Characterization of *Enterobacter* and *Citrobacter* spp. Isolates from United States Hospitals by Whole Genome Sequencing Analysis and Activity of Ceftazidime-Avibactam and Comparator Agents

IDWeek 2017
Poster #320

M Castanheira, RE Mendes, TB Doyle, AP Davis, HS Sader

JMI Laboratories, North Liberty, Iowa, USA

Contact Information:
Mariana Castanheira, PhD
JMI Laboratories
345 Beaver Kreek Centre, Suite A
North Liberty, IA 52317
Phone: (319) 665-3370
Fax: (319) 665-3371
Email: mariana-castanheira@jmilabs.com

INTRODUCTION

- Avibactam is a diazabicyclooctane β-lactamase inhibitor that demonstrates excellent inhibitory properties against β-lactamases belonging to Ambler classes A, C, and some class D
- Ceftazidime-avibactam is approved by the United States Food and Drug Administration (US FDA) for treating complicated urinary tract infections and for complicated intra-abdominal infections when combined with metronidazole
- We evaluated the presence of β-lactamases using whole genome sequencing analysis among 410 *Enterobacter* spp. and *Citrobacter* spp. isolates collected in US hospitals during 2016
  - The *in vitro* activity of ceftazidime-avibactam and comparator agents was analyzed

MATERIALS AND METHODS

- A total of 1,966 *Enterobacter* spp. and *Citrobacter* spp. clinical isolates collected during 2016 from 82 US hospitals participating in the INFORM program were susceptibility tested
- Species identification was confirmed by matrix-assisted laser desorption ionization-time of flight mass spectrometry
- Isolates were susceptibility tested against ceftazidime-avibactam (inhibitor at fixed 4 mg/L) and comparator agents using the reference broth microdilution method as described by the Clinical and Laboratory Standards Institute (CLSI)
  - Quality control (QC) was performed according to CLSI guidelines (M100-S27), and all QC MIC results were within acceptable ranges, as published in CLSI documents
  - Categorical interpretations for all comparator agents were those found in CLSI M100-S27 (2017) criteria, EUCAST breakpoint tables (version 7.0, January 2017), and/or US FDA package inserts
- A total of 410 isolates displaying MIC values ≥16 mg/L for ceftazidime and/or ≥2 mg/L cefepime were further evaluated
  - Selected isolates were submitted to whole genome sequencing on a MiSeq (Illumina, San Diego, California, US) instrument targeting a 30X coverage
  - Sequences were *de novo* assembled and searched for the presence of acquired β-lactamases using a curated library and applying criteria of >94% sequencing identity and 40% minimum length coverage

RESULTS

- Among 410 (20.9%) isolates screened for genes encoding β-lactamases, there were 70 (22.9% for this species) *Citrobacter freundii* species complex (herein referred to as *C. freundii*), 1 (0.5%) *C. koseri*, 81 (19.0%) *E. aerogenes*, and 258 (25.3%) *Enterobacter cloacae* species complex (herein referred to as *E. cloacae*)
- The most common acquired β-lactamase gene observed among *C. freundii*, *E. aerogenes*, and *E. cloacae* was bla_{CTX-M} (25 positive results; Figure 1)
  - Most isolates that yielded positive results for bla_{CTX-M} were *E. cloacae* (20 positive results; 2.0% of all *E. cloacae* isolates collected)
  - $bla_{CTX-M-15}$ was the most common variant (19 isolates; Figure 1)
  - Six other bla_{CTX-M} variants were observed and included 1 isolate each of $bla_{CTX-M-1}$, $bla_{CTX-M-2}$, $bla_{CTX-M-3}$, $bla_{CTX-M-9}$, $bla_{CTX-M-27}$, and $bla_{CTX-M-36}$ (Figure 1)
- Other ESBL genes detected among these isolates included: bla_{SHV}-like genes (39 positive results, 23 bla_{SHV-12}, and 5 other variants), $bla_{OXA-1/30}$ (20), and bla_{TEM-10} (3) (Figure 1)
- Transferrable cephalosporinases that were not intrinsic from these species were detected among 8 isolates and included 1 bla_{CMY-2} in *E. cloacae,* 2 bla_{FOX-5}, and 5 bla_{DHA-1} (Figure 1)
- Carbapenemase-encoding genes were observed among 5 *C. freundii*, 1 *E. aerogenes*, and 13 *E. cloacae*, comprising 5 bla_{KPC-2}, 11 bla_{KPC-3}, and 1 each of bla_{KPC-4}, bla_{KPC-6}, and bla_{NDM-1} in *E. cloacae* (Figure 1)
- Narrow-spectrum β-lactamases were also detected mostly among *C. freundii* and *E. cloacae* isolates tested and included bla_{CARB-2}, bla_{OXA}, and bla_{TEM} variants (2, 17, and 69 isolates) and 1 enzyme with spectrum of activity unknown ($bla_{OXA-224}$; data not shown)
- The majority of the *E. aerogenes* isolates resistant to cephalosporins did not carry acquired β-lactamases
- One *E. cloacae* isolate carrying bla_{KPC-6} exhibited meropenem, doripenem, and imipenem MIC values of 0.06, 0.12, and 0.5 mg/L, respectively; however, bla_{KPC-6} is intact and the transposon Tn*4401* carrying this gene had a promoter region that is consistent with other bla_{KPC}-carrying isolates
- Ceftazidime had limited activity against isolates screened for β-lactamase genes ($MIC_{50/90}$, >32/>32 mg/L for all species/genus); however, ceftazidime-avibactam was very active against these isolates ($MIC_{50/90}$ range 0.25 to 0.5/0.5 to 1 mg/L) (Table 1)

Figure 1. Genes encoding broad-spectrum β-lactamases detected among 410 *Enterobacter* spp. and *Citrobacter* spp. isolates collected during 2016 in USA hospitals displaying elevated MIC values for ceftazidime (≥16 mg/L) and/or cefepime (≥2 mg/L)

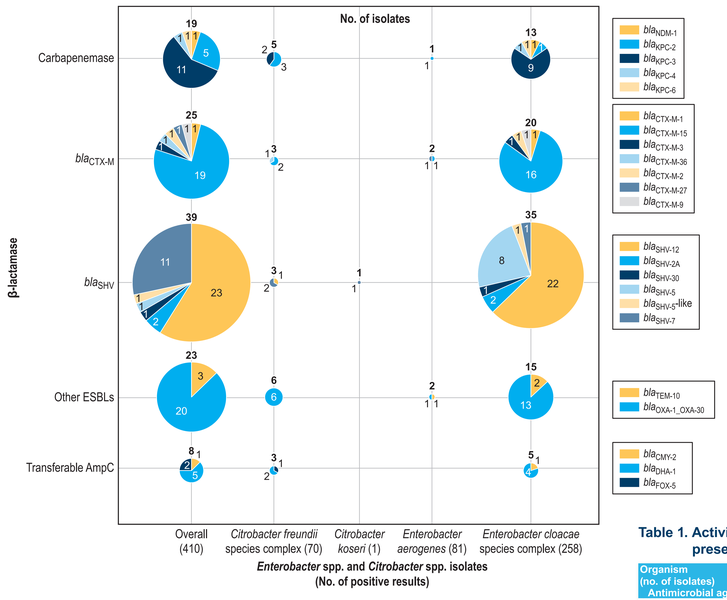
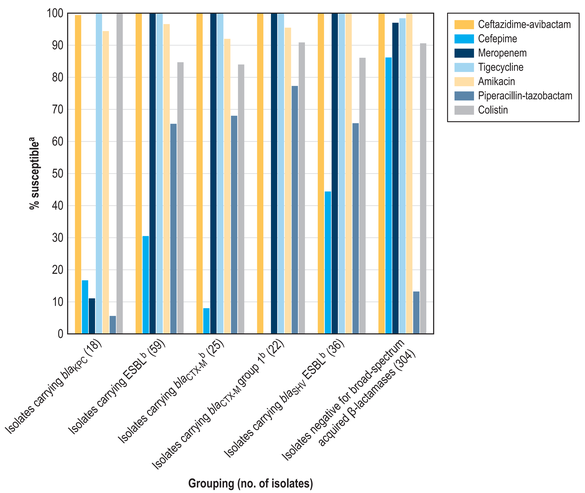


Figure 2. Activity of ceftazidime-avibactam and comparator agents against isolates producing β-lactamases



[a] CLSI 2017, with exception of colistin that was interpreted according to EUCAST criteria (2017)

[b] Includes only isolates without carbapenemases

- Ceftazidime-avibactam inhibited 99.4%–100.0% of *Enterobacter* and *Citrobacter* species isolates carrying β-lactamase genes, including bla_{KPC} (Figure 2)
  - Only 2 *E. cloacae* isolates in this collection displayed ceftazidime-avibactam MIC results >8 mg/L; 1 carrying bla_{NDM-1} (MIC, >32 mg/L) and another harboring bla_{KPC-4} and displaying a decreased expression of *ompF* and amino acid alterations on OmpC that might be incompatible with protein function (MIC, 16 mg/L; data not shown)
- Among comparator agents, tigecycline inhibited 98.1% to 100.0% of the isolates carrying different β-lactamases at the US FDA breakpoint regardless of enzymes present (Figure 2)
  - Meropenem displayed activity against 97.0%–100.0% of isolates that did not carry carbapenemases, but inhibited only 11.1% of isolates harboring bla_{KPC} (Figure 2)
  - Colistin and amikacin inhibited ≥84.0% and ≥92.0% of isolates carrying different β-lactamases, respectively (Figure 2)
  - The activity of other comparators varied based on the enzymes present (Figure 2)

Table 1. Activity of ceftazidime-avibactam and ceftazidime tested against all isolates screened for the presence of β-lactamase encoding genes

Organism (no. of isolates) / Antimicrobial agent	No. of isolates at MIC (mg/L; cumulative %)													MIC_{50}	MIC_{90}
	≤0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	> 32		
All isolates (410)															
Ceftazidime-avibactam	10 2.4	6 3.9	9 6.1	31 13.7	126 44.4	151 81.2	55 94.6	14 98.0	6 99.5	0 99.5	1 99.8	0 99.8	1 100.0	0.5	1
Ceftazidime						0 0.0	1 0.2	3 1.0	1 1.2	7 2.9	36 11.7	64 27.3	298 100.0	>32	>32
Enterobacter cloacae species complex (258)															
Ceftazidime-avibactam	4 1.6	2 2.3	1 2.7	14 8.1	76 37.6	101 76.7	45 94.2	11 98.4	2 99.2	0 99.2	1 99.6	0 99.6	1 100.0	0.5	1
Ceftazidime							0 0.0	2 0.8	1 1.2	4 2.7	27 13.2	37 27.5	187 100.0	>32	>32
Enterobacter aerogenes (81)															
Ceftazidime-avibactam	5 6.2	2 8.6	6 16.0	12 30.9	32 70.4	19 93.8	3 97.5	2 100.0						0.25	0.5
Ceftazidime									0 0.0	1 1.2	9 12.3	23 40.7	48 100.0	>32	>32
Citrobacter spp. (71)															
Ceftazidime-avibactam	1 1.4	2 4.2	2 7.0	5 14.1	18 39.4	31 83.1	7 93.0	1 94.4	4 100.0					0.5	1
Ceftazidime						0 0.0	1 1.4	1 2.8	0 2.8	2 5.6	0 5.6	4 11.3	63 100.0	>32	>32

CONCLUSIONS

- The most common acquired broad-spectrum β-lactamase genes/groups detected were bla_{CTX-M}, followed by bla_{SHV} and $bla_{OXA-1/30}$
  - The majority of these isolates were *E. cloacae* (55 isolates)
  - Only 4 of 77 *E. aerogenes* carried acquired broad-spectrum β-lactamase genes
- Carbapenemase-encoding genes were detected among 19 isolates and included 18 bla_{KPC} (4 variants) and 1 bla_{NDM-1}
- Differently from other clinically available β-lactamase inhibitors, avibactam inhibits class C cephalosporinases often overexpressed by *Enterobacter* and *Citrobacter* species
- Ceftazidime-avibactam displayed activity against 408 out of 410 (99.5%) isolates tested at the current breakpoint
  - Comparator agents displayed variable activity against isolates tested, but most comparators exhibited limited activity against isolates carrying carbapenemase-encoding genes

ACKNOWLEDGEMENTS

The authors acknowledge the contributions of the US hospitals participating in the International Network For Optimal Resistance Monitoring program.

This study was supported by Allergan. Allergan was involved in the design and decision to present these results, and JMI Laboratories received compensation for services in relation to preparing this presentation. Allergan had no involvement in the collection, analysis, or interpretation of data.

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