

# Daptomycin-Oxacillin Combinations in Treatment of Experimental Endocarditis Caused by Daptomycin-Nonsusceptible Strains of Methicillin-Resistant *Staphylococcus aureus* with Evolving Oxacillin Susceptibility (the “Seesaw Effect”)<sup>∇</sup>

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***In vivo* development of daptomycin resistance (DAP<sup>r</sup>) among *Staphylococcus aureus* strains, especially methicillin-resistant *S. aureus* (MRSA) strains, in conjunction with clinical treatment failures, has emerged as a major problem. This has raised the question of DAP-based combination regimens to enhance efficacy against such strains. We studied five recent DAP-susceptible (DAP<sup>s</sup>)/DAP<sup>r</sup> clinical MRSA strain pairs obtained from patients who failed DAP monotherapy regimens, as well as one DAP<sup>s</sup>/DAP<sup>r</sup> MRSA strain pair in which the resistant strain was generated by *in vitro* passage in DAP. Of note, we identified a DAP-oxacillin (OX) “seesaw” phenomenon *in vitro* in which development of DAP<sup>r</sup> was accompanied by a concomitant fall in OX resistance, as demonstrated by 3- to 4-fold decreases in the OX MIC, a susceptibility shift by population analyses, and enhanced early killing by OX in time-kill assays. In addition, the combination of DAP and OX exerted modest improvement in *in vitro* bactericidal effects. Using an experimental model of infective endocarditis and two DAP<sup>s</sup>/DAP<sup>r</sup> strain pairs, we demonstrated that (i) OX monotherapy was ineffective at clearing DAP<sup>r</sup> strains from any target tissue in this model (heart valve, kidneys, or spleen) and (ii) DAP-OX combination therapy was highly effective in DAP<sup>r</sup> strain clearances from these organs. The mechanism(s) of the seesaw effect remains to be defined but does not appear to involve excision of the staphylococcal cassette chromosome *mec* (SCC*mec*) that carries *mecA*.**

Daptomycin (DAP) is a cyclic lipopeptide antibiotic active against a wide range of Gram-positive organisms, including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin (VAN)-intermediate *S. aureus* (VISA), and vancomycin-resistant *S. aureus* (VRSA) strains (39, 54). However, an increasing number of reports has described the *in vivo* loss of DAP susceptibility in association with DAP clinical treatment failures in *S. aureus* infections (2, 17, 47). Clearly, innovative approaches to this problem need to be expeditiously applied to enhance/retain DAP efficacy, including dose escalations and combination therapy strategies.

It has been previously reported that in some clinical VISA and VRSA strains, as well as *in vitro*-selected VISA strains, as the VAN MIC was observed to rise, the concomitant MIC to semisynthetic antistaphylococcal penicillins (e.g., oxacillin [OX] or nafcillin) progressively fell. This phenomenon was termed the “seesaw effect” (42–44). This loss of methicillin resistance has most often been due to excision of the staphylococcal cassette chromosome *mec* element (SCC*mec*) that carries *mecA*, the gene encoding penicillin-binding protein 2a (PBP2a), which is responsible for the MRSA phenotype (11, 12, 37). Although uncommon *in vivo* (3), the seesaw effect was

recently observed in a clinical VISA isolate with a retained *mecA* gene obtained during VAN therapy; of interest, after discontinuation of VAN therapy, the methicillin resistance phenotype was restored (32). Importantly, the combination of VAN and nafcillin has been shown to exert synergistic killing activities *in vitro* and in an experimental model of infective endocarditis (IE) (8) with both VISA and VRSA strains (14).

We have found a similar seesaw relationship in *S. aureus* strains that progressively acquire DAP<sup>r</sup> during DAP exposure, and their respective OX MICs declined in parallel (29). In the current study, we investigated (i) the extent of this seesaw effect *in vitro* by utilizing five clinical DAP<sup>s</sup>/DAP<sup>r</sup> strain pairs and (ii) whether combination regimens of DAP and OX would enhance both the *in vitro* and *in vivo* efficacies of these single agents against DAP<sup>r</sup> strains.

Note that although the currently accepted term for reduced *in vitro* susceptibility to daptomycin is “nonsusceptible,” we use the terms “daptomycin resistant” (DAP<sup>r</sup>) and “daptomycin susceptible” (DAP<sup>s</sup>) in this paper for a more facile presentation.

## MATERIALS AND METHODS

**Bacterial strains and growth conditions.** The bacterial strains used in this study are listed in Table 1. A series of isogenic DAP<sup>s</sup>/DAP<sup>r</sup> MRSA strain pairs were used in this study, and represent (i) recent clinical isolates, (ii) *in vitro*-selected DAP<sup>r</sup> strain sets, and (iii) a range of the most common pulsotypes causing clinical infections in the United States (USA100, USA300, USA400, and USA500). Two of the clinically derived strain pairs and the one *in vitro*-derived

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TABLE 1. Strains used in this study

Strain (pulsotype) <sup>a</sup>	SCC <sub>mec</sub> type	Description	MIC (μg/ml)		Reference(s)
			DAP	OX	
CB1118 (USA400)	IV	Selected <i>in vitro</i> by serial passage in DAP	0.5	24	15, 29
CB2205 (USA400)	IV	Selected <i>in vitro</i> by serial passage in DAP	8	6	
MRSA 11/11 (USA300)*	IV	Clinical endocarditis isolate	0.38	32	31
REF2145 (USA300)*	IV	Clinical endocarditis isolate	4	6	
CB1482 (USA500)	IV	Clinical bloodstream isolate	0.5	64	15
CB184 (USA500)	IV	Clinical bloodstream isolate	2	16	
BMC1001 (USA500)	IV	Clinical bloodstream isolate	0.5	>256	This study
BMC1002 (USA500)	IV	Clinical bloodstream isolate	2	128	
CB5053 (USA100)*	II	Clinical bloodstream isolate	0.5	64	This study
CB5054 (USA100)*	II	Clinical bloodstream isolate	2	24	
CB5035 (USA100)	II	Clinical bloodstream isolate	0.38	192	This study
CB5036 (USA100)	II	Clinical bloodstream isolate	2	96	

<sup>a</sup> \*, strain used in the *in vivo* endocarditis studies.

strain pair have been previously reported (15, 29, 31). The remaining three clinically derived strain pairs were randomly selected from the Cubist Pharmaceuticals Registry collection of such bloodstream isolates (courtesy of Aileen Rubio, Cubist Pharmaceuticals). Molecular typing by pulsed-field gel electrophoresis using the SmaI restriction enzyme (data not shown) and SCC<sub>mec</sub> typing (Table 1) confirmed that each DAP<sup>r</sup> strain was clonal to the respective parental isolate.

All *S. aureus* strains were grown in either tryptic soy broth (TSB; Difco Laboratories, Detroit, MI) or Mueller-Hinton broth (MHB; Difco Laboratories).

**Antimicrobial agents and MIC testing.** DAP and OX were purchased from Cubist Pharmaceuticals (Lexington, MA) and Sigma Chemical Co. (St. Louis, MO), respectively. The MICs of DAP and OX were determined by standard micro-Etest according to the manufacturer's recommended protocols. A minimum of two independent experimental runs was performed. The Clinical and Laboratory Standards Institute (CLSI) has established MIC guidelines for DAP susceptibility ( $\leq 1$  μg/ml) only and not for DAP resistance (9). Thus, *S. aureus* strains with MICs of  $\geq 2$  μg/ml are termed DAP<sup>r</sup> in this report.

**Population analysis.** Population analysis of the strain sets was performed with both DAP (0 to 32 μg/ml) and OX (0 to 512 μg/ml) as described before (30, 57). A minimum of two independent experimental runs was performed.

***In vitro* time-kill curves.** Time-kill experiments (0, 2, 4, 6, and 24 h) were performed using MHB with an initial inoculum of 10<sup>6</sup> CFU/ml in the presence of sublethal to lethal concentrations of OX, based on individual strain Etest MIC data. This relatively high inoculum (10<sup>6</sup> CFU) was chosen to encompass bacterial counts commonly achieved in all target tissues of animals with experimental IE (52, 53, 55, 56). A minimum of two independent experimental runs was performed.

**Synergy kill curves.** Bactericidal synergy assays for DAP and OX were performed using MHB as described before (36). Briefly, we employed an initial inoculum of  $\sim 10^6$  CFU/ml in the presence of one-quarter of the MIC of OX in combination with one-quarter or one-half of the MIC for DAP (relative to each single agent). Synergy was defined as previously described (36). A minimum of two independent experimental runs was performed.

***mecA* retention and SCC<sub>mec</sub> typing analyses.** *mecA* is carried on an SCC<sub>mec</sub> genetic element that integrates into the chromosome (20, 21). Diversity in SCC<sub>mec</sub> architecture has led to the delineation of eight evolutionarily distinct types (20). Detection of *mecA* and SCC<sub>mec</sub> typing for the study strains were performed by PCR-based assays as described previously (5); SCC<sub>mec</sub> types were assigned using recent guidelines according to the types of *mec* class and *cer* complex detected (21).

***In vivo* experimental IE.** A well-characterized catheter-induced rabbit model of aortic infective endocarditis (IE) was used as described previously (52, 53, 55) to assess whether combination therapy regimens of DAP and OX would enhance single-drug efficacy against DAP<sup>r</sup> strains. In brief, 24 h after aortic catheter placement, we infected equivalent groups of animals intravenously with a 95% infective dose (ID<sub>95</sub>) inoculum for the respective infecting *S. aureus* strains as established in pilot studies ( $\sim 2 \times 10^5$  CFU/animal). The two strain sets selected

(MRSA 11/11-REF2145 and CB5053-CB5054) were prioritized based on the fact that (i) they were both clinical bacteremic or IE-causing isolates, (ii) each demonstrated the DAP-OX seesaw effect, and (iii) they were virulent in the IE model based on pilot studies.

At 24 h after induction of IE, animals were randomized into groups with the following treatments: none (untreated controls), DAP alone at 12 mg/kg once daily, OX alone at 200 mg/kg intramuscularly (i.m.) three times a day (t.i.d.), and DAP-OX combination at the doses listed above. The DAP dose regimen used mimics the currently approved regimen for treating serious infections in humans (38); the OX dose was used as in prior efficacy studies of *S. aureus* IE in the same model (19, 51). After 3 days of treatment, animals in each group were sacrificed and target organs (cardiac vegetations, kidneys, and spleen) quantitatively cultured. The mean log<sub>10</sub> CFU/g ( $\pm$  standard deviation [SD]) was calculated for each tissue in each group for statistical comparisons.

No serum antibiotic levels were obtained, as the pharmacokinetics of the above dose regimens in this model have been previously published (7, 19, 51).

**Statistical analysis.** To statistically compare *S. aureus* tissue densities among the various groups, we used the Kruskal-Wallis analysis of variance (ANOVA) test with the Tukey post hoc correction for multiple comparisons. Significance was determined at a *P* value of  $< 0.05$ .

## RESULTS

**DAP and OX MICs.** As shown in Table 1, we observed an *in vitro* DAP-OX seesaw relationship in all six MRSA strain pairs, in which the OX MICs declined in parallel with the emergence of DAP<sup>r</sup>. None of the OX MICs fell below the breakpoint for *in vitro* susceptibility ( $< 4$  μg/ml).

**Population analyses.** For all six DAP<sup>r</sup> strains, the DAP-associated population curves were shifted to the right (more resistant), while the OX-associated population curves were uniformly shifted to the left (more susceptible) (Fig. 1A to F).

***In vitro* OX time-kill curves.** As shown in Fig. 2A to F, in all strain pairs, the DAP<sup>r</sup> strains became more susceptible to the killing effect of OX by 4 or 6 h exposure. The maximal extent of the bactericidal effects at these time points generally ranged from 1 to 2 log<sub>10</sub> CFU/ml. Of note, OX prevented regrowth of the DAP<sup>r</sup> strains CB2205, REF2145, and BMC1002, even after 24 h of incubation at the highest OX concentrations tested (4, 32, and 256 μg/ml, respectively).

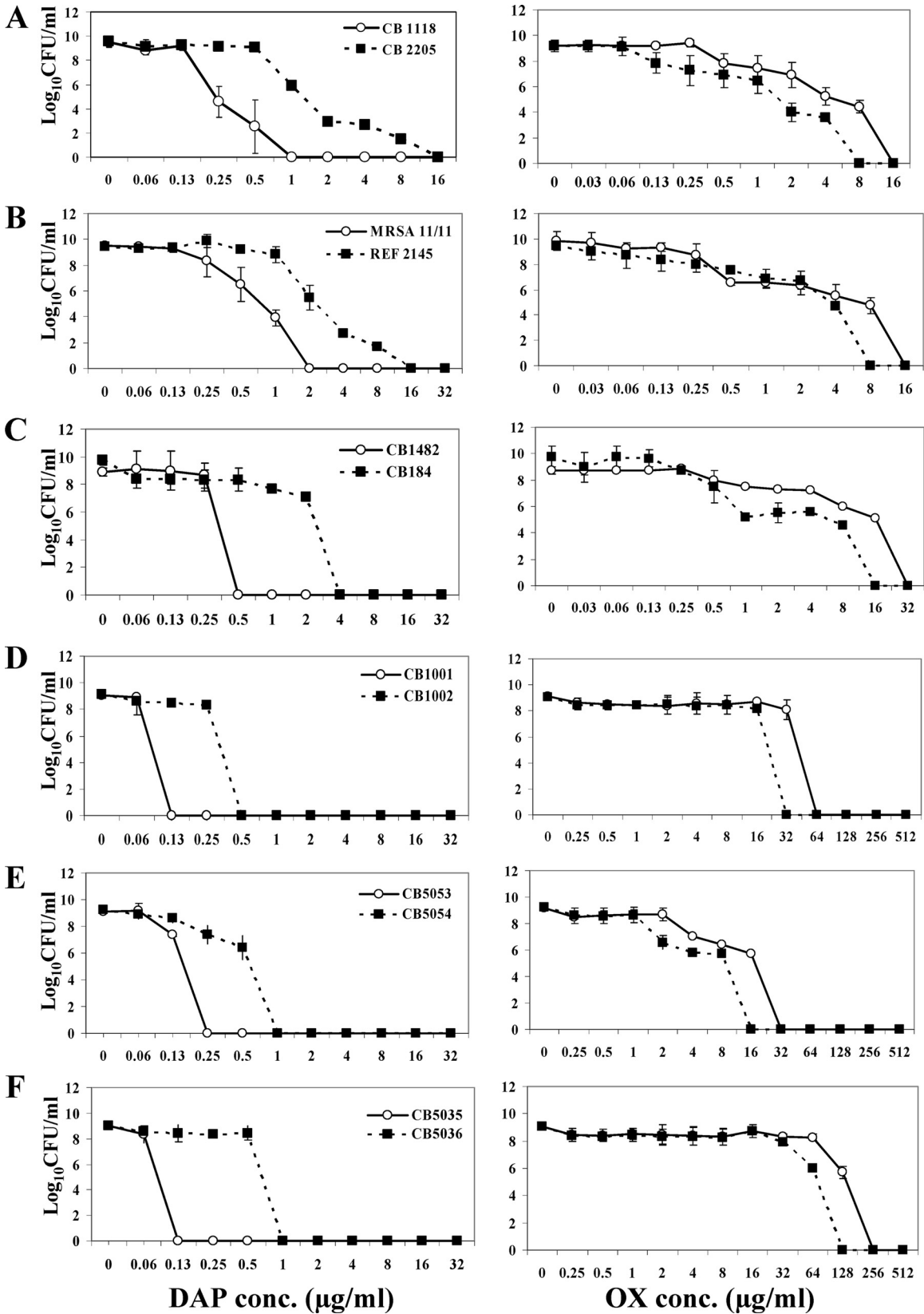


FIG. 1. Population analyses of study strains upon exposure to a range of DAP (left panel) or OX (right panel) concentrations. These data represent the means ( $\pm$  SD) for two separate assays. conc., concentration.

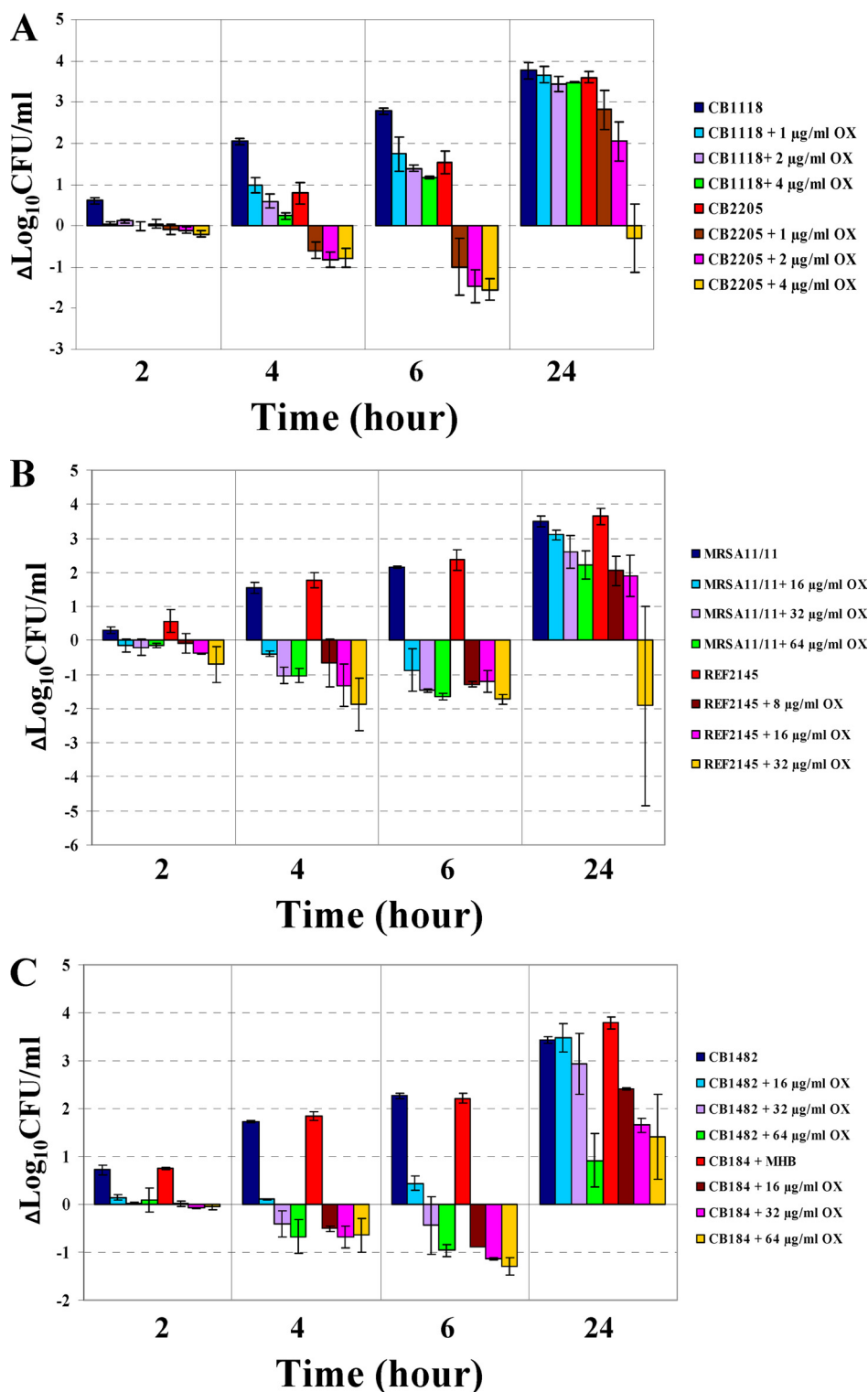


FIG. 2. OX time-kill analyses. Six DAP<sup>S</sup> and DAP<sup>F</sup> strain pairs were used for these experiments. (A) CB1118-CB2205; (B) MRSA 11/11-REF2145; (C) CB1482-CB184; (D) BMC1001-BMC1002; (E) CB5053-CB5054; (F) CB5035-CB5036. Time-kill experiments were performed using Mueller-Hinton broth with a 10<sup>6</sup>-CFU/ml inoculum in the presence of 0 to 256  $\mu\text{g/ml}$  OX.

**Synergy kill curves.** Two clinical DAP<sup>S</sup>/DAP<sup>F</sup> isogenic MRSA strain pairs were selected to determine the potential enhanced *in vitro* efficacy of DAP-OX combinations (compared to DAP or OX alone). The combination of DAP at

one-quarter of the MIC plus OX at one-quarter of the MIC increased the bactericidal activity in the DAP<sup>F</sup> MRSA strains REF2145 and CB5054 at 24 h (Fig. 3A and B). At one-half of the MIC of DAP plus one-quarter of the MIC of

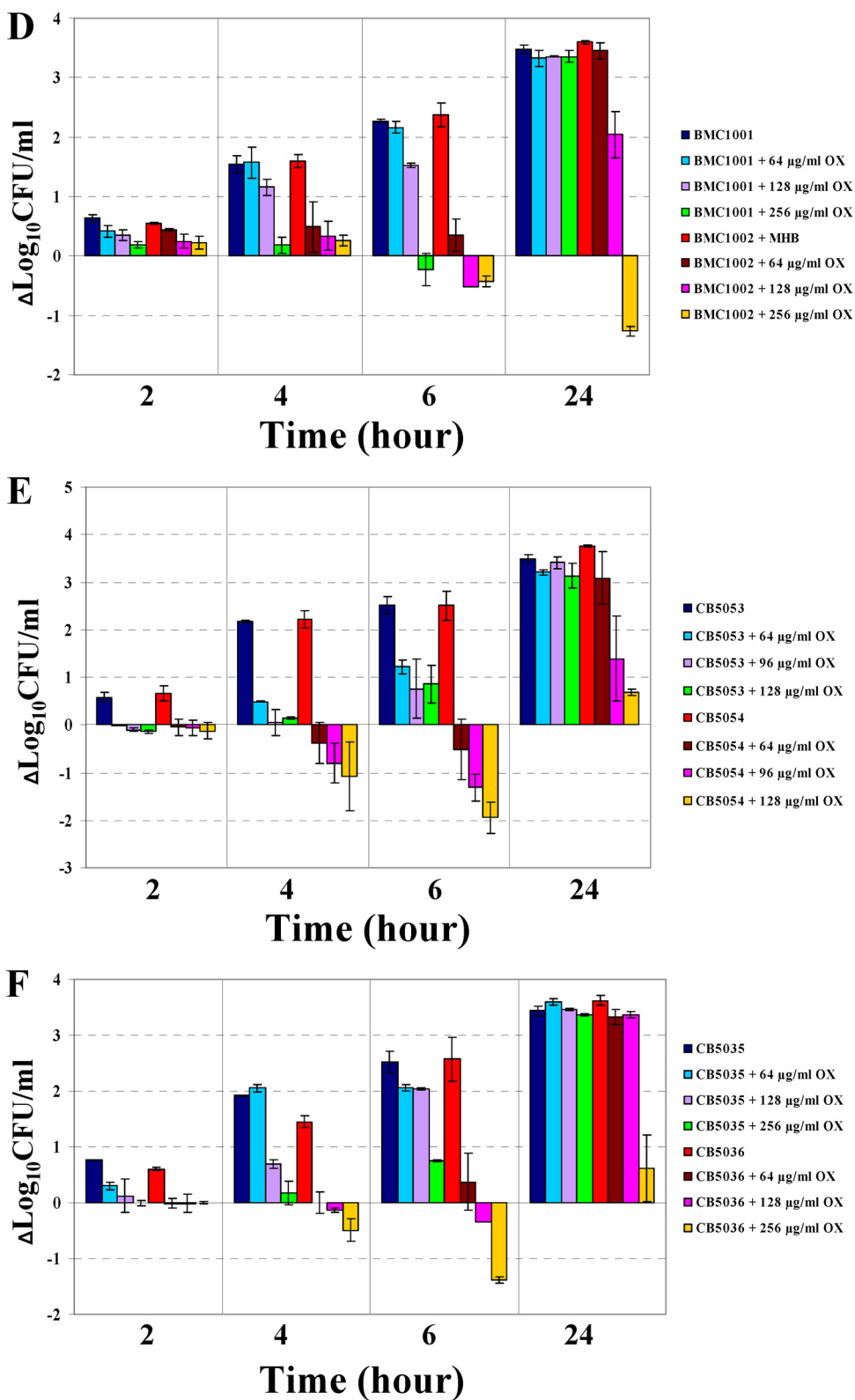


FIG. 2—Continued.

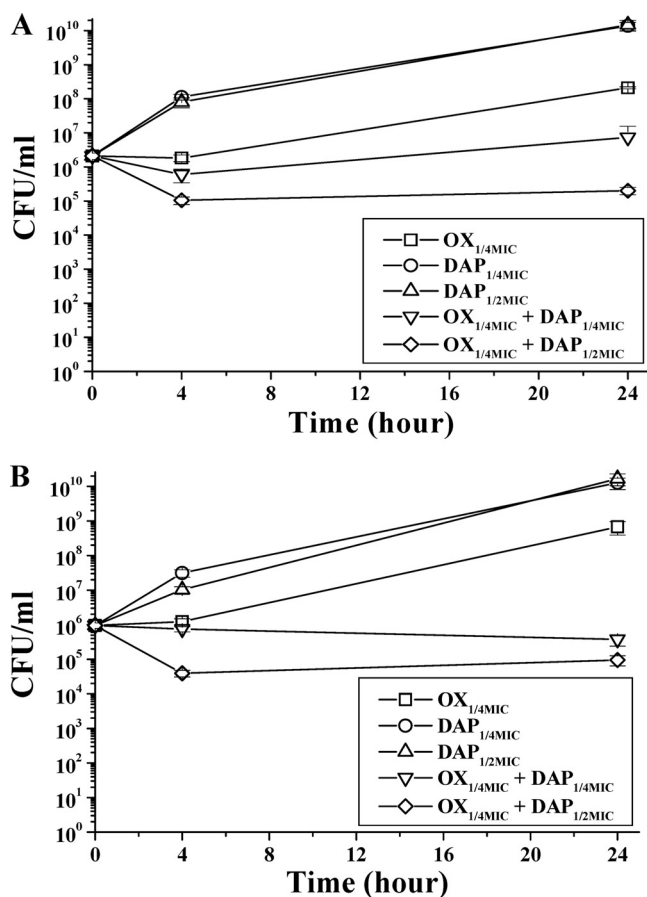


FIG. 3. *In vitro* synergy kill curves for DAP<sup>r</sup> REF2145 (A) and CB5054 (B) strains. These two strain sets were used in the experimental IE studies detailed in the text. The growth curve for the no-antibiotic growth control for these experiments is not shown but was identical to the growth curve for both strains with one-quarter of the MIC of DAP.

OX, there was a further increase in killing of both DAP<sup>r</sup> strains relative to that by the single agents.

***mecA* retention and SCC*mec* typing analyses.** We screened for both the retention of *mecA* and the SCC*mec* type present in each pair of isolates. As shown in Table 1, each DAP<sup>r</sup> isolate retained carriage of *mecA* like its parental isolate. Isolate pairs with pulsed-field gel types USA300, USA400, and USA500 carried SCC*mec* type IV (4). In contrast, USA100 isolates carried SCC*mec* type II. The respective SCC*mec* types remained the same in all isolate pairs.

***In vivo* IE model.** The two DAP<sup>s</sup> and DAP<sup>r</sup> pairs MRSA 11/11-REF2145 and CB5053-CB5054 had equivalent abilities to induce experimental IE and then disseminate hematogenously to kidneys and spleens. As shown in Table 2, for both DAP<sup>r</sup> strains, bacterial densities achieved in vegetations were significantly higher than those in kidneys and spleens for untreated control animals at 24 h postinfection ( $P < 0.05$ ). Following DAP treatment, the bacterial densities of DAP<sup>s</sup> parental strains MRSA 11/11 and CB5053 were significant decreased in all three target tissues, with each tissue nearly sterilized. Because of the high efficacy of DAP treatment alone against both DAP<sup>s</sup> parental strains, DAP-OX combinations were not evaluated. In contrast, the respective DAP<sup>r</sup> strains, REF2145 and CB5054, undergoing DAP monotherapy showed no significant differences in cell densities in any tissues relative to the untreated controls. In addition, although the DAP<sup>r</sup> strains exhibited decreased OX MICs *in vitro* relative to the DAP<sup>s</sup> parental strains, OX treatment alone caused no substantial reduction in bacterial densities in any target tissues. Importantly, the combination of DAP and OX exhibited the best efficacy overall in reducing DAP<sup>r</sup> cell densities in all three target tissues, better than DAP or OX monotherapy in these animals.

TABLE 2. MRSA 11/11-REF2145 and CB5053-CB5054 counts in target tissues with DAP and/or OX treatment in the IE model

Group (no. of animals) <sup>a</sup>	<i>S. aureus</i> density (log <sub>10</sub> CFU/g tissue) in <sup>b</sup> :		
	Vegetation	Kidney	Spleen
Control without treatment			
MRSA 11/11 (8)	7.74 ± 0.79	5.51 ± 0.63	5.28 ± 0.56
MRSA REF2145 (8)	8.84 ± 0.56	6.29 ± 0.67	6.15 ± 0.65
CB5053 (6)	7.74 ± 0.80	5.98 ± 0.98	5.84 ± 0.53
CB5054 (6)	8.76 ± 0.18	6.63 ± 0.75	5.95 ± 0.65
Treatment			
MRSA 11/11 (12 mg/kg DAP i.v. once daily) (7)	1.58 ± 0.59	0.53 ± 0.28	0.60 ± 0.16
MRSA REF2145 (12 mg/kg DAP i.v. once daily) (7)	7.93 ± 1.12*	6.74 ± 0.71*	5.52 ± 1.23*
MRSA 11/11 (200 mg/kg OX i.m. t.i.d.) (8)	5.96 ± 2.23	3.99 ± 1.43	4.07 ± 1.17
MRSA REF2145 (200 mg/kg OX i.m. t.i.d.) (8)	7.21 ± 1.39	5.09 ± 1.99	4.79 ± 1.51
MRSA REF2145 (DAP-OX) (8)	4.20 ± 1.61**	2.90 ± 1.50**	3.12 ± 0.81**
CB5053 (12 mg/kg DAP i.v. once daily) (6)	1.71 ± 0.96	0.95 ± 0.60	1.07 ± 0.92
CB5054 (12 mg/kg DAP i.v. once daily) (6)	8.34 ± 0.83*	6.32 ± 1.31*	5.78 ± 1.29*
CB5053 (200 mg/kg OX i.m. t.i.d.) (7)	6.18 ± 1.74	4.01 ± 1.82	3.88 ± 1.19
CB5054 (200 mg/kg OX i.m. t.i.d.) (6)	8.96 ± 0.44	6.35 ± 1.02	5.68 ± 0.80
CB5054 (DAP-OX) (10)	5.70 ± 1.98**	3.88 ± 1.25**	3.99 ± 0.95**

<sup>a</sup> 12 mg/kg DAP intravenously (i.v.) once daily is equivalent to a dose of 6 mg/kg once daily in humans (7).

<sup>b</sup> \*,  $P$  value of  $<0.001$  relative to the value for DAP<sup>s</sup> MRSA 11/11 or CB5053 with DAP treatment; \*\*,  $P$  value of  $<0.05$  relative to the value for REF2145 or CB5054 with OX-only or DAP-only treatment.

## DISCUSSION

DAP is a semisynthetic lipopeptide antibiotic approved by the FDA in 2003 for use in a wide variety of *S. aureus* infections (50). DAP targets the *S. aureus* cytoplasmic membrane to initiate its bactericidal activity in a calcium-dependent manner, resulting in potassium leakage, inhibition of DNA, RNA, and protein synthesis, and finally cell death (40, 49, 50). Due to its potent staphylocidal activity, DAP has become a clinical mainstay of anti-MRSA therapy, especially in patients with persistent bacteremia (2, 26, 39, 40). However, there have been a number of reports in which initially DAP<sup>s</sup> *S. aureus* strains have developed DAP<sup>r</sup> phenotypes in association with clinical treatment failures (17, 22, 23, 27, 34, 47). Clearly, innovative approaches to this problem, including dose escalations and/or combination therapy strategies, need to be expeditiously applied to enhance/retain DAP efficacy.

It was initially observed that in some *in vitro*-selected VISA mutants, gradual increases in VAN MICs were accompanied by parallel decreases in the levels of  $\beta$ -lactam resistance (44). This seesaw phenotype was subsequently also observed for some clinical VISA strains (44, 46). It appears that the seesaw phenomenon for VAN and  $\beta$ -lactams can be caused by multiple factors (e.g., *mecA*-dependent or *mecA*-independent factors), and its demonstration is reliant on the presence of VAN (37, 41, 45). Interestingly, by both MIC and population analyses, we found a similar seesaw relationship in MRSA strains which progressively acquired DAP<sup>r</sup> during DAP exposures, i.e., their respective OX MICs declined in parallel to increasing DAP MICs. The DAP-OX seesaw phenotype was observed in all five DAP<sup>s</sup>/DAP<sup>r</sup> clinical strain pairs tested, as well as an *in vitro*-selected DAP<sup>s</sup>/DAP<sup>r</sup> pair. Furthermore, all the DAP<sup>r</sup> strains became more susceptible to early killing by OX *in vitro* than their respective DAP<sup>s</sup> parental strains.

Recently, Rand and Houck (36) showed consistent synergy *in vitro* between DAP and OX by time-kill curve analyses. However, all strains were DAP<sup>s</sup>, and population analysis was not performed in their study. More recently, Credito et al. and others evaluated the synergistic effect of DAP and gentamicin or rifampin also by time-kill analyses and found synergy between DAP and gentamicin against methicillin-susceptible *S. aureus* (MSSA), MRSA, VISA, and VRSA strains (10, 48). In addition, LaPlante and Rybak (25), using an *in vitro* pharmacodynamic model which mimics IE, showed enhanced bactericidal activity of combinations of DAP and low-dose gentamicin. However, unlike for these *in vitro* results, the addition of gentamicin failed to enhance the effectiveness of DAP in the treatment of experimental MRSA-induced IE (28).

These findings and our own *in vitro* data led us to investigate whether combination therapy regimens of DAP-OX would enhance the *in vitro* and/or *in vivo* efficacy over that of each single agent against DAP<sup>r</sup> strains. In the current study, the combination of DAP and OX was found to increase the early *in vitro* bactericidal activity relative to that of DAP or OX alone in DAP<sup>r</sup> strains. Of note, using two clinical DAP<sup>s</sup>/DAP<sup>r</sup> isogenic pairs, we showed that the combination of DAP and OX yielded significantly enhanced bacterial clearance from all three target tissues in an experimental IE model relative to monotherapies in the context of only 3 days of treatment. However, despite the exhibition of a notable *in vitro* seesaw

effect of evolving OX susceptibility in each DAP<sup>r</sup> strain by both MIC and population analyses, OX treatment alone failed to reduce *S. aureus* cell densities in any target organ. The reason(s) for this *in vitro-in vivo* disparity may well be multifactorial. For example, a host factor(s) may impact the maintenance of the seesaw effect *in vivo*. Thus, the increased OX susceptibility phenotype may not be sustainable *in vivo*. Further, we have previously noted the coemergence of DAP<sup>r</sup> with resistance to several innate host defense cationic peptides from polymorphonuclear leukocytes (PMNs) and platelets that are important in the pathogenesis of endovascular infections (22, 57). It is conceivable that such microbial resistances may buttress organism survival and override any enhanced bactericidal effects of DAP and OX. In addition, it should be noted that despite the seesaw effect, the two DAP<sup>r</sup> strains used in the IE model still exhibited *in vitro* resistance to OX (MIC, >4  $\mu\text{g/ml}$ ). Therefore, the OX treatment regimen chosen may not have provided an adequate pharmacokinetic profile to exert an enhanced killing impact as monotherapy. We recognize that the magnitude of the enhanced bactericidal effect of DAP and OX *in vitro* against the DAP<sup>r</sup> strains we tested was modest, suggesting that the *in vivo* synergy detected may have involved key host defense factors.

The mechanism(s) of the seesaw effect are not well understood for either vancomycin-OX or DAP-OX. We ruled out the possibility of *mecA* excision in the DAP<sup>r</sup> isolates in the current study. Also, the SCC<sub>mec</sub> types remained identical within each DAP<sup>s</sup>/DAP<sup>r</sup> pair. There are many possibilities that may explain the decreased OX resistance phenotype of the DAP<sup>r</sup> isolates studied in this investigation. The mechanism of the MRSA phenotype is mainly due to the acquisition of an SCC<sub>mec</sub> that carries *mecA*, encoding PBP2a. This enzyme is a transpeptidase with low affinity for  $\beta$ -lactams, allowing it to carry out transpeptidation reactions required for peptidoglycan assembly in the presence of these usually lethal agents (21, 35). However, expression of the MRSA phenotype is multifactorial. In addition to PBP2a, the MRSA phenotype requires the transglycosylase activity supplied by the PBP2 housekeeping gene (35). Additionally, other cell wall biosynthesis enzymes, called factors essential for methicillin resistance (encoded by *fem*), are required for a *mecA*-mediated MRSA phenotype (1). In addition, the two-component regulatory system VraSR (24) is essential for the MRSA phenotype in a *mecA*-independent fashion (6). Finally, the dedicated regulators of *mecA* gene transcription, MecR1/MecI and BlaI/BlaR1, can also influence the MRSA phenotype via modulation of *mecA* expression (18). Thus, effective repression of *mecA* expression by MecI, with poor MecR1-mediated induction, can influence the MRSA phenotype (18). Further, the level of PBP2a produced does not necessarily correlate with the level of  $\beta$ -lactam resistance. For example, strains can express *mecA* but still exhibit a methicillin-susceptible phenotype (6, 33). SCC<sub>mec</sub> type IV lacks *mecI*, whereas SCC<sub>mec</sub> type II contains *mecI* and *mecR1*. However, most SCC<sub>mec</sub> type II-containing strains have defective *mecA* repression due to point mutations either in the *mecA* promoter or in the *mecI* open reading frame (ORF); the function of *mecI* and *mecR1* is replaced by *blaI* and *blaR1*, which has less effective repression and more effective induction of *mecA* (13, 16). It remains to be determined whether expression profiles of *mecA*, *mecI* and *mecR1*, or *blaI*

and *blaR1* differ between our DAP<sup>s</sup>/DAP<sup>r</sup> strain pairs. Given the complexity of factors which can ultimately determine the MRSA phenotype, many possibilities need to be explored in order to explain the decreased OX resistance phenotype commonly associated with evolving DAP<sup>r</sup>. These studies are in progress in our laboratories.

Collectively, these results suggest that combination therapy regimens of DAP and OX has enhanced *in vivo* efficacy relative to DAP monotherapy in DAP<sup>r</sup> strains which exhibit the DAP-OX seesaw phenomenon *in vitro*. This combination antibiotic approach may be relevant to salvaging DAP therapy in patients with evolving increases in DAP MICs during treatment, especially when OX MICs decrease in parallel.

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