Cefazolin inoculum effect predicts reduced susceptibility to other antibiotics and patient outcomes in MSSA endovascular infections

School of Pharmacy
UNIVERSITY OF WISCONSIN–MADISON

Dan F. Smelter, Sue McCrone, Warren E. Rose
University of Wisconsin-Madison School of Pharmacy

Background

- *MSSA* Infective endocarditis (IE) is inherently a high-burden infection with a mortality rate around 30%.
- Cefazolin is an appealing treatment option for IE with low toxicity and a favorable dosing interval. However, cefazolin has been associated with treatment failure in IE, likely attributable to a high-inoculum effect.
- The specific mechanism underlying the cefazolin inoculum effect (CIE) remains undetermined, but CIE has been linked to both *blaZ* expression and *agr* dysfunction.
- There are currently no published data focusing on the prevalence of CIE in IE and its effect on patient outcomes.
- The first-generation cephalosporin, cefazolin, and the penicillin antibiotic, nafcillin, exert bactericidal activity through similar mechanisms, though nafcillin is more resistant to beta-lactamases. Vancomycin is a glycopeptide antibiotic that inhibits cell wall synthesis through an alternative mechanism and is commonly used to treat MRSA.
- This study aims to determine whether CIE is linked to reduced susceptibility to other antibiotics and worse outcomes regardless of therapy in MSSA endovascular infections.

Hypothesis

We hypothesized that *in vitro* presence of the cefazolin inoculum effect in MSSA infective endocarditis correlates to reduced susceptibility to other antibiotics and is linked to worse patient outcomes.

Methods

Strains: 70 *MSSA* strains from patients with vascular infections not treated with cefazolin - 25 endovascular and 45 infective endocarditis
MICs determined for cefazolin, nafcillin, and vancomycin
- High-inocula (HI): 10^7 CFU/mL
- Standard-inocula (SI): 10^5 CFU/mL

Definitions of inoculum effect:
- CIE: ≥4x increase in MIC at HI compared to SI, with MIC of ≥4 mg/L at HI
- Nafcillin inoculum effect (Naf IE): MIC at HI of ≥4 mg/L with ≥4x increase in MIC from SI to HI
- Vancomycin inoculum effect (Van IE): MIC ≥4 mg/L at HI

Nitrocefin disks visualize presence of beta-lactamases, indicative of *blaZ* expression, illustrating increasing activity from left to right (left). Beta lysin disks on sheep blood agar visualize hemolytic activity, indicative of *agr* function (right). Accessory gene regulator (*agr*) is a determinant of virulence in *S. aureus* and dysfunction is linked to strong biofilm formation and drug resistance.

Results

Cefazolin Minimal Inhibitory Concentration

To determine the prevalence of CIE, MIC values were determined using microtiter dilution at varying inocula. A year-matched cohort of 85 MSSA strains from non-vascular sources was used to compare the prevalence. 26 of the 70 (37%) of the intravascular-sourced MSSA strains exhibit CIE. This rate is not significantly different than a cohort of year-matched MSSA strains from non-vascular sources with CIE in 31 of 85 strains (36%). This rate is consistent with rates published by other groups.

	CIE positive	MIC ≥ 32 mg/L at HI
Endovascular	12/25 (48%)	6/12 (50%)
Infective Endocarditis	14/45 (31%)	5/14 (36%)
Total Vascular	26/70 (37%)	11/26 (42%)
Non-vascular	31/85 (36%)	14/31 (45%)

Minimal Inhibitory Concentration Across Antibiotics

To determine the whether the presence of CIE is indicative of inoculum effects for reduced susceptibilities of other antibiotics at increasing CFU/mL, MIC values were determined using microtiter dilution assays.
Of the 70 vascular strains characterized, 37% were positive for CIE, 16% showed reduced susceptibility to nafcillin at HI, and 50% had reduced susceptibility to vancomycin at HI.

	Prevalence of inoculum effect
Cefazolin	26/70 (37%)
Nafcillin	11/70 (16%)
Vancomycin	35/70 (50%)

Interestingly, there was a trending correlation between CIE and presence of an inoculum effect for other antibiotics. For Naf IE, only 5 of the 11 with reduced susceptibility at HI also showed CIE. For Van IE, only 10 of the 35 strains with an inoculum effect also exhibit CIE.

An inoculum effect is not indicative of *agr* function

MSSA Source	Strains with *agr* dysfunction
Endovascular	7/25 (28%)
Infective Endocarditis	12/45 (27%)

	Strains with *agr* dysfunction
CIE positive	9/26 (35%)
CIE negative	10/44 (23%)
Naf IE positive	3/11 (27%)
Naf IE negative	16/59 (27%)
Van IE positive	7/35 (20%)
Van IE negative	12/35 (34%)

Playing a role in quorum sensing and virulence, *agr* dysfunction is believed to confer advantage to *S. aureus* in the hospital setting. It has been linked to CIE and increased persistence. Our data suggests that there is no correlation between decreased susceptibility to antibiotics at higher inocula and *agr* dysfunction.

Beta-lactamase expression correlates with CIE

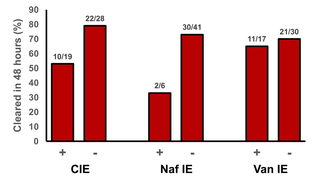
MSSA Source	Strains with *blaZ* expression
Endovascular	14/25 (56%)
Infective Endocarditis	17/45 (38%)

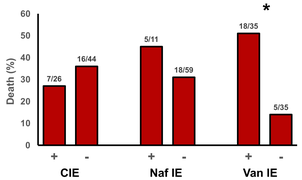
	Strains with *blaZ* espression
CIE positive	**19/26 (73%)**
CIE negative	**12/44 (27%)**
Naf IE positive	6/11 (55%)
Naf IE negative	25/59 (42%)
Van IE positive	11/35 (31%)
Van IE negative	20/35 (57%)

Using nitrocefin disks to assay the activity of beta-lactamase, indicating expression of *blaZ*, our data show that more endovascular sourced MSSA strains exhibit beta-lactamase activity compared to infective endocarditis strains. Presence of CIE and *blaZ* expression are linked (P = 0.0004). Interestingly, Van IE trends towards a negative correlation with *blaZ* expression (P = 0.05).

CIE vs. Van IE as a predictor of bacteremia duration and mortality

MSSA Source	Cleared within 48hrs	Death
Endovascular	12/19 (63%)	6/25 (24%)
Infective Endocarditis	20/28 (71%)	17/45 (38%)





Patients with IE had a death rate of 38%, while endovascular infections had fewer deaths at 24%.

Using the recently defined cutoff of 48 hours for persistent bacteremia, there was no difference in persistence between endovascular infections and IE. Interestingly, while CIE was associated with increased persistence, presence of the Van IE significantly correlated to increased mortality (P = 0.0019). Patients that died before posting a negative blood culture were removed from the analysis of infection duration.

Conclusions

- This is one of the largest studies of the prevalence and effect on outcomes of CIE in MSSA infective endocarditis and endovascular infections to date.
- Our data suggest that CIE is present in a minority of cases (37%) and is strongly associated with *BlaZ* expression and may not be linked to *agr* dysfunction.
- While nearly all patients were treated with oxacillin, our data show that CIE presence is linked to a longer time to clearance, but with a potentially reduced mortality rate.
- This study provides insight into the viability of cefazolin as a treatment option for IE.
- Further studies are needed to explore the role of a vancomycin inoculum effect in MSSA.

This work was supported by the National Institutes of Health (1R01AI132627 to WER) and the American Foundation for Pharmaceutical Education (DFS).