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ORIGINAL ARTICLE

Evaluation of telavancin susceptibility in isolates of *Staphylococcus aureus* with reduced susceptibility to vancomycin

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Abstract

Historically, vancomycin has been considered a primary therapeutic option for treating infections with *Staphylococcus aureus*, but isolates with reduced vancomycin susceptibility (SA-RVS) (MIC ≥ 4 μg/mL) have emerged. Telavancin, a semisynthetic lipoglycopeptide, is an alternative treatment option for *S. aureus*, but data examining telavancin activity against SA-RVS are limited. In the present study, we characterize 300 isolates of *S. aureus* isolates (50 vancomycin-susceptible (VSSA) isolates and 250 SA-RVS isolates) from a large tertiary care, academic medical center, 51.8% of which were methicillin resistant (MRSA). Sixteen (6.4%) SA-RVS isolates were non-susceptible to telavancin, whereas all VSSA isolates were susceptible. Additionally, 3.6% of SA-RVS isolates were non-susceptible to daptomycin, with three (1.2%) isolates testing non-susceptible to both telavancin and daptomycin. When tested against other classes of antimicrobials, there were no statistical differences in susceptibility of VSSA and SA-RVS isolates, except for the fluoroquinolones (ciprofloxacin and moxifloxacin). Molecular characterization of the isolates showed that SCC*mec* types II and IV together represented over half of the SA-RVS isolates; 12.0% of the VSSA isolates were SCC*mec* type II. Using RepPCR, we detected 16 distinct strain types in this isolate collection, and *tst-1* (gene encoding the *Staphylococcus* toxic shock syndrome super-antigen) carriage was low (5.4%). Overall, we show that in addition to reduced vancomycin susceptibility, a small, but clinically significant, proportion of SA-RVS isolates also demonstrate reduced susceptibility to both telavancin and daptomycin.

Keywords *Staphylococcus* · *Staphylococcus aureus* · Vancomycin · Telavancin · Susceptibility testing · SA-RVS

Introduction

Staphylococcus aureus isolates with a reduced susceptibility to vancomycin (SA-RVS) are emerging. To date, vancomycin-resistant *S. aureus* (VRSA) isolates (i.e., those with a vancomycin MIC ≥ 16 μg/mL) remain a very rare finding. The incidence of SA-RVS (such as vancomycin intermediate *S. aureus* isolates with MIC 4–8 μg/mL) is low overall but occurs commonly enough to be an important clinical concern [1–4]. While vancomycin resistance in *S. aureus* is conferred via the *vanA* gene, the intermediate susceptibility phenotype to vancomycin is mediated primarily by overproduction of cell wall precursors that sequester and overwhelm the antimicrobial [4]. Vancomycin is commonly considered the mainstay of therapy for the treatment of invasive infections with methicillin-resistant *S. aureus* (MRSA), but alternative agents (such as daptomycin, linezolid, and ceftaroline) are considered for the treatment of MRSA isolates that are not susceptible to vancomycin or for the treatment of patients who are not able to tolerate vancomycin. In addition to these alternative agents, telavancin is another antibacterial agent that can be considered for treatment of an infection with SA-RVS [5–7].

Telavancin is a semisynthetic lipoglycopeptide with two mechanisms of action. Like vancomycin, telavancin inhibits peptidoglycan synthesis, but also has bactericidal activity by binding bacterial cell membranes, leading to membrane depolarization [8, 9]. To date, there have been investigations

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demonstrating the effect of telavancin on various Gram-positive pathogens, including methicillin-resistant *S. aureus* [9]. However, in 2014, the FDA and CLSI revised both breakpoint data and methodology for telavancin susceptibility testing to allow for more accurate and reproducible results [10, 11]. These changes included using DMSO as the solvent and diluent for preparation of the antibiotic and using polysorbate-80 to limit the drug from binding to plastic. Since that time, there have been relatively few studies examining in vitro antimicrobial activity of this agent, and very little data on minimum inhibitory concentrations (MIC) for SA-RVS isolates. Herein, our objective was to characterize the epidemiology and antimicrobial susceptibility profiles of a large collection of SA-RVS isolates recovered at a tertiary care, academic medical center in order to provide a better understanding of these isolates and their possible treatment options.

Materials and methods

Bacterial isolates

Three hundred (300) *Staphylococcus aureus* strains were obtained from archived clinical isolates at Barnes-Jewish Hospital (St. Louis, Missouri) for evaluation. Two hundred and fifty (250) of the isolates originally tested with a vancomycin MIC of 4–8 μg/mL (SA-RVS), while the remaining 50 isolates, included as control strains, had a vancomycin MIC of ≤ 2 μg/mL (VSSA). Isolates evaluated represent the first 50 consecutive SA-RVS isolates from unique patients > 18 years of age from each year between 2011 and 2015. The VSSA isolates were all collected concurrently during a two-month period in 2015, also from unique patients > 18 years of age. Original clinical vancomycin susceptibility results were determined using vancomycin screening agar (BHI-V3, Remel, Lenexa, KS) as previously described, followed by a gradient diffusion test (ETEST, bioMéreiux, Durham, MC) [12]. Briefly, any isolate that did not grow on the BHI-V3 agar was considered to be vancomycin susceptible (VSSA), and any isolate with growth on the BHI-V3 agar was evaluated using a vancomycin ETEST. Isolates with MIC values of 4–8 μg/mL were considered to be SA-RVS, while isolates with MICs of ≤ 2 μg/mL were classified as VSSA.

For this investigation, SA-RVS isolates were recovered from frozen stocks (− 80 °C) and subcultured two times on BHI-V3 agar to maintain vancomycin selective pressure prior to evaluation. Isolates that did not grow on the subculture to BHI-V3 agar were subcultured twice on blood agar. The identification of each isolate was confirmed using matrix associated laser desorption- time of flight mass spectrometry (MALDI-TOF MS) (VITEK MS v2.0, bioMéreiux).

Susceptibility testing

Susceptibility testing was performed on all isolates using broth microdilution (Sensititre GPALL3F panels, Thermo Fisher) following the manufacturer's instructions and using interpretive criteria in the CLSI M100-26 document [13]. Methicillin resistance was determined by the cefoxitin (6 μg/mL) screening well on the Sensititre panel. Susceptibility testing for mupirocin was performed using gradient diffusion (ETEST, bioMéreiux). Quality control testing for all susceptibility testing was performed on each day of testing following CLSI and manufacturer recommendations: *S. aureus* ATCC 29213 was used for the Sensititre panels and the vancomycin ETEST; *S. aureus* ATCC 29213 and BAA-1708 was used for the mupirocin ETEST; *S. aureus* ATCC 25923 was used for cefoxitin disk diffusion testing.

Molecular characterization

Molecular characterization of the SA-RVS and VSSA isolates to determine strain relatedness included repetitive sequence PCR (repPCR) and *SCCmec* typing [14, 15]. For repPCR, isolates with a similarity index of ≥ 95% were considered related. Each strain grouping was assigned a letter (A–O).

High-level mupirocin resistance was detected using a real-time PCR assay that detects *mupA* as previously described [16, 17].

Two hundred ninety-three isolates underwent PCR testing for the determination of *tst*-1 gene carriage, using a modification of a previously published assay [18]. The *tst*-1 primers (forward 5′-ATGGCAGCATCAGCTTGATA-3′; reverse 5′-TTTCCAATAACCACCCGTTT-3′) were used to amplify 100 ng of target DNA (expected product size 350 bp) using illustra™ PuReTaq™ Ready-to-Go™ PCR beads (GE Healthcare, Buckinghamshire, UK). Ten microliters (10 μL) of each PCR product was visualized on a 2% agarose gel.

Statistical analyses

Statistical comparisons were conducted using Fisher's exact test or Student's *t* test, where appropriate, in R v.3.4.3 (cran.r-project.org).

Results

Bacterial isolates

The patient demographics and the specimen sources of the *S. aureus* isolates used in this study are shown in Table 1. Fifty-two percent (52%) of SA-RVS isolates and 66% of vancomycin-susceptible *S. aureus* (VSSA) isolates were recovered from males ($P > 0.05$). The majority (45.6% of SA-

Table 1 Patient demographics and specimen source of *Staphylococcus aureus* isolates

	SA-RVS, *n* (%) (*n* = 250)		VSSA, *n* (%) (*n* = 50)	
Sex				
Male	130	(52.0)	33	(66.0)
Female	120	(48.0)	17	(34.0)
Age				
< 20	6	(2.4)	0	(0.0)
20–39	81	(32.4)	15	(30.0)
40–59	77	(30.8)	17	(34.0)
60–79	73	(29.2)	17	(34.0)
> 80	13	(5.2)	1	(2.0)
Source				
Blood	24	(9.6)	3	(6.0)
Respiratory	59	(23.6)	10	(20.0)
Urine	30	(12.0)	1	(2.0)
Skin and soft tissue	114	(45.6)	30	(60.0)
Other, sterile	11	(4.4)	3	(6.0)
Other, non-sterile	12	(4.8)	3	(6.0)

SA-RVS, *S. aureus* isolates with reduced vancomycin susceptibility; *VSSA*, vancomycin-susceptible *S. aureus*

RVS and 60% of VSSA) of the isolates were derived from skin and soft tissue infections (45.6% SA-RVS, 60.0% VSSA), followed by respiratory sources (23.6% SA-RVS, 20% VSSA). Approximately 15% of the isolates were derived from sterile sources, including blood cultures. There were similar distribution of specimen sources and patient age ranges for SA-RVS and VSSA isolates ($P > 0.05$ for both).

Susceptibilities of SA-RVS isolates

Out of all isolates tested, 51.8% were MRSA and 48.2% were methicillin-susceptible *S. aureus* (MSSA). There were no statistically significant differences in the distribution of MRSA and MSSA among VSSA and SA-RVS isolates ($P > 0.05$). Out of the 250 SA-RVS isolates, 54% were MRSA (Table 2). Examining the susceptibilities of the SA-RVS isolates during our study, only 41 out of the 250 isolates (16.4%) (MIC of 4–8 μg/mL on initial clinical testing) had a vancomycin MIC of ≥ 4.0 μg/mL. Although the majority (83.6%) of the SA-RVS isolates had reverted to a vancomycin-susceptible phenotype after storage at − 80 °C, the mean MIC, MIC_{50}, and MIC_{90} were each elevated (2, 2, and 4 μg/mL, respectively) (Table 3). VSSA isolates had vancomycin mean MIC, MIC_{50}, and MIC_{90} of 1 μg/mL (Table 3).

Telavancin testing was performed and the MIC of isolates tested ranged from 0.03 to 0.25 μg/mL (Tables 3 and 4). Sixteen SA-RVS isolates (6.4%) had MICs of 0.25 μg/mL, and are classified as non-susceptible according to both FDA and CLSI breakpoints [10, 13]; 11 of these were MRSA and 5 were MSSA. All VSSA isolates were susceptible to telavancin with a geometric mean MIC, MIC_{50}, and MIC_{90} of 0.06 μg/mL. All isolates were also tested against other anti-staphylococcal agents as shown in Table 3. Nine SA-RVS isolates (3.6%) were non-susceptible to daptomycin, but all VSSA isolates were susceptible with a low MIC (≤ 1 μg/mL). There were seven isolates (6 (2.4%) SA-RVS and 1 (2.0%) VSSA) that were intermediate to ceftaroline, and one VSSA (2.0%) isolate that was resistant. Two SA-RVS isolates (0.8%) were resistant to linezolid and all VSSA isolates were susceptible to linezolid. Table 4 shows the susceptibilities for the same anti-staphylococcal antimicrobials but stratified by the susceptibility of the isolates to methicillin (MRSA vs MSSA).

In addition to these anti-staphylococcal drugs, the SA-RVS and VSSA isolates were tested against other antimicrobial agents, with the overall antibiogram shown in Table 2. Except for vancomycin, there were no statistically significant differences in the susceptibility rates between SA-RVS and VSSA, with the exception of ciprofloxacin ($P = 0.003$) and moxifloxacin ($P = 0.02$; Table 2). Of note, ciprofloxacin, levofloxacin, and moxifloxacin all showed reduced susceptibility overall (44%, 49%, and 48%, respectively) for SA-RVS isolates as compared with VSSA isolates (67%, 64%, and 66%, respectively).

Mupirocin resistance

High-level mupirocin resistance was measured both genotypically and phenotypically. Overall, there were 21 *S. aureus* (20 SA-RVS, 1 VSSA) isolates that were positive for the *mupA* gene by PCR. For SA-RVS isolates, the geometric mean, MIC_{50}, and MIC_{90} were 0.54 μg/mL, 0.25 μg/mL, and 1.0 μg/mL, respectively (Table 3). Fifteen (15) SA-RVS isolates demonstrated high-level resistance (HLMR) to mupirocin with MICs of ≥ 1024 μg/mL, while five SA-RVS isolates had low-level resistance (LLMR) (MICs of 16–32 μg/mL). Only one SA-RVS isolate with LLMR was found to contain the *mupA* gene using PCR (MIC = 32 μg/mL), while four isolates which tested susceptible to mupirocin were positive for the *mupA* gene (MICs of 0.125–0.5 μg/mL). For VSSA isolates, the geometric mean, MIC_{50}, and MIC_{90} were 0.45 μg/mL, 0.50 μg/mL, and 0.50 μg/mL, respectively (Table 3). The single *mupA*-positive VSSA isolate also demonstrated HLMR with an MIC of ≥ 1024 μg/mL. One additional VSSA isolate was positive for LLMR with an MIC of 64 μg/mL. Table 4 shows mupirocin susceptibilities for MRSA and MSSA isolates. MRSA isolates had a geometric mean, MIC_{50}, and MIC_{90} were 0.76 μg/mL, 0.50 μg/mL, and 16.0 μg/mL, respectively, while MSSA isolates had a geometric mean, MIC_{50}, and MIC_{90} were 0.35 μg/mL, 0.25 μg/mL, and 0.50 μg/mL, respectively.

Table 2 Antimicrobial susceptibility profiles for *S. aureus* isolates evaluated

Antimicrobial	SA-RVS			VSSA			*P* value
	All (%[$]) ($n = 250$)	MRSA (%) ($n = 138$)	MSSA (%) ($n = 112$)	All (%) ($n = 50$)	MRSA (%) ($n = 20$)	MSSA (%) ($n = 30$)	Comparing SA-RVS with VSSA
Oxacillin*	46	0	100	60	0	100	0.09
Ceftaroline	98	96	100	96	90	100	0.33
Daptomycin	97	95	99	100	100	100	0.61
Telavancin[&]	93	92	95	100	100	100	0.09
Ciprofloxacin	44	21	82	60	33	81	0.003
Clindamycin^	50	35	83	60	70	87	0.22
Erythromycin	29	6	57	40	15	57	0.14
Levofloxacin	49	27	75	64	40	73	0.06
Linezolid	99	100	98	100	100	100	1.00
Moxifloxacin	48	27	75	66	55	87	0.02
Rifampin	94	93	96	96	95	100	0.75
Tetracycline	91	89	94	94	95	97	0.78
TMP-SMX[‡]	98	97	99	100	100	100	0.59

SA-RVS, *S. aureus* isolates with reduced vancomycin susceptibility; *VSSA*, vancomycin-susceptible *S. aureus*; *MRSA*, methicillin-resistant *S. aureus*; *MSSA*, methicillin-susceptible *S. aureus*

[$] Percentages represent percent susceptible

*Oxacillin susceptibility determined using a screen well containing 6 μg/mL of cefoxitin

[&] Non-susceptibility based on CLSI and FDA breakpoints (MIC ≥ 0.25 μg/mL)

^Both constitutive and inducible resistance classified as resistant. Inducible resistance determined by the D-test broth screen

[‡] TMP-SMX, trimethoprim-sulfamethoxazole

Molecular characterization

To further characterize this collection of isolates, SCC*mec* typing and repPCR were performed for 293 isolates (Supplemental Table). For SA-RVS, SCC*mec* types II and IV represented over half of all the isolates tested with less than 5% of the isolates typing as type I and III each (Table 5). Nearly 1/3 of the isolates either were non-typable or had no detectable SCC*mec* cassette with 2.4% of these isolates being MRSA (Tables 5 and 6). For VSSA isolates, there was a smaller percentage of type II isolates (12.0%) compared with the SA-RVS isolates (Table 5). In all isolates studied, there was only one type V isolate, which was also a methicillin-susceptible SA-RVS (Table 5).

The repPCR analysis demonstrated 16 distinct strain types, with two strains accounting for over two-thirds of all the isolates (Supplemental Table). There were no shifts in SCC*mec* types over the five years of the study with the vast majority of MRSA isolates falling into SCC*mec* types II and IV in all years (Table 6).

Sixteen (16/294; 5.4%) isolates in this study were identified as being PCR positive for *tst*-1 (Table 6). Among MSSA, *tst*-1 PCR-positive isolates were either SCC*mec* III ($n = 4$) or non-typable/no cassette ($n = 3$), whereas among MRSA, *tst*-1-

Table 3 Susceptibility of anti-staphylococcal drugs against SA-RVS/VSSA isolates

	SA-RVS ($n = 250$) Mean/MIC_{50}/MIC_{90} (μg/mL) (range)	VSSA ($n = 50$) Mean/MIC_{50}/MIC_{90} (μg/mL) (range)
Vancomycin	2/2/4 (1–8)	1/1/1 (0.5–1)
Daptomycin	0.7/0.5/1 (< 0.5–> 4)	< 0.5/< 0.5/< 0.5 (< 0.5)
Telavancin	0.09/0.12/0.12 (0.06–0.25)	0.06/0.06/0.06 (0.03–0.06)
Linezolid	2.7/2.0/4 (1–> 8)	3.7/4/4 (2–4)
Ceftaroline	0.5/0.50/1 (< 0.12–2)	0.35/0.25/0.5 (< 0.12–4)
Mupirocin	0.54/0.25/1.0 (0.064–> 1024)	0.45/0.50/0.50 (0.125–> 1024)

SA-RVS, *S. aureus* isolates with reduced vancomycin susceptibility; *VSSA*, vancomycin-susceptible *S. aureus*

Table 4 Susceptibility of anti-staphylococcal drugs against MRSA/MSSA isolates

	MRSA ($n = 158$) Mean/MIC_{50}/MIC_{90} (μg/mL) (range)	MSSA ($n = 142$) Mean/MIC_{50}/MIC_{90} (μg/mL) (range)
Vancomycin	2/2/4 (1–4)	1.7/2/2 (0.5–4)
Daptomycin	1.1/< 0.5/1 (< 0.5–> 4)	1/< 0.5/1 (< 0.5–2)
Telavancin	0.09/0.06/0.12 (0.03–0.25)	0.08/0.06/0.12 (0.03–0.25)
Linezolid	2.8/2/4 (< 1–4)	2.9/4/4 (< 1–> 8)
Ceftaroline	0.8/1/1 (0.25–4)	0.3/0.25/0.25 (< 0.12–1)
Mupirocin	0.76/0.50/16.0 (0.064–> 1024)	0.348/0.25/0.50 (0.064–> 1024)

MRSA, methicillin-resistant *S. aureus*; *MSSA*, methicillin-susceptible *S. aureus*

positive isolates were SCC*mec* II ($n = 6$), III ($n = 1$), or IV ($n = 2$; Table 6).

Discussion

Herein, we examined 250 SA-RVS and 50 VSSA isolates and investigated the activity of telavancin and other anti-staphylococcal antibiotics on these strains. The rate of MRSA in our study (51.8%) closely resembled the normal MRSA epidemiology for the hospital, with overall hospital MRSA rates ranging between 48 and 50% from 2013 to 2015 (Barnes-Jewish Hospital annual antibiogram). Although 250 of the *S. aureus* isolates initially tested as having vancomycin MICs ≥ 4 μg/mL, over 80% reverted to a vancomycin-susceptible phenotype following freeze-thaw, even when subjected to vancomycin selective pressure (BHI-V3 Agar) for subculture. This decrease in vancomycin MICs following cryostorage and serial passaging has been demonstrated by others [19–21]. Charlton et al. (2014) showed that after 12 months of frozen storage, vancomycin MICs were reduced 0.74-fold, but there were minimal effects on MICs after serial passaging (up to 20 passages).

Following the updates to telavancin testing methodology and breakpoints, there have been relatively few studies examining SA-RVS isolates. In our study, 6.4% (16/250) of the SA-RVS isolates were non-susceptible (MIC ≥ 0.12 μg/mL) to telavancin. Using previous breakpoints of ≤ 1 μg/mL (VIBATIV package insert, revision 08/2009), all isolates would have been considered susceptible. Of these 16 SA-RVS isolates that had telavancin MICs of ≥ 0.25 μg/mL, nine isolates retained a SA-RVS phenotype with vancomycin MICs of 4–8 μg/mL upon testing in this study (after cryostorage and two subsequent passages). All VSSA isolates evaluated were telavancin susceptible.

Other studies examining telavancin activity in *S. aureus* using the updated breakpoints and methodology have shown mixed outcomes. Mendes et al. (2015) [22] studied telavancin activity in a large study of various Gram-positive bacteria and demonstrated that 50% of the vancomycin intermediate *S. aureus* (SA-RVS; vancomycin MICs of 4–8 μg/mL) isolates tested ($n = 6$) and all VRSA isolates ($n = 6$) (MIC > 16 μg/mL) were non-susceptible to telavancin. In two other studies by the same group with larger collections of SA-RVS isolates ($n = 115$ and $n = 90$), all of the SA-RVS isolates tested as susceptible to telavancin [23, 24]. Smart et al. (2016) discovered 0.06% (3/528) of *S. aureus* isolates with a vancomycin MIC ≥ 1 μg/mL had a telavancin non-susceptible phenotype [25]. In a study comparing broth microdilution (BMD) and Trek panel methodology, 10/100 (10%) SA-RVS isolates using the BMD method and 5/100 (5%) SA-RVS isolates using the Trek method were telavancin non-susceptible [26]. Finally, Saravolatz and Pawlak (2019) also showed telavancin non-susceptibility in SA-RVS using broth microdilution, Trek panels, and ETEST [27]. These last two studies most closely

Table 5 SCC*mec* typing for SA-RVS and VSSA isolates

SCC*mec* type	SA-RVS, *n* (%) ($n = 250$)	VSSA, *n* (%) ($n = 50$)
I	9 (3.6)	2 (4.0)
II	81 (32.4)	6 (12.0)
III	12 (4.8)	7 (14.0)
IV	66 (26.4)	15 (30.0)
V	1 (0.4)	0 (0.0)
Non-typable/no cassette	81 (32.4)*	20 (40.0)^

SA-RVS, *S. aureus* isolates with reduced vancomycin susceptibility; *VSSA*, vancomycin-susceptible *S. aureus*

*97.5% of isolates are MSSA

^100% of isolates are MSSA

Table 6 SCC*mec* typing and *tst*-1 PCR result for MSSA and MRSA isolates by year of the study

SCC*mec* type	2011		2012		2013		2014		2015	
	n (%)	*n tst*-1 pos.	*n* (%)	*n tst*-1 pos.	*n* (%)	*n tst*-1 pos.	*n* (%)	*n tst*-1 pos.	*n* (%)	*n tst*-1 pos.
MSSA										
I	1 (6.7)	0	1 (4.2)	0	2 (9.1)	0	3 (10.3)	0	4 (7.7)	0
II	1 (6.7)	0	2 (8.3)	0	1 (4.5)	0	2 (6.9)	0	1 (1.9)	0
III	2 (13.3)	1	0 (0.0)	0	4 (18.2)	1	3 (10.3)	0	8 (15.4)	2
IV	2 (13.3)	0	1 (4.2)	0	0 (0.0)	0	1 (3.4)	0	3 (5.8)	0
V	0 (0.0)	0	0 (0.0)	0	1 (4.5)	0	0 (0.0)	0	0 (0.0)	0
Non-typable/no cassette	9 (60.0)	0	20 (83.3)	1	14 (63.6)	0	20 (69.0)	0	36 (69.2)	2
MRSA										
I	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0
II	19 (52.8)	3	10 (40.0)	0	15 (55.6)	1	12 (54.5)	1	24 (50.0)	1
III	0 (0.0)	0	1 (4.0)	0	0 (0.0)	0	0 (0.0)	0	1 (2.1)	1
IV	17 (47.2)	1	13 (52.0)	0	12 (44.4)	0	10 (45.5)	0	22 (45.8)	1
V	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0
Non-typable/no cassette	0 (0.0)	0	1 (4.0)	0	0 (0.0)	0	0 (0.0)	0	1 (2.1)	0

MRSA, methicillin-resistant *S. aureus*; *MSSA*, methicillin-susceptible *S. aureus*; *pos.*, positive

resemble ours in both methodology and percentage of telavancin non-susceptible isolates.

In addition to telavancin, the comparison of the susceptibility profiles of the SA-RVS and VSSA isolates revealed few differences in antimicrobial susceptibility patterns to the other agents tested. The fluoroquinolones (ciprofloxacin, levofloxacin, and moxifloxacin) all demonstrated decreased susceptibilities in SA-RVS isolates as compared with VSSA isolates, but this could be attributed to the higher number of MRSA-SA-RVS isolates (54.4%) as compared with MRSA-VSSA isolates (38%) in our study. Ceftaroline and linezolid MICs did not appear to be directly affected by the SA-RVS phenotype, whereas daptomycin, an antimicrobial that is structurally and functionally related to vancomycin and telavancin, had increased mean MIC, MIC_{50}, and MIC_{90} in the SA-VRS isolates as compared with VSSA isolates. Additionally, all daptomycin non-susceptible isolates were also SA-RVS. When susceptibility profiles of isolates were examined based on methicillin susceptibility alone, the MICs for daptomycin and telavancin were similar for MRSA and MSSA, showing that the differences are most likely due to the SA-RVS phenotype and not the *mecA* gene.

It has been demonstrated that up to 30% of MRSA carriers develop infections and therefore decolonization of carriers is an important infection control practice [28]. One such strategy utilizes mupirocin ointment for nasal decolonization, but there are increasing reports of *S. aureus* developing mupirocin resistance. We demonstrate an increased MIC_{90} for mupirocin in MRSA isolates as compared with the MSSA isolates. To our knowledge, no other studies have examined mupirocin resistance specifically in SA-RVS isolates. Here, we demonstrate an increased rate of mupirocin resistance (presence of the *mupA* gene and phenotypic resistance) in our SA-RVS isolates as compared with the VSSA isolates (8.0% vs 2.0%), although the geometric mean, MIC_{50}, and MIC_{90} do not differ by more than one doubling dilution between SA-RVS and VSSA.

Molecular strain typing was performed to characterize the isolates in our investigation and although all the isolates were obtained from a single center, these isolates do not represent expansion of a single clonal population in our region as repPCR demonstrated 16 different types. The SA-RVS and VSSA isolates were distributed between the different SCC*mec* types. The predominant type was SCC*mec* type II in SA-RVS isolates and SCC*mec* type IV is VSSA isolates. Of note, 42 MSSA isolates contained a SCC*mec* type I-IV. These isolates most likely represent *mecA* "drop-out" strains. These isolates were distributed fairly equally among the different SCC*mec* types (Supplemental Table). Finally, the carriage rate of the *tst*-1 gene in this cohort is low (5.4%) with 50% of *tst*-1-positive isolates being MRSA. Finally, all susceptibility testing was performed using commercially available broth microdilution panels.

There are several limitations to our study, including that all isolates were collected from a single center, but multiple molecular typing methods demonstrate that the isolates do not represent a single clone. Our study does not include more recent data from 2016 to 2019 so we are unable to conclude if contemporary trends parallel the findings herein. Additionally, most of the isolates were stored at − 80 °C for long periods of time prior to testing, which can affect the susceptibility profiles of the isolates, but whenever possible selective pressure was maintained during recovery and

passaging to attempt to retain the original phenotype of the isolates.

With increasing rates of reduced susceptibility to vancomycin in *S. aureus* and the toxicity that can be associated with this antimicrobial agent, the utility of vancomycin for treatment of *S. aureus* infections is a topic of great debate. Two alternative treatments include daptomycin and telavancin. Our study demonstrated that, although very rare, isolates with a reduced in vitro susceptibility to vancomycin may also test as non-susceptible to these antimicrobials and that laboratories should perform antimicrobial susceptibility testing for these agents if they will be utilized for patient treatment, especially in SA-VRS. Finally, we also show that in our cohort, SA-RVS isolates were more likely to carry the *mupA* gene leading to HLMR than VSSA isolates. The presence of HLMR can further complicate patient care leading to the need for additional decolonization strategies. Further studies are needed to determine if SA-RVS isolates are more likely to also carry the *mupA* gene as compared with VSSA isolates. In conclusion, our study represents one of the largest longitudinal studies to examine the epidemiology and susceptibility profiles of SA-RVS isolates.

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Compliance with ethical standards

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee (Washington University Human Research Protection Office, IRB ID # 201508039) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Ethical approval retrospective studies Although retrospective studies are conducted on already available data or biological material (for which formal consent may not be needed or is difficult to obtain), ethical approval may be required dependent on the law and the national ethical guidelines of a country. Authors should check with their institution to make sure they are complying with the specific requirements of their country.

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