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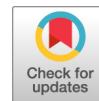
Cefazolin Inoculum Effect and Methicillin-Susceptible *Staphylococcus aureus* Osteoarticular Infections in Children

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ABSTRACT Select methicillin-susceptible *Staphylococcus aureus* (MSSA) strains may produce β-lactamases with affinity for first-generation cephalosporins (1GCs). In the setting of a high inoculum, these β-lactamases may promote the cleavage of 1GCs, a phenomenon known as the cefazolin inoculum effect (CzIE). We evaluated the prevalence and impact of CzIE on clinical outcomes among MSSA acute hematogenous osteomyelitis (AHO) cases. MSSA AHO isolates obtained from two children's hospitals between January 2011 and December 2018 were procured through ongoing surveillance studies. Isolates were tested for CzIE via a broth macrodilution assay using an inoculum of 10^7 CFU/ml; CzIE was defined as a cefazolin MIC of ≥ 16 μg/ml. Isolates were characterized by accessory gene regulator group (*agr*). The progression from acute to chronic osteomyelitis was considered an important outcome. A total of 250 cases with viable isolates were included. Notably, 14.4% of isolates exhibited CzIE with no observed temporal trend; and 4% and 76% of patients received a 1GC as an empirical and definitive therapy, respectively. CzIE isolates were more often resistant to clindamycin, belonged to *agr*III, and associated with the development of chronic osteomyelitis. In multivariable analyses, *agr*III, multiple surgical debridements, delayed source control, and CzIE were independently associated with progression to chronic osteomyelitis. A higher rate of chronic osteomyelitis was observed with CzIE isolates regardless of definitive antibiotic choice. CzIE is exhibited by 14.4% of MSSA AHO isolates in children. CzIE is independently associated with progression to chronic osteomyelitis in cases of AHO irrespective of final antibiotic choice. These data suggest that negative outcomes reported with CzIE may more accurately reflect strain-dependent virulence factors rather than true antibiotic failure.

KEYWORDS osteomyelitis, cefazolin inoculum effect, MSSA, pediatric, cephalosporin

The agents of choice in the treatment of methicillin-susceptible *Staphylococcus aureus* (MSSA) infections are β-lactam antimicrobials (1, 2). A number of recent studies have illustrated that the use of first-generation cephalosporins (1GCs; e.g., cefazolin) is associated with similar or, in some reports, improved outcomes compared with antistaphylococcal penicillins (e.g., nafcillin and oxacillin) in the treatment of serious MSSA infections (3–5). Moreover, both cefazolin and nafcillin/oxacillin are regarded as appropriate agents in national guidelines for the treatment of serious MSSA infections, such as bacteremia and endocarditis (2, 6, 7). Notably, 1GCs are associated with fewer drug-related adverse events than nafcillin/oxacillin, including less instances of infusion reactions, rash, neutropenia, acute kidney injury, and transaminase elevation (3, 8).

The vast majority of *S. aureus* strains possess a β-lactamase encoded by *blaZ* (9–11).

S. aureus β-lactamases were initially characterized serologically into types A to D. The various β-lactamase types have subtle differences in kinetics and substrate affinity (12, 13). Some *S. aureus* β-lactamase types have a degree of affinity for 1GCs, with the highest affinity among types A and C (13, 14). Despite this, drug inactivation *in vivo* is not believed to typically occur to a clinically meaningful degree.

As early as the 1970s, a subset of MSSA isolates was noted to exhibit striking elevations in 1GC MICs when a greater than standard inoculum was used in susceptibility testing (15, 16). This phenomenon, which affects 1GCs (including cefazolin and cephalexin) but not nafcillin/oxacillin, is referred to as the cephalosporin (or cefazolin) inoculum effect (CzIE) (17, 18). In a recent study of Argentinian adults with MSSA bacteremia treated with 1GC, it was noted that CzIE was an independent predictor of 30-day all-cause mortality (9). Such observations continue to raise questions regarding the efficacy of 1GCs in severe MSSA infections.

There are no data available on the prevalence or clinical significance of CzIE in a pediatric population. Acute hematogenous osteomyelitis (AHO) is the most common invasive staphylococcal infection in children. *S. aureus* AHO is frequently associated with large deep-seated purulent collections which require surgical debridement (19, 20) and as such may be regarded as a high inoculum infection. As the proportion of invasive *S. aureus* disease attributable to MSSA among children in the United States continues to rise (19, 21, 22), understanding the impact of CzIE on clinical outcomes will be of increasing importance.

The primary goals of this study were to (i) describe the frequency and characteristics of CzIE among MSSA AHO isolates at two children's hospitals and (ii) assess the impact of CzIE on AHO outcomes. The progression from acute to chronic osteomyelitis was considered a clinically significant outcome which is regarded as a surrogate for treatment failure.

RESULTS

A total of 250 viable MSSA AHO isolates from January 2011 to December 2018 met all inclusion criteria. Of those, 208 isolates were obtained from Texas Children's Hospital (TCH) and 42 were from St. Louis Children's Hospital (SLCH). The median patient age was 9.3 years (interquartile range [IQR], 5.4 to 12.2 years) (Table 1). Overall, 64.4% of cases were associated with isolated osteomyelitis and 35.6% with osteomyelitis and concomitant septic arthritis. A total of 57.6% of cases had bacteremia. Surgical drainage/debridement was performed in 63.2% of cases and 18% underwent ≥2 temporally distinct surgical procedures during the index admission.

Significant differences in patient demographics, disease presentation, and management were noted between contributing institutions (see Table S1 in the supplemental material). MSSA isolates from both institutions were similar in terms of clindamycin resistance, accessory gene regulator (*agr*) group, and Panton-Valentine leucocidin (PVL) carriage.

Medical therapy. Overall, the most common empirical antibiotic choices were clindamycin monotherapy (87, 34.5%) and vancomycin monotherapy (75, 30%); 38 patients (15.2%) received empirical vancomycin and nafcillin combination therapy. Only 10 subjects (4%) received a 1GC for empirical therapy with or without other agents. A full list of empirical therapy regimens is provided in Table S2 in the supplemental material.

A total of 189 patients (76%) received a 1GC for definitive therapy, 114 patients (45.6%) were prescribed cephalexin, 74 patients (29.6%) received cefazolin, and 1 patient received cefadroxil. Patients received a median of 4 days of non-1GC therapy (IQR, 3 to 6 days) prior to transitioning to 1GC. Specific dosing regimens of cephalosporins are provided in Table S3 in the supplemental material; patients were most commonly prescribed 100 mg/kg of body weight per day of cefazolin or cephalexin divided every 8 h. Other commonly used agents for definitive therapy included nafcillin (37, 14.8%) and clindamycin (14, 5.6%).

TABLE 1 Characteristics of study group[a]

Characteristic	Clinical values[b]
Age (yrs)	9.3 (5.4–12.2)
Female gender	100 (40)
Race	
White	183 (73.2)
Black	48 (19.2)
Asian	6 (2.4)
Other[c]	5 (2)
Unknown/not disclosed	8 (3.2)
Hispanic ethnicity	71 (28.4)
Chronic medical comorbidities	15 (6)
Duration of symptoms on presentation (days)	5 (3–7)
Isolated osteomyelitis	162 (64.4)
Osteomyelitis with concomitant septic arthritis	88 (35.2)
Subperiosteal/intraosseous abscess	108 (43.2)
Surgery performed	158 (63.2)
Positive blood culture	144 (57.6)
ICU[d] admission	13 (5.2)
Length of stay (days)	6 (5–9)
Total duration of therapy (days)	42 (31–56)
Total duration of follow-up (days)	92.5 (37–324)
Progression to chronic osteomyelitis	13 (5.2)

[a]$n = 250$.
[b]All continuous variables are presented as medians with interquartile ranges; categorical variables are presented as n (%).
[c]Other with regard to race includes Native American/Alaskan Native, Native Hawaiian/Pacific Islander, and self-identified multiple races given the relatively small number of patients.
[d]ICU, intensive care unit.

Overall, 127 patients (50.8%) were discharged on oral antibiotics. These patients received a median of 6 days of intravenous antibiotics prior to transition to oral antibiotics (IQR, 4 to 8 days). The rate of oral antibiotic use increased during the study period from 19% in 2011 to 75% in 2018 ($P = 0.003$).

Cefazolin inoculum effect. Using a standard inoculum (10^5 CFU/ml), all isolates had a cefazolin MIC of ≤ 2 μg/ml (Fig. 1). When tested with a high inoculum (10^7 CFU/ml), 90 isolates (36%) exhibited a $\geq$4-fold increase in cefazolin MIC, although the majority had a high inoculum MIC of ≤ 4 μg/ml (53/90, 58.9%). Overall, 36 MSSA isolates (14.4%) exhibited significant CzIE *in vitro*, defined as a high inoculum MIC of ≥ 16 μg/ml (range, 16 to 128 μg/ml) (9, 23). CzIE isolates were more likely to be clindamycin resistant (25% versus 9.3%, $P = 0.02$) and to belong to *agr*III (52.7% versus 9.8%, $P < 0.001$) than isolates not exhibiting CzIE (Table 2). There was no temporal trend with regard to the proportion of isolates exhibiting CzIE in the study period. A similar proportion of isolates from each contributing hospital exhibited CzIE. Additionally, among the subset tested, CzIE isolates exhibited higher cephalexin MICs when using a 10^7-CFU/ml inoculum than non-CzIE isolates (Fig. 2).

Infections caused by isolates with and without CzIE were similar in terms of patient age, demographics, the presence of bone abscesses, and the need for surgery and medical management (Table 2). Patients with CzIE isolate infections less often underwent multiple surgical debridement procedures (5.6% versus 20.1%, $P = 0.04$). A similar proportion of patients in both groups received definitive treatment with a 1GC. A greater proportion of infections caused by isolates with CzIE evolved into chronic osteomyelitis by the time of last follow-up (13.8% versus 3.7%, $P = 0.03$).

Chronic osteomyelitis. Overall, 13 (5.2%) MSSA osteoarticular infections (OAIs) progressed to chronic osteomyelitis. With regard to characteristics of the infecting organisms themselves, the development of chronic osteomyelitis was associated with isolates exhibiting CzIE and those belonging to *agr*III in univariable analyses (Table 3); there was no association with the carriage of PVL and development of chronic osteomyelitis.
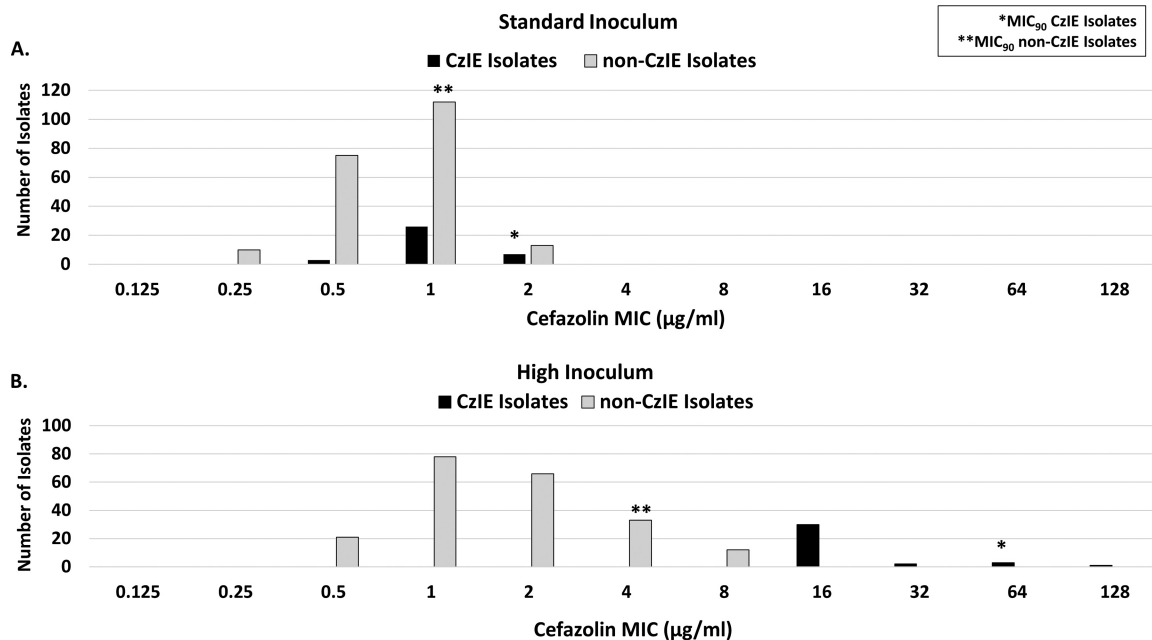
FIG 1 Cefazolin MICs. Broth macrodilution MICs for cefazolin using standard (10^5 CFU/ml) (A) and high inocula (10^7 CFU/ml) (B). CzIE, cefazolin inoculum effect.

Patients who developed chronic osteomyelitis more often had subperiosteal/intraosseous abscesses (69.2% versus 41.8%, $P = 0.08$, Table 4), bacteremia (84.6% versus 56.1%, $P = 0.048$), multiple surgical debridements during the period of acute infection (53.8% versus 15.8%, $P = 0.003$), and experienced a delay in first source control (>3 days until source control [24]; 30.7% versus 9.2%, $P = 0.03$). There were no significant associations observed between antibiotic choices, route or duration of therapy, and progression to chronic osteomyelitis. With regard to 1GCs, there was no observed association with specific agents or dosing regimens and chronic osteomyelitis (see Table S4 in the supplemental material).

The results of multivariable analyses revealed significant associations between progression to chronic osteomyelitis and multiple surgical debridements ($P = 0.01$), delayed source control ($P = 0.03$), agrIII ($P = 0.04$), and CzIE ($P = 0.03$) (Table 5). When CzIE alone was substituted with CzIE and 1GC treatment and forced into the logistic regression model, we observed no association with chronic osteomyelitis ($P = 0.3$).

Antibiotic choice, inoculum effect, and outcomes. Subjects were stratified by inoculum effect status to assess the effect of definitive antibiotic choice on the development of chronic osteomyelitis. A 1GC was definitive therapy in 30/36 (83.3%) CzIE subjects and 159/214 (74.2%) of non-CzIE subjects ($P = 0.29$). Isolates with CzIE were associated with higher rates of progression to chronic osteomyelitis than those without CzIE regardless of whether treatment was with a 1GC or a non-1GC regimen (Fig. 3).

Whole-genome sequencing. Whole-genome sequencing was performed on all 36 isolates exhibiting CzIE to determine sequence types (STs) and β-lactamase types (Fig. 4). The majority of cases were ST30 (19/36, 52.7%), with the next most common being ST8 (5/36, 13.9%). Among ST30, 15/19 (78.9%) belonged to agrIII. Type A β-lactamase was most common, being noted in 27/36 cases (75%), followed by type C (8/36, 22.2%) and type B (1/36, 2.7%). Type A β-lactamase predominated in ST30 strains (18/19, 94.7%), while all ST8 strains possessed type C β-lactamase.

DISCUSSION

In the treatment of serious MSSA infections outside the central nervous system, 1GCs are generally regarded as equally efficacious as antistaphylococcal penicillins (5).

TABLE 2 Comparison of infections by isolates with and without CzIE

| Characteristic | Clinical and microbiologic values[a] related to isolates: | | P value |
	With CzIE (n = 36)	Without CzIE (n = 214)	
Age (yrs)	10.4 (6.9–11.9)	9.1 (5.1–12.2)	0.38
Female gender	13 (36.1)	87 (40.6)	0.71
Race			0.82
White	30 (83.3)	153 (71.4)	
Black	5 (13.8)	43 (20.1)	
Asian	0	6 (2.8)	
Other[b]	0	5 (2.3)	
Unknown/not disclosed	1 (2.7)	7 (3.3)	
Hispanic ethnicity	10 (27.8)	61 (28.5)	1
Duration of symptoms on presentation (days)	4.5 (2–7)	5 (3–7)	0.52
Admission CRP[c] (mg/dl)	4.6 (3.8–7.7)	6.6 (3.7–15.5)	0.21
Multifocal infection	3 (8.3)	7 (3.3)	0.16
Subperiosteal/intraosseous abscess	17 (47.2)	85 (39.7)	0.46
Maximum abscess diameter[d] (cm)	2.9 (1.1–4)	3 (1.5–5.8)	0.3
Surgical procedure performed	20 (55.5)	136 (63.5)	0.36
≥2 surgical procedures performed[e]	2 (5.6)	43 (20.1)	0.04
Delayed first source control[f]	5 (13.8)	23 (10.7)	0.57
ICU admission	1 (2.7)	12 (5.6)	0.7
Positive blood culture	26 (72.2)	118 (55.1)	0.07
Duration of bacteremia (days)	1 (1–2)	1 (1–2)	0.39
Duration of fever after admission (days)	3 (2–4)	3 (2–4)	0.37
Definitive treatment with 1GC	30 (83.3)	159 (74.3)	0.29
Discharge on oral antibiotics	20 (55.6)	105 (49.1)	0.58
Length of stay (days)	6 (5–10)	6 (5–9)	0.9
Duration of follow-up (days)	68 (39–494)	77 (35–247)	0.32
Progression to chronic osteomyelitis	5 (13.8)	8 (3.7)	0.03
Clindamycin resistance	9 (25)	20 (9.3)	0.01
PVL[g] positive	5 (13.8)	47 (21.9)	0.38
Vancomycin MIC of >1 µg/ml	12 (33.3)	100 (46.7)	0.15
agrI[h]	13 (36.1)	140 (65.4)	0.001
agrII	2 (5.6)	31 (14.5)	0.19
agrIII	19 (52.7)	21 (9.8)	<0.001
agrIV	1 (2.7)	8 (3.7)	1
agr nontypeable	1 (2.7)	14 (6.5)	0.7

[a]All continuous variables are presented as medians with interquartile ranges; categorical variables are presented as n (%).
[b]Other with regard to race includes Native American/Alaskan Native, Native Hawaiian/Pacific Islander, and self-identified multiple races.
[c]CRP, C-reactive protein.
[d]Only 61 cases had abscess size documented.
[e]The number of surgical procedures performed refers only to those performed during the index admission.
[f]Delayed source control was defined as >3 calendar days from the time of admission until first surgical source control.
[g]PVL, Panton-Valentine leucocidin.
[h]agr refers to accessory gene regulator group.

Controversy has surrounded the clinical impact of CzIE and how this phenomenon should influence therapeutic decisions (25). A number of studies in animal models as well as at least one clinical study in adults suggest that the presence of CzIE reduces the efficacy of 1GC therapy (9, 26). However, other observational clinical studies have not illustrated an increased risk of treatment failure or attributable mortality with CzIE infection (10, 27). Here, we present the first study of the epidemiology and impact of CzIE among pediatric osteoarticular infections, demonstrating an association between infections caused by CzIE strains and progression to chronic osteomyelitis, a surrogate for treatment failure.

Across published studies, the definition of CzIE varies. A cefazolin MIC of ≥16 µg/ml has previously been used by CLSI to define cefazolin resistance in *S. aureus* (28) and may represent a clinically meaningful measure; this definition was used in our study as by others (9). In our study population, 14.4% of MSSA isolates exhibited CzIE. In contrast, 55% of MSSA bloodstream isolates exhibited CzIE in a study from Argentina (9) using an identical definition. When applying the definition of a ≥4-fold increase in MIC comparing high and standard inoculums, 36% of our isolates exhibited CzIE. In a
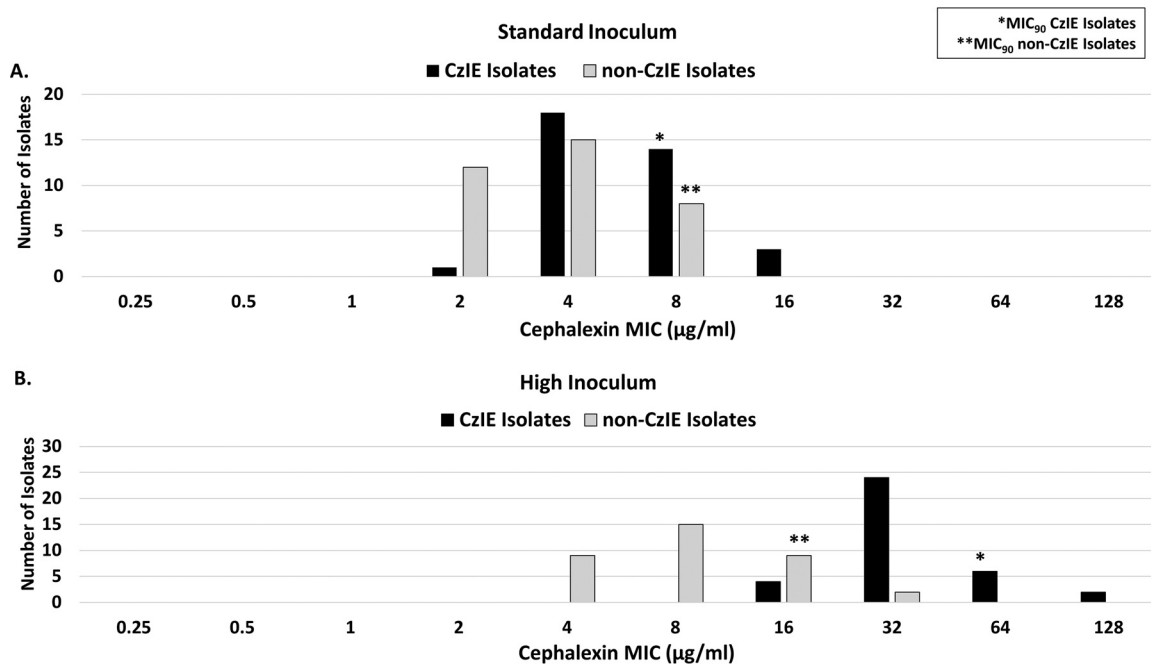
FIG 2 Cephalexin MICs. Broth macrodilution MICs for cefazolin using standard (10^5 CFU/ml) (A) and high inocula (10^7 CFU/ml) (B).

recent study of MSSA isolates from four hospitals in the Chicago area, Wang et al. reported that 16.7% of all isolates exhibited a 4-fold increase in cefazolin MIC comparing high and standard inoculums (29). Using a similar definition of CzIE, investigators at Emory University reported CzIE in 27% of MSSA bloodstream isolates obtained from adults, although only 4.3% had a cefazolin MIC of $\geq 16\ \mu$g/ml (11). Such findings suggest a degree of geographic variability in CzIE prevalence.

We also observed an association with CzIE and clindamycin resistance, occurring in 25% of isolates. Even though β-lactam antibiotics are clearly preferred for MSSA, this is notable, as clindamycin is commonly used empirically in suspected AHO. While the exact reasons for this association are unclear, such a finding was previously reported by investigators in Korea who found that 21% of CzIE isolates exhibited concomitant clindamycin resistance; this was particularly common among CzIE isolates with type A β-lactamase occurring in 44% of them (27). These findings may be associated with the underlying strain type. Wi et al. reported that among Korean CzIE isolates, 75% belonged to agrIII (30). Similar to these data, we found that CzIE was disproportionately occurring in agrIII isolates in our North American population.

TABLE 3 Univariable associations of chronic osteomyelitis and microbiologic and molecular characteristics of MSSA

Characteristic of MSSA	No. (%) of patients with:		P value
	Progression to chronic osteomyelitis ($n = 13$)	No progression to chronic osteomyelitis ($n = 237$)	
Clindamycin resistant	3 (23.1)	26 (10.9)	0.18
CzIE[a]	5 (38.5)	31 (13.1)	0.02
$\geq$ 4-fold increase in cefazolin MIC with high inoculum	6 (46.2)	84 (35.4)	0.55
Vancomycin Etest MIC of $>1\ \mu$g/ml[b]	3 (23.1)	109 (45.9)	0.15
PVL positive	5 (38.5)	47 (19.8)	0.15
agrI	6 (46.1)	147 (62)	0.26
agrII	2 (15.4)	31 (13.1)	0.67
agrIII	4 (30.7)	36 (15.2)	0.1
agrIV	0	9 (3.8)	1
agr nontypeable	1 (7.6)	14 (5.9)	0.56

[a]CzIE was defined as a high-inoculum cefazolin MIC of $\geq 16\ \mu$g/ml.
[b]No isolate had a vancomycin Etest MIC of $>2\ \mu$g/ml.

TABLE 4 Univariable associations of chronic osteomyelitis, patient characteristics, and management

Characteristic	Values[a] for patients with:		P value
	Progression to chronic osteomyelitis (n = 13)	No progression to chronic osteomyelitis (n = 237)	
Age (yrs)	8.9 (4.2–13.9)	8.3 (3.1–11.6)	0.26
Female gender	4 (30.7)	96 (40.5)	0.57
Race			0.9
White	10 (76.9)	173 (72.9)	
Black	3 (23.1)	45 (18.9)	
Asian	0	6 (2.5)	
Other[b]	0	5 (2.1)	
Unknown/not disclosed	0	8 (3.4)	
Hispanic ethnicity	2 (15.4)	69 (29.1)	0.35
Duration of symptoms on presentation (days)	5 (4–20)	5 (3–7)	1
Multifocal infection	0	11 (4.6)	1
Subperiosteal/intraosseous abscess	9 (69.2)	99 (41.8)	0.08
Maximum abscess diameter[c] (cm)	4.8 (2.9–6.1)	3 (1.4–4.4)	0.21
ICU admission	2 (15.4)	11 (4.6)	0.14
Positive blood culture	11 (84.6)	133 (56.8)	0.048
Duration of bacteremia (days)	1 (1–1)	1 (1–2)	0.9
Duration of fever after admission (days)	1 (0–2)	2 (0–3)	0.11
Medical management			
Empiric therapy			0.18
Clindamycin monotherapy	3 (23.1)	84 (35.4)	
Vancomycin monotherapy	6 (46.2)	69 (29.1)	
1GC monotherapy	1 (7.7)	9 (3.8)	
Vancomycin + nafcillin	3 (23.1)	35 (14.8)	
Other	0	40 (16.8)	
Definitive therapy			0.26
1GC	8 (61.5)	181 (76.4)	
Nafcillin	5 (38.4)	32 (13.5)	
Clindamycin	0	14 (5.9)	
Other	0	10 (4.2)	
Oral antibiotic at discharge	5 (38.5)	120 (50.6)	0.57
Duration of intravenous antibiotics (days)	22.5 (10–55)	14.5 (6–34)	0.17
Total duration of antibiotics (days)	46 (32–61)	42 (31–53)	0.71
Surgical management			
Surgical procedure performed	11 (84.6)	145 (61.2)	0.14
≥2 surgical procedures	7 (53.8)	38 (15.8)	0.003
Delayed first source control	4 (30.7)	22 (9.2)	0.03
Duration of follow-up (days)	61 (32–82)	79 (36–285)	0.15

[a]All continuous variables are presented as medians with interquartile ranges; categorical variables are presented as n (%).
[b]Other with regard to race includes Native American/Alaskan Native, Native Hawaiian/Pacific Islander, and self-identified multiple races.
[c]Only 61 cases had abscess size documented.

In multivariable analyses, CzIE was an independent predictor for the progression of acute osteomyelitis to chronic infection, a proxy for treatment failure. This finding is consistent with that of a recent study in adults with MSSA bacteremia which suggested an association with CzIE and mortality (9). Importantly, we found that children with

TABLE 5 Multivariable analyses of risk factors for MSSA chronic osteomyelitis[a]

Factor	Multivariable P value	Adjusted OR	95% CI
Bone abscess	0.54	1.58	0.37–6.7
Positive blood culture	0.09	4.1	0.8–20.26
≥2 surgical procedures	0.01	6.99	1.58–31.1
Delayed first source control	0.03	2.59	1.16–11.11
agrIII	0.04	1.71	1.03–7.81
CzIE	0.03	13.4	1.1–18.21

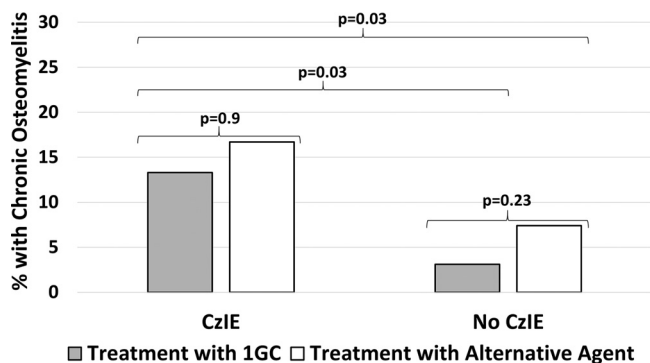[a]OR, odds ratio; CI, confidence interval.

FIG 3 Relationship between CzIE, definitive antibiotic choice, and development of chronic osteomyelitis. Higher rates of chronic osteomyelitis were observed among CzIE isolates regardless of whether patients were treated with a 1GC or an alternative agent. Comparisons performed with Fisher's exact test.

AHO caused by CzIE MSSA have similar rates of progression to chronic osteomyelitis when treated with either 1GC or non-1GC regimens. These results suggest that poor outcomes previously observed with CzIE strains may more accurately reflect some intrinsic virulence factor rather than antibiotic failure *per se*, as negative outcomes occurred irrespective of antibiotic choice. Such findings may explain why 1GCs and antistaphylococcal penicillins have been found to have a similar overall efficacy in the treatment of serious MSSA infections (3–5) despite the existence of CzIE in 10% to 20% of North American MSSA isolates (29). Based on the results of the present study, clinicians may be able to successfully use 1GCs to treat CzIE osteoarticular MSSA infections, although careful follow-up is urged. Arguably, 1GCs may be the preferred agent even in the setting of CzIE, given their favorable side effect profile relative to antistaphylococcal penicillins; the rate of adverse drug reactions was almost 3-fold higher among adults receiving nafcillin than those receiving cefazolin in one study (8).
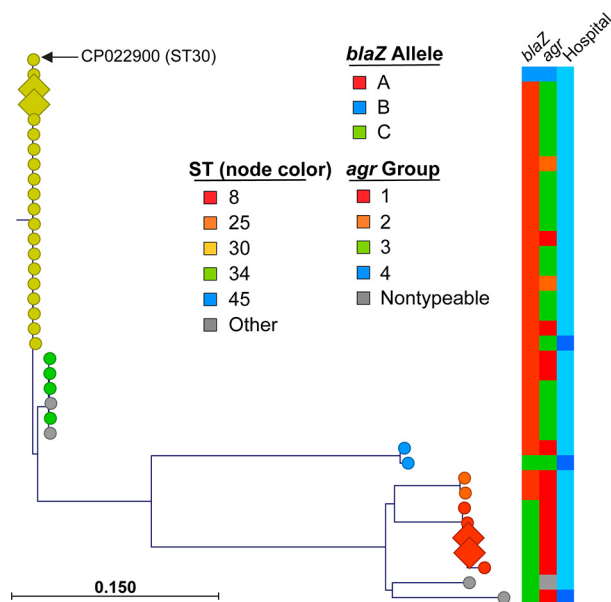

FIG 4 Phylogenetic tree of CzIE isolates. Whole-genome sequencing from a total of 36 CzIE strains was used to identify single nucleotide polymorphisms relative to the ST30 reference strain (GenBank accession number CP022900) core genome, as described in the Materials and Methods. Node shape (diamonds versus circles) corresponds to isolates with progression to chronic osteomyelitis, while color indicates sequence type (ST; legend). Metadata layers are indicated and defined in the legend; final metadata layer indicates geographic site of isolate, with light blue corresponding to TCH and dark blue representing SLCH.

Our findings must be interpreted with caution given the relatively small number of patients in the present study who received definitive treatment with a non-1GC regimen. Furthermore, these results are in direct contrast to work in adults by Lee et al. (31) which showed that CzIE MSSA infections treated with cefazolin were associated with higher rates of treatment failure than those treated with nafcillin. Interestingly, these investigators found that CzIE and non-CzIE MSSA infections have overall similar outcomes. In addition, studies in rat endocarditis models demonstrate that the use of cefazolin in a CzIE MSSA infection is associated with a more severe disease course which may be ameliorated by the addition of a β-lactamase inhibitor with the 1GC (26, 32). Such discrepancies could in part be related to differences in populations studied (adults with comorbidities versus otherwise healthy children), as well as measures of treatment success/failure. Definitively addressing this question would require a clinical trial comparing the outcomes of CzIE MSSA infections treated with antistaphylococcal penicillins versus those with 1GC; however, significant challenges would exist for the execution of such a study. Of note, the majority of patients in our study receiving a 1GC were treated with cephalexin at an every-8-hour dosing interval with a median dose of 100 mg/kg/day. Studies from the 1970s found that cephalexin peak serum concentrations in children following a 25 mg/kg dose were 20 to 25 μg/ml (33). Given the cephalexin MIC$_{90}$ of 8 μg/ml, concerns may exist about the potential failure of this particular therapy; however, the observed MIC range for cephalexin was similar to that found by other investigators (18). Furthermore, a recent observational study at Rady Children's found that patients with MSSA AHO treated with cephalexin at a median of 91 mg/kg/day had similar cure rates when doses were administered with either every 8 h or every 6 h (34). For our study, all patients received some duration of intravenous therapy and many underwent surgical drainage procedures prior to starting cephalexin; these interventions may have reduced the bacterial load enough to obviate CzIE.

The agrIII strains were also associated with chronic osteomyelitis. agr is a complex quorum sensing regulatory system controlling the expression of numerous staphylococcal adhesins and virulence factors (35). Isolates exhibiting agr dysfunction and dysregulation of downstream gene products have previously been associated with progression to chronic infection in animal models of osteomyelitis (36). While we did not specifically assess for agr dysfunction, differences in agr-associated gene regulation attributable to specific agr polymorphisms may partially explain our findings. Interestingly, agrIII is rarely associated with community-acquired methicillin-resistant $S.$ $aureus$ (MRSA) in the United States, with most USA300 strains (either MRSA or MSSA) being agrI (21, 24). Approximately 25% of invasive non-USA300 MSSA strains belong to agrIII (21) and thus may represent a small but virulent subset of MSSA. USA300 strains have previously been associated with large purulent collections (19) that often require multiple debridement procedures; this may explain in part why CzIE strains (which are most frequently agrIII) are less often associated with multiple debridements.

Based on whole-genome sequencing studies, the most common sequence type among CzIE isolates was ST30, accounting for nearly half of all strains, which is consistent with work from South America (9). Interestingly, previous research examining all invasive MSSA in children found that ST30 strains only account for ~10% of all disease (21). Taken together, these data suggest that strain background may be important in at least a subset of CzIE isolates. Notably, the overwhelming majority of ST30 CzIE isolates possessed type A β-lactamase, which is known to have the highest affinity for 1GCs (13, 14).

Additional limitations to this study should be acknowledged. Foremost, the retrospective nature of the study limits the degree to which conclusions can be made regarding therapeutic decisions and outcomes. The unequal contribution of cases by the two sites may have potentially introduced bias, and moreover, all MSSA osteomyelitis may not have been captured. Notably, the proportion of cases with CzIE was similar at both study institutions. The rate of chronic osteomyelitis in this study (5.2%) was higher than that reported in some centers but was overall consistent with the range reported across North American studies (20, 37); our study utilized a broad

definition of chronic osteomyelitis that incorporated clinical, pathological, and radiographic criteria (24) to provide a thorough capture of sequelae. The high degree of surgical source control at the participating study sites may not accurately reflect wider pediatric practice and may have influenced the impact of CzIE on outcomes. Similarly, the fact that the majority of patients received a non-1GC regimen empirically may have impacted disease burden sufficiently to influence the observed relationship between CzIE, 1GC use, and outcome. It is difficult retrospectively to account for the potential impact of postdischarge medication adherence on outcomes. Furthermore, clinical microbiology laboratories do not routinely screen MSSA isolates for CzIE, limiting the impact our findings have on management at the bedside. The relatively labor-intensive nature of the broth macrodilution assay may make implementation difficult for many busy clinical laboratories. The study is underpowered to explore the relationship between dosing of 1GCs, CzIE, and outcome. Finally, as this study focused on AHO, the findings may not necessarily be extrapolated to other invasive MSSA infections in children.

In conclusion, CzIE is exhibited by 14% of MSSA isolates at 2 geographically distinct pediatric centers. CzIE is associated with clindamycin resistance as well as specific genotypes which include *agr*III/ST30. CzIE, along with delayed source control and the need for multiple surgical procedures in MSSA AHO, is associated with progression to chronic osteomyelitis. Further work is needed to better understand the relationship between CzIE and negative outcomes, as well as how or if this phenomenon should impact therapeutic decisions.

MATERIALS AND METHODS

Isolates and patients were identified through two separate ongoing surveillance studies at Texas Children's Hospital (TCH; affiliated with Baylor College of Medicine, Houston, TX, comprising 724 inpatient beds) (38) and St. Louis Children's Hospital (SLCH; affiliated with Washington University School of Medicine, St. Louis, MO, with 390 inpatient beds) (39). At TCH, since 2001, all *S. aureus* isolates identified by the clinical microbiology laboratory in the routine course of care are subcultured and stored in horse blood at −80°C in the Edward O. Mason, Jr., Infectious Diseases Research Laboratory (IDRL), and basic clinical and demographic data are recorded on a standardized case report form (19). In previous studies at our institution, MSSA accounted for 43% of all osteomyelitis cases, with the surveillance study capturing >70% of these isolates (40). During the same time period, at SLCH, *S. aureus* isolates recovered from the blood, bone, or synovial fluid were obtained from the clinical microbiology laboratory and stored at −80°C, and similar clinical data were recorded. Only one isolate per patient per episode of infection was collected, and isolates were not serially passaged. For the purposes of this study, only patients with culture-confirmed MSSA AHO with or without concomitant septic arthritis identified from 1 January 1 2011 to 31 December 2018 were included. The diagnosis of AHO was defined by a constellation of physical examination, radiology, and microbiological findings as previously described (19, 40, 41). Patients with open or penetrating trauma, orthopedic hardware *in situ*, or osteomyelitis secondary to a contiguous focus or a surgical procedure (such as sternal osteomyelitis after cardiac surgery) were excluded. Data from a subset of these patients have been reported in other publications (19, 24, 39, 42). All medical records were reviewed from the time of initial hospital admission with AHO until time of last follow-up with infectious diseases or orthopedics. The institutional review boards of Baylor College of Medicine and Washington University School of Medicine approved this study. A full description of study definitions and the genomic and statistical analyses are provided in Appendix S1 in the supplemental material.

Microbiology studies. The clinical microbiology labs at TCH and SLCH performed initial isolate identification as well as susceptibility testing to oxacillin, vancomycin, and clindamycin in accordance with CLSI guidelines (43). Additional characterization of isolates as well as microbiology studies were performed in the IDRL at TCH. All isolates underwent testing for the presence of CzIE using paired cefazolin broth macrodilution assays with an inoculum of 10^5 CFU/ml (standard inoculum to confirm susceptibility) and 10^7 CFU/ml (high inoculum) (additional detail in Appendix S1). Current CLSI guidelines do not define 1GC breakpoints for *S. aureus*. CzIE was defined as a cefazolin MIC of ≥16 μg/ml using the 10^7 CFU/ml inoculum (9, 23); this value was regarded as a clinically significant MIC, as previous 2012 guidelines defined cefazolin susceptibility in *S. aureus* as an MIC of ≤8 μg/ml (28). All isolates exhibiting CzIE as well as an equivalent number of random non-CzIE isolates were also subjected to cephalexin MIC determinations using high and low inocula, as described above. Laboratory personnel performing CzIE testing were blind to all clinical data.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.
SUPPLEMENTAL FILE 1, PDF file, 0.3 MB.

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