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EXPERIMENTAL THERAPEUTICS

Evaluation of Omadacycline Alone and in Combination with Rifampin against *Staphylococcus aureus* and *Staphylococcus epidermidis* in an *In Vitro* Pharmacokinetic/Pharmacodynamic Biofilm Model

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ABSTRACT Biofilm-associated infections lead to substantial morbidity. Omadacycline (OMC) is a novel aminomethylcycline with potent *in vitro* activity against *Staphylococcus aureus* and *Staphylococcus epidermidis*, but data surrounding its use in biofilm-associated infections are lacking. We investigated the activity of OMC alone and in combination with rifampin (RIF) against 20 clinical strains of staphylococci in multiple *in vitro* biofilm analyses, including an *in vitro* pharmacokinetic/pharmacodynamic (PK/PD) CDC biofilm reactor (CBR) model (simulating human exposures). The observed MICs for OMC demonstrated potent activity against the evaluated strains (0.125 to 1 mg/L), with an increase of MICs generally observed in the presence of biofilm (0.25 to >64 mg/L). Furthermore, RIF was shown to reduce OMC biofilm MICs (bMICs) in 90% of strains, and OMC plus RIF combination in biofilm time-kill analyses (TKAs) exhibited synergistic activity in most of the strains. Within the PK/PD CBR model, OMC monotherapy primarily displayed bacteriostatic activity, while RIF monotherapy generally exhibited initial bacterial eradication, followed by rapid regrowth likely due to the emergence of RIF resistance (RIF bMIC, >64 mg/L). However, the combination of OMC plus RIF produced rapid and sustained bactericidal activity in nearly all the strains (3.76 to 4.03 $\log_{10}$ CFU/cm^2 reductions from starting inoculum in strains in which bactericidal activity was reached). Furthermore, OMC was shown to prevent the emergence of RIF resistance. Our data provide preliminary evidence that OMC in combination with RIF could be a viable option for biofilm-associated infections with *S. aureus* and *S. epidermidis*. Further research involving OMC in biofilm-associated infections is warranted.

KEYWORDS biofilm, omadacycline, rifampin

Despite enhancements in bioengineering and perioperative antimicrobial prophylaxis, bacterial infections associated with indwelling medical devices represent a substantial cause of morbidity and lead to significant health care expenditures (1). *Staphylococcus aureus* and coagulase-negative staphylococci, predominately *Staphylococcus epidermidis*, are two of the most common pathogen types associated with orthopedic implant infections and other medical device infections (MDIs) (1–5). Methicillin-resistant *S. aureus* (MRSA) has increased steadily since its clinical debut in the 1960s, and the Centers for Disease Control and Prevention (CDC) lists MRSA as a serious threat with greater than 300,000 cases in hospitalized patients and 10,000 deaths reported annually (6). *S. epidermidis* is less frequently pathogenic but can cause opportunistic infections in particular scenarios (7). Given that

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these organisms are human skin commensals, the insertion of prostheses or other medical devices through the skin can allow adherence, imposing the risk of MDIs (8).

Medical device infections are commonly associated with microorganisms that grow in biofilm, which is a phenotypic resistance mechanism composed of a complex bacterial community enclosed within a polymeric matrix that shields bacteria from the host immune system and antimicrobials (1, 9, 10). Importantly, *S. aureus* and *S. epidermidis* have been shown to be among the most common bacteria that produce biofilm (11). Given the complexities in treating infections associated with staphylococci biofilm, adjunctive antimicrobial therapy with rifampin (RIF) or RIF derivatives are commonly recommended and employed in the clinical realm due to their role in biofilm penetration (12–15). Furthermore, RIF has been shown to be synergistic with other commonly used antibiotics for methicillin-resistant *Staphylococcus* sp. infections embedded in biofilm, including tetracycline derivatives (16–20).

Omadacycline (OMC) is a novel aminomethylcycline within the tetracycline class that is Food and Drug Administration (FDA) approved for community-acquired bacterial pneumonia and acute bacterial skin and skin structure infections (21). OMC has demonstrated potent *in vitro* activity against many Gram-positive organisms, including *S. aureus* and *S. epidermidis* (22). Given its availability in both an oral and intravenous (i.v.) formulation, potency against Gram-positive organisms commonly associated with MDIs, and relative safety, OMC represents an attractive option for treating biofilm-associated infections (21).

To better understand the potential of using OMC to treat biofilm-associated infections, we evaluated OMC in a set of *in vitro* biofilm experiments. Specifically, we assessed OMC, both alone and in combination with RIF, in terms of potency, bacterial eradication potential, and the ability to prevent the emergence of resistance. This assessment was accomplished throughout multiple *in vitro* biofilm analyses, including combination biofilm MICs (bMICs), biofilm time-kill analyses (TKAs), and pharmacokinetic/pharmacodynamic (PK/PD) CDC biofilm reactor (CBR) models (simulating human antibiotic exposures) with two types of materials commonly found in medical devices (polyurethane and titanium).

RESULTS

Susceptibility testing. To evaluate the susceptibility of 20 randomly selected *Staphylococcus* sp. clinical isolates (*S. aureus*, $n = 10$; and *S. epidermidis*, $n = 10$) against OMC and RIF, we determined the susceptibilities in the presence and absence of biofilm. OMC demonstrated potent activity against the evaluated strains in the planktonic state (0.125 to 1 mg/L), with an increase in MIC generally observed in the presence of biofilm (0.25 to >64 mg/L). RIF planktonic MICs ranged from <0.0019 to >64 mg/L with an increase also observed in the presence of biofilm (0.0039 to >64 mg/L). Next, OMC bMICs were performed in the presence of RIF to determine the potential for RIF to lower the OMC bMIC. RIF was shown to reduce OMC MICs in 100% of *S. aureus* strains (2- to >32-fold) and 80% of *S. epidermidis* strains (4- to 128-fold). Specific details regarding susceptibility information for OMC in both the planktonic and biofilm state, as well as combination bMICs, are shown in Table 1.

Biofilm time-kill analyses. The eight isolates (*S. aureus*, $n = 4$; *S. epidermidis*, $n = 4$) with the greatest fold reduction demonstrated in combination bMICs were chosen for evaluation in biofilm TKAs. Overall, for the OMC and RIF monotherapy regimens, the biofilm TKAs generally demonstrated initial bacterial kill with regrowth observed, while the combination of OMC plus RIF demonstrated synergistic activity in 6/8 (75%) of evaluated isolates and bactericidal activity in 5/8 (62.5%) of isolates. When combining OMC plus RIF for *S. aureus* isolates R8015 and N315, the combination demonstrated synergistic activity against both strains while also demonstrating bactericidal activity against R8015. The combination of OMC plus RIF demonstrated both synergistic and bactericidal activity against *S. epidermidis* isolates R145 and R4101. Data for the TKAs can be found in Fig. S1 in the supplemental material.

***In vitro* PK/PD model.** The four isolates (*S. aureus*, $n = 2$; and *S. epidermidis*, $n = 2$) that demonstrated the greatest synergistic effects and/or bactericidal activity in the biofilm TKAs were chosen for evaluation in the CDC PK/PD biofilm reactor model. To determine the impact of humanized exposures of OMC, alone and in combination with

TABLE 1 MIC and bMIC values[a] for *Staphylococcus aureus* and *Staphylococcus epidermidis* isolates

Isolate	OMC MIC	OMC bMIC	OMC + RIF bMIC
S. aureus			
N315	0.125	0.5	<0.03
MW2	0.25	0.5	0.06
JH1	0.25	0.5	0.06
494	0.5	1	0.06
SA113	0.125	0.5	0.25
SH1000	0.25	1	<0.03
R8014	0.25	0.5	0.06
R8015	0.125	0.5	0.06
D592	0.25	0.5	0.25
D712	0.125	1	0.25
S. epidermidis			
R145	0.25	8	0.06
R227	0.125	0.25	0.06
R263	0.125	0.25	0.06
R271	0.25	0.25	0.06
BC1004	0.25	0.25	0.06
NRS 122	1	>64	>64
NRS 7	0.5	0.5	0.5
NRS 101	0.125	1	0.125
R4101	0.125	0.5	0.06
R7724	0.125	0.25	0.06

[a]All values have units of mg/L.

RIF, we conducted a series of experiments in the biofilm PK/PD model over 96 h which included biofilm-embedded *S. aureus* and *S. epidermidis* on both polyurethane and titanium coupons. Overall, there were no major observable differences in colony counts between the two different coupons for all regimens over the duration of the study (Fig. 1 and 2).

Against *S. aureus* isolates N315 and R8015, the quantitative changes in $\log_{10}$ CFU/cm^2 are shown in Fig. 1. N315 is an isolate with an OMC bMIC of 0.5 mg/L and RIF bMIC of 0.0078 mg/L. OMC and RIF monotherapy demonstrated bacteriostatic effects with final reductions observed at 96 h being $-\Delta 0.15$ $\log_{10}$ CFU/cm^2 and $+\Delta 0.38$ $\log_{10}$ CFU/cm^2, respectively. The combination of OMC plus RIF demonstrated both an enhancement of activity with reductions of $-\Delta 3.70$ $\log_{10}$ CFU/cm^2 from the most active single agent and nearly bactericidal effects with a 96-h bacterial reduction of $-\Delta 2.91$ $\log_{10}$ CFU/cm^2 from the starting inoculum. R8015 is an isolate with an OMC bMIC of 0.5 mg/L and RIF bMIC of 0.015 mg/L. OMC and RIF monotherapy demonstrated bacteriostatic effects with final reductions observed at 96 h being $-\Delta 1.23$ $\log_{10}$ CFU/cm^2 and $-\Delta 0.51$ $\log_{10}$ CFU/cm^2, respectively. The combination of OMC plus RIF demonstrated both an enhancement of activity with reductions of $-\Delta 2.93$ $\log_{10}$ CFU/cm^2 from the most active single agent and sustained bactericidal effects with a 96-h bacterial reduction of $-\Delta 3.76$ $\log_{10}$ CFU/cm^2 from the starting inoculum.

Against *S. epidermidis* isolates R145 and R4101, the quantitative changes in $\log_{10}$ CFU/cm^2 are shown in Fig. 2. R145 is an isolate with an OMC bMIC of 8 mg/L and RIF bMIC of 0.125 mg/L. No significant activity was observed with OMC or RIF monotherapy, except for rapid reductions in bacterial counts with RIF, followed by a rapid regrowth. Despite initial bactericidal activity that was observed with OMC plus RIF at 24 to 32 h with reductions in $-\Delta 3.53$-3.72 $\log_{10}$ CFU/cm^2, bacterial regrowth occurred. R4101 is an isolate with an OMC bMIC of 0.5 mg/L and RIF bMIC of 0.0078 mg/L. Overall, OMC monotherapy demonstrated bacteriostatic effects with final reductions observed at 96 h being $-\Delta 1.54$ $\log_{10}$ CFU/cm^2. RIF monotherapy demonstrated rapid reductions in bacterial counts with rapid regrowth. The combination of OMC plus RIF demonstrated both enhancement with reductions of $-\Delta 2.46$ $\log_{10}$ CFU/cm^2 from the most active single agent and sustained bactericidal effects with a 96-h bacterial reduction of $-\Delta 4.03$ $\log_{10}$ CFU/cm^2 from the starting inoculum.

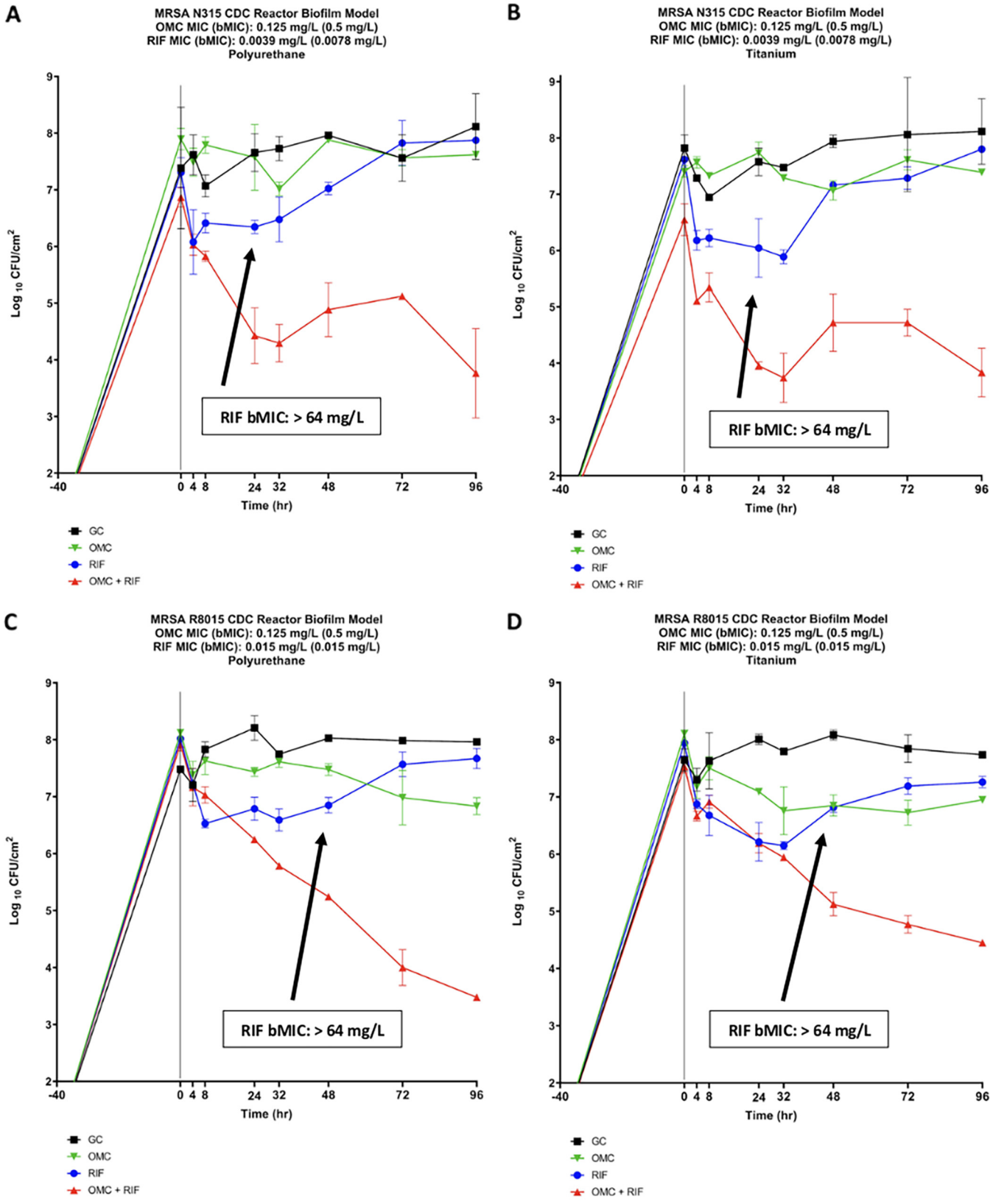

FIG 1 *In vitro* PK/PD biofilm model results for all 4 regimens (growth control [GC], OMC monotherapy, RIF monotherapy, and OMC plus RIF combination therapy). Data are shown for methicillin-resistant *Staphylococcus aureus* isolate N315 with polyurethane (A) and titanium (B) coupons and for methicillin-resistant *Staphylococcus aureus* isolate R8015 with polyurethane (C) and titanium (D) coupons. Black arrows signify the time frame of the first-observed elevated RIF bMIC.

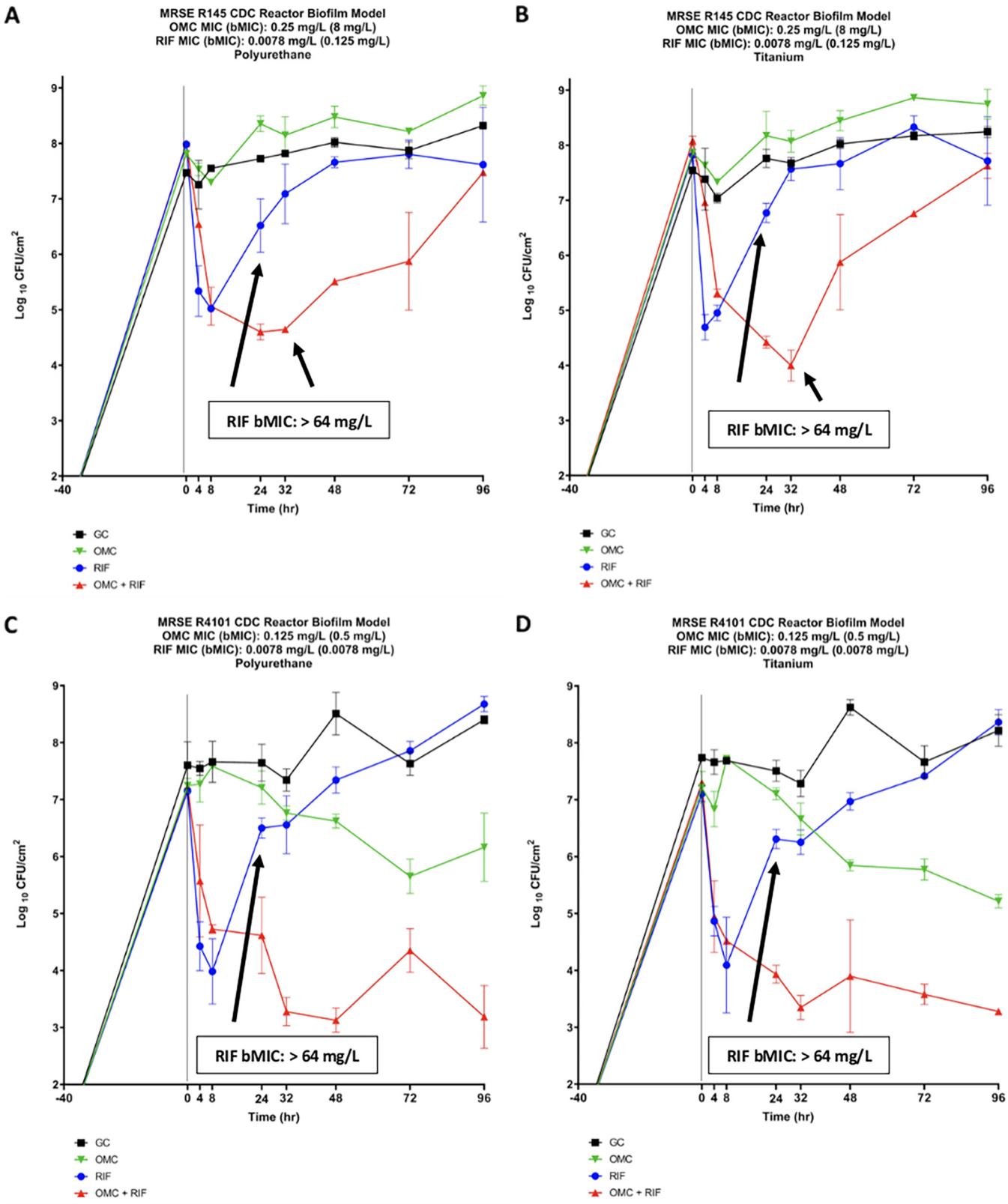

FIG 2 *In vitro* PK/PD biofilm model results for all 4 regimens (GC, OMC monotherapy, RIF monotherapy, and OMC plus RIF combination therapy). Data are shown for methicillin-resistant *Staphylococcus epidermidis* isolate R145 with polyurethane (A) and titanium (B) coupons and for methicillin-resistant *Staphylococcus epidermidis* isolate R4101 with polyurethane (C) and titanium (D) coupons. Black arrows signify the time frame of the first-observed elevated RIF bMIC.

Pharmacokinetics. To ensure that the targeted humanized exposures of OMC and RIF were obtained in the PK/PD model, antimicrobial samples were quantified via bioassay analysis. Intraday coefficients of variance were less than 4%. Overall, the sampled and measured PK concentrations were similar to target values, as shown with the following observed values: loading dose free maximum concentration of drug (fC_{max}) of 2.77 ± 0.20 mg/L and half-life ($t_{1/2}$) of 15.1 h and maintenance dose fC_{max} of 1.39 ± 0.20 mg/L and $t_{1/2}$ of 15.1 h for OMC; fC_{max} of 2.74 ± 0.32 mg/L and $t_{1/2}$ of 2.7 h for RIF. Antimicrobial exposures can be found in Fig. S2 in the supplemental material.

Changes in susceptibility. The potential for the emergence of antimicrobial resistance via elevations in bMICs were evaluated by sampling the biofilm PK/PD model at time points identical to those obtained for PD analysis throughout the experiment. No elevations in OMC bMICs were found when OMC was used as monotherapy. When RIF was utilized as monotherapy, elevations in RIF bMICs (>64 mg/L) emerged throughout every model between 24 and 48 h. OMC was shown to prevent the emergence of RIF resistance for 3/4 (75%) isolates throughout the combination models. Increases in RIF bMICs were noted at 32 h for the only isolate in which the combination of OMC plus RIF did not lead to sustained bacterial eradication (R145).

DISCUSSION

Biofilm-associated infections house bacteria within a complex of phenotypic resistance against antimicrobials and the host immune system, are often challenging to eradicate and have very limited therapeutic options (1, 10). Our study conducted preliminary experiments evaluating the ability of RIF to reduce the bMICs of OMC and the ability of this combination to produce bactericidal and/or synergistic effects in biofilm TKAs. Based on the promising results observed in these initial experiments, humanized exposures of OMC and RIF, both alone and in combination, were evaluated against *S. aureus* isolates N315 and R8015 and *S. epidermidis* isolates R145 and R4101. The novel aminomethylcycline OMC was shown to have promising *in vitro* effects in combination with RIF, as well as preventing the emergence of RIF resistance, against two of the most common pathogen culprits of MDI associated with biofilm.

To our knowledge, OMC has limited data on its activity against biofilm-producing *S. aureus* and has yet to be evaluated against biofilm-producing *S. epidermidis*. Karau and colleagues (23) evaluated OMC and RIF alone and in combination in an experimental rat model of MRSA osteomyelitis. The combination of OMC and RIF exhibited significant reductions compared with OMC monotherapy. Furthermore, RIF-resistant isolates emerged within the RIF monotherapy group, while no resistant isolates were observed with OMC-RIF combination therapy. Diehl and colleagues (24, 25) evaluated OMC against biofilm-producing *Escherichia coli* and indicated that OMC exhibited dose-dependent activity against an established biofilm and reduced the bioburden of *E. coli* at concentrations near the MIC and that *E. coli* biofilms did not proliferate at concentrations at sub-MICs. These results provided evidence for the activity of OMC against *E. coli* isolates with biofilm production. These data in combination with the results of this study provide preliminary evidence that OMC may be an attractive option to further evaluate *in vivo* for biofilm-associated infections.

When used as monotherapy within the PK/PD CBR, OMC was shown to be bacteriostatic against the organisms evaluated, which was expected due to the bacteriostatic nature of the tetracyclines and tetracycline derivatives for most organisms (25–28). Although rapid reductions in bacterial counts were generally observed with RIF when utilized as monotherapy, they were followed by rapid regrowth likely due to increases that were observed with the RIF bMICs that occurred around the time of the regrowth. This regrowth was also anticipated, as RIF used as monotherapy has been documented to be prone to a rapid development of resistance (18, 29, 30). Although one could argue that alternative dosage regimens of RIF could have been modeled (i.e., 600 mg every 24 h [q24h] and 600 mg q12h), multiple studies have shown no difference in patient outcomes when comparing various dosages of rifampin-based regimens for MDIs caused by *Staphylococcus*

spp. (31–33). Furthermore, higher exposures have been associated with increased rates of gastrointestinal distress that have led to discontinuation within the clinical realm (32).

Importantly, many guidance documents that provide recommendations for the treatment of particular MDI, such as the Infectious Disease Society of America (IDSA) Prosthetic Joint Infection Guidelines, IDSA Clinical Practice Guidelines for Healthcare-Associated Ventriculitis and Meningitis, and the American Heart Association (AHA) Infective Endocarditis Scientific Statement, endorse the addition of RIF to antimicrobial regimens for retained medical devices (13–15). Not only did this evaluation show sustained bactericidal activity when utilizing OMC plus RIF but also OMC was shown to prevent the emergence of resistance of RIF that emerged throughout the models on common materials (polyurethane and titanium) associated with MDIs. Given that it is commonplace to use RIF-containing regimens for a retained medical device with biofilm-associated infections caused by staphylococci species, the preliminary *in vitro* evidence observed throughout this study could provide another therapeutic option with the combination of OMC and RIF, if proven efficacious in the real-world setting. Important for the bedside clinician, as OMC and RIF are both available as oral formulations, this combination could help facilitate transitions of care to the outpatient setting.

There are limitations of this study that are worth discussing. First and foremost, the *in vitro* PK/PD CBR model within this analysis evaluated durations of single and combination antimicrobial therapy only for 96 h. Infections involving retained hardware generally require weeks to months of therapy. Therefore, it is unknown whether the *in vitro* effects and the prevention of resistance that were observed with OMC and RIF in this study would be sustained over these longer periods of time. Another limitation of this study is the limited number of evaluated organisms within the humanized PK/PD model. However, preliminary experiments began with testing the synergistic potential of the OMC plus RIF combination against 20 randomly selected staphylococcal strains. Furthermore, the combination of OMC plus RIF did not lead to bactericidal and/or enhancement of activity in all strains evaluated within the PK/PD CBR model. However, for one strain (R145), the initial OMC bMIC was high (8 mg/L), and it is very unlikely that PK/PD targets were attained in this experiment (34). Also, antibiotics within our experiments were injected into the CBR model via bolus administration, which is unlikely to reflect the intravenous infusion times that occur in humans. Finally, although there were no relevant differences noted in bacterial eradication between the two materials of coupons utilized (polyurethane and titanium), our results may not be applicable to other common materials associated with MDIs, such as Teflon or steel.

In summary, we have shown preliminary evidence that OMC in combination with RIF seems to be an attractive therapeutic option for biofilm-associated MDIs caused by *S. aureus* and *S. epidermidis*. The combination was bactericidal, and OMC plus RIF improved the activity against biofilm-embedded staphylococci compared with monotherapy against most strains tested. Moreover, although RIF resistance was noted in all models when used alone, increases in OMC bMICs were not observed when OMC was utilized as monotherapy, and the use of OMC prevented elevations in RIF bMICs in the majority of combination models. The combination of OMC plus RIF may be a consideration for biofilm-associated MDIs caused by *S. aureus* and *S. epidermidis*. Further *in vitro* and *in vivo* experiments, and real-world evidence, of OMC in combination with RIF are warranted.

METHODS

Bacterial strains and antimicrobials. A total of 10 *S. aureus* and 10 *S. epidermidis* clinical isolates were randomly selected from the Anti-Infective Research Laboratory (ARL; Detroit, MI) strain library and were evaluated in this study. The majority of these strains are well-characterized and well-referenced (information regarding these strains listed in Table 1 can be found in various catalogs) (35). OMC was provided by its manufacturer (Paratek Pharmaceuticals, Inc., Boston, MA). RIF was obtained commercially from Sigma Chemical Company (St. Louis, MO).

Susceptibility testing. Planktonic susceptibility testing of OMC and RIF was performed by broth microdilution on all organisms following Clinical & Laboratory Standards Institute (CLSI) guidelines in cation-adjusted Mueller-Hinton broth (MHB) (36). Biofilm susceptibility testing of OMC and RIF was performed using the well-established pin-lid method as described previously (37). Briefly, bacteria tested were grown in glucose-supplemented tryptic soy broth (gSTSB) in the presence of a pin-lid that served as the biofilm surface. After an incubation step for 24 h at 37°C, MIC testing was performed. Following the determination of biofilm MIC values for each isolate, OMC biofilm MICs were determined again in the presence of RIF at a 0.5× biofilm

MIC or maximum concentration of free drug achieved in human serum (whichever was lower) to determine the potential for synergy between OMC and RIF. All susceptibility testing was performed in duplicate to ensure reproducibility.

Biofilm time-kill analyses. Four *S. aureus* and four *S. epidermidis* strains were selected from the organisms tested previously for susceptibility to perform biofilm time-kill analyses (TKAs). Isolates were chosen based on the greatest fold reduction of OMC biofilm MIC seen in combination biofilm susceptibility testing. Biofilm TKA methodology that has been described previously was utilized to evaluate synergy against biofilm-producing organisms (38). Briefly, biofilm TKAs were performed by inoculating test organisms in 1% glucose-supplemented tryptic soy broth (gSTSB) for 24 h in 2-mL macrowells containing polyurethane beads in a shaker incubator at 37°C to allow for biofilm formation. The following day, the gSTSB was aspirated, and the beads were carefully removed and placed into wells containing cation-adjusted MHB and exposed to OMC alone, RIF alone, and OMC plus RIF at a 0.5× biofilm MIC or maximum concentration of free drug achieved in human serum (whichever was lower). A bead was removed aseptically using sterile forceps from each well at 0, 4, 8, and 24 h; placed into 1 mL of cold normal saline; and run through three 60-s cycles of vortexing and sonicating to recover the organism from the biofilm matrix. The vortexed and sonicated samples were serially diluted in cold normal saline and plated on tryptic soy agar (TSA) using an automated spiral sampler (easySpiral; Interscience for Microbiology, Saint Nom la Breteche, France). After an 18- to 24-h incubation at 37°C, bacterial colonies were counted using a laser colony counter (Scan 1200; Interscience for Microbiology, Saint Nom la Breteche, France). Time-kill curves were generated by plotting mean ± standard deviation colony counts ($\log_{10}$ CFU/cm^2) versus time to compare 24-h killing effects of antimicrobial exposures. Synergy between OMC and RIF was defined as ≥2-$\log_{10}$ CFU/cm^2 reduction compared with the most effective agent alone at 24 h, while bactericidal activity was defined as ≥3-$\log_{10}$ CFU/cm^2 reduction at 24 h compared with the starting inoculum. All biofilm TKAs were performed in duplicate to ensure reproducibility.

***In vitro* PK/PD model.** Two *S. aureus* and two *S. epidermidis* strains were selected from the organisms tested within biofilm TKAs for evaluation in a previously described and robust PK/PD CBR model (BioSurface Technologies, Bozeman, MT) equipped with titanium and polyurethane coupons inserted into eight rods per model, with simulated human PK to evaluate the *in vitro* activity of OMC and RIF, alone and in combination (39, 40). The strains chosen for the models were determined based on those which showed the most synergistic and/or bactericidal effects within the biofilm TKAs. The entirety of the experimental procedure took place in a 37°C incubator. Briefly, a 40-h biofilm conditioning phase was performed prior to evaluation of antimicrobial efficacy and consisted of a 24-h incubation of the test organism that was inoculated in 1% gSTSB, followed by a 16-h continuous flow with 1/10 concentration gSTSB that was performed with peristaltic pumps (Masterflex; Cole-Parmer Instrument Co., Chicago IL). After completion of this 40-h biofilm preparation phase, cation-adjusted MHB was used for the remainder of the experiment and antimicrobial boluses simulating human antibiotic exposure were injected into the CBR compartment over a 96-h period. The peristaltic pumps were set to simulate the adult human half-life ($t_{1/2}$) of the antimicrobials, with fresh MHB supplied continuously and removed from the compartment along with the drug. A total of four regimens were evaluated on each isolate over the 96-h treatment period, as follows: antimicrobial-free growth control; 200 mg OMC for one dose (fC_{max}, 2.88 mg/L; assuming protein binding of 20% and targeting an average adult half-life of 16.0 h), followed by 100 mg q24h OMC (fC_{max}, 1.44 mg/L; assuming protein binding of 20% and targeting an average adult half-life of 16.0 h; given the dose-proportional and linear PK profile that has been reported with OMC over a dosage range from 25 to 600 mg); 450 mg q12h RIF (fC_{max}, 2.9 mg/L; assuming protein binding of 80% and targeting an average adult half-life of 2.9 h); and 200 mg OMC for one dose plus 450 mg q12h RIF, followed by 100 mg q24h OMC plus 450 mg q12h RIF (PK parameters unchanged from those listed previously) (41–44). With OMC plus RIF combination models, supplemental OMC was added at an appropriate rate to compensate for the higher flow rate required to simulate RIF clearance. All CBR experiments were performed in duplicate to ensure reproducibility, with mean ± SD computed for the sum of the duplicates from both coupons.

PD analysis. One rod (containing two polyurethane and two titanium coupons) was aseptically removed from each model at 0, 4, 8, 24, 32, 48, 72, and 96 h. To remove excess planktonic cells, each coupon was rinsed twice in sterile normal saline. Bacteria within the biofilm were recovered by three alternating 60-s cycles of vortexing and sonicating in a 10-mL volume of normal saline. From this solution, 1 mL of recovered biofilm cells was collected and further serially diluted in cold normal saline. Biofilm-embedded bacterial cell concentrations (mean ± SD CFU/cm^2) were determined by spiral plating appropriate dilutions using an automatic spiral plater (easySpiral; Interscience for Microbiology, Saint Nom la Breteche, France), incubating the plates at 37°C for 18 to 24-h, and by computing by using a laser colony counter (Scan 1200; Interscience for Microbiology, Saint Nom la Breteche, France). The limit of detection through these methods of bacterial colony count determination was 2 $\log_{10}$ CFU/cm^2. Reductions in $\log_{10}$ CFU/cm^2 were plotted against time to construct curves to determine the overall killing activity of OMC, RIF, and OMC plus RIF against biofilm-embedded *S. aureus* and *S. epidermidis*. Bactericidal activity was defined as a ≥3-$\log_{10}$ CFU/cm^2 reduction in colony count compared with the starting inoculum baseline, while an enhancement of activity was defined as a ≥2-$\log_{10}$ CFU/cm^2 reduction in bacterial eradication compared with the most active single antimicrobial. Cell concentration (mean ± SD $\log_{10}$ CFU/cm^2) was computed for each coupon in duplicate.

PK analysis. PK samples (1 mL) were obtained through the injection port of each model for verification of target antibiotic concentrations and PK parameters. All samples were stored at −80°C until analysis. RIF and OMC concentrations were determined via bioassays using *Kocuria rhizophila* (formerly *Micrococcus luteus*) strain ATCC 9394 and *Staphylococcus aureus* ATCC 29213 (40). Briefly, blank 0.25-in disks were placed on preswabbed agar plates and spotted with 10 μL of the standards (linear over the concentration range of 0.5 to 4 mg/L and 0.725 to 11.6 mg/L for OMC and RIF, respectively) and PK samples. Each standard and PK sample were tested in duplicate. Plates were incubated for 24 h at 37°C, at

which time the sizes of the zones were measured using an automated plate reader (Scan 1200; Interscience for Microbiology, Saint Nom la Breteche, France). Standard curves were created using the zone of inhibition sizes of known antibiotic concentrations (standards), and the inhibition zone size at each PK sample time point was plotted against this curve to obtain the sampled concentrations from the CBR models.

Emergence of resistance. Evaluation of the emergence of resistance was performed for each sample taken throughout the 96-h model by plating 100 μL of samples on brain heart infusion (BHI) agar (Difco, Detroit, MI) plates supplemented with OMC or RIF at a concentration of 3$\times$ the bMIC. Plates were examined for growth after a 48-h incubation at 37°C. Resistant colonies growing on screening plates were evaluated by bMIC testing methods.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

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