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Prevalence of *blaZ* Gene Types and the Inoculum Effect with Cefazolin among Bloodstream Isolates of Methicillin-Susceptible *Staphylococcus aureus*

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We sought to define the prevalence of *blaZ* gene types and the inoculum effect to cefazolin among methicillin-susceptible *Staphylococcus aureus* (MSSA) bloodstream infections. The *blaZ* gene was present in 142/185 (77%) isolates. A total of 50 (27%) isolates had a ≥4-fold increase in the cefazolin MIC from a standard to a high inoculum, and 8 (4%) demonstrated a nonsusceptible cefazolin MIC, all type A *blaZ* strains. The efficacy of cefazolin in the presence of the inoculum effect requires further study.

The relative efficacy of different β-lactam antibiotics for the treatment of methicillin-susceptible *Staphylococcus aureus* (MSSA) bloodstream infections is not well defined. Although cefazolin and nafcillin have never been compared in a controlled trial, several cases of cefazolin failure in MSSA endocarditis have been reported (2, 9, 16, 18, 20). In two reports, the infecting MSSA strain was found to have high MICs to cefazolin, but not to nafcillin, in the presence of a large bacterial inoculum, e.g., 10^7 CFU/ml (18, 20). Such a high inoculum of bacteria may better approximate the density of bacteria within an infected valvular vegetation or an undrained abscess (24). The phenomenon among bacterial isolates of antibiotic susceptibility at a standard inoculum but resistance at a high inoculum is called the inoculum effect (1).

The inoculum effect observed with cefazolin correlates with inactivation of the drug by staphylococcal β-lactamases (4, 22) and has been largely associated with strains producing the type A β-lactamase, which can efficiently hydrolyze cefazolin *in vitro* (12, 18). The clinical relevance of the type A β-lactamase or the inoculum effect is unclear.

We sought to define the prevalence of β-lactamases and the inoculum effect among MSSA isolates causing bloodstream infections. Between 1 January 2010 and 31 December 2010, bloodstream MSSA isolates were collected from five Emory-affiliated facilities, including a pediatric hospital, a public hospital, a veterans' medical center, a community-based hospital, and a tertiary care hospital. The study was approved by the institutional review board at Emory University, and a waiver for written consent was granted.

A total of 217 unique index MSSA isolates were collected. Thirty-two isolates were excluded for the following reasons: ≥2 different microbes grew in the blood culture (24), the isolate was deemed a "contaminant" by the treating physician (7), or the medical record was not accessible (1). Of the remaining 185 unique bloodstream isolates, the mean age of cases was 44 years (range, 3 weeks to 87 years); adults accounted for 81% of cases and children (<18 years) for 19%; 63% of cases were male; 51% were black, and 38% were white. The most common types of infections were osteomyelitis and catheter-associated bloodstream infections, with a catheter-associated bloodstream infection defined as a documented exit site infection or an MSSA-positive catheter tip culture (Table 1).

To detect the presence of the *blaZ* gene, which encodes β-lactamase, DNA was prepared using InstaGene Matrix (Bio-Rad, Hercules, CA) according to the manufacturer's instructions with an additional 30-min incubation at 65°C followed by 30 min at 37°C with 20 μg/ml of lysostaphin (Sigma-Aldrich, St. Louis, MO) between step 3 and step 4. An 861-bp segment of the *blaZ* gene was amplified by PCR (18) and sequenced (Beckman Coulter Genomics, Beverly, MA) using the following *S. aureus* β-lactamase reference strains as controls: type A, PC1; type B, 22260; type C, RN98; type D, FAR19 (13, 14). Lasergene (DNASTAR, Inc., Madison, WI) sequence analysis software was used. Southern blot hybridization (23) was performed to confirm the absence of the *blaZ* gene for strains that were negative by PCR on pulsed-field gel electrophoresis (PFGE) gels (17) that were probed with the 861-bp *blaZ* gene segment labeled with digoxigenin by PCR (Roche Diagnostics, Indianapolis, IN), according to the manufacturer's protocol (Roche Diagnostics, Indianapolis, IN).

The *blaZ* gene was detected in 142 isolates (77%): 48 (34%) type A, 43 (30%) type B, 49 (35%) type C, and 2 (1%) type D. Forty-three isolates (23%) were negative for *blaZ* by both PCR and Southern blot assays and do not appear to be clonal by PFGE. The presence or absence of β-lactamase was confirmed by Cefinase β-lactamase detection discs (BD, Franklin Lakes, NJ) (8) and corresponded to the presence or absence of the *blaZ* gene for 182/185 isolates (98%), with the remaining 3 isolates indeterminate.

Broth microdilution antimicrobial susceptibility testing was performed according to CLSI guidelines to determine the MIC for cefazolin (Sigma-Aldrich, St. Louis, MO) and nafcillin (MP Biomedicals, Solon, OH) at a standard inoculum (5×10^5 CFU/ml) and at a high inoculum (5×10^7 CFU/ml) (6). For the high inoculum, the broth at a 0.5 McFarland standard was concentrated by centrifugation and resuspended in one-fifth the original volume

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TABLE 1 Types of infections and management of MSSA bacteremia in adults and children

Parameter	Result for: Adults	Children
Infection type[a]		
Total no. of cases	150	35
Bacteremia without known focus	66 (44%)	19 (54%)
Catheter-associated bloodstream infection	21 (14%)	0
Osteomyelitis	19 (13%)	10 (29%)
Endocarditis	14 (9%)	0
Pneumonia	11 (7%)	2 (6%)
Septic arthritis	9 (6%)	3 (9%)
AV[c] graft infection	5 (3%)	0
Septic thrombophlebitis	5 (3%)	0
Skin and soft tissue infection	5 (3%)	2 (6%)
Septic pulmonary emboli	4 (3%)	0
Management[b]		
No. of cases	84	24
Repeat blood cultures to document clearance	79 (94%)	22 (92%)
Echocardiogram	69 (82%)	7 (29%)
Central line removed	29/34 (85%)	5/12 (42%)
Total parenteral antibiotics	35.7 days	11.8 days
Total antibiotic course	37.4 days	18.0 days
Predominantly oral antibiotics	3 (4%)	9 (38%)
Surgical drainage or debridement	25 (30%)	4 (17%)
Outcome[b]		
No. of cases	84	24
Recurrences	12 (14%)	1 (4%)

[a] Infection types are not mutually exclusive.
[b] In patients with documented follow-up at 12 weeks; excludes patients who died.
[c] AV, arteriovenous.

TABLE 2 MICs (μg/ml) of cefazolin by *blaZ* gene type and size of the bacterial inoculum

blaZ gene type (*n*) and parameter	Cefazolin MIC (μg/ml) Standard inoculum[b]	High inoculum[c]	No. (%) of strains exhibiting inoculum effect ≥4-fold increase[d]	≥4-fold increase to nonsusceptible MIC[e]
Type A (48)				
Mean (GM)[a]	1.2 (1.1)	7.6 (3.7)	22 (46)	8 (17)
Mode	1	2		
Range	0.5–2	1–32		
MIC_{50}	1	4		
MIC_{90}	2	16		
Type B (43)				
Mean (GM)	1.2 (1.1)	2.1 (1.9)	3 (7)	0
Mode	1	2		
Range	0.5–2	1–4		
MIC_{50}	1	2		
MIC_{90}	2	4		
Type C (49)				
Mean (GM)	1.1 (1.0)	3.2 (2.8)	22 (45)	0
Mode	1	4		
Range	0.5–2	1–8		
MIC_{50}	1	4		
MIC_{90}	2	8		
Type D (2)				
Mean (GM)	1.0 (1.0)	2.0 (2.0)	0	0
Mode	1	2		
Range	1–1	2–2		
MIC_{50}	1	2		
MIC_{90}	1	2		
blaZ negative (43)				
Mean (GM)	0.8 (0.77)	1.4 (1.2)	3 (7)	0
Mode	1	1		
Range	0.25–2	0.25–2		
MIC_{50}	1	1		
MIC_{90}	1	2		

[a] GM, geometric mean.
[b] Standard inoculum, 10^5 CFU/ml.
[c] High inoculum, 10^7 CFU/ml.
[d] Isolates exhibiting ≥4-fold increase in cefazolin MIC between standard and high inocula.
[e] Isolates exhibiting ≥4-fold increase in cefazolin MIC between standard and high inocula that resulted in a nonsusceptible cefazolin MIC (≥16 μg/ml) at the high inoculum.

of saline. All broth microdilution plates were read by two members of the study staff, and current CLSI susceptibility breakpoints were used. For cefazolin, an MIC of ≤8 μg/ml was considered susceptible, 16 was intermediate, and ≥32 was resistant; for nafcillin, an MIC of ≤2 μg/ml was considered susceptible and ≥4 was resistant. It was common for bacterial pellets to be observed in all wells when performing broth microdilution testing using high bacterial inocula. When this occurred, the lowest antibiotic concentration at which a significant reduction of visible turbidity was noted and beyond which the bacterial pellet size remained constant despite increasing antibiotic concentrations was designated the MIC endpoint. To validate the endpoints, serial dilution colony counts plated on Trypticase soy agar with 5% sheep blood (Becton, Dickinson and Company, Sparks, MD) were performed from a sample of wells containing antibiotic concentrations higher than the MIC endpoint and demonstrating stable bacterial pellets. A 99.9% reduction in colony counts from all test wells beyond the MIC endpoint was noted, compared to that from the control well containing no antibiotics (equivalent to ≥3 $\log_{10}$ drop in CFU/ml).

All 185 isolates were susceptible to nafcillin and cefazolin at the standard inocula. Using a high inoculum, eight (4%) isolates were nonsusceptible to cefazolin. The mean, geometric mean, mode, range, MIC_{50}, and MIC_{90} to cefazolin by *blaZ* type are shown in Table 2. Cefazolin, but not nafcillin, MICs at high inocula differed according to the presence and type of *blaZ* (Table 2). Cefazolin MICs of the type A strains determined using a high inoculum were significantly higher than those of type C strains, the *blaZ* type with the next highest MICs (mean MICs of 7.6 versus 3.2 μg/ml; $P < 0.01$). The inoculum effect was defined as a ≥4-fold increase in MIC from a standard to a high inoculum. A total of 50 (27%) isolates demonstrated the inoculum effect: 22 (46%) type A, 3 (7%) type B, 22 (45%) type C, and 3 (7%) negative for *blaZ* (Table 2). Of those isolates demonstrating an inoculum effect, 8 (17%) isolates were nonsusceptible to cefazolin (4 with MICs of 16 μg/ml, 4 with MICs of 32 μg/ml). All 8 of these isolates carried the type A *blaZ* gene.

To determine the impact of *blaZ* and the inoculum effect on clinical outcomes, medical records were reviewed retrospectively up to 12 weeks after the index blood culture for all 185 cases. Excluding 36 cases without follow-up, 41 of 149 (28%) patients

died. A total of 26 (63%) deaths occurred during the initial hospitalization, and 15 (37%) occurred after hospital discharge. The overall mortality rate was 11% for children and 31% for adults. Among the 108 patients who survived and had follow-ups at 12 weeks, recurrent MSSA bacteremia occurred in 13 (12%) cases. Of the 13 recurrences, 8 had both the index and recurrent isolates available for characterization by PFGE: 6 of the recurrent isolates were identical to the index isolate, and 2 were 80 to 85% similar to the index isolate.

Use of a β-lactam antibiotic instead of vancomycin for >50% of the treatment course was protective against treatment failure (odds ratio [OR], 0.21; 95% confidence interval [95% CI], 0.06 to 0.80). Cases who received cefazolin for >50% of the treatment course were more likely to have treatment failure than those who received nafcillin (12 g continuous infusion every 24 h), but the difference was not significant (OR, 2.07; 95% CI, 0.35 to 12.27).

There were 11 patients infected with type A *blaZ* strains who were treated with cefazolin for >50% of the treatment course. Three patients were lost to follow-up, and one patient died after hospital discharge. There were no treatment failures in the remaining seven patients, who were treated with either 2 g of cefazolin intravenously (i.v.) every 8 h (four cases with a CrCl >30 ml/min) or 2 g of cefazolin i.v. 3 times per week after hemodialysis (three cases with end-stage renal disease [ESRD]). Two of these seven cases demonstrated the inoculum effect, and both were without failure at 12 weeks: one case had vertebral osteomyelitis, and one case had an arteriovenous graft infection, which was excised. All seven patients were treated with vancomycin initially, which may have reduced the inoculum size to such a degree that the inoculum effect was no longer clinically relevant.

The reasons some strains demonstrate the inoculum effect and others do not may be severalfold. The type of β-lactamase is important, as nonsusceptible cefazolin MICs were seen only with type A strains in this cohort. In addition, the level of β-lactamase production has been shown to influence the inoculum effect (4) by differential expression of the *blaZ* gene (5, 11) and/or *blaZ* gene dosage (21). For *blaZ* types A, C, and D, which are all located on plasmids, multiple copies of the same gene could be carried within the same bacterium.

Compared to a recent report, this cohort demonstrated a lower prevalence of both *blaZ*-positive strains (87% versus 77%) and the inoculum effect (19% versus 4%) (19). The prevalence of *blaZ* and the inoculum effect may vary across geographic regions. Older studies using different methods of surveillance have also reported different rates, suggesting that the prevalence of these factors is not static over time and may vary by body site (12, 13, 26). Furthermore, we noted the occurrence of trailing endpoints in some strains when using a high inoculum (3, 7). Since there is no standardized definition for this phenomenon, the interpretation of trailing endpoints may vary across studies.

We found the *blaZ* gene to be common among our MSSA bloodstream isolates. However, the clinical impact of β-lactamase production, the type of *blaZ* gene, and the inoculum effect is unclear. In animal studies, higher mortality rates were seen when infections due to β-lactamase-producing strains were treated with cefazolin (4, 10). Data from human studies are more variable (19, 25). A retrospective propensity score-matched case-control study found no difference in the clinical efficacy of cefazolin and nafcillin for MSSA bacteremias, but the prevalence of *blaZ* and the inoculum effect were not described (15).

The inoculum effect is likely to be most relevant when cefazolin is used to treat complicated MSSA infections with high densities of bacteria. Further research is needed to define the prevalence of the inoculum effect and assess the efficacy of cefazolin when present.

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