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Activity of Meropenem-Vaborbactam and Comparator Agents against Carbapenemase-Negative, Carbapenem-Resistant *Enterobacterales* from US Hospitals

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Introduction

- Carbapenemase-producing *Enterobacterales* (CRE) are a threat to human health.
- Most CRE isolates produce carbapenemases, but some of these isolates have a combination of increased production of β-lactamases with limited carbapenemase activity and alteration of outer membrane proteins and/or overexpressed efflux of the β-lactam.
- Carbapenemase-negative CRE isolates have been reported, but the activity of newer agents against these isolates is still not well understood.
- Meropenem-vaborbactam (MVB) is an important addition to the armamentarium to treat infections caused by CREs.
- We evaluated the in vitro activity of meropenem-vaborbactam and comparator agents against carbapenemase-negative CREs collected during 6 years of surveillance in US hospitals.

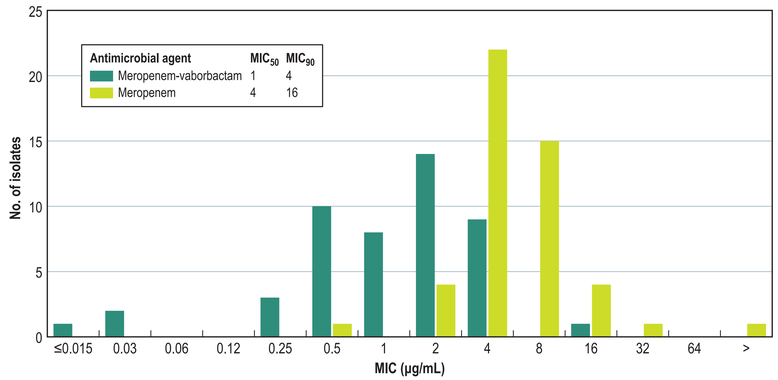
Materials and Methods

- A total of 27,968 *Enterobacterales* isolates were collected in US hospitals from 2014–2019.
  - Isolates were collected from infections in the bloodstream, intra-abdominal, skin/soft tissue, or urinary tract or from patients hospitalized with pneumonia.
  - Only 1 isolate per patient episode was included.
- Isolates were susceptibility tested against meropenem-vaborbactam and comparator agents using the reference broth microdilution method as described by the Clinical and Laboratory Standards Institute (CLSI) M07 (2018) and M100 (2021) documents.
- Quality control (QC) was performed according to the CLSI M100 (2021) criteria.
  - All QC MIC results were within acceptable ranges.
- Categorical interpretations for all comparator agents are from the CLSI M100 (2021) or the US Food and Drug Administration (FDA) website for tigecycline.
- Carbapenem-resistant *Enterobacterales* (CRE) isolates were defined as any isolate exhibiting doripenem, imipenem and/or meropenem MIC values of >2 µg/mL.
  - *Proteus mirabilis* and indole-positive Proteeae were categorized as CRE if doripenem and/or meropenem MIC values were at >2 µg/mL due to intrinsically elevated imipenem MIC values.
  - These isolates were submitted to β-lactamase screening.
- CRE isolates collected during 2014–2015 were screened for acquired carbapenemase encoding genes by PCR using custom primers.
  - Amplicons were sequenced on both strands, and nucleotide sequences obtained were analyzed using the Lasergene® software package (DNAStar; Madison, Wisconsin, USA) and compared to available sequences via NCBI BLAST search (http://www.ncbi.nlm.nih.gov/blast/)
- Whole Genome Sequencing was performed for isolates collected from 2016–2019 on a MiSeq (Illumina, San Diego, California, USA) instrument targeting a 30X coverage.
  - Sequences were *de novo* assembled.
  - Analysis of β-lactam resistance mechanisms was performed *in silico*.
  - Genes encoding resistance were searched using a curated library and a criteria of >94% sequencing identity and 40% minimum length coverage was applied.
- Selected species were evaluated for expression levels of the constitutive AmpC gene.
  - Expression levels were determined by in triplicate quantitative real-time PCR using high quality RNA samples, as previously described.
  - Transcription levels were considered significantly different if at least a 10-fold difference was noted compared with the baseline susceptible isolate.

Results

- Among 357 (1.3% of all isolates) CRE isolates identified during 6 years of surveillance, 48 (13.4% of the CRE) isolates did not produce known carbapenemases.
- Carbapenemase-negative CRE isolates belonged to 7 bacterial species/complex (Table 1).
  - The most common species were *K. pneumoniae* (16 isolates), followed by *E. cloacae* (9), *E. coli* (8), and *K. aerogenes* (8).
- Among the 27 isolates collected from 2016–2019 that were submitted to WGS, 15 harbored CTX-M encoding genes.
  - CTX-M-15 was observed in 12 isolates, including 9 of 10 *K. pneumoniae* and 2 of 5 *E. coli*.
  - CTX-M-14, CTX-M-71, and CTX-M-193 were also observed in 1 isolate each.
- One *P. mirabilis* isolates harbored the ESBL TEM-155 and 1 *E. coli* carried the gene encoding the transferrable AmpC CMY-2.
- Three *K. aerogenes* isolates, 3 *E. cloacae*, and 1 *C. freundii* overexpressed AmpC.
- Twenty isolates had OmpC/OmpK36 disrupted, including 15 isolates that displayed premature stop codons and 5 that had insertions and/or deletions.
- Three isolates exhibited OmpF/OmpK35 insertions and deletions and 7 other isolates showed premature stop codons with OmpC/OmpK36 disruptions.
- Eight *Klebsiella* spp. isolates had insertions and/or deletions in OmpK37.
- All but 1 isolate was inhibited by meropenem-vaborbactam at an MIC of 4 mg/L or 8 mg/L (Figure 1).
  - The only isolate displaying a resistant MIC for meropenem-vaborbactam was a *P. mirabilis* (MIC, 16 mg/L) collected in 2015.
  - Meropenem alone inhibited only 2.1% of the isolates.
- Forty-seven of 48 (97.9%) of the isolates tested were inhibited by meropenem-vaborbactam, the most active agent tested against these isolates (Figure 2).
  - Other β-lactams inhibited 4.2 to 14.6% of the isolates.
  - Among non-β-lactam comparator agents, tigecycline and amikacin inhibited 93.8 and 91.7% of the isolates, respectively, when applying CLSI or US FDA breakpoints. A total of 89.6% of the isolates had intermediate colistin MIC values.

Figure 1. MIC distributions for meropenem-vaborbactam and meropenem tested against 48 carbapenemase-negative CRE isolates collected in US hospitals from 2014 to 2019



Conclusions

- Carbapenemase-negative CRE isolates accounted from over 13% of the CRE isolates during the study period.
- Most isolates had a combination of β-lactam resistance mechanisms, including production of β-lactamases and alterations of outer membrane proteins that could impair the entry of β-lactam agents into the cell.
- All 27 isolates which were identified as non-carbapenemase-producing CRE and analyzed by WGS tested susceptible to meropenem-vaborbactam (≤ 4 mg/L).
- Meropenem-vaborbactam was the most active agent tested against carbapenemase-negative CRE isolates from US hospitals despite of elevated meropenem MIC values in these isolates
  - The inhibition of ESBL and cephalosporinases by vaborbactam might have contributed to these results.

References

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Table 1. Characteristics of 27 carbapenemase-negative CRE isolates collected in US hospitals from 2016 to 2019

Organism	Study Year	State	MIC (mg/L) Meropenem-vaborbactam	Meropenem	Imipenem	Beta-lactamase	AmpC expression	OmpC/OmpK36	OmpF/OmpK35	OmpK37
C. freundii	2018	TX	4	8	8	TEM-1	Elevated expression (>1000X)			
E. cloacae	2017	NY	0.5	8	2		Elevated expression (652X)	insertions/deletions	insertions/deletions	
E. cloacae	2018	KY	0.5	4	4		Did not amplify	insertions/deletions	insertions/deletions	
E. cloacae	2016	NY	2	8	4		Elevated expression (>1000X)	premature stop	insertions/deletions	
E. cloacae	2016	CO	4	8	8		Elevated expression (295X)	premature stop	premature stop	
E. coli	2018	LA	0.25	2	4	**CMY-2**, TEM-1	Similar to baseline	insertions/deletions	premature stop	
E. coli	2018	FL	0.25	4	1	**CTX-M-15**	Similar to baseline	premature stop		
E. coli	2019	TX	0.25	4	1	**CTX-M-15**, TEM-1	not tested	premature stop		
E. coli	2016	MI	0.5	4	1	**CTX-M-193**, TEM-1	Similar to baseline	premature stop	premature stop	
E. coli	2017	TX	2	8	2	**CTX-M-14**	Similar to baseline	insertions/deletions		
K. aerogenes	2016	NJ	1	8	8		Elevated expression (>1000X)	premature stop		insertions/deletions
K. aerogenes	2016	TX	1	8	4		Elevated expression (>1000X)	premature stop	premature stop	
K. aerogenes	2018	CO	1	4	8		Elevated expression (>1000X)	premature stop		
K. aerogenes	2019	TX	1	4	8		not tested	premature stop		
K. aerogenes	2019	CO	2	8	>8		not tested	premature stop		insertions/deletions
K. pneumoniae	2019	LA	0.03	16	>8	**CTX-M-15**, SHV-11, OXA-1	not tested	insertions/deletions		
K. pneumoniae	2019	VA	0.5	4	0.5	**CTX-M-15**, SHV-11, TEM-1, OXA-1	not tested	insertions/deletions	premature stop	
K. pneumoniae	2017	NY	1	4	1	**CTX-M-15**, SHV-1, TEM-1, OXA-1	not tested	insertions/deletions		insertions/deletions
K. pneumoniae	2018	NY	2	4	1	**CTX-M-15**, SHV-11, TEM-1, OXA-1	not tested	premature stop		insertions/deletions
K. pneumoniae	2019	MA	2	8	1	**CTX-M-15**, SHV-11	not tested	insertions/deletions		
K. pneumoniae	2019	TX	2	4	1	**CTX-M-15**, SHV-187, TEM-1, OXA-1	not tested	premature stop		
K. pneumoniae	2019	TX	2	4	0.5	**CTX-M-71**, SHV-11, TEM-1	not tested	insertions/deletions		insertions/deletions
K. pneumoniae	2018	FL	2	8	4	**CTX-M-15**, SHV-11, OXA-1	not tested	premature stop	premature stop	insertions/deletions
K. pneumoniae	2019	NY	4	8	2	**CTX-M-15**, SHV-28, TEM-1, OXA-1	not tested	premature stop	premature stop	insertions/deletions
K. pneumoniae	2017	TX	4	4	2	**CTX-M-15**, SHV-1, TEM-1, OXA-1	not tested	premature stop		insertions/deletions
P. mirabilis	2017	NY	0.5	0.5	8	**TEM-155**, TEM-2	not tested	insertions/deletions		
S. marcescens	2017	OH	4	16	4	**CTX-M-15**, TEM-1, OXA-1	Similar to baseline			

Figure 2. Activity of meropenem-vaborbactam and comparator agents tested against 48 carbapenemase-negative CRE isolates collected in US hospitals from 2014 to 2019