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Comparative Activities of Daptomycin, Linezolid, and Tigecycline against Catheter-Related Methicillin-Resistant *Staphylococcus* Bacteremic Isolates Embedded in Biofilm▽

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In the setting of catheter-related bloodstream infections, intraluminal antibiotic lock therapy could be useful for the salvage of vascular catheters. In this in vitro study, we investigated the efficacies of the newer antibiotics daptomycin, linezolid, and tigecycline, in comparison with those of vancomycin, minocycline, and rifampin, against methicillin-resistant *Staphylococcus aureus* (MRSA) embedded in biofilm. We also assessed the emergence of MRSA strains resistant to these antibiotics, alone or in combination with rifampin, after 4-hour daily use for catheter lock therapy. Minocycline, daptomycin, and tigecycline were more efficacious in inhibiting MRSA in biofilm than linezolid, vancomycin, and the negative control ($P < 0.001$) after the first day of exposure to these antibiotics, with minocycline being the most active, followed by daptomycin and then tigecycline, and with vancomycin and linezolid lacking activity, similar to the negative control. After 3 days of 4-hour daily exposures, daptomycin was the fastest in eradicating MRSA from biofilm, followed by minocycline and tigecycline, which were faster than linezolid, rifampin, and vancomycin ($P < 0.001$). When rifampin was used alone, it was the least effective in eradicating MRSA from biofilm after 5 days of 4-hour daily exposures, as it was associated with the emergence of rifampin-resistant MRSA. However, when rifampin was used in combination with other antibiotics, the combination was significantly effective in eliminating MRSA colonization in biofilm more rapidly than each of the antibiotics alone. In summary, daptomycin, minocycline, and tigecycline should be considered further for antibiotic lock therapy, and rifampin should be considered for enhanced antistaphylococcal activity but not as a single agent.

Intraluminal colonization of the central venous catheter (CVC) is universal and is often caused by methicillin-resistant staphylococci that embed themselves in the biofilm layer in the lumen of the catheter, resulting in catheter-related bloodstream infections (CRBSI) (21). Given the cost, difficulty, and complications associated with the removal of a long-term CVC and the insertion of a new CVC at a different site, recent guidelines have suggested the use of antibiotic catheter lock therapy (ALT) for the prevention and salvage treatment of CRBSI in high-risk patients (16).

Few antibiotics with activity against methicillin-resistant *Staphylococcus aureus* (MRSA) may be used for intraluminal ALT (6, 8, 10, 14, 27). However, MRSA organisms that are susceptible to glycopeptides, such as vancomycin in suspension, become resistant to this antibiotic when embedded in biofilm on catheter surfaces (6, 8, 9, 23, 27). In the present study, we used an in vitro silicone disk biofilm colonization model to determine the activities of novel antistaphylococcal antibiotics (daptomycin, linezolid, and tigecycline) against catheter-related bacteremic MRSA embedded in biofilm, in comparison with one another and with older agents, such as vancomycin, minocycline, and rifampin. Furthermore, we determined the risk of developing organisms resistant to such antibiotics used, alone or in combination with rifampin, for catheter lock therapy on a daily basis.

MATERIALS AND METHODS

Antibiotic activity in biofilm. The abilities of various antibiotics to eradicate MRSA organisms embedded in biofilm were determined by the silicone disk biofilm colonization in vitro model as previously described (13). Sterile silicone disks were placed in human plasma and incubated with shaking for 24 h at 37°C. The plasma was then replaced with 1 ml of bacterial inoculum and incubated with shaking for 24 h at 37°C. Bacterial inoculum was made by diluting 30 MRSA isolates that had caused CRBSI to 5.5×10^5 cells/ml in Mueller-Hinton broth (MHB). Organisms were tested in quadruplet against each drug. The inoculated broth was then removed, and the silicone disks were washed with 0.9% saline shaking for 30 min.

The silicone disks were then transferred to new tubes containing either MHB or 2-mg/ml solutions of the following drugs: (i) daptomycin (supplemented to a physiologic level of 50 mg/liter Ca^{2+}), (ii) linezolid, (iii) minocycline, (iv) tigecycline, (v) rifampin, and (vi) vancomycin. After 24 h of incubation at 37°C, the disks were placed in 5 ml of 0.9% saline and sonicated for 15 min. Finally, the tubes were vortexed for 30 seconds, a 100-μl aliquot of saline was plated on Trypticase soy agar with 5% sheep blood, and the plates were incubated overnight at 37°C. The colonies were then counted, and final counts were calculated, with the dilution factor taken into account.

Antibiotic daily lock. To test the cyclic short-term activities of the antibiotics in a manner that simulates daily antibiotic catheter lock for 4 hours, the same procedure, whereby the colonized disks were immersed in the drug solution for 4 hours every day, followed by incubation in MHB for 24 h at 37°C, was repeated for 10 MRSA isolates. This procedure was repeated for 5 consecutive days. Every day, the silicone disks were exposed to the antibiotics listed above, alone or in combination with rifampin, for 4 hours. At the end of 4 hours, four to six silicone

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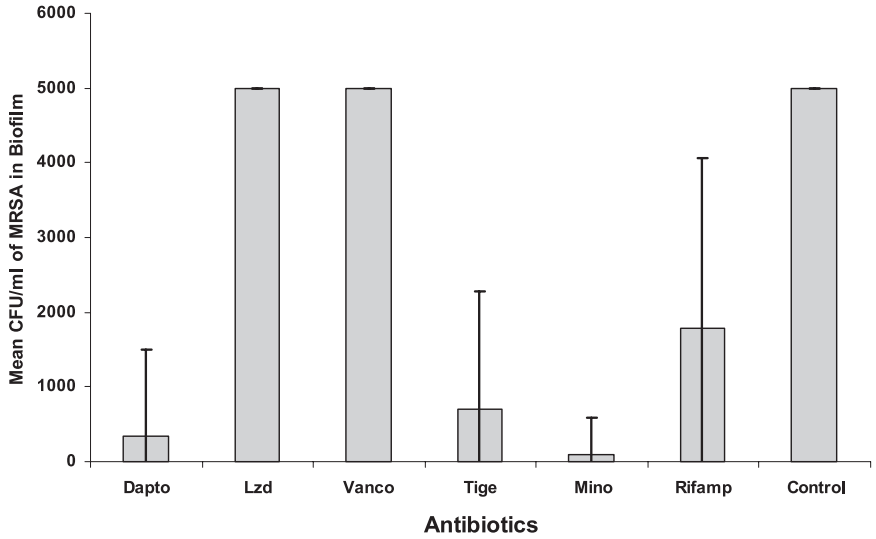

FIG. 1. Activities of antibiotics against MRSA bacteria embedded in biofilm after 24 h of exposure. Dapto, daptomycin; Lzd, linezolid; Vanco, vancomycin; Tige, tigecycline; Mino, minocycline; Rifam, rifampin. The standard deviations for linezolid, vancomycin, and the control are 0 because our upper limit of detection was 5,000 CFU/ml, and any value greater than that was considered 5,000 in analysis.

disks from each group were cultured as described above and the other silicone disks were reincubated for another 24 h in MHB before they were reexposed to the same antibiotic alone or in combination with rifampin for a total of 5 consecutive days.

Susceptibility testing. The susceptibilities of the 10 MRSA isolates exposed on a daily basis to all six antibiotics (as measured by MICs) were determined by the broth microdilution method recommended by the CLSI (formerly NCCLS) (18). A baseline MIC was obtained prior to exposure and thereafter on a daily basis until day 5, the last day of growth. Decreased susceptibility was considered to have occurred if the MIC increased at least fourfold after repeated exposures compared to the baseline level.

Statistical methods. The inhibitory activities against catheter-related MRSA in biofilm (indicated by numbers of CFU) were compared by the Kruskal-Wallis test (P values of <0.05 were considered statistically significant). When a significant result was found for the test, we used Wilcoxon rank sum tests for pairwise comparisons to locate the significant differences. The α levels of the post hoc pairwise comparisons were adjusted using a sequential Bonferroni adjustment method to control for type 1 error. All computations were performed with the Statistical Package for Social Sciences (version 12.0 for Windows; SPSS, Inc., Chicago, IL).

RESULTS

As shown in Fig. 1, minocycline, daptomycin, and tigecycline were highly efficacious in inhibiting MRSA in biofilm after 24 h of exposure to the antibiotics compared to linezolid, vancomycin, and the negative control (all P values were <0.001). Minocycline was significantly more active than all other antibiotics (all P values were ≤ 0.022), followed by daptomycin and tigecycline. Vancomycin and linezolid had no reductive impact on MRSA growth in biofilm after a one-time 24-h exposure and were comparable to the broth control (Fig. 1).

However, upon short-term (4-hour) daily exposures, daptomycin was the most rapidly active antibiotic, followed by minocycline and tigecycline, resulting in a significant elimination of MRSA colonization in biofilm by day 3 compared to what was found for linezolid, rifampin, and vancomycin ($P < 0.001$). Figure 2A shows that daptomycin and minocycline resulted in complete eradication of MRSA in biofilm after two 4-hour daily exposures. Tigecycline required three brief 4-hour daily exposures to eradicate MRSA in biofilm, whereas MRSA persisted in biofilm despite five 4-hour daily exposures to linezolid, rifampin, or vancomycin (all P values were <0.01), as shown in Fig. 2B.

Rifampin was unique compared to all other antibiotics. On day 1, after a brief (4-hour) exposure, rifampin was the most effective antibiotic in decreasing the microbial burden of MRSA colonization in biofilm (Fig. 2B). However, after 5 days of daily 4-hour exposures, rifampin failed to decrease the density of MRSA colonization further and was the least effective among all antibiotics, including vancomycin and linezolid (Fig. 2A and B) (all P values were <0.01).

This failure to decrease the microbial burden of MRSA in biofilm by the daily exposure to rifampin was associated with the emergence of resistant organisms when rifampin was used alone on a daily basis (Table 1). Seven of the nine MRSA isolates that were initially highly susceptible to rifampin became resistant to this antibiotic after cyclic 4-hour exposures on a daily basis over a 5-day period (Table 1). Decreased susceptibility to linezolid was also noted for 3 of the 10 MRSA isolates tested. Decreased susceptibilities to other antibiotics did not occur, particularly for daptomycin, minocycline, and tigecycline, which eliminated colonization in biofilm by day 3 of the cyclic 4-hour exposure (Fig. 2A).

On the other hand, the addition of rifampin to daptomycin, minocycline, or tigecycline resulted in enhanced activity and complete eradication of MRSA in biofilm on day 1 following a 4-hour exposure of the 10 MRSA isolates to these combinations including rifampin. Furthermore, the addition of rifampin to vancomycin or linezolid resulted in the complete eradication of MRSA by day 2 of the daily 4-hour exposures, demonstrating that rifampin functions in an enhanced manner with these antibiotics, thereby significantly expediting the eradication of MRSA in biofilm (Fig. 3A and B) (all P values were <0.001).

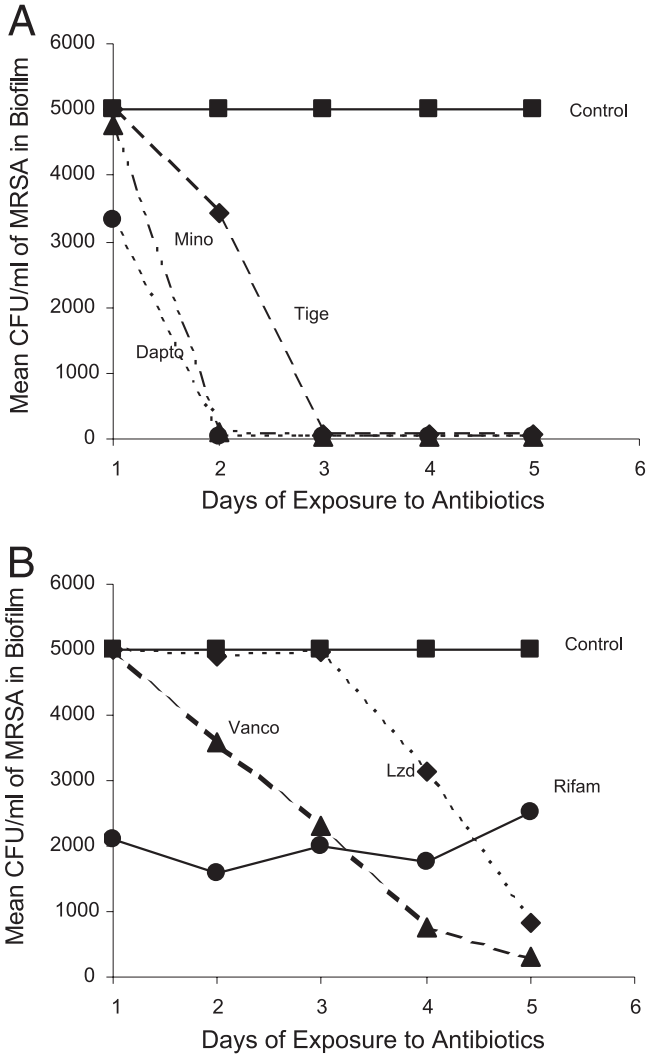

FIG. 2. Time-kill activities of antibiotics against 10 strains of MRSA embedded in biofilm after 4-hour daily exposures over 5 days. (A) Activities of daptomycin (Dapto), minocycline (Mino), and tigecycline (Tige). (B) Activities of rifampin (Rifam), vancomycin (Vanco), and linezolid (Lzd). Our lower limit of detection was 50 CFU/ml, and our upper limit of detection was 5,000 CFU/ml.

DISCUSSION

This study shows that daptomycin, minocycline, and tigecycline were significantly more effective than vancomycin or linezolid in inhibiting MRSA organisms embedded in biofilm. The use of rifampin alone was associated with the emergence of rifampin-resistant MRSA, making this drug the least effective in decreasing the microbial burden of MRSA colonization in biofilm after 5 days of daily 4-hour exposures. However, when rifampin was used in combination with other antibiotics, the combination expedited the elimination of MRSA colonization in biofilm.

The Infectious Diseases Society of America (IDSA) guidelines on the management of CRBSI suggested the use of ALT for the salvage of CVC associated with CRBSI (16). However, these guidelines failed to specify which antibiotics are more active in the biofilm environment and how long the antibiotic should be locked in. For hospitalized patients, dwell time prolonged beyond 12 h is neither practical nor feasible. Furthermore, the choice as to which antibiotic should be used for ALT is often based on the in vitro susceptibilities of the organisms in suspension, which might not necessarily indicate that the antibiotic is active against the same organisms embedded in biofilm.

The relative lack of efficacy of vancomycin in eradicating MRSA organisms embedded in biofilm is consistent with prior data. Vancomycin has been shown to have limited activity against staphylococci embedded in biofilm (6, 8, 9, 19, 27). Although some studies showed favorable results related to the use of vancomycin for ALT, several clinical studies have reported low salvage rates associated with the use of vancomycin and heparin for ALT in the management of staphylococcal CRBSI (1, 12, 15). Furthermore, prolonged therapy with vancomycin for CRBSI caused by MRSA was found to possibly be associated with the emergence of vancomycin-resistant *S. aureus* (3). Hence, vancomycin on its own might not be the best choice for ALT that would consistently lead to catheter salvage.

Linezolid also failed to eradicate MRSA isolates embedded in biofilm that was attached to the silicone disks. This finding is also consistent with prior in vitro studies. Curtin et al. showed that for linezolid to be effective against *Staphylococcus epidermidis* embedded in biofilm, a dwell time of greater than 72 h is required, which is often not clinically feasible (6). Wiederhold et al. showed that both linezolid and vancomycin lacked activity against staphylococci embedded in biofilm (27). The emergence of MRSA isolates with decreased susceptibilities to linezolid upon the repeated daily exposure of these organisms to linezolid should further deter the use of linezolid alone as an antibiotic catheter lock solution.

Minocycline, on the other hand, was highly active against MRSA isolates embedded in biofilm. We have previously shown that minocycline was significantly more effective than vancomycin or vancomycin plus heparin in reducing the colonization of *S. aureus* or *S. epidermidis* embedded in biofilm on catheter surfaces in an in vitro model (20). In the same in vitro model, the antistaphylococcal activity of minocycline was further enhanced by the addition of EDTA. The combination of minocycline and EDTA resulted in enhanced activity and was highly successful in eradicating staphylococcal organisms em-

TABLE 1. MIC comparison of MRSA strains on day 1 and day 5

MRSA strain	MIC[a] (μg/ml) for indicated antibiotic and day			
	Rifampin		Linezolid	
	Day 1	Day 5	Day 1	Day 5
4803	<0.06	128	0.5	NG
4913	128	128	0.5	8
4930	<0.06	<0.06	0.5	NG
5098	<0.06	128	0.25	4
5004	<0.06	128	0.5	NG
859	<0.06	32	0.5	2
4875	<0.06	128	0.5	NG
4342	<0.06	128	1	NG
293	<0.06	128	1	NG
789	<0.06	NG	0.5	NG

[a] NG, no growth on day 5.

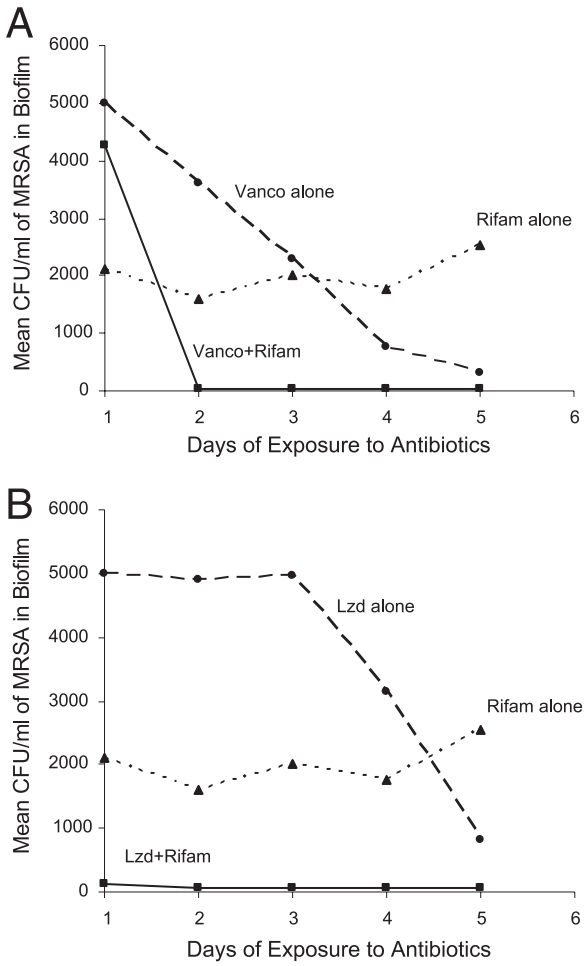

FIG. 3. Time-kill activities of rifampin (Rifam) in combination with either vancomycin (Vanco) (A) or linezolid (Lzd) (B) against 10 strains of MRSA embedded in biofilm after 4-hour daily exposures over 5 days.

bedded in biofilm (20). Subsequent clinical studies have shown that minocycline-EDTA, compared with heparin, was significantly more effective in decreasing the risk of colonization and CRBSI in hemodialysis patients and pediatric patients with cancer, respectively (2, 5).

In the present study, the activity of minocycline was further enhanced by the addition of rifampin. The combination of minocycline and rifampin eliminated MRSA colonization in biofilm for all of the 10 bacteremic isolates tested. This enhanced activity against MRSA organisms embedded in biofilm has previously been demonstrated in a different in vitro model of the modified Robbins device (23). Furthermore, four prospective, randomized clinical trials showed that CVCs coated with minocycline and rifampin were highly efficacious and superior to uncoated catheters or other antiseptic catheters in preventing CRBSI (4, 7, 11, 22). Therefore, minocycline alone or in combination with other antistaphylococcal enhancers, such as EDTA or rifampin, could be highly efficacious in ALT for the prevention of intraluminal colonization of the CVC with staphylococci.

Tigecycline is a derivative of minocycline with activities against MRSA and glycopeptide-intermediate *S. aureus* bacteria (19), including staphylococci embedded in biofilm (14). In a systemic murine model, tigecycline was as efficacious as daptomycin and superior to vancomycin (19).

In the present study, daptomycin was also highly effective against MRSA in biofilm. Time-kill studies have demonstrated that daptomycin is more rapidly bactericidal than vancomycin and linezolid (10). However, it is limited in that it requires a concurrent high concentration of calcium, which would prohibit its concurrent use with a calcium chelator and antibiotic enhancer, such as EDTA. The alternative is to use it with heparin. However, heparin has recently been shown to enhance the biofilm formation of MRSA (25).

Rifampin was shown to significantly decrease the MRSA colonization of silicone disks following a brief 4-hour exposure or a prolonged exposure of 24 h. However, after repeated daily exposures to rifampin, most of the MRSA isolates developed resistance to this antibiotic (Table 1) and the MRSA colonization was maintained despite daily exposure to rifampin over a 5-day period (Fig. 2B and 3A and B). These data are not inconsistent with prior studies that have shown that rifampin initially decreases the microbial burden of staphylococci embedded in biofilm on catheter surfaces (9, 23, 24). Several other studies have shown that the repeated exposure of biofilm-forming staphylococcal organisms to rifampin alone is associated with the development of resistance to this antibiotic (17, 26). However, when rifampin was used in combination with other antibiotics, resistance to rifampin failed to develop (17).

In the present study, rifampin was highly active with other antibiotics in eradicating MRSA colonization in biofilm on silicone disk surfaces. As shown in Fig. 3A and B, rifampin was highly efficacious in enhancing the activities of all antibiotics tested, particularly linezolid against MRSA embedded in biofilm, and such rifampin combinations prevented the emergence of resistant organisms. This enhancement of the antistaphylococcal activities of other antibiotics against staphylococci embedded in biofilm has previously been demonstrated for vancomycin, minocycline, ciprofloxacin, fusidic acid, and clindamycin (23, 24). Saginur et al. have recently shown that rifampin was the most common constituent of antibiotic combinations active against staphylococci in biofilms (24). Hence, rifampin is best used in combination with other antistaphylococcal antibiotics against staphylococci embedded in biofilm.

In conclusion, antibiotics to be included in the ALT regimen should be active against organisms embedded in biofilm. Minocycline, daptomycin, and tigecycline were significantly more active than vancomycin and linezolid against MRSA embedded in biofilm. Rifampin, when used alone in a model that simulates antibiotic catheter lock solution for 4 hours on a daily basis, was associated with the emergence of resistant organisms and ultimately failed to eradicate MRSA colonization in biofilm. However, rifampin could serve as an antistaphylococcal antibiotic enhancer. When used with other tested antibiotics, it achieved rapid eradication of MRSA in biofilm.

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