**JAC-
Antimicrobial
Resistance**

Characterization of *Enterobacter cloacae* and *Citrobacter freundii* species complex isolates with decreased susceptibility to cephalosporins from United States hospitals and activity of ceftazidime/avibactam and comparator agents

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Objectives: To evaluate the antimicrobial susceptibility and resistance mechanisms to β-lactams among *Enterobacter cloacae* and *Citrobacter freundii* from United States medical centres.

Methods: 2571 *E. cloacae* and 1008 *C. freundii* species complex isolates were consecutively collected from 77 medical centres and susceptibility tested by broth microdilution method. Isolates displaying MIC values ≥ 16 mg/L for ceftazidime or ≥ 2 mg/L for cefepime ($n = 914$) were tested for β-lactamase-encoding genes using whole genome sequencing.

Results: Overall susceptibility to ceftazidime and cefepime were 73.9% and 91.2% among *E. cloacae* and 74.2% and 93.5% among *C. freundii*, respectively. Sixty-three isolates harboured a carbapenemase gene, including 56 bla_{KPC}, 2 bla_{NMC-A}, and 5 metallo-β-lactamase genes. Among non-carbapenemase producers, 121 isolates had at least one ESBL-encoding gene, mainly bla_{SHV} (81) or bla_{CTX-M} (61), and 15 had a transferable AmpC gene, mainly bla_{DHA-1} (8) or bla_{FOX-5} (6). Carbapenemase, ESBL, or transferable AmpC-encoding genes were not identified among 718 of 914 (78.6%) isolates sequenced. The most active agents against isolates with a decreased susceptibility to ceftazidime and/or cefepime were ceftazidime/avibactam ($MIC_{50/90}$, 0.5/1 mg/L; 99.3% susceptible), amikacin ($MIC_{50/90}$, 1/4 mg/L; 99.5% susceptible), and meropenem ($MIC_{50/90}$, 0.06/0.5 mg/L; 92.9% susceptible). The isolates resistant to ceftazidime/avibactam were the five MBL producers and one *E. cloacae* isolate with a reduced expression of OmpF and overexpression of AcrAB-TolC.

Conclusions: Hyperproduction of chromosomal AmpC appears to be the most common mechanism of resistance to ceftazidime and/or cefepime in *E. cloacae* and *C. freundii*. Ceftazidime/avibactam remained highly active against most isolates showing decreased susceptibility to ceftazidime and/or cefepime.

Introduction

Enterobacter cloacae species complex (*E. cloacae*) is an important nosocomial pathogen that has emerged as one of the most commonly found nosocomial pathogen in neonatal units, with several infection outbreaks reported.[1] *Citrobacter freundii* species complex (*C. freundii*) causes a broad spectrum of infections as an opportunistic pathogen, including infections of the urinary tract (UTI), respiratory tract, wounds, and bloodstream. Limited outbreaks involving clonal antimicrobial-resistant isolates also have been observed in healthcare settings.[2–4]

The main mechanism of resistance to broad-spectrum β-lactams among *E. cloacae* and *C. freundii* is the overexpression of

chromosomal *ampC* β-lactamase genes, but other mechanisms have been increasingly reported among these organisms. The acquisition of plasmid-mediated ESBL or carbapenemase genes, mainly of the KPC or OXA-48 type or metallo-β-lactamases (MBLs) of the VIM-, IMP-, and NDM-1 types have been reported among *E. cloacae* and *C. freundii*. However, the frequency of these resistance genes among *E. cloacae* and *C. freundii* isolates causing infections in United States medical centres has not been evaluated in large multicentre investigations.[3–7]

In the present study, we evaluated the *in vitro* activity of ceftazidime/avibactam and many comparator agents tested against 2571 *E. cloacae* and 1008 *C. freundii* isolates consecutively collected in United States medical centres from 2017 to 2019.

Isolates displaying elevated MIC values of ceftazidime and/or cefepime were screened for the presence of β-lactamases using whole genome sequencing (WGS) analysis.

Materials and methods

Organism collection

A total of 2571 *E. cloacae* and 1008 *C. freundii* isolates were consecutively collected (1 per infection episode) from 77 US medical centres distributed across 36 states and all nine US census divisions from 2017 to 2019 as part of the International Network for Optimal Resistance Monitoring (INFORM) Program. *E. cloacae* isolates were collected mainly from patients hospitalized with skin and skin structure infections (SSSI; 25.6%), pneumonia (24.2%), complicated urinary tract infections (cUTI; 21.8%), and bloodstream infections (BSI; 18.0%). *C. freundii* isolates were collected primarily from patients with cUTI (45.9%), SSSI (19.3%), BSI (11.0%), and pneumonia (10.5%). Only bacterial isolates determined to be significant by local criteria as the reported probable cause of an infection were included in this investigation. Species identification, when necessary, was confirmed by MALDI-TOF mass spectrometry using the Bruker Daltonics MALDI Biotyper (Billerica, Massachusetts, US) following the manufacturer's instructions.

Antimicrobial susceptibility testing

Antimicrobial susceptibility was evaluated by reference broth microdilution methods following CLSI procedures (M07).[8] Ceftazidime/avibactam and ceftolozane/tazobactam were tested with the β-lactamase inhibitor at a fixed concentration of 4 mg/L. Concurrent quality control (QC) testing was performed to ensure proper test conditions and procedures. QC strains included *Escherichia coli* ATCC 25922 and NCTC 13353, *K. pneumoniae* ATCC 700603 and ATCC BAA 1705, and *P. aeruginosa* ATCC 27853. When available, CLSI and EUCAST susceptibility interpretive criteria were used to determine the susceptibility/resistance rates for antimicrobial agents.[9,10]

Screening for β-lactamases

Isolates displaying MIC values ≥16 mg/L for ceftazidime or ≥2 mg/L for cefepime were tested for β-lactamase-encoding genes using WGS. Briefly, total genomic DNA was extracted using the fully automated ThermoScientific™ KingFisher™ Flex Magnetic Particle Processor (Cleveland, Ohio, USA). Libraries were normalized using the bead-based normalization procedure (Illumina) and sequenced on the MiSeq. FASTQ format files for each sample set were assembled independently using the *de novo* assembler SPAdes 3.11.1 with K-values of 21, 33, 55, 77, and 99 and careful mode on to reduce the number of mismatches. This process produced a FASTA format file of contiguous sequences with the best N50 value. An in-house proprietary bioinformatics pipeline and a JMI Laboratories-curated resistance gene database (Version 3; uses Python v2.7.9, SPAdes v3.11.1, and BBMap v36.x) based on the NCBI Bacterial Antimicrobial Resistance Reference Gene Database (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA313047) was used for the *in silico* analysis that screened for β-lactamase genes to align β-lactamase resistance determinants against the target assembled sequences. Hits with identities greater than 94% and with 40% minimum coverage length were selected for further analysis and the final assignment of β-lactamase alleles.[11,12]

Results

Based on the current CLSI breakpoint criteria (CLSI M100), ceftazidime was active against 73.9% of *E. cloacae* and 74.2% of *C. freundii* isolates and cefepime was active against 91.2% of *E. cloacae* and 93.5% of *C. freundii* isolates collected by the INFORM program in 2017–19 (Table 1). The most active

compounds against these organisms were ceftazidime/avibactam (MIC$_{50/90}$, 0.12–0.25/0.5 mg/L; 99.8%–99.9% susceptible by CLSI and EUCAST) and amikacin (MIC$_{50/90}$, 1–2/2–4 mg/L; 99.7%–99.8% susceptible by CLSI). Meropenem inhibited 98.1% of *C. freundii* and 98.2% of *E. cloacae* at their respective CLSI susceptible breakpoints (MIC$_{50/90}$, 0.03/0.12 mg/L for both organisms; Table 1).

Among the 2571 *E. cloacae* and 1008 *C. freundii* isolates, 652 (25.4%) *E. cloacae* and 262 (26.0%) *C. freundii* exhibited decreased susceptibility to ceftazidime (MIC ≥16 mg/L) and/or cefepime (MIC ≥2 mg/L). These isolates were mainly from patients with cUTI (29.1%), pneumonia (24.7%), SSSI (18.4%), and BSI (15.5%).

The most active agents against the collection of isolates with decreased susceptibility to ceftazidime or cefepime were ceftazidime/avibactam (MIC$_{50/90}$, 0.5/1 mg/L; 99.3% susceptible by CLSI and EUCAST) and amikacin (MIC$_{50/90}$, 1/4 mg/L; 99.5%/98.0% susceptible by CLSI/EUCAST; Table 2). Meropenem (MIC$_{50/90}$, 0.06/0.5 mg/L) was active against 92.9% and 94.9% of isolates with decreased susceptibility to β-lactams (MIC values ≥16 mg/L for ceftazidime and ≥2 mg/L for cefepime) at the CLSI and EUCAST breakpoints, respectively. Ceftolozane/tazobactam (MIC$_{50/90}$, 8/>16 mg/L) was active against 23.7% of those isolates at the CLSI and EUCAST breakpoints (Table 2). Notably, ertapenem was active against only 75.2% of meropenem-susceptible isolates from this collection, with 14.6% of isolates categorized as ertapenem-intermediate and 10.3% as ertapenem-resistant; additionally, meropenem was active against 76.3% of ertapenem-non-susceptible isolates (MIC$_{50/90}$, 0.12/8 mg/L; data not shown).

Carbapenemase genes were detected in 63 isolates (1.8% of total): 20 *C. freundii* (2.0% of total) and 43 *E. cloacae* (1.7% of total). The most common carbapenemase gene was *bla*$_{KPC}$ type (56 isolates; 88.9% of carbapenemase-producing isolates); a metallo-β-lactamase (MBL) gene was observed in only five isolates (0.14% of total; Table 3 and Figure 1). Among isolates that did not harbour a carbapenemase gene, 121 isolates (3.4% of total) harboured ESBL genes and 15 isolates (0.6% of total) harboured transferable AmpC genes that were not intrinsic to that species. The most common ESBL gene types were *bla*$_{SHV}$ (81 isolates; 2.3% of total and 66.9% of ESBL-producing isolates). Genes encoding CTX-M enzymes were noted among 61 isolates (1.7% of total and 50.4% of ESBL-producing isolates), with CTX-M-15 being the most common enzyme found. A total of 47 isolates carried the gene encoding OXA-1, which is also known as OXA-30. This gene was mostly observed among isolates carrying other ESBLs, mainly CTX-M-15. *bla*$_{DHA-1}$ (eight isolates) and *bla*$_{FOX-5}$ (six isolates) were the most common transferable AmpC genes detected among isolates without a carbapenemase gene (Table 3 and Figure 1).

Ceftazidime/avibactam showed complete activity against isolates producing ESBLs, KPCs, and/or transferable AmpC (Table 2). The only isolates resistant to ceftazidime/avibactam were the five MBL producers and one *E. cloacae* isolate with porin alterations and no carbapenemase, ESBL, or transferable AmpC gene. Meropenem exhibited potent activity against isolates producing ESBLs (99.2% susceptible), including SHV producers (100.0% susceptible) and CTX-M producers (98.2% susceptible), as well as isolates producing transferable AmpC (100.0% susceptible). However, meropenem showed limited activity against KPC producers (MIC$_{50/90}$, 4/16 mg/L; 12.5% susceptible; Table 2). Ceftolozane/tazobactam demonstrated limited activity against carbapenemase-negative isolates that produced an ESBL

Table 1. Activity of ceftazidime/avibactam and comparator antimicrobial agents tested against 2571 *Enterobacter cloacae* species complex and 1008 *Citrobacter freundii* species complex isolates collected from United States medical centres (2017–19)

Antimicrobial agent	MIC (mg/L)		CLSI[a]		EUCAST[a]	
	MIC_{50}	MIC_{90}	%S	%R	%S	%R
E. cloacae (2571)						
Ceftazidime/avibactam	0.25	0.5	99.8	0.2	99.8	0.2
Ceftolozane/tazobactam	0.25	8	81.1	15.3	81.1	18.9
Ceftriaxone	0.25	>8	68.8	28.6	68.8	28.6
Ceftazidime	0.5	>32	73.9	24.7	70.1	26.1
Cefepime	≤0.12	2	91.2[b]	3.6	85.6	5.4
Piperacillin/tazobactam	2	128	79.7	11.1	75.9	20.3
Meropenem	0.03	0.12	98.2	1.4	98.6	0.6
Ertapenem (1719)	0.06	1	89.9	5.2	89.9	10.1
Levofloxacin	≤0.03	0.5	92.1	5.9	92.1	5.9
Gentamicin	0.25	0.5	95.7	3.7	95.3[c]	4.7
Amikacin	1	2	99.8	0.1	99.3[c]	0.7
Colistin	0.12	>8		18.3	81.7	18.3
C. freundii (1008)						
Ceftazidime/avibactam	0.12	0.5	99.9	0.1	99.9	0.1
Ceftolozane/tazobactam	0.25	16	79.0	18.5	79.0	21.0
Ceftriaxone	0.25	>8	72.2	26.6	72.2	26.6
Ceftazidime	0.5	>32	74.2	24.8	69.3	25.8
Cefepime	≤0.12	2	93.5[b]	2.7	88.4	4.3
Piperacillin/tazobactam	4	128	77.2	14.5	72.8	22.8
Meropenem	0.03	0.06	98.1	1.1	98.9	0.5
Ertapenem (697)	0.015	0.25	96.6	2.3	96.6	6.7
Levofloxacin	0.06	1	86.6	9.0	86.6	9.0
Gentamicin	0.5	1	93.6	6.0	93.3[c]	6.7
Amikacin	2	4	99.7	0.1	99.4[c]	0.6
Colistin	0.25	0.25		0.1	99.9	0.1

[a]Criteria as published by CLSI[9] and EUCAST.[10]
[b]Intermediate is interpreted as susceptible-dose-dependent.
[c]For infections originating from the urinary tract. For systemic infections, aminoglycosides must be used in combination with another active therapy.

($MIC_{50/90}$, 1/>16 mg/L; 67.8% susceptible), including SHV producers ($MIC_{50/90}$, 1/>16 mg/L; 58.1% susceptible) and CTX-M producers ($MIC_{50/90}$, 1/16 mg/L; 80.4% susceptible), and against isolates producing transferable AmpC ($MIC_{50/90}$, 1/>16 mg/L; 60.0% susceptible; Table 2). The five MBL-producing isolates were resistant to all antimicrobials tested, except amikacin (MIC_{50}, 4 mg/L; 100.0%/80.0% susceptible by CLSI/EUCAST), colistin (MIC_{50}, 0.12 mg/L; 100.0% susceptible per EUCAST), and gentamicin (MIC_{50}, >16 mg/L; 40.0% susceptible by CLSI and EUCAST; data not shown).

Carbapenemase, ESBL, or transferable AmpC-encoding genes were not identified among 718 of 914 (78.6%) isolates submitted to WGS. This collection was very susceptible to ceftazidime/avibactam ($MIC_{50/90}$, 0.5/1 mg/L; 99.9% susceptible by CLSI and EUCAST), meropenem ($MIC_{50/90}$, 0.06/0.25 mg/L; 98.9% and 99.2% susceptible by CLSI and EUCAST, respectively), and amikacin ($MIC_{50/90}$, 1/2 mg/L; 100.0% and 99.7% susceptible by CLSI and EUCAST, respectively), but showed elevated MIC results for ceftolozane/tazobactam ($MIC_{50/90}$, 8/>16 mg/L; 18.1% susceptible) and ceftazidime ($MIC_{50/90}$, >32/>32 mg/L; 0.7% susceptible; Table 2). Ertapenem was active against 72.9% of these isolates ($MIC_{50/90}$,

0.5/2 mg/L; Table 2). The susceptibility results for ceftazidime tested alone indicate that these isolates expressed derepressed AmpC, which seems to markedly affect the activity of ceftolozane/tazobactam. Moreover, these isolates exhibited higher susceptibility to cefepime compared with ESBL producers. The percentages of isolates inhibited at ≤2 mg/L and ≤8 mg/L of cefepime were 26.7% and 52.5%, respectively, among the ESBL producers, and 80.1% and 96.8%, respectively, among the collection of isolates where carbapenemase, ESBL, or transferable AmpC-encoding genes were not identified (data not shown).

Discussion

Antimicrobial treatment of systemic infections caused by *E. cloacae, C. freundii*, and other Enterobacterales species that produce inducible AmpC is controversial. The emergency of resistance to third-generation cephalosporins during therapy is relatively high among these organisms, especially when the initial site of isolation is blood.[13,14] Hence, the use of third-generation cephalosporins is not recommended for the treatment of severe infections caused by Enterobacterales species that produce inducible AmpC,

Table 2. Activity of ceftazidime/avibactam and comparator antimicrobial agents tested against *Enterobacter cloacae* species complex and *Citrobacter freundii* species complex isolates with decreased susceptibility to β-lactams (MIC values ≥16 mg/L for ceftazidime and ≥2 mg/L for cefepime) from United States medical centres (2017–19)

Antimicrobial agent	MIC (mg/L)		CLSI[a]		EUCAST[a]	
	MIC_{50}	MIC_{90}	%S	%R	%S	%R
All *C. freundii* and *E. cloacae* (914)[b]						
Ceftazidime/avibactam	0.5	1	99.3	0.7	99.3	0.7
Ceftolozane/tazobactam	8	>16	23.7	63.5	23.7	76.3
Ceftriaxone	>8	>8	0.2	99.1	0.2	99.1
Ceftazidime	>32	>32	1.5	96.9	0.3	98.5
Cefepime	2	16	68.1	13.1	46.8	19.9
Piperacillin/tazobactam	64	>128	18.9	46.9	11.4	81.1
Meropenem	0.06	0.5	92.9	5.1	94.9	2.2
Ertapenem[c]	0.5	>2	69.8	16.7	69.8	30.2
Levofloxacin	0.06	8	76.2	19.0	76.2	19.0
Gentamicin	0.5	16	86.9	10.9	85.9[d]	14.1
Amikacin	1	4	99.5	0.1	98.0[d]	2.0
Colistin	0.25	8		11.4	88.6	11.4
KPC producers (56)[e]						
Ceftazidime/avibactam	1	4	100.0	0.0	100.0	0.0
Ceftolozane/tazobactam	>16	>16	1.8	94.6	1.8	98.2
Ceftazidime	>32	>32	0.0	91.1	0.0	100.0
Cefepime	>16	>16	5.4	64.3	1.8	82.1
Piperacillin/tazobactam	>128	>128	1.8	94.6	0.0	98.2
Meropenem	4	16	12.5	60.7	39.3	26.8
Ertapenem[c]	>2	>2	2.6	97.4	2.6	97.4
Levofloxacin	16	>16	10.7	85.7	10.7	85.7
Gentamicin	8	>16	46.4	30.4	39.3[d]	60.7
Amikacin	2	16	94.6	0.0	87.5[d]	12.5
Colistin	0.12	0.5		8.9	91.1	8.9
ESBL producers (121)[f]						
Ceftazidime/avibactam	0.5	1	100.0	0.0	100.0	0.0
Ceftolozane/tazobactam	1	>16	67.8	27.0	67.8	32.2
Ceftazidime	>32	>32	7.4	88.4	1.7	92.6
Cefepime	8	>16	27.3	47.1	16.5	57.9
Piperacillin/tazobactam	8	>128	64.5	23.1	50.4	35.5
Meropenem	0.03	0.12	99.2	0.8	99.2	0.0
Ertapenem	0.12	1	87.5	3.4	87.5	12.5
Levofloxacin	1	>16	44.2	45.8	44.2	45.8
Gentamicin	16	>16	43.8	51.2	41.3[d]	58.7
Amikacin	2	8	98.3	0.8	93.4[d]	6.6
Colistin	0.12	0.25		5.0	95.0	5.0
SHV producers (64)[g]						
Ceftazidime/avibactam	0.5	1	100.0	0.0	100.0	0.0
Ceftolozane/tazobactam	1	>16	58.1	35.5	58.1	41.9
Ceftazidime	>32	>32	1.6	98.4	0.0	98.4
Cefepime	4	>16	40.6	25.0	28.1	37.5
Piperacillin/tazobactam	8	>128	60.9	26.6	51.6	39.1
Meropenem	0.03	0.12	100.0	0.0	100.0	0.0
Ertapenem	0.06	1	81.8	4.5	81.8	18.2
Levofloxacin	0.5	16	53.1	42.2	53.1	42.2
Gentamicin	4	>16	51.6	40.6	46.9[d]	53.1
Amikacin	1	8	98.4	0.0	90.6[d]	9.4
Colistin	0.12	0.25		4.7	95.3	4.7

Continued

Table 2. *Continued*

Antimicrobial agent	MIC (mg/L)		CLSI[a]		EUCAST[a]	
	MIC$_{50}$	MIC$_{90}$	%S	%R	%S	%R
CTX-M producers (55)[h]						
Ceftazidime/avibactam	0.25	1	100.0	0.0	100.0	0.0
Ceftolozane/tazobactam	1	16	80.4	15.7	80.4	19.6
Ceftazidime	32	>32	12.7	80.0	1.8	87.3
Cefepime	>16	>16	5.5	81.8	0.0	90.9
Piperacillin/tazobactam	8	128	72.7	16.4	52.7	27.3
Meropenem	0.03	0.12	98.2	1.8	98.2	0.0
Ertapenem	0.12	0.5	92.9	2.4	92.9	7.1
Levofloxacin	1	>16	38.9	44.4	38.9	44.4
Gentamicin	>16	>16	30.9	67.3	30.9[d]	69.1
Amikacin	2	8	98.2	1.8	96.4[d]	3.6
Colistin	0.12	0.25		5.5	94.5	5.5
Transferable AmpC (15)[i]						
Ceftazidime/avibactam	0.5	1	100.0	0.0	100.0	0.0
Ceftolozane/tazobactam	1	>16	60.0	40.0	60.0	40.0
Ceftazidime	32	>32	6.7	86.7	0.0	93.3
Cefepime	1	16	66.7	13.3	66.7	26.7
Piperacillin/tazobactam	16	>128	53.3	46.7	40.0	46.7
Meropenem	0.06	0.5	100.0	0.0	100.0	0.0
Ertapenem	0.25	—[j]	77.8	11.1	77.8	22.2
Levofloxacin	1	>16	46.7	46.7	46.7	46.7
Gentamicin	8	>16	46.7	46.7	40.0[d]	60.0
Amikacin	4	16	93.3	6.7	86.7[d]	13.3
Colistin	0.12	0.5		0.0	100.0	0.0
Isolates with no ESBL, no transferable AmpC, and no carbapenemase (718)[k]						
Ceftazidime/avibactam	0.5	1	99.9	0.1	99.9	0.1
Ceftolozane/tazobactam	8	>16	18.1	66.8	18.1	81.9
Ceftazidime	>32	>32	0.7	98.7	0.1	99.3
Cefepime	1	4	79.9	3.2	55.2	8.2
Piperacillin/tazobactam	64	>128	12.4	46.7	5.4	87.6
Meropenem	0.06	0.25	98.9	0.8	99.2	0.0
Ertapenem	0.5	2	72.9	15.5	72.9	27.1
Levofloxacin	0.06	1	87.5	8.5	87.5	8.5
Gentamicin	0.25	0.5	98.1	1.9	97.9[d]	2.1
Amikacin	1	2	100.0	0.0	99.7[d]	0.3
Colistin	0.25	>8		12.7	87.3	12.7

[a]Criteria as published by CLSI[9] and EUCAST.[10]

[b]Organisms include: *Citrobacter freundii* species complex (262) and *E. cloacae* species complex (652).

[c]Ertapenem was only tested in 2018 and 2019.

[d]For infections originating from the urinary tract. For systemic infections, aminoglycosides must be used in combination with another active therapy.

[e]Organisms include *Citrobacter freundii* species complex (19) and *Enterobacter cloacae* species complex (37).

[f]Excludes isolates with carbapenemases. Organisms include *Citrobacter freundii* species complex (21) and *Enterobacter cloacae* species complex (100).

[g]Excludes isolates with carbapenemases. Organisms include *Citrobacter freundii* species complex (9) and *Enterobacter cloacae* species complex (55).

[h]Excludes isolates with carbapenemases. Organisms include *Citrobacter freundii* species complex (7) and *Enterobacter cloacae* species complex (48).

[i]Excludes isolates with carbapenemases. Organisms include *Citrobacter freundii* species complex (6) and *Enterobacter cloacae* species complex (9).

[j]Only 9 isolates tested (2018–19).

[k]No carbapenemase, ESBL, or transferable AmpC gene was detected. Organisms include *Citrobacter freundii* species complex (221) and *E. cloacae* species complex (509).

Table 3. β-Lactamase genes identified among 914 *Enterobacter cloacae* species complex and *Citrobacter freundii* species complex isolates displaying elevated MIC values for ceftazidime (≥16 mg/L) and/or cefepime (≥2 mg/L)

Gene results	No. of positive results		
	Overall	*C. freundii*	*E. cloacae*
Carbapenemases			
Serine carbapenemases	58	19	39
KPC total	56	19	37
KPC-2	21	12	9
KPC-3	30	6	24
KPC-4	3	1	2
KPC-6	2	0	2
NMC-A	2	0	2
Metallo-β-lactamases	5	1	4
IMP-4	1	0	1
NDM-1	3	1	2
VIM-1	1	0	1
ESBLs			
SHV total	81	11	70
SHV-12	56	7	49
SHV-7	13	1	12
SHV-30	7	2	5
SHV-5	2	1	1
SHV-12-like	1	0	1
SHV-2-like	1	0	1
SHV-7-like	1	0	1
CTX-M total	61	7	4
CTX-M-15	48	6	42
CTX-M-9	6	0	6
CTX-M-3	4	0	4
CTX-M-14	2	0	2
CTX-M-1	1	1	0
OXA total	49	13	36
OXA-17-like	1	1	0
OXA-1/OXA-30	47	11	36
OXA-4	1	1	0
GES-7	1	1	0
TEM-12	1	0	1
Transferable AmpC			
CMY-109	1	0	1
DHA-1	8	3	5
FOX-5	6	3	3

regardless of *in vitro* susceptibility.[15,16] A recent report of the British Society for Antimicrobial Chemotherapy/Healthcare Infection Society/British Infection Association Joint Working Party suggested that cefepime could be used when the organism is susceptible by EUCAST criteria (MIC ≤1 mg/L), but strongly recommended the use of carbapenems instead. This document also stated that temocillin could be used to treat UTI and ceftazidime/avibactam could be used as an alternative to the carbapenems.[17]

Although *E. cloacae* and *C. freundii* represent important causes of healthcare-associated infections and may express high rates of antimicrobial resistance, we could not find any study that properly evaluated the frequency of occurrence of acquired β-lactamases among these organisms or other Enterobacterales species that produce inducible chromosomal AmpC in the United States. There have been sporadic reports of *E. cloacae* producing KPC-3 or ESBLs, such SHV-7,[18,19] but the frequency of acquired carbapenemases or ESBLs among *E. cloacae* and *C. freundii* in the United States is unknown.

In the present study, we evaluated 3579 contemporary isolates of *E. cloacae* and *C. freundii* from US medical centres. Approximately one-fourth of these isolates (n = 914; 25.5%) exhibited resistance to ceftazidime (MIC, ≥16 mg/L) and/or decreased susceptibility to cefepime (MIC, ≥2 mg/L). The most common mechanism responsible for this resistance pattern appears to be hyperproduction of chromosomal AmpC, since carbapenemases, ESBLs, or transferable AmpC-encoding genes were not identified in 78.6% (718/914) of isolates submitted to WGS. Among isolates producing β-lactamases that hydrolyse broad-spectrum cephalosporins or carbapenems, the most common β-lactamase type observed was SHV, followed by KPC and CTX-M. In summary, 6.9% (63/914) of the collection with decreased susceptibility to ceftazidime and/or cefepime produced a carbapenemase, mainly of the KPC-type, and 13.2% (121/914) produced an ESBL, mainly of the SHV-type and CTX-M-type.

The inclusion criteria for performing WGS (MIC values of ≥16 mg/L for ceftazidime or ≥2 mg/L for cefepime) was selected to optimize the detection of ESBLs, transferable AmpCs, and carbapenemases. Since it is thought that most isolates that produce chromosomally derepressed AmpC usually remain susceptible to cefepime unless they express an additional resistance mechanism,[15] we could have selected only isolates with elevated MIC values for both ceftazidime and cefepime. However, when the WGS results were stratified according to the MIC values for ceftazidime and cefepime, we observed that 32 of 428 (7.5%) isolates with ceftazidime MICs ≥16 mg/L *and* cefepime MICs ≤1 mg/L harboured an acquired β-lactamase gene (20 ESBL producers, 10 transferable AmpC producers, and 2 carbapenemase producers). Moreover, 314 of 428 (68.6%) isolates with a ceftazidime MIC ≥16 mg/L *and* a cefepime MIC ≥2 mg/L did not harbour an ESBL, a transferable AmpC, or a carbapenemase gene.

As a limitation of the study, we did not evaluate the expression of AmpC genes or porin alterations. Although the assessment of mutations in several genes involved in AmpC regulation and the porins would bring additional value to the investigation, the goal of our study was to evaluate the presence of acquired β-lactamases among *C. freundii* and *E. cloacae* isolates displaying decreased susceptibility to broad-spectrum cephalosporins, which has not been systematically evaluated among these species. In addition to information on the occurrence of acquired β-lactamases, our study provides valuable information on the activities of ceftazidime/avibactam and ceftolozane/tazobactam against these organisms.

The results of this investigation also showed that ceftazidime/avibactam remained highly active against the vast majority of *E. cloacae* and *C. freundii* isolates with decreased susceptibility to ceftazidime and/or cefepime (99.3% susceptibility). Amikacin also retained activity against this collection of organisms, with a susceptibility rate of 99.5% per CLSI criteria (98.0% per EUCAST criteria), and may represent an option for treatment of cUTI; however,
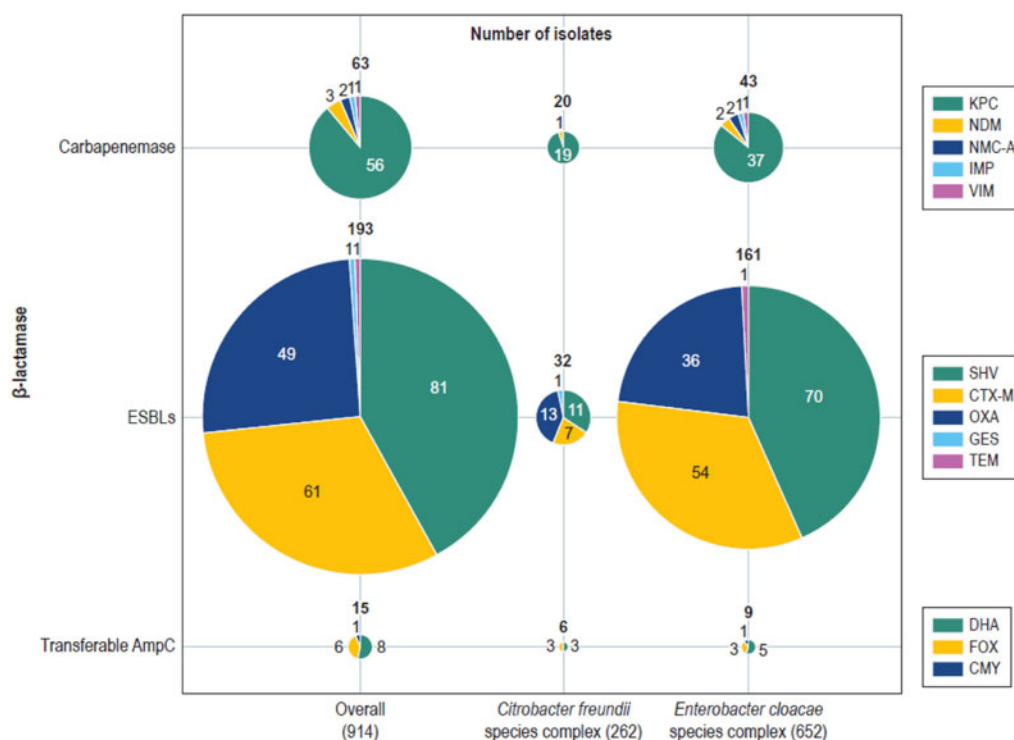
Figure 1. Genes encoding broad-spectrum β-lactamases detected among for 914 *Enterobacter cloacae* species complex and *Citrobacter freundii* species complex isolates that displayed elevated MIC values for ceftazidime (≥16 mg/L) and/or cefepime (≥2 mg/L) collected in 2017–19 from United States medical centres.

its use as monotherapy should be avoided for systemic infections.[20] Meropenem remains active against isolates that do not produce a carbapenemase.

Transparency declarations

JMI Laboratories contracted to perform services in 2019 and 2020 for Albany College of Pharmacy and Health Sciences, Allecra Therapeutics, Allergan, AmpliPhi Biosciences Corp., Amicrobe Advanced Biomaterials, Amplyx, Antabio, American Proficiency Institute, Arietis Corp., Arixa Pharmaceuticals, Inc., Astellas Pharma Inc., Athelas, Basilea Pharmaceutica Ltd., Bayer AG, Becton, Dickinson and Company, bioMérieux SA, Boston Pharmaceuticals, Bugworks Research Inc., CEM-102 Pharmaceuticals, Cepheid, Cidara Therapeutics, Inc., CorMedix Inc., DePuy Synthes, Destiny Pharma, Discuva Ltd., Dr Falk Pharma GmbH, Emery Pharma, Entasis Therapeutics, Eurofarma Laboratorios SA, US Food and Drug Administration, Fox Chase Chemical Diversity Center, Inc., Gateway Pharmaceutical LLC, GenePOC Inc., Geom Therapeutics, Inc., GlaxoSmithKline plc, Harvard University, Helperby, HiMedia Laboratories, F. Hoffmann-La Roche Ltd., ICON plc, Idorsia Pharmaceuticals Ltd., Iterum Therapeutics plc, Laboratory Specialists, Inc., Melinta Therapeutics, Inc., Merck & Co., Inc., Microchem Laboratory, Micromyx, MicuRx Pharmaceuticals, Inc., Mutabilis Co., Nabriva Therapeutics plc, NAEJA-RGM, Novartis AG, Oxoid Ltd., Paratek Pharmaceuticals, Inc., Pfizer, Inc., Polyphor Ltd., Pharmaceutical Product Development, LLC, Prokaryotics Inc., Qpex Biopharma, Inc., Roivant Sciences, Ltd., Safeguard Biosystems, Scynexis, Inc., SeLux Diagnostics, Inc., Shionogi and Co., Ltd., SinSa Labs, Spero Therapeutics, Summit Pharmaceuticals International Corp., Synlogic, T2 Biosystems, Inc., Taisho Pharmaceutical Co., Ltd., TenNor Therapeutics Ltd., Tetraphase Pharmaceuticals, Theravance Biopharma, University of Colorado, University of Southern California-San Diego, University of North Texas Health Science Center, VenatoRx Pharmaceuticals, Inc., Viosera Therapeutics, Vyome Therapeutics Inc., Wockhardt, Yukon Pharmaceuticals, Inc., Zai Lab, Zavante Therapeutics, Inc. There are no speakers' bureaus or stock options to declare.

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