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MAJOR ARTICLE

# Enhanced Killing of Methicillin-Resistant *Staphylococcus aureus* With Ceftaroline or Vancomycin in Combination With Carbapenems

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***Background.*** Methicillin-resistant *Staphylococcus aureus* (MRSA) bacteremia is associated with high rates of treatment failure, even when antibiotics showing in vitro susceptibility are used. Early optimization of therapy is crucial to reduce morbidity and mortality. Building on our previous research on carbapenem therapy for methicillin-susceptible *S aureus* bacteremia, we examined the utility of adjunctive carbapenems (ertapenem or meropenem) to enhance the efficacy of ceftaroline or vancomycin for treatment of MRSA.

***Methods.*** The effectiveness of combination therapy versus monotherapy against MRSA was assessed using checkerboard, time-kill, and human whole blood killing assays, as well as a murine bacteremia model. Additionally, we performed transcriptomic analysis and conducted human platelet and antimicrobial peptide killing assays on MRSA pretreated with subtherapeutic concentrations of ceftaroline and carbapenems. The supernatants from these MRSA isolates were used to treat platelets, and cytotoxicity was assessed via lactate dehydrogenase release assays.

***Results.*** Although not used for MRSA, we identified striking in vitro and in vivo synergy between carbapenems and ceftaroline or vancomycin. MRSA pretreated with subtherapeutic ceftaroline-carbapenem therapy revealed transcriptional shifts indicative of reduced antibiotic resistance, virulence, and host immune evasion. Supernatants from these MRSA isolates also caused less platelet injury compared to monotherapy. Furthermore, MRSA pretreated with ceftaroline and carbapenems demonstrated increased susceptibility to killing by human platelets and the antimicrobial peptide LL-37.

***Conclusions.*** The therapeutic success of adjunctive carbapenems appears driven by multiple mechanisms, including direct drug–drug synergy with first-line anti-MRSA agents, attenuation of resistance and virulence factors, and enhancement of immune-mediated killing, each warranting further investigation.

**Keywords.** MRSA; bacteremia; endocarditis; ceftaroline; vancomycin; carbapenems; ertapenem; meropenem; combination therapy.

*Staphylococcus aureus* is a leading cause of bacteremia and infective endocarditis, particularly in developed countries [1]. Persistent *S aureus* bacteremia refractory to first-line therapy is a strong predictor of mortality [2, 3]. This underscores the need for earlier use of antibiotic combination regimens in high-risk patients, rather than as salvage therapy after treatment failure [4–6]. Our recent studies have demonstrated the efficacy of combining ertapenem with cefazolin or other anti-staphylococcal β-lactams in refractory methicillin-susceptible *S aureus* (MSSA) bacteremia and endocarditis [7, 8], with validation in a rat model of MSSA endocarditis [8]. Notably, while in vitro studies showed only modest synergy, the high bactericidal activity observed in vivo suggests potential immunological or virulence-attenuating effects of carbapenems [9, 10].

Given the limitations of current treatments for methicillin-resistant *S aureus* (MRSA), exploring alternative options is critical. Ceftaroline, a cephalosporin with excellent in vitro and in vivo activity against MRSA, offers potential for narrowing clinical outcome disparities between MSSA and MRSA endovascular infections, especially in combination regimens [11]. Although not US Food and Drug Administration–approved for MRSA bacteremia, ceftaroline has been increasingly used by clinicians due to its lower toxicity profile and adjunctive properties that enhance immune function [12, 13]. We hypothesized that combining first-line MRSA agents (ceftaroline or vancomycin) with carbapenems (ertapenem or meropenem) could enhance antimicrobial activity

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against MRSA, mirroring the efficacy of cefazolin-carbapenem therapy against MSSA. This approach has not been adequately studied either clinically or in the laboratory.

This study evaluated the impact of adjunctive carbapenem therapy against MRSA both in vitro and in a murine model of MRSA bacteremia. Our investigation included clinical isolates where this combination therapy was successfully employed as a salvage regimen in challenging cases of MRSA bacteremia. Importantly, we also assessed the effects of adjunctive carbapenems on host immune clearance of MRSA.

## MATERIALS AND METHODS

### Informed Consent and Institutional Approval

Studies were approved by the University of California, Irvine (UCI) Institutional Review Board and the Institutional Animal Care and Use Committee. Informed consent was obtained from healthy human subjects for the collection of venous blood specimens.

### Antibiotics

Antibiotics were purchased from the UCI Medical Center Pharmacy. For in vitro studies, we used the active form of ceftaroline (MedChemExpress). For the murine studies, we administered ceftaroline fosamil, the prodrug that is rapidly converted to ceftaroline in the body. Antibiotic stock solutions and the human antimicrobial peptide LL-37 (Biotech Peptide) were prepared in molecular-grade water (Corning Cellgro) and stored at −20°C.

### Bacterial Strains and In Vitro Susceptibility Tests

All experiments were conducted using clinical isolates obtained from patients with persistent MRSA bacteremia. Bacteria were grown overnight in Todd-Hewitt broth (Hardy Diagnostics) and stored with 40% glycerol at −80°C. Fresh colonies were streaked weekly onto Todd-Hewitt agar (THA; Hardy Diagnostics) plates for experiments. Broth microdilution antimicrobial susceptibility testing was conducted under both standard ($10^5$ colony-forming units [CFU]/mL) and high ($10^7$ CFU/mL) inoculum conditions using cation-adjusted Mueller-Hinton broth (CA-MHB; Difco), following Clinical and Laboratory Standards Institute (CLSI) guidelines [14]. Minimum bactericidal concentrations were determined as previously described and defined as the lowest antibiotic concentration that kills ≥99.9% of the test inoculum [15]. Checkerboard assays were performed in CA-MHB to assess antibiotic interactions, as defined by fractional inhibitory concentration indices (FICIs) 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 [15].

### Time-Kill Curves and Whole Blood Killing Assays

Kill curve and whole blood killing assays were performed as previously described [16]. In brief, bacteria ($2 \times 10^7$ CFU/mL) were incubated with shaking at 37°C in CA-MHB, with or without subtherapeutic ceftaroline ± ertapenem or meropenem in 96-well plates. For whole blood killing assays, bacteria and antibiotics were similarly added to heparinized human blood in 2-mL low-binding tubes (Eppendorf) and rotated at 37°C. Aliquots were collected at 6 and 24 hours and serially diluted in phosphate-buffered saline (PBS) for CFU enumeration. Synergy was defined as a ≥2 $\log_{10}$ CFU/mL reduction of the combination over the most active single agent and a ≥1 $\log_{10}$ CFU/mL reduction from baseline.

### Platelet Lactate Dehydrogenase Release Assays, Platelet Killing, and LL-37 Kill Curves

Platelets were isolated as previously described from healthy volunteers [17, 18]. Overnight cultures were washed and diluted to a final inoculum of $2 \times 10^7$ CFU/mL in CA-MHB with or without subtherapeutic concentrations of ceftaroline (1/8 minimum inhibitory concentration [MIC] relative to the inoculum) ± ertapenem (1/8 MIC) or meropenem (1/8 MIC) in 14-mL tubes at 37°C for 6 hours with shaking. Bacteria were subsequently normalized and diluted to a final inoculum of $2 \times 10^6$ CFU/mL per condition. Additional antibiotics were not added. For the lactate dehydrogenase (LDH) release assays, bacteria were spun down and the bacterial supernatant was collected. For each condition, 50 µL of supernatant was added to $2 \times 10^6$ platelets with 20% pooled human serum in 2-mL tubes and rotated at 37°C for 90 minutes. LDH release was then quantified using the LDH-Glo Cytotoxicity Luminescence Assay (Promega) according to the manufacturer's instructions. The percentage of LDH release from platelets was calculated as follows: 100 × (LDH release per condition – medium background) divided by (maximum LDH release control [no antibiotic condition] – medium background). For the platelet killing assays, bacteria ($2 \times 10^6$ CFU/mL) from each condition were opsonized with 20% pooled human serum for 5–10 minutes in 2-mL tubes with rotation at 37°C, and subsequently inoculated at a multiplicity of infection of 1 with platelets ($2 \times 10^6$/mL) in a final volume of 200 µL. After incubation for 90 minutes at 37°C, the platelets were lysed with 0.3% cold saponin (1:5 dilution), and the bacteria were serially diluted in PBS for CFU enumeration. The percent bacterial survival was calculated based on the average number of CFU/mL noted for each condition divided by the input inoculum. For the LL-37 kill curves, bacteria ($2 \times 10^6$ CFU/mL) from each condition were incubated at 37°C with rotation with or without LL-37 8 µM in 2-mL tubes, and serially diluted in PBS for CFU enumeration at 2 and 4 hours.

### Murine Bacteremia Model

Murine studies were performed as previously described [18]. In brief, overnight MRSA-AS cultures were diluted 1:50 in fresh tryptic soy broth (TSB) and grown to an optical density at 600 nm of 0.40. Bacteria were washed and resuspended in PBS,

and $1 \times 10^9$ CFU/mL was injected intravenously via retro-orbital vein into outbred female CD1 mice (8–10 weeks old, Charles River Laboratories). Mice were then randomly divided into treatment and control groups. Two hours after infection, intraperitoneal doses of antibiotics were administered: ceftaroline (1.56 mg/kg every 8 hours [q8h]) or vancomycin (3.125 mg/kg q8h), and either ertapenem (12.5 mg/kg q8h) or meropenem (50 mg/kg q8h) [19–22]. Mice were euthanized with carbon dioxide 26 hours after infection, followed by cervical dislocation. The kidneys were then harvested for CFU enumeration, weighed, and placed in a 2-mL sterile microtube (Sarstedt) containing 1 mL of PBS and 1-mm-diameter silica beads (Biospec). They were subsequently homogenized by shaking twice at 6000 rpm for 60 seconds, using a MagNA Lyser (Roche). Specimens were placed on ice as soon as they were harvested. Aliquots from each tube were serially diluted in PBS for CFU enumeration on THA plates. CFUs were normalized to CFU/g of tissue for 1 colony, which was considered the limit of detection.

#### RNA Sequencing and Analysis

MRSA-AS was grown overnight in TSB and subcultured in CA-MHB under the same conditions as previously detailed. Cultures were diluted to an inoculum of $2 \times 10^7$ CFU/mL in CA-MHB and grown for 6 hours with no antibiotics (control) or with subtherapeutic concentrations (1/4 MIC relative to the inoculum) of ceftaroline ± ertapenem or meropenem. Each condition ($5 \times 10^6$ CFUs) was centrifuged, resuspended in TRIzol for RNA isolation, and processed using the Direct-zol-96 RNA kit (Zymo Research) according to the manufacturer's instructions. The cells were mechanically lysed using an Omni Bead Ruptor for 7 minutes, with 1-minute intervals (including cooling steps). DNase I treatment was performed during RNA purification. RNA purity was assessed using NanoDrop (260/280 ratio of ~2.0), and RNA quality and integrity was evaluated with an Agilent Bioanalyzer. Per each sample, 500 ng of RNA was used to deplete ribosomal RNA (rRNA) using the NEBNext Bacteria rRNA Depletion Kit. The remaining RNA was fragmented at 94°C for 4 minutes and used to construct a complementary DNA (cDNA) library for sequencing with the NEBNext Ultra II Directional RNA Library Prep Kit. The generated cDNA libraries were sent for Illumina sequencing on a NovaSeq in a pair-ended read format. RNA sequencing (RNA-seq) reads were quality filtered using Trim Galore with a minimum phred score of 30. Quality reports were generated with the FastQC function. Quality filtered reads were aligned to the MRSA-AS genome using HISAT2. Uniquely mapped reads were counted using FeatureCounts in strand-specific mode. Principal component analysis (PCA) was built using DeSeq2, and differentially expressed gene (DEG) analysis was performed using EdgeR. DEGs were defined as 2-fold change in expression with a false discovery rate–corrected $P$ value <.05. The data are available at GenBank under accession number PRJNA1164784.

#### Statistical Analyses

All data were collected from at least 3 biological replicates performed in at least technical triplicate. Statistical analyses were performed using GraphPad Prism version 10.3.0 software. $P$ values <.05 were considered statistically significant.

## RESULTS

#### Clinical Isolates and In Vitro Activity

Four clinical isolates from patients with persistent MRSA bacteremia, representing different MRSA lineages encountered in clinical settings, were used in this study (Table 1). Among these, sequence type (ST) 8 (USA300) is commonly associated with community-acquired infections, while ST22 and ST45 are found in both community and healthcare settings. ST22 is more frequently linked to healthcare-acquired infections in some regions [23]. Persistent MRSA bacteremia was defined as positive blood cultures for ≥3 days despite appropriate antibiotic therapy based on in vitro susceptibility testing [24].

We assessed the in vitro activity of several antibiotics against these persistent MRSA isolates, and the results for vancomycin, ceftaroline, ertapenem, and meropenem are shown in Table 2. Given the association between high-inoculum staphylococcal infections, such as infective endocarditis, and clinical treatment failures [25, 26], antibiotic activities were assessed under

**Table 1. Clinical Isolates From Patients With Persistent Methicillin-Resistant *Staphylococcus aureus* Bacteremia**

| MRSA Isolate | MLST | Age | Sex | Source | Comments |
|---|---|---|---|---|---|
| AS | 22 | <2 y | Female | Blood | Necrotizing pneumonia with MRSA bacteremia requiring ECMO. |
| GS4 | 8 | 70s | Male | Blood | MRSA bacteremia with pacemaker endocarditis cleared within 24 h when meropenem was added to ceftaroline. |
| WRSA7 | 8 | 30s | Male | Blood | AIDS, MRSA bacteremia with mitral valve endocarditis. |
| BS | 45 | 70s | Female | Blood | MRSA bacteremia recurrence with aortic valve endocarditis, hemodialysis catheter–associated thrombophlebitis of the internal jugular vein. MRSA bacteremia 6 d that cleared within 24 h when meropenem was added to ceftaroline. |

Abbreviations: ECMO, extracorporeal membrane oxygenation; MLST, multilocus sequence type.

**Table 2. Minimum Inhibitory Concentration and Minimum Bactericidal Concentration (mg/L) Under Standard ($10^5$) and High ($10^7$) Inoculum in Cation-Adjusted Mueller-Hinton Broth for Vancomycin, Ceftaroline, Ertapenem, and Meropenem Across the 4 Clinical Isolates of Methicillin-Resistant *Staphylococcus aureus* Used in This Study**

| | Vancomycin | | | | Ceftaroline | | | | Ertapenem | | | | Meropenem | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | MIC | | MBC | | MIC | | MBC | | MIC | | MBC | | MIC | | MBC | |
| MRSA Isolate | $10^5$ | $10^7$ | $10^5$ | $10^7$ | $10^5$ | $10^7$ | $10^5$ | $10^7$ | $10^5$ | $10^7$ | $10^5$ | $10^7$ | $10^5$ | $10^7$ | $10^5$ | $10^7$ |
| AS | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 64 | 2 | 32 | 8 | 64 | 2 | 16 | 32 | 64 |
| GS4 | 1 | 2 | 1 | 4 | 1 | 2 | 2 | 64 | 2 | 32 | 4 | 64 | 2 | 32 | 16 | >64 |
| WRSA7 | 1 | 2 | 1 | 2 | 1 | 2 | 2 | 64 | 2 | 32 | 4 | 64 | 2 | 32 | 16 | 64 |
| BS | 1 | 2 | 1 | 4 | 1 | 2 | 1 | 4 | 1 | 8 | 2 | 32 | 2 | 16 | 4 | 32 |

Abbreviations: MBC, minimum bactericidal concentration; MIC, minimum inhibitory concentration; MRSA, methicillin-resistant *Staphylococcus aureus*.

**Table 3. Checkerboard Assays Under Standard ($10^5$) and High ($10^7$) Inoculum in Cation-Adjusted Mueller-Hinton Broth Across the 4 Clinical Isolates of Methicillin-Resistant *Staphylococcus aureus* Used in This Study**

| | Ceftaroline + Ertapenem | | | | Ceftaroline + Meropenem | | | |
|---|---|---|---|---|---|---|---|---|
| | $10^5$ | | $10^7$ | | $10^5$ | | $10^7$ | |
| MRSA Isolate | FICI | Interpretation | FICI | Interpretation | FICI | Interpretation | FICI | Interpretation |
| AS | 0.50 | Synergy | 0.25 | Synergy | 0.375 | Synergy | 0.375 | Synergy |
| GS4 | 0.50 | Synergy | 0.25 | Synergy | 0.375 | Synergy | 0.25 | Synergy |
| WRSA7 | 0.50 | Synergy | 0.156 | Synergy | 0.50 | Synergy | 0.25 | Synergy |
| BS | 0.50 | Synergy | 0.375 | Synergy | 0.50 | Synergy | 0.25 | Synergy |
| | Vancomycin + Ertapenem | | | | Vancomycin + Meropenem | | | |
| | $10^5$ | | $10^7$ | | $10^5$ | | $10^7$ | |
| MRSA Isolate | FICI | Interpretation | FICI | Interpretation | FICI | Interpretation | FICI | Interpretation |
| AS | 1 | Additivity | 0.625 | Additivity | 0.75 | Additivity | 0.625 | Additivity |
| GS4 | 0.375 | Synergy | 0.625 | Additivity | 0.375 | Synergy | 0.75 | Additivity |
| WRSA7 | 0.50 | Synergy | 0.625 | Additivity | 0.375 | Synergy | 0.625 | Additivity |
| BS | 0.75 | Additivity | 1 | Additivity | 0.50 | Synergy | 0.75 | Additivity |

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.
Abbreviations: FICI, fractional inhibitory concentration index; MRSA, methicillin-resistant *Staphylococcus aureus*.

standard ($5 \times 10^5$ CFU/mL) and high ($2 \times 10^7$ CFU/mL) inocula. All isolates were susceptible to vancomycin regardless of inoculum, whereas ceftaroline susceptibility was observed only at standard inoculum. At high inoculum, ceftaroline MICs shifted to the susceptible dose-dependent category (2–4 mg/L) per CLSI breakpoints [14]. No inoculum effect was seen with vancomycin or ceftaroline. Although no clinical breakpoints exist for ertapenem or meropenem against MRSA due to their lack of accepted activity [27, 28], both antibiotics exhibited significant inoculum effects, with MIC increases (≥3 dilutions) at high inoculum (Table 2).

### Checkerboard Assays and MRSA Kill Curves

Checkerboard assays revealed synergy between ceftaroline and either ertapenem or meropenem across all isolates, irrespective of inoculum (Table 3). Additivity was seen between vancomycin and either carbapenem at high inoculum, with some synergy at standard inoculum (Table 3). MRSA kill curves with subtherapeutic antibiotic concentrations (1/4 MIC relative to the inoculum) revealed no bactericidal activity by single agents (ceftaroline, ertapenem, or meropenem) under high-inoculum conditions. However, combination therapy (ceftaroline with ertapenem or meropenem) significantly enhanced killing across all isolates (Figure 1*A*). When antibiotic concentrations were reduced to 1/8 MIC (Figure 1*B*), synergy diminished in CA-MHB but remained significant in human whole blood, with a 2 $\log_{10}$ reduction after 24 hours compared to the most active single agent (Figure 1*C*).

### In Vivo Murine Model of MRSA Bacteremia

Carbapenem sensitization of MRSA to killing, through both direct drug–drug synergy with ceftaroline and by human blood–mediated effects, suggested potential in vivo utility despite the lack of monotherapy activity. In a pilot murine bacteremia model using a high inoculum of MRSA-AS ($1 \times 10^9$ CFU/mL), >50% mortality occurred within 72 hours (Supplementary Figure 1). Mice treated with subtherapeutic humanized antibiotic regimens exhibited a 2.5 $\log_{10}$ reduction in bacterial counts from kidneys

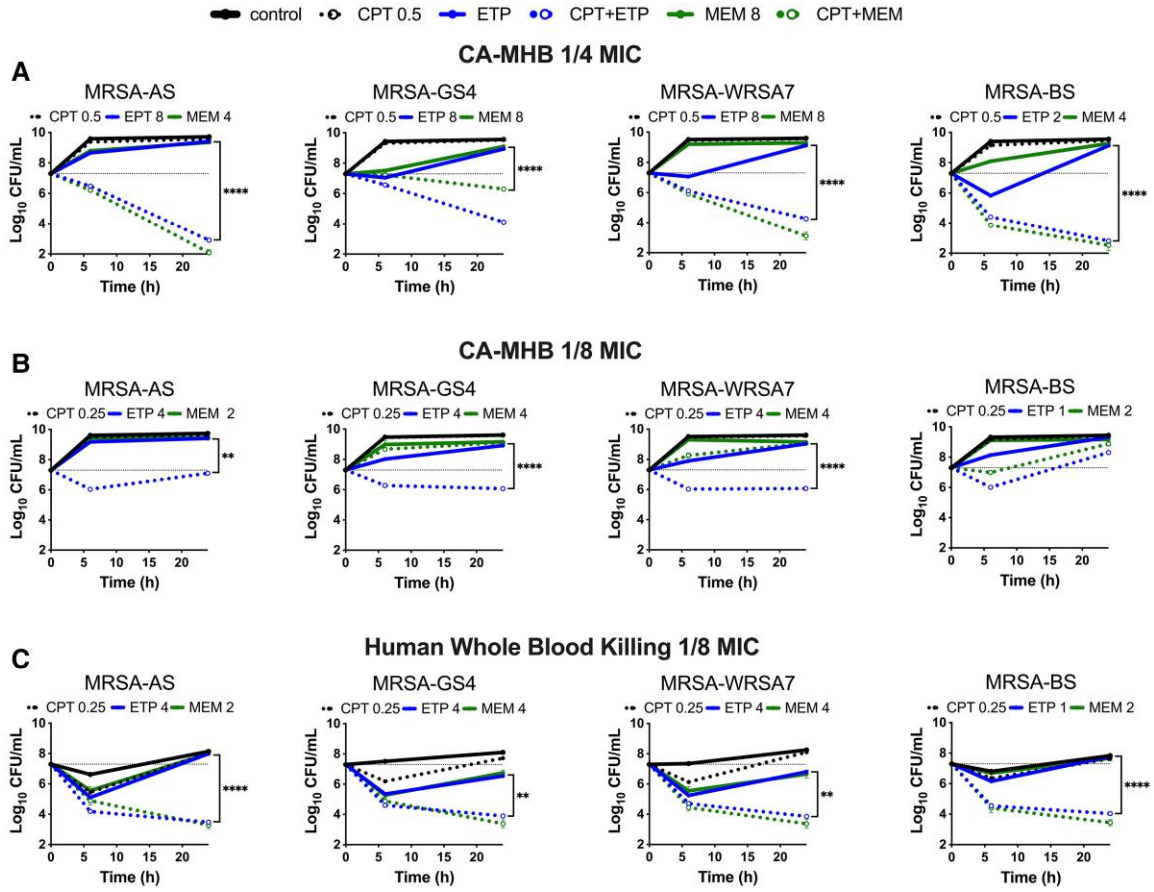


**Figure 1.** Synergistic effects of ceftaroline-carbapenem combinations against methicillin-resistant *Staphylococcus aureus* (MRSA) under various conditions. *A*, Ertapenem (ETP) or meropenem (MEM) synergizes with ceftaroline (CPT) at subtherapeutic concentrations (1/4 minimum inhibitory concentration [MIC], determined at $2 \times 10^7$ colony-forming units [CFU]/mL) to kill 4 isolates of MRSA after 6 and 24 h of treatment under high-inoculum conditions ($2 \times 10^7$ CFU/mL). *B*, When antibiotic concentrations are decreased to 1/8 MIC, synergy between CPT and ETP or MEM is significantly diminished in cation-adjusted Mueller-Hinton broth (CA-MHB) against the same 4 MRSA isolates. *C*, Notably, synergy with combination therapy at 1/8 MIC is still observed in human whole blood killing assays, which mimic physiological conditions. This effect is sustained for up to 24 h. Horizontal lines represent the input inoculum ($2 \times 10^7$ CFU/mL). Subtherapeutic concentrations (1/4 and 1/8 MIC), as detailed in the figure, are expressed in mg/L. $^{**}P \leq .01$, $^{***}P \leq .001$, $^{****}P \leq .0001$, by unpaired 2-tailed *t* test.

at 24 hours with ceftaroline plus ertapenem or meropenem, compared to ceftaroline alone (Figure 2). Similar results were observed for vancomycin-carbapenem combinations, despite weaker in vitro activity (Table 3).

#### Transcriptome Analysis and Differential Gene Expression

RNA-seq analysis of MRSA-AS after 6 hours of growth in CA-MHB identified DEGs in response to subtherapeutic concentrations of ceftaroline, either alone or in combination with ertapenem or meropenem (all at 1/4 MIC, as determined at $2 \times 10^7$ CFU/mL). The data were adjusted for cell count ($5 \times 10^6$ CFUs per condition) to mitigate the impact of differences in bacterial numbers across conditions. The data were then analyzed for signatures of adaptation, including transcriptional shifts in resistance, virulence, or host immune-modulating factors. For a full list of DEGs, please see the Supplementary Excel File. PCA showed distinct clustering of the combination therapy groups, accounting for 70% of variance along principal component 1, with carbapenem therapy contributing an additional 10% along principal component 2 (Figure 3*A*).

#### Resistance and Virulence Factor Modulation

Monotherapy increased expression of blaR1, blaI, blaZ, and PBP2a, associated with β-lactam resistance [29]. In contrast, combination therapy reduced expression of blaZ and PBP2a resistance factors (Figure 3*B*). Additionally, key virulence factors, including leukotoxins (lukD, lukG, lukH, lukF-PV, lukS-PV) [30], were downregulated with combination therapy.

Adjunctive carbapenem therapy resulted in decreased expression of global virulence regulators, including the accessory gene regulator quorum sensing system (agrA-D), transcriptional accessory regulator (sarA), and sigma factor (sigB). The latter is responsible for intracellular persistence and phenotypic changes associated with small colony variants (Figure 3*C*) [31]. Genes essential for staphylococcal biofilm formation, such as the bifunctional autolysin (atl), were also

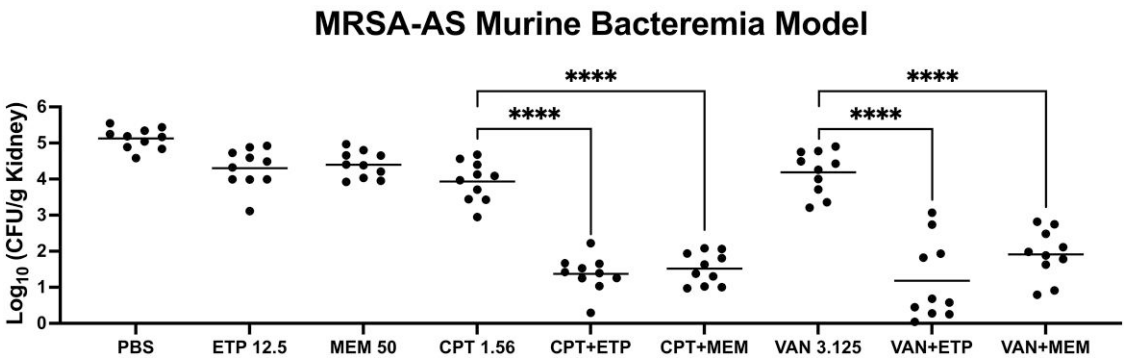


**Figure 2.** Efficacy of adjunctive carbapenem therapy against methicillin-resistant *Staphylococcus aureus* (MRSA-AS) in a murine bacteremia model. Bacterial counts from kidneys (colony-forming units [CFU]/g) after 24 h of treatment with ceftaroline (CPT; 1.56 mg/kg every 8 hours [q8h]), vancomycin (VAN; 3.125 mg/kg q8h), ertapenem (ETP; 12.5 mg/kg q8h), meropenem (MEM; 50 mg/kg q8h), or combination therapy versus no antibiotics (phosphate-buffered saline [PBS] control) are shown. Combinations of CPT or VAN with either ETP or MEM significantly reduce recoverable MRSA from kidneys compared to both monotherapies and the PBS control in a murine bacteremia model (n = 10). Horizontal bars represent the mean. $^{****}P \leq .0001$, by Welch's *t* test.

downregulated [32]. Key staphylococcal factors that promote evasion of host phagocytic responses were also reduced, including adhesins, leukocidins, and proteins like the γ-hemolysins (hlgA-C), the extracellular serine protease V8 (sspA) that cleaves human immunoglobulins by degrading the Fc region [33], and the cysteine protease staphopain B (sspB). Adjunctive carbapenems had contrasting effects on cell wall components. They increased expression of genes targeting the cell wall peptidoglycan matrix, including the peptidoglycan hydrolase or autolysin LytM and the putative transglycosylase SceD involved in peptidoglycan remodeling (Figure 3*D*) [34]. Simultaneously, expression of the antiphagocytic virulence factor type 8 capsular polysaccharides (cap8D-P) was decreased.

The binding of bacteria to platelets is a postulated central event in the pathogenesis of infective endocarditis. Loss of staphylococcal surface glycoproteins, such as serine-rich adhesin for platelets (SraP), attenuates virulence in an animal model of endocarditis, suggesting that this interaction is important for the pathogenesis of endovascular infection [35]. Combination therapy downregulated several staphylococcal virulence factors involved in platelet binding and killing (Figure 3*E*), such as SraP, the accessory Sec system (secA2, gtfA, gtfB) that modifies and exports SraP [36], the serine-rich glycoprotein adhesin (sasA), clumping factor A (clfA), and the pore-forming α-toxin (Hla). There was also a downregulation of the multiple peptide resistance factor protein (mprF) and D-alanine ligase (dltA). These virulence factors are known to alter cell surface charge by modifying phosphatidylglycerol and lipoteichoic acids, which increases resistance to cationic peptides, including platelet microbicidal proteins. Such modifications are associated with increased virulence in a rabbit endocarditis model [37–40]. Furthermore, blaI has been shown to render *S aureus* more resistant to antimicrobial peptides (Figure 3*B*) [29].

### Platelet and Antimicrobial Peptide Interactions With Antibiotic-Pretreated MRSA

Given these findings, we examined the killing of MRSA-AS by human platelets and antimicrobial peptides (LL-37) after pretreating the bacteria for 6 hours with or without subtherapeutic (1/8 MIC) antibiotics under high-inoculum conditions ($2 \times 10^7$ CFU/mL). All experiments were adjusted to a cell count of $2 \times 10^6$ CFU/mL per condition. Human platelets were treated with supernatants taken from the different conditions, with no additional antibiotics added. Supernatants from the ceftaroline and ertapenem or meropenem conditions inhibited human platelet cytotoxicity, as measured by LDH release (Figure 4*A*). Additionally, pretreatment with combination therapy enhanced platelet killing of MRSA-AS (Figure 4*B*). Since platelets exert their antimicrobial effects in part through the release of antimicrobial peptides, we examined LL-37–mediated killing of antibiotic-pretreated MRSA-AS and observed enhanced killing with combination therapy (Figure 4*C*).

## DISCUSSION

Despite significant advancements in many fields of medicine, mainstream treatment strategies for MRSA bloodstream infections have remained largely unchanged over the past several decades, with little improvement in patient outcomes [41]. Therefore, novel treatment strategies must be explored and translated into clinical practice to advance patient care.

In *S aureus* bacteremia, effective treatment strategies can be informed by the salvage regimens used to treat the most complicated cases. The combination of ertapenem with cefazolin has shown great success in managing refractory MSSA bacteremia and endocarditis [7, 8]. Our previous work with this combination suggests that its powerful efficacy stems from both

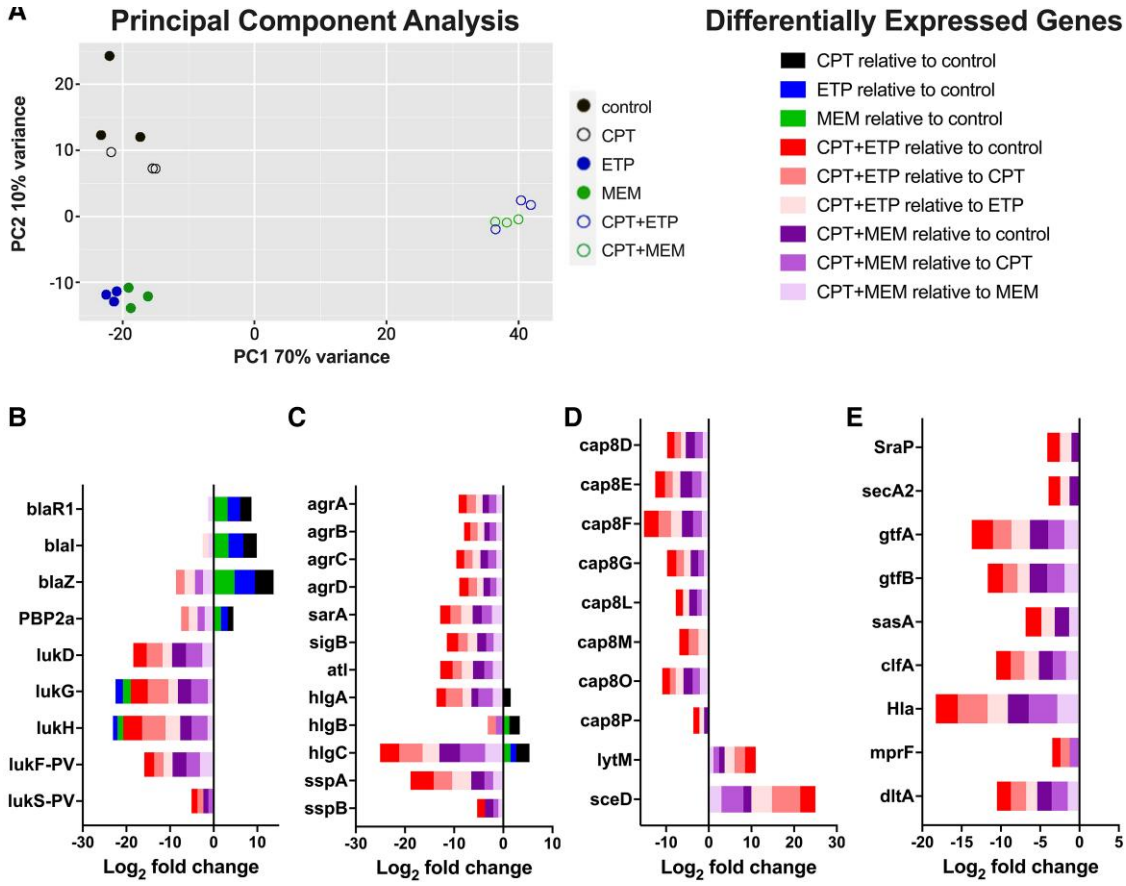


**Figure 3.** Transcriptomic analysis for methicillin-resistant *Staphylococcus aureus* (MRSA-AS) reveals transcriptional shifts indicative of reduced resistance, virulence, and host immune evasion in the presence of ceftaroline (CPT)–carbapenem combination therapy. MRSA-AS was grown for 6 h under high-inoculum conditions with or without subtherapeutic antibiotics (1/4 minimum inhibitory concentration, determined at $2 \times 10^7$ colony-forming units [CFU]/mL) under high-inoculum conditions ($2 \times 10^7$ CFU/mL). Treatments included CPT (0.5 mg/L), ertapenem (ETP, 8 mg/L), or meropenem (MEM, 4 mg/L) as monotherapy, or CPT-carbapenem combination therapy. Data were adjusted for cell count ($5 \times 10^6$ CFU per condition). *A*, Principal component (PC) analysis of differentially expressed genes (DEGs) showed that 70% of the variance was due to combination therapy, while 10% was due to carbapenem therapy (ETP or MEM). *B*, DEGs related to antibiotic resistance were upregulated with CPT or carbapenem monotherapy (blaR1, blaI, blaZ, PBP2a), while combination therapy downregulated resistance genes (blaZ and PBP2a) and reduced expression of leukocidins (lukD, lukG, lukH), including Panton-Valentine leukocidin (lukF-PV and lukS-PV). *C*, Combination therapy also resulted in decreased expression of important global regulators of virulence (agrA-D, sarA, sigB), biofilm (atl), γ-hemolysins (hlgA-C), and proteases (sspA and sspB) important for immune evasion. *D*, Expression of capsular polysaccharides (cap8D-P) was reduced, whereas genes targeting the cell wall peptidoglycan matrix (lytM and sceD) were upregulated. *E*, Genes involved in platelet killing (SraP, secA2, gtfA, gtfB, sasA, clfA) and the pore-forming α-toxin (Hla) were downregulated with combination therapy, as were genes that increase resistance to antimicrobial peptides (mprF and dltA). DEGs were defined as a 2-fold change in expression with a false discovery rate–corrected *P* value <.05. Expression fold changes are presented as positive (upregulated) or negative (downregulated) values. For further details, refer to the Supplementary Excel File.

direct antibacterial activity and augmented immune-mediated clearance [9]. Building on this concept, we hypothesized that an analogous approach using ceftaroline, a cephalosporin with established anti-MRSA activity, combined with a carbapenem could yield similar success. This study provides preliminary yet multidimensional evidence that ceftaroline plus carbapenems represent a potent strategy for treating MRSA infections, as evidenced by (1) in vitro synergy observed in checkerboard and kill curve assays; (2) attenuation of MRSA resistance and virulence; and (3) superior microbiological outcomes in a murine MRSA bacteremia model. The latter also demonstrated significant bacterial killing with lower-than-human-equivalent antibiotic concentrations, suggesting the potential for effective treatment strategies even at subtherapeutic drug dosages.

The RNA expression changes observed are particularly noteworthy, suggesting potential disruptions in the pathogenesis of MRSA endovascular infections. Key findings include (1) reduced expression of leukocidins (lukD, lukG, and lukH), including Panton-Valentine leukocidin, which contributes to endothelial injury; (2) decreased production of agr, sarA, and α-toxin, a key virulence factor involved in anti-platelet activity [42]; (3) downregulation of sraP and the Sec system, which contribute to anti-platelet virulence; (4) reduced expression of clfA, a critical MSCRAMM adhesin involved in endovascular infection [43]; (5) downregulation of atl, which plays a role in

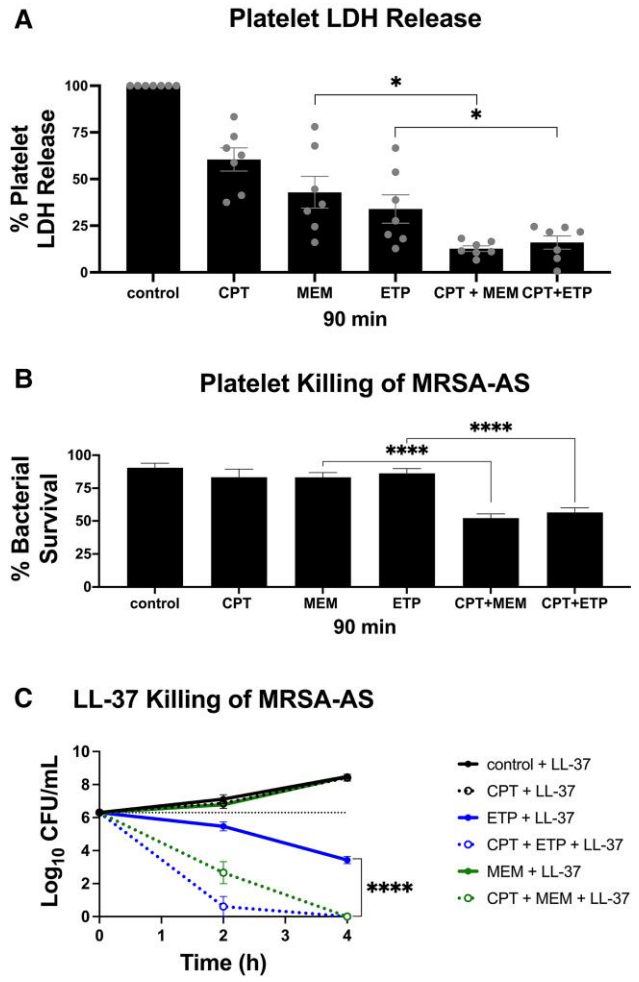


**Figure 4.** Effects of combination antibiotic therapy on methicillin-resistant *Staphylococcus aureus* (MRSA-AS) susceptibility to host defense mechanisms. MRSA-AS was grown for 6 h with or without subtherapeutic antibiotics (1/8 minimum inhibitory concentration, determined at $2 \times 10^7$ colony-forming units [CFU]/mL) under high-inoculum conditions ($2 \times 10^7$ CFU/mL). Treatments included ceftaroline (CPT, 0.25 mg/L), ertapenem (ETP, 4 mg/L), or meropenem (MEM, 2 mg/L) as monotherapy, or CPT-carbapenem combination therapy. *A*, Lactate dehydrogenase (LDH) release from human platelets exposed to supernatants from pretreated MRSA-AS cultures. Combination therapy reduced platelet cytotoxicity. *B*, Killing of pretreated MRSA-AS by human platelets. Combination therapy increased bacterial sensitivity to platelet-mediated killing. *C*, Killing of pretreated MRSA-AS by the human antimicrobial peptide LL-37 (8 μμ). Combination therapy enhanced LL-37–mediated killing. The horizontal line represents the input inoculum ($2 \times 10^6$ CFU/mL). Statistical significance was determined by paired 2-tailed *t* test (*A*) and unpaired 2-tailed *t* test (*B* and *C*). $^*P \leq .05$, $^{****}P \leq .0001$.

biofilm formation [32]; and (6) decreased expression of mprF and dltA, which mediate cell surface charge changes that confer resistance to antimicrobial peptides [39]. While additional effects were observed, such as a reduction in capsular polysaccharide, their role in endocarditis pathogenesis is less well-established.

Since platelets are central to endovascular immunity, neutralizing key *S aureus* virulence factors, like α-toxin, may enhance therapeutic effects beyond direct antibacterial activity [44–46]. In line with this, we demonstrated improved platelet survival and enhanced platelet-mediated killing of MRSA when bacteria were pretreated with subtherapeutic concentrations of the ceftaroline-carbapenem regimen. Furthermore, accelerated killing of MRSA pretreated with combination therapy was observed in the presence of LL-37, an antimicrobial peptide that enhances platelet interactions with bacteria and exhibits potent antimicrobial activity against MRSA [47].

Given the success of cefazolin or nafcillin combined with carbapenems in treating MSSA bacteremia and endocarditis, our findings underscore the need for more extensive clinical evaluations of these combination regimens in *S aureus* bacteremia and endocarditis, including MRSA. However, clinical trials may face challenges, particularly in enrolling the most critically ill patients, such as those with endocarditis. Despite these hurdles, clinicians managing challenging MRSA bacteremia and endocarditis cases may consider these data when devising salvage treatment regimens.

This study has several limitations. The small number of isolates studied and the limited clinical experience with ceftaroline-carbapenem therapy are key constraints. Furthermore, the RNA expression data should be considered preliminary until validated with additional strains and confirmed at the protein level, along with phenotypic assessments of the observed changes.

In conclusion, adding a carbapenem, such as ertapenem or meropenem, to standard MRSA therapies like ceftaroline or vancomycin appears to be a potent treatment strategy for serious MRSA bloodstream infections. While traditional in vitro synergy testing shows only modest results, our murine model and limited clinical cases demonstrate that adjunctive carbapenem therapy enhances anti-MRSA activity through resistance and virulence attenuation, along with potential immune system enhancement. Further investigation into carbapenem adjunctive therapy for *S aureus* bacteremia, particularly in MRSA, is warranted.

## Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

## Notes

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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.

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