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CLINICAL THERAPEUTICS

# Prevalence of a Cefazolin Inoculum Effect Associated with *blaZ* Gene Types among Methicillin-Susceptible *Staphylococcus aureus* Isolates from Four Major Medical Centers in Chicago

**Sheila K. Wang,[a,b] Annette Gilchrist,[c] Anastasia Loukitcheva,[a] Balbina J. Plotkin,[d] Ira M. Sigar,[d] Alan E. Gross,[e] J. Nicholas O'Donnell,[a,f*] Natasha Pettit,[g] Amy Buros,[h] Tristan O'Driscoll,[a,b*] Nathaniel J. Rhodes,[a,f] Cindy Bethel,[i] John Segreti,[j] Angella Charnot-Katsikas,[i] Kamaljit Singh,[j*] Marc H. Scheetz[a,f]**

[a]Department of Pharmacy Practice, Midwestern University, Downers Grove, Illinois, USA
[b]Department of Pharmacy, Rush University Medical Center, Chicago, Illinois, USA
[c]Department of Pharmaceutical Sciences, Midwestern University, Downers Grove, Illinois, USA
[d]Department of Microbiology and Immunology, Midwestern University, Downers Grove, Illinois, USA
[e]Department of Pharmacy Practice, University of Illinois at Chicago, Chicago, Illinois, USA
[f]Department of Pharmacy, Northwestern Memorial Hospital, Chicago, Illinois, USA
[g]Department of Pharmacy, University of Chicago Medical Center, Chicago, Illinois, USA
[h]Midwestern University, Glendale, Arizona, USA
[i]Department of Pathology, University of Chicago Medical Center, Chicago, Illinois, USA
[j]Division of Infectious Diseases, Rush University Medical Center, Chicago, Illinois, USA

**ABSTRACT** The efficacy of cefazolin with high-inoculum methicillin-susceptible *Staphylococcus aureus* (MSSA) infections remains in question due to therapeutic failure inferred as being due to an inoculum effect (InE). This study investigated the local prevalence of a cefazolin InE (CInE) and its association with staphylococcal *blaZ* gene types among MSSA isolates in the Chicago area. Four medical centers in Chicago, IL, contributed MSSA isolates. Cefazolin MICs (C-MIC) were determined at 24 h by the broth microdilution method using a standard inoculum (SI; $5 \times 10^5$ CFU/ml) and a high inoculum (HI; $5 \times 10^7$ CFU/ml). The CInE was defined as (i) a ≥4-fold increase in C-MIC between SI and HI and/or (ii) a pronounced CInE, i.e., a nonsusceptible C-MIC of ≥16 μg/ml at HI. PCR was used to amplify the *blaZ* gene, followed by agarose gel electrophoresis and sequencing to determine the gene type. Approximately 269 MSSA isolates were included. All but one isolate were susceptible to cefazolin at SI, and 97% remained susceptible at HI. A total of 196 isolates (73%) were *blaZ* positive, with the *blaZ* types led by gene type C (40%). CInE was seen in 45 *blaZ*-positive isolates (23%), with 44 (22%) presenting a ≥4-fold increase in C-MIC (SI to HI) and 5 (3%) a pronounced CInE. Four of the five met both definitions of CInE, two of which expressed the type A gene. The prevalence of a pronounced CInE associated with the type A *blaZ* gene from MSSA isolates in Chicago is low. Our predilection for cefazolin use, even early in the management of hospitalized MSSA infections, is tenable.

**KEYWORDS** MSSA, *Staphylococcus aureus*, beta-lactamases, *blaZ*, cefazolin, inoculum effect

According to the 2017 *WHO Model List of Essential Medicines*, cefazolin is designated a key ACCESS β-lactam, a widely available, affordable and quality-assured antibacterial (1). This first-generation intravenous cephalosporin is also the most frequently recommended antimicrobial agent for surgical prophylaxis (2). Compared to vancomycin, treatment with either cefazolin or an antistaphylococcal penicillin (ASPCN), such as nafcillin or oxacillin, has been associated with lower rates of treatment failure and

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Address correspondence to Sheila K. Wang, swangx@midwestern.edu.

* Present address: J. Nicholas O'Donnell, Albany College of Pharmacy and Health Sciences, Albany, New York, USA; Tristan O'Driscoll, Aspirus Wausau Hospital, Wausau, Wisconsin, USA; Kamaljit Singh, NorthShore University Health System, Evanston, Illinois, USA.

mortality for methicillin-susceptible *Staphylococcus aureus* (MSSA) bloodstream infections (BSI) (3–6). Moreover, recent retrospective comparative effectiveness studies have gone on to demonstrate similar outcomes between cefazolin and ASPCNs for MSSA infections complicated by bacteremia (7–12). Given its dissimilar side chain and the improbability of cross-reactivity, cefazolin also appears to be a safe substitute for individuals who experience a non-IgE-mediated hypersensitivity reaction to penicillins (13, 14). As a result, cefazolin is a recommended alternative to ASPCNs for several complicated MSSA infections (15–18). Compared to ASPCNs, cefazolin is better tolerated, allows for less frequent dosing, and has a cost advantage (19–27). However, in the setting of high-inoculum (HI) MSSA infections, such as infected endocarditis and undrained abscesses, the efficacy of cefazolin remains in question.

Several *in vitro* and *in vivo* studies corroborate the degradation of cefazolin in serum by certain staphylococcal β-lactamase-producing strains (28–40). However, predicting cefazolin activity against potent β-lactamases in high-inoculum settings while using a standard inoculum (SI) size for *in vitro* susceptibility testing ($10^5$ CFU/ml) has been contested. As a result, the assessment of quantitative differences in the amounts of β-lactamase produced, otherwise known as the inoculum effect (InE), was introduced. The InE has been described as "a significant rise in the MIC when the inoculum size is increased" (41). Bacteria that present as susceptible at a low inoculum ($<10^4$ CFU/ml) would become significantly resistant at a higher inoculum ($10^6$ CFU/ml). Therefore, in the absence of controlled clinical trials, several studies explored the InE and its influence on staphylococcal β-lactamase stability as a means of differentiating the clinical advantages between penicillins and cephalosporins for severe staphylococcal infections (29, 30, 37–40, 42–45). However, the methods of interpreting significant resistance through the rise in MIC to define a clinically relevant InE have differed (46–51), and a standard definition remains to be established.

Quantitative differences in inoculum size relative to changes in cefazolin MICs have been shown to correlate with the degradation of cefazolin by staphylococcal β-lactamase (29, 30, 37–40, 42–45). This observation is less significant with the ASPCNs that have been tested (30, 37, 38, 42). Also, notable differences in the magnitudes of the effect of staphylococcal isolates in causing changes in MICs through rises in inoculum size were seen, suggesting that qualitative differences in staphylococcal β-lactamases may further explain the variation in sensitivity to cefazolin. Four distinct serotypes of staphylococcal β-lactamases encoded by the *blaZ* gene (types A, B, C, and D) have been identified and their kinetic differences evaluated (45, 52–54). The type A *blaZ* gene has been shown to be the most efficient at hydrolyzing cefazolin *in vitro*, contrasted by the type C variant, which showed almost a 4-fold-lower relative efficiency of cefazolin hydrolysis (53). A correlation between the type A variant and a pronounced InE, expressed as a significant rise in cefazolin MIC at high inoculum, has been described previously (31–33). Therefore, this phenotypic characteristic of a high-efficiency type A β-lactamase has been hypothesized to be associated with cefazolin degradation and clinical failure, but not without doubt (36, 40).

Our own assessment of cefazolin versus oxacillin use at two Chicago medical centers showed no significant difference in rates of treatment failure even in patients with deep-seated infections, including infective endocarditis (10). As a result, both institutions have successfully implemented a clinical pathway supporting the use of cefazolin for the treatment of MSSA BSI (55). However, aversion to the use of cefazolin for high-inoculum MSSA infections persists due to the concerns over the presence of an InE. The focus of this study was to identify the local prevalence of the cefazolin inoculum effect (CInE) and determine its association with high-efficiency staphylococcal *blaZ* gene types among MSSA isolates collected from four major medical centers in the Chicago area.

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## RESULTS

A total of 308 MSSA isolates were collected from the participating medical centers, referred to herein as sites 1 to 4. Thirty-nine isolates were excluded due to unsuccessful *blaZ* gene sequencing ($n = 31$), negative 16S rRNA gene results ($n = 6$), or blank samples ($n = 2$). Two hundred sixty-nine MSSA isolates were included. Sixty-nine (26%) were from site 1, 63 (23%) from site 2, 78 (29%) from site 3, and 59 (22%) from site 4 ($P = 0.38$). Cultures of samples from wounds and abscesses comprised approximately two-thirds of the sources reported, whereas a quarter of the samples documented were collected from sputum or tracheal aspirates, blood, and other bodily fluids.

Site-specific cefazolin MICs of isolates at standard inoculum (SI) and high inoculum (HI) along with associated *blaZ* gene types and CInE are shown in Table 1. Isolates from each site were susceptible to cefazolin at SI except for one isolate from site 1 (MIC = 8 $\mu$g/ml). A difference in ranked MICs at SI was observed between the sites ($P = 0.0001$), with the greatest differences seen for site 1 compared to sites 2, 3, and 4 (adjusted *P* values of 0.02, 0.0035, and 0.0001, respectively). Most MSSA isolates from each site remained susceptible at HI (97%), except for seven isolates. Four isolates with nonsusceptible cefazolin MICs of $\geq$8 $\mu$g/ml at HI were collected from site 1 (MIC = 8 $\mu$g/ml, $n = 1$; MIC = 16 $\mu$g/ml, $n = 3$), and the other three came from site 3 (MIC = 8 $\mu$g/ml, $n = 1$; MIC = 16 $\mu$g/ml, $n = 1$; MIC = 32 $\mu$g/ml, $n = 1$). The ranked MICs at HI were also found to be significantly different between sites ($P = 0.012$), with the greatest difference observed between site 1 and site 4 (adjusted $P = 0.006$). A total of 196 isolates (73%) were positive for the *blaZ* gene, averaging 49 isolates (73%) per site ($P = 0.52$). The most prevalent *blaZ* serotype among all isolates included was gene type C (40%), followed by type A (23%), type B (9%), and type D (1%) (Table 2). Seventy-three (27%) were *blaZ* negative. Only the type B strains trended toward a significant difference when stratified by site ($P = 0.06$), being isolated predominantly from site 2 (13%) and site 3 (14%) and less frequently from site 1 (4%) and site 4 (5%).

A total of 45 isolates (23%) carrying a *blaZ* gene displayed a CInE using either definition, averaging 11 isolates (16%) per site ($P = 0.25$). The majority of these isolates carried the type C *blaZ* gene ($n = 25$), followed by type A ($n = 17$). Forty-four *blaZ*-positive isolates (22%) presented with a $\geq$4-fold increase in cefazolin MIC between SI and HI. A pronounced CInE, representing a nonsusceptible cefazolin MIC of $\geq$16 $\mu$g/ml at HI for an isolate with a susceptible MIC at SI, was observed in five (3%) *blaZ*-positive isolates (MIC = 16 $\mu$g/ml, $n = 3$ type A and $n = 1$ type D; MIC = 32 $\mu$g/ml, $n = 1$ type C). The same five nonsusceptible isolates described above were collected from sites 1 and 3, exclusively from abscess and wound specimen cultures. Four of the five isolates (2%) presented both an $\geq$4-fold increase in cefazolin MIC between SI and HI and a nonsusceptible cefazolin MIC of $\geq$16 $\mu$g/ml at HI (MIC = 16 $\mu$g/ml, $n = 2$ type A and $n = 1$ type D; MIC = 32 $\mu$g/ml, $n = 1$ type C). A significant difference between specific *blaZ* gene types associated with a pronounced CInE was not detected ($P = 0.45$).

Five isolates did display a CInE but were negative for the *blaZ* gene by our PCR analysis. Four of these isolates exhibited a $\geq$4-fold increase in cefazolin MIC, with one having a pronounced CInE. Our reverse PCR primer was the same as that used by Nannini et al. (32). However, BLAST analysis of the forward primer from this publication (5′-TACAACTGTAATATCGGAGGG-3′) showed multiple divergent strains. Thus, we used a novel forward primer (5′-ATTTTGAAAAAGTTAATATTTTTAATTG-3′), recognizing that while better, there were *S. aureus* strains that could not be amplified, including GN1, GN3, GD1677, and JH4899.

## DISCUSSION

The present study follows the findings by Rao et al., who reviewed the treatment outcomes of cefazolin versus oxacillin for deep-seated MSSA BSI at two Chicago medical centers (10). They demonstrated that treatment failure did occur more often in their patient population with deep-seated infections than in those without ($P = 0.005$). However, the failure rates for cefazolin and oxacillin were not found to be significantly different ($P = 0.72$). As a result, institution-based clinical pathways promoting the use of cefazolin for the

**TABLE 1** Site-specific ranked cefazolin MICs at SI and HI with associated *blaZ* gene types and CInE[a]

| Site | No. (%) of isolates: | | | Cefazolin $MIC_{24}$ value for: | | | | No. (%) of isolates: | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | | Presenting InE[b]: | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | Using either definition [$n$ = 45 (23)] | | | | | | Using each definition[c]: | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | ≥4-fold increase in MIC [$n$ = 44 (22)] | | | | | Pronounced InE [$n$ = 5 (3)] | | | | |
| | Collected ($n$ = 308) | Excluded ($n$ = 39) | Included ($n$ = 269) | SI ($10^5$ CFU/ml); HI ($10^7$ CFU/ml) | | | | *bla* positive [$n$ = 196 (73)] | With *blaZ* gene type: | | | | | | | | | | | | | | | | | | | | |
| | | | | Median | Mode | $MIC_{90}$ | Range | | A | B | C | D | Neg | Total | A | B | C | D | Neg | A | B | C | D | Neg | A | B | C | D | Neg |
| 1 | 81 (26) | 12 | 69 (26) | 0.5; 1.0 | 0.5; 0.5 | 2.0; 4.0 | 0.25–8; 0.25–16 | 52 (75) | 17 (25) | 3 (4) | 30 (43) | 2 (3) | 17 (25) | 14 (20) | 8 (12) | 0 (0) | 5 (7) | 1 (1) | 0 (0) | 7 | | 5 | 1 | | 2[d] | | | 1[e] | | |
| 2 | 68 (22) | 5 | 63 (23) | 0.5; 1.0 | 0.5; 2 | 1.0; 2.0 | 0.125–2; 0.125–4 | 48 (76) | 14 (22) | 8 (13) | 26 (41) | 0 (0) | 15 (24) | 11 (17) | 4 (6) | 1 (2) | 6 (10) | 0 (0) | 0 (0) | 4 | 1 | 6 | | | | | | | | |
| 3 | 81 (26) | 3 | 78 (29) | 0.5; 1.0 | 0.5; 1 | 1.0; 2.0 | 0.0625–4; 0.25–32 | 55 (71) | 14 (18) | 11 (14) | 30 (39) | 0 (0) | 23 (29) | 14 (18) | 3 (4) | 0 (0) | 11 (14) | 0 (0) | 4 (5) | 3 | | 11 | | 4 | 1[e] | | 1[e] | | 1[e] | |
| 4 | 78 (26) | 19 | 59 (22) | 0.5; 1.0 | 0.5; 0.5 | 1.0; 2.0 | 0.625–2; 0.25–2 | 41 (69) | 16 (27) | 3 (5) | 22 (37) | 0 (0) | 18 (31) | 6 (10) | 2 (3) | 1 (2) | 3 (5) | 0 (0) | 1 (2) | 2 | 1 | 3 | | 1 | | | | | | |
| *P* value | 0.66 | | 0.38 | | | | 0.0001; 0.012[f] | 0.52 | | | | | | 0.25 | | | | | | | | | | | | | | | |

[a]CInE, cefazolin inoculum effect; $MIC_{24}$, MIC read at 24 h; SI, standard inoculum; HI, high inoculum; *bla*, β-lactamase gene.
[b]Instances of InE associated with *bla*-positive isolates.
[c]Definitions of InE used are as follows: isolates presenting (i) a ≥4-fold increase in cefazolin MIC between SI and HI and/or (ii) a pronounced InE represented by a nonsusceptible cefazolin MIC of ≥16 μg/ml at HI.
[d]Isolated from an abscess.
[e]Isolated from a wound.
[f]For SI, the ranked MIC was significantly higher at site 1 than at sites 2, 3, and 4 (adjusted *P* values of 0.02, 0.0035, and 0.0001, respectively); for HI, the ranked MIC was significantly higher at site 1 than at site 4 (adjusted *P* value of 0.006).

**TABLE 2** Related prevalence studies of cefazolin InE associated with *blaZ* gene types[a]

| Study information; CInE definition(s) used | *bla* type or status | No. (%) of isolates: | | |
|---|---|---|---|---|
| | | With indicated *bla* status | Meeting indicated CInE definition | |
| | | | ≥4-fold increase SI to HI | ≥16 μg/ml at HI |
| This study, 4 hospitals in Chicago, IL, 269 isolates from all sources except CSF and urine; ≥4-fold increase in cefazolin MIC (SI to HI) and/or a pronounced InE with a nonsusceptible MIC of ≥16 μg/ml at HI | A | 61 (23) | 16 (26) | 3 (5) |
| | B | 25 (9) | 2 (8) | 0 |
| | C | 108 (40) | 25 (23) | 1 (1) |
| | D | 2 (1) | 1 (50) | 1 (50) |
| | Total *bla* positive | 196 (73) | 44 (22)[b] | 5 (3)[b] |
| | *bla* negative | 73 (27) | 5 (7) | 1 (1) |
| Song et al. (46), 10 hospitals in South Korea, 303 isolates from blood; an increased cefazolin MIC of ≥16 μg/ml at HI from a susceptible MIC at SI | A | 41 (13.5) | | 23 (56) |
| | B | 80 (26) | | 1 (1) |
| | C | 132 (44) | | 37 (28) |
| | D | 1 (0.5) | | 0 |
| | Total *bla* positive | 254 (84) | | 61 (24)[b] |
| | *bla* negative | 49 (16) | | 0 |
| Wi et al. (47), 9 hospitals in South Korea, 146 isolates from blood; cefazolin MIC of ≥16 μg/ml at HI | A | 60 (41) | | 16 (3) |
| | B | 32 (22) | | 0 |
| | C | 30 (21) | | 0 |
| | D | 3 (2) | | 0 |
| | Total *bla* positive | 125 (86) | | 16 (13)[b] |
| | *bla* negative | 21 (14) | | 0 |
| Chong et al. (59), 1 hospital in South Korea, 218 isolates from blood; ≥4-fold increase in MIC (SI to HI) resulting in a nonsusceptible MIC at HI | A | 38 (17) | | 23 (61) |
| | B | 43 (20) | | 2 (5) |
| | C | 117 (54) | | 4 (3) |
| | D | 3 (1) | | 0 |
| | Total *bla* positive | 201 (92) | | 29 (14)[b] |
| | *bla* negative | 17 (8) | | 0 |
| Lee et al. (60), 3 hospitals in South Korea, 113 isolates from blood; ≥4-fold increase in MIC (SI to HI) and an increased MIC of ≥16 μg/ml at HI | A | 17 (15) | 16 (94) | 11 (65) |
| | B | 24 (21) | 4 (17) | 0 |
| | C | 46 (41) | 44 (96) | 12 (26) |
| | D | 1 (1) | 0 | 0 |
| | Total *bla* positive | 88 (78) | 65 (74)[b] | 23 (26)[b] |
| | *bla* negative | 25 (22) | 1 (4) | 0 |
| Livorsi et al. (57), 5 hospitals in Georgia, 185 isolates from blood; ≥4-fold increase in MIC (SI to HI) and a nonsusceptible MIC of ≥16 μg/ml at HI | A | 48 (26) | 22 (46) | 8 (17) |
| | B | 43 (23) | 3 (7) | 0 |
| | C | 49 (27) | 22 (45) | 0 |
| | D | 2 (1) | 0 | 0 |
| | Total *bla* positive | 142 (77) | 42 (30)[b] | 8 (6)[b] |
| | *bla* negative | 43 (23) | 3 (7) | 0 |
| Nannini et al. (58), multiple countries, 98 isolates from cSSTI, HAP, IE, and blood; pronounced InE with MIC of ≥16 μg/ml at HI | A | 25 (26) | | 9 (36) |
| | B | 15 (15) | | 0 |
| | C | 45 (46) | | 10 (22) |
| | D | 0 | | 0 |
| | Total *bla* positive | 85 (87) | | 19 (22)[b] |
| | *bla* negative | 13 (13) | | 0 |

[a]CInE, cefazolin inoculum effect; *bla*, β-lactamase gene; SI, standard inoculum; HI, high inoculum; CSF, cerebrospinal fluid; cSSTI, complicated skin-soft tissue infection; HAP, hospital-acquired pneumonia; IE, infective endocarditis.
[b]Percent CInE was calculated using the total number of *bla*-positive isolates as the denominator.

majority of MSSA infections have been successfully implemented (55). And yet, deliberations have resumed, particularly on the clinical outcomes of using cefazolin early in therapy where potential influences of a CInE are linked to high-inoculum MSSA infections. Gaining knowledge of the latter led us to investigate the prevalence of the CInE and its association with known high-efficiency *blaZ* gene types among our MSSA isolates. The current study included 269 MSSA isolates from four major academic medical centers in the Chicago area. Our study findings prompt several points of discussion.

First, only 3% ($n = 7$) of our MSSA isolates were observed to have a nonsusceptible MIC of ≥8 μg/ml at HI. Moreover, just 4 (2%) of these MSSA isolates that were nonsusceptible at HI agreed with both study definitions of a CInE, i.e., (i) a ≥4-fold increase in cefazolin MIC between SI and HI and (ii) a pronounced InE represented by a nonsusceptible cefazolin MIC of ≥16 μg/ml at HI. Furthermore, the most pronounced CInE or rise in cefazolin MIC at HI was 32 μg/ml. The definition of a significant or pronounced rise in MIC from SI to HI to suggest an InE with clinical concerns for treatment failure with cefazolin has not been determined. Quinn et al. were the first to report a pronounced increase in cefazolin MIC (0.4 μg/ml for SI to >50 μg/ml for HI), in a heroin addict with *S. aureus* endocarditis experiencing recurrent bacteremia while receiving 2 to 4 g of cefazolin daily (34). Kernodle et al. also described rises in cefazolin MICs at HI (median MIC = 64 μg/ml) among 18 MSSA isolates, mostly expressing the type A *blaZ* gene, collected from deep sternal wound infections associated with cefazolin prophylaxis failure (52). Finally, Nannini et al. presented an aortic native valve endocarditis patient who presumably failed cefazolin therapy with a MSSA strain expressing the type A *blaZ* gene showing a significant rise in MIC, to 128 μg/ml at HI (32). In contrast, Fields et al. observed a more modest rise in the MIC (1 μg/ml for SI to 32 μg/ml for HI) within rabbit abscess models inoculated with an *S. aureus* strain carrying type A *blaZ*, but with successful clearance of infection (40). The authors concluded that the maintenance of a mean cefazolin concentration above the MIC could effectively reduce the bacterial concentrations within localized purulent MSSA infections exhibiting a less pronounced CInE. The cases of cefazolin failure all demonstrated an appreciable CInE, displaying MICs of >50 μg/ml at HI and possibly offering a greater production of the type A β-lactamase, which is known to be more efficient at hydrolyzing cefazolin. Our most pronounced CInE displayed a modest rise in cefazolin MIC to 32 μg/ml at HI, and of interest, the *blaZ* gene was serotype C. Therefore, reduction of the bacterial concentration at the local level with adequate clearance of MSSA infection could be achieved through optimal cefazolin dosing when a less pronounced CInE is involved.

Second, coupled with our preponderance of susceptible MSSA isolates and low prevalence of a pronounced CInE, standard use of high-dose cefazolin (≥6 g daily, adjusted for renal function) for complicated MSSA infections deserves attention. Bryant et al. recommended caution in the use of cefazolin after presenting two cases of treatment failure with cefazolin involving IV drug addicts with *S. aureus* endocarditis (28). The authors concluded that insufficient doses of cefazolin (4 to 6 g daily) may have influenced the treatment failure, resulting in inadequate concentrations at the site of the vegetation despite achieving sterile blood cultures. However, it is also important to note that both cases were complicated by microabscesses within the spleen and one had central nervous system (CNS) involvement, achieving clinical cure only after surgical source control was obtained. Kaye et al. later rebutted the cautionary use of cefazolin recommended by Bryant et al., noting that their extensive experience with cefazolin for *S. aureus* endocarditis had been no different from their experience with ASPCNs (51). Their use of nafcillin and oxacillin also resulted in cases of failure, relapse, and persistent bacteremia, leading to the conclusion that the experience of failure may be more suggestive of the severity of *S. aureus* endocarditis than of the antibiotic selected. Goldman and Petersdorf reported a survival rate of 35% after using cefazolin in 20 rabbits with endocarditis infected by a single β-lactamase-producing *S. aureus* strain with a pronounced CInE (MIC = 125 μg/ml at HI) (29). The authors noted their use of a lower cefazolin dose to rationalize the disparity in survival rates compared to those of a similar study conducted by Carrizosa et al., where 86% of the rabbits survived to the end of the experiment (42). Optimizing the dose of cefazolin (≥6 g daily), whether it be by intermittent or continuous infusion, has been shown to attain its target percentage of time above the MIC (%$T >$ MIC) for hospitalized MSSA infection cases (56), offering an ideal dosing strategy when CInE is a concern.

Third, the frequency of *blaZ*-positive MSSA strains observed in our analysis (73%) was comparable to rates reported from other, similar prevalence studies (Table 2).

However, the frequencies of *blaZ* subtypes across studies appear to be more distinct. The type C *blaZ* gene prevailed in our study (40%), as it did with the others, except for that of Wi et al. (47), where the type A gene led. Our study, along with those of Livorsi et al. (57) and Nannini et al. (58), observed type A to be the second most common *blaZ* gene, whereas Chong et al. (59), Lee et al. (60), and Song et al. (46) found type B to be the next most prevalent gene. To offer consistency between studies and given concerns about cefazolin treatment failure in high-burden MSSA infections, we analyzed the frequency of CInE as a fraction of *bla*-positive MSSA isolates across studies, using the pronounced-InE definition. Although 23% of our *bla*-positive MSSA isolates carried the type A *blaZ* gene, just 5% of strains expressing this high-efficiency $\beta$-lactamase displayed a pronounced CInE. Only Wi et al. demonstrated a lower rate of 3% of type A strains showing CInE (47). In contrast, the South Korea study by Lee et al. (60) showed that over half of their type A MSSA strains (65%) achieved a pronounced CInE. Most notably, compared to other studies, we observed the lowest prevalence of a pronounced CInE among all *bla*-positive MSSA isolates, at just 3%.

Finally, there has been a steady growth of studies comparing cefazolin to ASPCNs for MSSA BSI, with outcomes suggesting a lack of difference between the two agents with regard to treatment failure and mortality (7–11). Although the studies are not without limitations, we have yet to see a single clinical study proposing that ASPCNs are indeed superior to cefazolin for MSSA infections with or without the presence of a CInE. Even so, a clinical shortcoming of cefazolin use continues to be its contentious effect on MSSA when associated with highly inoculated infections. Therapeutic failure with cefazolin has been reported (28–34), mostly extrapolated from case reports and *in vitro* studies with animal experiments. However, contrary findings of clinical success with cefazolin under similar conditions and study limitations have also been described but less frequently referenced (34–36, 42, 50). Nonetheless, caution in the use of cefazolin for high-inoculum staphylococcal infections has been professed over time, with it even being deemed "a regimen for mothers-in-law" (61), even among conflicting outcomes and in the absence of controlled clinical trials to support this hypothesis.

Our study is not without limitations. First, given that the collection of MSSA isolates was conducted during the months of October 2014 to January 2015 with the inclusion of only four local hospitals in the Chicago area, seasonality and clonal relatedness were considered but not assessed as potential covariates in this study. However, we believe our isolates are representative of our region. Additional studies are needed to define CInE over a broader geographic distribution. Second, although the source of the MSSA isolate was not available for all specimens collected, we did know the infectious sources of the five *blaZ*-positive isolates with pronounced CInE, which were collected from abscesses ($n = 2$, both type A) and wound cultures ($n = 3$, types A, C, and D) prior to attempting source control. Third, limitations in amplification and sequencing of the *blaZ* subtype arose because of divergence in MSSA sequences. Finally, the significance of the underlying disease or clinical status of the source patient was not included, as the clinical impact of the CInE in our study was not a targeted objective.

Generalizing the clinical relevance of a pronounced CInE and its association with the high-efficiency type A *blaZ* gene of MSSA isolates is difficult, as a pronounced CInE is yet to be defined and the prevalence of the type A gene appears to vary widely (5% to 65%). High-inoculum MSSA infections are often dynamic, involving several other significant factors that can also influence clinical outcomes, as opposed to antibiotic selection alone. Furthermore, differences according to geographic region, host characteristics, and even *blaZ* polymorphisms may influence CInE variability, suggesting that cefazolin treatment failure in high-inoculum MSSA infections may be a complementary effect. Examples of moderately pronounced CInE (MIC $<50$ $\mu$g/ml for HI) may have clinical significance where maintaining cefazolin concentrations above the MIC could assemble enough protection against cefazolin degradation by the targeted $\beta$-lactamases. Regardless, pragmatic approaches to cefazolin dosing to maximize pharmacokinetic and pharmacodynamic

efficiencies should always be considered for complicated MSSA infections (≥6 g daily, adjusted for renal function), particularly in regions where ASPCNs are not readily available. The majority of our MSSA isolates, including the five found to be β-lactamase positive with a pronounced CInE, were collected from either wound or abscess cultures prior to attempting source control. We believe this finding offers a likely representation of our MSSA isolates as to where they typically reside and the prevalence with which they exhibit a *blaZ* gene with a pronounced CInE. We recognize the prospect that more complicated MSSA cases involving infective endocarditis with or without cerebral embolic events or high-grade bacteremia lacking source control may justify the early use of ASPCN therapy over cefazolin. However, for these unique cases, we advise that an infectious diseases or antimicrobial stewardship consultation be initiated to best determine antibiotic appropriateness, opportunities for surgical intervention or source control, and antibiotic duration.

Our investigation of MSSA isolates collected from four major medical centers in the Chicago metropolitan area reveals that the prevalence of a pronounced CInE associated with *bla*-positive strains, including the high-efficiency type A *blaZ* gene, is low. The majority of our *bla*-positive strains expressed the type C variant, which has been associated with a lower efficiency of cefazolin hydrolysis than has the type A *blaZ* gene. These findings support the sustainability of our current clinical practice, which is the predilection for and assurance of early cefazolin use for the management of hospitalized MSSA infection cases.

## MATERIALS AND METHODS

**Samples and setting.** A maximum of 100 MSSA isolates each were requested from four major academic medical centers in the Chicago area from October 2014 to February 2015. Site 1 is a 664-bed medical center located on the West Side of Chicago, site 2 is an 894-bed hospital based in Central Chicago, site 3 is also located on the West Side and supports 495 inpatient beds, and site 4, with 811 beds, is located on the South Side of Chicago. Specimens were collected from any source, with the exception of urine and cerebral spinal fluid (CSF), and then transferred from each participating institution to Midwestern University in Downers Grove, IL, for susceptibility testing and DNA extraction. Institutional Review Board approval was established by each participating site.

**Susceptibility testing.** Cefazolin (Sigma, St. Louis, MO, USA) MICs, read at 24 h, were determined by a broth microdilution method using cation-adjusted Mueller-Hinton II broth (Becton Dickinson) and in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines (62). Cefazolin MICs were determined using a standard inoculum (SI; $5 \times 10^5$ CFU/ml) and a high inoculum (HI; $5 \times 10^7$ CFU/ml). The inocula were confirmed by colony counts. Both the site of origin and inoculum of the isolate were coded, and the colony counts performed independently in a blinded fashion by two study members. Two definitions have been used in the literature for CInE: (i) isolates presenting with a ≥4-fold increase in cefazolin MIC between SI and HI (57, 59) and (ii) a pronounced CInE where isolates present an increase to a nonsusceptible cefazolin MIC of ≥16 μg/ml at HI from a susceptible MIC at SI (46, 47, 58, 60). Both definitions were adopted in this study.

**Sequence analysis.** DNA was extracted from each sample using a rapid lysis method (48). Briefly, the inoculum (1 ml) was centrifuged for 5 min at 14,000 × *g*, the supernatant was discarded, and the cell pellet resuspended in 20 μl of sterile water. Lysostaphin solution (50 μl, 100 μg/ml) was added to each sample, followed by an incubation at 37°C for 10 min. Proteinase K (50 μl, 100 μg/ml) and resuspension buffer (150 μl, 0.1 M Tris, pH 7.5) were added to each sample. Samples were incubated at 37°C for 10 min and then boiled for 5 min before being placed on ice. The DNA concentration was determined for each sample, and samples were stored at −20°C until needed. NCBI BLAST was used to design the following primers that amplify most variants of the β-lactamase gene: forward, 5′-ATTTTGAAAAAGTTAATATTTTTAATTG-3′, and reverse, 5′-CATTACACTCTTGGCGGTTTC-3′. The 50-μl PCR mixture included 0.5 μl Phusion polymerase, 1 μl each forward/reverse primers (10 μM), 10 μl 5× Phusion buffer, 1 μl deoxynucleoside triphosphates (dNTPs) (10 mM), 1 μl DNA template (concentrations ranged from 40 to 100 ng/μl), and 35.5 μl $H_2O$. The following thermocycling parameters were used: preheating for 2 min at 95°C, 30 cycles of 94°C for 15 s, 55°C for 30 s, and 72°C for 30 s, an additional extension at 72°C for 10 min, and then maintenance at 4°C for up to 24 h. After thermocycling, 5 μl was removed and subjected to agarose gel electrophoresis to determine the quantity, quality, purity, and appropriate size of products. PCR products were run at 120 V for 30 min on a 1.5% agarose gel alongside a molecular weight marker (Life Technologies). The agarose gel was visualized using ethidium bromide and a UV light box. Positive and negative controls were performed alongside the samples. As controls, we used *S. aureus* strains ATCC 29213, known to produce small amounts of the type A *blaZ* gene, ATCC 25923, a *blaZ*-negative strain, and TX0117, a high-level producer of the type A *blaZ* gene. After PCR, samples that were positive for the *blaZ* gene (band at 700 bp) were sent to Northwestern University for sequencing (sequencing primer, 5′-GCTCATATTGGTGTTTATG-3′). Sequence analysis of the *blaZ* gene type was performed by examining

the amino acids present at residues 128 and 216 (49). Type A *blaZ* genes have a threonine at position 128 and serine at position 216, type B *blaZ* genes have a lysine at position 128 and asparagine at position 216, type C *blaZ* genes have a threonine at position 128 and asparagine at position 216, and type D *blaZ* genes have an alanine at position 128 and serine at position 216.

**Statistical analysis.** Descriptive statistics were calculated for all isolates with regard to site, cefazolin MIC, ClnE, and β-lactamase gene type. Isolates were stratified according to site (Table 1) and then based on MIC, ClnE, and β-lactamase gene type. Comparisons of the included isolates, occurrence of ClnE, and β-lactamases across sites were analyzed using the $\chi^2$ test. The ranked MICs were compared across the four sites with the nonparametric Kruskal-Wallis test. If the Kruskal-Wallis test showed significant differences, the Dunn *post hoc* test was run to confirm where the differences occurred between sites, using the Holm method for *P* value adjustment for multiple comparisons. Correlation between *blaZ* gene types and a pronounced ClnE was analyzed using either Fisher's exact test or the $\chi^2$ test. An *a priori* level of alpha was set at 0.05 for statistical significance. Analysis was done using Excel and R statistical software (50).

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S.K.W. designed the project and drafted the manuscript; A.G. and A.L. performed and analyzed *bla* PCRs; B.J.P. and I.M.S. performed and analyzed all the MIC and minimal bactericidal concentration (MBC) testing, assisted by T.O.; A.E.G., J.N.O., N.P., A.B., N.J.R., C.B., J.S., A.C.-K., K.S., and M.H.S. collected, isolated, and characterized MSSA isolates and participated in manuscript drafting and critical review; and all authors provided final review and approval of the manuscript.

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