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Comparative Activities of Ceftazidime-Avibactam and Ceftolozane-Tazobactam against *Enterobacteriaceae* Isolates Producing Extended-Spectrum β-Lactamases from U.S. Hospitals

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ABSTRACT The activities of ceftazidime-avibactam, ceftolozane-tazobactam, and comparators were evaluated for 733 isolates displaying resistance to broad-spectrum cephalosporins and carrying extended-spectrum β-lactamase (ESBL) genes detected by whole-genome sequencing analysis. Isolates were collected during 2017 in U.S. hospitals. The ESBL producers were 486 *Escherichia coli*, 190 *Klebsiella pneumoniae*, and 42 *Enterobacter cloacae* isolates and isolates from 3 other species. The most common groups of ESBL-encoding genes were $bla_{\text{CTX-M-15}}$-like ($n = 491$ isolates) and $bla_{\text{CTX-M-15}}$ alone ($n = 168$) or plus $bla_{\text{OXA-1}}$ ($n = 260$), followed by $bla_{\text{CTX-M-14}}$-like ($n = 162$), which included $bla_{\text{CTX-M-27}}$ and $bla_{\text{CTX-M-14}}$ (104 and 51 isolates, respectively), and $bla_{\text{SHV-12}}$ and $bla_{\text{SHV-7}}$ (48 and 22 isolates, respectively). ESBL producers carried other β-lactamases, including 1 *E. cloacae* harboring $bla_{\text{KPC-3}}$. All ESBL-producing isolates were susceptible to ceftazidime-avibactam, and 90.2/83.9% (CLSI/EUCAST breakpoints) were susceptible to ceftolozane-tazobactam. Tigecycline (98.1/95.8% susceptible) and colistin (99.2%) were comparators that displayed the greatest activity against these isolates. Ceftolozane-tazobactam inhibited 91.4/83.9% of isolates carrying $bla_{\text{CTX-M-15}}$-like and 97.5/95.1% of isolates carrying $bla_{\text{CTX-M-14}}$-like, and its activity was more limited against the 91 isolates carrying bla_{SHV} (66.7/61.1% susceptible). Ceftolozane-tazobactam inhibited 95.5% of the *E. coli* isolates but only 83.0%, 64.3%, and 80.0% of *K. pneumoniae*, *E. cloacae*, and other species harboring ESBL-encoding genes (CLSI breakpoints), respectively. Outer membrane protein sequences for ceftolozane-tazobactam-nonsusceptible isolates did not exhibit significant differences compared to those in genetically related ceftolozane-tazobactam-susceptible isolates. Ceftazidime-avibactam was more active than other agents tested, including ceftolozane-tazobactam, and the activity of this combination was stable regardless of species or ESBL gene carried.

KEYWORDS CTX-M, ESBL, ceftazidime-avibactam, ceftolozane-tazobactam

Isolates producing extended-spectrum β-lactamases (ESBLs) have increased in the hospital and community settings over the past 2 decades, with a dominance of isolates carrying CTX-M-encoding genes (1–6) over the ESBL variants of TEM and SHV that were predominant in the early 1990s (7). Initially, isolates harboring $bla_{\text{CTX-M}}$ were mainly *Escherichia coli*, but now this gene has been detected in *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Enterobacter*, *Citrobacter*, and *Proteus* species among others. *Escherichia coli* isolates harboring $bla_{\text{CTX-M}}$ often belong to the fluoroquinolone-resistant ST131 lineage, are resistant to cefepime due to the coproduction of OXA-1 (also known as OXA-30), and harbor other resistance genes transferred in the same plasmids (1, 2, 8). The combination of these traits confers a multidrug-resistant (MDR) profile to

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isolates carrying bla_{CTX-M} that can be observed in other species carrying bla_{CTX-M} or other ESBL genes (9, 10).

Due to resistance to cephalosporins and other antimicrobial classes, carbapenems are considered the drug of choice and have been broadly used for the treatment of infections caused by organisms producing ESBLs (11). These agents are recommended for empirical therapy of serious infections in geographic areas where ESBL rates are known to be elevated (12). However, the increased use of carbapenems has led to resistance against these antimicrobial agents due to selective pressure, and an increase in the carbapenem-resistant *Enterobacteriaceae* rates have been noted in the past 20 years (13).

Carbapenem-sparing therapies have been broadly discussed, and other active agents against ESBL-producing isolates include classic and newer β-lactam/β-lactamase inhibitor (BLBLI) combinations, cephamycins, temocillin, aminoglycosides, tigecycline, and less often due to elevated resistance rates, fluoroquinolones and trimethoprim-sulfamethoxazole (5, 11, 13). Among these agents, (i) aminoglycosides are recommended for urinary tract infections, but the efficacy of these agents in combination is not supported for serious infections (13); (ii) although cephamycins and temocillin are stable against most ESBL and AmpC enzymes, these agents have conflicting efficacy data available against serious infections (14–16); and (iii) tigecycline achieves a low plasma concentration and is not indicated for bloodstream infections (17).

Classic and newer BLBLIs, including piperacillin-tazobactam, ceftolozane-tazobactam, and ceftazidime-avibactam, seem to be valuable alternatives for the treatment of serious infections caused by ESBL-producing organisms (13). In this study, we compared the activities of ceftazidime-avibactam, ceftolozane-tazobactam, and other agents against 733 carbapenem-susceptible *Enterobacteriaceae* isolates carrying ESBL genes identified using a whole-genome sequencing analysis approach. These isolates were collected in 69 U.S. hospitals during 2017.

RESULTS

Occurrence of ESBL-encoding genes. Among 7,026 isolates from selected species collected during 2017 in U.S. hospitals and tested, 1,095 isolates resistant to broad-spectrum cephalosporins that were not resistant to carbapenems were screened for β-lactamase genes; 733 carried ESBL-encoding genes. Carbapenem-resistant isolates were excluded, since ceftolozane-tazobactam has limited activity against these isolates (18).

ESBL-producing isolates comprised 486 *E. coli* (14.2% for this species), 190 *K. pneumoniae* (10.4%), 42 *Enterobacter cloacae* (4.6%), 8 *K. oxytoca* (2.1%), 5 *Citrobacter freundii* (1.7%), and 2 *Citrobacter koseri* (1.1%) isolates. Isolates harboring ESBL genes were mainly recovered from urinary tract infections (286 isolates; 39.0% overall), bloodstream infections (159 isolates; 21.7% overall), and pneumonia in hospitalized patients (172 isolates; 23.5% overall), but also from skin and skin structure infections (80 isolates; 10.9% overall) and intra-abdominal infections (33 isolates; 4.5% overall). Only 3 isolates were recovered from other or unknown sources (0.4% overall).

A total of 447 isolates carried $bla_{CTX-M-15}$, but this gene was detected without other ESBL-encoding genes among only 168 isolates (Table 1). Most commonly, this gene was carried along with bla_{OXA-1} alone (260 isolates) or with bla_{SHV} or bla_{TEM} (10 isolates). Overall, isolates harboring $bla_{CTX-M-15}$ were 291 *E. coli* and 138 *K. pneumoniae* isolates but also 13 *E. cloacae*, 4 *K. oxytoca*, and 1 *C. freundii* isolates (Table 1). Following $bla_{CTX-M-15}$, the most common ESBL genes in U.S. hospitals detected during 2017 were $bla_{CTX-M-27}$ (104 isolates), $bla_{CTX-M-14}$ (51 isolates), bla_{SHV-12} (48 isolates), $bla_{CTX-M-55}$ (32 isolates), and bla_{SHV-7} (22 isolates). All these CTX-M-encoding genes were mostly detected among *E. coli* isolates, whereas bla_{SHV-12} was most commonly noted among *K. pneumoniae* and *E. cloacae* (20 isolates each).

The isolates evaluated carried other β-lactamase genes that included transferable cephalosporinases (AmpC), mainly bla_{CMY-2} and various penicillinases (see Table S1).

TABLE 1 Extended-spectrum β-lactamase enzymes detected among 733 isolates from U.S. hospitals

Enzyme	No. of positive results (% of isolates by total)						
	Total (*n* = 733)	*C. freundii* (*n* = 5)	*C. koseri* (*n* = 2)	*E. coli* (*n* = 486)	*E. cloacae* (*n* = 42)	*K. oxytoca* (*n* = 8)	*K. pneumoniae* (*n* = 190)
CTX-M-1 group	491 (67.0)	1 (20.0)	0	328 (67.5)	15 (35.7)	5 (62.5)	142 (74.7)
CTX-M-1	4 (0.5)	0	0	3 (0.6)	0	0	1 (0.5)
CTX-M-15	447 (61.0)	1 (20.0)	0	291 (59.9)	13 (31.0)	4 (50.0)	138 (72.6)
CTX-M-3	8 (1.1)	0	0	4 (0.8)	2 (4.8)	1 (12.5)	1 (0.5)
CTX-M-55	32 (4.4)	0	0	30 (6.2)	0	0	2 (1.1)
CTX-M-2 group	1 (0.1)	0	0	0	0	0	1 (0.5)
CTX-M-115	1 (0.1)	0	0	0	0	0	1 (0.5)
CTX-M-9 group	162 (22.1)	0	0	153 (31.5)	2 (4.8)	0	7 (3.7)
CTX-M-134	1 (0.1)	0	0	1 (0.2)	0	0	0
CTX-M-14	51 (7.0)	0	0	45 (9.3)	1 (2.4)	0	5 (2.6)
CTX-M-19	1 (0.1)	0	0	1 (0.2)	0	0	0
CTX-M-24	3 (0.4)	0	0	3 (0.6)	0	0	0
CTX-M-27	104 (14.2)	0	0	102 (21.0)	0	0	2 (1.1)
CTX-M-9	2 (0.3)	0	0	1 (0.2)	1 (2.4)	0	0
OXA-1	281 (38.3)	2 (40.0)	0	174 (35.8)	9 (21.4)	4 (50.0)	92 (48.4)
SHV	91 (12.4)	3 (60.0)	2 (100.0)	6 (1.2)	27 (64.3)	3 (37.5)	50 (26.3)
SHV-106	1 (0.1)	0	0	0	0	0	1 (0.5)
SHV-12	48 (6.5)	3 (60.0)	0	5 (1.0)	20 (47.6)	0	20 (10.5)
SHV-154	1 (0.1)	0	0	0	0	0	1 (0.5)
SHV-2	3 (0.4)	0	0	0	0	0	3 (1.6)
SHV-27	7 (1.0)	0	0	0	0	0	7 (3.7)
SHV-2A	1 (0.1)	0	0	0	0	0	1 (0.5)
SHV-30	3 (0.4)	0	0	0	1 (2.4)	0	2 (1.1)
SHV-38	1 (0.1)	0	0	0	0	0	1 (0.5)
SHV-5	6 (0.8)	0	0	1 (0.2)	1 (2.4)	0	4 (2.1)
SHV-7	22 (3.0)	0	2 (100.0)	0	5 (11.9)	3 (37.5)	12 (6.3)
TEM	3 (0.4)	0	0	2 (0.4)	1 (2.4)	0	0
TEM-12	1 (0.1)	0	0	0	1 (2.4)	0	0
TEM-169	1 (0.1)	0	0	1 (0.2)	0	0	0
TEM-19	1 (0.1)	0	0	1 (0.2)	0	0	0

One *E. cloacae* isolate carried bla_{KPC-3} despite susceptible carbapenem MIC values (1 mg/liter for both imipenem and meropenem).

Activities of ceftazidime-avibactam, ceftolozane-tazobactam, and comparator agents. All isolates were susceptible to ceftazidime-avibactam, and ceftolozane-tazobactam inhibited 90.2% of the isolates at the CLSI susceptible breakpoint criteria. The isolates tested carrying ESBL genes displayed low susceptibility rates against ceftriaxone (0.1% susceptible for CLSI), ceftazidime (19.9%), cefepime (11.3%), and aztreonam (10.5%). Piperacillin-tazobactam inhibited 84.5% of these isolates when applying the CLSI breakpoint. Carbapenems inhibited 99.5% of the ESBL-producing isolates at CLSI breakpoints, but carbapenem-resistant isolates were excluded from this analysis and carbapenem activity will not be further discussed (Table 2).

Isolates carrying ESBL genes exhibited low susceptibility rates against ciprofloxacin (21.7% susceptible when applying CLSI breakpoints), levofloxacin (21.3%), gentamicin (57.4%), and trimethoprim-sulfamethoxazole (27.8%). These isolates were mostly susceptible to amikacin (97.4%), tigecycline (98.1%; U.S. FDA/EUCAST breakpoints), and colistin (99.2%; EUCAST breakpoint).

The activity of ceftazidime-avibactam was stable regardless of the bacterial species tested (Fig. 1); however, susceptibility rates of comparator agents varied according to the organism. Ceftolozane-tazobactam inhibited 95.5% of the 486 ESBL-carrying *E. coli* isolates but only inhibited 83.0% of the 190 *K. pneumoniae*, 64.3% of the 42 *E. cloacae*, and 80.0% of the other species isolates tested when applying the CLSI breakpoint. Piperacillin-tazobactam susceptibility rates were also lower among isolates from *K. pneumoniae*, *E. cloacae*, and other species (74.7%, 64.3%, and 60.0%, respectively) than

TABLE 2 Activities of ceftazidime-avibactam, ceftolozane-tazobactam, and comparators tested against 733 *Enterobacteriaceae* isolates carrying ESBL genes

Antimicrobial agent	MIC (mg/liter)			CLSI[a]		EUCAST	
	50%	90%	Range	%S	%R	%S	%R
All isolates carrying ESBLs (n = 733)							
Ceftazidime-avibactam	0.25	0.5	≤0.015 to 4	100.0	0.0	100.0	0.0
Ceftolozane-tazobactam	0.5	2	≤0.12 to >16	90.2	7.3	83.9	16.1
Ceftazidime	16	>32	0.25 to >32	19.9	67.1	4.5	80.1
Aztreonam	>16	>16	0.5 to >16	10.5	78.7	1.0	89.5
Ceftriaxone	>8	>8	1 to >8	0.1	98.9	0.1	98.9
Cefepime	>16	>16	≤0.12 to >16	11.3	71.2[b]	5.7	80.9
Piperacillin-tazobactam	4	64	0.25 to >128	84.4	7.6	71.2	15.6
Meropenem	0.03	0.06	≤0.015 to 2	99.5	0.0	100.0	0.0
Levofloxacin	8	>16	≤0.03 to >16	29.9	65.9	21.3	72.9
Gentamicin	1	>16	≤0.12 to >16	57.4	40.1	56.2	42.6
Amikacin	2	8	0.5 to >32	97.4	0.7	93.2	2.6
Trimethoprim-sulfamethoxazole	>8	>8	≤0.5 to >8	27.8	72.2	27.8	71.5
Tigecycline	0.25	1	≤0.06 to 8	98.1	0.1[c]	95.8	1.9
Colistin	0.12	0.25	≤0.06 to >8	99.2[d]		99.2	0.8
Isolates carrying bla_{CTX-M} (n = 650)							
Ceftazidime-avibactam	0.12	0.5	≤0.015 to 4	100.0	0.0	100.0	0.0
Ceftolozane-tazobactam	0.5	2	≤0.12 to >16	92.9	4.6	86.6	13.4
Ceftazidime	16	>32	0.25 to >32	21.5	64.6	4.9	78.5
Aztreonam	>16	>16	0.5 to >16	10.5	78.3	0.6	89.5
Ceftriaxone	>8	>8	8 to >8	0.0	100.0	0.0	100.0
Cefepime	>16	>16	1 to >16	5.5	78.2[b]	0.8	87.8
Piperacillin-tazobactam	4	32	0.25 to >128	86.6	5.7	73.2	13.4
Meropenem	0.03	0.06	≤0.015 to 2	99.4	0.0	100.0	0.0
Levofloxacin	16	>16	≤0.03 to >16	25.8	69.8	19.1	76.5
Gentamicin	1	>16	≤0.12 to >16	58.3	40.5	57.8	41.7
Amikacin	4	8	0.5 to >32	98.0	0.6	94.3	2.0
Trimethoprim-sulfamethoxazole	>8	>8	≤0.5 to >8	25.7	74.3	25.7	73.5
Tigecycline	0.25	1	≤0.06 to 8	98.3	0.2[c]	96.6	1.7
Colistin	0.12	0.25	≤0.06 to >8	99.2[d]		99.2	0.8
Isolates carrying $bla_{CTX-M-15}$ (n = 491)							
Ceftazidime-avibactam	0.25	0.5	≤0.015 to 4	100.0	0.0	100.0	0.0
Ceftolozane-tazobactam	0.5	2	≤0.12 to >16	91.4	5.7	83.9	16.1
Ceftazidime	32	>32	1 to >32	9.0	81.3	0.8	91.0
Aztreonam	>16	>16	2 to >16	4.5	92.1	0.0	95.5
Ceftriaxone	>8	>8	8 to >8	0.0	100.0	0.0	100.0
Cefepime	>16	>16	1 to >16	3.9	90.0[b]	0.6	94.3
Piperacillin-tazobactam	8	32	0.25 to >128	83.1	6.9	65.4	16.9
Meropenem	0.03	0.06	≤0.015 to 2	99.2	0.0	100.0	0.0
Levofloxacin	8	>16	≤0.03 to >16	27.9	67.6	19.8	75.2
Gentamicin	4	>16	≤0.12 to >16	50.1	48.7	49.7	49.9
Amikacin	4	8	0.5 to >32	97.4	0.8	92.7	2.6
Trimethoprim-sulfamethoxazole	>8	>8	≤0.5 to >8	25.1	74.9	25.1	73.9
Tigecycline	0.25	1	≤0.06 to 8	98.0	0.2[c]	95.9	2.0
Colistin	0.12	0.25	≤0.06 to >8	99.4[d]		99.4	0.6
Isolates carrying $bla_{CTX-M-14}$-like (n = 162)							
Ceftazidime-avibactam	0.12	0.25	≤0.015 to 1	100.0	0.0	100.0	0.0
Ceftolozane-tazobactam	0.25	1	≤0.12 to >16	97.5	1.2	95.1	4.9
Ceftazidime	4	32	0.25 to >32	59.3	15.4	17.3	40.7
Aztreonam	8	>16	0.5 to >16	28.4	37.0	2.5	71.6
Ceftriaxone	>8	>8	8 to >8	0.0	100.0	0.0	100.0
Cefepime	8	>16	1 to >16	10.5	42.6[b]	1.2	68.5
Piperacillin-tazobactam	2	4	0.5 to >128	96.9	1.9	96.3	3.1
Meropenem	0.03	0.03	≤0.015 to 1	100.0	0.0	100.0	0.0
Levofloxacin	16	>16	≤0.03 to >16	20.4	76.5	17.9	79.6
Gentamicin	1	>16	0.25 to >16	83.3	15.4	82.7	16.7
Amikacin	2	4	1 to >32	99.4	0.6	98.8	0.6
Trimethoprim-sulfamethoxazole	>8	>8	≤0.5 to >8	27.2	72.8	27.2	72.8
Tigecycline	0.25	0.5	≤0.06 to 4	99.4	0.0 [c]	99.4	0.6
Colistin	0.12	0.25	≤0.06 to 4	98.8[d]		98.8	1.2

(Continued on next page)

TABLE 2 (Continued)

Antimicrobial agent	MIC (mg/liter)			CLSI[a]		EUCAST	
	50%	90%	Range	%S	%R	%S	%R
Isolates carrying bla_{SHV} ($n = 91$)							
Ceftazidime-avibactam	0.25	1	≤0.015 to 2	100.0	0.0	100.0	0.0
Ceftolozane-tazobactam	0.5	>16	≤0.12 to >16	66.7	28.9	61.1	38.9
Ceftazidime	>32	>32	2 to >32	5.5	91.2	0.0	94.5
Aztreonam	>16	>16	0.5 to >16	8.8	85.7	1.1	91.2
Ceftriaxone	>8	>8	2 to >8	0.0	93.4	0.0	93.4
Cefepime	4	>16	≤0.12 to >16	45.1	31.9[b]	38.5	40.7
Piperacillin-tazobactam	8	>128	0.5 to >128	67.0	23.1	56.0	33.0
Meropenem	0.03	0.06	≤0.015 to 1	100.0	0.0	100.0	0.0
Levofloxacin	1	>16	≤0.03 to >16	61.5	34.1	40.7	42.9
Gentamicin	8	>16	≤0.12 to >16	44.0	44.0	37.4	56.0
Amikacin	2	16	0.5 to >32	91.2	1.1	83.5	8.8
Trimethoprim-sulfamethoxazole	>8	>8	≤0.5 to >8	40.7	59.3	40.7	59.3
Tigecycline	0.5	2	0.12 to 4	95.6	0.0 [c]	89.0	4.4
Colistin	0.12	0.25	≤0.06 to >8	96.4[d]		96.7	3.3
Isolates carrying bla_{OXA-1} ($n = 281$)							
Ceftazidime-avibactam	0.25	0.5	≤0.015 to 4	100.0	0.0	100.0	0.0
Ceftolozane-tazobactam	0.5	2	≤0.12 to >16	90.7	7.1	82.1	17.9
Ceftazidime	32	>32	1 to >32	10.7	80.8	1.8	89.3
Aztreonam	>16	>16	0.5 to >16	6.4	89.3	0.4	93.6
Ceftriaxone	>8	>8	1 to >8	0.4	99.6	0.4	99.6
Cefepime	>16	>16	≤0.12 to >16	8.2[b]	84.7	1.8	89.7
Piperacillin-tazobactam	16	64	1 to >128	73.3	10.0	44.1	26.7
Meropenem	0.03	0.06	≤0.015 to 2	98.6	0.0	100.0	0.0
Levofloxacin	16	>16	≤0.03 to >16	14.6	81.9	14.6	81.9
Gentamicin	>16	>16	0.25 to >16	35.6	63.7	35.2	64.4
Amikacin	4	16	1 to >32	96.4	1.1	87.5	3.6
Trimethoprim-sulfamethoxazole	>8	>8	≤0.5 to >8	18.9	81.1	18.9	80.4
Tigecycline	0.25	1	≤0.06 to 4	97.5[c]	0.0		
Colistin	0.12	0.25	≤0.06 to >8	98.9[d]		98.9	1.1

[a]Clinical and Laboratory Standards Institute (CLSI) criteria (21). S, susceptible; R, resistant.
[b]Intermediate interpreted as susceptible-dose dependent.
[c]U.S. FDA breakpoint (https://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/ucm575163.htm).
[d]Percentage of wild type based on epidemiological cutoff value (CLSI M100 [21]).

among *E. coli* isolates (90.7% susceptible). Rates for gentamicin displayed a similar trend, and the activity of this aminoglycoside was lower in other species (50.0% to 20.0%) than in *E. coli* (63.8%). The activities of cefepime and levofloxacin were lower in *E. coli* (8.6% and 14.4%, respectively) than in other species/groups.

Isolates carrying bla_{CTX-M} ($n = 650$) were inhibited at ≤4 mg/liter and categorized as susceptible to ceftazidime-avibactam; this collection included 491 $bla_{CTX-M-15}$-like genes composed of $bla_{CTX-M-1}$, $bla_{CTX-M-3}$, and $bla_{CTX-M-15}$ among others and 162 $bla_{CTX-M-14}$-like genes, mainly $bla_{CTX-M-27}$ and $bla_{CTX-M-14}$ (104 and 51 isolates, respectively). Ceftolozane-tazobactam inhibited 92.9% of the isolates harboring bla_{CTX-M}, 91.4% of the isolates carrying $bla_{CTX-M-15}$, and 97.5% of the isolates carrying $bla_{CTX-M-14}$. Low susceptibility rates were noted among isolates carrying bla_{CTX-M} for levofloxacin (25.8% susceptible per CLSI) and trimethoprim-sulfamethoxazole (25.7%). Gentamicin was active against 58.3% of the isolates, and piperacillin-tazobactam was active against 86.6% of the isolates. Amikacin, colistin, and tigecycline were the most active agents against these isolates (≥98.0% susceptible for CLSI).

The highest ceftazidime-avibactam MIC value observed for 91 isolates carrying bla_{SHV} encoding ESBL enzymes was 2 mg/liter. Ceftolozane-tazobactam inhibited only 66.7% of these isolates, and piperacillin-tazobactam inhibited 67.0% at the CLSI breakpoints. Tigecycline and colistin were the most active comparators against these isolates.

Against isolates carrying bla_{OXA-1} ($n = 281$), ceftolozane-tazobactam and piperacillin-tazobactam inhibited 90.7% and 73.3% of the isolates, respectively, at respective CLSI breakpoints.

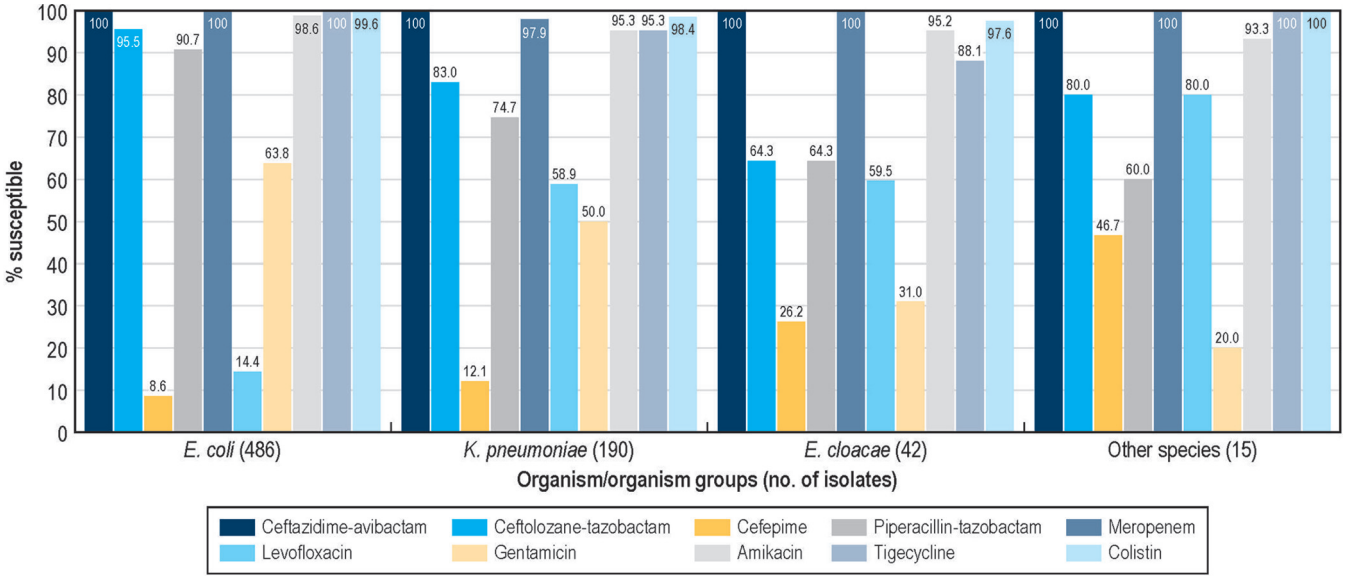

FIG 1 Activities of ceftazidime-avibactam, ceftolozane-tazobactam, and comparator agents using CLSI breakpoints against bacterial species carrying ESBL genes. ESBL, extended-spectrum β-lactamase.

Overall, the activities of ceftolozane-tazobactam and piperacillin-tazobactam were lower against *K. pneumoniae* isolates carrying common ESBL genes than against *E. coli* isolates harboring the same genes (Table S2).

OMP analysis of ceftolozane-nonsusceptible isolates. A total of 72 (9.8%) ceftolozane-tazobactam-nonsusceptible isolates, when applying the CLSI breakpoint criteria, were observed in this collection of ESBL producers. These isolates were 32 *K. pneumoniae*, 22 *E. coli*, 15 *E. cloacae*, 2 *K. oxytoca*, and 1 *C. freundii* carrying $bla_{\text{CTX-M}}$ variants (46/72 isolates; 39 harbored $bla_{\text{CTX-M-15}}$) or bla_{SHV} (23 isolates), mostly *K. pneumoniae* or *E. cloacae* harboring $bla_{\text{SHV-12}}$ (12 and 10 isolates, respectively).

Ten *K. pneumoniae*, 10 *E. coli*, and 4 *E. cloacae* ceftolozane-tazobactam-nonsusceptible isolates were matched with isolates susceptible to this combination that belonged to the same sequence type (ST) (Table 3). This approach was chosen to decrease the number of polymorphisms in the outer membrane protein (OMP) sequences that might not impact resistance.

The OMP protein sequence comparison of the ceftolozane-tazobactam-nonsusceptible and susceptible pairs revealed differences in only 2 isolates. One ceftolozane-tazobactam-resistant *K. pneumoniae* belonging to ST307 (MIC, >16 mg/liter) had a nonsense mutation in OmpK36 compared to that in its susceptible counterpart (ceftolozane-tazobactam MIC, 1 mg/liter) (Table 3). Additionally, 1 *E. coli* isolate belonging to ST167 (MIC, 16 mg/liter) displayed multiple alterations in OmpC compared to that in an isolate exhibiting a ceftolozane-tazobactam MIC of 0.5 mg/liter (see Fig. S1).

Among the paired isolates, 5 *E. coli* and 8 *K. pneumoniae* isolates carried the same $bla_{\text{CTX-M}}$ gene and 4 *E. coli* and 1 *K. pneumoniae* isolates carried different $bla_{\text{CTX-M}}$ variants. Two *E. cloacae* groups harbored different bla_{SHV} variants. Among 1 *E. cloacae*, 5 *K. pneumoniae*, and 3 *E. coli* pairs, $bla_{\text{OXA-1}}$ was observed in the ceftolozane-tazobactam-nonsusceptible isolate but not in the susceptible pair; however, this gene was present in both isolates from other nonsusceptible/susceptible pairs.

DISCUSSION

We screened the most common *Enterobacteriaceae* genus/species collected in 70 U.S. hospitals during 2017 that displayed susceptibility to carbapenems and resistance to broad-spectrum cephalosporins for the presence of ESBL-encoding genes. Our results demonstrated that the activities of ceftazidime-avibactam and ceftolozane-tazobactam are comparable for most *E. coli* isolates carrying different ESBL enzymes

TABLE 3 Comparison of OMP sequences among ceftolozane-tazobactam-nonsusceptible and susceptible isolates from the same sequence type

Organism sequence type	MIC (mg/liter)[a]				OMP sequence alteration[b,c]		
	Ceftolozane-tazobactam	Ceftazidime-avibactam	Piperacillin-tazobactam	β-lactamase(s) present[b]	OmpC/ OmpK36	OmpF/ OmpK35	OmpK37
E. cloacae							
78	16 (R)	1	>128	DHA-1, **OXA-1**	WT	WT	NA
	1 (S)	0.5	4	CTX-M-15, TEM-1			
113	16 (R)	0.5	64	SHV-12, TEM-1	WT	WT	NA
	0.25 (S)	0.25	2	SHV-7			
114	16 (R)	1	64	SHV-12, TEM-1	WT	WT	NA
	0.5 (S)	0.12	1	CTX-M-15, TEM-1			
133	>16 (R)	1	128	SHV-12	WT	WT	NA
	2 (S)	0.5	16	SHV-7			
E. coli							
38	4 (I)	0.5	2	CTX-M-19, TEM-1	WT	WT	NA
	1 (S)	0.25	4	CTX-M-15, TEM-1			
131	16 (R)	0.12	32	CTX-M-14, TEM-1	WT	WT	NA
	0.25 (S)	0.06	2	CTX-M-134			
167	16 (R)	2	128	CTX-M-55, SHV-12, TEM-1	Multiple alterations	WT	NA
	0.5 (S)	≤0.015	8	CTX-M-15, **OXA-1**			
354	4 (I)	0.5	4	CTX-M-24, TEM-1	Q349L	WT	NA
	1 (S)	0.5	4	CTX-M-27, TEM-1			
405	16 (R)	2	64	CTX-M-15, **OXA-1**, TEM-1	WT	WT	NA
	0.5 (S)	0.25	4	CTX-M-15			
410	16 (R)	2	>128	CTX-M-15, **OXA-1**	WT	WT	NA
	0.5 (S)	0.06	4	CTX-M-15, TEM-1			
617	>16 (R)	4	>128	CTX-M-15, **OXA-1**	WT	WT	NA
	0.5 (S)	0.12	8	CTX-M-15, TEM-1			
636	4 (I)	0.12	>128	CTX-M-15, TEM-1	Q37K, D138G	WT	NA
	0.5 (S)	0.25	4	CTX-M-15, TEM-1			
977	4 (I)	0.5	16	CMY-2, CTX-M-15, TEM-1	WT	WT	NA
	1 (S)	0.25	8	CMY-2, CTX-M-15, TEM-1			
1196	8 (R)	≤0.015	128	CTX-M-55, TEM-1	WT	WT	NA
	1 (S)	≤0.015	8	CTX-M-55, TEM-1			
K. pneumoniae							
14	>16 (R)	1	>128	OXA-10, SHV-12, SHV-28	WT	WT	WT
	0.5 (S)	0.12	16	CTX-M-15, OXA-1, SHV-28, TEM-1			
15	8 (R)	0.5	128	CARB-2, CTX-M-15, FOX-5, SHV-28, TEM-1	WT	WT	WT
	1 (S)	0.25	4	CTX-M-115, SHV-28			
16	4 (I)	0.5	32	CTX-M-15, OXA-1, SHV-1, TEM-1	WT	WT	WT
	1 (S)	0.25	16	CTX-M-15, OXA-1, SHV-1			
17	>16 (R)	0.5	>128	OXA-1, SHV-11-like, SHV-12	WT	WT	WT
	1 (S)	0.12	8	CTX-M-15, OXA-1, SHV-11, TEM-1			
29	16 (R)	0.5	>128	CTX-M-15, SHV-1-like, TEM-1	WT	WT	T15A, A209T
	1 (S)	0.25	4	CTX-M-15, SHV-187-like, TEM-1			
37	16 (R)	0.5	>128	CTX-M-15, OXA-1, SHV-11, TEM-1	WT	WT	WT
	0.25 (S)	0.12	8	CTX-M-15, OXA-1-like, SHV-11, TEM-1			
307	>16 (R)	0.5	128	CTX-M-15, **OXA-1**, SHV-28-like, TEM-1	WT	**X125 (stop)**	WT
	1 (S)	0.25	2	CTX-M-15, SHV-28, TEM-1			
392	4 (I)	0.25	4	CTX-M-15, SHV-11	WT	WT	WT
	0.5 (S)	0.12	1	CTX-M-15, SHV-11			
405	4 (I)	0.5	16	CTX-M-15, **OXA-1**, SHV-76, TEM-1	WT	WT	WT
	2 (S)	0.25	4	CTX-M-15, SHV-76, TEM-1			
788	4 (I)	0.5	>128	CTX-M-15, **OXA-1**, SHV-52, TEM-1	WT	WT	WT
	0.5 (S)	0.25	4	CTX-M-15, SHV-52, TEM-1			

[a]MIC interpretation by CLSI for ceftolozane tazobactam. S, susceptible; I, intermediate; R, resistant.
[b]Results in boldface font were considered important and are addressed in the OMP analysis in section 3.
[c]OMP, outer membrane protein (grouped according to homology of sequences); NA, not applicable (OmpK37 has no homologous OMP in *E. coli* or *E. cloacae*); WT, wild type. Mutations not bolded were observed in comparison with the ceftolozane-tazobactam-susceptible isolate belonging to the same sequence type but not in a susceptible ATCC sequence also compared.

(100.0% versus 95.5% applying CLSI criteria); however, ceftazidime-avibactam was active against all *K. pneumoniae* and *E. cloacae* isolates carrying common ESBL-encoding genes, such as $bla_{CTX\text{-}M}$ or bla_{SHV}, in the U.S. hospitals, while only 83.0% and 64.3% of these respective species were inhibited by ceftolozane-tazobactam when applying the U.S. FDA breakpoints.

Comparisons of the activities of ceftazidime-avibactam and ceftolozane-tazobactam against *Enterobacteriaceae* isolates have been performed in small data sets that might not be representative of organisms causing serious infections in U.S. hospitals. In a study by Alatoom et al. (19), the authors concluded that ceftazidime-avibactam and ceftolozane-tazobactam had comparable activities against the ESBL isolates (100% versus 97%, respectively); however, among the 29 ESBL-producing isolates analyzed, only 6 were *K. pneumoniae*.

Similar to our results, Bouxom et al. (20) observed that the activity of ceftazidime-avibactam was greater than the activity of ceftolozane-tazobactam against 50 *K. pneumoniae* isolates collected in a French hospital during 2016. All isolates tested were inhibited by ceftazidime-avibactam at ≤8 mg/liter (CLSI, U.S. FDA, and EUCAST breakpoints), whereas only 52.0% of the isolates were inhibited by ≤1 mg/liter of ceftolozane-tazobactam, which is the EUCAST breakpoint for this combination, and 70.0% were inhibited at ≤2 mg/liter, which is the CLSI/U.S. FDA breakpoint.

In a literature review, van Duin and Bonomo (18) evaluated the differences between ceftazidime-avibactam and ceftolozane-tazobactam and presented the activities of these combinations against different collections of *E. coli* and *K. pneumoniae* isolates displaying an ESBL-phenotype criterion according to CLSI (21). Ceftazidime-avibactam displayed activity against all isolates of both species and ceftolozane-tazobactam inhibited 93.4% and 78.7% of isolates belonging to these common species, respectively.

The ceftolozane-tazobactam resistance mechanisms in ESBL-producing isolates are not well understood. Outer membrane sequence alterations that could play a role in resistance to ceftolozane-tazobactam were not observed among resistant *K. pneumoniae*, *E. coli*, and *E. cloacae* isolates compared to those in genetically similar isolates susceptible to this combination; however, decreased expression of OMP-encoding genes or posttranslational modifications that could play a role in resistance to β-lactams (22–24) were not evaluated and present a limitation of this study. Additionally, efflux extrusion of the antimicrobial or the inhibitor and overexpression of β-lactamases could also be important in resistance to ceftolozane-tazobactam. Regardless of the cause of ceftolozane-tazobactam resistance, ceftazidime-avibactam seems to not be affected by the same resistance mechanisms.

The presence of bla_{OXA-1} was noted among some isolates that were resistant to ceftolozane-tazobactam, but >90% of the isolates harboring this gene were inhibited by this combination. Despite the evidence in the literature that ceftolozane-tazobactam is active *in vivo* against isolates carrying bla_{OXA-1} (25), studies have shown that tazobactam can be overwhelmed by the hyperexpression of penicillinases (26, 27), and this might require additional evaluations to understand the effect on ceftolozane-tazobactam.

Several studies evaluating infections caused by ESBL-producing organisms demonstrated that early initiation of appropriate antimicrobial therapy reduces mortality and morbidity rates, reduces hospital stay times, and significantly decreases hospital costs (4, 13, 28). Thus, starting an antimicrobial regimen that demonstrated activity against isolates carrying ESBL-encoding genes from that institution or region is most important. Understanding the epidemiology and monitoring the activity of novel antimicrobial agents against current clinical isolates is needed, and these studies should include broad side-by-side comparisons of new agents to demonstrate the strengths and weaknesses of each until enough clinical experience is accumulated to guide decisions on empirical therapies.

Ceftazidime-avibactam and ceftolozane-tazobactam have similarities in the activity profiles that include *E. coli* and *Pseudomonas aeruginosa* clinical isolates (18); however, these combinations have important differences that need to be highlighted. From our results, it is important to emphasize that ceftazidime-avibactam activity against this contemporary collection of clinically relevant *Enterobacteriaceae* species from U.S. hospitals was unchanged regardless of the ESBL enzyme and species tested.

MATERIALS AND METHODS

Bacterial isolates. A total of 7,058 isolates of *E. coli*, *Citrobacter* spp., *Enterobacter cloacae* species complex (here, *E. cloacae*), and *Klebsiella* spp. were collected from 70 U.S. hospitals during 2017; among these, 733 carbapenem-susceptible isolates carrying ESBL-encoding genes were analyzed. Species identification was confirmed by matrix-assisted laser desorption ionization–time of flight mass spectrometry using the Bruker Daltonics MALDI Biotyper (Billerica, MA, USA) according to the manufacturer's instructions.

Susceptibility testing. Antimicrobial susceptibility was evaluated by reference broth microdilution methods performed according to CLSI procedures (29). Avibactam was provided by Allergan (Irvine, CA, USA) and ceftolozane stock solution was obtained from Thermo Fisher Scientific (Cleveland, OH, USA) and combined with tazobactam (United States Pharmacopeia [USP]). Avibactam and tazobactam were tested at a fixed concentration of 4 mg/liter. All other compounds were obtained from USP or Sigma-Aldrich (St. Louis, MO, USA). Quality control (QC) testing was performed using *E. coli* ATCC 25922 and NCTC 13353, *K. pneumoniae* ATCC 700603 and ATCC BAA-1705, and *P. aeruginosa* ATCC 27853. CLSI (21), European Committee on Antimicrobial Susceptibility Testing (EUCAST) (30), and U.S. FDA (https://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/ucm575163.htm) susceptibility interpretive criteria were applied.

Whole genome sequencing analysis of β-lactamase and outer membrane protein genes. *E. coli* and *Klebsiella* species isolates displaying MIC values of ≥2 mg/liter for at least 2 β-lactams (ceftazidime, ceftriaxone, aztreonam, and cefepime) and *Enterobacter* species and *Citrobacter* species isolates displaying MIC values of ≥16 mg/liter for ceftazidime and/or ≥2 mg/liter for cefepime were submitted for whole genome sequencing. The Nextera XT library construction protocol and index kit (Illumina, San Diego, CA, USA) were used according to the manufacturer's instructions and sequenced on a MiSeq Sequencer (Illumina) with a target coverage of 30×. FASTQ format files for each sample set were assembled independently using *de novo* assembler SPAdes 3.11.1 (31) with K-values of 21, 33, 55, 77, and 99 and careful mode on to reduce the number of mismatches, producing a FASTA format file of contiguous sequences with the best N_{50} value. An in-house designed software using the target assembled sequences as queries (32, 33) to align against over 500 ESBL genes from the National Center for Biotechnology Information Bacterial Antimicrobial Resistance Reference Gene Database (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA313047) was used to search for resistance genes, and potential matches were generated with the criteria of >94% identity and 40% minimum coverage length.

ESBL-carrying isolates nonsusceptible to ceftolozane-tazobactam were analyzed for alterations in OMPs. These isolates were matched with ceftolozane-tazobactam-susceptible isolates belonging to the same multilocus sequence type, and OMP sequences were compared. The ceftolozane-tazobactam-susceptible isolates *E. cloacae* ATCC 13047, *E. coli* ATCC 25922, and *K. pneumoniae* ATCC 13883 were also compared.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.00160-19.

SUPPLEMENTAL FILE 1, PDF file, 0.8 MB.

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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, The Medicines Company, Theravance Biopharma, University of Colorado, University of Southern California—San Diego, University of North Texas Health Science Center, VenatoRx Pharmaceuticals, Inc., Vyome Therapeutics Inc., Wockhardt, Yukon Pharmaceuticals, Inc., Zai Lab, and Zavante Therapeutics, Inc. There are no speakers' bureaus or stock options to declare.

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