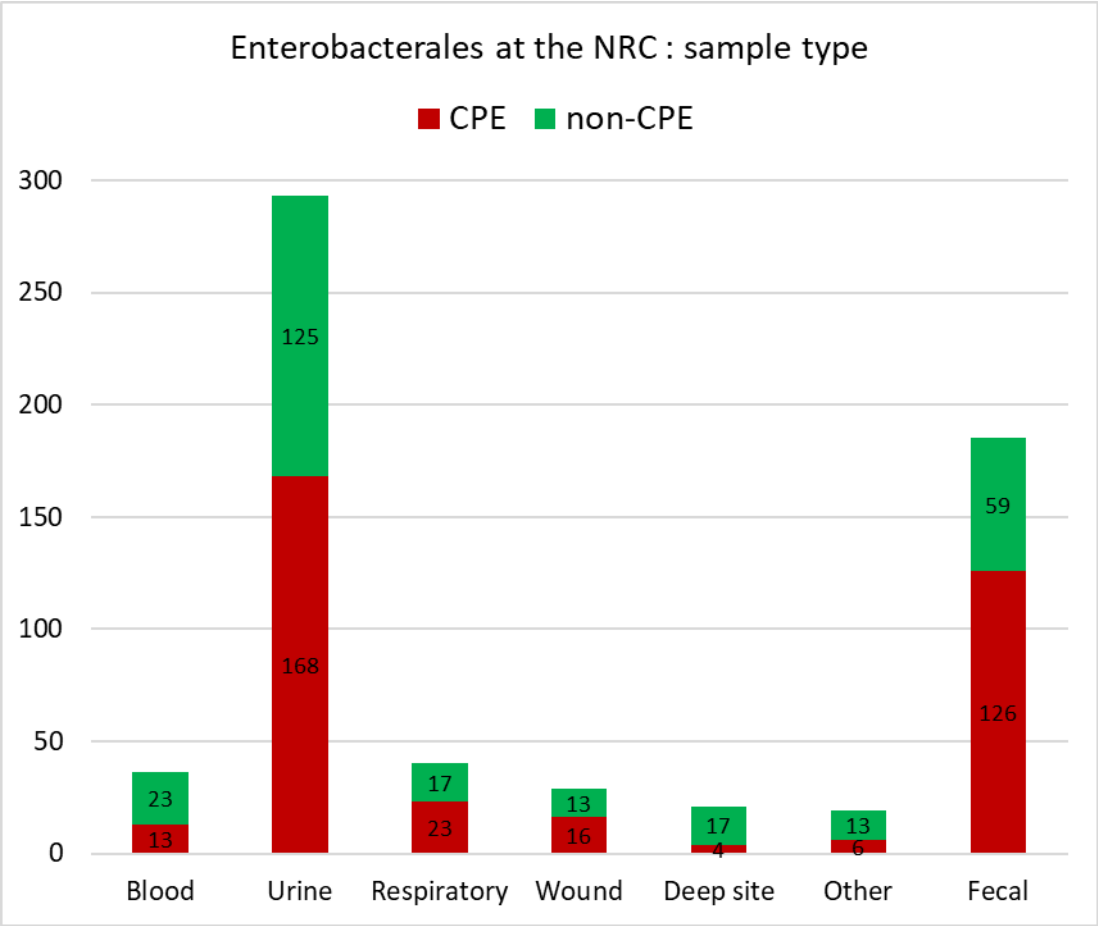
In 2023, 66 (80%) of the 82 clinical laboratories that referred isolates to the NRC (including 51 hospital-based and 15 serving general practitioners in the community) had at least one isolate confirmed as CPE.

Figure 2. Number of Enterobacterales isolates per gender and per age group in 2023.



In 2023, the NRC received more isolates (52%) collected from male patients (no difference between CPE and non-CPE) and mainly (76%) from patients above 60 years old.

Figure 3. Sample types from which Enterobacterales were isolated in 2023. 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.

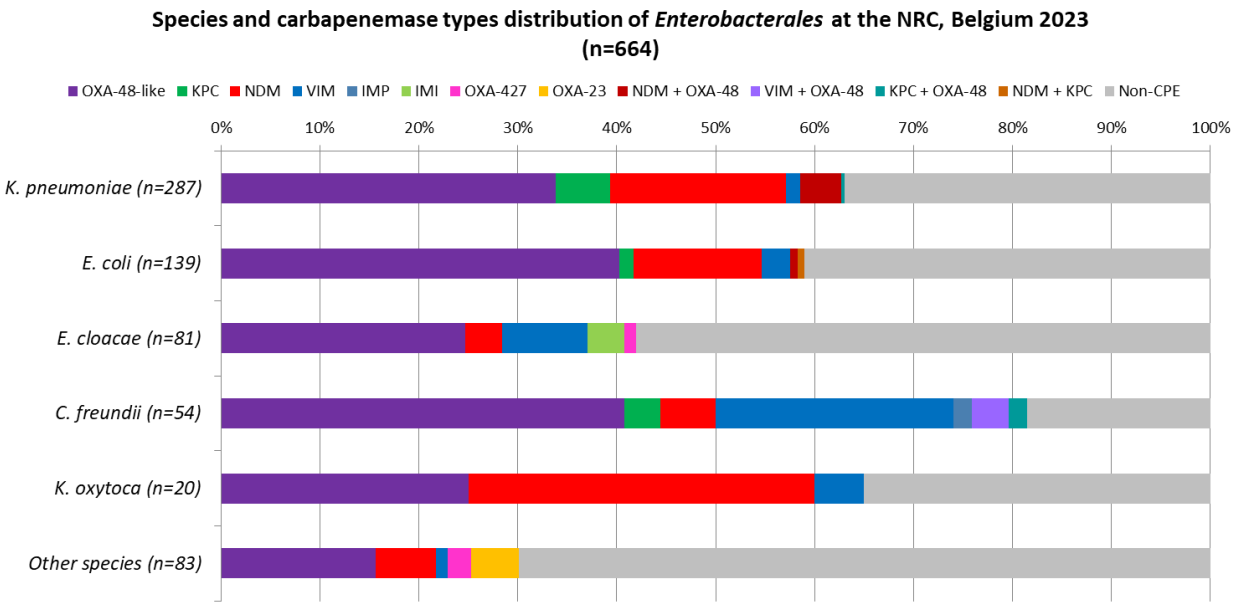


Of the 623 isolates with indicated sample nature, 30% were cultured from fecal screening samples, while urinary specimens represented the large majority (67%) of sample origins for the 438 clinical isolates. Of the 36 isolates causing bloodstream infections, 13 were confirmed as carbapenemase producers.

2.2. Carbapenemase-producing *Enterobacterales*

2.2.1. Bacterial species and resistance mechanisms

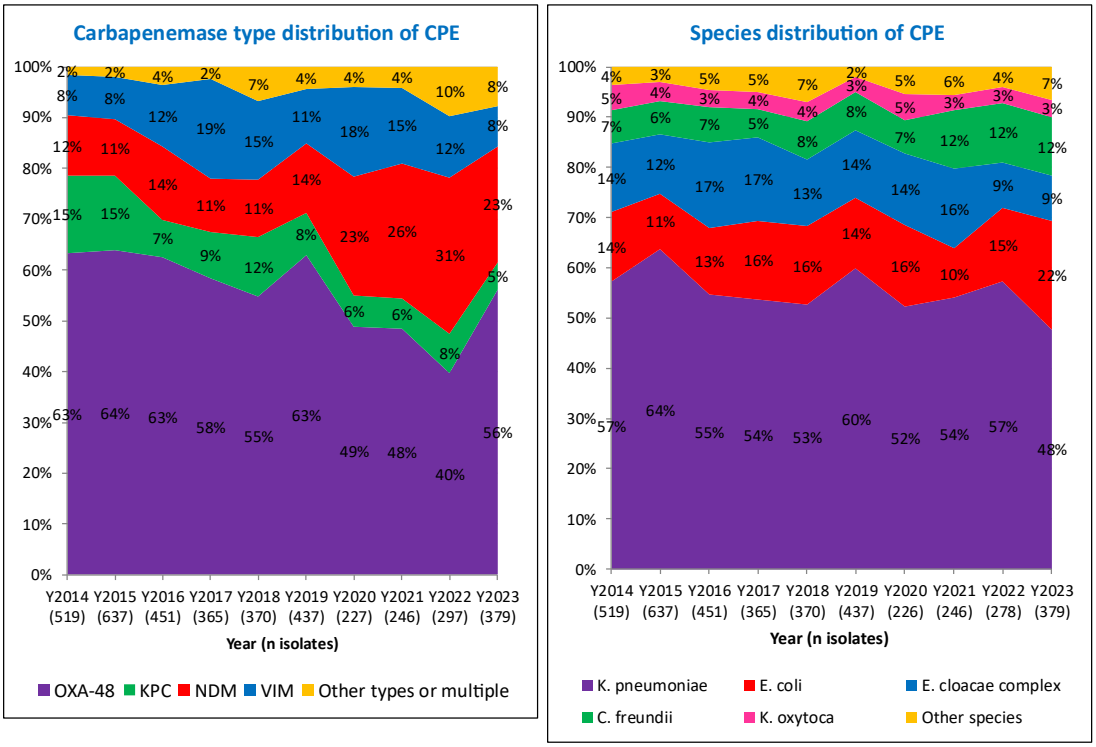
Figure 4. Species and carbapenemase types distribution of Enterobacterales isolates



In 2023, *K. pneumoniae, E. coli, E. cloacae* complex*, C. freundii* and *K. oxytoca* remained the top 5 species among the total 664 Enterobacterales isolates analyzed for the production of carbapenemase at the NRC. Among each of these top 5 species, the majority of the isolates were confirmed as carbapenemase producers, except for *E. cloacae* complex for which most (58%) were not CPE.

Figure 5. Yearly distribution of species and carbapenemase types among CPE

Yearly distribution of CPE

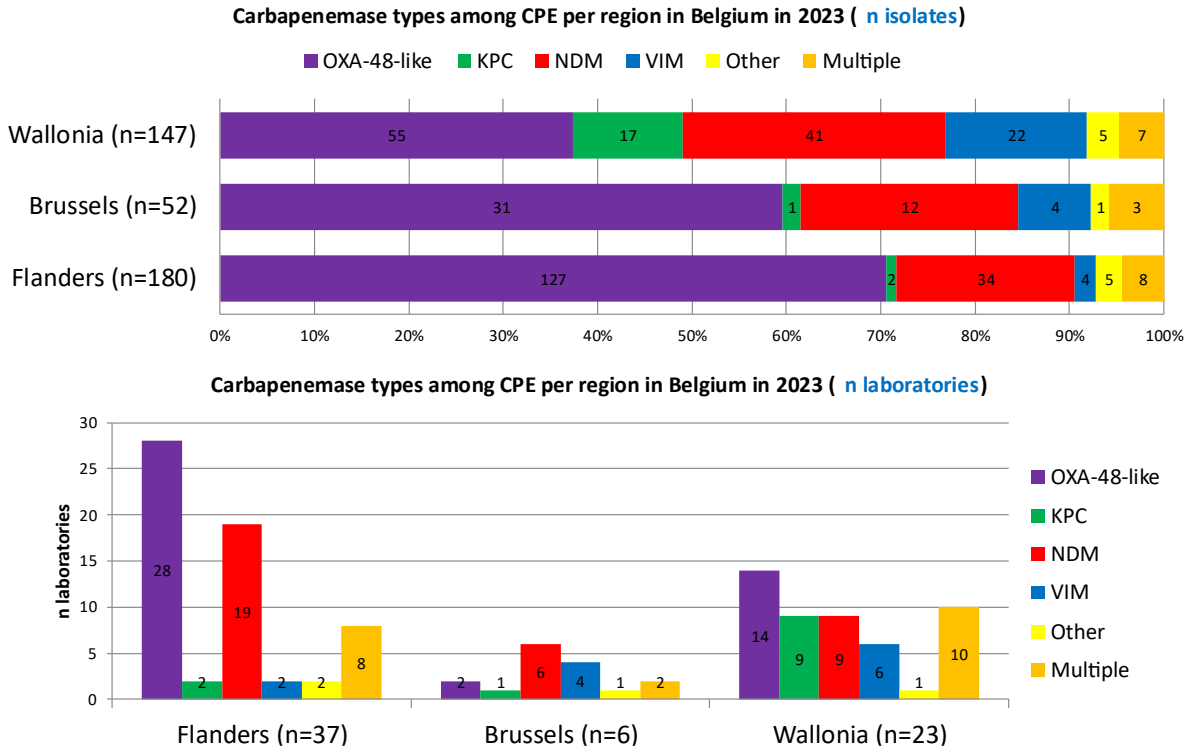


Among the 379 confirmed CPE isolates in 2023, OXA-48-like enzymes remain the predominant common carbapenemase (56% in 2023), followed by NDM (23%), while KPC (8%) and VIM (5%) were less prevalent. The other carbapenemase types include OXA-23-producing Proteus mirabilis (n=4), IMI-producing E. cloacae complex (n=3), OXA-427 CPE (n=3) and IMP-positive *C. freundii* (n=1). A trend towards diversification of carbapenemase types is evident, with isolates coproducing multiple enzymes being increasingly reported (26 in 2022 and 18 in 2023). 80% 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=2), KPC + OXA-48 (n=2) and NDM + KPC (n=1) enzymes.

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

Regional distribution of laboratories referring 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, and isolates coproducing multiple carbapenemases are reported in 10/23 laboratories.

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

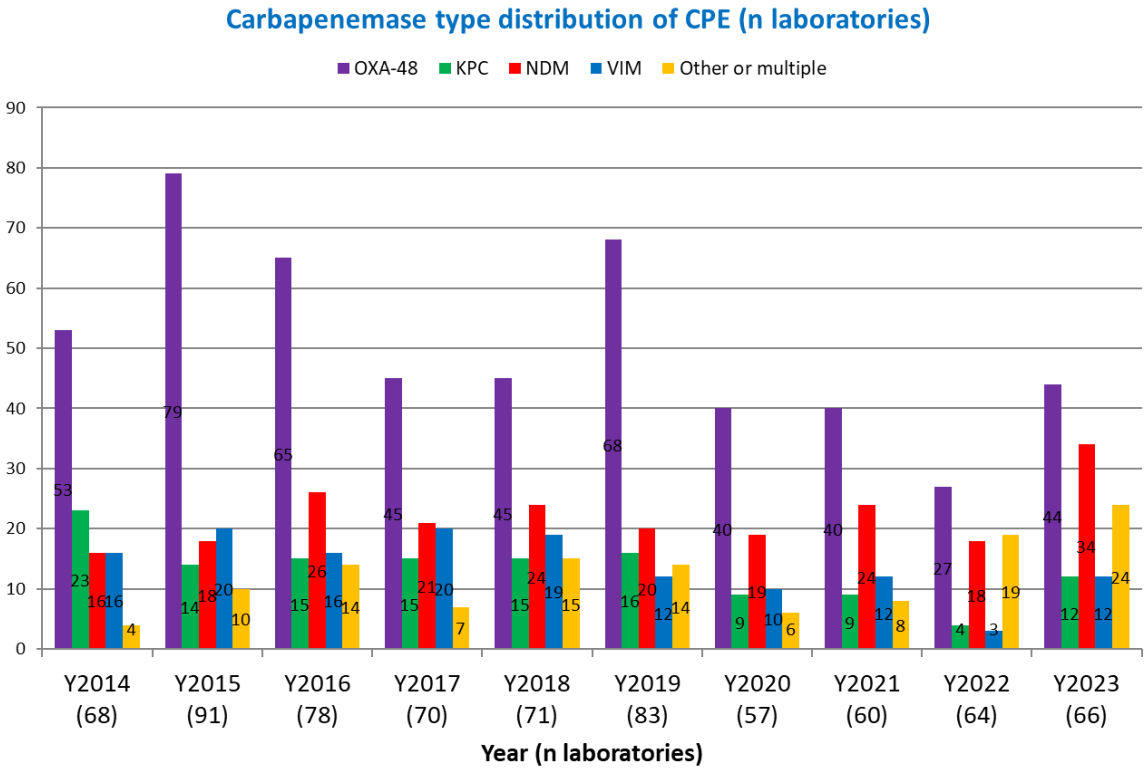
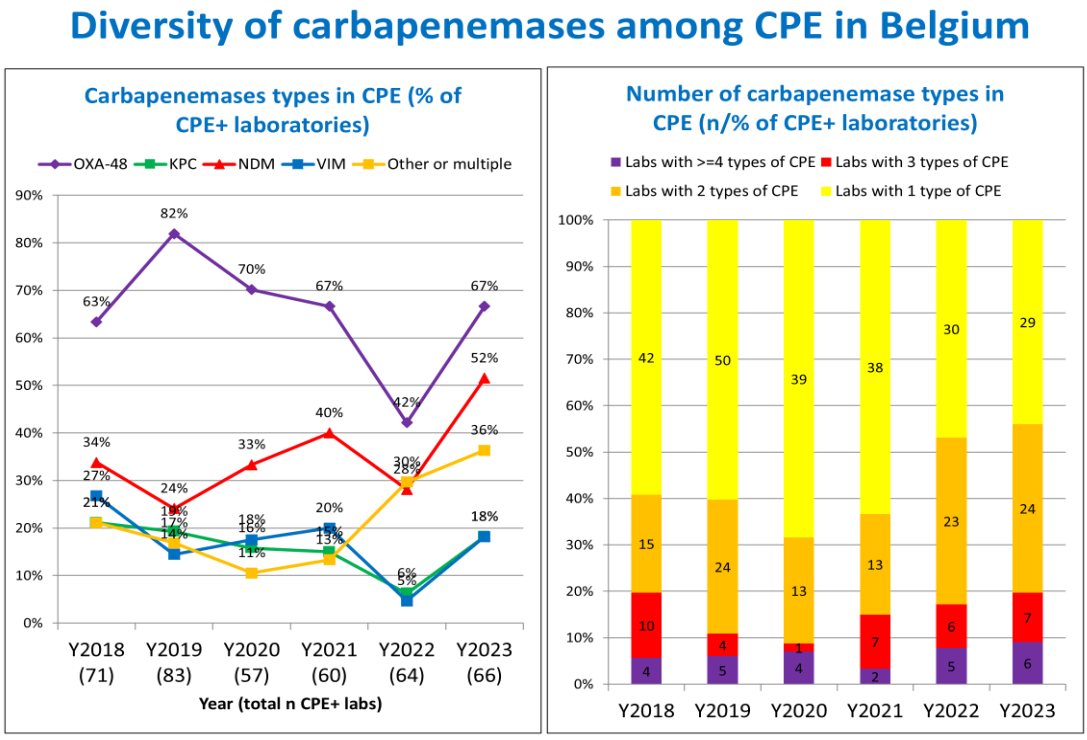


Figure 8. Yearly proportion (%) of CPE-reporting laboratories for each carbapenemase type among CPE

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



OXA-48 remains in 2023 the main carbapenemase type reported by the highest number (44 in 2023) and proportion (67% in 2023) of the laboratories. When comparing 2023 to 2018, while there is a lower number of laboratories reporting KPC (12 vs 19) and VIM (12 vs 19) types CPE, we observe a steady increase in the number (34 vs 16) and proportion (52% vs 34%) of laboratories reporting NDM type CPE reaching the peak in 2023.

In addition, the number (37 vs 29) and proportion (56% vs 41%) of laboratories reporting more than one types of carbapenemase types among CPE also increased comparing 2023 to 2018. 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. Carbapenemase enzymes (sequenced bla gene) among CPE in 2023

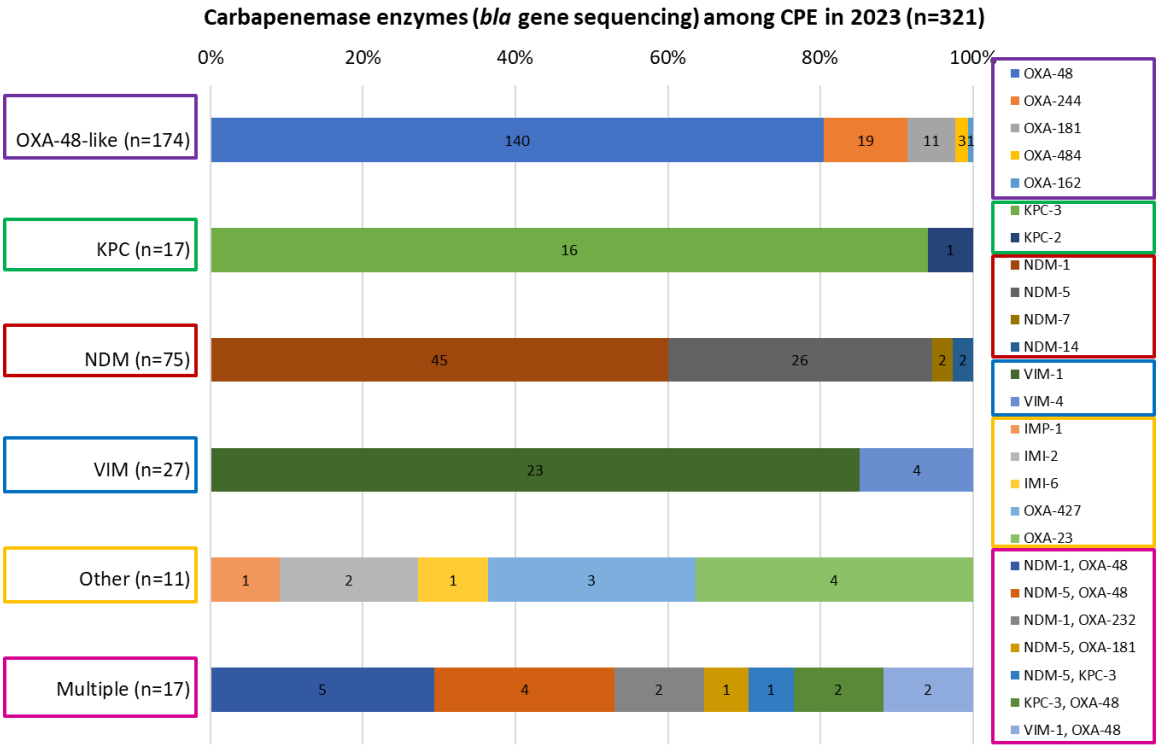


Figure 11. summarized the distribution of carbapenemase enzyme variants based on bla gene sequence determined by WGS performed on 339 CPE isolates in 2023.

Among OXA-48-like CPE, OXA-48 represents the predominant (80%) enzyme, followed by OXA-244 variant (11%), which were all detected only in *E. coli*.

Among NDM CPE, while NDM-1 represents the main (60%) enzyme present among Enterobacterales, NDM-5 represents by far the most frequent variant (88%, 15/17) among NDM-producing E. coli isolates.

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=3) and E. cloacae complex (n=1) CPE.

Figure 12. MLST clonal distribution of CPE among K. pneumoniae and E. coli in 2023

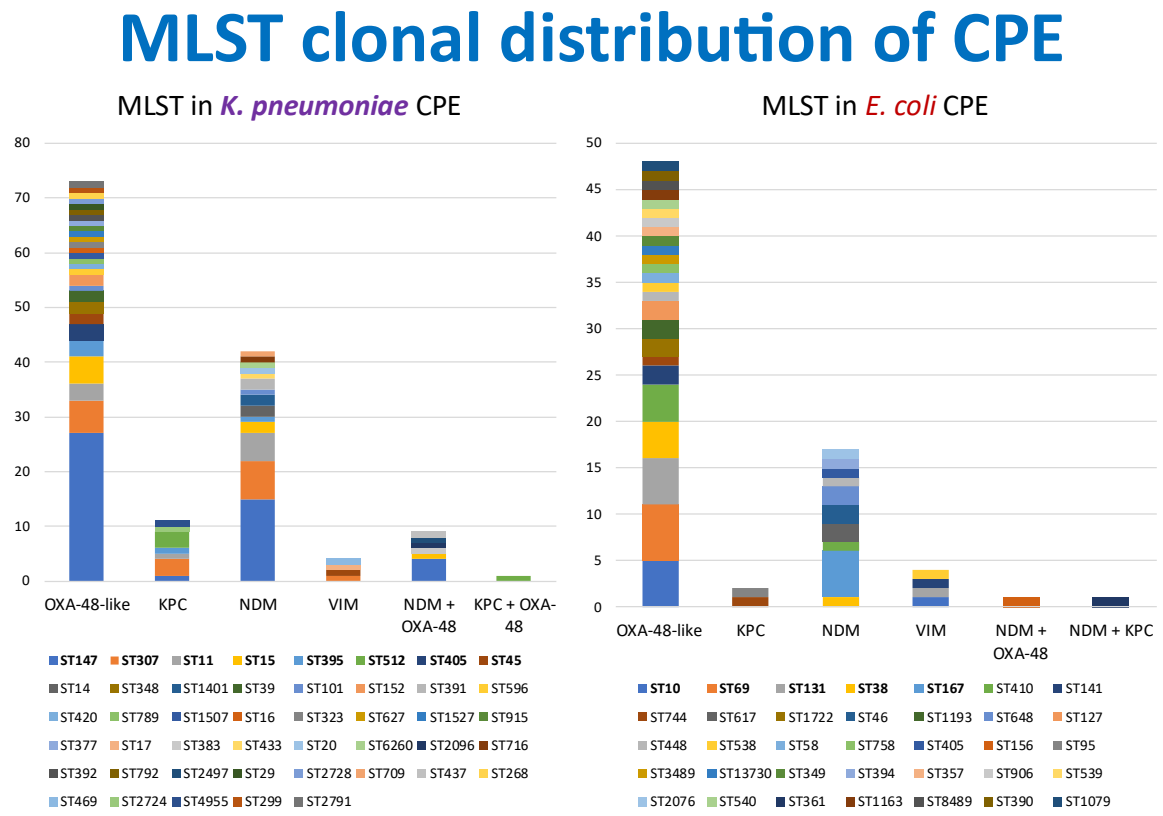


Figure 13. detailed the distribution of sequence types (ST) based on the multilocus sequence typing (MLST) determined by WGS on K. pneumoniae and E. coli CPE isolates in 2023.

The 140 *K. pneumoniae* CPE isolates belonged to 45 different ST, including notably ST147 (n=47), ST307 (n=17), ST11 (n=9), ST15 (n=8), ST395 (n=5), ST512 (n=4), ST405 (n=3), ST45 (n=3), which were the top 8 ST clones with at least 3 isolates reported. The predominance of ST147 (n=47) and ST307 (n=17) supports the dissemination of these well-recognized high-risk clones (HRC) in Belgium. Of note, 4/9 *K. pneumoniae* CPE coproducing OXA-48 and NDM-1 (n=2) / NDM-5 (n=2) carbapenemases belonged to ST147.

The 73 *E. coli* CPE isolates showed higher clonal diversity and belonged to 35 different ST, including ST10 (n=6), ST69 (n=6), ST131 (n=6), ST38 (n=5), ST167 (n=5), ST410 (n=5), ST141 (n=3), which were the top 7 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 4 STs (ST10 (n=4), ST69 (n=3), ST131 (n=4), ST38 (n=4)), suggesting the potential clonal 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-48-like) and NDM-5 enzymes, with ST167 (n=4) and ST410 (n=4), which are two recently recognized HRC in *E. coli*.

Figure 14. Geographical distribution of carbapenemases among CPE in 2023

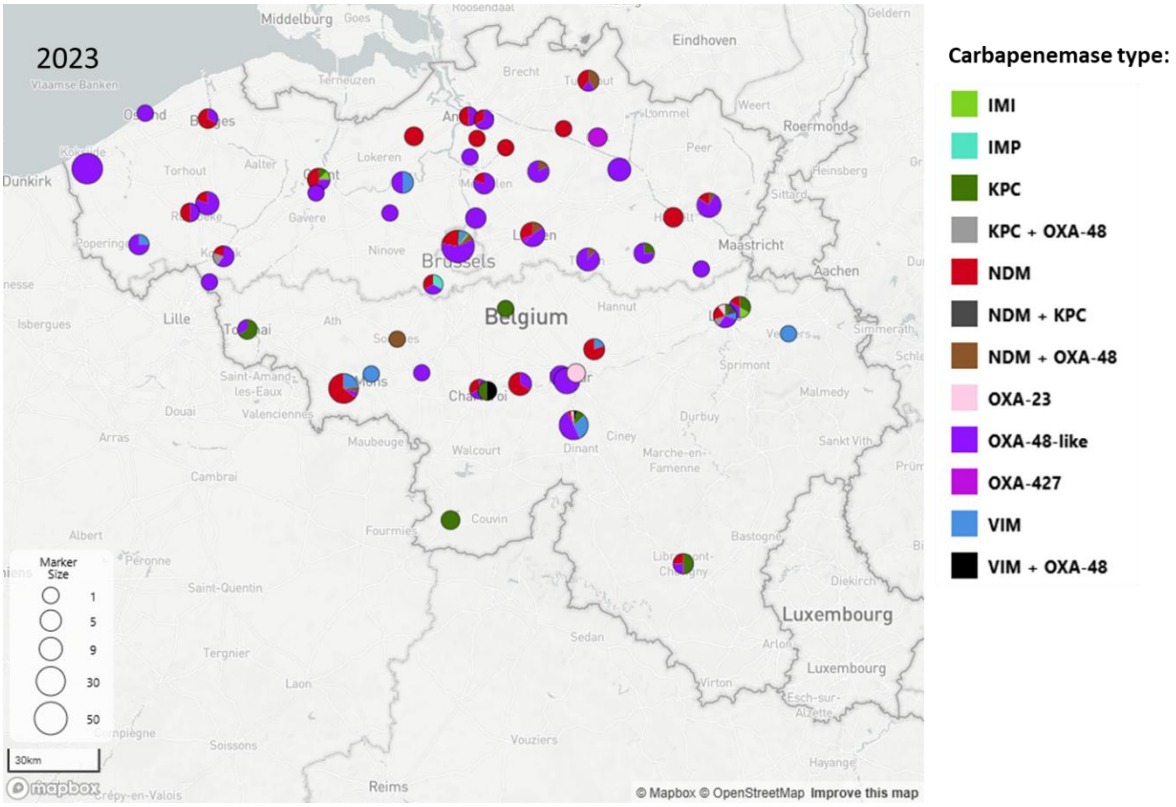


Figure 15. Geographical distribution of top 8 MLST clones among K. pneumoniae CPE in 2023

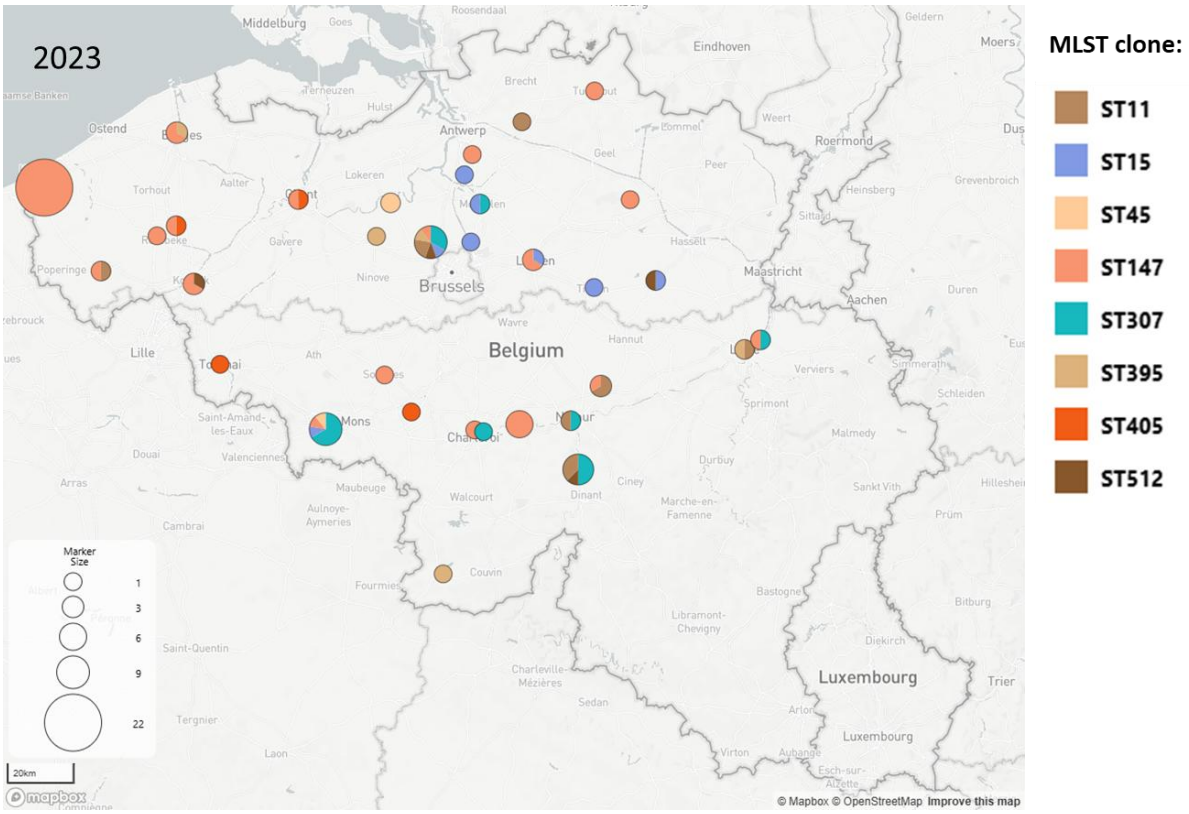
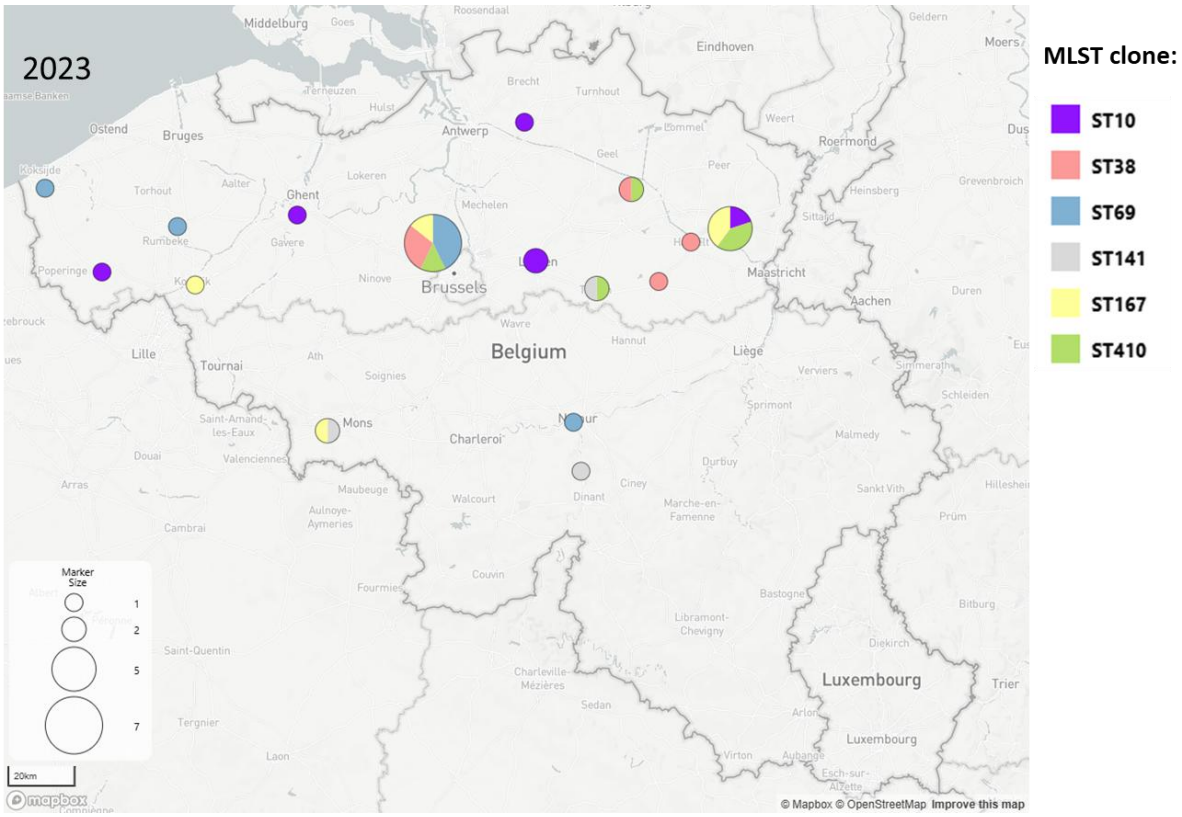


Figure 16. Geographical distribution of top 6 MLST clones among E. coli CPE in 2023



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 2021-2023 (n=951)

Enterobacterales (total n=951)	OXA-48	KPC	NDM	VIM	Multiple	non-CPE
**Antibiotic ** n isolates	407	55	219	94	50	117
Temocillin	2%	18%	6%	1%	2%	61%
Piperacillin/tazobactam	1%	0%	0%	0%	2%	42%
Aztreonam	48%	0%	12%	49%	14%	45%
Cefotaxime	36%	4%	0%	0%	2%	40%
Ceftazidime	46%	2%	0%	3%	2%	46%
Cefepime	56%	4%	0%	25%	6%	58%
Meropenem	89%	45%	28%	93%	12%	88%
Ceftolozane/tazobactam	38%	2%	0%	0%	0%	54%
Meropenem/vaborbactam	NA	98%	NA	NA	15%	89%
Ceftazidime/avibactam	100%	100%	NA	NA	10%	94%
Aztreonam/avibactam (n=251)	100%	100%	97%	100%	94%	50%
Cefiderocol (n=798)	97%	77%	76%	89%	73%	89%
Cotrimoxazole (DD)	47%	57%	42%	32%	43%	54%
Ciprofloxacin	30%	9%	7%	46%	6%	36%
Gentamicin	67%	60%	43%	74%	30%	65%
Amikacin	93%	51%	50%	97%	38%	86%
Fosfomycin IV	72%	63%	83%	86%	55%	68%
Tigecycline	71%	65%	67%	72%	54%	68%
Colistin	93%	81%	87%	93%	82%	44%

*Multiple: isolates producing multiple carbapenemases

Figure 17. Meropenem cumulative MIC by carbapenemase type among CPE

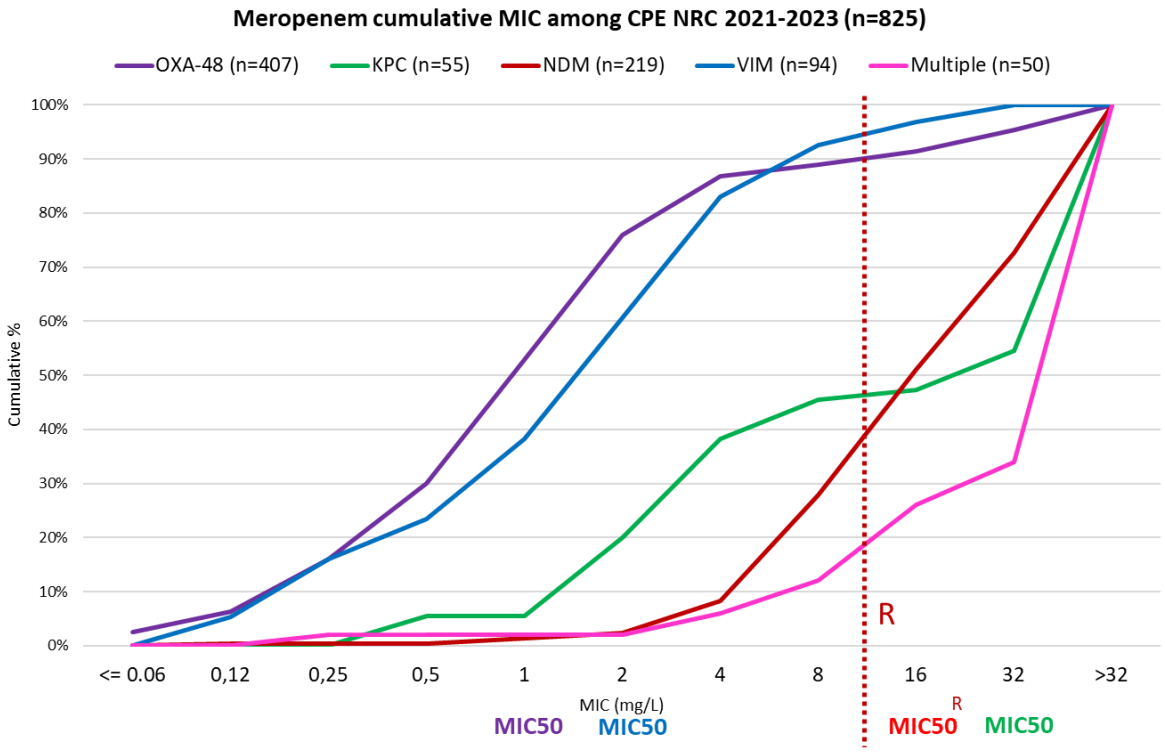


Table 2. shows the antibiotic susceptibility profile according to carbapenemase types for putative CPE isolates analyzed by BMD at the NRC with aggregated data for the 2021-2023 period.

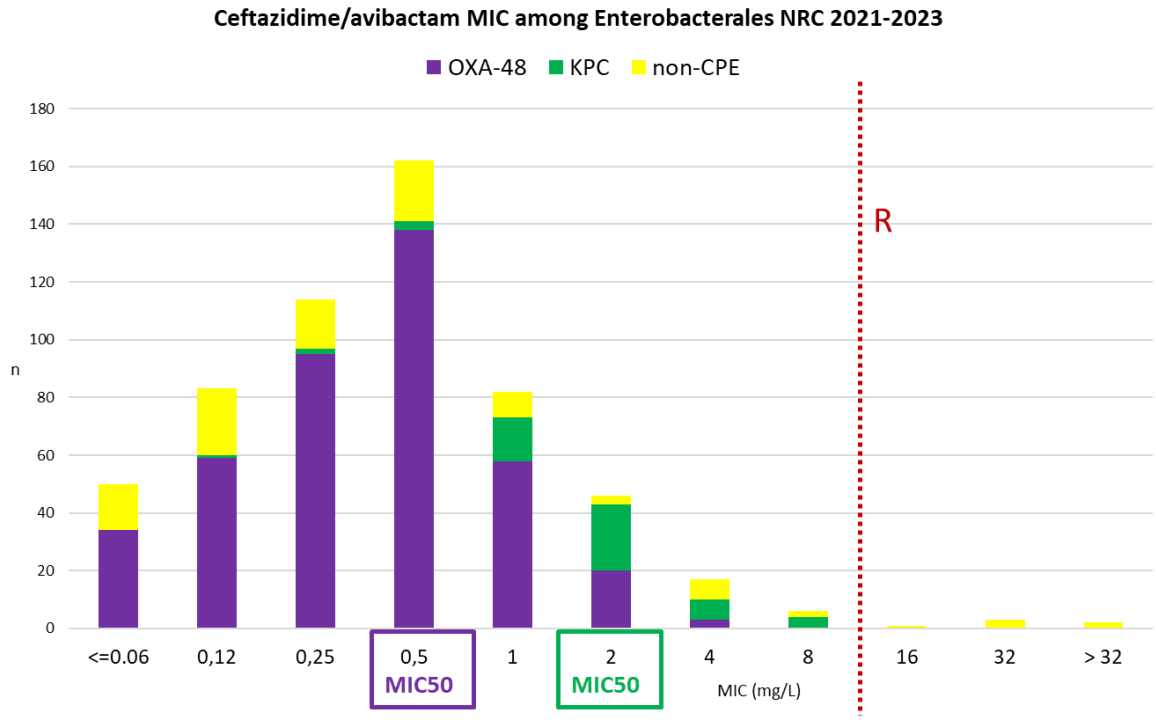
The resistance levels to carbapenems are highly variable depending on the type of carbapenemase produced (and also modulated by other non-enzymatic resistance mechanisms such as porin deficiency not characterized genotypically). While the majority of the KPC and NDM are resistant to meropenem, 89% of OXA-48 and 93% of VIM CPE are susceptible 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 (96%) and piperacillin/tazobactam (98%), which can serve as excellent additional phenotypical resistance markers for the suspicion of CPE.

While most NDM and KPC isolates are resistant to third and fourth generation cephalosporins (3GC and 4GC), OXA-48-like CPE can retain susceptibility 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.

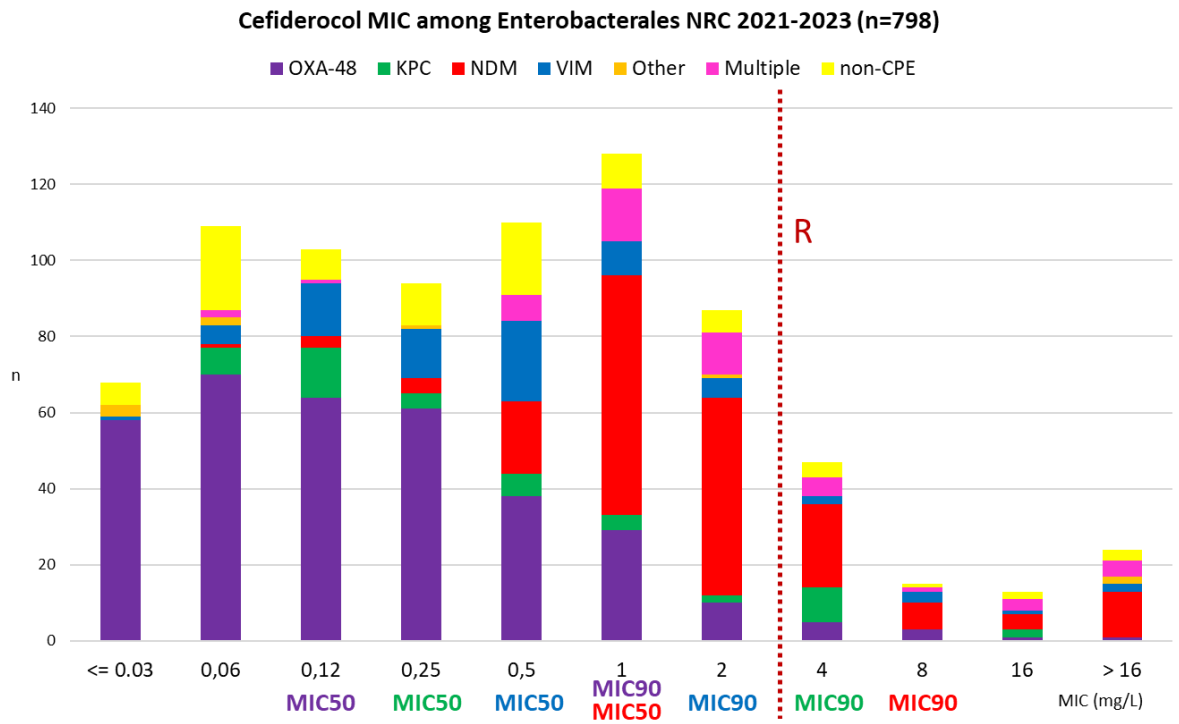
Susceptibility of CPE against new anti-CPE agents including recent beta-lactam/beta-lactamase inhibitor (BLBLI) combinations are summarized in Table and detailed in the following figures.

Figure 18. Ceftazidime/avibactam MIC distribution among CPE



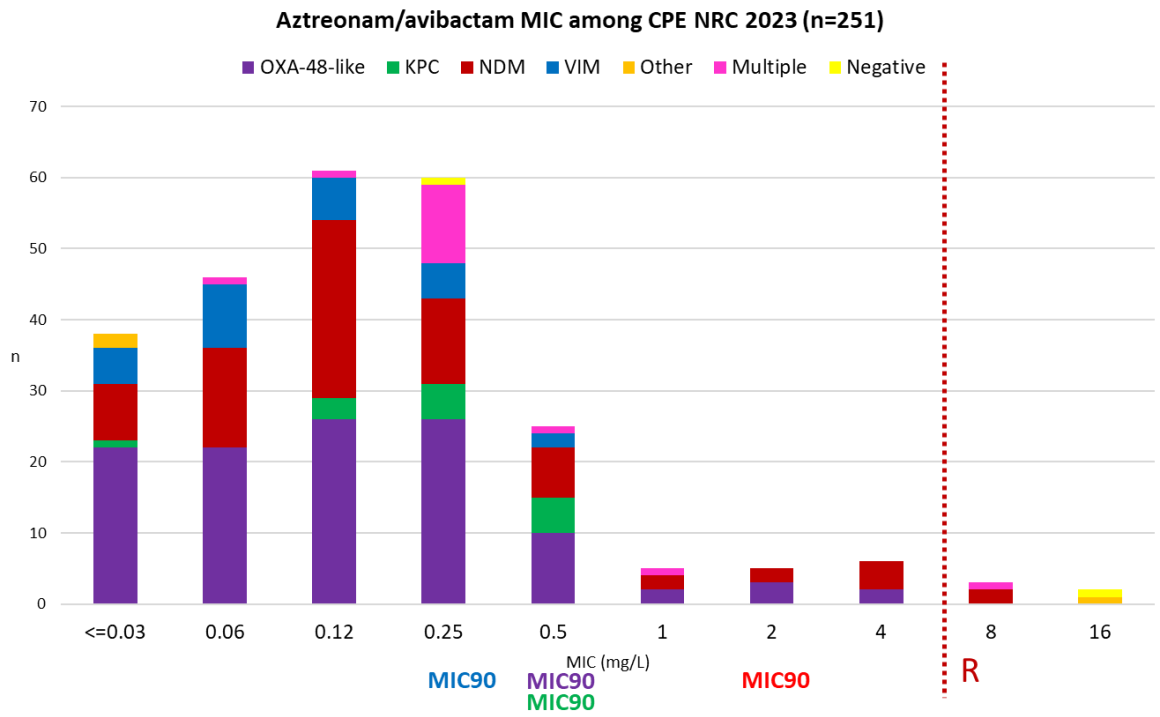
Ceftazidime/avibactam maintains perfect activity of against 100% of OXA-48 and KPC CPE isolates. The MIC distribution shows higher mean MIC for KPC compared to OXA-48 with 4 KPC strains having ceftazidime/avibactam MIC close to the R breakpoint of 8 mg/l. Meropenem/vaborbactam were equally active (98%) with even lower MIC50 of 0.12 mg/l (data not shown) compared to the MIC50 of 2 mg/l for ceftazidime/avibactam against KPC CPE.

Figure 19. 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 89% against OXA-48 and VIM CPE, respectively, it has lower activity rates against KPC (77%) and NDM (76%) strains, including 21 isolates (mainly NDM producers) showing high MIC >16 mg/l. These observations are particularly of concerns as cefiderocol resistance is already commonly present in CPE isolates, despite the unavailability of the drug currently in the Belgian market, especially for Ambler class B (e.g. NDM) CPE for which cefiderocol represents the potential last-line treatment.

Figure 20. Aztreonam/avibactam MIC distribution among CPE

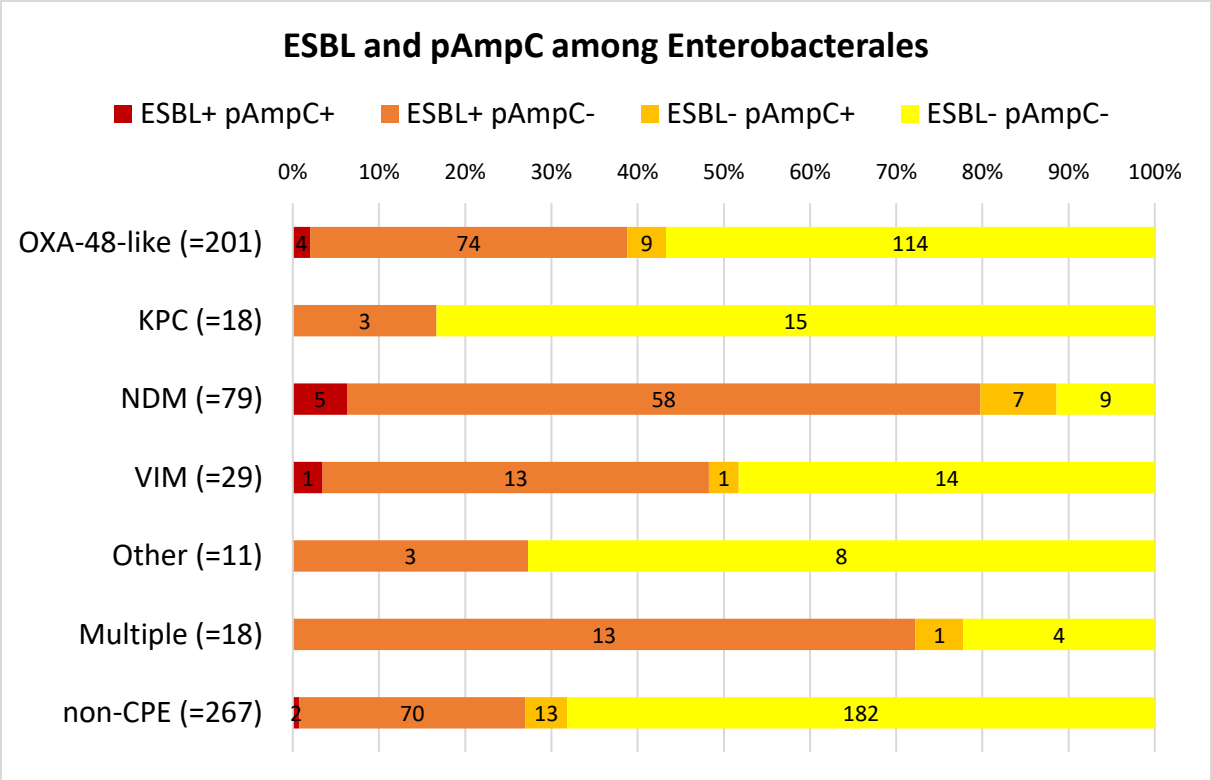


Aztreonam/avibactam is another novel BLBLI with very wide spectrum of activity against MDR *Enterobacterales* including CPE strains producing KPC and OXA-48-like, but also those producing Ambler class B enzymes. While aztreonam/avibactam shows the highest overall activity (94%-100%) against all four major families of CPE (including strains coproducing multiple carbapenemases), few isolates display higher MIC >=1 mg/l or even above the resistance breakpoint of 4 mg/l.

Acquired colistin resistance (MIC >2 mg/l) was observed in 44 (out of 430 colistin-tested) Enterobacterales isolates. Plasmid-mediated mobile colistin resistance (MCR) was detected in 10 (50% of colistin-R) *E. coli* and 1 (out of 18 colistin-R) *K. pneumoniae* isolates. All isolates carried *mcr-1* gene and none showed multidrug-resistant phenotype.

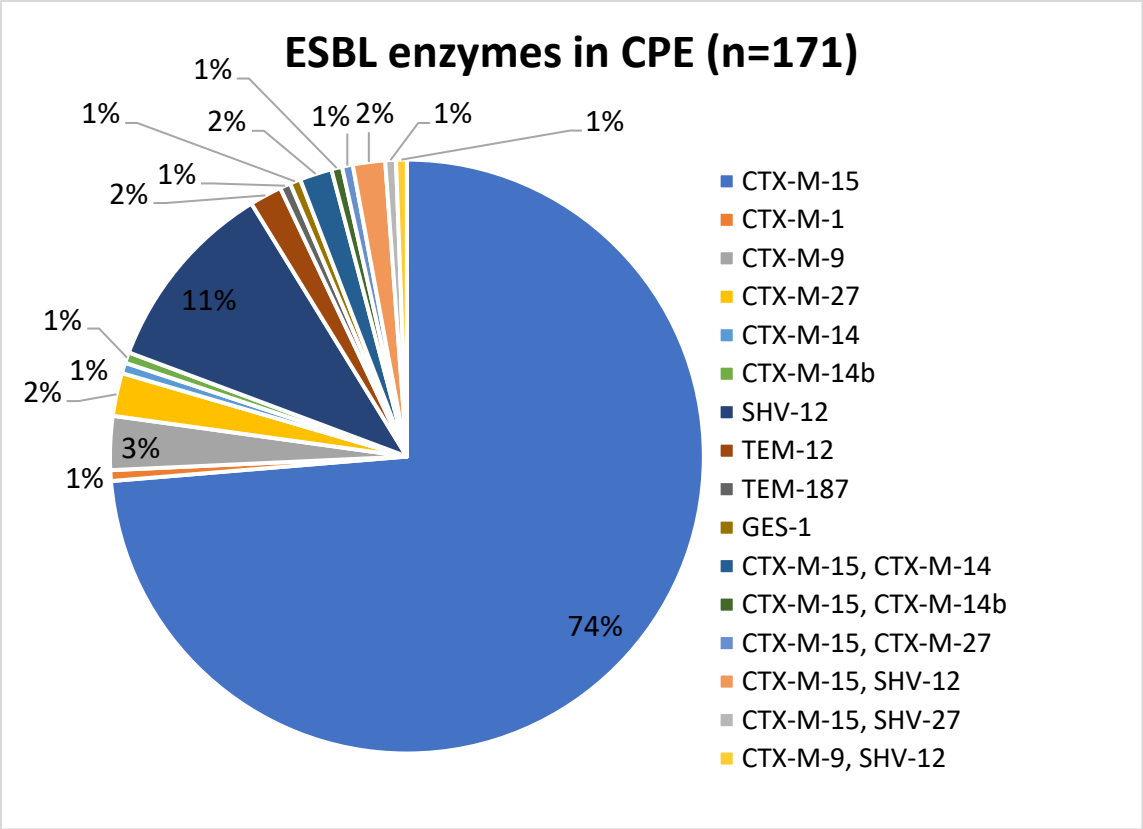
2.3. Extended-spectrum beta-lactamases (ESBL) and plasmidic AmpC cephalosporinases (pAmpC)

Figure 21. Distribution of the presence of ESBL and/or of plasmidic AmpC cephalosporinase (pAmpC) among CPE (per carbapenemase type) and non-CPE in 2023



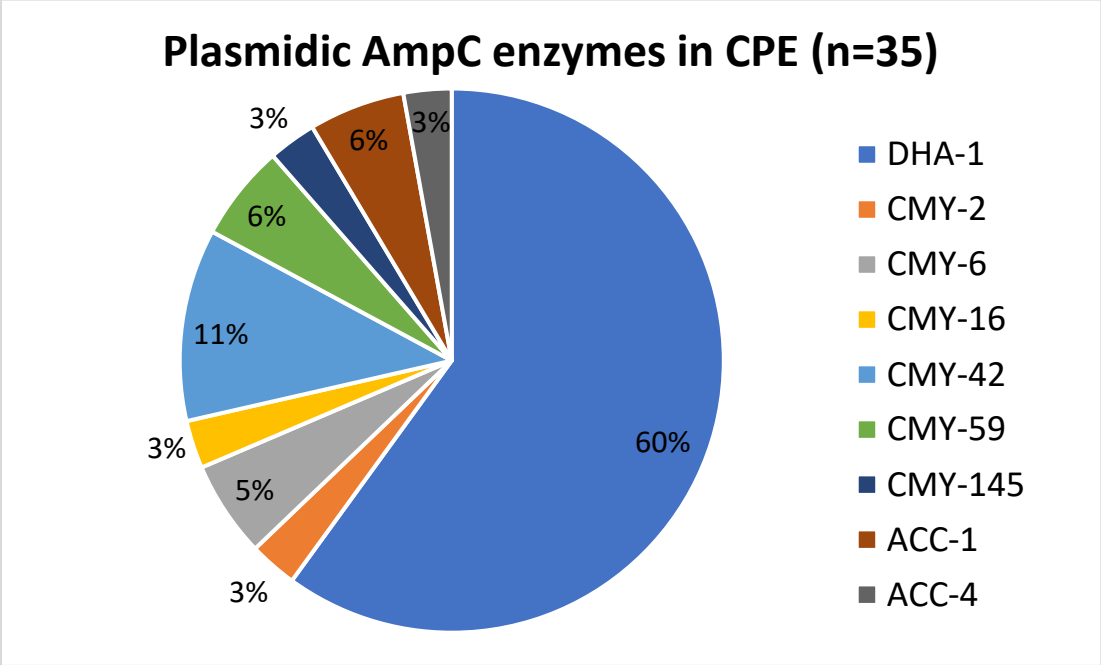
Among CPE, most (89%) of the NDM CPE produce also an ESBL and/or AmpC, while VIM, OXA-48-like and KPC types CPE, coproduce less frequently (52%, 43% and 17%, respectively) an ESBL and/or AmpC.

Figure 22. Distribution of ESBL enzymes (based on sequenced blaESBL genes) among CPE



CTX-M-15 represented by far the most frequent (74%) ESBL enzymes among CPE, followed by SHV-12 ESBL (11%).

Figure 23. Distribution of plasmidic AmpC cephalosporinases (based on sequenced blaAmpC genes) among CPE

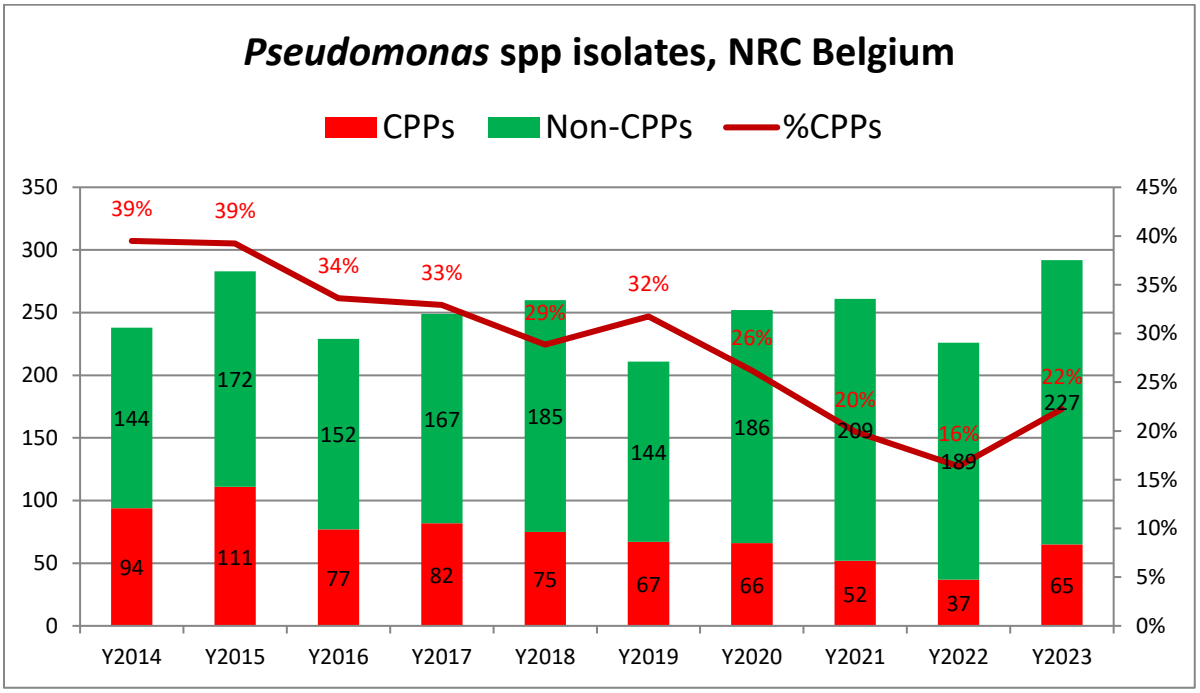


DHA-1 (60%) and CMY-2-like (31%) were the main plasmidic AmpC cephalosporinases detected among CPE.

3. Multidrug-resistant *Pseudomonas*

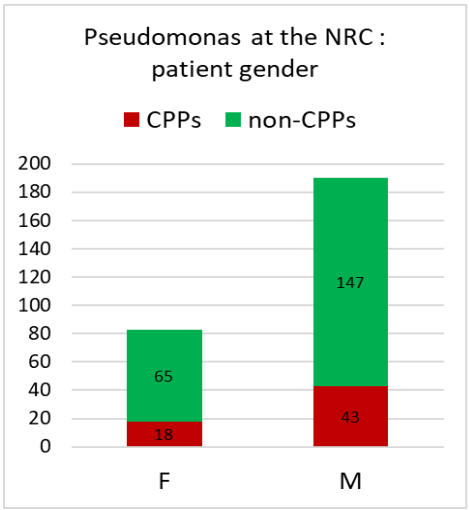
3.1. Characteristics of samples and patients related to isolates

Figure 24. Number of 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 250 *Pseudomonas* isolates. The number remained stable over the past 10 years, although reaching the highest peak of 292 isolates in 2023. The number and the proportion 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 25. Number of Pseudomonas isolates per gender and per age group in 2023.



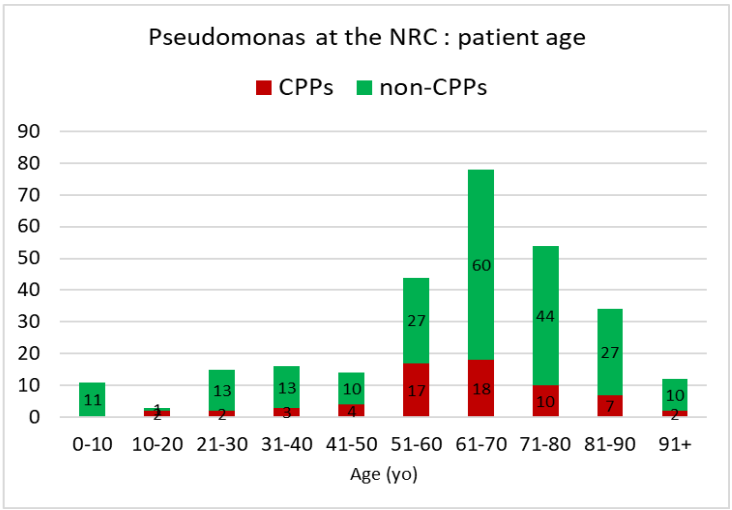
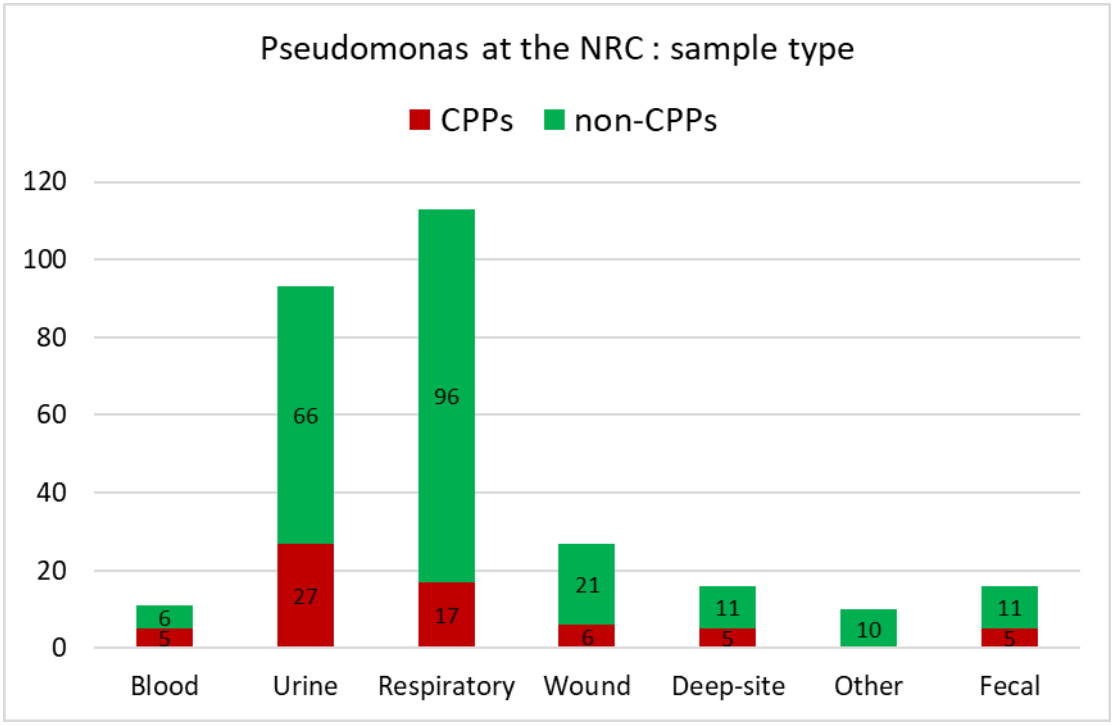


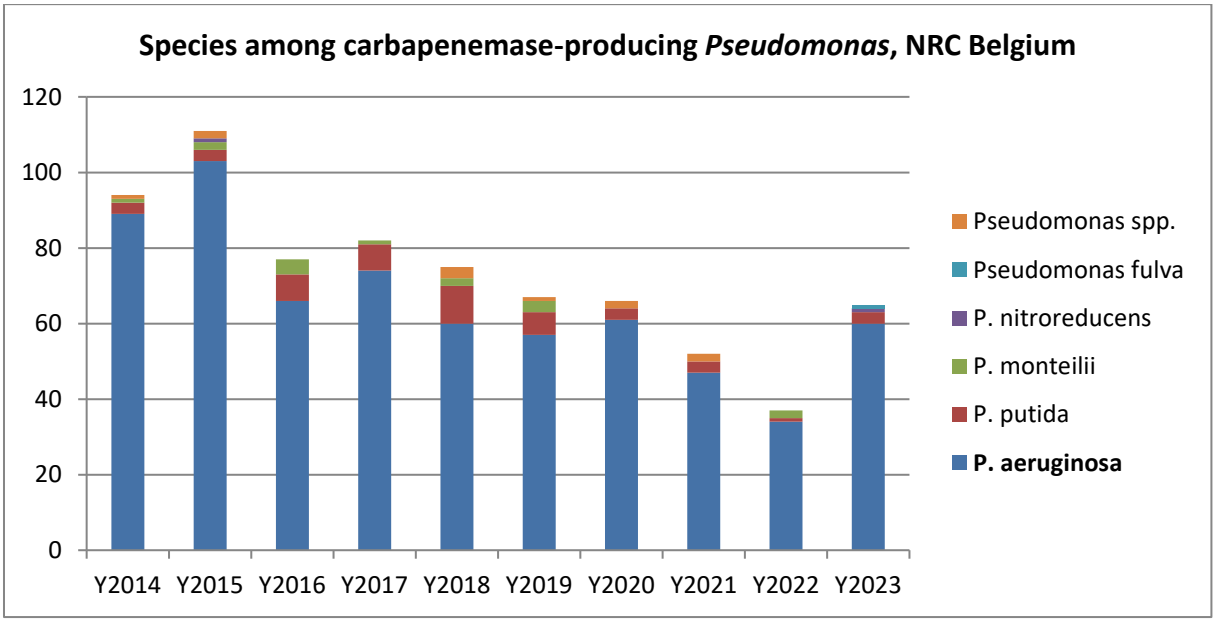
Figure 26. Sample types from which Pseudomonas were isolated in 2023. The 'deep site' category includes fluid and tissue specimens other than superficial or orifical sample sites. The 'other' category includes genital samples, percutaneous catheters and samples of unknown origin.

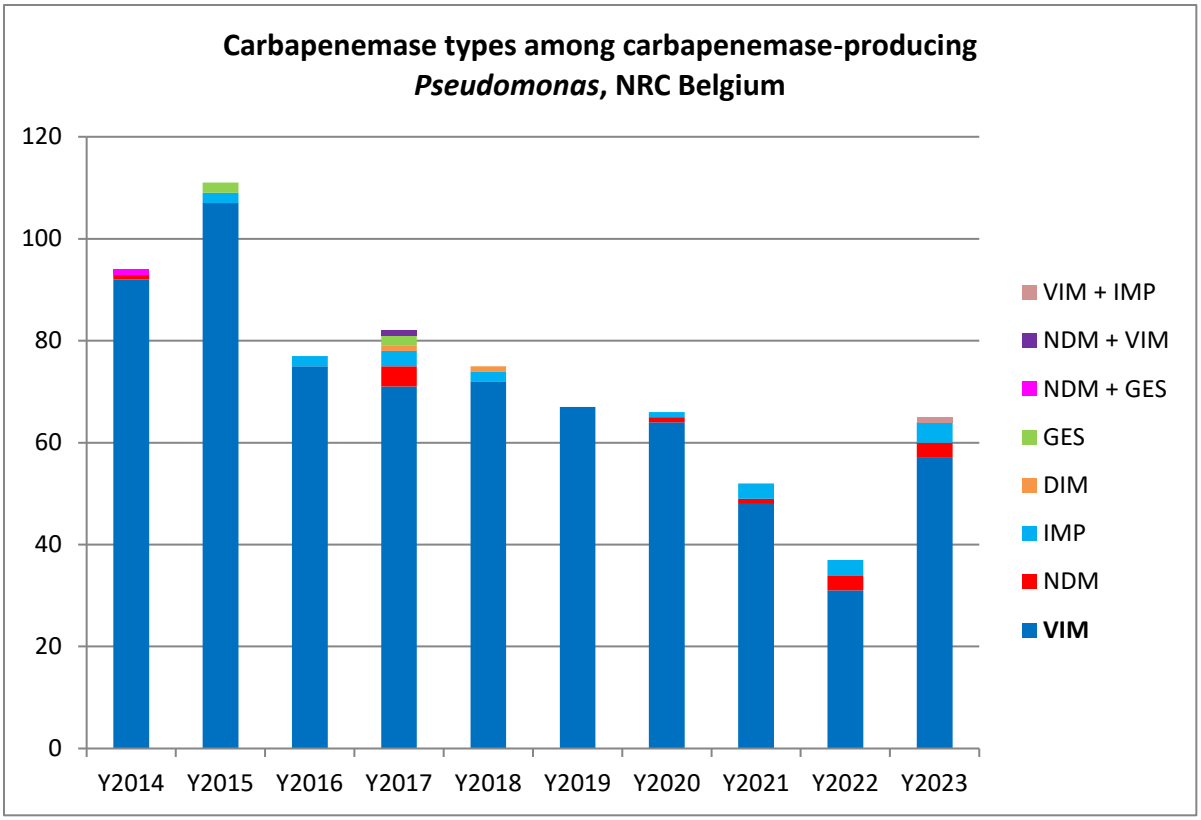


3.2. Carbapenemase-producing *Pseudomonas*

3.2.1. Bacterial species and resistance mechanisms

Figure 27. Species and carbapenemase types distribution of Pseudomonas isolates

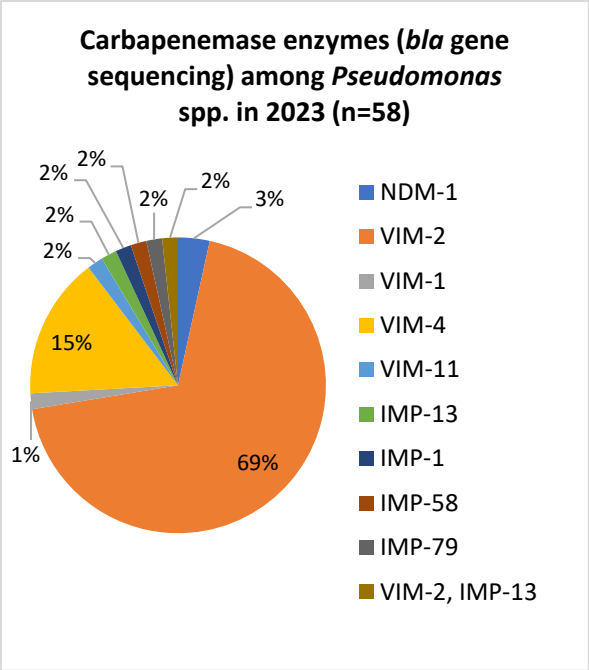


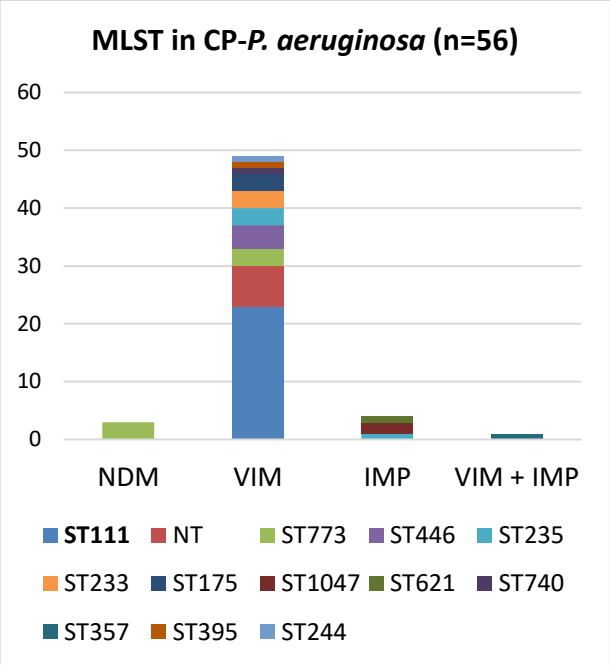


Among the 65 carbapenemase-producing *Pseudomonas* (CPPs) confirmed in 2023, *P. aeruginosa* represented by far the predominant species (92%), with few sporadic isolates of *P. putida* as the other CPPs species (similar to the data of the previous years). VIM-type carbapenemase remains by far the predominant carbapenemase family (89% in 2023) 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 each year since 2020, while they were reported previously only once as a single cluster of 4 isolates in 2017. No class A carbapenemase in *Pseudomonas* has been detected since 2017 (GES-5 carbapenemase).

3.2.2. Genomic surveillance

Figure 28. Carbapenemase enzymes (sequenced bla gene) among CPPs and the MLST clonal distribution among carbapenemase-producing P. aeruginosa (CPPA) in 2023





Among the 58 CPPs genome-sequenced, VIM-2 (69%) and VIM-2 (15%) were the two predominant carbapenemase enzymes, while VIM-1 and VIM-11 were produced by one isolate each. IMP CPPs had more diversified carbapenemase variants with one isolate each expressing IMP-1, IMP-13, IMP-58 and IMP-79. One ST357 *P. aeruginosa* coproduced VIM-2 and IMP-13 carbapenemases.

Among the 56 carbapenemase-producing *P. aeruginosa* (CPPA) determined for their MLST genotype, 50 isolates belonged to 12 different MLST (6 were not typeable (NT)) and ST111 were by far the predominant clone (41%). Of the 12 determined MLST among CPPA, 7 (ST111, ST175, ST233, ST235, ST244, ST357, 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.

The 3 NDM-1 CPPA belonged to the same ST773 clone, an emerging NDM-linked HRC that is increasingly reported worldwide. Interestingly, these NDM-1 CPPA were recovered from different patients at 3 geographically distanced hospitals, and two of the carriers had travelled to Ukraine or to Morocco, suggesting independent importations abroad.

Figure 29. Geographical distribution of carbapenemases among CPPA in 2023

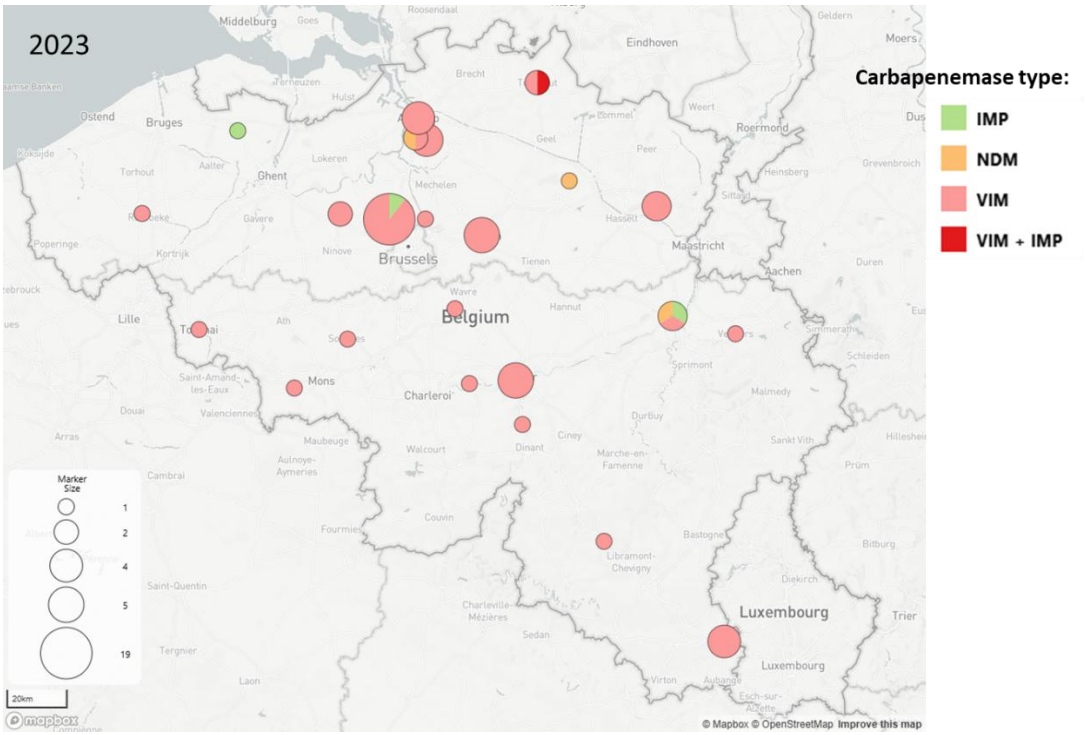
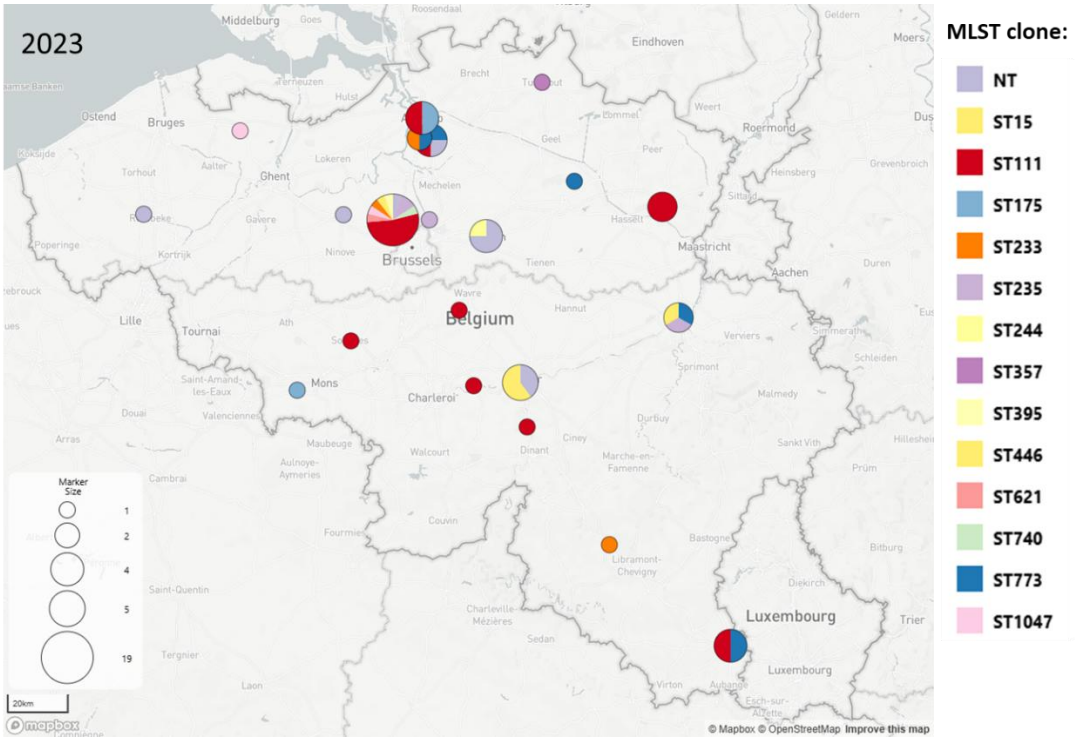


Figure 30. Geographical distribution of MLST clones among CPPA in 2023



3.2.3. 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 P. aeruginosa at the NRC for 2021-2023 (n=315)

%S/I_BMD_EUCAST Total n *P. aeruginosa*	NDM 7	VIM 104	IMP 7	non-CPPA 196	Total 315
Piperacillin/tazobactam	0%	1%	14%	29%	18%
Aztreonam	71%	75%	14%	36%	50%
Ceftazidime	0%	0%	0%	26%	16%
Cefepime	0%	3%	0%	27%	17%
Meropenem	0%	4%	0%	55%	34%
Ceftolozane/tazobactam	0%	1%	0%	57%	35%
Ceftazidime/avibactam	0%	0%	0%	59%	36%
Aztreonam/avibactam (n=47)	50%	23%	0%	27%	23%
Cefiderocol	17%	100%	100%	94%	94%
Ciprofloxacin	0%	7%	0%	31%	21%
Amikacin	0%	19%	14%	84%	57%
Fosfomycin (ECOFF)	57%	91%	100%	83%	86%
Colistin	100%	100%	100%	97%	98%

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 75% of VIM CPPA that do not express other resistance mechanisms to aztreonam. Recent beta-lactam/beta-lactam inhibitors are not active against CPPA. Ciprofloxacin and amikacin also displayed poor sensitivity rates as most CPPA carry 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 31. MIC distribution of carbapenemase-negative P. aeruginosa isolates for ceftolozane/tazobactam and for ceftazidime/avibactam

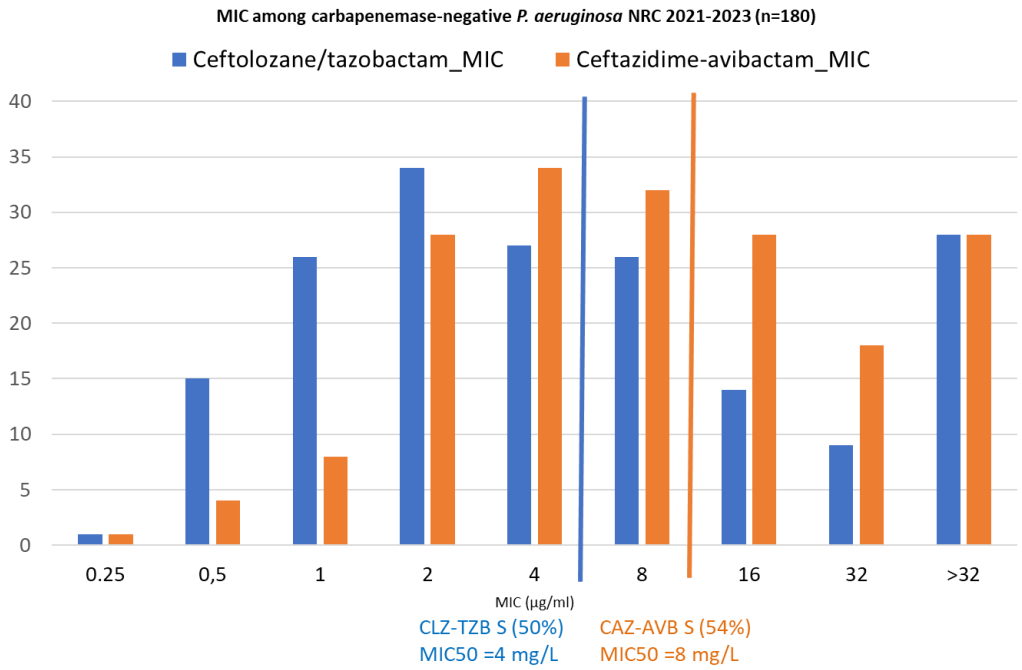
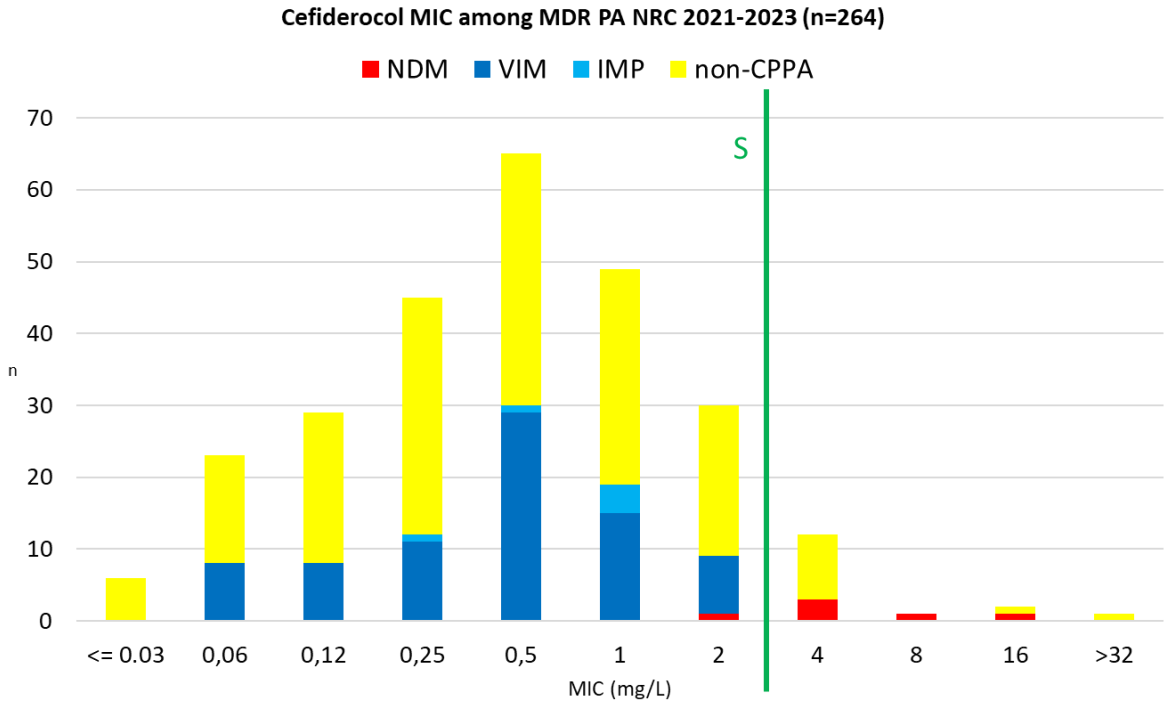


Figure 32. MIC distribution of multidrug-resistant P. aeruginosa isolates for cefiderocol



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.

Cefiderocol, the recently developed siderophore cephalosporin, retained high activity (>94%) overall against MDRPA, except for NDM producers (17%) which showed much higher MIC (2-16 mg/l). Aztreonam/avibactam had only limited activity (23% on CPPA

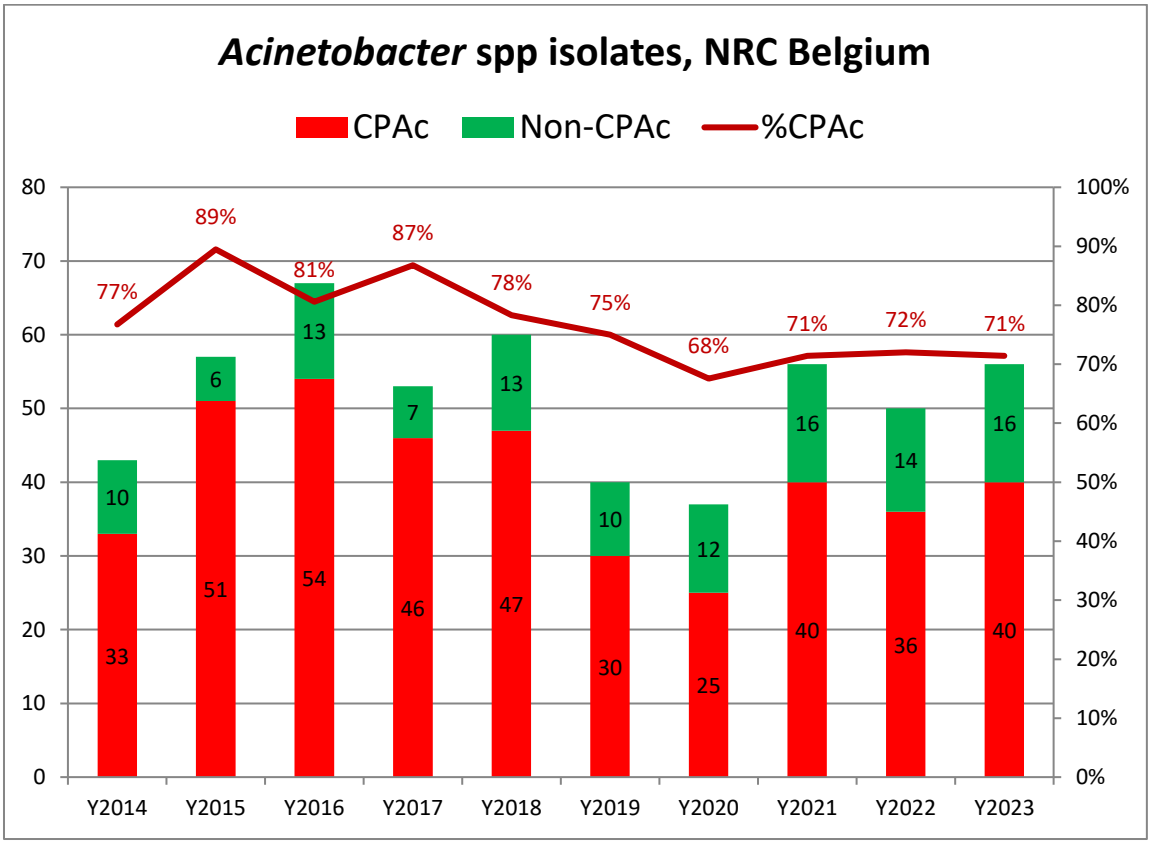
and 27% on non-CPPA) as avibactam cannot restore the activity of aztreonam if the strain is aztreonam resistant by other non-enzymatic resistance mechanisms such as efflux pumps, which are frequently overexpressed in *P. aeruginosa*.

Colistin retained the highest activity rate overall against MDRPA with resistance detected only among few (3%) sporadic 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*

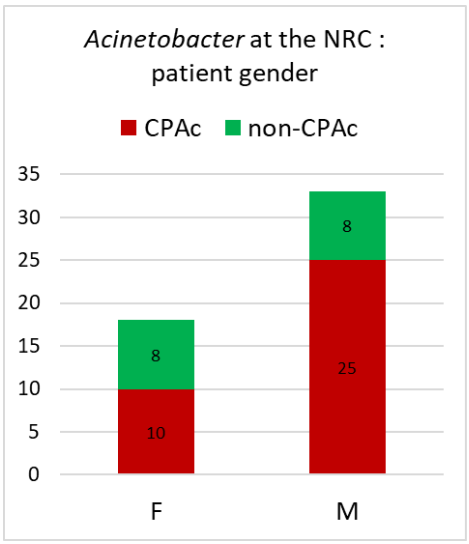
4.1. Characteristics of samples and patients related to isolates

Figure 33. Number of 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. The number remained stable and fluctuated over the past 10 years. In 2023, the NRC received 56 *Acinetobacter* isolates for confirmation of carbapenem resistance, but also 18 additional multi-susceptible isolates as control strains for surveillance. The proportion of confirmed carbapenemase producers (CPAc) have tended to decrease slightly in the past decade.

Figure 34. Number of Acinetobacter isolates per gender and per age group in 2023.



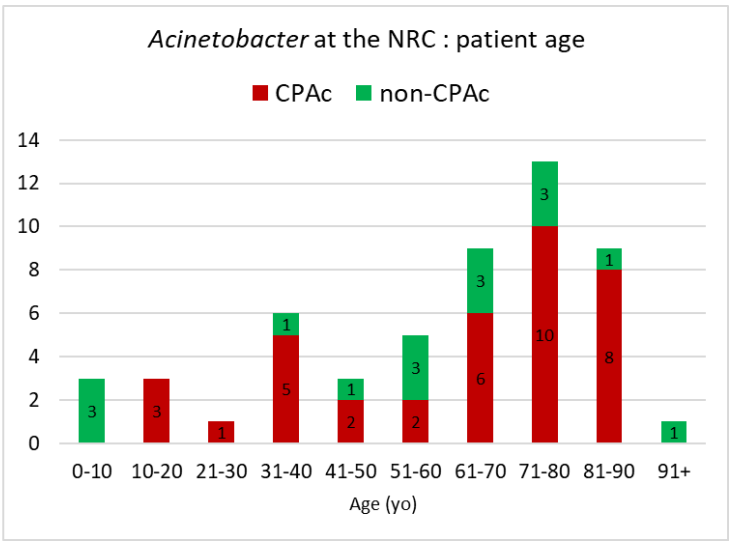
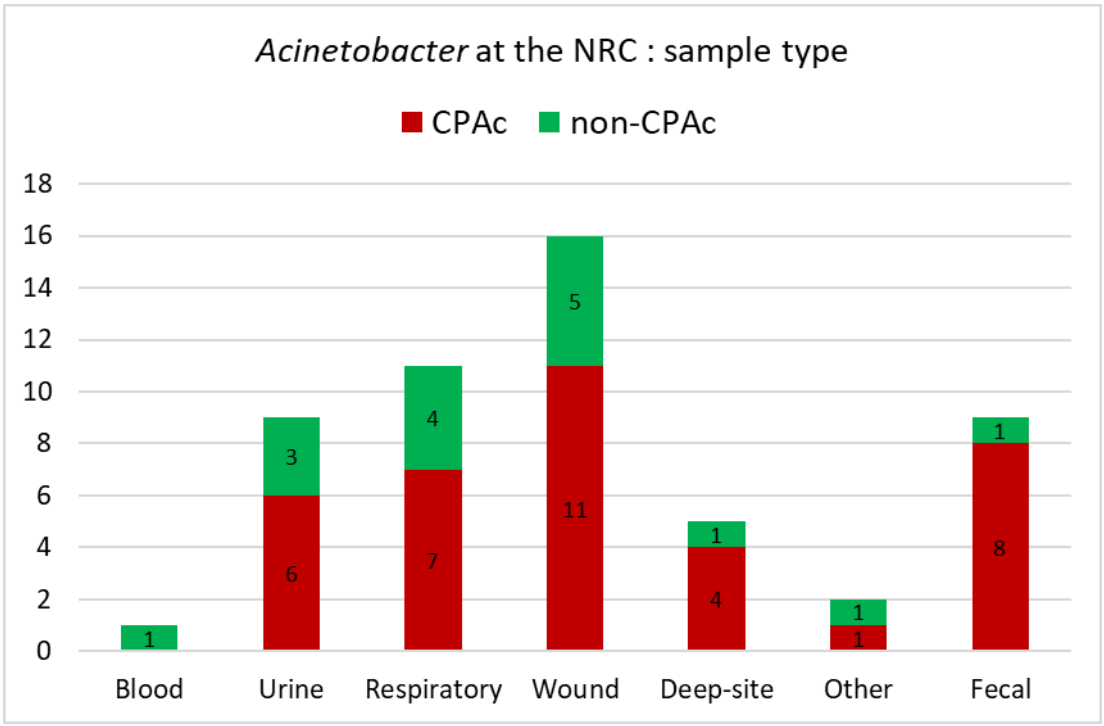


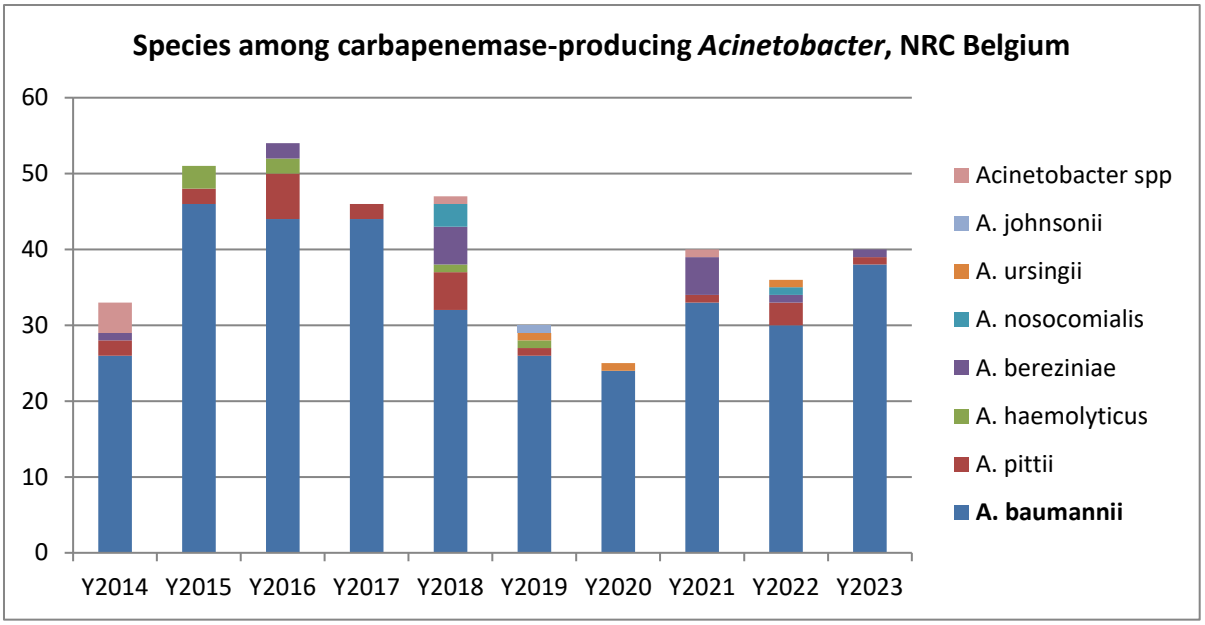
Figure 35. Sample types from which Acinetobacter were isolated in 2023. The 'deep site' category includes fluid and tissue specimens other than superficial or orifical sample sites. The 'other' category includes genital samples, percutaneous catheters and samples of unknown origin.

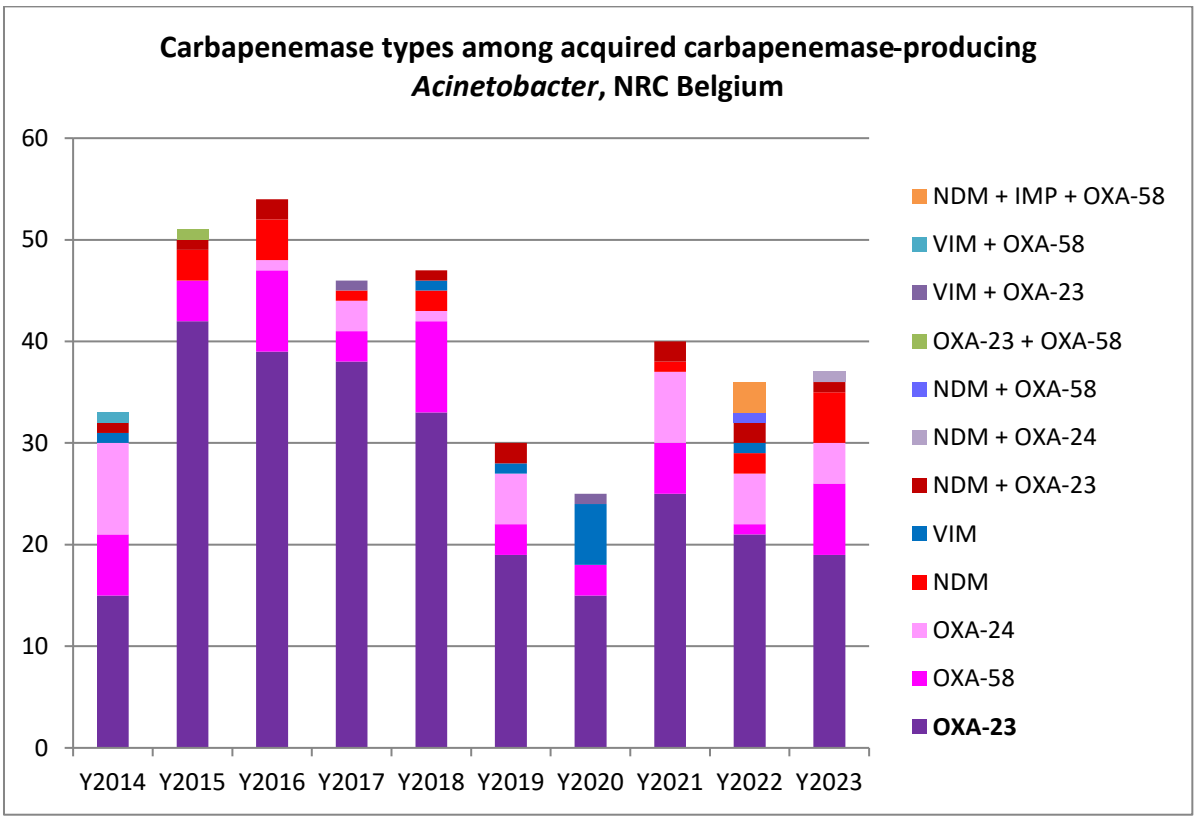


4.2. Carbapenemase-producing *Acinetobacter*

4.2.1. Bacterial species and resistance mechanisms

Figure 36. Species and carbapenemase types distribution of Acinetobacter isolates



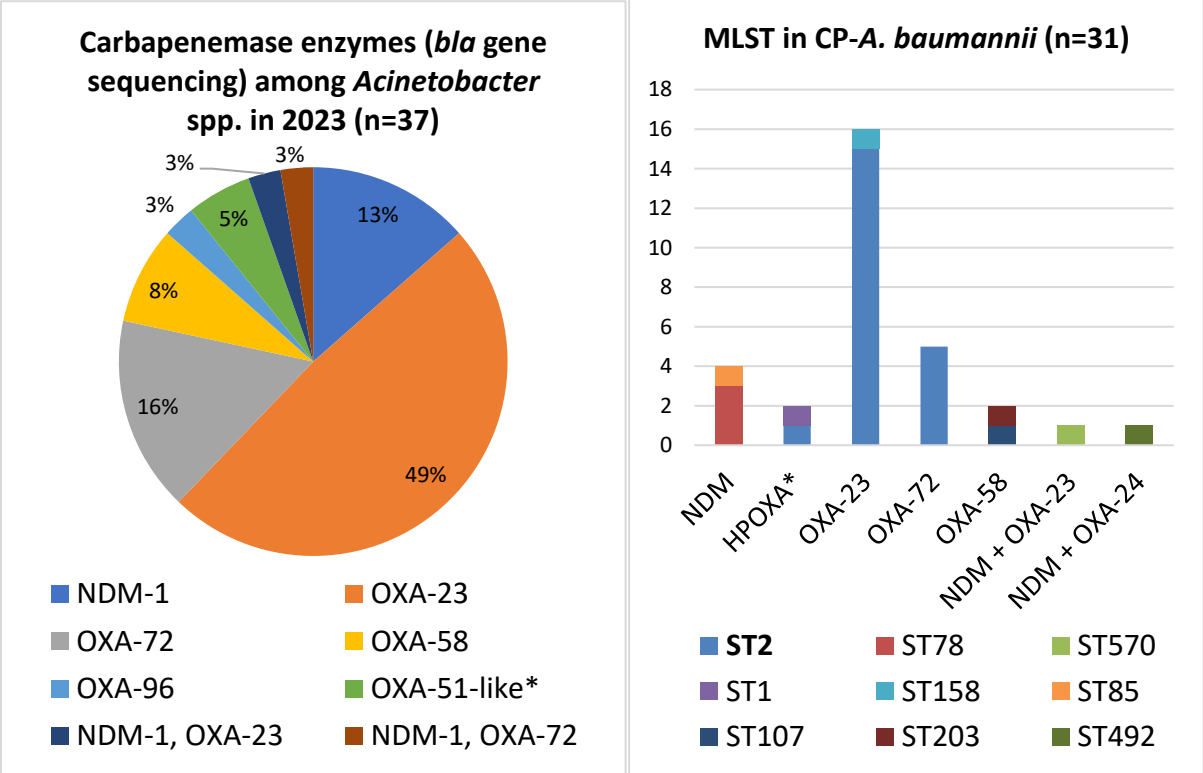


Among the 40 carbapenemase-producing *Acinetobacter* (CPAc) confirmed in 2023, *A. baumannii* represented by far the predominant species (95%). One OXA-24-producing *A. pittii* and one OXA-58-producing *A. bereziniae* were the only two other CPAc species detected in 2023. OXA-23 carbapenemase remained the predominant acquired carbapenemase family (49% in 2023) over the years, while OXA-58-type, OXA-24-type and NDM producers have been reported almost each year over the past years (n=7, n=4 and n=5 in 2023, respectively). CPAc coproducing NDM and OXA-23 have also been

detected nearly each year, and the first CPAc isolates associating NDM with OXA-58 or OXA-24 were confirmed in 2022 and 2023, respectively. VIM CPAc were sporadically detected, except a cluster of 5 VIM-producing *A. baumannii* 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 37. Carbapenemase enzymes (sequenced bla gene) among CPAc and the MLST clonal distribution of carbapenemase-producing A. baumannii (CPPA) in 2023



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

Figure 38. Geographical distribution of carbapenemases among CPAB in 2023

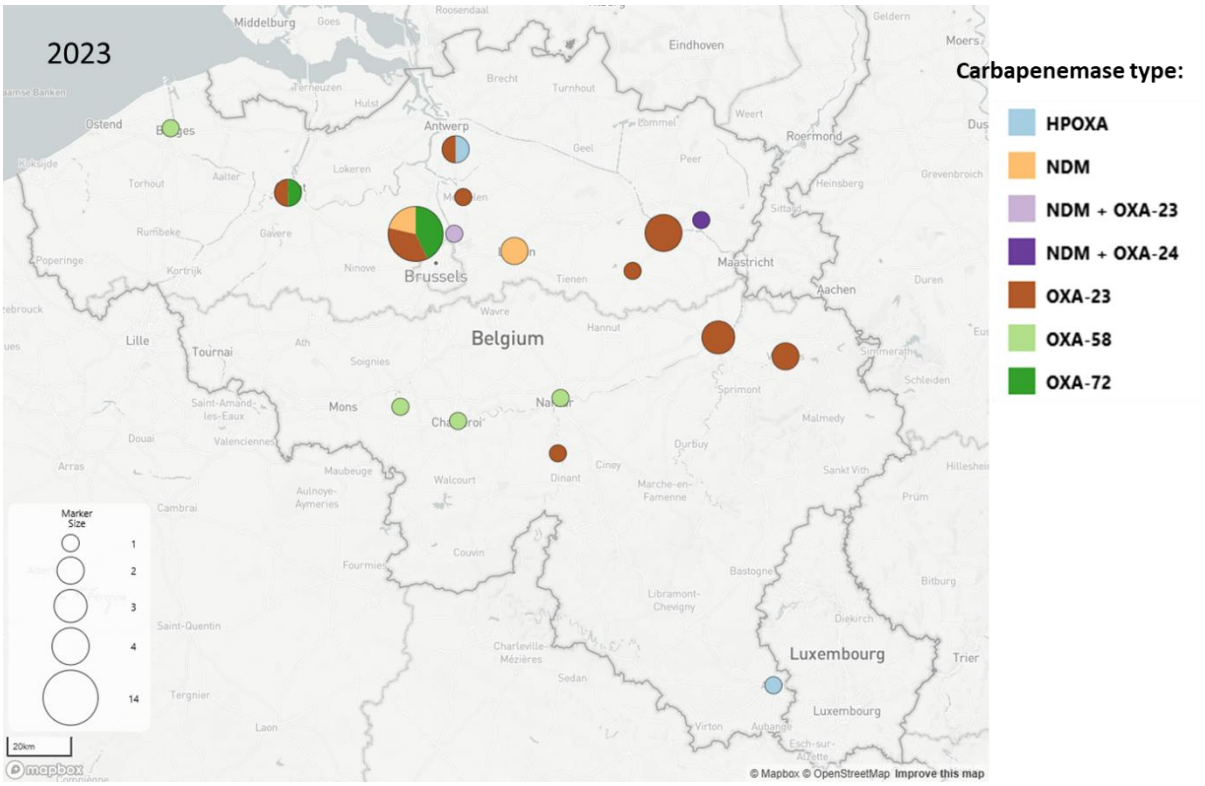
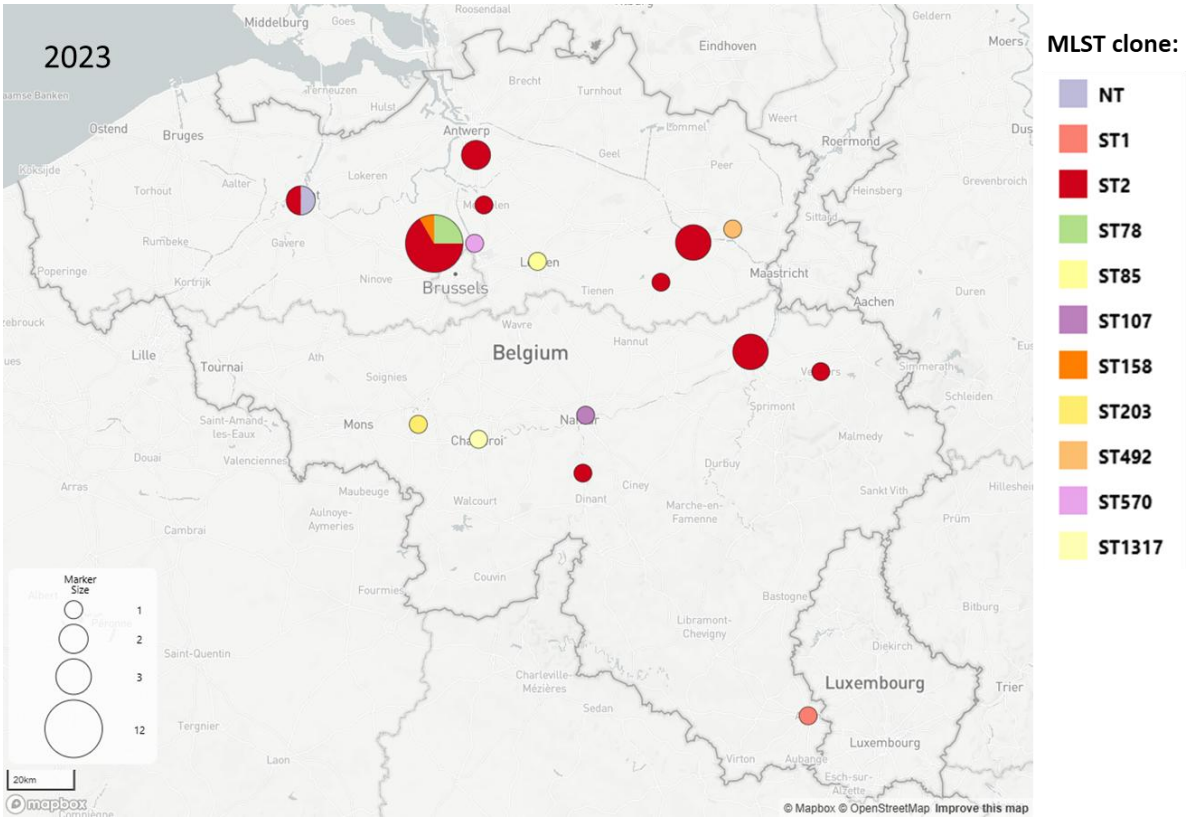


Figure 39. Geographical distribution of MLST clones among CPAB in 2023



Among the 37 CPAc genome-sequenced, OXA-23 (49%), OXA-72 (OXA-24-like enzyme, 16%), NDM-1 (14%), OXA-58 (8%) were the main acquired carbapenemase enzymes identified, while OXA-96 (an OXA-58-like variant), NDM-1 + OXA-23 and NDM-1 + OXA-72

were produced by one isolate each. Of note, in two *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 31 carbapenemase-producing *A. baumannii* (CPPA) determined for their MLST genotype (Pasteur nomenclature), 31 isolates belonged to 9 different MLST (4 isolates were not typeable (NT)) and ST2 were by far the predominant clone (60%), well-recognized as the most prevalent international high-risk clone (HRC) in Europe. Three of the 4 NDM-1 CPAB isolates (which expressed an additional PER-7 ESBL enzyme) belonged to a same clone (ST78) and were recovered from a cluster of 3 patients hospitalized in a same intensive care unit of a hospital.

4.2.3. Antimicrobial susceptibility

Table 4. 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 2019-2023 (n=132)

%S/I_BMD_EUCAST Total n *Acinetobacter* spp.	OXA-23-like 85	OXA-24-like 14	OXA-58-like 9	VIM 5	NDM 5	Multiple* 12	Total 132
Meropenem	0%	7%	44%	0%	0%	0%	4%
Cefiderocol PKPD (n=77)	79%	100%	50%	NA	40%	67%	77%
Ciprofloxacin	1%	14%	44%	80%	20%	25%	11%
Gentamicin	12%	29%	44%	0%	20%	0%	14%
Amikacin	7%	29%	67%	0%	80%	17%	18%
Tigecycline PKPD	21%	43%	44%	100%	80%	67%	34%
Colistin	86%	100%	78%	60%	80%	100%	87%

* Includes NDM+OXA-23 (n=6), NDM+OXA-72 (n=1), NDM+OXA-58 (n=1), VIM+OXA-23 (n=1), NDM+IMP+OXA-23 (n=3)

Nearly all the CPAc were highly resistant to meropenem, except 44% 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 PKPD breakpoint of 0.5 mg/l) against OXA-23-like CPAc, while there more variable activity for these classes against the other types of carbapenemase.

For cefiderocol (using PKPD breakpoint of 2 mg/l), the in vitro activity reached 79-100% against OXA-23-like and OXA-24-like 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.

Colistin retained a globally high activity rate (87%) against CPAc without notable differences between carbapenemase types produced.

Figure 40. MIC distribution of carbapenemase-producing Acinetobacter for cefiderocol