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Activity of Ceftazidime-Avibactam against Carbapenemase-negative Carbapenem-resistant *Enterobacterales* (CRE) isolates from US Hospitals

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CONCLUSIONS

CRE isolates that did not produce carbapenemase had multiple resistance mechanisms that included acquired β-lactamases, changes in permeability, and overexpression of intrinsic enzymes.

Non-carbapenemase-producing CRE isolates were resistant to most β-lactams. Most comparator agents had limited activity against these isolates.

Ceftazidime-avibactam demonstrated *in vitro* activity against all carbapenemase-negative CRE carrying multiple resistance mechanisms. Meropenem-vaborbactam inhibited 71.4% of the 35 meropenem-resistant carbapenemase-negative CRE isolates.

RESULTS

A total of 304 (1.1%) CREs were observed in the study period; 45 (14.8%) isolates did not carry carbapenemases (Figure 1).
- These isolates mainly were *Klebsiella aerogenes, Enterobacter cloacae* species complex, and *Klebsiella pneumoniae* (11, 11 and 10 isolates, respectively).
- Five other species also were included.

Acquired β-lactamase genes, including ESBLs and transferable cephalosporinases, were detected among 18 isolates (Figure 1).
- $bla_{\text{CTX-M-15}}$ was the most common gene and was detected among 14 isolates.
- $bla_{\text{CTX-M-15}}$ was accompanied by $bla_{\text{OXA-1}}$ in 11 isolates.
- $bla_{\text{CTX-M-14}}$, $bla_{\text{CTX-M-2}}$, and $bla_{\text{SHV-12}}$ each were observed in 1 isolate.
- One *P. mirabilis* carried 2 ESBLs, $bla_{\text{TEM-155}}$, and $bla_{\text{TEM-2}}$.
- One *E. coli* isolate harbored $bla_{\text{CMY-2}}$.

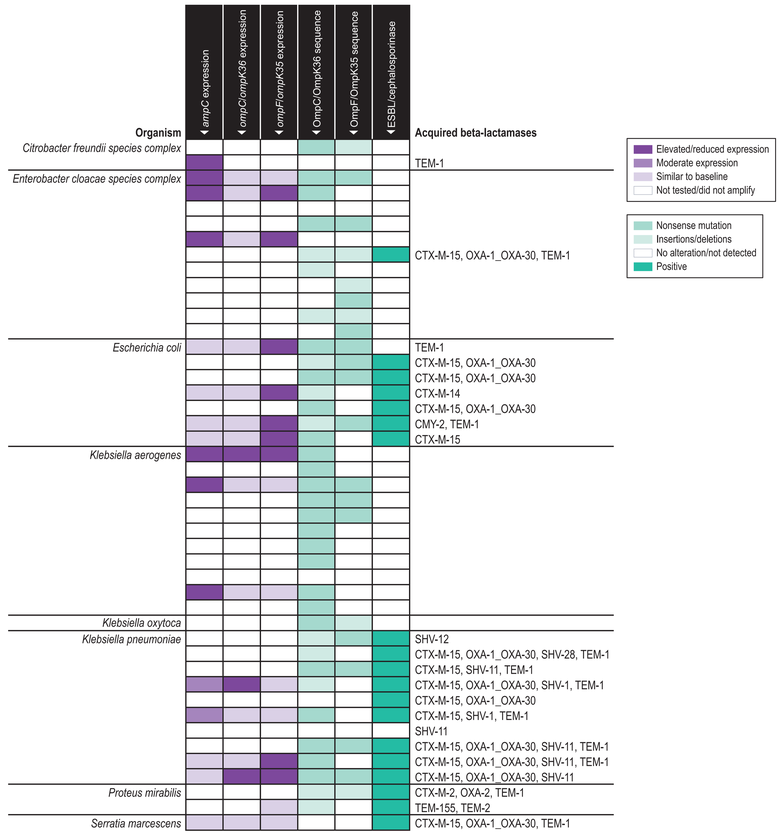
All *K. aerogenes*, 1 *K. oxytoca*, and 10 of 11 *E. cloacae* did not carry acquired β-lactamase genes (Figure 1).
- Among the 2 *C. freundii* species complex isolates analyzed, one carried $bla_{\text{TEM-1}}$, but neither harbored ESBLs or transferable cephalosporinases (Figure 1).

Analysis of outer membrane proteins (OMPs) demonstrated that 18 isolates had both OmpC/OmpK36 and OmpF/OmpK35 disrupted (nonsense or insertions and deletions), including 10 isolates with nonsense mutations in both OMPs (Figure 1).
- Isolates with both OMPs disrupted were detected among 7 species.
- 3 and 17 isolates had either OmpF/OmpK35 or OmpC/OmpK36 disrupted, respectively.

AmpC was overexpressed among 7 of the 17 isolates tested.
- Among the 17 isolates tested for expressions, 3 and 7 isolates had reduced expression of OMPs.

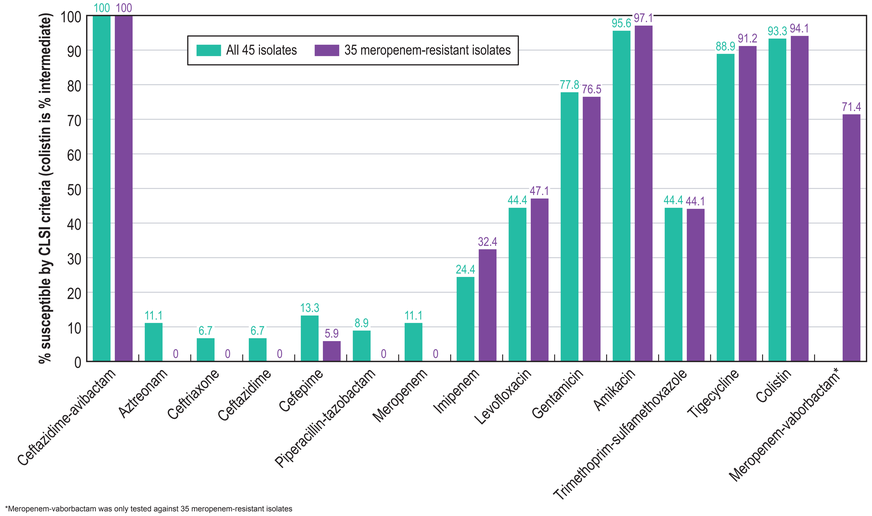
Ceftazidime-avibactam (100% susceptible) inhibited all isolates at the current CLSI breakpoint (Figure 2).
- β-lactam agents had limited activity, inhibiting 11.1% to 24.4% of these isolates.
- Tigecycline and amikacin inhibited 88.9% and 95.6% of the isolates at the current breakpoint, respectively.
- A total of 93.3% had intermediate MIC values for colistin (CLSI breakpoints).
- Other comparators inhibited 44.4% to 77.8% of the non-carbapenemase-producing CRE isolates.

Figure 1. Resistance mechanisms to β-lactam agents detected among 45 carbapenemase-negative CRE



Meropenem-vaborbactam inhibited 71.4% of the 35 meropenem-resistant isolates (Figure 2).
- Ceftazidime-avibactam was active against all meropenem-resistant isolates.
- Meropenem-resistant isolates were more resistant to other β-lactams, except for imipenem and gentamicin, when compared to the 45 non-carbapenemase-producing CRE isolates.

Figure 2. Activity of ceftazidime-avibactam and comparator agents tested against 45 carbapenemase-negative CRE



*Meropenem-vaborbactam was only tested against 35 meropenem-resistant isolates

INTRODUCTION

Carbapenem-resistant *Enterobacterales* isolates emerged worldwide. Most of these carry carbapenemases, such as metallo β-lactamases (MBLs), oxacillinases with carbapenemase activity (OXA-48-like), and KPCs.

Carbapenem-resistant isolates that do not carry carbapenemases are not perceived as widespread as carbapenemase producers, but they remain a challenge for treatment with carbapenem agents. These isolates usually have elevated expression β-lactamases associated with permeability alterations and/or penicillin-binding protein (PBP) alterations.

Ceftazidime-avibactam is active against ESBLs, cephalosporinases, serine-carbapenemases, and some oxacillinases. Despite being affected by non-enzymatic resistance mechanisms, these resistance mechanisms against ceftazidime might be different from those mechanisms affecting carbapenems.

We investigated the prevalence, resistance mechanisms, and activity of ceftazidime-avibactam and comparator agents against CRE that did not carry carbapenemase genes from US hospitals.

Meropenem-resistant isolates were tested for meropenem-vaborbactam.

MATERIALS AND METHODS

A total of 28,904 *Enterobacterales* isolates were collected in 70 US hospitals during 2016–2018.
- Isolates were identified as the cause of infection.
- Isolates were limited to 1 per patient per infection episode.

Isolates were susceptibility tested using the reference broth microdilution method described by the Clinical and Laboratory Standards Institute (CLSI).
- Categorical interpretations for all comparator agents were those criteria found on CLSI M100 document.
- Quality control (QC) was performed according to CLSI guidelines (M07, 2018). All QC minimal inhibitory concentration (MIC) results were within acceptable ranges as published in CLSI documents.
- Meropenem-vaborbactam was tested using lyophilized broth microdilution panels (ThermoFisher Scientific) according to manufacturer instructions.

Carbapenem-resistant *Enterobacterales* (CRE) isolates were defined as any isolate exhibiting imipenem and/or meropenem MIC values of ≥2 µg/mL.
- These isolates were submitted to whole genome sequencing (WGS).
- *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.

MATERIALS AND METHODS

WGS was performed on a MiSeq (Illumina, San Diego, California, USA) instrument targeting a 30X coverage.
- Sequences were *de novo* assembled.
- Analysis of β-lactam resistance mechanisms and MLST 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 isolates were evaluated for expression levels of intrinsic resistance genes associated with resistance to β-lactams.
- Expression levels were determined by in triplicate quantitative real-time PCR using high quality RNA samples.
- Genes tested were the chromosomal *ampC, ompC,* and *ompF* (non-*Klebsiella* species) and *ompK35* and *ompK36* (*Klebsiella* spp.).
- Transcription levels were considered different if at least a 10-fold for AmpC and a 5-fold for other genes increase was noted compared to the baseline susceptible isolate.

DISCLOSURES

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