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# Activity of meropenem/vaborbactam and comparators against non-carbapenemase-producing carbapenem-resistant Enterobacterales isolates from Europe

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**Background:** Carbapenem-resistant Enterobacterales (CRE) isolates have disseminated worldwide. CREs usually produce a carbapenemase; however, some isolates are negative for known carbapenemases. In this study, we evaluated the activity of meropenem/vaborbactam and comparators against CREs without a carbapenemase (nonCP CREs) collected from European hospitals from 2016 to 2019.

**Materials and methods:** 23 043 Enterobacterales clinical isolates were collected in 41 hospitals located in 20 countries. Susceptibility (S) testing was performed using the broth microdilution method. CLSI/EUCAST (2021) interpretive criteria were used. 978 CREs were identified with MICs >2 mg/L to meropenem or imipenem. Whole-genome sequencing was performed on each CRE isolate. 125 isolates were negative for carbapenemase genes, including $bla_{KPC}$, $bla_{NDM}$, $bla_{IMP}$, $bla_{VIM}$ and $bla_{OXA-48-like}$. NonCP CRE isolates were analysed for the presence of other β-lactamases, multilocus sequence types (ST) and mutations in outer membrane protein (OMP) sequences.

**Results:** Most nonCP CRE were *Klebsiella pneumoniae* (KPN; $n = 97/125$). 84.0% of nonCP CRE ($n = 105$) were from Poland, including 88 KPN. The most common β-lactamase was $bla_{CTX-M-15}$ in 92/125 isolates. OMP disruptions or alterations were noted among 76 KPN. Among KPN isolates that had MLST typing, 30 belonged to ST11, 18 to ST152 and 17 to ST147, while 13 other STs were observed. Susceptibility to meropenem/vaborbactam was 96.0/97.6% (CLSI/EUCAST) while meropenem was 2.4/8.0%S.

**Conclusions:** Meropenem/vaborbactam had potent *in vitro* activity against CRE isolates that lacked known carbapenemases. Resistance mechanisms observed among nonCP CREs included acquired β-lactamases and OMP alterations. These results indicate that meropenem/vaborbactam may be a useful treatment for infections caused by nonCP CREs.

## Introduction

Infections caused by antimicrobial-resistant bacterial pathogens were globally associated with 4.95 million deaths in 2019, and resistance to first-line therapies is continuing to increase.[1] Carbapenems have been a common first-line therapy for serious Gram-negative infections; as a result, carbapenem-resistant Enterobacterales (CRE) isolates are a growing global concern.[2,3] Among the carbapenemases detected in Enterobacterales species, *Klebsiella pneumoniae* serine carbapenemases (KPCs) have disseminated worldwide and are now endemic in many hospitals across a wide range of countries.[4,5] Metallo-β-lactamases have

also spread globally, with New Dehli metallo-β-lactamase (NDM) the most common metallo-β-lactamase.[6] Isolates producing Class D OXA-48 carbapenemases are also increasingly common in Europe.[6] Some CRE isolates do not produce a known carbapenemase and are referred to as non-carbapenemase-producingCRE (nonCP CRE).[7,8] These isolates usually produce multiple acquired β-lactamases, may have increased expression of chromosomal cephalosporinases and/or possess outer membrane protein (OMP) dysfunction.[9]

In response to increasing numbers of CREs, β-lactam/β-lactamase inhibitor combinations with activity against serine carbapenemases, meropenem/vaborbactam, ceftazidime/avibactam

and imipenem/relebactam were developed and approved for use in the USA and Europe.[10–12] Vaborbactam is a cyclic boronic acid β-lactamase inhibitor that was developed to inhibit Ambler class A serine carbapenemases, including KPCs and class C β-lactamases. When combined with meropenem, vaborbactam restored the activity of this carbapenem against KPC-producing isolates in comparison to meropenem alone. Vaborbactam, like other currently approved β-lactamase inhibitors, has no activity against class B metallo-β-lactamases.[13–15] Meropenem/vaborbactam has been approved in Europe for the treatment of the following infections in adults: complicated urinary tract infection (cUTI), including acute pyelonephritis; complicated intra-abdominal infection (cIAI); hospital-acquired bacterial pneumonia and ventilator-associated pneumonia; as well as bacteraemia (BSI) occurring in association with or suspected to be associated with any of the infections listed before.[11] Meropenem/vaborbactam is also approved by the European Medicines Agency for the treatment of infections due to aerobic Gram-negative organisms in adults with limited treatment options. The US FDA has approved meropenem/vaborbactam for treatment of cUTI, including pyelonephritis.[12]

In this study, we evaluated the activity of meropenem/vaborbactam and comparators against nonCP CREs collected from European hospitals from 2016 to 2019. We determined other possible mechanisms of carbapenem resistance, including presence of acquired β-lactamases and/or disruptions or alterations of OMPs.[9]

## Materials and methods

A total of 23 043 Enterobacterales clinical isolates were consecutively collected from 41 European hospitals in 20 countries over the 4-year period (2016–2019). Participating laboratories were asked to submit one isolate per patient per infection episode.[16] Each isolate was considered the probable cause of the infection by the submitting site. No medical chart reviews were performed. The number of sites per country ranged from 1 to 6.[17]

Susceptibility testing was performed using the broth microdilution method.[18] Clinical Laboratory and Standards Institute (CLSI, 2022) and European Committee for Antimicrobial Susceptibility Testing (EUCAST, 2022) interpretive criteria were used.[19,20] CLSI and EUCAST quality control organisms were tested as appropriate for the tested agents and all MIC results were within these specified ranges. The meropenem/vaborbactam EUCAST breakpoints are: susceptible ≤8 mg/L; no intermediate; and resistant, >8 mg/L, which reflects the higher dose of the meropenem component and the maximal inhibitory effect of the vaborbactam component. The CLSI breakpoints are: susceptible, ≤4 mg/L; intermediate, 8 mg/L; resistant, ≥16 mg/L.

There were 978 CREs identified using the criteria of an MIC >2 mg/L to doripenem, imipenem and/or meropenem as defined by CLSI.[19] Imipenem MIC values were not used to categorize *Proteus*, *Providencia* or *Morganella* spp.

Whole-genome sequencing was performed on each CRE isolate as previously described.[9,21,22] A total of 125 CRE isolates were identified that did not have known carbapenemase genes, including $bla_{KPC}$, $bla_{NDM}$, $bla_{IMP}$, $bla_{VIM}$ and $bla_{OXA-48-like}$. NonCP CRE isolates were analysed for the presence of other β-lactamases and mutations in the protein-coding regions of OMP, as previously described.[9,21] An OMP gene was considered disrupted when a premature stop codon was identified within the protein coding sequence, while other insertions or deletions were considered alterations.[13] Ninety-two of 97 nonCP CRE *K. pneumoniae* isolates were also analysed for their multilocus sequence type (ST) as previously described.[23]

## Results

The most common infections from which nonCP CRE were isolated were pneumonia in hospitalized patients ($n=37$), urinary tract infection (UTI; $n=26$), intra-abdominal infection (IAI; $n=23$) and bloodstream infection (BSI; $n=22$). Of the 978 CRE identified, 12.8% ($n=125$) of these isolates lacked a known carbapenemase gene. The nonCP CREs were *Klebsiella pneumoniae* ($n=97$, 77.6%), *Enterobacter cloacae* complex ($n=11$, 8.8%), 10 *K. aerogenes*, three *Escherichia coli*, two *Hafnia alvei*, one *K. oxytoca* and one *Serratia marcescens* (Table 1). 84.0% of nonCP CRE ($n=105$) were from Poland, including 90.7% of *K. pneumoniae* ($n=88$; Table 1).

Among the 92 *K. pneumoniae* isolates with an ST identified, 30 belonged to ST11, 18 to ST152 and 17 to ST147, but at least 13 other STs were observed (Table 2). Twenty-nine of 30 ST11 isolates were from Poland; other STs from Poland were ST152 ($n=18$) and ST147 ($n=17$). The distribution of STs by year did not

**Table 1.** Country and species distributions of nonCP CREs in Europe (2016–2019)

| Country/organism | Year | | | | Grand total |
|---|---|---|---|---|---|
| | 2016 | 2017 | 2018 | 2019 | |
| Belarus | | 1 | | | 1 |
| *Enterobacter cloacae* species complex | | 1 | | | 1 |
| France | | 1 | | | 1 |
| *Klebsiella aerogenes* | | 1 | | | 1 |
| Germany | 1 | | | | 1 |
| *Klebsiella aerogenes* | 1 | | | | 1 |
| Ireland | 1 | 2 | | | 3 |
| *Klebsiella aerogenes* | | 1 | | | 1 |
| *Klebsiella pneumoniae* | 1 | | | | 1 |
| Serratia *marcescens* | | 1 | | | 1 |
| Italy | | 1 | 2 | | 3 |
| *Klebsiella pneumoniae* | | 1 | 2 | | 3 |
| Poland | 35 | 28 | 20 | 22 | 105 |
| *Enterobacter cloacae* species complex | 6 | 1 | 2 | 1 | 10 |
| *Escherichia coli* | | 1 | | | 1 |
| *Hafnia alvei* | | 1 | 1 | | 2 |
| *Klebsiella aerogenes* | | | | 3 | 3 |
| *Klebsiella oxytoca* | | | 1 | | 1 |
| *Klebsiella pneumoniae* | 29 | 25 | 16 | 18 | 88 |
| Russia | | | 2 | 1 | 3 |
| *Klebsiella pneumoniae* | | | 2 | 1 | 3 |
| Spain | | | | 2 | 2 |
| *Klebsiella aerogenes* | | | | 2 | 2 |
| Turkey | | 1 | | 3 | 4 |
| *Escherichia coli* | | | | 2 | 2 |
| *Klebsiella pneumoniae* | | 1 | | 1 | 2 |
| UK | 1 | 1 | | | 2 |
| *Klebsiella aerogenes* | 1 | 1 | | | 2 |
| Grand Total | 38 | 35 | 24 | 28 | 125 |

**Table 2.** *K. pneumoniae* multilocus sequence type (MLST) distribution of nonCP CRE isolates by country and year

| | Year | | | | |
|---|---|---|---|---|---|
| Country/MLST | 2016 | 2017 | 2018 | 2019 | Grand total |
| Ireland | 1 | | | | 1 |
| 25 | 1 | | | | 1 |
| Italy | | 1 | 2 | | 3 |
| 13 | | | 1 | | 1 |
| 307 | | | 1 | | 1 |
| 377 | | 1 | | | 1 |
| Poland | 27 | 23 | 15 | 18 | 83 |
| 11 | 12 | 7 | 4 | 6 | 29 |
| 15 | | | | 1 | 1 |
| 76 | | 2 | | | 2 |
| 101 | 3 | 2 | 1 | 1 | 7 |
| 147 | 4 | 6 | 3 | 4 | 17 |
| 152 | 7 | 3 | 5 | 3 | 18 |
| 196 | | 1 | | | 1 |
| 392 | 1 | 2 | 1 | 2 | 6 |
| 437 | | | 1 | 1 | 2 |
| Russia | | | 2 | 1 | 3 |
| 11 | | | 1 | | 1 |
| 23 | | | 1 | | 1 |
| 86 | | | | 1 | 1 |
| Turkey | | 1 | | 1 | 2 |
| 25 | | | | 1 | 1 |
| 1593 | | 1 | | | 1 |
| Grand total | 28 | 25 | 19 | 20 | 92 |

indicate changes in prevalence of the most common STs. There was a slight decrease in the number of nonCP CREs through the study period from 28 in 2016 to 20 in 2019 (Table 2).

OMP disruptions or alterations, as determined by the presence of premature stop codons or insertions and/or deletions in the protein coding sequences, were noted mostly among *K. pneumoniae*. Seventy-six *K. pneumoniae* had OMP disruptions or alterations: 24 isolates had disruptions of both OmpK35 and OmpK36, six had only OmpK35 disrupted, 44 had only OmpK36 disrupted and two had only OmpK35 alterations. There were four *E. cloacae* complex, one *H. alvei* and one *K. aerogenes* with disrupted OmpC and/or OmpF.

The susceptibilities of the nonCP CRE are shown in Table 3. Meropenem/vaborbactam susceptibility was 96.0/97.6% (CLSI/EUCAST) while susceptibility to meropenem was 2.4/8.0% (CLSI/EUCAST; Table 3). Susceptibility to imipenem was higher than meropenem at 28.0/48.8% (CLSI/EUCAST; Table 3). Three isolates were resistant to meropenem/vaborbactam (MIC ≥16 mg/L); two of the three were *K. pneumoniae* and had alterations or disruptions in both OmpK35 and 36 (Table 4). These *K. pneumoniae* isolates, both ST-76 from Poland, also contained $bla_{CTX-M-15}$, $bla_{SHV-12}$, $bla_{OXA-1}$, $bla_{OXA-10}$ and $bla_{TEM-57}$. The third meropenem/vaborbactam-resistant isolate, from the UK, was a *K. aerogenes* with TEM-1, chromosomal AmpC and an OmpC disruption (Table 4).

Multiple acquired β-lactamases were detected in the nonCP CRE as shown in Table 4. Overall, 72.8% of these isolates carried $bla_{CTX-M-15}$, including 86 of 97 *K. pneumoniae* isolates. Other β-lactamases commonly identified were $bla_{SHV-1, SHV-11, SHV-12}$; $bla_{OXA-1\_OXA-30}$ and $_{OXA-9}$; $bla_{TEM-1}$ and $_{TEM-57}$; and plasmid-

**Table 3.** Activity of meropenem/vaborbactam and comparator antimicrobial agents tested against 125 CRE, nonCP European isolates (2016–2019)

| | | mg/L | | | CLSI[a] | | | EUCAST[a] | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Antimicrobial agent | No. of isolates | $MIC_{50}$ | $MIC_{90}$ | MIC range | %S | %I | %R | %S | %SIE | %R |
| Meropenem/vaborbactam | 125 | 1 | 4 | 0.03 to 16 | 96.0 | 1.6 | 2.4 | 97.6 | | 2.4 |
| Meropenem | 125 | 8 | 16 | 0.12 to 32 | 2.4 | 5.6 | 92.0 | 8.0[b] | 80.0 | 92.0 |
| | | | | | | | | 8.0[c] | | 12.0 |
| Imipenem | 125 | 4 | >8 | 0.5 to >8 | 28.0 | 20.8 | 51.2 | 48.8 | 24.0 | 27.2 |
| Amikacin | 125 | 8 | 32 | 0.5 to >32 | 82.4 | 8.0 | 9.6 | 65.6[d] | | 34.4 |
| Aztreonam | 125 | >16 | >16 | 2 to >16 | 3.2 | 1.6 | 95.2 | 0.0 | 3.2 | 96.8 |
| Cefepime | 125 | >16 | >16 | 0.5 to >16 | 3.2 | 10.4[e] | 86.4 | 0.8 | 8.8 | 90.4 |
| Ceftazidime | 125 | >32 | >32 | 2 to >32 | 2.4 | 0.8 | 96.8 | 0.0 | 2.4 | 97.6 |
| Colistin | 123[f] | 0.25 | >8 | ≤0.06 to >8 | [g] | 74.8 | 25.2 | 74.8 | | 25.2 |
| Gentamicin | 125 | 2 | >8 | ≤0.12 to >8 | 56.8 | 0.8 | 42.4 | 56.0[d] | | 44.0 |
| Levofloxacin | 125 | >4 | >4 | ≤0.03 to 4 | 11.2 | 5.6 | 83.2 | 11.2 | 5.6 | 83.2 |
| Piperacillin-tazobactam | 125 | >64 | >64 | 8 to >64 | 1.6 | 13.6 | 84.8 | 0.8 | | 99.2 |

[a]Criteria as published by CLSI (2022) and EUCAST (2022). SIE, susceptible increased exposure.
[b]Using meningitis breakpoints (≤2 mg/L susceptible and >2 mg/L resistant).
[c]Using non-meningitis breakpoints (≤2 susceptible, 4–8 mg/L intermediate, >8 mg/L resistant).
[d]For infections originating from the urinary tract. For systemic infections, aminoglycosides must be used in combination with other active therapy.
[e]Intermediate is interpreted as susceptible-dose dependent.
[f]2 *K. pneumoniae* isolates did not have a colistin MIC and were not retested.
[g]CLSI does not have a susceptible breakpoint for colistin.
Organisms include *Enterobacter cloacae* species complex (11), *Escherichia coli* (3), *Hafnia alvei* (2), *Klebsiella aerogenes* (10), *K. oxytoca* (1), *K. pneumoniae* (97) and *Serratia marcescens* (1).

**Table 4.** List of β-lactam resistance mechanisms correlated with meropenem/vaborbactam MIC values for all isolates

| OMP disruptions | Meropenem/vaborbactam MIC (mg/L) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| β-lactamases present | 0.03 | 0.06 | 0.12 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | Grand total |
| OMP K36 disrupted/K35 disrupted or altered | 1 | | 1 | 1 | 14 | 26 | 14 | 10 | | | 67 |
| CMY-2 | | | 1 | | | | | | | | 1 |
| CMY-48-like, CTX-M-15, SHV-11, TEM-1 | | | | | | | 1 | | | | 1 |
| CTX-M-15, CTX-M-15-like, CTX-M-3-like, DHA-1, OXA-1_OXA-30, SHV-11, TEM-32 | | | | | | 1 | | | | | 1 |
| CMY-16, CTX-M-15, OXA-10, OXA-1_OXA-30, SHV-1, TEM-1 | | | | | | | 1 | | | | 1 |
| CTX-M-15, CTX-M-9, OXA-1_OXA-30, SHV-11, SHV-12, TEM-1 | | | | | | | | 1 | | | 1 |
| CTX-M-15, DHA-1, OXA-1_OXA-30, OXA-9, SHV-11, TEM-1 | | | | | 1 | 1 | | | | | 2 |
| CTX-M-15, DHA-1, OXA-1_OXA-30, SHV-1 | | | | | | | | 1 | | | 1 |
| CTX-M-15, DHA-1, OXA-1_OXA-30, SHV-11 | | | | | 1 | 5 | 1 | | | | 7 |
| CTX-M-15, DHA-1, OXA-1_OXA-30, SHV-11, TEM-1 | | | | | 1 | 1 | | | | | 2 |
| CTX-M-15, DHA-1, OXA-9, SHV-11, TEM-1 | | | | | 2 | 1 | 2 | | | | 5 |
| CTX-M-15, DHA-1, SHV-11 | | | | | 1 | | | 1 | | | 2 |
| CTX-M-15, OXA-1_OXA-30-like, SHV-11, TEM-1 | | | | | | | | 1 | | | 1 |
| CTX-M-15, OXA-1_OXA-30, OXA-9, SHV-1, TEM-1 | | | | | | | | 1 | | | 1 |
| CTX-M-15, OXA-1_OXA-30, SHV-1 | | | | | 1 | 1 | 1 | | | | 3 |
| CTX-M-15, OXA-1_OXA-30, SHV-1, SHV-11, TEM-1 | | | | | | | 1 | | | | 1 |
| CTX-M-15, OXA-1_OXA-30, SHV-1, TEM-1 | | | | | 4 | 5 | 1 | | | | 10 |
| CTX-M-15, OXA-1_OXA-30, SHV-11 | | | | | | 1 | | | | | 1 |
| CTX-M-15, OXA-1_OXA-30, SHV-11, SHV-155-like, TEM-1 | | | | | | | 1 | | | | 1 |
| CTX-M-15, OXA-1_OXA-30, SHV-11, TEM-1 | | | | | | 3 | 4 | 2 | | | 9 |
| CTX-M-15, OXA-9, SHV-12, SHV-28, TEM-1 | 1 | | | | | | | | | | 1 |
| CTX-M-15, SHV-1 | | | | | | 1 | | | | | 1 |
| CTX-M-15, SHV-1, TEM-1 | | | | | | 2 | 1 | | | | 3 |
| CTX-M-15, SHV-11 | | | | | 1 | | | 1 | | | 2 |
| CTX-M-15, SHV-11, TEM-1 | | | | | | 1 | | 1 | | | 2 |
| CTX-M-27, DHA-1, SHV-12 | | | | | | 1 | | | | | 1 |
| CTX-M-3, DHA-1, OXA-1_OXA-30, OXA-9, SHV-11, TEM-1 | | | | | 1 | | | | | | 1 |
| CTX-M-3, OXA-1_OXA-30, SHV-1 | | | | | | 1 | | | | | 1 |
| CTX-M-3, OXA-9, SHV-11, TEM-1 | | | | | | 1 | | | | | 1 |
| CTX-M-33, OXA-1_OXA-30, SHV-11 | | | | | | | | 1 | | | 1 |
| DHA-1, OXA-1_OXA-30, SHV-11 | | | | | 1 | | | | | | 1 |
| DHA-1, SHV-11 | | | | 1 | | | | | | | 1 |
| SHV-11, TEM-1 | | | | | | | 1 | | | | 1 |
| No Omp disruptions or alterations | | | 1 | 2 | 10 | 18 | 6 | 4 | | | 41 |
| CMY-2, TEM-1 | | | | | | | 1 | | | | 1 |
| CTX-M-15, DHA-1, OXA-1_OXA-30, OXA-9, SHV-11, TEM-1 | | | | | 1 | 1 | | 1 | | | 3 |
| CTX-M-15, OXA-1_OXA-30-like, SHV-11, TEM-1 | | | | | | 1 | | | | | 1 |
| CTX-M-15, OXA-1_OXA-30, OXA-9, SHV-1 | | | | | | | 1 | | | | 1 |
| CTX-M-15, OXA-1_OXA-30, OXA-9, SHV-11, TEM-1 | | | | | | 1 | | | | | 1 |
| CTX-M-15, OXA-1_OXA-30, SHV-1 | | | | | | 1 | | | | | 1 |
| CTX-M-15, OXA-1_OXA-30, SHV-1, TEM-1 | | | | | | 2 | | | | | 2 |
| CTX-M-15, OXA-1_OXA-30, SHV-11 | | | | | | | | 1 | | | 1 |
| CTX-M-15, OXA-1_OXA-30, SHV-11, TEM-1 | | | | | | 3 | 1 | | | | 4 |
| CTX-M-15, OXA-1_OXA-30, SHV-110, TEM-1 | | | | | | 1 | | | | | 1 |
| CTX-M-15, OXA-1_OXA-30, SHV-28 | | | | | 1 | | | | | | 1 |
| CTX-M-15, OXA-1_OXA-30, TEM-1 | | | | | 1 | 2 | | | | | 3 |
| CTX-M-15, SHV-11 | | | | | | 2 | 1 | | | | 3 |
| CTX-M-15, SHV-11, TEM-1 | | | | | 1 | | | | | | 1 |
| CTX-M-3, TEM-1, TEM-1-like | | | | 1 | | | | | | | 1 |

*Continued*

**Table 4.** *Continued*

| OMP disruptions | Meropenem/vaborbactam MIC (mg/L) | | | | | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| β-lactamases present | 0.03 | 0.06 | 0.12 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | Grand total |
| CTX-M-33, DHA-1, SHV-11 | | | | | | 1 | | | | | 1 |
| OXA-1_OXA-30 | | | | | | 2 | | 2 | | | 4 |
| SRT-like | | | 1 | | | | | | | | 1 |
| (no acquired β-lactamases detected) | | | | 1 | 6 | 1 | 2 | | | | 10 |
| Only Omp K35 disrupted | | | | 1 | 2 | | 2 | 1 | | 1 | 7 |
| CTX-M-15, DHA-1, OXA-1_OXA-30, OXA-9, SHV-1, TEM-1 | | | | | | | 1 | | | | 1 |
| CTX-M-15, DHA-1, OXA-9, SHV-1 | | | | 1 | | | | | | | 1 |
| CTX-M-15, OXA-1_OXA-30, OXA-9, SHV-1 | | | | | 1 | | | | | | 1 |
| CTX-M-15, OXA-1_OXA-30, OXA-9, SHV-1, TEM-1 | | | | | | | 1 | | | | 1 |
| CTX-M-15, OXA-1_OXA-30, SHV-1 | | | | | 1 | | | | | | 1 |
| CTX-M-15, OXA-10, OXA-1_OXA-30, SHV-1, SHV-12, TEM-57 | | | | | | | | | | 1 | 1 |
| CTX-M-2, OXA-2, TEM-1 | | | | | | | | 1 | | | 1 |
| Only Omp K35 alterations | | | | 1 | | | 1 | | | 1 | 3 |
| CTX-M-15, OXA-10, OXA-1_OXA-30, SHV-12, TEM-57 | | | | | | | | | | 1 | 1 |
| DHA-1, OXA-1_OXA-30, SHV-11 | | | | 1 | | | | | | | 1 |
| (no β-lactamases detected) | | | | | | | 1 | | | | 1 |
| OmpC/F disrupted | | | | | | | 2 | 1 | 1 | 1 | 5 |
| CTX-M-15, OXA-1_OXA-30 | | | | | | | 1 | | | | 1 |
| TEM-1 | | | | | | | | | | 1 | 1 |
| (no acquired β-lactamases detected) | | | | | | | 1 | 1 | 1 | | 3 |
| OmpC/F alterations | | | | | | | | | | 1 | 1 |
| CTX-M-15, OXA-1_OXA-30, TEM-1 | | | | | | | | | | 1 | 1 |
| Grand total | 1 | | 2 | 5 | 26 | 44 | 26 | 16 | 2 | 3 | 125 |

mediated AmpC $bla_{DHA-1}$. 96.8% of the nonCP CRE isolates had two or more acquired β-lactamases. Of the 10 nonCP CRE *K. aerogenes*, only one had an acquired β-lactamase, $bla_{TEM-1}$ (Table 4). The other nine *K. aerogenes* without acquired β-lactamases detected had imipenem MIC values of 4–8 mg/L, meropenem MIC values of 2–4 mg/L and did not have OMP disruption. Three *E. cloacae* complex and two *H. alvei* also were negative for acquired β-lactamases.

The imipenem, meropenem and meropenem/vaborbactam MIC distributions of all isolates, and those with or without OMP disruptions or alterations, are shown in Table 5. All isolates with OMP dysfunction also produced one or more β-lactamase enzymes (Table 4). The inhibition of these β-lactamases by vaborbactam is demonstrated by the lower $MIC_{50/90}$ of meropenem/vaborbactam ($MIC_{50}$ and $_{90}$ values of 1 and 4 mg/L) compared to meropenem alone ($MIC_{50}$ and $_{90}$ values of 8 and 16 mg/L; Table 5). A correlation of meropenem and meropenem/vaborbactam MIC values is shown in Supplemental Figure S1 (available as Supplementary data at *JAC* Online). This correlation also demonstrates higher MIC values for meropenem for 120/125 isolates due to the presence of β-lactamases that are inhibited by vaborbactam. Isolates without OMP changes had lower $MIC_{50}$ and $_{90}$ values, with $MIC_{50/90}$ values of 1/2 mg/L to meropenem/vaborbactam and $MIC_{50/90}$ values of 4/8 mg/L to meropenem alone. The $MIC_{50/90}$ values suggest a contribution of OMP mutations to meropenem resistance in the presence of multiple β-lactamases.

The isolates in this study were mostly resistant to the other agents tested, including the β-lactams and piperacillin/tazobactam, with <5.0% susceptibility for each of these agents (Table 3). Susceptibility to levofloxacin was 11.2%. The most active comparators were colistin (74.8% susceptible, EUCAST) and amikacin (82.4/65.6%, CLSI/EUCAST).

## Discussion

In this collection of European nonCP CRE, *K. pneumoniae* was the most common species, accounting for 77.6% overall. Most isolates, including most of the *K. pneumoniae*, were from Poland. The nonCP CR *K. pneumoniae* from Poland were received throughout the 4-year period and contained nine different ST types, suggesting that this overall pattern was not an outbreak caused by a single strain. This is consistent with the EuSCAPE multinational surveillance on carbapenemase-producing *E. coli* and *K. pneumoniae* conducted from 2013 to 2014, where 88.2% of CR *K. pneumoniae* from Poland were negative for carbapenemases.[24] The most common clone in the current study was ST-11, which is considered an international high-risk clone. ST-11 was associated with an NDM-1 outbreak in Poland from 2012 to 2018.[25–27] The other two most frequent STs in Poland were ST-147 and ST-152. ST-147 has also been called an international high-risk clone with broad dissemination, particularly in the Mediterranean, and has been associated with NDM-1.[28] ST-152 was described initially in Saudi Arabia and more recently in Poland.[29–31]

**Table 5.** MIC distributions and cumulative % at MIC, of meropenem/vaborbactam, meropenem and imipenem tested against all nonCP CRE isolates, isolates with, and without OMP alterations or disruptions

| Organism/antimicrobial agent | MIC (mg/L) | | | | | | | | | | | | MIC$_{50}$ | MIC$_{90}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0.015 | 0.03 | 0.06 | 0.12 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | >$^a$ | | |
| **All nonCP CRE (n=125)** | | | | | | | | | | | | | | |
| Meropenem/vaborbactam | 0 | 1 | 0 | 2 | 5 | 26 | 44 | 26 | 16 | **2** | 3 | | 1 | 4 |
| | 0.0% | 0.8% | 0.8% | 2.4% | 6.4% | 27.2% | 62.4% | 83.2% | 96.0% | **97.6%** | 100.0% | | | |
| Meropenem | | | 0 | 1 | 0 | 0 | 2 | **7** | 51 | 49 | 13 | 2 | 8 | 16 |
| | | | 0.0% | 0.8% | 0.8% | 0.8% | 2.4% | **8.0%** | 48.8% | 88.0% | 98.4% | 100.0% | | |
| Imipenem | | | | | 0 | 9 | 26 | **26** | 30 | 17 | | 17 | 4 | >8 |
| | | | | | 0.0% | 7.2% | 28.0% | **48.8%** | 72.8% | 86.4% | | 100.0% | | |
| **Isolates with OMP alterations or disruptions (n=84)** | | | | | | | | | | | | | | |
| Meropenem/vaborbactam | 0 | 1 | 0 | 1 | 3 | 16 | 26 | 20 | 12 | **2** | 3 | | 1 | 4 |
| | 0.0% | 1.2% | 1.2% | 2.4% | 6.0% | 25.0% | 56.0% | 79.8% | 94.0% | **96.4%** | 100.0% | | | |
| Meropenem | | | | | | 0 | 2 | **3** | 29 | 37 | 11 | 2 | 8 | 16 |
| | | | | | | 0.0% | 2.4% | **6.0%** | 40.5% | 84.5% | 97.6% | 100.0% | | |
| Imipenem | | | | | 0 | 8 | 15 | **17** | 16 | 14 | | 14 | 4 | >8 |
| | | | | | 0.0% | 9.5% | 27.4% | **47.6%** | 66.7% | 83.3% | | 100.0% | | |
| **Isolates without OMP alterations or disruptions (n=41)** | | | | | | | | | | | | | | |
| Meropenem/vaborbactam | | | 0 | 1 | 2 | 10 | 18 | 6 | 4 | | | | 1 | 2 |
| | | | 0.0% | 2.4% | 7.3% | 31.7% | 75.6% | 90.2% | 100.0% | | | | | |
| Meropenem | | | 0 | 1 | 0 | 0 | 0 | **4** | 22 | 12 | 2 | | 4 | 8 |
| | | | 0.0% | 2.4% | 2.4% | 2.4% | 2.4% | **12.2%** | 65.9% | 95.1% | 100.0% | | | |
| Imipenem | | | | | 0 | 1 | 11 | **9** | 14 | 3 | | 3 | 2 | 8 |
| | | | | | 0.0% | 2.4% | 29.3% | **51.2%** | 85.4% | 92.7% | | 100.0% | | |

$^a$>, greater than highest dilution tested.
EUCAST susceptible breakpoints are indicated in bold font.

Resistance mechanisms observed among the nonCP CRE isolates in this study included multiple acquired β-lactamases and disruption of OmpC/F in *K. aerogenes* and *E. cloacae* or OmpK35/K36 in *K. pneumoniae*. Most isolates produced CTX-M-15. To determine whether this gene had a role in carbapenem resistance, in a previous study Castanheira *et al.* cloned and expressed CTX-M-15 in *E. coli*.[9] This experiment suggested that CTX-M-15 production alone was not sufficient to cause carbapenem resistance as meropenem MICs increased only 2-fold with wild-type CTX-M-15 expressed on a plasmid.[9] Other studies have looked at the contribution of OMP mutations to carbapanem resistance and found that disruption of both OmpK35 and OmpK36 were associated with an increase in meropenem MIC, although neither were sufficient to cause meropenem resistance.[32,33] Our data support the conclusion by Castanheira *et al.* that a combination of extended-spectrum β-lactamases with or without the presence of OMP disruption are capable of causing carbapenem resistance in the absence of a specific carbapenemase. The *K. aerogenes* that lacked an acquired β-lactamase or OMP changes suggest that the CRE phenotype in this species may be due to increased expression of chromosomal AmpC.[34]

In this study, we found that the susceptibility rate of imipenem was higher (28.0/48.8%, CLSI/EUCAST) than that of meropenem (2.4/8.0%, CLSI/EUCAST). The isolates that were imipenem susceptible and meropenem resistant had meropenem MIC values of 4–8 mg/L. The mechanism(s) for the differences in activities of imipenem and meropenem for these isolates is unknown. It is possible that meropenem was more susceptible to hydrolysis by the multiple β-lactamase enzymes produced by the isolates in this study. The three meropenem/vaborbactam resistant isolates in this study had either multiple β-lactamases and disrupted OmpK35-K36 (*K. pneumoniae*) or disrupted OmpC, TEM-1 and chromosomal AmpC (*K. aerogenes*), suggesting that both porin disruption and the production of multiple β-lactamases are needed for nonCP CRE to develop resistance to meropenem/vaborbactam.[9,33]

Our study has several limitations that should be noted. First, we cannot draw any conclusions regarding the prevalence of nonCP CRE in any one country or across Europe as a whole due to the small number of sites in each country from which the described isolates were submitted. Second, due to the lack of medical chart review, we do not know patient antimicrobial treatment or treatment outcomes. Third, we cannot rule out the possibility of outbreaks at any of these sites during the study period. Fourth, absence of known carbapenemase genes does not necessarily rule out the presence of unknown carbapenemases. Fifth, the identification of OMP disruptions/alterations were based on mutations in the protein-coding sequences only.

These results demonstrate meropenem/vaborbactam was the most active drug tested in this study against CRE isolates

that lack known carbapenemases, as 96.0/97.6% (CLSI/EUCAST) of these isolates were susceptible to meropenem/vaborbactam while only 2.4/8.0% were susceptible to meropenem alone. The activity of meropenem/vaborbactam against these isolates demonstrates that inhibition of the non-carbapenemase β-lactamases by vaborbactam restored the activity of meropenem. These *in vitro* results indicate that meropenem/vaborbactam may be a useful treatment for infections caused by CREs that lack a known carbapenemase.

## Funding

## Transparency declaration

## Supplementary data

Figure S1 is available as Supplementary data at *JAC* Online.

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