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CLINICAL THERAPEUTICS

Piperacillin-Tazobactam versus Other Antibacterial Agents for Treatment of Bloodstream Infections Due to AmpC β-Lactamase-Producing *Enterobacteriaceae*

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ABSTRACT *In vivo* induction of AmpC beta-lactamases produces high-level resistance to many beta-lactam antibiotics in *Enterobacteriaceae*, often resulting in the need to use carbapenems or cefepime (FEP). The clinical effectiveness of piperacillin-tazobactam (TZP), a weak inducer of AmpC beta-lactamases, is poorly understood. Here, we conducted a case-control study of adult inpatients with bloodstream infections (BSIs) due to *Enterobacter*, *Serratia*, or *Citrobacter* species from 2009 to 2015 to assess outcomes following treatment with TZP compared to FEP or meropenem (MEM). We collected clinical data and screened all isolates for the presence of *ampC* alleles by PCR. Primary study outcomes were 30-day mortality and persistent bacteremia at ≥72 h from the time of treatment initiation. Of 493 patients with bacteremia, 165 patients met the inclusion criteria, of which 88 were treated with TZP and 77 with FEP or MEM. To minimize differences between covariates, we carried out propensity score matching, which yielded 41 matched pairs. Groups only differed by age, with patients in the TZP group significantly older ($P = 0.012$). There were no significant differences in 30-day mortality, persistent bacteremia, 7-day mortality, or treatment escalation between the two treatment groups, including in the propensity score-matched cohort. PCR amplification and sequencing of *ampC* genes revealed the presence of *ampC* in isolates with cefoxitin MICs below 16 μg/ml, in particular in *Serratia* spp., and demonstrated that these alleles were highly genetically diverse. Taken together, TZP may be a valuable treatment option for BSIs due to AmpC beta-lactamase-producing *Enterobacteriaceae*, diminishing the need for broader-spectrum agents. Future studies are needed to validate these findings.

KEYWORDS AmpC beta-lactamases, piperacillin-tazobactam, bacteremia

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Gram-negative organisms are particularly adept at acquiring antimicrobial drug resistance and pose a major challenge to health care (1–4). AmpC beta-lactamases represent a unique inducible mechanism of Gram-negative resistance (5). They are chromosomally encoded in certain *Enterobacteriaceae*, such as *Enterobacter* spp., *Citrobacter freundii*, and *Serratia* spp. (1, 5). In these organisms, AmpC production is controlled by transcription factors that respond variably under the influence of beta-lactam exposure (1, 5–7). Exposure to beta-lactams can induce high-level AmpC expression, leading to resistance to some beta-lactams, most notably third-generation cephalosporins. In addition, mutations in genes that affect AmpC regulation or tran-

scription can result in constitutive expression of AmpC upon exposure to beta-lactams. This process, known as derepression, can also result in resistance to beta-lactams, and it is enhanced by selection of resistant mutants during antibiotic therapy (8). Despite initial susceptibility, treatment with third-generation cephalosporins can lead to relapsed infection and development of resistance (9).

Historically, carbapenems have been the primary agents used to treat infections caused by AmpC-producing bacteria. With increasing use of carbapenems and associated antimicrobial resistance (4), there has been a growing interest in using alternative agents in recent years. Cefepime is a zwitterion with a net neutral charge that can rapidly enter bacterial outer membranes and has been shown to be more stable against AmpC beta-lactamases. Retrospective studies have shown that cefepime has an efficacy similar to that of carbapenems for treatment of *Enterobacter* spp. bacteremia (10, 11). In these analyses, which included propensity score-matched pairwise comparisons, cefepime use resulted in no difference in duration of bacteremia (11), mortality, or length of stay compared to meropenem use (10). Based on these results, cefepime has become an important carbapenem-sparing option for the treatment of these organisms. Other antibiotic agents, such as broad-spectrum beta-lactam/beta-lactamase inhibitors (BLBLI), have also been suggested as alternative therapies. A survey of infectious disease practitioners and microbiologists revealed that though there was a preference to treat *Enterobacter* spp. bacteremias with carbapenems (58%) and cefepime (19%), a small minority of prescribers relied on piperacillin-tazobactam (8%) (12). A recent retrospective study found that the use of BLBLIs in bacteremia caused by *Enterobacter* spp. was not significantly associated with microbiological failure (13). Piperacillin-tazobactam may be an attractive alternative, because both agents are weak inducers of AmpC enzymes. However, the effectiveness of this combination in the treatment of infections due to AmpC-producing organisms has not been fully elucidated (14). Thus, the objective of this study was to evaluate outcomes in patients receiving piperacillin-tazobactam compared to outcomes for patients receiving cefepime and meropenem for bloodstream infections (BSIs) due to AmpC beta-lactamase-producing *Enterobacteriaceae*.

RESULTS

Description of the cohort. Over the 7-year study period, we identified 493 patients who had BSIs caused by *Enterobacter* spp., *Serratia* spp., or *Citrobacter* spp. Of these, 201 patients met the initial inclusion criteria (Fig. 1). The major reasons for exclusion were age less than 18 years ($n = 135$, 41.2%) and polymicrobial BSI ($n = 100$, 30.5%). After excluding patients treated with an alternative therapy (monotherapy with aminoglycosides, aztreonam, or levofloxacin) or combination therapy (aminoglycosides for greater than 72 h), 165 patients were included in the final analysis.

Phenotypic and molecular typing of isolates. Ninety-seven percent of patients had clinical isolates for which cefoxitin MICs were available (Table 1). The majority of *Enterobacter* spp. ($n = 97/100$, 97%) and *Serratia* spp. ($n = 36/44$, 82%) showed cefoxitin-intermediate or -resistant MICs (MICs ≥ 16 μg/ml). Less than half ($n = 7/16$, 44%) of *Citrobacter* spp. had elevated MICs to cefoxitin. This was mainly accounted for by *C. koseri*, for which only 1 of 10 isolates was cefoxitin resistant. Of note, all susceptible *Serratia* spp. isolates had an MIC of 8 μg/ml, which is 1 dilution below the breakpoint.

A total of 152 isolates collected from the 165 bacteremia episodes were available for further molecular typing (Table 1). Among these, we detected 54 different alleles (Fig. 2A), including 31 that were detected using novel primer combinations. Of 96 *Enterobacter* spp. isolates tested, 88 (92%) were positive for *ampC* genes. These isolates were highly heterogeneous and clustered into 39 alleles. ACT-7 was the most common allele and was present in 10 (13%) *Enterobacter cloacae* isolates. When *E. cloacae ampC* alleles were grouped based on their phylogenetic relationship, the majority of detected alleles belonged to group 2 (*E. cloacae*; 52%), followed in frequency by allelic group Easb (*E. cloacae*; 15%) (Fig. 2A). All but three of the *ampC*-positive *E. cloacae* isolates and all

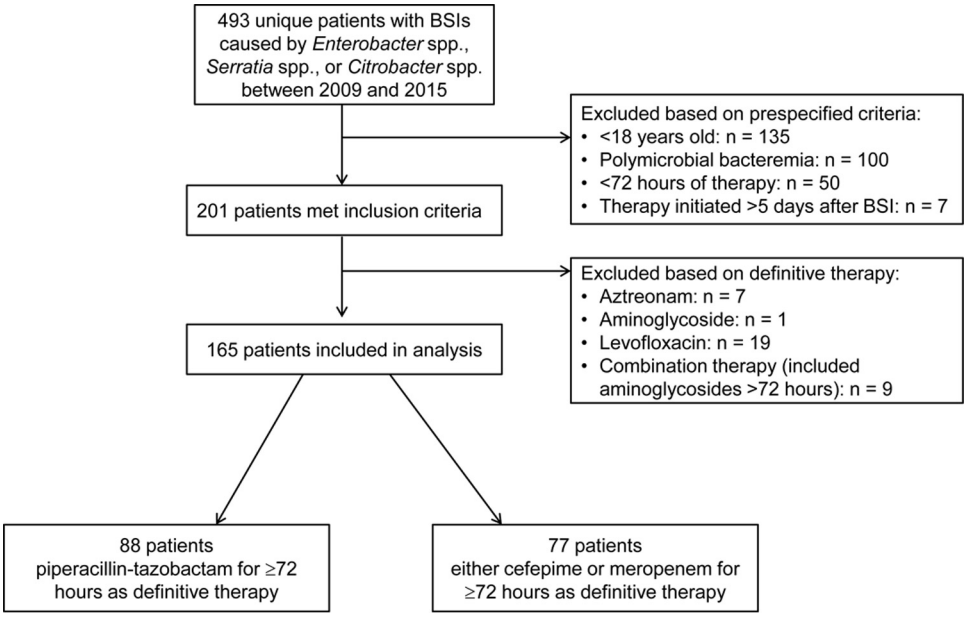

FIG 1 Enrollment flow chart and inclusion and exclusion criteria.

Enterobacter aerogenes, *Enterobacter asburiae*, and *Enterobacter cancerogenus* isolates were intermediately resistant or resistant to cefoxitin (MICs, ≥16 μg/ml) (Fig. 2B). However, all 8 *ampC*-negative *Enterobacter* isolates (*E. cloacae* [*n* = 6], *E. aerogenes* [*n* = 1], *E. cancerogenus* [*n* = 1]) were also resistant to cefoxitin and had MICs of ≥64 μg/ml.

All 38 available *Serratia marcescens* isolates were positive for *ampC*, including 7 isolates with an MIC of 8 μg/ml, which is considered in the susceptible range. We identified 12 different *S. marcescens ampC* alleles, and these all clustered separately from *Enterobacter* and *Citrobacter ampC* genes (Fig. 2A). We were unable to identify *ampC* genes in the two *Serratia liquefaciens* isolates.

Of the seven cefoxitin-resistant *Citrobacter* spp. isolates, three isolates harbored three different *ampC* genes, as shown using previously established primers (Fig. 2A) (15). All cefoxitin-susceptible isolates were PCR negative.

TABLE 1 Isolate susceptibilities and genotypes

Genus and species	*n*	No. (%) of isolates with[a]: Cefoxitin MIC ≥ 16 μg/ml	Cefepime MIC ≥ 16 μg/ml	Meropenem MIC ≥ 4 μg/ml	TZP MIC ≥ 128 μg/ml	No. (%) of isolates in which *ampC* was detected[b]
Enterobacter species	103	97/100 (97)	4/102 (4)	0/103 (0)	15/102 (15)	88/96 (92)
E. aerogenes	15	15/15 (100)	0/15 (0)	0/15 (0)	3/15 (20)	13/14 (93)
E. asburiae	2	2/2 (100)	0/2 (0)	0/2 (0)	0/2 (0)	2/2 (100)
E. cancerogenus	1	1/1 (100)	0/1 (0)	0/1 (0)	1/1 (100)	0/1 (0)
E. cloacae	85	79/82 (96)	4/84 (5)	0/85 (0)	11/84 (13)	73/79 (92)
Serratia species	45	36/44 (82)	0/45 (0)	0/45 (0)	0/29 (0)	38/40 (95)
S. marcescens	43	35/42 (83)	0/43 (0)	0/43 (0)	0/28 (0)	38/38 (100)
S. liquefaciens	2	1/2 (50)	0/2 (0)	0/2 (0)	0/1 (0)	0/2 (0)
Citrobacter species	17	7/16 (44)	0/16 (0)	0/16 (0)	2/14 (14)	3/16 (19)
C. braakii	2	2/2 (100)	0/2 (0)	0/2 (0)	1/2 (50)	0/2 (0)
C. freundii	3	3/3 (100)	0/3 (0)	0/3 (0)	1/1 (100)	2/3 (67)
C. koseri	11	1/10 (10)	0/10 (0)	0/10 (0)	0/10 (0)	0/10 (0)
C. youngae	1	1/1 (100)	0/1 (0)	0/1 (0)	0/1 (0)	1/1 (100)

[a]Percentages were calculated based on the number of isolates for which MIC data were available (*n* = 160).
[b]Percentages were calculated based on the number of isolates available for genotyping (*n* = 152), including nontypeable isolates (for *Enterobacter* spp., *n* = 1; *Serratia* spp., *n* = 3; *Citrobacter* spp., *n* = 0).

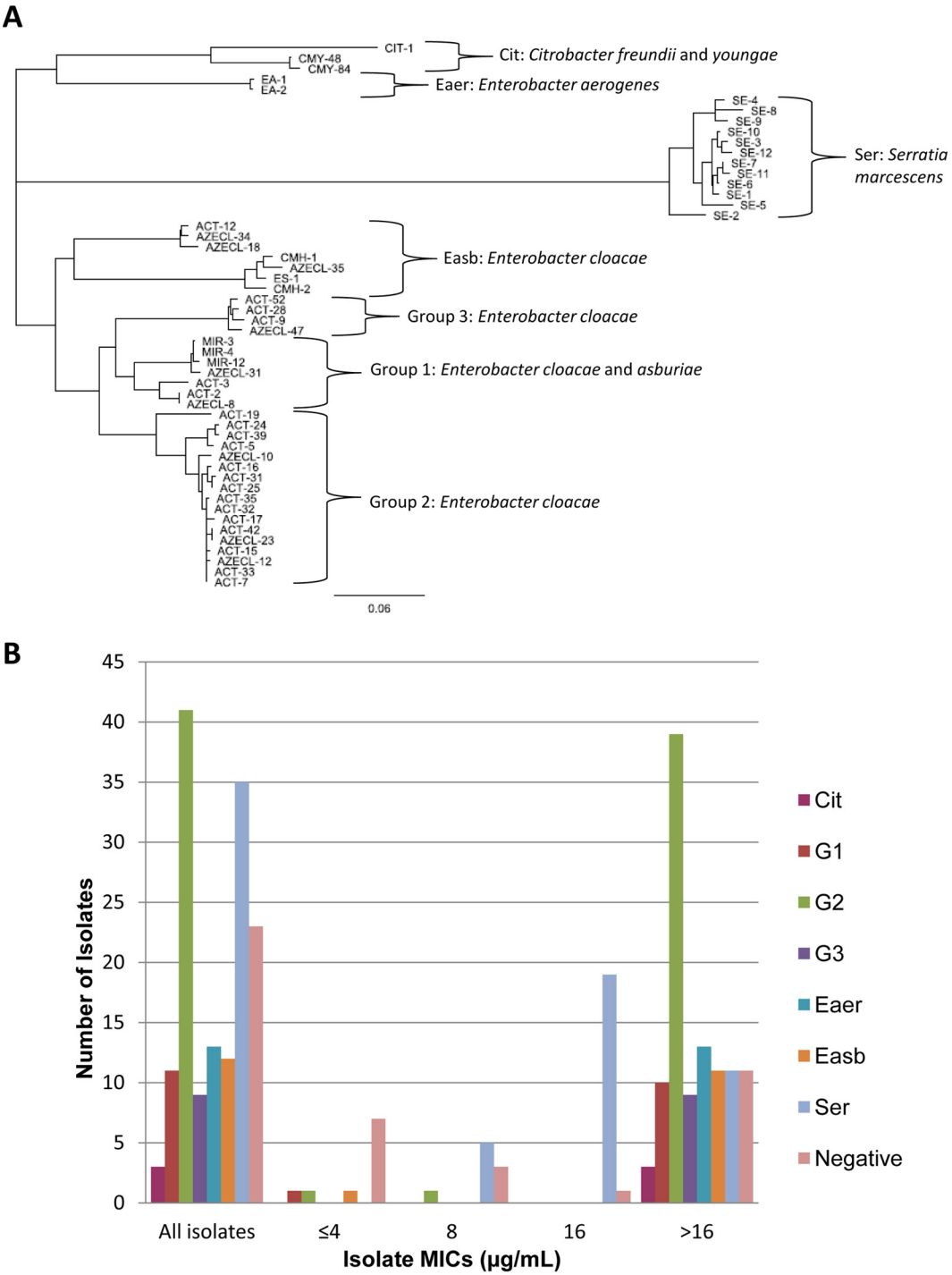

FIG 2 (A) Allelic variability, based on the distribution of isolate MICs by *ampC* allele group. (B) AmpC allelic groups, demonstrating the substantial allelic variability. Species abbreviations correspond to those shown in panel A.

Translation of aligned 563-bp internal sequences of *ampC* alleles demonstrated the presence of nonsynonymous single nucleotide polymorphisms within and between allele groups. Among *Enterobacter* spp. groups, when we used *act-7* (group 2) as the reference sequence, group 2 alleles differed by a median of 2 amino acids (aa; interquartile range [IQR], 1.5 to 3), whereas groups 1 and 3 differed by a median of 13 and 15 aa (IQRs, 11.5 to 13 and 14.75 to 15.3, respectively) and the Eaer and Easb allele groups differed by a median of 40 and 25 aa (IQRs, 40 to 40 and 21.3 to 25, respectively).

TABLE 2 Baseline patient characteristics

Covariate	No. of patients (%) with the characteristic in[a]:					
	Overall cohort comparison ($n = 165$)			Propensity score-matched cohort comparison ($n = 82$)		
	TZP ($n = 88$)	FEP/MEM ($n = 77$)	*P* value	TZP ($n = 41$)	FEP/MEM ($n = 41$)	*P* value
Age [median yr (range)]	65 (52, 75)	65 (47, 75)	0.41	68 (59, 78)	57 (40, 69)	0.012
Male sex	50 (57)	48 (62)	0.58	25 (61)	26 (63)	0.84
Neutropenia	3 (3)	7 (9)	0.26	0	3 (7)	0.99
Immunosuppression	18 (21)	25 (33)	0.12	7 (17)	9 (22)	0.34
Charlson comorbidity score [median (range)]	3 (1, 7)	3 (2, 5)	0.65	3 (1, 6)	3 (2, 4)	0.47
Days to bacteremia (median [range])	1 (1, 9)	5 (1, 15)	0.002	1 (1, 6)	2 (1, 10)	0.11
Renal replacement therapy	14 (16)	14 (18)	0.39	2 (5)	3 (7)	0.65
ICU stay	30 (34)	36 (60)	0.002	17 (41)	15 (37)	0.34
Septic shock	14 (16)	26 (34)	0.07	7 (17)	7 (17)	1
Pitt bacteremia score [median (range)]	1 (0, 3)	2 (0, 6)	0.012	2 (0, 4)	1 (0, 3)	0.4
Presumed source of infection			0.83			0.6
Urinary tract	19 (22)	12 (21)		10 (24)	8 (20)	
LRTI/VAP	12 (14)	16 (21)		6 (15)	8 (20)	
Surgery related/SSTI	8 (9)	7 (9)		5 (12)	4 (10)	
Catheter related	10 (11)	12 (16)		3 (7)	4 (10)	
Intra-abdominal	20 (23)	13 (17)		10 (24)	5 (12)	
Gut translocation	7 (8)	7 (9)		2 (5)	5 (12)	
Multiple	1 (1)	2 (3)		1 (2)	2 (5)	
Unknown	11 (13)	8 (10)		4 (10)	5 (12)	
Responsible pathogen			0.12			0.54
Enterobacter spp.	51 (58)	52 (68)		23 (56)	23 (56)	
Serratia spp.	24 (27)	21 (27)		12 (29)	15 (37)	
Citrobacter spp.	13 (15)	4 (5)		6 (15)	3 (7)	

[a]Data are the number (percentage) of patients with the indicated characteristic, unless otherwise indicated (i.e., median number and range). Abbreviations: LRTI/VAP, lower respiratory tract infection/ventilator-associated pneumonia; SSTI, skin/soft tissue infection.

Serratia alleles differed by a median of only 2 aa (compared to SE-1; IQR, 1 to 2.5), whereas *Citrobacter* alleles differed by a median of 21 aa (compared to CIT-1; IQR, 20.5 to 21.5). There were no premature stop codons identified to directly infer functional differences between alleles.

Patient characteristics. In our study cohort, the median patient age was 65 years (IQR, 49 to 75 years), and 59% of patients were male (Table 2). At the initial detection of the BSI, 46% of patients were in an intensive care unit (ICU), and 24% were in septic shock. Of the 165 patients, 88 were treated with piperacillin-tazobactam, and 77 were treated with cefepime or meropenem (cefepime [$n = 41$], meropenem [$n = 36$]). All patients received antibiotic therapy within the first 48 h of the first positive blood culture, except for one patient whose blood culture was initially reported to show Gram-positive cocci but later the report was corrected to *Serratia* spp. at 96 h, at which time piperacillin-tazobactam was started. Concomitant aminoglycosides were administered for less than 72 h in 32% of patients, including 31 patients (35%) in the piperacillin-tazobactam group and 22 patients (29%) in the cefepime/meropenem group. For five patients in the piperacillin-tazobactam group, MICs were unavailable, partly due to the FDA regulations regarding Vitek reporting for *Serratia marcescens* since 2012. Otherwise, all patients were infected with organisms susceptible to their respective treatment agent, and they received appropriate drug dosing as outlined in the hospital's antibiotic guidelines (10).

For most variables, the two groups were comparable at baseline; however, patients who received cefepime or meropenem were more likely to have had a longer hospital stay prior to onset of BSI than those who received piperacillin-tazobactam (median, 5 days versus 1 day; $P = 0.002$), develop septic shock (26 of 77 [34%] versus 14 of 88 [16%]; $P = 0.07$), require ICU stay (60% versus 34%; $P = 0.002$), and have a higher Pitt bacteremia score (PBS; median, 2 versus 1; $P = 0.012$) (Table 2). The presumed source of infection and responsible pathogens did not differ between the two treatment

TABLE 3 Clinical outcomes, according to treatment category

Outcome	No. (%) of patients with outcome in:							
	Overall cohort comparison				Propensity score-matched cohort comparison			
	TZP ($n = 88$)	FEP/MEM ($n = 77$)	*P* value	OR (95% CI)	TZP ($n = 41$)	FEP/MEM ($n = 41$)	*P* value	OR (95% CI)
30-Day mortality	9 (10)	9 (12)	0.96	1.16 (0.44, 3.09)	6 (15)	3 (7)	0.33	0.50 (0.13, 2.0)
Persistent bacteremia	14 (16)	10 (13)	0.66		8 (20)	4 (10)	0.26	
7-Day mortality	1 (1)	3 (4)	0.34		0	1 (2)	0.99	
Treatment escalation	12 (14)	8 (10)	0.63		6 (15)	6 (15)	1	

groups. No significant difference was observed in the distribution of MICs or *ampC* allele groups among isolates in the two groups. Propensity score matching yielded 41 matched pairs, of which 24 patients received cefepime and 17 received meropenem. Most of the differences between the covariates were minimized after matching, except that patients were older in the piperacillin-tazobactam group (median age, 68 years [IQR, 59 to 78 years] versus 57 years [IQR, 40 to 69 years]; $P = 0.012$).

Clinical outcomes. For the primary outcome of mortality, there were 9 deaths (10%) among patients who received piperacillin-tazobactam, compared to 9 deaths (12%) in patients who received cefepime or meropenem ($P = 0.96$) in the overall cohort (Table 3). Additionally, there was no significant difference in 30-day mortality between the two groups in the matched sample (15% versus 7%; $P = 0.33$). In the univariate analysis, the odds ratios (ORs) for mortality in patients receiving piperacillin-tazobactam versus those receiving cefepime or meropenem were 1.16 (95% confidence interval [CI], 0.44 to 3.09) and 0.5 (95% CI, 0.13 to 2.0) in the overall cohort and the matched sample, respectively. For a total of 149 patients, follow-up blood cultures were performed, and 14 patients (16%) receiving piperacillin-tazobactam and 10 patients (10%) receiving cefepime or meropenem had a positive blood culture growing the same species 72 h after treatment initiation. In the matched cohort, there were more episodes of persistent bacteremia in the piperacillin-tazobactam group (8/41) than the control group (4/41); however, the difference was not statistically significant ($P = 0.26$). Half of the patients with persistent bacteremia in each group died within 30 days, and the overall mortality rates were low in each group. Likewise, there were no significant differences in either primary outcome among *ampC* allele groups (overall $P = 0.4$ for 30-day mortality; overall $P = 0.2$ for persistent bacteremia) (Table 4). However, a higher proportion of patients infected with group 2 *ampC* gene-harboring organisms died within 30 days ($n = 7/41$, 17%) or had persistent bacteremia ($n = 9/41$, 22%) compared to all other *ampC* allele groups (Table 4).

For the secondary outcomes, 12 (14%) patients receiving piperacillin-tazobactam and 8 (10%) patients receiving cefepime or meropenem required escalation of the antibacterial agent(s) dose due to persistent bacteremia within 7 days of active therapy ($P = 0.63$). A total of four patients died during that time period (one in the piperacillin-tazobactam group and three in the cefepime/meropenem group; $P = 0.34$). There were

TABLE 4 Clinical outcomes, by *ampC* allele group

ampC allele group (n)[a]	No. (%) with outcome in allele group			
	30-Day mortality	Persistent bacteremia	7-Day mortality	Treatment escalation
Cit (3)	0	0	0	1 (33)
Group 1 (11)	0	1 (9)	0	2 (18)
Group 2 (41)	7 (17)	9 (22)	1 (2)	7 (17)
Group 3 (9)	1 (11)	1 (11)	1 (11)	0
Easb (12)	2 (17)	0	1 (8)	1 (8)
Eaer (13)	2 (15)	0	1 (8)	3 (23)
Ser (35)	4 (11)	7 (20)	0	5 (14)

[a]Allele group abbreviations are defined in Fig. 2A.

no differences in 7-day all-cause mortality or treatment failure in the matched cohort. Although a higher percentage of patients with group 2 *ampC* infections died or had treatment failure within 7 days ($n = 14/41$, 34%) than in other groups, the overall difference in outcomes between treatment groups was not statistically significant ($P = 0.4$).

None of the 20 patients who were infected with cefoxitin-susceptible isolates (12 in the piperacillin-tazobactam group and 8 in the cefepime/meropenem group) died within 30 days of onset of bacteremia. One patient had persistent bacteremia but was able to achieve clearance on day 6 of piperacillin-tazobactam treatment. One patient in the piperacillin-tazobactam group for whom a piperacillin-tazobactam MIC was not available died, but this patient was not included in the matched cohort analysis. None of the patients in the cohort demonstrated development of resistance to their antibacterial agent(s).

DISCUSSION

In this retrospective, propensity score-matched case-control study, we did not observe a significant difference in treatment failure or 7-day or 30-day mortality between *Enterobacteriaceae*-infected patients treated with piperacillin-tazobactam and those treated with cefepime or meropenem. These findings support the use of piperacillin-tazobactam as an additional valuable treatment option for BSIs due to AmpC beta-lactamase-producing organisms. Our results are based on propensity matching, resulting in comparable groups, including the distribution of critically ill patients and those with septic shock (16). The only variable which was not accounted for by propensity matching was age.

Our findings are consistent with those of other recent studies (16–18). A Spanish study spanning a 16-year time period (1991 to 2006) analyzed 377 episodes of *Enterobacter* spp. bacteremia and found piperacillin-tazobactam-treated patients had a lower mortality rate than those who received third-generation cephalosporins, carbapenems, ciprofloxacin, or gentamicin (17). However, only 38 patients received piperacillin-tazobactam in this cohort, and for some the treatments were changed to other definitive therapies. A 2016 meta-analysis by Harris and colleagues evaluated 11 studies comparing carbapenems with noncarbapenems as treatment for bacteremia from AmpC-producing *Enterobacteriaceae* (primarily *Enterobacter* spp.) (18). In an unadjusted analysis, there was no difference in mortality between BLBLIs and carbapenems. After adjusting for potential confounders such as age and severity of illness, the nonsignificant trend toward increased mortality in the carbapenem group was reduced (18). Most recently, a 2016 retrospective cohort study by Moy and colleagues (19) compared carbapenems to noncarbapenems for the treatment of bacteremia and urinary tract infections due to SPICE organisms (*Serratia*, *Pseudomonas*, indole-positive *Proteus*, *Citrobacter*, and *Enterobacter*). Piperacillin-tazobactam was the most common noncarbapenem agent used. The study found no significant differences in clinical response or microbiological cure. However, the noncarbapenem group appeared to have a higher severity of illness overall. Additionally, the inclusion of *Pseudomonas* spp., which have multiple mechanisms of resistance, makes it difficult to extrapolate these findings to other organisms that produce AmpC beta-lactamases (12).

Our study has several key differences from the preceding studies. First, we included only patients with monomicrobial bacteremia, in an attempt to minimize the inclusion of additive clinical effects of non-AmpC-producing organisms. We also only included organisms that are known to possess inducible *ampC* genes. The most common pathogens in our study were *Enterobacter* spp. (of which we found 92% harbored an *ampC* gene) and *S. marcescens* (of which 100% harbored an *ampC* gene). We conducted propensity matching to minimize differences between patients who received cefepime/carbapenems and those who did not. We also uniquely confirmed the presence of *ampC* genes in bloodstream isolates and carried out molecular typing to identify alleles.

We detected *ampC* in most cefoxitin-resistant isolates, with several exceptions. First, a number of *ampC*-positive, cefoxitin-susceptible isolates, in particular *Serratia* spp., had

cefoxitin MICs of 8 μg/ml, 1 dilution below the breakpoint. The clinical significance of this remains unclear but raises concerns that the presence of *ampC* may be underrecognized in this organism. The widespread presence of *ampC* in *Serratia* spp. isolates that were linked with preserved susceptibility to cephalosporins was recently described in a large genomic survey from the United Kingdom (20). Additional mutations, for example in the *ampC* regulators AmpD and AmpR (6), may be needed to manifest the AmpC phenotype in *Serratia* spp. However, we also noted that the *Serratia* alleles showed significant divergence from *Enterobacter* alleles, raising the possibility that these are functionally different enzymes. Further studies are needed to more comprehensively characterize the mechanisms of *ampC* induction in clinical isolates. Second, we did not detect *ampC* alleles in a number of isolates with high cefoxitin MICs. Despite our expanded set of primers, this finding likely represented additional allelic variation of the target gene that was not captured by our approach.

While our study did not reveal an association between specific alleles and cefoxitin MIC values, differences between *Enterobacteriaceae* species were apparent. *E. cloacae* isolates had the highest proportion of cefoxitin-resistant isolates (defined by an MIC of 16 μg/ml or greater), followed by *Serratia* spp. and *Citrobacter* spp. The relatively low prevalence of cefoxitin resistance in *Citrobacter* spp. was mainly accounted for by *C. koseri* isolates. We also did not detect significant differences in the primary or secondary outcomes among allele groups, although group sizes were small. However, it is notable that a relatively high proportion of patients infected with organisms harboring group 2 *ampC* alleles had a poor outcome.

Limitations to our study need to be considered. Our study represents a single-center, retrospective study, and the findings might not be generalizable to other settings. Initially, the cefepime/meropenem group had a higher severity of illness and longer hospital stays. We attempted to account for these differences by using propensity matching. The resulting groups were small, and thus the study may have lacked sensitivity to detect differences in treatment outcomes. As such, this could affect generalizability, but numbers were comparable to those in other studies of treatment of AmpC-producing infections. The high allelic diversity in our study decreased our power to detect possible differences in primary or secondary outcomes based on *ampC* genotype. While we included isolates with cefoxitin MICs in the susceptible range, which may have influenced our analyses, these isolates were evenly distributed between the two groups.

Taken together, our study findings support the use of piperacillin-tazobactam for the treatment of bloodstream infections with AmpC-producing *Enterobacter*, *Serratia*, and *Citrobacter* species. Prospective studies and meta-analyses are needed to further delineate patient populations that might benefit from this treatment approach and to delineate specific risk factors warranting use of carbapenem or cefepime instead. For the species studied here, *ampC* genes may be present even in isolates with lower cefoxitin MICs. In particular, patients with infections caused by *Serratia* spp. with MICs of ≥8 μg/ml should be closely monitored for treatment failure. However, detection of these genes was challenging due to extensive allelic variation, which may limit further assessments of their clinical implications.

MATERIALS AND METHODS

Study design. This retrospective cohort study evaluated all adult patients with a BSI due to *Enterobacter* spp., *Serratia* spp., or *Citrobacter* spp. who were hospitalized between January 2009 and December 2015 at a tertiary care medical center comprised of a large academic hospital and a smaller community hospital. The study was reviewed and approved by the Columbia University Irving Medical Center New York Institutional Review Board. Patients were eligible for inclusion if they received any *in vitro* active antibiotic therapy for at least 72 h within 5 days of the first positive blood culture. Exclusion criteria consisted of age of <18 years or polymicrobial bacteremia within 7 days of the initial positive blood culture, with the exception of a single positive culture for coagulase-negative staphylococci. Patients were also excluded if they received antibiotic agents other than cefepime, meropenem, or piperacillin-tazobactam for definitive therapy, which was defined as the primary treatment agent used for 72 h or more. *In vitro* susceptibility to these agents was demonstrated using current Clinical and Laboratory Standards Institute (CLSI) interpretative breakpoints (21). Combination therapy with amino-

glycosides for Gram-negative double coverage during the first 72 h of therapy was allowed and patients who had received such therapy were included in the study.

The primary outcomes evaluated were 30-day mortality and persistent bacteremia. Time to mortality was determined starting from the onset of infection, defined as the time of collection of the first positive blood culture. Persistent bacteremia was defined as positive blood cultures for greater than 72 h after the start of active therapy as outlined above. Secondary outcomes included 7-day all-cause mortality and treatment failure. Treatment failure was defined as the need for antibiotic escalation within the first 7 days of active therapy, as assessed by the treating physician and recorded in the electronic medical record.

Data points collected at the time of admission included patient demographics, the Charlson comorbidity index (CCI) (22), and presence of immunosuppression (defined as the receipt of chemotherapeutic agent[s] within 90 days or receipt of corticosteroids at doses equivalent to >5 mg/day prednisone or other immunosuppressive agents for at least 14 days in the past 30 days). Additional data points collected at the time of BSI and over the course of therapy included presumed source of BSI (as documented by the treating physician), the PBS (23), use of renal replacement therapy, serum creatinine, stay in the ICU, presence of septic shock (24), and date of discharge or death.

Microbiology and molecular typing. All isolates were identified by the clinical microbiology laboratory located within the study center. Susceptibility testing was performed via Kirby-Bauer disc diffusion and the Vitek 2 system (bioMérieux). Numeric isolate MIC values for cefotixin, cefepime, meropenem, and piperacillin-tazobactam were collected from laboratory records, except where unavailable either because susceptibilities were determined using Kirby-Bauer disc-diffusion testing or suppressed or changed to a classification of resistant with the Vitek 2 Advanced Expert system. We did not perform conventional confirmatory phenotypic testing, as it is not routinely done by the laboratory. Patients who had isolates with cefoxitin MICs in the susceptible range were included in the analysis.

Molecular typing for *ampC* genes was carried out with PCR and subsequent sequencing of PCR products. Each isolate was tested using five sets of previously published primers (7–9). In addition, we designed four novel primer combinations (see Table S1 in the supplemental material), based on nucleotide alignments of existing ACT and *Serratia ampC* sequences in the NCBI database and using Geneious bioinformatics software (version 8.1.4). We then aligned 563-bp internal sequences of identified *ampC* alleles and constructed a phylogenetic tree using the neighbor-joining algorithm to assess relatedness.

Statistics. Patients who received piperacillin-tazobactam were analyzed as cases, and patients who received cefepime or meropenem served as controls. We performed an unmatched case-control analysis with all patients meeting criteria for inclusion in the study, and we also used propensity scoring to create well-matched groups by using 1:1 nearest-neighbor matching without replacement. Covariates included in the propensity score-matched analysis were the following: duration of hospital stay prior to bacteremia, use of immunosuppressive agents, CCI score, PBS, presumed source of infection, responsible pathogen, ICU stay, and development of septic shock. Cases without a match within 0.25 propensity score standard deviations were excluded from the analysis. Baseline characteristics of cases and controls in the overall cohort and propensity score-matched sample were compared to ensure similarity of the two treatment groups. For the primary and secondary clinical outcomes, univariate analyses were performed. We used the chi-square test or Fisher's exact test to compare categorical variables and the two-sample Wilcoxon rank-sum test for continuous variables for analyzing unmatched patients, as appropriate. Conditional logistic regression was used to compare variables between cases and controls in matched patients. For the primary clinical outcome of 30-day mortality, due to small sample size, number, percentage, univariate Odd's ratio with 95% confidence intervals, and *P* value were reported for both unmatched and matched case-control patients. For the secondary clinical outcomes, number, percentage, and *P* values from univariate association tests were reported. All statistical tests were two tailed, and a *P* value of less than 0.05 was considered statistically significant. Data were analyzed using SAS/STAT software (version 9.4; SAS Institute Inc., Cary, NC, USA).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.00276-17.

TABLE S1, PDF file, 0.1 MB.

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