

The Cefazolin Inoculum Effect Is Associated With Increased Mortality in Methicillin-Susceptible *Staphylococcus aureus* Bacteremia

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Background. Recent studies have favored the use of cefazolin over nafcillin for the treatment of methicillin-susceptible *Staphylococcus aureus* (MSSA) bacteremia. The clinical influence of the cefazolin inoculum effect (CzIE) in the effectiveness of cephalosporins for severe MSSA infections has not been evaluated.

Methods. We prospectively included patients from 3 Argentinian hospitals with *S. aureus* bacteremia. Cefazolin minimum inhibitory concentrations (MICs) were determined at standard (10^5 colony-forming units [CFU]/mL) and high (10^7 CFU/mL) inoculum. The CzIE was defined as an increase of MIC to ≥ 16 $\mu\text{g}/\text{mL}$ when tested at high inoculum. Whole-genome sequencing was performed in all isolates.

Results. A total of 77 patients, contributing 89 MSSA isolates, were included in the study; 42 patients (54.5%) had isolates with the CzIE. In univariate analysis, patients with MSSA exhibiting the CzIE had increased 30-day mortality ($P = .034$) and were more likely to have catheter-associated or unknown source of bacteremia ($P = .033$) compared with patients infected with MSSA isolates without the CzIE. No statistically significant difference between the groups was observed in age, clinical illness severity, place of acquisition (community vs hospital), or presence of endocarditis. The CzIE remained associated with increased 30-day mortality in multivariate analysis (risk ratio, 2.65; 95% confidence interval, 1.10–6.42; $P = .03$). MSSA genomes displayed a high degree of heterogeneity, and the CzIE was not associated with a specific lineage.

Conclusions. In patients with MSSA bacteremia where cephalosporins are used as firstline therapy, the CzIE was associated with increased 30-day mortality. Clinicians should be cautious when using cefazolin as firstline therapy for these infections.

Keywords. cephalosporins; endocarditis; inoculum effect; methicillin-susceptible *Staphylococcus aureus*.

Staphylococcus aureus is one of the most important human pathogens and causes a variety of infections with diverse clinical presentations [1]. Morbidity and mortality associated with severe infections caused by *S. aureus* are substantial [2, 3], and in several parts of the world (including some areas of the United States), the frequency of methicillin-susceptible *S. aureus* (MSSA) infections is surpassing that of methicillin-resistant *S. aureus* (MRSA) [4, 5]. Despite causing an important burden of disease, there have not been major recent

advances in the treatment of MSSA infections. Indeed, the mortality associated with MSSA infective endocarditis (IE) has not substantially changed in the last 20 years according to the International Collaboration on Endocarditis database (ca. 23%) [6]. In the mid-1960s, cephalothin and cephaloridine were introduced as the main drugs against MSSA. However, early on, it was noted that cephaloridine activity was impaired against penicillin-resistant *S. aureus* strains when tested at high inoculum [7, 8]. A case of failure of cephaloridine in the treatment of MSSA endocarditis supported the initial concerns of cephalosporin treatment in deep-seated infections caused by MSSA [9].

The cephalosporin inoculum effect is defined as a prominent rise in the antibiotic minimum inhibitory concentration (MIC; to ≥ 16 $\mu\text{g}/\text{mL}$) when the susceptibility test is performed with an inoculum higher than the standard recommended bacterial inoculum ($\sim 10^7$ colony-forming units [CFU]/mL vs $\sim 10^5$ CFU/mL) [10]. When cefazolin came into commercial use in the United States in 1971, it was noted that several penicillinase-producing MSSA strains were able to readily hydrolyze

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this drug [11–14]. Subsequently, cases of *S. aureus* endocarditis failing cefazolin therapy were described [15–17]. Of note, in 1 of these cases, the MSSA strain exhibited high MICs of cefazolin when a large inoculum was used (cefazolin inoculum effect [CzIE]) [18]. Initial studies [19, 20] carried out in the 1970s and 1980s focused on the characterization of the *S. aureus* β -lactamases. Initially, typing of such enzymes (BlaZ) was based on immunological methods with 4 different serotypes (A, B, C, and D) described [19]. Subsequently, typing was performed based on limited sequences of BlaZ and some support by kinetic studies that involved hydrolysis of different β -lactams [21, 22]. From those studies, type A BlaZ seemed to be more efficient in hydrolyzing cefazolin, and the preferred substrate for type C BlaZ was cephalothin.

Despite this limitation of the cephalosporins, the availability of isoxazolyl penicillins (at least in the United States) with excellent stability against BlaZ has made such concerns less clinically relevant. Cefazolin has been used as second-line therapy, often after patients have received a course of isoxazolyl penicillins or, rarely, as firstline treatment if severe penicillin allergy is suspected. In these scenarios, the clinical importance of the inoculum effect has been difficult to assess. However, in recent years, several studies have suggested that cefazolin has clinical efficacy similar to nafcillin with a lower rate of adverse events, greater ease of administration, lower costs, and, most importantly, decreased mortality [23–29]. These data have prompted some clinicians to use cefazolin now as firstline therapy for severe MSSA infections.

Unlike the United States, isoxazolyl penicillins are not available in Argentina, and the vast majority of MSSA infections are treated with cephalosporins, making this country an ideal setting to evaluate the effect of the CzIE in patients with deep-seated MSSA infections. Our previous published data indicate that there is a strong association between the CzIE and relapses in deep-seated MSSA infections [17] and failure to eradicate MSSA from the bloodstream in patients undergoing hemodialysis [30]. Moreover, using a rat endocarditis model, we have clearly shown that the CzIE has important consequences for the efficacy of cefazolin in vivo [31], a finding that has been replicated by others [32]. Here, using a prospective cohort of patients with *Staphylococcus aureus* bacteremia in Argentina, we assessed the clinical impact of the CzIE with an emphasis on mortality.

METHODS

Study Design

We included adult patients admitted to 3 hospitals located in Buenos Aires, Argentina, from January 2011 to July 2014 with bacteremia caused by *S. aureus* (at least 1 positive blood culture was required). These patients were part of a previously published observational prospective cohort study of *S. aureus* bacteremia in Latin America [33]. Each patient contributed 1

episode of bacteremia, defined as the 14-day period after the first positive blood culture was obtained, and all isolates collected during this period were included in the genomic analysis. Excluded patients included those with polymicrobial bacteremia, a previous episode of bacteremia (relapse outside the 14-day period), those who were transferred from other institutions with bacteremia at the time of transfer, and those who were discharged from the hospital or died within the first 48 hours of the initial diagnosis. The institutional review boards of Universidad Peruana Cayetano Heredia in Lima, Peru (Data Collection Center), and of participating hospitals in Argentina approved the study. Demographic information was collected for all patients and included age, sex, source of bacteremia, place of acquisition (hospital, community, or health care associated according to the Centers for Disease Control and Prevention definition [34]), Charlson comorbidity score, Pitt bacteremia score, presence or absence of complicated bacteremia (as previously defined [33]) or endocarditis (as per the treating physician), and antibiotics used for definitive therapy (Table 1). The dosing of antimicrobial agents was at the discretion of the treating physicians, and this information was not collected specifically for logistical reasons. The usual dosing of cefazolin and cephalotin (2 cephalosporins available in Argentina intravenously) for severe MSSA infections in Argentina is 2 grams every 8 hours and 2 grams every 6 hours, respectively, adjusted for renal function. The major outcomes of interest were all-cause mortality at 7 and 30 days after the initial blood culture.

Microbiological, Molecular, and Genomic Characterization of *S. aureus* Isolates

All *S. aureus* isolates were identified by standard microbiological techniques at the local hospital and subsequently sent to a reference laboratory (Universidad El Bosque, Bogota, Colombia) for additional characterization and confirmation of identification using a previously described multiplex polymerase chain reaction (PCR) assay [35]. Minimal inhibitory concentrations (MICs) of cefazolin were determined with standard ($\sim 5 \times 10^5$ CFU/mL) and high ($\sim 5 \times 10^7$ CFU/mL) inocula via the broth microdilution method, as previously described, and the maximum antibiotic concentration tested for cefazolin was 64 μ g/mL [36]. The presence of the CzIE was defined as an increase in the MIC to ≥ 16 μ g/mL when performed at high inoculum. If patients had more than 1 MSSA isolate recovered from the same set of blood cultures, the presence of the CzIE in any isolate was sufficient to categorize the patient as having an infection with an organism exhibiting the CzIE. All MIC determinations were performed in triplicate and read by 3 independent observers. The following were included as controls: *S. aureus* TX0117, a CzIE-positive isolate with type A β -lactamase, *S. aureus* ATCC 29213, a strain possessing a type A β -lactamase without the CzIE; and *S. aureus* ATCC 25923, a BlaZ-negative strain.

Table 1. Comparison of Clinical Characteristics of Patients With MSSA Bacteremia Exhibiting and Not Exhibiting the Cefazolin High Inoculum Effect^a

Characteristic	No CzIE	CzIE	PValue
No. (%)	35 (45.5)	42 (54.5)	
Age, mean (SD), y	66.9 (11.7)	66.9 (19.6)	.992
Male gender	23 (65.7)	24 (57.1)	.488
Source of bacteremia			.033
Primary	8 (22.9)	20 (47.6)	
Secondary	27 (77.1)	22 (52.4)	
Central venous catheter–related primary bacteremia	7 (20.0)	15 (35.7)	.129
Source of secondary bacteremia			
Respiratory	7 (20.0)	9 (20.0)	.878
Skin	3 (8.6)	2 (4.8)	.499
Surgical	1 (2.9)	3 (7.1)	.398
Endovascular	8 (22.9)	8 (19.0)	.682
Bone/joint	5 (14.3)	4 (9.6)	.517
Urinary	1 (2.9)	1 (2.4)	.896
Abdominal	4 (11.4)	1 (2.4)	.227
Central nervous system	0 (0.0)	0 (0.0)	—
Place of acquisition			.795
Community	8 (22.9)	7 (16.7)	
Hospital	14 (40.0)	17 (40.5)	
Health care related	13 (37.1)	18 (42.9)	
Comorbidities in the 3-mo period before diagnosis of bacteremia			
Cancer	12 (34.3)	12 (28.6)	.628
Transplant	0 (0.0)	0 (0.0)	—
AIDS	1 (2.9)	0 (0.0)	.455
Myocardial infarction	1 (2.9)	2 (4.8)	1.000
Heart failure	7 (20.0)	6 (14.3)	.553
Peripheral vascular disease	6 (17.1)	9 (21.4)	.775
Diabetes	6 (17.1)	11 (26.2)	.414
Lung disease	7 (20.0)	8 (19.1)	1.000
Burn	1 (2.9)	0 (0.0)	.455
Liver disease	4 (11.4)	3 (7.1)	.695
Kidney disease	9 (25.7)	18 (42.9)	.152
Neurological disorder	2 (5.7)	2 (4.8)	1.000
Gastric/duodenal ulcer	0 (0.0)	0 (0.0)	—
Connective tissue disease	0 (0.0)	4 (9.5)	.121
Previous surgery	10 (28.6)	12 (28.6)	1.000
Immunosuppressive therapy	7 (20.0)	10 (23.8)	.786
Other comorbidities	10 (28.6)	10 (23.8)	.795
Previous <i>S. aureus</i> infection	0 (0.0)	3 (7.1)	.246
Hospitalization within prior 3 mo	19 (54.3)	13 (31.0)	.062
Use of antimicrobials 30 d before admission	12 (34.3)	7 (16.7)	.111
Ward of admission			1.000
Medical	28 (80.0)	34 (81.0)	
Surgical	4 (11.4)	5 (11.9)	
Obstetrics–gynecology	0 (0.0)	0 (0.0)	
Emergency room	3 (8.6)	3 (7.1)	
Other	0 (0.0)	0 (0.0)	
Clinical condition 48 h before blood sampling			
Mechanical ventilation	1 (2.9)	4 (9.5)	.369
Central venous catheter in place	10 (28.6)	16 (38.1)	.470
Parenteral nutrition	1 (2.9)	3 (7.1)	.621
Surgical procedure	0 (0.0)	1 (2.4)	1.000
Dialysis	4 (11.4)	11 (26.2)	.150
Severe sepsis	6 (17.1)	12 (28.6)	.287
Charlson comorbidity scale, median (IQR)	2 (1–3)	2 (1–3)	.818
Score >2	14 (40)	15 (35.7)	.814

Table 1. Continued

Characteristic	No CzIE	CzIE	PValue
McCabe Jackson scale			.689
Rapidly fatal	4 (11.4)	5 (11.9)	
Ultimately fatal	14 (40.0)	21 (50.0)	
Nonfatal	17 (48.6)	16 (38.1)	
APACHE II score, median (IQR)	10 (0–12)	4 (0–13)	.629
Score >0	8 (22.9)	9 (21.4)	1.000
Pittsburg score, median (IQR)	1 (0–1)	1 (0–2)	.440
Score >1	8 (22.9)	13 (31.0)	.454
Intensive care unit admission during current hospitalization	13 (37.1)	18 (42.9)	.647
Trans-thoracic echocardiography performed	30 (85.7)	31 (73.8)	.263
Duration of fever, median (IQR), d	3 (2–4)	3 (2–4)	.398
Total white blood cells × 10 ³ , median (IQR) ^e	11.0 (6.9–14.6)	9.6 (7.4–15.1)	.701
Creatinine, median (IQR), mg/dl	0.9 (0.6–1.4)	1.4 (0.9–3.5)	.005
Glucose, mean (SD), mg/dl	116.6 (66.6)	131.4 (81.1)	.391
Complicated bacteremia	7 (20.0)	8 (19.1)	1.000
Focus of complicated bacteremia			
Infective endocarditis	2 (5.7)	6 (14.3)	.280
Others	5 (14.3)	2 (5.7)	.139
7-d all-cause mortality	2 (5.7)	5 (11.9)	.445
30-d all-cause mortality	5 (15.2)	15 (39.5)	.034
Antibiotics used for definitive treatment			.261
Vancomycin monotherapy	2	4	
Cephalosporins	22	31	
Combination therapy	8	3	
Other	3	4	

Abbreviations: CzIE, cefazolin inoculum effect; IQR, interquartile range; MSSA, methicillin-susceptible *Staphylococcus aureus*.

^eValues are No. (%) unless noted otherwise.

Genomic DNA libraries were prepared using the NexteraXT kit (Illumina) and whole-genome sequencing performed with 250-bp paired-end reads on an Illumina MiSeq sequencer. Assemblies were done using Spades v3.11 [37]. We performed in silico multilocus sequence typing (MLST) with the mlst-tool (<https://github.com/tseemann/mlst>) using the scheme for *Staphylococcus aureus* from PUBMLST [38]. Identification of the BlaZ protein sequence was done with BlastX using the reference sequence WP_000733289.1 from the NCBI. Typing of BlaZ was carried out by identification of the amino acid residues in positions 128 and 216 [22]. Phylogenetic reconstruction of all sequenced genomes using the core genome was performed with RAXML, based on RAST annotations [39, 40]. The best tree of 20 runs using a general time-reversible evolution model and a gamma model of rate heterogeneity with 100 bootstrap resampling was rooted at the midpoint and plotted with iTOL [41].

Statistical Analyses

Data analyses were carried out using Stata v 8.2 (College Station, TX). Univariate associations comparing patients with isolates exhibiting the CzIE vs those who did not exhibit this phenotype were explored using the Student *t*, Kruskal-Wallis, and Fisher exact test. Risk ratios were calculated to compare cumulative mortality between patients infected with MSSA

isolates exhibiting the CzIE vs no CzIE. Stratified analysis was carried out using Mantel-Haenszel. Adjusted risk ratios were calculated to detect confounders, and the Breslow and Day tests were used to detect potential effect modifiers of the association between the presence of the CzIE and 30-day all-cause mortality. As no variable had a significant value in the test of homogeneity of risk ratio, no further evaluation of effect modification was performed. All variables that changed the crude risk ratio by more than 10% were considered potential confounders and were included in a multivariate model using a generalized linear fixed model by link “log.” Variables with the highest *P* values were removed in a stepwise fashion, until no variables with *P* values higher than .05 remained in the model. Age in years was categorized as ≤60, 61–80, and ≥81 for the stratified analysis. In the multivariate analysis, age was evaluated as a linear and as a quadratic term.

RESULTS

A total of 77 patients with MSSA bacteremia contributing 89 MSSA isolates were included in the analysis. Overall, 67 patients contributed an initial bloodstream isolate, 8 patients had 2 recovered isolates, and 2 patients had 3 isolates. The cefazolin MIC₉₀ for all isolates when tested at standard inoculum was

1 µg/mL. A total of 42 (54.5%) patients harbored isolates that exhibited the CzIE. Among the CzIE patients, 7 had more than 1 isolate recovered from blood cultures, but genomic analyses indicated that each set of isolates belonged to the same sequence type (ST) and that they were closely related in the phylogenetic reconstruction (Figure 1). The average increase in the cefazolin MIC for isolates that exhibited the CzIE when tested at high inoculum was 85.3-fold (isolates with cefazolin MIC >64 µg/mL were given a value of 128 µg/mL).

Table 1 shows that patients infected with isolates that exhibited the CzIE had similar characteristics to those who did not.

Notable exceptions were that a higher number of patients in the CzIE group developed a primary bacteremia associated with a catheter or from an unknown source ($P = .03$) and that the CzIE group had a significantly higher baseline creatinine level (0.9 vs 1.4, $P = .005$). Importantly, baseline comorbidities (Charlson comorbidity index [42]) severity of bacteremia (assessed by APACHE II [43], McCabe Jackson score [44], and Pittsburgh bacteremia score [45]), diagnosis of infective endocarditis (IE), admission to the intensive care unit, and prescribed antibiotics for definite therapy were similar between the groups. Although no difference in 7-day all-cause mortality was seen between

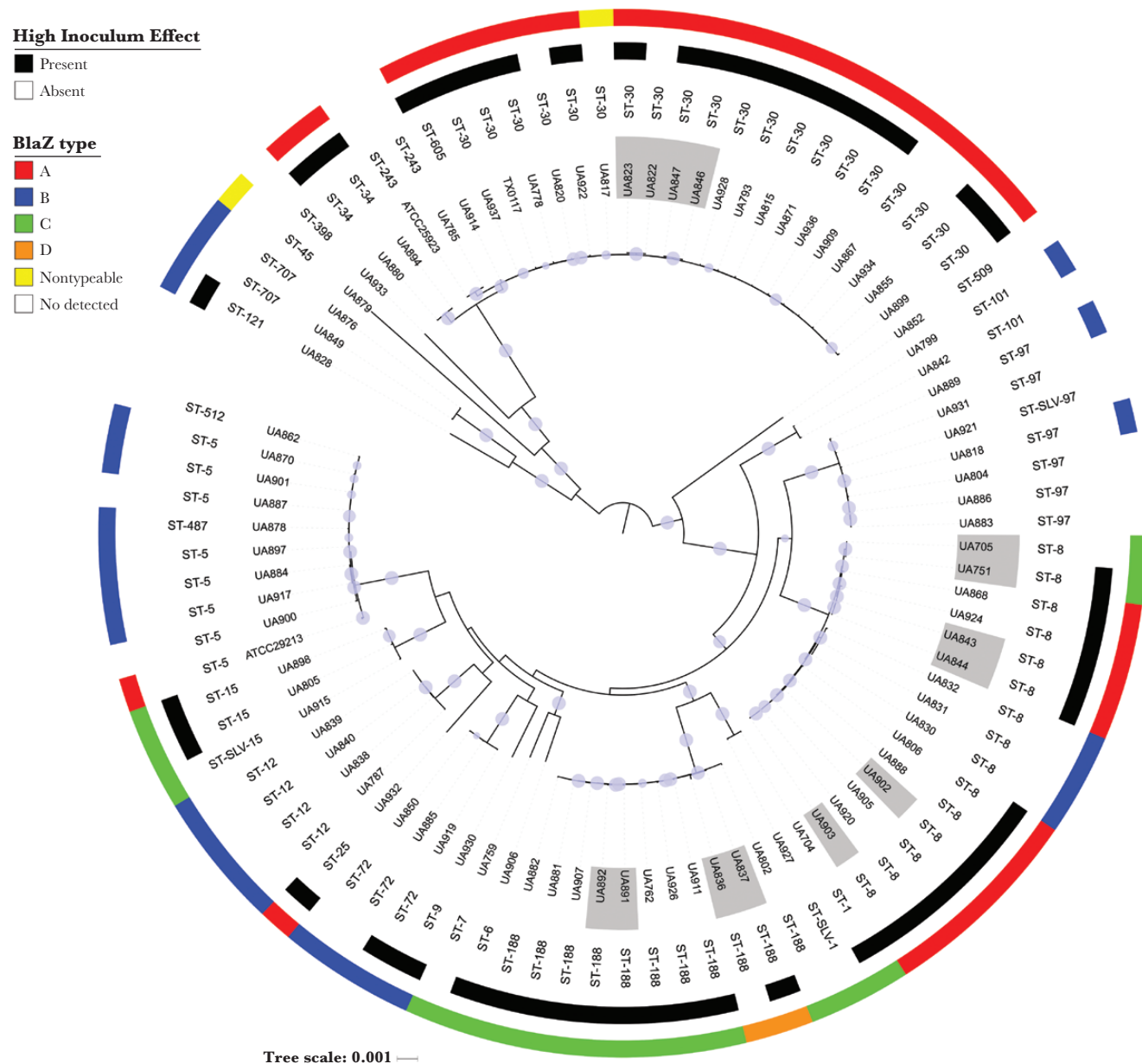


Figure 1. Phylogenetic tree of the core genome of 89 Argentinian methicillin-susceptible *Staphylococcus aureus* isolates and 3 control sequences. The outermost circle denotes the BlaZ type for each β-lactamase; isolates without a β-lactamase are left blank. The outer-middle circle shows the presence (black) or absence (white) of the cefazolin inoculum effect (CzIE) in each isolate. The inner-middle circle lists the sequence type, and the innermost circle lists the name for each isolate; single isolates are colored white, and paired isolates from the same patient are colored gray. The light blue circles at each branch point show the bootstrap support values, varying from 70 to 100.

the groups, patients infected with isolates exhibiting the CzIE had a statistically significantly higher 30-day mortality compared with those patients infected with MSSA lacking the CzIE (39.5% vs 15.2%, $P = .034$).

To explore clinical factors associated with 30-day all-cause mortality, we performed bivariate and multivariate analyses using a generalized linear fixed model (Table 2). In the multivariate model, the presence of the CzIE, when adjusted for potential confounders, continued to be associated with increased 30-day all-cause mortality (risk ratio [RR], 2.65; 95% confidence interval [CI], 1.10–6.42; $P = .03$). The only other significant factor in the multivariate analysis was an association between increased 30-day all-cause mortality and a secondary source of bacteremia (RR, 2.15; 95% CI, 1.01–4.57; $P = .047$). After performing a stratified analysis for age, a trend toward increased 30-day mortality was seen with age >81 years, though this did not reach significance (RR, 4.77; 95% CI, 0.97–23.44; $P = .055$). As a significant difference in baseline creatinine was seen on univariate analysis and acute kidney injury has been independently associated with mortality [46], we included serum creatinine >1.0 mg/dL as a variable in the analysis. In both bivariate and multivariate models, a serum creatinine level of >1.0 mg/dL was not associated with increased risk of 30-day mortality.

Figure 1 shows the core genome phylogenetic reconstruction of the 89 MSSA isolates recovered from the 77 patients and the 3 control strains ($n = 92$) using whole-genome sequencing. Approximately one-third of the total recovered isolates belonged to CC5, CC30, and CC8, lineages whose members include the successful MRSA epidemic clones described in Argentina [47]. However, the overall population structure of MSSA displayed more heterogeneity as compared with a recent analysis of MRSA strains from this same region [47]. The presence of the CzIE was not restricted to a limited set of sequence types, suggesting that this phenomenon is not due to the clonal spread of a particular hospital-adapted clone. As reported previously, the CzIE was associated most strongly with type A and

type C β -lactamase. Interestingly, almost all isolates (8/9, 88.9%) from ST188 possessed a type C β -lactamase and displayed the CzIE; in addition, a large proportion of ST30 (15/20, 75%) and ST8 (11/15, 73.3%) isolates (which predominately carried type A enzymes) were also CzIE positive. None of the 8 ST5 isolates (harboring predominately type B β -lactamase) sequenced were found to be resistant to cefazolin at high inoculum. Of note, we also identified 3 isolates with type B β -lactamase and 1 with type D β -lactamase that display the inoculum effect, an association that has not been described in previous studies.

DISCUSSION

In this prospective cohort study of patients with MSSA bacteremia, the presence of the CzIE in the infecting isolate was associated with a statistically significant increase in 30-day all-cause mortality. Unlike previous reports, our study directly addresses the question of whether the presence of the CzIE negatively affects patient survival. Prior studies of cefazolin vs nafcillin for MSSA bacteremia have largely focused on retrospective clinical data, with limited or no microbiologic information, including evaluation of the presence of the CzIE [23–25, 29]. One retrospective study analyzed 113 patients with MSSA bacteremia whose isolates were available for testing. In this study, 57.5% of isolates were positive for the CzIE (defined by the authors of the study as a 4-fold or greater increase in cefazolin MIC at high inoculum), and although there was a trend toward increased treatment failures in CzIE-positive isolates (48% vs 25%), this did not reach statistical significance [27]. The failure to reach statistical significance may be due to the fact that the CzIE as defined in this study did not require the isolate to be above the susceptibility cutoff. A recent prospective study of nafcillin vs cefazolin by the same group included a small subset of patients treated with cefazolin for which the presence of CzIE in the isolate was assessed [28]. Overall, 22.4% of isolates were positive for the CzIE, and those treated with cefazolin, but not nafcillin, were statistically more likely to have clinical failure or death within 1 month of positive culture when infected with a

Table 2. Bivariate and Multivariate Analysis of Factors Associated With 30-Day All-Cause Mortality

Variable	Crude RR	95% CI	P Value	Adjusted RR	95% CI	P Value
Presence of the CzIE	2.61	0.91–7.45	.074	2.65	1.10–6.42	.030
Age, y						
≤60	Ref			Ref		
61–80	2.11	0.32–13.82	.436	1.63	0.32–8.29	.554
≥81	5.94	0.98–36.14	.053	4.77	0.95–23.44	.055
High Charlson score	1.23	0.58–2.60	.594	1.99	0.95–4.19	.069
Previous hospitalization	1.12	0.53–2.37	.771	0.90	0.46–1.74	.751
Pittsburgh bacteremia score	1.17	1.00–1.35	.045	1.09	0.93–1.27	.292
Previous <i>S. aureus</i> infection	1.19	0.24–5.89	.828	1.25	0.44–3.57	.676
Secondary source of bacteremia	1.43	0.60–3.45	.423	2.15	1.01–4.57	.047
Serum creatinine >1.0 mg/dl	2.20	0.79–6.12	.133	1.59	0.76–3.31	.219

Abbreviations: CI, confidence interval; CzIE, cefazolin inoculum effect; RR, risk ratio.

CzIE-positive isolate. Our results provide further evidence that use of cephalosporins for MSSA isolates that display the CzIE may be associated with worse clinical outcomes. Additionally, our study reiterates the importance of source control in managing staphylococcal infections, as a secondary source of bacteremia was the only other significant variable associated with treatment failure. In the context of the CzIE, source control would provide a reduction of the bacterial inoculum, as well as potentially improving efficacy of antibiotics to the site of infection.

Of note, the number of isolates that tested positive for the CzIE was 54.5%, which is higher than the rates of 19% to 36% described in previous surveys [30, 36]. This increased prevalence may have allowed us to detect a difference between the 2 groups that would have gone unrecognized with a smaller number of isolates. Interestingly, we did not note a significant difference in the distribution of CzIE-positive MSSA isolates between community and hospital settings as compared with CzIE-negative isolates. This may reflect the significant heterogeneity observed in the population of MSSA isolates and the widespread presence of the CzIE. It is unclear if the higher prevalence of the CzIE in Argentina is associated with the use of cephalosporins for MSSA and represents an adaptation to the resultant selective pressure of these antibiotics. This could become an important question for active surveillance if cefazolin becomes front-line therapy in areas currently using isoxazolyl penicillins.

An important contribution of our study was the use of genomics to study the population structure of MSSA exhibiting the CzIE. Consistent with previous studies [48], there is a much wider heterogeneity of isolates represented as compared with MRSA infections, although sequence types ST8 and ST30 were predominant. Of particular interest, ST188–BlaZ type C was associated with the CzIE in almost all isolates we analyzed (8/9), though this could have been a consequence of nonrandom sampling. Interestingly, the presence of type A β -lactamase seems to be more associated with ST30 and ST8 whereas type C was more associated with ST188. Previous studies have linked the CzIE to dysfunction of the accessory gene regulator (*agr*) locus, an important density-dependent quorum sensing system that regulates genes that encode autolysins and secreted virulence factors, among others [49]. In vitro studies have shown that methicillin-resistant strains deficient in *agr* function more readily form biofilm on polystyrene plates and display increased resistance to host-derived cationic antimicrobial peptides [50, 51]. Clinically, *agr* dysfunction has been associated with prolonged bacteremia and mortality in *S. aureus* infections [52, 53]. Thus, further comparison of the genomic sequence data and phenotypic characterization of these isolates with other sequence types, such as ST5, which was not associated with the CzIE, may provide insight into the factors that drive this complex phenotype.

There are several limitations of our study. First, the patients were enrolled from a limited geographic area, and the collected isolates may not be representative of MSSA circulating in other parts of the world. In locations where a lower prevalence of the CzIE is present, the clinical impact might be less evident. Thus, the local prevalence of the CzIE must be taken into account when generalizing these results. Although the use of cephalosporins in Argentina provides a unique opportunity to evaluate the clinical impact of the CzIE, it is unknown whether the use of other agents active against MSSA before initiation of cefazolin therapy would have a protective effect. Second, patient selection and treatment regimens were nonrandomized, thus introducing potential confounders to the data. One potential source of bias was the significant difference in renal function between the 2 groups. Though the mean serum creatinine was noted to be different between the 2 groups, other measures of clinical comorbidities or illness severity were not different, and inclusion of creatinine did not result in a significant impact on mortality in either the bivariate or multivariate models. Although we attempted to control for this and other confounders by statistical methods, further prospective randomized data are needed to confirm these initial findings. Third, we used the end points of 7-day and 30-day all-cause mortality to assess outcomes for the patients enrolled in this study. In the prior Latin American cohort [33], investigator-determined attributable mortality differed significantly from the per-protocol definitions, so this information was not used for the calculations in the current study to avoid subjective bias. Mortality data on 6 patients (4 with and 2 without the CzIE) were not available. Due to the small number of outcomes, a conservative sensitivity analysis (assuming survival in those patients with CzIE and death in those without the effect) resulted in an unstable model. Thus, further prospective studies with a larger number of patients are needed to confirm these findings. Other potentially important outcomes, such as duration of bacteremia and microbiologic clearance, were not assessed in the current study.

In conclusion, the CzIE was associated with an increased risk of 30-day all-cause mortality in Argentinian patients with MSSA bacteremia. The unique clinical setting of Argentina, where cephalosporins are the firstline therapy for MSSA infections and isoxazolyl penicillins are not available, provides an ideal scenario in which to study the impact of the CzIE on clinical outcomes. Although cefazolin may be acceptable as initial therapy in uncomplicated MSSA bacteremia with adequate source control, we urge caution in the selection of cefazolin for serious MSSA infections when a large burden of organisms or an unaddressed secondary focus of infection may be present. Rapid diagnostic tools to easily identify isolates with the CzIE in the clinical lab and further studies on which patients are at risk of failing cefazolin therapy in the presence of the CzIE are urgently needed.

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References

- Tong SY, Davis JS, Eichenberger E, et al. *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clin Microbiol Rev* **2015**; *28*:603–61.
- Cosgrove SE, Sakoulas G, Perencevich EN, et al. Comparison of mortality associated with methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* bacteremia: a meta-analysis. *Clin Infect Dis* **2003**; *36*:53–9.
- van Hal SJ, Jensen SO, Vaska VL, et al. Predictors of mortality in *Staphylococcus aureus* bacteremia. *Clin Microbiol Rev* **2012**; *25*:362–86.
- Dantes R, Mu Y, Belflower R, et al. National burden of invasive methicillin-resistant *Staphylococcus aureus* infections, United States, 2011. *JAMA Intern Med* **2013**; *173*:1970–9.
- Hadler JL, Petit S, Mandour M, Cartter ML. Trends in invasive infection with methicillin-resistant *Staphylococcus aureus*, Connecticut, USA, 2001–2010. *Emerg Infect Dis* **2012**; *18*:917–24.
- Miro JM, Anguera I, Cabell CH, et al; International Collaboration on Endocarditis Merged Database Study Group. *Staphylococcus aureus* native valve infective endocarditis: report of 566 episodes from the International Collaboration on Endocarditis Merged Database. *Clin Infect Dis* **2005**; *41*:507–14.
- Barber M, Waterworth PM. Penicillinase-resistant penicillins and cephalosporins. *Br Med J* **1964**; *2*:344–9.
- Ridley M, Phillips I. Relative instability of cephaloridine to staphylococcal penicillinase. *Nature* **1965**; *208*:1076–8.
- Burgess HA, Evans RJ. Failure of cephaloridine in a case of staphylococcal endocarditis. *Br Med J* **1966**; *2*:1244.
- Luria S. A test for penicillin sensitivity and resistance in *Staphylococcus*. *Proc Soc Exp Biol Med* **1946**; *61*:46–51.
- Regamey C, Libke RD, Engelking ER, et al. Inactivation of cefazolin, cephaloridine, and cephalothin by methicillin-sensitive and methicillin-resistant strains of *Staphylococcus aureus*. *J Infect Dis* **1975**; *131*:291–4.
- Sabath LD, Garner C, Wilcox C, Finland M. Effect of inoculum and of beta-lactamase on the anti-staphylococcal activity of thirteen penicillins and cephalosporins. *Antimicrob Agents Chemother* **1975**; *8*:344–9.
- Laverdiere M, Welter D, Sabath LD. Use of a heavy inoculum in the in vitro evaluation of the anti-staphylococcal activity of 19 cephalosporins. *Antimicrob Agents Chemother* **1978**; *13*:669–75.
- Fong IW, Engelking ER, Kirby WM. Relative inactivation by *Staphylococcus aureus* of eight cephalosporin antibiotics. *Antimicrob Agents Chemother* **1976**; *9*:939–44.
- Bryant RE, Alford RH. Unsuccessful treatment of staphylococcal endocarditis with cefazolin. *JAMA* **1977**; *237*:569–70.
- Kaye D, Hewitt W, Remington JS, Turck M. Cefazolin and *Staphylococcus aureus* endocarditis. *JAMA* **1977**; *237*:2601.
- Nannini EC, Singh KV, Murray BE. Relapse of type A beta-lactamase-producing *Staphylococcus aureus* native valve endocarditis during cefazolin therapy: revisiting the issue. *Clin Infect Dis* **2003**; *37*:1194–8.
- Quinn EL, Pohlod D, Madhavan T, et al. Clinical experiences with cefazolin and other cephalosporins in bacterial endocarditis. *J Infect Dis* **1973**; *128*:S386–91.
- Richmond MH. Wild-type variants of exopenicillinase from *Staphylococcus aureus*. *Biochem J* **1965**; *94*:584–93.
- Rosdahl VT. Penicillinase production in *Staphylococcus aureus* strains of clinical importance. *Dan Med Bull* **1986**; *33*:175–84.
- Zygmunt DJ, Stratton CW, Kernodle DS. Characterization of four beta-lactamases produced by *Staphylococcus aureus*. *Antimicrob Agents Chemother* **1992**; *36*:440–5.
- Voladri RK, Kernodle DS. Characterization of a chromosomal gene encoding type B beta-lactamase in phage group II isolates of *Staphylococcus aureus*. *Antimicrob Agents Chemother* **1998**; *42*:3163–8.
- Li J, Echevarria KL, Hughes DW, et al. Comparison of cefazolin versus oxacillin for treatment of complicated bacteremia caused by methicillin-susceptible *Staphylococcus aureus*. *Antimicrob Agents Chemother* **2014**; *58*:5117–24.
- Pollett S, Baxi SM, Rutherford GW, et al. Cefazolin versus nafcillin for methicillin-sensitive *Staphylococcus aureus* bloodstream infection in a California Tertiary Medical Center. *Antimicrob Agents Chemother* **2016**; *60*:4684–9.
- Vardakas KZ, Apiranthiti KN, Falagas ME. Antistaphylococcal penicillins versus cephalosporins for definitive treatment of methicillin-susceptible *Staphylococcus aureus* bacteraemia: a systematic review and meta-analysis. *Int J Antimicrob Agents* **2014**; *44*:486–92.
- Forsblom E, Ruotsalainen E, Järvinen A. Comparable effectiveness of first week treatment with anti-staphylococcal penicillin versus cephalosporin in methicillin-sensitive *Staphylococcus aureus* bacteremia: a propensity-score adjusted retrospective study. *PLoS One* **2016**; *11*:1–13.
- Lee S, Kwon KT, Kim HI, et al. Clinical implications of cefazolin inoculum effect and β -lactamase type on methicillin-susceptible *Staphylococcus aureus* bacteremia. *Microb Drug Resist* **2014**; *20*:568–74.
- Lee S, Song K, Jung S, et al. Comparative outcomes of cefazolin versus nafcillin for methicillin-susceptible *Staphylococcus aureus* bacteraemia: a prospective multicentre cohort study in Korea. *Clin Microbiol Infect* **2018**; *24*:152–8.
- McDaniel JS, Roghmann MC, Perencevich EN, et al. Comparative effectiveness of cefazolin versus nafcillin or oxacillin for treatment of methicillin-susceptible *Staphylococcus aureus* infections complicated by bacteremia: a nationwide cohort study. *Clin Infect Dis* **2017**; *65*:100–6.
- Nannini EC, Stryjewski ME, Singh KV, et al. Inoculum effect with cefazolin among clinical isolates of methicillin-susceptible *Staphylococcus aureus*: frequency and possible cause of cefazolin treatment failure. *Antimicrob Agents Chemother* **2009**; *53*:3437–41.
- Nannini EC, Singh KV, Arias CA, Murray BE. In vivo effects of cefazolin, daptomycin, and nafcillin in experimental endocarditis with a methicillin-susceptible *Staphylococcus aureus* strain showing an inoculum effect against cefazolin. *Antimicrob Agents Chemother* **2013**; *57*:4276–81.
- Chapman SW, Steigbigel RT. Staphylococcal beta-lactamase and efficacy of beta-lactam antibiotics: in vitro and in vivo evaluation. *J Infect Dis* **1983**; *147*:1078–89.
- Seas C, Garcia C, Salles MJ, et al. *Staphylococcus aureus* bloodstream infections in Latin America: results of a multinational prospective cohort study. *J Antimicrob Chemother* **2017**; *73*:212–22.
- Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. *Am J Infect Control* **2008**; *36*:309–32.
- Martineau F, Picard FJ, Lansac N, et al. Correlation between the resistance genotype determined by multiplex PCR assays and the antibiotic susceptibility patterns of *Staphylococcus aureus* and *Staphylococcus epidermidis*. *Antimicrob Agents Chemother* **2000**; *44*:231–8.
- Rincón S, Reyes J, Carvajal LP, et al. Cefazolin high-inoculum effect in methicillin-susceptible *Staphylococcus aureus* from South American hospitals. *J Antimicrob Chemother* **2013**; *68*:2773–8.
- Bankevich A, Nurk S, Antipov D, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* **2012**; *19*:455–77.
- Jolley KA, Maiden MC. BIGSdb: scalable analysis of bacterial genome variation at the population level. *BMC Bioinformatics* **2010**; *11*:595.
- Stamatakis A. RAXML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **2014**; *30*:1312–3.
- Overbeek R, Olson R, Pusch GD, et al. The SEED and the rapid annotation of microbial genomes using subsystems technology (RAST). *Nucleic Acids Res* **2014**; *42*:206–14.
- Leticia I, Bork P. Interactive Tree Of Life (iTOL) v3: an online tool for the display and annotation of phylogenetic and other trees. *Nucleic Acids Res* **2016**; *44*:W242–5.

42. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *J Chronic Dis* **1987**; 40:373–83.
43. Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. *Crit Care Med* **1985**; 13:818–29.
44. McCabe W, Jackson G. Gram-negative bacteremia. I. Etiology and ecology. *Arch Intern Med* **1962**; 110:847–53.
45. Chow JW, Fine MJ, Shlaes DM, et al. Enterobacter bacteremia: clinical features and emergence of antibiotic resistance during therapy. *Ann Intern Med* **1991**; 115:585–90.
46. Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. *Kidney Inter Suppl* **2013**; 3:1–150.
47. Arias CA, Reyes J, Carvajal P, et al. A prospective cohort multicenter study of molecular epidemiology and phylogenomics of *Staphylococcus aureus* bacteremia in nine Latin American countries. *Antimicrob Agents Chemother* **2017**; 61:1–12.
48. Miko BA, Hafer CA, Lee CJ, et al. Molecular characterization of methicillin-susceptible *Staphylococcus aureus* clinical isolates in the United States, 2004 to 2010. *J Clin Microbiol* **2013**; 51:874–9.
49. Wi YM, Park YK, Moon C, et al. The cefazolin inoculum effect in methicillin-susceptible *Staphylococcus aureus* blood isolates: their association with dysfunctional accessory gene regulator (*agr*). *Diagn Microbiol Infect Dis* **2015**; 83:286–91.
50. Vuong C, Saenz HL, Götz F, Otto M. Impact of the *agr* quorum-sensing system on adherence to polystyrene in *Staphylococcus aureus*. *J Infect Dis* **2000**; 182:1688–93.
51. Seidl K, Bayer AS, Fowler VG Jr, et al. Combinatorial phenotypic signatures distinguish persistent from resolving methicillin-resistant *Staphylococcus aureus* bacteremia isolates. *Antimicrob Agents Chemother* **2011**; 55:575–82.
52. Fowler VG Jr, Sakoulas G, McIntyre LM, et al. Persistent bacteremia due to methicillin-resistant *Staphylococcus aureus* infection is associated with *agr* dysfunction and low-level in vitro resistance to thrombin-induced platelet microbicidal protein. *J Infect Dis* **2004**; 190:1140–9.
53. Schweizer ML, Furuno JP, Sakoulas G, et al. Increased mortality with accessory gene regulator (*agr*) dysfunction in *Staphylococcus aureus* among bacteremic patients. *Antimicrob Agents Chemother* **2011**; 55:1082–7.