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In Vitro Activity of Retapamulin and Antimicrobial Susceptibility Patterns in a Longitudinal Collection of Methicillin-Resistant *Staphylococcus aureus* Isolates from a Veterans Affairs Medical Center

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Mupirocin is a topical antimicrobial used to decolonize patients who carry methicillin-resistant *Staphylococcus aureus* (MRSA), and the topical agent retapamulin may be a potential alternative therapy. The goal of this study was to determine the *in vitro* activity of retapamulin as well as a panel of 15 antimicrobial agents, including mupirocin, for 403 MRSA isolates collected longitudinally from a naive population at the Veterans Affairs Puget Sound Health Care System. The MICs for retapamulin had a unimodal distribution, ranging from 0.008 to 0.5 μg/ml. One isolate had an MIC of >16 μg/ml, was also resistant to clindamycin and erythromycin, and was recovered from the nares of a patient undergoing hemodialysis. Twenty-four isolates (6%) and 11 isolates (3%) demonstrated low-level resistance (MICs of 8 to 64 μg/ml) and high-level resistance (MICs of ≥512 μg/ml), respectively, to mupirocin. Isolates were recovered from 10 patients both before and after mupirocin therapy. Of those, isolates from 2 patients demonstrated MIC changes postmupirocin therapy; in both cases, however, strain typing demonstrated that the pre- and postmupirocin strains were different. A total of 386 isolates (96%) had vancomycin MICs of ≤1.0 μg/ml; 340 isolates (84%) were resistant to levofloxacin, 18 isolates (4.5%) were resistant to trimethoprim-sulfamethoxazole, and 135 isolates (33%) had elevated MICs of 4 μg/ml for linezolid. The baseline levels of resistance were low for mupirocin (9%) and even lower for retapamulin (0.25%) Although the use of mupirocin is currently the standard therapy for decolonization practices, the activity of retapamulin warrants its consideration as an alternative therapy in MRSA decolonization regimens.

Retapamulin is a semisynthetic derivative of the compound pleuromutilin, which binds to and prevents formation of the active 50S ribosomal subunit, thus inhibiting bacterial protein synthesis (1). Approved in 2007 and licensed as Altabax in the United States, retapamulin is currently indicated for the topical treatment of impetigo due to methicillin-susceptible *Staphylococcus aureus* and *Streptococcus pyogenes* (2–4). It has not been approved for use against methicillin-resistant *Staphylococcus aureus* (MRSA), as *in vitro* susceptibility data did not correlate with clinical efficacy for open wounds (5). Its use against MRSA as a decolonization agent has not been widely investigated (6).

Mupirocin selectively binds bacterial isoleucyl-tRNA, and antimicrobial resistance is phenotypically categorized into two groups. MICs for low-level resistance, mediated by point mutations of *ileS*, range from 8 to 128 μg/ml (isolates with MICs of 128 or 256 μg/ml are uncommon) (7). MICs for high-level resistance, resulting from the acquisition of *mupA*, are ≥512 μg/ml. Mupirocin is a topical antimicrobial that is commonly used for decolonization among patients who carry MRSA, and several studies demonstrated increases in the rates of mupirocin resistance after the introduction of extensive decolonization practices (7–9). Decolonization using mupirocin is not a standardized practice within the Veterans Affairs (VA) health care system, and baseline levels of mupirocin resistance have never been determined at our facility.

As an alternative to mupirocin, the topical agent retapamulin may be a potential decolonization therapy option (5). The purpose of this study was to determine the *in vitro* activities of retapamulin and mupirocin for MRSA isolates that we considered extensively drug-resistant (XDR) (defined on the basis of resistance to one or more drug classes in addition to β-lactams, macrolides/lincosamides, and fluoroquinolones) or from groups of patients for whom topical therapy might be beneficial. In addition, we evaluated these isolates using a panel of antimicrobials to establish a baseline "antibiogram" for these patient groups at our facility, as many of our patients have had repeated hospital exposures and may be at risk for nosocomial acquisition of MRSA.

MATERIALS AND METHODS

Bacterial isolates. MRSA isolates from both clinical and surveillance samples from a 1-year time frame were selected for evaluation from a larger, longitudinal, frozen stock collection kept at −70°C. Surveillance isolates were acquired through the VA active MRSA surveillance program, in which patient samples were cultured for detection of MRSA upon admission to the hospital, transfer within the hospital, or discharge from the hospital. Isolates were selected based on the type of specimen, the clinical service where the patient was being cared for at the time of isolate recovery, isolates with a temporal relationship to the patient, and/or an extensively drug-resistant (XDR) antibiogram. Clinical service groups included inpatients, outpatients, spinal cord injury patients (in an inpatient unit), outpatient dermatology clinic patients, and hemodialysis patients. Tem-

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poral categories included nares conversion/transmission isolates (defined as isolates with positive nares MRSA screening results preceded by negative results after hospital admission), paired clinical and surveillance isolates (collected within 48 h of each other), longitudinal isolates (defined as isolates recovered from the same patient ≥30 days after the initial isolate), and postmupirocin isolates. *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* ATCC 43300 (for cefoxitin resistance), and *Enterococcus faecalis* ATCC 29212 were used for quality control, and the results were interpreted using Clinical and Laboratory Standards Institute (CLSI) guidelines (10). The quality control MIC ranges were 0.12 to 0.5 for mupirocin (ATCC 25923), 0.125 to 0.5 μg/ml for fusidic acid (ATCC 25923), and 0.06 to 0.25 μg/ml for retapamulin (ATCC 29213). In addition, 6 isolates from an outside laboratory, with known MIC values for mupirocin determined by Etest, were tested for comparison. Five of these strains were known carriers of high-level resistance (MICs of >512 μg/ml) to mupirocin.

Determination of *in vitro* antimicrobial susceptibility. Antimicrobial susceptibility data were generated using customized frozen Sensititre plates (Trek Diagnostics, Cleveland, OH). Retapamulin powder was provided directly to Trek Diagnostics by GlaxoSmithKline. The broth microdilution panels contained cation-adjusted Mueller-Hinton broth, according to CLSI recommendations, and were inoculated according to the manufacturer's instructions. MICs were determined for the following drugs: erythromycin, clindamycin, daptomycin, gentamicin, linezolid, rifampin, vancomycin, trimethoprim (TMP)-sulfamethoxazole (SXT), levofloxacin, quinupristin-dalfopristin, tigecycline, moxifloxacin, fusidic acid, mupirocin, and retapamulin. The panels also contained three growth controls, a screening test for cefoxitin, and inducible clindamycin resistance (D1 and D2). EUCAST criteria were used for interpretation of retapamulin, fusidic acid, and mupirocin data, FDA criteria were used for interpretation of tigecycline data, and CLSI guidelines (10) were used for interpretation of data for all other drugs. Isolates that were resistant to antimicrobials in one or more drug classes in addition to β-lactams, macrolides/lincosamides, and fluoroquinolones were categorized as extensively drug resistant (XDR).

Determination of strain type and clonality. Selected strains were evaluated using repetitive sequence-based PCR (Rep-PCR) (DiversiLab; bioMérieux, Durham, NC), according to the manufacturer's instructions, to determine strain type and clonality, as reported previously (1). The relatedness of isolates was determined with DiversiLab software. Strains with the same banding patterns were considered indistinguishable. Strains with <95% similarity and 1 or 2 band differences were considered to be not related (1). Classification of strains as USA types on the basis of Rep-PCR data was performed using the R74 DL MRSA library provided by the DiversiLab software for reference (11).

Molecular characterization of *vgaA* gene. An isolated colony from strain 142 was resuspended in 50 mM EDTA buffer, washed twice in the same buffer, and lysed with 10 μg/ml lysostaphin for 30 min at 37°C. Total DNA was isolated using a Qiagen DNeasy kit (Qiagen, Germantown, MD), according to the manufacturer's instructions. One microliter of eluted DNA was used as the template DNA and added to 48 μl of PCR Supermix High Fidelity (Invitrogen, Carlsbad, CA) and 0.5 μl each of 100 pmol/μl *vgaA*-specific diagnostic primers (5′-TCACATGATCGCGCTTTTTTAGAT-3′ and 5′-TCGCTCTCCACCACTTAAGACACT-3′) that anneal internal to the *vgaA* gene of *S. aureus*. PCR was carried out using an initial denaturation step of 95°C for 2 min, 30 cycles of 95°C for 30 s, 50°C for 3 min, and 70°C for 2 min, and a final extension step of 70°C for 7 min. The PCR DNA fragment was purified using a QiaQuick PCR purification kit (Qiagen), and DNA was sequenced using the same primers as used for amplification. The PCR mixture was analyzed by agarose gel electrophoresis and yielded a single DNA product of ~770 bp. tBLAST analysis of the compiled DNA sequence of the amplicon was also performed.

Statistical analysis. Statistical analysis of data was performed with Fisher's exact test, using GraphPad QuickCalcs software.

TABLE 1 Categorization of isolates by clinical characteristics and extensively drug-resistant patterns

Category	Total no. of isolates	No. (%) XDR	*P*
Specimen type			
Wound	134	6 (5)	0.0039
Blood	41	14 (34)	0.0008
Nares	174	20 (12)	
Urine	14	3 (21)	
Respiratory	40	8 (20)	
Clinical service			
Inpatient	226	41 (18)	0.04
Outpatient	92	1 (1)	<0.0001[b]
Spinal cord injury	30	8 (27)	
Hemodialysis[a]	30	5 (17)	
Dermatology	25	0 (0)	
Temporal relationship to patient[c]			
Nares conversion isolates	71	9 (13)	
Paired isolates	113[d]	10 (9)	
Longitudinal isolates	39	6 (15)	

[a] Twenty hemodialysis isolates were from inpatients and 10 were from outpatients.
[b] *P* value for combined data from outpatients and dermatology clinic patients.
[c] Nares conversion isolates are defined as isolates with positive nares MRSA screen test results preceded by negative results after hospital admission. Paired clinical and surveillance isolates are defined as isolates collected from the same patient within 48 h of each other. Longitudinal isolates are defined as isolates recovered from the same patient ≥30 days after collection of the initial isolate.
[d] Fifty-four pairs; for 5 pairs, an isolate from a third anatomic site was available.

Human subjects. This study was approved by the institutional review board of the VA Puget Sound Health Care System.

RESULTS

Categorization and *in vitro* activity of MRSA isolates. A total of 403 isolates from 288 individual patients were tested for antimicrobial susceptibility and categorized based on the type of specimen, clinical service, temporal relationship to the patient, and/or extensively drug-resistant (XDR) antibiogram (Table 1); 69% of the isolates were recovered from hospitalized patients. Fifteen percent of the isolates in our study were from patients who had repeated hospital exposures, specifically patients from the spinal cord injury unit and those undergoing hemodialysis; in contrast, 29% of the isolates were from patients who typically had limited contact with hospital exposures, including patients from the outpatient dermatology service. Nares (43%) and wound (33%) specimens provided the majority of the isolates, with respiratory and blood isolates (10% each) being fewer but equally common.

The MIC distributions for the 403 isolates are listed in Tables 2 and 3. The MICs for retapamulin had a unimodal distribution, ranging from 0.008 to 0.5 μg/ml; these values were considered to indicate susceptibility, using a defined epidemiological cutoff value of ≤0.5 mg/liter, as recommended by the EUCAST. One additional isolate had an MIC of >16 μg/ml. This isolate, which had been recovered from the nares of a patient undergoing hemodialysis, was also resistant to clindamycin and erythromycin but was susceptible to fluoroquinolones and all other antimicrobials. Twenty-four isolates (6%) demonstrated low-level resistance to mupirocin (MICs of 8 to 64 μg/ml), and 11 isolates (3%) demonstrated high-level resistance (MICs of ≥512 μg/ml). A total of 367 isolates (91%) were above the epidemiological cutoff value for

TABLE 2 Summary of susceptibility data

Antimicrobial agent	MIC range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	% susceptible
Retapamulin	0.008 to ≥16	0.12	0.25	99
Mupirocin	≤0.25 to ≥512	0.25	1	91
Fusidic acid	≤0.12 to 2	0.12	0.25	99
Vancomycin	≤0.5 to 8	0.5	1	99
Trimethoprim-sulfamethoxazole	≤0.5 to ≥4	0.5	0.5	96
Tigecycline	0.06 to ≥1	0.12	0.25	92
Linezolid	≤1 to 4	2	4	100
Daptomycin	≤0.5 to 4	0.5	0.5	99
Quinupristin-dalfopristin	≤0.5 to 2	≤0.5	≤0.5	99
Moxifloxacin	≤0.25 to ≥4	2	4	16
Levofloxacin	≤0.25 to ≥4	4	4	15
Rifampin	≤0.5 to ≥4	0.5	0.5	98
Erythromycin	≤0.25 to ≥8	8	8	6
Clindamycin	≤0.5 to ≥4	≤0.5	≥4	54
Gentamicin	≤2 to ≥16	≤2	≤2	98
Inducible clindamycin resistance[a]				12

[a] Clindamycin-sensitive isolates only.

mupirocin susceptibility (MICs of ≤1.0 mg/liter) for topical use, as set by the EUCAST; 397 isolates (99%) were susceptible to vancomycin, and 386 (96%) isolates had vancomycin MICs of ≤1.0 μg/ml. Intermediate vancomycin susceptibility, along with fusidic acid resistance and daptomycin nonsusceptibility, were found only in isolates from a single patient from a unique bacteremic episode. High rates of resistance were found for erythromycin (93%), levofloxacin (85%), and moxifloxacin (84%). Forty-six percent of isolates were resistant to clindamycin, and 12% of the clindamycin-susceptible isolates demonstrated inducible clindamycin resistance. Low rates of resistance were found for TMP-SXT (4%), rifampin (2%), and gentamicin (2%). Eight percent of isolates were found to be nonsusceptible to tigecycline, and 1% of isolates showed intermediate resistance to quinupristin-dalfopristin (no strains were fully resistant). Although 100% of the isolates were susceptible to linezolid, 33% had MICs of 4 μg/ml (Table 3). Eighty-one percent of the isolates (110/135 isolates) with elevated linezolid MICs were from patients associated with nosocomial exposure, including isolates from spinal cord injury patients, respiratory isolates, nares conversion isolates, and longitudinal isolates ($P < 0.001$).

Fifty-one isolates (13%) were found to be resistant to one or more drug classes in addition to β-lactams, macrolides/lincosamides, and fluoroquinolones (defined as extensively drug resistant [XDR] for this study) (Table 1). Isolates from blood (34%; $P = 0.0008$) and inpatients (18%; $P = 0.04$) had higher percentages of XDR isolates in our collection, compared to lower percentages for isolates from wounds (5%; $P = 0.0039$) and outpatient clinics (1%; $P < 0.0001$). Isolates from outpatient clinics were also vastly more susceptible than the entire population. They demonstrated 12% clindamycin resistance (with no inducible resistance among susceptible isolates) ($P < 0.001$) and 67% levofloxacin and moxifloxacin resistance ($P < 0.04$). There were limited XDR isolates among the nares isolates, although they accounted for almost one-half of the isolates in the entire study population (43%). Nares conversion/transmission isolates demonstrated increased resistance to TMP-SXT (10% resistant; $P < 0.002$) and gentamicin (6% resistant; $P < 0.04$), compared to the entire population. In the other categories, we did not find significant differences in rates, compared to those of the study population as a whole.

Isolates from patients exposed to mupirocin and differences in susceptibility by strain type. Rates of mupirocin resistance among the 54 isolates from patients who had received mupirocin therapy were not significantly different from those for the study population as a whole. Sixteen patients with pre- and postmupirocin cultures were identified. Of those, 6 patients (37%) had negative nares cultures after receiving mupirocin therapy; for 10 patients (63%), isolates were recovered both prior to initiation of mupirocin therapy and during or after receipt of mupirocin therapy. Of the patients in our study who experienced recolonization after mupirocin therapy, 7/10 (70%) were recolonized with the same strain that was originally recovered, which was susceptible to mupirocin. For 2 patients, the strain isolated subsequent to mupirocin therapy differed from the premupirocin strain in strain type and, not surprisingly, in antibiotic susceptibility. In one case, a more susceptible isolate (MIC of ≤0.25 μg/ml) was replaced by a resistant isolate (MIC of 16 μg/ml). In the other case, a resistant isolate (MIC of 8 μg/ml), recovered from the nares, was replaced by a more susceptible isolate (MIC of ≤0.25 μg/ml), also from the nares. Interestingly, the isolate that recolonized the nares postmupirocin was the same strain that was recovered from the patient's wound prior to initiation of mupirocin therapy.

Of the 153 isolates that underwent Rep-PCR strain typing analysis, 93 (61%) were classified as USA300 (composed of two Rep-PCR types) and 54 (35%) were classified as USA100 (composed of two Rep-PCR types) (12). The remaining isolates in unique Rep-PCR types were classified as USA400 (1 isolate), USA800 (4 isolates), and USA1000 (1 isolate). Significant differences in susceptibility data for the two clonal groups were found and are presented in Table 4. Interestingly, the USA300 isolates were significantly less resistant to fluoroquinolones than were the USA100 isolates ($P = 0.003$). Only 6% of the USA300 isolates demonstrated inducible clindamycin resistance ($P < 0.0001$), and 48 (52%) were isolated from hospitalized patients; 94% of the USA100 isolates demonstrated inducible clindamycin resistance

TABLE 3 *In vitro* activities of mupirocin, retapamulin, and linezolid against 403 MRSA isolates

Drug	No. with MIC (μg/ml) of:																		
	≤0.004	0.008	0.02	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	>512
Mupirocin							356[a]	6	4	1	1	3	9	10	2	0	0	2	9
Retapamulin	0	1	2	13	69	200	102	16	0	0	0	0	1						
Linezolid									12[b]	256	135								

[a] ≤0.25 μg/ml.
[b] ≤1 μg/ml.

TABLE 4 Strain type and susceptibility data for selected isolates

Strain type	% (no. of isolates/total no. of isolates)		
	Fluoroquinolone resistant	Clindamycin resistant	Elevated linezolid MIC (4 μg/ml)
USA300	67 (64/93)	6 (6/93)	28 (13/47)
USA100	100 (54/54)	94 (51/54)	66 (31/47)

($P < 0.0001$), and 50 (93%) were isolated from hospitalized patients. Of the typed isolates with elevated linezolid MICs, 66% were USA100 and 28% were USA300 (Table 4), which differs from the distribution of isolates of the collection as a whole ($P < 0.0001$).

Investigation of elevated retapamulin MIC. A single isolate had an elevated retapamulin MIC of >16 μg/ml. When this isolate was further analyzed by PCR using *vgaA*-specific diagnostic primers, agarose gel electrophoresis yielded a single DNA product of ~770 bp, consistent with the expected size of a *vga*A-amplified product. tBLAST analysis of the compiled DNA sequence of the amplicon showed 100% amino acid identity to *vga*A of *S. aureus* across the entire length of the sequence. VgaA, which is a member of the ATP-binding cassette family of proteins, is likely involved in drug efflux and has been shown to confer decreased sensitivity to lincosamides and streptogramins (14, 15). This isolate had a clindamycin MIC of >2 μg/ml (resistant) and a quinupristin-dalfopristin MIC of 1 μg/ml, which is considered susceptible but at the breakpoint. In addition, confirmatory antibiotic panels demonstrated ≥8-fold higher MICs for pleuromutilins (retapamulin and tiamulin), lincosamides (clindamycin and lincomycin), and streptogramin A (virginiamycin M1), relative to the reference laboratory strain RN4220 (data not shown).

DISCUSSION

We determined the *in vitro* activity of retapamulin, mupirocin, and a select panel of antimicrobials against MRSA isolates specifically selected from targeted groups of veteran patients, including patients who had significant infections (i.e., bloodstream or respiratory infections) caused by MRSA, were at risk for long-term colonization or recolonization with MRSA, and/or were potential candidates for or were receiving decolonization treatment. We included nares isolates collected from our hospital-wide MRSA surveillance program and isolates from specific hospitalized patient groups at risk for MRSA infections, including chronically catheterized patients from the spinal cord injury unit, patients on ventilators, and patients with bloodstream infections. We included isolates from an outpatient population group (specifically, patients from the dermatology clinic) for comparison. The overall susceptibility rates for our isolates were similar to those found across the United States and at other VA medical centers, with high rates of fluoroquinolone and macrolide resistance, moderate rates of lincosamide resistance, and low rates of trimethoprim-sulfamethoxazole resistance (16, 17). Isolates from the outpatient group, nares conversion isolates, and isolates classified as strain types USA100 and USA300 demonstrated significant differences from the overall rates. Although USA300 remains a predominant strain type in the United States, the distribution of strain types varies among VA hospitals (18, 19).

Our original hypothesis was that longitudinal isolates (i.e., defined as isolates recovered from the same patient ≥30 days after collection of the initial isolate) and isolates from patients with repeated hospital exposures (i.e., hemodialysis patients and patients in the spinal cord injury unit) would demonstrate significant differences in antimicrobial resistance patterns, compared to the baseline population. The data did not bear this out; however, evaluating the data for a single antimicrobial revealed an interesting finding. Although 100% of the isolates in our study were considered susceptible to linezolid, 135 (33%) had elevated MICs of 4 μg/ml. Greater than 80% of those isolates were from patients with nosocomial exposure, including isolates from spinal cord injury patients, respiratory isolates, nares conversion isolates, and longitudinal isolates. Given that the worldwide rates of linezolid resistance and elevated MICs for MRSA are low (<0.8% in 2002 to 2010) (20), our findings were unexpected. These isolates were not tested for the presence of the *cfr* gene but, after reviewing the strain typing analysis, we found that the majority of these isolates were segregated into two larger clonal groups (12). Based on the rarity of MRSA isolates with elevated MICs and the clonality of the isolates in this study, the data suggest the possibility that these organisms were nosocomially acquired.

Routine decolonization of nasal carriers of MRSA is a common practice in many hospitals worldwide, although it is not a standardized practice across the United States or within the Veterans Affairs health care system. Its use has not been systematically implemented at our facility. Several studies demonstrated that patients are frequently recolonized after treatment (9, 21), and they also associated the use of mupirocin with increased rates of resistance (7). In our facility, the baseline level of mupirocin resistance in isolates from patients who had received mupirocin therapy was low (9%), which was comparable to reports from other VA hospitals (19). Of the patients in our study who were recolonized after mupirocin therapy, 30% were recolonized with a different strain. In addition, mupirocin MICs did not appear to predict whether patients would become recolonized. Although the number of patient isolates evaluated was small, the postmupirocin strain was different from the premupirocin strain for the isolates from nares cultures that demonstrated MIC changes after mupirocin therapy. Previously reported data confirmed that patients may be colonized with more than one strain of MRSA at different anatomical sites and that surveillance of the nares alone may not be the best predictor of subsequent wound infection (12, 13). Colonization by multiple strain types and variations in colonization at different anatomical sites have the potential to affect the decolonization process (22). A recent study by Huang et al. demonstrated the effectiveness of universal decolonization (nasal decolonization and chlorhexidine bathing for all patients) versus targeted decolonization (screening, isolation, and decolonization of MRSA carriers) in reducing the rates of MRSA clinical isolates and bloodstream infections from any pathogen (23). Given the published data, it is reasonable to conclude that surveillance of the nares alone is inadequate to assess whether a patient is at risk for clinical infection by MRSA, and it is also inadequate to assess whether the patient is carrying a strain resistant to part of the decolonization regimen (mupirocin). These examples demonstrate the necessity for additional analysis (for example, strain typing) to evaluate the development of mupirocin (or chlorhexidine) resistance and the usefulness of susceptibility data for evaluating "treatment failure," as recolonization does not necessarily indicate resistance to the decolonization therapy or failure of the therapy. It may instead be colonization by a new isolate, as recolonization is an opportunistic

event. Many laboratories do not have the technical capability to perform rapid strain typing or do not have access to such methods. In such cases, mupirocin MICs (determined by Etest; bioMérieux, Durham, NC) can substitute as preliminary screens for changes in strain type if the isolates are available. In any case, when therapy failure is suspected, we recommend that mupirocin MICs be determined.

The use of retapamulin as a topical agent for nares decolonization is under investigation. A 2008 clinical trial evaluated the efficacy of retapamulin against isolates of *S. aureus* isolated from nasal and pharyngeal swab specimens. In a randomized, double-blind, placebo-controlled, clinical trial, retapamulin was found to be highly active *in vitro*, with 86% of surveillance cultures being negative after 4 weeks. However, only 8% of *S. aureus* isolates were methicillin resistant (24). Our evaluation involved exclusively MRSA isolates, and our findings demonstrated that 99% of our isolates were considered susceptible. We identified only one isolate that had an elevated MIC. The reduced retapamulin (and pleuromutilin) and other drug susceptibilities for this isolate suggest that the elevated MIC may be linked to the acquisition of *vga*A.

Our study has several limitations. This study was performed at a single site with a targeted selection of isolates (as opposed to concurrent isolates) of MRSA. There is inherent bias in our study population, due to both the availability of certain isolates and our selection process. Although this study was performed at one VA hospital, the VA Puget Sound Health Care System is the referral site for a 5-state region encompassing Washington, Alaska, Montana, Idaho, and Oregon, with approximately 80,000 veterans. Because we care for patients from a large region, these data could be considered to be generalizable to the Northwest region. Another limitation was that we were able to assess the activity of retapamulin only *in vitro*. Our study demonstrates that retapamulin has excellent activity *in vitro*, regardless of the patient population or other patterns of resistance, as we identified only 1 isolate with an elevated MIC. Given that there are only a limited number of reported strains outside the wild-type distribution, it is difficult to know how these isolates would respond to the agent *in vivo*.

We think there are several points from this study to be considered. First, this is the largest systematic evaluation of MRSA isolates from North America and suggests that retapamulin may be a viable candidate for clinical evaluation as an alternative therapy for MRSA decolonization. Hospitals will likely need more than one antimicrobial option if universal decolonization is to become the standard of care. Second, in the creation of a baseline antibiogram for a subset of our stock collection, we found that antimicrobial susceptibility testing provided interesting clues regarding the dynamics of the potential transmission of MRSA. While our collection was composed largely of two clonal types, uncommon susceptibility patterns have the potential to add discriminatory power for tracking transmission in an institution. Although we would need to widen our scope to make definitive conclusions regarding the transmission of MRSA at our institution, the number of isolates with elevated linezolid MICs is a potential indicator that transmission has occurred. Third, the mere recovery of a MRSA isolate is not enough to indicate treatment failure in decolonization. Although our numbers were small, we identified cases in which the MRSA strain isolated after mupirocin therapy not only was different from the strain recovered prior to therapy but also was susceptible to the decolonizing agent. Although we would need to expand our investigation further to make definitive conclusions, this initial finding potentially calls into question the accuracy of study findings of elevated rates of mupirocin resistance after treatment. Accurate baseline data are essential for proper evaluation of the effectiveness of infection prevention programs.

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