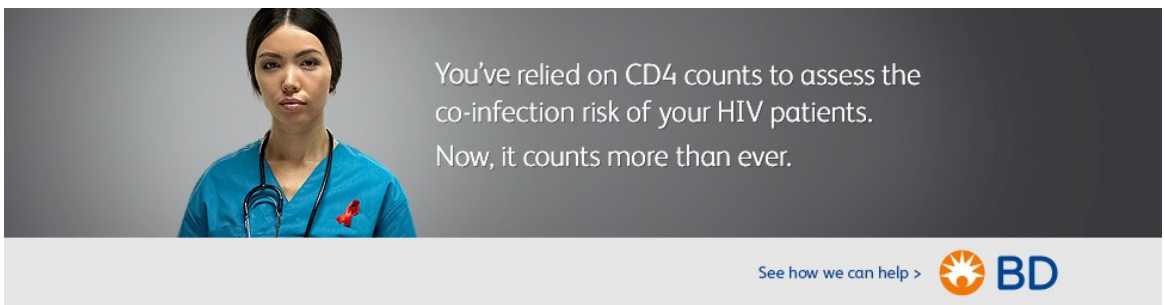
Cefazolin and Ertapenem Salvage Therapy Rapidly Clears Persistent Methicillin-Susceptible Staphylococcus aureus Bacteremia

Erlinda R Ulloa, Kavindra V Singh, Matthew Geriak, Fadi Haddad, Barbara E Murray, Victor Nizet, George Sakoulas



Downloaded from https://academic.oup.com/cid/article-abstract/doi/10.1093/cid/ciz995/5644098 by University of Edinburgh user on 28 July 2020

Clinical Infectious Diseases

MAJOR ARTICLE

Cefazolin and Ertapenem Salvage Therapy Rapidly Clears Persistent Methicillin-Susceptible *Staphylococcus aureus* Bacteremia

Erlinda R. Ulloa,[1,2] Kavindra V. Singh,[3,4] Matthew Geriak,[5] Fadi Haddad,[6] Barbara E. Murray,[3,4] Victor Nizet,[1,7] and George Sakoulas[1,5]

[1]Collaborative to Halt Antibiotic-Resistant Microbes, Department of Pediatrics, University of California, San Diego School of Medicine, La Jolla, California, USA, [2]Division of Infectious Disease, Department of Pediatrics, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, USA, [3]Division of Infectious Diseases, Department of Internal Medicine, McGovern Medical School, University of Texas Health Science Center at Houston, Houston, Texas, USA, [4]Center for Antimicrobial Resistance and Microbial Genomics, McGovern Medical School, University of Texas Health Science Center at Houston, Houston, Texas, USA, [5]Sharp Memorial Hospital, San Diego, California, USA, [6]Sharp Grossmont Hospital, La Mesa, California, USA, and [7]Skaggs School of Pharmacy, University of California, San Diego, La Jolla, California, USA

Cefazolin and ertapenem combination therapy was used successfully to salvage 11 cases (6 endocarditis) of persistent methicillin-susceptible *Staphylococcus aureus* (MSSA) bacteremia, including immediate clearance (≤24 hours) in 8 cases. While in vitro synergy was modest, cefazolin plus ertapenem exhibited synergistic action in a rat model of MSSA endocarditis. The combination of cefazolin and ertapenem provides potent in vivo activity against MSSA beyond what is predicted in vitro and warrants further clinical study in the treatment of refractory MSSA bacteremia and endocarditis.

Keywords. *Staphylococcus aureus*; bacteremia; endocarditis; cefazolin; ertapenem.

Staphylococcus aureus is a major cause of bacteremia, causing significant morbidity and mortality in high-risk patients [1, 2]. Management of persistent methicillin-susceptible *Staphylococcus aureus* (MSSA) bacteremia is grounded in surgical source control and early initiation of β-lactam therapy with classical antistaphylococcal agents (eg, oxacillin, nafcillin, flucloxacillin) or cefazolin [3]. While the latter is being increasingly utilized, infections with high MSSA inoculum, as in infective endocarditis, have been associated with clinical treatment failure in patients receiving cefazolin [4–7]. Although suboptimal therapy against MSSA may have catastrophic clinical consequences, currently there is no guidance on optimal treatment regimens for high-inoculum MSSA infections refractory to standard treatment regimens.

We previously described that adding ertapenem to cefazolin led to in vitro and in vivo synergy against an MSSA bloodstream isolate from a patient for whom this drug combination was used successfully in salvage therapy for persistent bacteremia without a surgical focus [8]. We have continued to experience high success with this salvage regimen for refractory MSSA bacteremia. Here we report our clinical experience using cefazolin plus ertapenem as salvage therapy for 11 cases with refractory MSSA bacteremia, and explore this combination therapy in vitro and in an established rat model of endocarditis.

METHODS

Patient Cases

Patients with persistent MSSA bacteremia were identified through treating physicians (authors G. S. and F. H.), and the following data were collected retrospectively: patient age, source of bacteremia, duration of bacteremia, hematology and chemistry laboratory tests, and prior antibiotics administered that failed to clear bacteremia. Of the 11 patients in the case report, 9 had daily blood cultures as part of their routine clinical management, which allowed for bacteremia duration assessment. For 2 of the patients, blood cultures were separated by 3 days from the time of the last positive to first negative, such that specific bacteremia duration could not be measured. These details are provided in the clinical summary (Table 1). Note that all patients treated with ertapenem plus cefazolin were included and no patients were excluded from the case report. Expedited approval for data collection was granted by the Sharp Healthcare Internal Review Board.

Bacterial Strains and In Vitro Assays

MSSA isolates (isolated and identified by the clinical microbiology laboratory via routine workup of clinical specimens via MicroScan) from the initial blood culture were obtained from 6 clinical cases and evaluated for in vitro susceptibility to cefazolin, ertapenem, and nafcillin under both standard Clinical and Laboratory Standards Institute (10^5 colony-forming units

Received 20 May 2019; editorial decision 27 September 2019; accepted 20 October 2019; published online November 27, 2019.

Correspondence: E. R. Ulloa, Collaborative to Halt Antibiotic-Resistant Microbes (CHARM), UC San Diego School of Medicine, Biomedical Research Facility II, 9500 Gilman Drive, Mail Code 0760, La Jolla, CA 92093-0760 (erulloa@ucsd.edu).

Clinical Infectious Diseases® 2019;XX(XX):1–6

© The Author(s) 2019. Published by Oxford University Press for the Infectious Diseases Society of America. All rights reserved. For permissions, e-mail: journals.permissions@oup.com.

DOI: 10.1093/cid/ciz995

Downloaded from https://academic.oup.com/cid/article-abstract/doi/10.1093/cid/ciz995/5644098 by University of Edinburgh user on 28 July 2020

Table 1. Use of Cefazolin Plus Ertapenem as Salvage Therapy for Persistent Methicillin-Susceptible *Staphylococcus aureus* Bacteremia

Case No.	Age/Sex	Prior Therapies (No. of Days)	Days of Bacteremia: Prior to ETP/CZ	Days of Bacteremia: After ETP/CZ	Source(s)	Comorbidities	IL-10, pg/mL	ICU	Comments
1	70s/F	PIP-TAZ (1) CZ (5)	6	0	Osteomyelitis Epidural abscess Lumbar spine	CHF/atrial fibrillation DM 2 COPD Obesity Eczema	5	No	Bedridden, morbid obesity, limited workup. This case was previously published [8].
2	60s/F	PIP-TAZ (1) Nafcillin (3)	4	0	AV fistula Pneumonia	Renal transplant DM 2	9	Yes	AV fistula placed 2 weeks prior.
3	20s/F	CZ (4 + 5)[a]	16 9 on antibiotics	0	Tricuspid valve 2 × 2 cm vegetation	IVDU	Not done	No	Vegetation grew to 2.5 × 2.3 cm, but improved clinically to surgical candidacy (initially declined due to poor medical condition). Tricuspid valve repair 10 days after bacteremia clearance.
4	30s/M	Vancomycin + ceftriaxone (1), nafcillin (3)	4	0	Triscuspid valve, 2.3 × 3.3 cm and 1.2 × 1.4 cm vegetations	IVDU	14	Yes	AngioVac 3 days after bacteremia clearance. Partial success in vegetation debulking with 1.9 × 2 cm residual vegetation. Signed out against medical advice 25 days after bacteremia clearance.
5	50s/F	Vancomycin + ceftriaxone (1), nafcillin (4)	5	0	Psoas and L3–L1 epidural abscess	None	7	No	48 hours into bacteremia developed neck pain, found to have C5–C7 cervical pachymeningitis/discitis; vulvar abscess.
6	60s/M	Vancomycin + PIP-TAZ (1), nafcillin (4)	5	2	Cervical paraspinous abscess and thigh abscess	DM 2 (HbA1C = 12 mg/dL)	6	No	Secondary suppurative pericarditis 2 weeks into treatment.
7	30s/M	Vancomycin + PIP-TAZ (1) Vancomycin + ceftriaxone (1), nafcillin (5)	7	≤3[b]	Aortic valve Meningitis	IVDU	Not done	Yes	Cardioembolic shower with multiple septic emboli to brain. Cardiogenic shock with heart failure. Aortic valve replacement on hospital day 23.
8	30s/M	Vancomycin + PIP-TAZ (1) Vancomycin + ceftriaxone (1), nafcillin (3) Nafcillin + rifampin (3)	8	0	Tricuspid valve	IVDU Hepatitis C	Not done	No	Multiple septic pulmonary emboli. Empyema requiring decortication.
9	70s/F	Nafcillin (5)	6	0	Sacroiliac joint	ESRD DM 2	7	No	ESRD patient admitted with uremia to initiate hemodialysis via AV fistula. Patient developed right hip/buttock pain and leukocytosis but remained afebrile and was discharged. She was called back to the hospital for positive blood cultures.
10	70s/F	Vancomycin + ceftriaxone (4), CZ (2)	6	≤3[b]	Mitral valve Aortic valve	Hypertension Hyperlipidemia	Not done	Yes	Multiple brain emboli, intracranial hemorrhage, lumbar discitis, and osteomyelitis.
11	60s/M	Vancomycin + PIP-TAZ (2) CZ + gentamicin + rifampin (4)	6	0	Aortic valve 1.3 × 1 and 1 × 1 cm vegetation Aortic graft infection	Hypertension Atrial fibrillation Seizures Aortic aneurysm repair Cerebral aneurysm clipping Prior rectal cancer	Not done	No	Hospital day 8, patient underwent sternotomy, hemiarch and ascending aorta repair, aortic valve replacement (Edwards Magna), mediastinal debridement. Negative blood cultures 48 hours preoperatively on CZ plus ETP. Intraoperative tissue cultures + MSSA. Postoperative reinitiated CZ, gentamicin, and rifampin.

Abbreviations: AV, arteriovenous; CHF, congestive heart failure; COPD, chronic obstructive pulmonary disease; CZ, cefazolin; DM2, type 2 diabetes mellitus; ESRD, end-stage renal disease; ETP, ertapenem; F, female; HbA1C, hemoglobin A1C; ICU, intensive care unit; IL-10, interleukin 10; IVDU, intravenous drug use; M, male; MSSA, methicillin-susceptible *Staphylococcus aureus*; PIP-TAZ, piperacillin-tazobactam.

[a]Patient eloped after 5 days, despite positive blood cultures, but returned to the hospital on day 7 with ongoing bacteremia.

[b]Follow-up blood cultures were obtained 3 days after CZ plus ETP salvage therapy.

Downloaded from https://academic.oup.com/cid/article-abstract/doi/10.1093/cid/ciz995/5644098 by University of Edinburgh user on 28 July 2020

[CFU]/mL) and high-inoculum (10^7 CFU/mL) conditions using both standard Mueller-Hinton broth (MHB) bacteriological media and Roswell Park Memorial Institute (RPMI) physiological cell culture media supplemented with 5% Luria-Bertani (LB) broth [9]. Checkerboard assays were also performed using standard and high inoculums in MHB [10]. For kill curve studies, bacteria were grown overnight in Todd-Hewitt broth at 37°C with shaking to stationary phase and diluted in MHB or RPMI + 5% LB to an optical density at 600 nm = 0.40. Cultures were diluted in MHB or RPMI + 5% LB to an initial inoculum of 1×10^7 CFU/mL. Antibiotics were added at one-fourth the MIC, and tubes were placed in a shaking incubator at 37°C. Aliquots were collected at 6 hours and 24 hours and serially diluted for CFU enumeration. These data were collected from at least 3 biological replicates performed in at least technical triplicate. All antibiotics were purchased from the Sharp Memorial Hospital pharmacy (San Diego, California), supplied as vials available for clinical use and administration to patients.

Disk diffusion synergy assays between cefazolin and ertapenem were performed as previously described [8]. In brief, a bacterial suspension of 0.5 McFarland (10^8 CFU/mL) was streaked as a lawn on brain-heart infusion agar plates. A cefazolin or ertapenem disk was placed in the center of the plate and was replaced with a fresh cefazolin disk 1 hour later. Diameter of the zone of inhibition was measured after incubation at 37°C for 24 hours. Synergy was defined as >3-mm increase in zone size when sequential disks of different agents were used, as compared to a single antimicrobial disk. Disks were purchased from Hardy Diagnostics (Santa Maria, California).

Rat Aortic Valve Endocarditis Model

The well-characterized MSSA strain TX0117 (harboring a type A β-lactamase and exhibiting a cefazolin inoculum effect) [4, 11] was studied in an established model of endocarditis using male Sprague-Dawley rats (weight ~200 g) [4, 11, 12]. Thirty hours after bacterial inoculation, therapy was started with ertapenem 30 mg/kg SC every 8 hours, cefazolin 50 mg/kg intramuscularly every 8 hours, or a combination of cefazolin plus ertapenem at the above doses and intervals. Dosages were selected based on prior use of this model to assess antimicrobial therapeutics (authors K. V. S., B. E. M.). Antibiotic therapy was administered for 3 days, animals were killed approximately 15 hours after the last antibiotic dose, and vegetations formed on the aortic valve and surrounding tissues were aseptically removed, weighed, and homogenized in 1 mL of 0.9% saline solution. Sequential dilutions of the homogenized tissues were carried out and subsequently, the entire volume of each dilution (including the undiluted sample) was plated onto BHI agar. The geometric mean $\log_{10}$ CFU/g and standard deviations were calculated from colonies recovered from vegetations, and treatment groups were compared to untreated controls. Animals were included in the final analysis only if the catheters were found across the aortic valve in the left ventricle, and only rats that survived beyond the first 24 hours of therapy were included in the treatment group. The minimum detection limit of bacteria by this method was 10 CFU/g of tissue. Results were analyzed as previously described [4, 11, 12].

Statistical Analyses

Statistical analyses were performed using GraphPad Prism, version 7.0d. *P* values <.05 were considered significant.

RESULTS

A total of 11 cases of persistent MSSA bacteremia were successfully cleared with cefazolin plus ertapenem combination therapy (Table 1). All isolates were methicillin susceptible per the clinical microbiology laboratory. The first case listed in Table 1 is from the previous case report [8]. Median duration of bacteremia was 6 days (range, 4–9 days) while on antibiotics prior to cefazolin plus ertapenem salvage therapy. Infective endocarditis was definitively identified by echocardiography in 6 of these cases, including 2 cases of tricuspid valve endocarditis where cardiac vegetations were ≥2 cm in size. Remarkably, in these 2 cases, bacteremia cleared within 24 hours after the initiation of the salvage regimen. Among the 9 cases where blood cultures were drawn daily, bacteremia cleared within 24 hours in 8 cases (88%). In 2 cases, blood cultures were not obtained until day 3 of salvage therapy, so it was not possible to define the exact duration of bacteremia. While the patients included in this case report were not enrolled in a clinical trial for which outcome metrics were prespecified, all patients survived to hospital discharge.

In vitro assessment of cefazolin, ertapenem, and nafcillin activity for the 6 available isolates is shown in Table 2. Three of the 6 isolates exhibited a significant inoculum effect with cefazolin, with MIC >3 dilutions higher when susceptibility testing was done using 10^7 CFU/mL vs 10^5 CFU/mL (Table 2). The high-inoculum cefazolin MICs ranged from 8 mg/L to 32 mg/L. Nafcillin and ertapenem showed no inoculum effect.

Checkerboard testing revealed general additivity with some synergy between ertapenem and cefazolin or nafcillin based on fractional inhibitory concentration index calculations (Table 2). Disk diffusion assays compared zones of inhibition using a cefazolin disk with or without agar priming by prior placement of an ertapenem disk for 1 hour (Table 2). All the isolates showed at least a 4-mm increase in cefazolin inhibition zone with ertapenem priming.

We and others have recently appreciated that susceptibility testing results obtained in bicarbonate-buffered bacteriological media or physiologically relevant tissue culture–based media can be more reflective of antibiotic activity in vivo [13–15]. Therefore, susceptibility testing for cefazolin, nafcillin, and ertapenem was also performed in RPMI media supplemented with 5% LB under both standard and high inoculum

Downloaded from https://academic.oup.com/cid/article-abstract/doi/10.1093/cid/ciz995/5644098 by University of Edinburgh user on 28 July 2020

Table 2. In Vitro Studies in Mueller-Hinton Broth Under Low or High Inocula Against Nafcillin, Cefazolin, and Ertapenem From Methicillin-Susceptible *Staphylococcus aureus* Obtained From 6 Clinical Cases

	MIC, mg/L						Checkerboard, FICI				Disk Diffusion, mm			
	NAF		CZ		ETP		CZ + ETP		NAF + ETP		ETP	CZ	ETP→CZ	Δ
Case	10^5	10^7	10^5	10^7	10^5	10^7	10^5	10^7	10^5	10^7				
1	0.25	0.50	0.25	1	0.06	0.06	0.56	**0.50**	0.56	0.75	30	27	32	+5
2	0.50	1	1	**16**	0.13	0.25	**0.50**	**0.38**	0.53	**0.50**	29	24	29	+5
3	0.25	0.50	0.50	**8**	0.06	0.06	0.75	0.56	**0.38**	0.56	29	26	29	+3
4	0.25	0.50	0.25	1	0.06	0.06	**0.50**	0.63	0.63	0.56	30	28	32	+4
5	0.25	0.50	0.50	**32**	0.03	0.06	0.75	0.53	0.50	0.63	30	24	28	+4
6	0.25	0.25	0.25	0.50	0.03	0.06	1	0.63	0.75	0.63	30	30	34	+4

Data in bold indicate isolates that exhibited an inoculum effect with cefazolin based on MIC; or synergy between cefazolin or nafcillin + ertapenem based on checkerboard, FICI. FICIs were interpreted as follows: synergy, FICI of ≤0.50; additivity, FICI of >0.50 to ≤1.0; no interaction (indifference), FICI of >1 to ≤4; antagonism, FICI of >4. ETP→CZ: ertapenem disk placed for 1 hour, then replaced by CZ disk for overnight. This was compared to CZ where a blank disk was placed for an hour and replaced with CZ for overnight incubation. The difference (Δin zone size between CZ and ETP→CZ was measured (mm) and synergy defined as >3 mm.

Abbreviations: CZ, cefazolin; ETP, ertapenem; FICI, fractional inhibitory concentration indices; MIC, minimum inhibitory concentration; NAF, nafcillin.

conditions (Table 3). For cefazolin and nafcillin, MICs obtained in RPMI + 5% LB under standard inoculum conditions were similar to results obtained in standard MHB. Isolates from cases 2 and 5 showed 8-fold decreased cefazolin MIC in RPMI + 5% LB compared to MHB when tested under high inoculum. Only the MSSA isolate from case 5, which had a very high cefazolin MIC (32 mg/L) in MHB and high bacterial inoculum, showed a significant inoculum effect in RPMI + 5% LB (cefazolin MIC 4 mg/L). However, much higher ertapenem MICs were seen for all of isolates under both standard and high inoculum testing in RPMI + 5% LB media. Using standard bacterial inocula, the ertapenem MICs observed in RPMI + 5% LB were 15–133 times higher than in MHB.

To simulate the most challenging high-inoculum MSSA infections, kill curves were performed at a starting inoculum of 10^7 CFU/mL in addition to standard inoculum (10^5 CFU/mL) with ertapenem and cefazolin, alone or in combination, against clinical strains from cases 1–6 listed in Table 1. Results are shown in Supplementary Figure 1, with data from experiments in MHB on the left and those done in RPMI + 5% LB on the right. In both MHB and RPMI + 5% LB media, ertapenem and cefazolin provided heterogenous results across the 6 strains, with some strains showing synerg (≥2 $\log_{10}$ killing of the combination as compared to the most active antibiotic alone) [16] at the 24-hour time point while others did not. Most strains showed regrowth at 24 hours. Two of the strains were also examined in a similar fashion using ertapenem and nafcillin, alone or in combination in both MHB and RPMI + 5% LB (Supplementary Figure 2). Both strains showed synergism with the combination of ertapenem plus nafcillin in RPMI + 5% LB, with approximately 2 $\log_{10}$ killing at 24 hours but only at high inoculums (10^7 CFU/mL).

The results of cefazolin and ertapenem, alone and in combination, in the endocarditis model are shown in Figure 1. In the presence of ertapenem, the bacterial inoculum in all but 1

Table 3. Minimum Inhibitory Concentration Under Low and High Inocula for Nafcillin, Cefazolin, and Ertapenem Across the 6 Clinical Isolates of Methicillin-Susceptible *Staphylococcus aureus* Used in This Study in Roswell Park Memorial Institute 1640 Tissue Culture Medium Supplemented With 5% Luria-Bertani Broth

	MIC, mg/L					
	Nafcillin		Cefazolin		Ertapenem	
Case	10^5	10^7	10^5	10^7	10^5	10^7
1	0.13	0.25	0.25	1	4	2
2	0.25	0.50	0.50	2	2	4
3	0.25	0.50	1	4	4	2
4	0.25	0.25	0.50	1	2	2
5	0.13	0.13	0.25	**4**	4	4
6	0.13	0.25	0.25	1	4	1

Data in bold indicate isolates that exhibited an inoculum effect with cefazolin based on MIC.

Abbreviation: MIC, minimum inhibitory concentration.

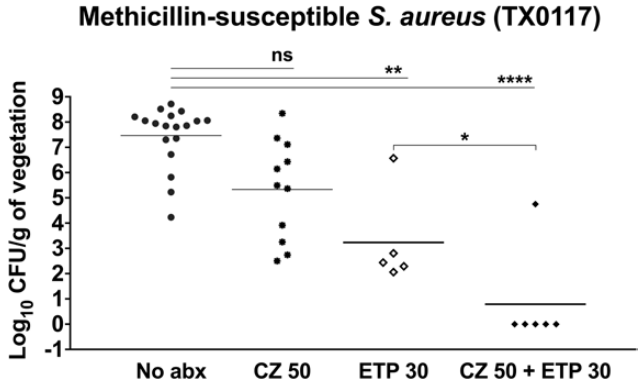


Figure 1. Efficacy of antibiotic therapy in a rat endocarditis model of infection with methicillin-susceptible *Staphylococcus aureus* TX0117. The results of therapy with cefazolin, ertapenem, and cefazolin-ertapenem for TX0117-infected rats are shown. Horizontal bars represent the geometric mean colony-forming unit titers. No antibiotics, filled circles; cefazolin, filled asterisks; ertapenem, open diamonds; cefazolin + ertapenem, closed diamonds. *P < .05, by 2-tailed Mann-Whitney test. **P = .005, ****P < .0001, by Kruskal-Wallis 1-way analysis of variance. Abbreviations: abx, antibiotics; CFU, colony-forming units; CZ, cefazolin; ETP, ertapenem; ns, not significant.

Downloaded from https://academic.oup.com/cid/article-abstract/doi/10.1093/cid/ciz995/5644098 by University of Edinburgh user on 28 July 2020

valve was reduced below the limit of detection. The addition of cefazolin to ertapenem trended toward increased activity ($P < .0001$) compared to cefazolin ($P = .0591$) or ertapenem ($P = .005$) alone. Additionally, the results achieved statistical significance when the ertapenem treatment group was compared to the cefazolin plus ertapenem condition ($P < .05$).

DISCUSSION

Cefazolin has been increasingly used and recommended for treatment of serious MSSA infections such as bacteremia, endocarditis, and osteomyelitis. In fact, recent studies have shown increased tolerability and perhaps better outcomes in patients receiving cefazolin compared to classical antistaphylococcal β-lactams such as oxacillin and nafcillin [17–19]. These favorable data for cefazolin are somewhat limited as they were retrospectively collected and, therefore, vulnerable to bias (eg, higher-risk patients may be biased toward receiving antistaphylococcal β-lactams over cefazolin). Yet, they do speak to the more favorable drug tolerability of cefazolin, particularly with longer treatment durations of >4 weeks called upon by these more serious infections, especially in the elderly. For example, myelosuppression and acute kidney injury are much less common with cefazolin than with the classical antistaphylococcal β-lactams [17–19]. However, the inferior activity of cefazolin under high inoculum conditions against some MSSA (eg, type A β-lactamase–producing strains) raises concern for treatment failure, which has been documented in the literature [4–7]. High-level population data may not highlight individual cases caused by MSSA exhibiting the cefazolin inoculum effect if such isolates are not very common, and recent studies suggest that these isolates may be common in some settings but quite rare in other hospital centers [20–23]. *Staphylococcus aureus* inoculum effects have recently been shown for additional β-lactam drugs, including β-lactam/β-lactamase inhibitor combinations [24]. Suboptimal antimicrobial therapy against MSSA may have catastrophic clinical consequences in very severe infections such as endocarditis or epidural abscess. It is noteworthy that 3 of the 6 MSSA isolates examined in this study showed a cefazolin inoculum effect, although only case 5 showed a cefazolin inoculum effect in RPMI + 5% LB media. We highlight that the patient from this case experienced overt clinical failure requiring readmission to the hospital a few days after ertapenem was discontinued and cefazolin remained as monotherapy. Whether clinically significant cefazolin inoculum effects warrant testing in physiological media such as RPMI is a potential topic for future study, especially if cefazolin comes to replace classical antistaphylococcal β-lactams as the treatment of choice for complex MSSA infections.

In this study, we have shown that cefazolin plus ertapenem combination salvage therapy resulted in rapid MSSA bacteremia clearance in patients failing standard monotherapy, even in cases with large-burden endovascular infections on echocardiogram. A modest synergy or additivity of cefazolin plus ertapenem against MSSA was observed in vitro utilizing both bacteriologic (MHB) and physiologic (RPMI) media. Considerable discordance was seen between in vitro synergy testing by the disk diffusion, checkerboard, and time-kill assays, overall raising questions as to how clinically relevant results of these assays are in assessing what appears to be a strong synergy between cefazolin and ertapenem in vivo, corroborated by significant synergy in the rat model of endocarditis. Based on these cumulative results, cefazolin plus ertapenem appears to offer a viable salvage regimen option in patients with MSSA bacteremia refractory to standard β-lactam therapy, provided appropriate surgical source control has been performed. While the clinical data and the rat endocarditis model data obtained with 1 bacterial strain support the hypothesis that ertapenem plus cefazolin combination has greater efficacy, the in vitro data were less convincing, with limited correlation between strains or assays.

The initial rationale for selecting this combination was to provide therapy with 2 β-lactam antibiotics with complementary penicillin binding protein (PBP)–binding proclivities, thus simultaneously targeting multiple steps in cell wall synthesis to provide enhanced killing [25]. Specifically, carbapenem antibiotics have exceptional affinity to the essential PBP of *S. aureus*, PBP1, exceeding even that of the antistaphylococcal β-lactams [25, 26]. This would complement the relative PBP2 proclivity of cefazolin [27]. A similar rationale has been the basis for use of ampicillin plus ceftriaxone to treat *Enterococcus faecalis* endocarditis and, due to its better tolerability, is phasing out toxic aminoglycoside therapy in this disease [28]. Our in vitro studies demonstrate additivity or synergy, without evidence of antagonism against the isolates tested. However, clearing bacteremia in <24 hours in patients with cardiac vegetations >2 cm in size far exceeds the predicted expectations from the in vitro studies, suggesting that additional factors may be involved that require further study.

One possibility for the profound effect in vivo may be the sensitization of MSSA exposed to both antibiotics to the innate immune system. We illustrated this phenomenon in our prior study, wherein MSSA from case 1 was more effectively killed by the human cationic host defense peptide, cathelicidin LL-37, or by neutrophils that produce many antimicrobial factors, when exposed to sub-MIC concentrations of both cefazolin and ertapenem compared to either drug alone [8]. Another possibility is that very potent interference of PBP1, the only essential PBP in *S. aureus*, by the addition of ertapenem may surpass some cellular viability threshold that cannot be compensated by the other PBPs, particularly if another β-lactam is also interfering with their functions. Finally, our extensive review of the literature has revealed that expression of PBP2, the primary target for cefazolin, is diminished in the presence of neutrophils [27]. Thus, our assessment of antimicrobial activity in artificially contrived bacterial media may be ill-equipped to examine dynamic changes to cell wall that occur in vivo. In reference

Downloaded from https://academic.oup.com/cid/article-abstract/doi/10.1093/cid/ciz995/5644098 by University of Edinburgh user on 28 July 2020

to the cefazolin-ertapenem interaction, ertapenem may serve to "rescue" the relatively attenuated activity of cefazolin that may be occurring in microenvironments such as bacterial endocarditis vegetations. More studies will be needed to examine the relative activities of cefazolin in the presence of components of innate immunity present in vegetations.

In summary, we present a case series of consecutively treated patients with refractory MSSA bacteremia who achieved prompt bacteremia clearance with cefazolin plus ertapenem combination therapy. Synergy or additivity were observed in vitro between cefazolin and ertapenem against MSSA available from 6 of the treated patients and corroborated by studies in a rat endocarditis model. Based on the potent effects observed in vivo beyond what is predicted by in vitro assays, we hypothesize that cooperativity with innate immunity in vivo may also aid in bacterial killing when cefazolin and ertapenem are used together, as previously described [8]. Ertapenem with either cefazolin or other anti-staphylococcal β-lactams should be more extensively evaluated in the clinical setting to establish the role of these combination regimens in refractory cases of MSSA bacteremia and endocarditis.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Financial support. This research was supported by the National Institutes of Health: the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD; Pediatric Scientist Development Program Fellowship K12-HD000850 to E. R. U.) and the National Institute of Allergy and Infectious Diseases (grant numbers U01-AI124316-01 and U54-HD090259 to V. N. and G. S.).

Potential conflicts of interest. G. S. has received speaking honoraria from Allergan and Melinta Pharmaceuticals, and consulting fees from Allergan and Paratek Pharmaceuticals. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Fowler VG Jr, Olsen MK, Corey GR, et al. Clinical identifiers of complicated *Staphylococcus aureus* bacteremia. Arch Intern Med **2003**; 163:2066–72.
2. Naber CK. *Staphylococcus aureus* bacteremia: epidemiology, pathophysiology, and management strategies. Clin Infect Dis **2009**; 48(Suppl 4):S231–7.
3. Holland TL, Arnold C, Fowler VG Jr. Clinical management of *Staphylococcus aureus* bacteremia: a review. JAMA **2014**; 312:1330–41.
4. Nannini EC, Singh KV, Murray BE. Relapse of type A beta-lactamase-producing *Staphylococcus aureus* native valve endocarditis during cefazolin therapy: revisiting the issue. Clin Infect Dis **2003**; 37:1194–8.
5. Bryant RE, Alford RH. Unsuccessful treatment of staphylococcal endocarditis with cefazolin. JAMA **1977**; 237:569–70.
6. Quinn EL, Pohlod D, Madhavan T, Burch K, Fisher E, Cox F. Clinical experiences with cefazolin and other cephalosporins in bacterial endocarditis. J Infect Dis **1973**; 128(Suppl):S386–9.
7. Fernandez-Guerrero ML, de Gorgolas M. Cefazolin therapy for *Staphylococcus aureus* bacteremia. Clin Infect Dis **2005**; 41:127.
8. Sakoulas G, Olson J, Yim J, et al. Cefazolin and ertapenem, a synergistic combination used to clear persistent *Staphylococcus aureus* bacteremia. Antimicrob Agents Chemother **2016**; 60:6609–18.
9. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 26th ed. CLSI Supplement M100S. Wayne, PA: CLSI, **2016**.
10. Moody J, Knapp C. Tests to assess bactericidal activity, in clinical microbiology procedures handbook. In: LS Garcia, ed. Washington, DC: ASM Press, **2010**.
11. Nannini EC, Singh KV, Arias CA, Murray BE. In vivo effects of cefazolin, daptomycin, and nafcillin in experimental endocarditis with a methicillin-susceptible *Staphylococcus aureus* strain showing an inoculum effect against cefazolin. Antimicrob Agents Chemother **2013**; 57:4276–81.
12. Singh KV, Tran TT, Nannini EC, Tam VH, Arias CA, Murray BE. Efficacy of ceftaroline against methicillin-susceptible *Staphylococcus aureus* exhibiting the cefazolin high-inoculum effect in a rat model of endocarditis. Antimicrob Agents Chemother **2017**; 61. doi:10.1128/AAC.00324-17.
13. Lin L, Nonejuie P, Munguia J, et al. Azithromycin synergizes with cationic antimicrobial peptides to exert bactericidal and therapeutic activity against highly multidrug-resistant gram-negative bacterial pathogens. EBioMedicine **2015**; 2:690–8.
14. Kumaraswamy M, Lin L, Olson J, et al. Standard susceptibility testing overlooks potent azithromycin activity and cationic peptide synergy against MDR *Stenotrophomonas maltophilia*. J Antimicrob Chemother **2016**; 71:1264–9.
15. Ersoy SC, Heithoff DM, Barnes L 5th, et al. Correcting a fundamental flaw in the paradigm for antimicrobial susceptibility testing. EBioMedicine **2017**; 20:173–81.
16. Doern CD. When does 2 plus 2 equal 5? A review of antimicrobial synergy testing. J Clin Microbiol **2014**; 52:4124–8.
17. Li J, Echevarria KL, Hughes DW, Cadena JA, Bowling JE, Lewis JS 2nd. Comparison of cefazolin versus oxacillin for treatment of complicated bacteremia caused by methicillin-susceptible *Staphylococcus aureus*. Antimicrob Agents Chemother **2014**; 58:5117–24.
18. McDanel JS, Roghmann MC, Perencevich EN, et al. Comparative effectiveness of cefazolin versus nafcillin or oxacillin for treatment of methicillin-susceptible *Staphylococcus aureus* infections complicated by bacteremia: a nationwide cohort study. Clin Infect Dis **2017**; 65:100–6.
19. Weis S, Kesselmeier M, Davis JS, et al. Cefazolin versus anti-staphylococcal penicillins for the treatment of patients with *Staphylococcus aureus* bacteraemia. Clin Microbiol Infect **2019**; 25:818–27.
20. Nannini EC, Stryjewski ME, Singh KV, et al. Inoculum effect with cefazolin among clinical isolates of methicillin-susceptible *Staphylococcus aureus*: frequency and possible cause of cefazolin treatment failure. Antimicrob Agents Chemother **2009**; 53:3437–41.
21. Wang SK, Gilchrist A, Loukitcheva A, et al. Prevalence of a cefazolin inoculum effect associated with blaZ gene types among methicillin-susceptible *Staphylococcus aureus* isolates from four major medical centers in Chicago. Antimicrob Agents Chemother **2018**; 62. doi:10.1128/AAC.00382-18.
22. Chong YP, Park SJ, Kim ES, et al. Prevalence of blaZ gene types and the cefazolin inoculum effect among methicillin-susceptible *Staphylococcus aureus* blood isolates and their association with multilocus sequence types and clinical outcome. Eur J Clin Microbiol Infect Dis **2015**; 34:349–55.
23. Rincón S, Reyes J, Carvajal LP, et al. Cefazolin high-inoculum effect in methicillin-susceptible *Staphylococcus aureus* from South American hospitals. J Antimicrob Chemother **2013**; 68:2773–8.
24. Song KH, Jung SI, Lee S, et al; Korea Infectious Diseases (KIND) Study Group. Inoculum effect of methicillin-susceptible *Staphylococcus aureus* against broad-spectrum beta-lactam antibiotics. Eur J Clin Microbiol Infect Dis **2019**; 38:67–74.
25. Chambers HF, Sachdeva M. Binding of beta-lactam antibiotics to penicillin-binding proteins in methicillin-resistant *Staphylococcus aureus*. J Infect Dis **1990**; 161:1170–6.
26. Dumitrescu O, Choudhury P, Boisset S, et al. Beta-lactams interfering with PBP1 induce Panton-Valentine leukocidin expression by triggering sarA and rot global regulators of *Staphylococcus aureus*. Antimicrob Agents Chemother **2011**; 55:3261–71.
27. Bamberger DM, Herndon BL, Fitch J, Florkowski A, Parkhurst V. Effects of neutrophils on cefazolin activity and penicillin-binding proteins in *Staphylococcus aureus* abscesses. Antimicrob Agents Chemother **2002**; 46:2878–84.
28. Pericas JM, Cervera C, del Rio A, et al; Hospital Clinic Endocarditis Study Group. Changes in the treatment of *Enterococcus faecalis* infective endocarditis in Spain in the last 15 years: from ampicillin plus gentamicin to ampicillin plus ceftriaxone. Clin Microbiol Infect **2014**; 20:O1075–83.

Downloaded from https://academic.oup.com/cid/article-abstract/doi/10.1093/cid/ciz995/5644098 by University of Edinburgh user on 28 July 2020