International Journal of Antimicrobial Agents 55 (2020) 105864

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: www.elsevier.com/locate/ijantimicag

Short Communication

In vitro activity of meropenem/piperacillin/tazobactam triple combination therapy against clinical isolates of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus pseudintermedius* and vancomycin-resistant *Enterococcus* spp

Aki Yoneda[a,1,*], Robert Thänert[a,1], Carey-Ann D. Burnham[b,c,d,*], Gautam Dantas[a,b,d,e,*]

[a] The Edison Family Center for Genome Sciences and Systems Biology, Washington University in St. Louis School of Medicine, St. Louis, Missouri, USA
[b] Department of Pathology and Immunology, Washington University in St. Louis School of Medicine, St. Louis, Missouri, USA
[c] Department of Pediatrics, Washington University in St. Louis School of Medicine, St. Louis, Missouri, USA
[d] Department of Molecular Microbiology, Washington University in St. Louis School of Medicine, St. Louis, Missouri, USA
[e] Department of Biomedical Engineering, Washington University in St. Louis, St. Louis, Missouri, USA

ARTICLE INFO

Article history:
Received 10 July 2019
Accepted 14 December 2019

Editor: Stefania Stefani

Keywords:
MRSA
Staphylococci
Whole-genome sequencing
Clinical isolates
Antibiotic combination
Antibiotic resistance

ABSTRACT

Objectives: To evaluate the activity of the reported synergistic and collaterally sensitive antibiotic combination, meropenem/piperacillin/tazobactam (ME/PI/TZ), against a panel of methicillin-resistant *Staphylococcus aureus* (MRSA) and other methicillin-resistant *Staphylococcus* species; and to investigate the relationship between ME/PI/TZ susceptibility and the genomic background of clinical isolates of MRSA.

Methods: ME/PI/TZ combination and single drug minimum inhibitory concentrations (MICs) were determined for 207 strains (including 121 MRSA, 4 methicillin-sensitive *S. aureus* [MSSA], 37 vancomycin-intermediate *S. aureus* [VISA], 6 ceftaroline non-susceptible MRSA, 29 coagulase-negative staphylococci [CoNS], 5 *S. pseudointermedius* and 5 vancomycin-resistant Enterococci [VRE]) by broth microdilution. Whole genomes of 168 *S. aureus* strains were sequenced, assembled, and comparatively analysed.

Results: USA300-SCC*mec* type IV isolates, clonal complex 8 (CC8)-MRSA isolates, including some VISA and ceftaroline (CPT)-intermediate strains, and all tested methicillin-resistant *S. epidermidis* isolates were highly susceptible to ME/PI/TZ. Isolates with elevated MICs (MICs of >16/16/16 mg/L) clustered with the USA100-SCC*mec* type II strain. Susceptibility of MRSA to ME/PI/TZ was correlated with susceptibility to ME. No obvious cross-resistance to CPT was observed among high-ME/PI/TZ MIC isolates.

Conclusions: The ME/PI/TZ combination is effective against a variety of clinical MRSA isolates, particularly of the USA300 lineage, which is expanding worldwide. ME/PI/TZ is also effective against drug-resistant CoNS and *S. pseudintermedius* clinical isolates.

1. Introduction

Staphylococcus aureus is a Gram-positive bacterium frequently associated with community-acquired pneumonia, bacteremia, and nosocomial infections, resulting in a significant burden on healthcare systems [1]. The initial effectiveness of antibiotics in overcoming mortality associated with *S. aureus* infections has been lost because of the rise in antibiotic resistance [2]. Drug-resistant *S. aureus* strains, particularly methicillin-resistant *S. aureus* (MRSA), account for almost half the deaths caused by infections with drug-resistant bacteria in the United States [1]. A critical bridge between ineffective antibiotics and limiting antibiotic use to preserve efficacy involves combining existing drugs in novel ways. We have reported about an antibiotic combination (meropenem/piperacillin/tazobactam; ME/PI/TZ) that synergistically kills MRSA and suppresses development of drug resistance [3]. The most effective ratio against MRSA N315 using checkerboard assays was 1:1:1 [3]. This combination simultaneously targets β-lactamases and multiple penicillin-binding proteins (PBPs), including drug-resistant PBP2a in MRSA, in a collaterally sensitive manner. In addition to inhibiting the cell-division protein

* Corresponding authors.
E-mail addresses: cburnham@wustl.edu (C.D. Burnham), dantas@wustl.edu (G. Dantas).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.ijantimicag.2019.105864
0924-8579/

PBP1, ME binds PBP2a at a site distal to the transpeptidase active site binding pocket, causing allosteric opening of the binding pocket to enable attack by PI or ME. TZ inhibits β-lactamase, thus protecting PI, and disrupts genetic cross-regulation in cell wall generation in MRSA [3]. The in vivo relevance of ME/PI/TZ activity was confirmed by complete clearance of an otherwise lethal MRSA systemic infection in neutropenic mice [3].

The current study investigated the spectrum of ME/PI/TZ activity in a diverse panel of clinical isolates from patients at Barnes Jewish Hospital (BJH), a tertiary-care academic medical center. To understand the genomic and phylogenetic basis of resistance and susceptibility, whole-genome sequencing (WGS) of 138 clinical *S. aureus* isolates from BJH was performed and these strains were compared to 30 isolates from outside sources, including reference "type" strains representing major strain types. Resistance genes were also identified that could explain variable patterns of resistance to the combination therapy.

2. Materials and methods

2.1. Bacterial strains and antibiotic susceptibility testing

De-identified clinical strains of MRSA, coagulase-negative *Staphylococcus* (CoNS), VISA (both MRSA and MSSA), and vancomycin-resistant *Enterococcus* (VRE) were obtained from the BJH Clinical Microbiology Laboratory (St. Louis, MO), and strains ATCC 29213 (MSSA), 43300 (MRSA), 29212 (vancomycin-susceptible *E. faecalis*), and 51299 (vancomycin-resistant *E. faecalis*) were obtained from the American Type Culture Collection (ATCC; Manassas, VA). Fourteen VISA strains were obtained from the CDC & FDA AR Bank (https://wwwn.cdc.gov/arisolatebank/ Atlanta, GA). USA type strains (USA100 through USA1100) were a gift from Dr. Andrew Tomaras (St. Louis, MO). Strains were streaked out on tryptic soy agar before use. ME, PI, and TZ were purchased from Sigma-Aldrich (St. Louis, MO). Drugs/combinations were solubilized in DMSO at 51.2 g/L and diluted in cation-adjusted Mueller-Hinton broth (CAMHB; Sigma-Aldrich, St Louis, MO) to a final concentration of 128, 256, 1024, and 2048 mg/L. Reference broth microdilution testing was performed according to the CLSI guidelines using appropriate quality control strains [4]. Strain N315 was used as an internal standard.

2.2. 3-D checkerboard analysis

ME, PI and TZ were dissolved in DMSO at 256 mg/L (ME) or 1024 mg/L (PI, TZ) to make master plates for PI (diluted 2-fold column-wise in CAMHB) and TZ (diluted 2-fold row-wise in CAMHB). 50 µL of serially diluted PI were aliquoted to 24 U-bottom 96-well plates. To the 24 plates, 25 µL of serially diluted TZ was added. ME (starting at 256 mg/L) was 2-fold serially diluted in 10 mL CAMHB in 8 levels, and 25 µL of each concentration was dispensed into triplicate plates that already contained PI-TZ 2D gradient. A bacterial suspension was made by resuspending freshly streaked colonies in CAMHB and adjusted to an optical density (OD) corresponding to McFarland Standard 5.0. The bacterial suspension was then diluted 1/1000 in 250 mL CAMHB, and 100 µL of the cell suspension was dispensed into each well using a multichannel pipette. Quality control plates for ATCC 29213 were made using drug stock solutions and inoculated. After inoculation, plates were sealed with Breathe-easy membranes and incubated at 37 °C for 16-20 h. The presence/absence of bacteria in each well was determined by visual inspection.

2.3. Whole-genome sequencing, bioinformatics, and statistical analysis

Genomic DNA extraction, Illumina sequencing library preparation, raw read processing and assembly were performed as previously described [5]. Staphylococcal Chromosome Cassette (SCC) *mec* types were determined using SCC*mec*Finder [6]. Clinical *S. aureus* isolates were contextualized within the broader taxonomy by constructing a core-genome containing genes shared by 99% of all isolates, and maximum-likelihood phylogenetic trees were constructed and visualized using previously described programs and methods [5]. To increase phylogenetic resolution for the two dominant genomic clusters (mostly consisting of *S. aureus* CC5 and CC8 isolates), additional core-genomes and maximum-likelihood trees were constructed.

Antimicrobial resistance genes (ARGs) were annotated in silico, retaining ARGs that covered at least 90% of the reference sequence, with >90% sequence identity using previously described methods [5]. These thresholds were selected taking into account the frequently polymorphic nature of central elements of *S. aureus* antimicrobial resistance [7].

3. Results

3.1. Susceptibility of clinical isolates to meropenem/piperacillin/tazobactam

The ME/PI/TZ combination at 1:1:1 mass ratio was empirically determined as the optimal ratio for MRSA N315 in our previous study [3]. The present study confirmed that this ratio offers broad coverage for isolates with various resistance profiles (Table S1) and was effective against a spectrum of MRSA and methicillin-resistant (MR) *Staphylococcus* species: 82% (99/121) of vancomycin and CPT-susceptible MRSA isolates (Fig. 1A) and all (29/29) MR-CoNS (28 *S. epidermidis* and 1 *S. simulans*) were inhibited at concentrations of 16/16/16 mg/L (Table 1). The majority of MR-*S. pseudintermedius* were resistant to PI but, like MSSA, highly susceptible to ME and ME/PI/TZ (Tables 1, S2E). MR-*S. pseudintermedius* causes similar infections to MRSA and is an increasingly recognized pathogen that was commonly misidentified as MRSA in the pre-MALDI-TOF era [8]. Some multidrug-resistant MRSAs, such as VISA (17/27, Table 1) and CPT-intermediate and -resistant isolates (4/6, Tables 1, 2, S2B, S2C), were susceptible to ME/PI/TZ, with no cross-resistance to CPT observed (Table S2H, Figure S5).

Three of five VRE strains had ME/PI/TZ MICs of ≤16/16/16 mg/L, although the efficacy of ME/PI/TZ against VREs appeared to be linked to PI susceptibility for *E. faecalis* (Tables 1, S2G). The triple combination inhibited (fractional inhibitory concentration index = 0.30 at 16/16/16 mg/L) one *E. faecium* strain ("PT20") that showed high levels of ME and PI resistance synergistically (MICs of 64 mg/L and >512 mg/L, respectively, Table S2G).

For MRSA isolates, susceptibility to ME (correlation coefficient 0.85, Fig. 1B) and not PI susceptibility (correlation coefficient 0.18, Fig. 1C) was strongly positively correlated with susceptibility to the ME/PI/TZ combination. The β-lactamase inhibitor TZ did not have antimicrobial activity alone.

3.2. Comparative genomics reveals a strong link between ME/PI/TZ susceptibility and strain background

To explore the relationship between ME/PI/TZ susceptibility and the genomic background of the clinical isolates, whole genomes of strains tested for ME/PI/TZ susceptibility were sequenced to reconstruct the population structure at BJH (strain IDs "BJH###" denote isolates collected prior to 2013; "BJH18_###" denotes strains isolated after 2018) and identify associations between phylogenetic

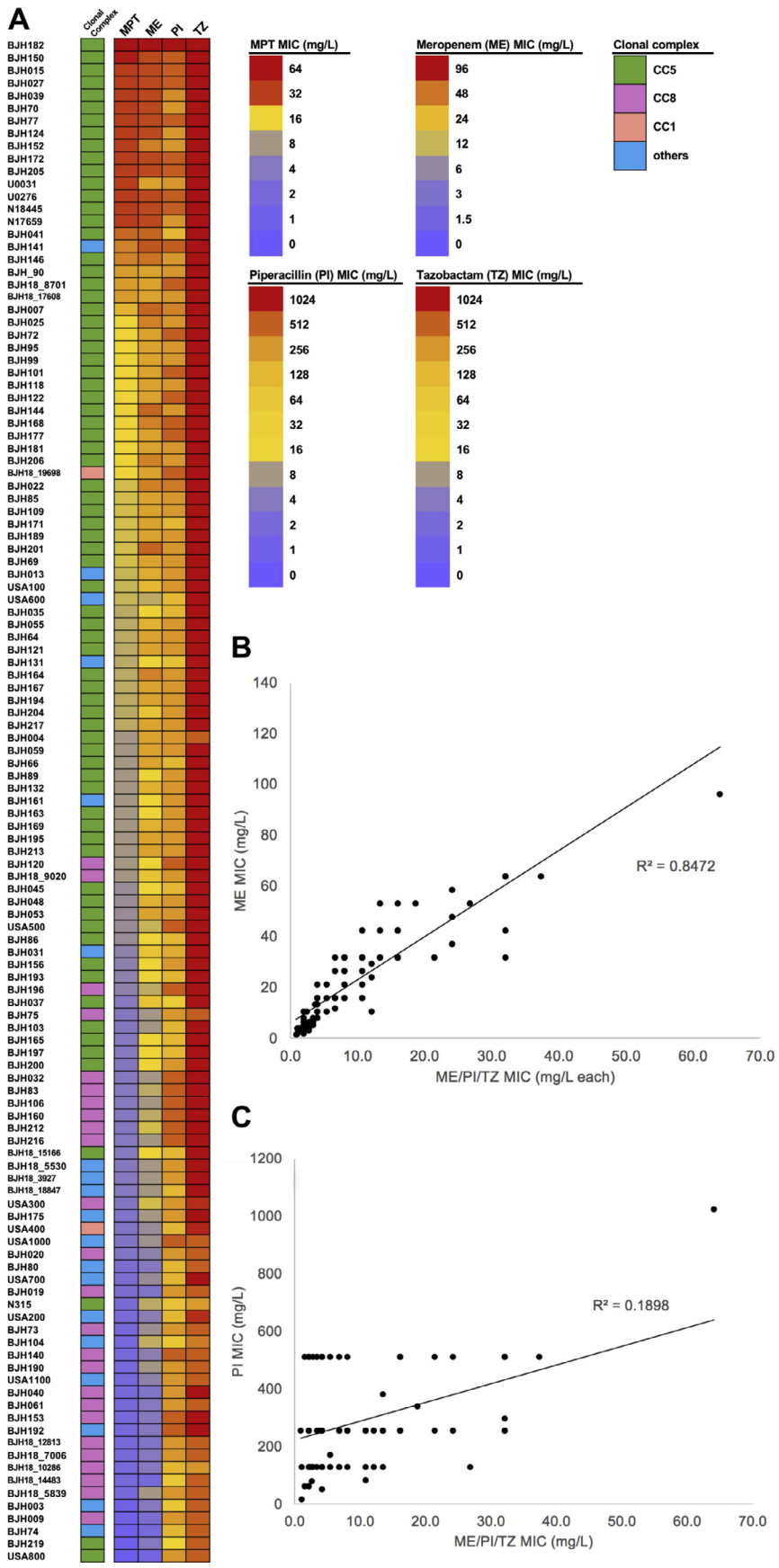

Fig. 1. Minimum inhibitory concentrations of MRSA strains. (A) Heatmap of MICs of all 121 MRSA isolates against the triple combination of ME/PI/TZ (labeled as "MPT") as well as single treatment with each drug. (B) Scatterplot of MICs of MRSA isolates for meropenem against MICs for ME/PI/TZ. The linear regression line is plotted. (C) Scatterplot of MICs of MRSA isolates for piperacillin against MICs for ME/PI/TZ. The linear regression line is plotted.

Table 1
Distribution of ME/PI/TZ MICs by various clinical isolate groups[a]

ME/PI/TZ concentration g/L (each)	MRSA[b]	VISA (MRSA)	VISA (MSSA)	CPT non-susceptible MRSA	MR-*S. pseudintermedius*	MR-CoNS	VRE
>32	2						2
32	13	5		2			
>16 <32	7	5		1			
16	13	4				3	1
>8 <16	20	1					
8	12	2		1		3	
>4 <8	9	0		1		1	
4	16	4				4	1
>2 <4	10	1		1			
2	14	3				2	
<2	5	2	10		5	16	1
Total	121	27	10	6	5	29	5

CPT, ceftaroline; ME, meropenem; MIC, minimum inhibitory concentration; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-sensitive *Staphylococcus aureus*; PI, piperacillin; TZ, tazobactam; VISA, vancomycin-intermediate *Staphylococcus aureus*; VRE, vancomycin-resistant Enterococci.
[a] MICs of non-VISA and CPT-susceptible MSSA not shown.
[b] VISA (MRSA) and CPT-non-susceptible MRSA not included.

background and ME/PI/TZ resistance. Strains from external sources, including USA-types, N315, 4 MSSAs and 13 VISAs, were included in our analysis to place our clinical isolates in the larger epidemiological context of multidrug-resistant *S. aureus* lineages. Overall, genomes of 138 clinical isolates were sequenced and assembled. The core genome, constructed from all clinical isolates and reference strains, contained 1381 genes shared at 95% nucleotide identity. The relatively large accessory genome (5485 genes) highlights genetic plasticity of clinical *S. aureus* isolates, enabling its success as a human pathogen [9]. Maximum Likelihood phylogenetic trees generated showed that the majority of clinical isolates (92/138 clones) clustered with the USA100 type-strain (Fig. 2, S3). In silico MLST and SCC*mec* typing indicated that these isolates, as characteristic for the USA100 lineage, predominately belonged to CC5 and carried the SCC*mec* type II cassette harboring a complete version of the *mec*-operon (*mecI, mecR1,* and *mecA*). A total of 77 of these isolates were characterized as MRSA, 10 were VISA, 4 were CPT non-susceptible, and one clone had an MSSA phenotype. Two isolates clustering with the USA100 type strain, BJH003 and BJH74, harbored the SCC*mec* type IV cassette and were highly susceptible to ME/PI/TZ, indicating an apparent link between SCC*mec* type and resistance to the combination (Figure S3). This assumption is supported by reports that link SCC*mec* types and antibiotic susceptibilities of MRSA [10]. The majority of MRSA clones were associated with the USA100 lineage, which is consistent with the epidemiological trends in the United States during the time of their isolation before 2013 [11]. Generally, isolates clustering with USA100 tended to have higher ME/PI/TZ MICs than isolates that clustered with the USA300 type strain (Fig. 2), the second epidemic strain present in our cohort. In silico MLST and SCC*mec* typing of isolates from the USA300 cluster identified SCC*mec* type IV and CC8 for most clones. The majority of isolates from this cluster were MRSA (24/26), and two clones were VISA. Interestingly, all MRSA isolates clustering with USA300 were highly susceptible to ME/PI/TZ (Fig. 2, S4), indicating that detectable genetic signatures associated with MRSA isolates might contribute to resistance against the triple combination.

3.3. The absence of mecA regulatory genes is associated with ME/PI/TZ susceptibility

This study showed that susceptible isolates clustering with the USA300 type strain harbored the SCC*mec* IV cassette (Fig. 2, S4). Interestingly, isolates with the SCC*mec* type IV lack a functional *mec* regulatory system, consisting of a *mecA*-repressor encoded by *mecI* and a β-lactam-sensing transmembrane protein encoded by *mecR1* that induces the PBP2a-encoding gene, *mecA*. Clones carrying the SCC*mec* type IV cassette have a truncated version of *mecR1* and lack *mecI*, altering the regulatory patterns of *mecA* [12]. Altered expression of PBP2a, which provides resistance to ME, could explain increased susceptibility of SCC*mec* IV-positive clones and is consistent with reports of higher susceptibility of SCC*mec* IV strains to other drugs [11,13]. Notably, resistance against the triple combination was not correlated with the presence of other resistance genes, indicating that SCC*mec* status might be the defining factor for resistance against the triple combination (Figure S6).

4. Discussion

Novel antibiotic drug development is a slow process; therefore, combining established drugs that regain treatment efficacy through synergistic interaction is a promising avenue in the fight against rising antibiotic resistance [14]. In the present study, ME/PI/TZ was evaluated against a range of clinical isolates and was shown to be effective against a wide variety of *S. aureus* and MR-*Staphylococcus* species (Tables 1, S2). These findings, combined with our previous observation that the triple combination prevents low-MIC MRSA from becoming resistant to β-lactams, highlight the clinical potential of ME/PI/TZ [3]. The majority of clinical isolates included in the analysis were genetically similar to the USA100 type strain (Fig. 2). USA100 has historically been considered a “hospital-associated” lineage, whereas the USA300 lineage, representing the second cluster of isolates, has traditionally been considered “community-associated” [15]. In recent years, these demarcations have blurred, with strains previously classified as community-associated now frequently causing nosocomial infections. In the clinical setting, USA300 is increasingly important, partly due to its superior infectivity compared with the USA100 lineage, and MRSA infections in the US are now predominantly caused by the USA300 strains [13,16]. Most of the isolates in the present study were collected prior to 2013, which may explain the relatively higher abundance of clones grouping with USA100 compared with isolates of the USA300 lineage (Fig. 2). Isolates of CC5 were observed to be more resistant to the triple combination than isolates of CC8. Considering the trend for increasing USA300 strains in the US and elsewhere [10,13], ME/PI/TZ may be an effective therapeutic option for severe MRSA infections.

ME is a broad-spectrum antibiotic of the carbapenem family. MRSA is usually resistant to ME because of its alternative penicillin-binding protein, PBP2a [3]. The data in the present study indicate that ME susceptibility is a major determinant of MRSA susceptibility to ME/PI/TZ. Combining ME with PI and TZ lowers drug concentrations via synergy, rendering ineffective antibiotics effective against MRSA. Surprisingly, ME/PI/TZ MICs were corre-

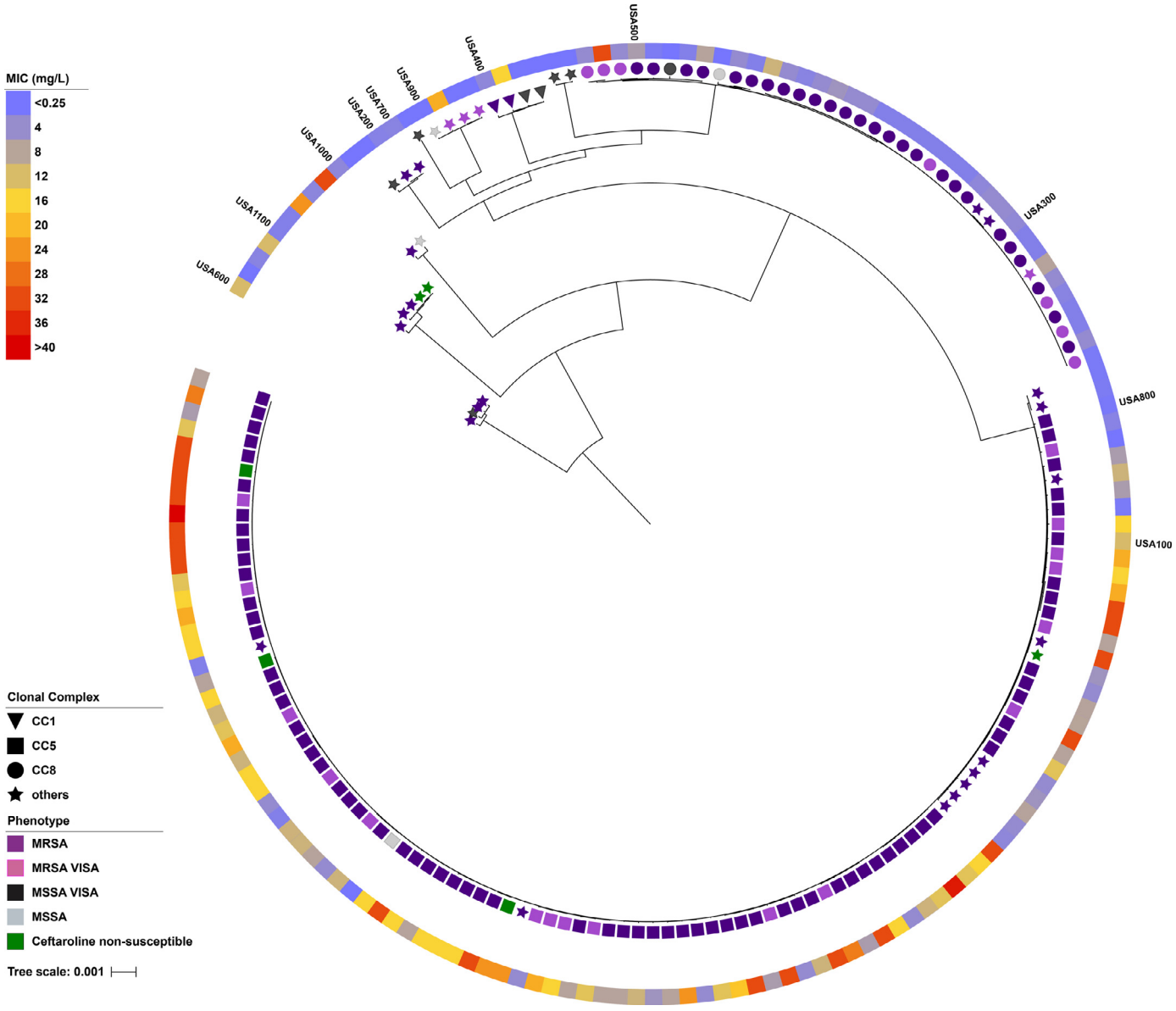

Fig. 2. Rooted core genome phylogeny of *S. aureus* isolates. The phylogeny is based on 1381 genes shared by all isolates at 95% identity. The overlaid heatmap depicts MICs for the ME/PI/TZ combination. Symbol shapes associated with each isolate symbolize the clonal complex as identified by in silico MLST analysis. Symbol colors highlight clinically determined resistance phenotypes (MRSA/MRSA-VISA/MSSA-VISA/MSSA/CPT).

lated to a similar extent with ME and PI resistance in *S. epidermidis* (Figure S2). This might be an effect of the generally lower resistance of *S. epidermidis* isolates to PI compared with MRSA isolates (Tables S2A, F).

The study data indicate that resistance to ME/PI/TZ may be tied to *mecA*/PBP2a activity. Clones carrying the SCC*mec* type IV cassette, which lacks *mecI* and harbors a truncated version of *mecR1*, exhibited increased susceptibility to the triple combination. Previous studies have shown that crosstalk between the *bla*-locus and *mec*-locus additionally affects *mecA* expression, with presence of both operons resulting in high levels of PBP2a activity in the presence of antibiotic stress [17,18]. However, there were no differences in susceptibility to the triple combination between isolates harboring and isolates lacking the *bla*-operon (Figure S6). This observation was independent of whether *mecI*/*R1* were present in the isolate genome. As TZ inhibits the β-lactamase activity encoded by *blaZ*, and disrupts *blaZ* gene expression, the regulatory impact of the *bla*-operon on *mecA* expression may be inhibited by the triple combination [3]. However, this disruption is seemingly counteracted by a functional *mecI*/*mecR1* regulatory system, which may drive *mecA* expression even under *bla*-inactivated conditions and lead to high levels of resistance to ME/PI/TZ. Taken together, these observations indicate that *mecA* expression or overall PBP2a activity may be responsible for the differential resistance observed between clinical isolates.

As previously described, ME/PI/TZ targets multiple nodes in the cell wall machinery, limiting evolution of resistance [3]. High-dose ME has been used to treat technically "resistant" bacterial isolates with high ME MICs, and it is expected that constant unbound serum levels of ME, PI, and TZ of ~20 mg/L can be achieved through continuous infusion, maximizing the time above MIC for these time-dependent drugs [19]. Additional studies are necessary to determine a breakpoint for clinical therapy; however, the present data show that achievable serum concentrations would be effective against >80% of all investigated clinical MRSA isolates (Table 1). The data also indicate that ME/PI/TZ can be particularly

effective against virulent clones of the USA300 lineage for which MICs <12 mg/L (each) were detected. Furthermore, a recent study indicates that USA300 isolates are susceptible to β-lactam + β-lactamase combination if they possess mutations both within the mecA promoter and mecA itself [20]. As this lineage has become a relevant problem in the epidemiology of nosocomial *S. aureus* infections, ME/PI/TZ holds promise for controlling this worrisome trend [13,16].

Acknowledgements

We would like to thank Dr. Andrew Tomaras for *S. aureus* strains, and National Center for Emerging Zoonotic and Infectious Diseases Centers for Disease Control and Prevention for providing the VISA isolates (VISA01-14). We would like to thank Alaric D'Souza for advice for the data analysis, and members of the Dantas lab for helpful discussions of the manuscript. We would like to thank Jessica Hoisington-Lopez, Maria Lynn Jaeger, Brian Koebbe, and Eric Martin of The Edison Family Center for Genome Sciences & Systems Biology, for assistance with high-throughput sequencing and computing.

Declarations

Funding: This work is supported in part by awards to G.D. through the Edward Mallinckrodt, Jr. Foundation (Scholar Award), and from the National Institute of Allergy and Infectious Diseases, and the Eunice Kennedy Shriver National Institute of Child Health & Human Development of the National Institutes of Health (NIH) under award numbers R01AI123394 and R01HD092414, respectively. The content is solely the responsibility of the authors and does not necessarily represent the official views of the funding agencies. The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

Competing Interests: G.D. is a co-founder of Viosera Therapeutics, which is evaluating the meropenem, piperacillin, tazobactam triple combination as a potential commercial therapeutic. A.Y., C-A.D.B., and G.D. have consulting and advisory relationships with Viosera Therapeutics. However, Viosera Therapeutics was not involved in any aspect of the work described herein, including funding or evaluation of any data or analyses.

Ethical Approval: Not required

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2019.105864.

References

[1] Dantes R, Mu Y, Belflower R, Aragon D, Dumyati G, Harrison LH, et al. National burden of invasive methicillin-resistant *Staphylococcus aureus* infections, United States, 2011. JAMA Intern Med 2013;173(21):1970–8.
[2] Lowy FD. Antimicrobial resistance: the example of *Staphylococcus aureus*. J Clin Invest 2003;111(9):1265–73.
[3] Gonzales PR, Pesesky MW, Bouley R, Ballard A, Biddy BA, Suckow MA, et al. Synergistic, collaterally sensitive beta-lactam combinations suppress resistance in MRSA. Nat Chem Biol 2015;11(11):855–61.
[4] Clinical and Laboratory Standards Institute. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. CLSI standard M07 Wayne, PA, USA. 11th ed.; 2018.
[5] Potter RF, Lainhart W, Twentyman J, Wallace MA, Wang B, Burnham CA, et al. Population structure, antibiotic resistance, and uropathogenicity of *Klebsiella variicola*. MBio 2018;9(6).
[6] Kaya H, Hasman H, Larsen J, Stegger M, Johannesen TB, Allesee RL, et al. SCCmecFinder, a web-based tool for typing of staphylococcal cassette chromosome mec in *Staphylococcus aureus* using whole-genome sequence data. mSphere 2018;3(1).
[7] Kobayashi N, Taniguchi K, Urasawa S. Analysis of diversity of mutations in the mecI gene and mecA promoter/operator region of methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis*. Antimicrob Agents Chemother 1998;42(3):717–20.
[8] Yarbrough ML, Lainhart W, Burnham CA. Epidemiology, clinical characteristics, and antimicrobial susceptibility profiles of human clinical isolates of *Staphylococcus intermedius* group. J Clin Microbiol 2018;56(3).
[9] Fuda CC, Fisher JF, Mobashery S. Beta-lactam resistance in *Staphylococcus aureus*: the adaptive resistance of a plastic genome. Cell Mol Life Sci 2005;62(22):2617–33.
[10] Harada D, Nakaminami H, Miyajima E, Sugiyama T, Sasai N, Kitamura Y, et al. Change in genotype of methicillin-resistant *Staphylococcus aureus* (MRSA) affects the antibiogram of hospital-acquired MRSA. J Infect Chemother 2018;24(7):563–9.
[11] Tickler IA, Goering RV, Mediavilla JR, Kreiswirth BN, Tenover FC, Consortium HAI. Continued expansion of USA300-like methicillin-resistant *Staphylococcus aureus* (MRSA) among hospitalized patients in the United States. Diagn Microbiol Infect Dis 2017;88(4):342–7.
[12] Noto MJ, Kreiswirth BN, Monk AB, Archer GL. Gene acquisition at the insertion site for SCCmec, the genomic island conferring methicillin resistance in *Staphylococcus aureus*. J Bacteriol 2008;190(4):1276–83.
[13] Diekema DJ, Richter SS, Heilmann KP, Dohrn CL, Riahi F, Tendolkar S, et al. Continued emergence of USA300 methicillin-resistant *Staphylococcus aureus* in the United States: results from a nationwide surveillance study. Infect Control Hosp Epidemiol 2014;35(3):285–92.
[14] Roemer T, Boone C. Systems-level antimicrobial drug and drug synergy discovery. Nat Chem Biol 2013;9(4):222–31.
[15] Klevens RM, Morrison MA, Nadle J, Petit S, Gershman K, Ray S, et al. Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. JAMA 2007;298(15):1763–71.
[16] Pasquale TR, Jabrocki B, Salstrom SJ, Wiemken TL, Peyani P, Haque NZ, et al. Emergence of methicillin-resistant *Staphylococcus aureus* USA300 genotype as a major cause of late-onset nosocomial pneumonia in intensive care patients in the USA. Int J Infect Dis 2013;17(6):e398–403.
[17] Liu P, Xue H, Wu Z, Ma J, Zhao X. Effect of bla regulators on the susceptible phenotype and phenotypic conversion for oxacillin-susceptible mecA-positive staphylococcal isolates. J Antimicrob Chemother 2016;71(8):2105–12.
[18] Hackbarth CJ, Chambers HF. blaI and blaR1 regulate beta-lactamase and PBP 2a production in methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 1993;37(5):1144–9.
[19] Kuti JL, Moss KM, Nicolau DP, Knauft RF. Empiric treatment of multidrug-resistant Burkholderia cepacia lung exacerbation in a patient with cystic fibrosis: application of pharmacodynamic concepts to meropenem therapy. Pharmacotherapy 2004;24(11):1641–5.
[20] Harrison EM, Ba X, Coll F, Blane B, Restif O, Carvell H, et al. Genomic identification of cryptic susceptibility to penicillins and beta-lactamase inhibitors in methicillin-resistant *Staphylococcus aureus*. Nat Microbiol 2019;4(10):1680–91.