Decreased Susceptibilities to Retapamulin, Mupirocin, and Chlorhexidine among *Staphylococcus aureus* Isolates Causing Skin and Soft Tissue Infections in Otherwise Healthy Children

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Topical antimicrobial and antiseptic agents are commonly used in the management of minor skin and soft tissue infections (SSTIs). Resistance to mupirocin has been documented in *Staphylococcus aureus* isolates causing SSTIs. Data are limited, however, on the prevalence of retapamulin resistance or tolerance to antiseptics. We sought to determine the prevalence of decreased susceptibility to retapamulin and mupirocin as well as the potential for decreased chlorhexidine susceptibility of *S. aureus* isolates from SSTIs in children. Two hundred isolates from patients with a single SSTI and 200 isolates from patients with ≥ 3 previous episodes from the years 2010 to 2012 were selected from an *S. aureus* surveillance study. Screening for retapamulin resistance was performed by the broth macrodilution method; mupirocin MICs were determined by Etest. PCR was performed for the presence of the *smr* gene associated with elevated MICs/minimum bactericidal concentrations (MBCs) to chlorhexidine. Among the isolates screened, 38 isolates (9.5%) exhibited retapamulin resistance, of which 22 (57.9%) were methicillin-resistant *S. aureus* (MRSA). Two isolates (0.5%) displayed cross-resistance to retapamulin and linezolid. Thirty-nine isolates (9.8%) were found to have mupirocin resistance. *smr*-positive *S. aureus* accounted for 14% of isolates. The proportion of *smr*-positive organisms increased during the study ($P = 0.005$). The prevalence of *in vitro* resistance to topical antimicrobials among *S. aureus* isolates causing SSTI in healthy children in our community is almost 10%. Retapamulin resistance was associated with cross-resistance to linezolid in 0.5% of isolates. In addition, there was an increase in the proportion of *smr*-positive isolates. Further research including clinical correlations with these findings is warranted.

Staphylococcus aureus is the most common cause of bacterial skin and soft tissue infection (SSTI) among both children and adults and accounts for up to 76% of all purulent SSTIs (1). While there is the potential for severe illness with these infections, many cases can be treated with topical agents alone. In fact, topical antimicrobial agents are recommended by the Infectious Disease Society of America for the management of minor skin infections due to methicillin-resistant *S. aureus* (MRSA) (2).

Mupirocin is a topical antimicrobial agent used for the treatment of minor SSTI due to Gram-positive agents. Previous work at our center evaluated *S. aureus* isolates causing recurrent SSTI in children for the presence of mupirocin resistance and the *mupA* gene conferring high-level resistance (3). Among isolates causing recurrent infection in children in the Houston area, 14.7% were resistant to mupirocin and often exhibited coresistance to clindamycin (3). The finding of coresistance to other agents has been found in other centers as well (4).

Topical antiseptics, such as chlorhexidine gluconate, are often used in the prevention of health care-associated infections (5) as well as to decolonize patients known to be carriers of MRSA (2). Previous investigations of health care-associated staphylococcal infections in our hospital revealed a higher-than-expected prevalence of isolates with elevated MICs/minimum bactericidal concentrations (MBCs) to chlorhexidine (6, 7). Numerous genes can confer this phenotype, and notably the *smr* gene (also called *qacC/D* or *ebr* [8]) is among the most commonly occurring antiseptic tolerance genes in *S. aureus* in the United States (9). Given that some experts recommend chlorhexidine and other antiseptics to combat recurrent skin infections (10, 11), antiseptic-tolerant *S. aureus* may emerge as a significant infectious disease challenge in the community.

Pleuromutilins are a class of semisynthetic antimicrobials with activity against a spectrum of Gram-positive agents. Pleuromutilin resistance in *S. aureus* has been previously documented through the acquisition of efflux pumps, such as *vgaA/Av* (12), or the *cfr* methyltransferase, leading to a modification of the drug target (13). Mutations within the ribosomal protein L3 and the 23S rRNA have also been associated with pleuromutilin resistance (12, 14). Retapamulin is the only drug of the pleuromutilin class approved for use in humans for the topical treatment of minor SSTIs. Data on the prevalence of retapamulin resistance in clinical isolates of *S. aureus* are limited, with rates of resistance ranging from <1 to 2.6% (15, 16). There is no information on this issue in a pediatric population.

We determined the prevalence of retapamulin resistance among *S. aureus* isolates causing SSTI, as well as associated clinical risk factors, in the pediatric population served at Texas Children's Hospital (TCH). Furthermore, we examined the presence of the *smr* gene among *S. aureus* isolates causing community-onset infections.

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MATERIALS AND METHODS

Patients. All isolates were selected from a prospective *S. aureus* surveillance study ongoing at TCH since 2001 (17). TCH is a tertiary teaching hospital in Houston, TX, with 639 licensed inpatient beds. All clinical cultures from which *S. aureus* is isolated by the clinical microbiology laboratory are subcultured and stored in horse blood at -80°C in the Infectious Diseases Laboratory. Patient-level clinical data, including demographics, diagnosis, and underlying conditions, are catalogued in a secure central database. This database was searched for isolates obtained from children with a diagnosis of SSTI from 2010 to 2012. For purposes of this study, an SSTI was regarded as skin and soft tissue abscesses, cellulitis, folliculitis, pustulosis, impetigo, and lymphadenitis. Two-hundred isolates from patients with a single SSTI and 200 isolates from patients with at least three previous episodes were selected for inclusion with the aid of a random-number generator. Children with an immunocompromising condition (such as primary immunodeficiency, malignancy, or HIV) were excluded. Children with eczema were included in the study, but other chronic skin diseases were excluded (such as epidermolysis). Isolates associated with invasive *S. aureus* infection, such as bacteremia, pneumonia, or bone or joint infection, or those obtained beyond 48 hours of hospitalization were excluded. Medical records of all patients were reviewed retrospectively, and clinical information was recorded on a standard data collection form. In particular, documentation of a hospital admission or emergency center visit and topical or systemic antimicrobial use in the preceding 3 months was recorded. Informed consent was waived for this retrospective study, and patients' families were not contacted by study personnel. Treatment failure was defined by Hyun et al. as an *S. aureus* infection at the same site requiring additional antimicrobial therapy or a drainage procedure within 1 month of the original infection (18). This study was approved by the institutional review board of Baylor College of Medicine.

Antibiotic susceptibility testing. Isolates underwent susceptibility testing to oxacillin, vancomycin, clindamycin, and trimethoprim-sulfamethoxazole by the TCH clinical microbiology laboratory during the routine course of care. Isolates were initially screened for retapamulin resistance with an agar dilution method (19). Retapamulin powder (provided by Stiefel Laboratories, a GlaxoSmithKline company) was dissolved in pH-adjusted saline and incorporated into Mueller-Hinton agar. Overnight cultures of isolates were used to make a 0.5 McFarland suspension, which was adjusted to a final concentration of 1×10^4 CFU per spot and inoculated onto retapamulin agar dilution plates using a Steers replicator. Isolates identified as resistant or intermediate to retapamulin were then confirmed by broth macrodilution (19). For this procedure, retapamulin powder was suspended in dimethyl sulfoxide and diluted in 10% β -cyclodextrin; the stock solution was used to make Mueller-Hinton broth macrodilution tubes (20). MICs were interpreted after overnight incubation at 37°C as the minimum concentration that inhibited visible growth of organisms. Retapamulin susceptibility breakpoints used were those described by Traczewski and Brown: MICs of ≤ 0.5 $\mu\text{g/ml}$ were susceptible, MICs of 1 $\mu\text{g/ml}$ were intermediate, and MICs of ≥ 2 $\mu\text{g/ml}$ were resistant (20). In case of discrepancy between agar and broth dilution, the lower MIC was regarded as the true value. A *cfr*-positive retapamulin-resistant isolate provided generously by Stiefel Laboratories was used as a positive control. Mupirocin and linezolid MICs were determined by the Etest (bioMérieux, Sweden). High-level mupirocin resistance (HMR) was defined as mupirocin MICs of ≥ 512 $\mu\text{g/ml}$, and low-level resistance (LMR) was defined as mupirocin MICs of 8 to 256 $\mu\text{g/ml}$ (21). Chlorhexidine MICs and minimum bactericidal concentrations (MBC) were determined as previously described (7). *S. aureus* ATCC 29213 was used as a quality control in antimicrobial susceptibility testing.

Molecular analyses. All isolates had genomic DNA extracted with the use of the microbial DNA isolation kit (Mol Bio Laboratories, Carlsbad, CA). The *smr* gene complex was detected by PCR (primers *smr* F, ATGC CTATATTTTATTTAATAATAGCC; *smr* R, ATGCGATGTTCCGAAAA TGTTTAAC) (22). Retapamulin-nonsusceptible isolates and a subset of

TABLE 1 Demographics and selected clinical features

Variable	No. of patients out of 400 total (%) ^a
Median age, mo (IQR ^b)	27 (16–75.5)
Female gender	232 (58)
African American race	100 (25)
Hispanic ethnicity	161 (40.3)
Chronic conditions	121 (30.3)
Eczema	44 (11)
Asthma	18 (4.5)
Congenital heart disease	12 (3)
Hospitalization in prior 3 mo	36 (9)
Antibiotic use in prior 3 mo	190 (47.5)
Trimethoprim-sulfamethoxazole	80 (42.1)
Clindamycin	68 (35.7)
Mupirocin	28 (14.7)
Topical antiseptic	9 (2.3)
Presentation to emergency center	354 (88.5)
Requirement for drainage procedure	334 (83.5)
Requirement for hospital admission	172 (43)

^a Values are numbers and percentages of patients unless stated otherwise.

^b IQR, interquartile range.

susceptible isolates matched 1:1 for year of isolation were characterized by pulsed-field gel electrophoresis (PFGE); retapamulin-nonsusceptible MRSA isolates further underwent staphylococcal cassette chromosome *mec* element (SCC*mec*) typing by a multiplex PCR assay (23). Isolates identified as retapamulin resistant underwent screening for the *vgaA/Av*, *vgaC*, *vgaE*, and *cfr* genes by PCR as previously described (12, 13, 24, 25). Select retapamulin-resistant isolates underwent sequencing of the *rplC* gene, encoding ribosomal protein L3 (12), and the 23S rRNA (14); sequences were analyzed using BLAST algorithms. Mupirocin-resistant isolates were screened for the presence of the *mupA* and *mupB* genes through a PCR assay (26, 27).

Statistical analyses. Continuous variables were examined with the Student *t* test and Mann-Whitney U test; dichotomous variables were analyzed by chi-square, Fisher exact, and mid-*P* exact tests. Two-tailed *P* values of <0.05 were considered statistically significant. Select variables that were significant in univariate analyses were examined in a multivariate logistic regression model using STATA version 12 (StataCorp, College Station, TX).

RESULTS

Demographics. During the study period, 5,121 patient encounters occurred for SSTI at TCH. One hundred and eight encounters occurred in patients with an underlying immunocompromising condition and were excluded. A total of 4,949 patients comprised the remaining encounters, with 258 patients with 2 SSTIs excluded. From the remaining data set of 3,672 patients with a single SSTI and 247 patients with multiple SSTIs, 200 patients were selected at random for inclusion in each group. The median age of patients was 27 months, and the ethnic and racial makeup was similar to the overall demographics of the city of Houston (Table 1). Patients resided in 147 postal ZIP codes within the TCH referral area. Chronic medical conditions existed in 121 (30.3%) patients, the most common of which was eczema. Patients with eczema had similar rates of antibiotic exposure as children without eczema (21/44 [47.2%] compared to 169/356 [47.4%], *P* = 0.9).

TABLE 2 Characteristics of retapamulin-resistant and -susceptible infections

Variable	No. of patients (%) ^c		P value
	Retapamulin resistant (n = 38)	Retapamulin susceptible (n = 344)	
Median age, mo (IQR)	42.5 (18–104)	27 (16–73.5)	0.09
Female gender	20 (52.6)	201 (58.4)	0.5
African American race	10 (26.3)	86 (25)	0.8
Hispanic ethnicity	16 (42.1)	139 (40.4)	0.8
History of ≥3 SSTIs	28 (73.6)	163 (47.4)	0.002
History of chronic medical conditions	12 (31.5)	105 (30.5)	0.8
Hospitalization in prior 3 mo	8 (21.1)	27 (7.8)	0.01
Antibiotic use in prior 3 mo	34 (89.5)	147 (42.7)	<0.001
Clindamycin	17 (50)	48 (32.7)	<0.001
TMP-SMX ^a	13 (38)	61 (17.7)	0.03
Retapamulin	1 (2.6)	0	0.09
Linezolid	1 (2.6)	1 (0.3)	0.1
Mupirocin	8 (21.1)	18 (5.2)	0.002
Other topical antimicrobial ^b	3 (7.8)	6 (1.7)	0.1
≥2 classes of antibiotics	19 (55.9)	28 (19)	<0.001
≥3 classes of antibiotics	10 (29.4)	9 (6.1)	<0.001
Drainage procedure performed	33 (86.8)	184 (82.6)	0.5
Need for hospital admission	14 (36.8)	150 (43.6)	0.4
Treatment failure	4 (10.5)	11 (3.2)	0.06
Antibiogram			
Methicillin resistant	22 (57.9)	234 (68)	0.2
Clindamycin resistant	9 (23.7)	46 (13.4)	0.1
Mupirocin resistant	8 (21.1)	31 (9)	0.05
Linezolid resistant	2 (5.6)	0	0.01
TMP-SMX resistant	0	6 (1.7)	0.5
smr positive	5 (13.2)	50 (14.5)	0.8

^a TMP-SMX, trimethoprim-sulfamethoxazole.^b Other topical antimicrobials used by patients for treatment included bacitracin (n = 3), iodine (n = 3), gentamicin (n = 2), and “unknown antibiotic ointment” (n = 1).^c Values are numbers and percentages of patients unless stated otherwise.

Among patients with recurrent infections, the mean number of SSTIs during the study period was 3 (range, 3 to 6). The majority of patients presented to the TCH emergency center for care. Methicillin-resistant *S. aureus* (MRSA) accounted for 269 (67.3%) isolates, and 57 isolates were resistant to clindamycin (14.3%).

Retapamulin susceptibility. Thirty-eight patients (9.5%) were identified as having isolates with retapamulin MICs of ≥2 µg/ml; retapamulin-resistant isolates occurred in 5% of patients with a single SSTI and 14% of patients with a history of multiple SSTIs. An additional 18 isolates (4.5%) had an intermediate MIC. No isolate had a retapamulin MIC of >64 µg/ml. The 38 patients with retapamulin-resistant isolates resided in 30 different Houston area ZIP codes.

Patients with retapamulin-resistant infections were of similar demographics as those with susceptible infections (Table 2). In addition, patients with retapamulin-resistant isolates were more likely to have a history of multiple *S. aureus* infections (28/38 [73.6%] compared to 163/344 [47.4%], *P* = 0.002), recent hospitalization (8/38 [21.1%] compared to 27/344 [7.8%], *P* = 0.01), and antibiotic exposure (34/38 [89.5%] compared to 147/344 [42.7%], *P* < 0.001) than patients with susceptible isolates.

Among patients with recurrent infections, the proportion of patients with retapamulin-resistant isolates did not increase with >3 SSTIs. Only one patient in the study had documented previous use of retapamulin, and this patient later had a retapamulin-resistant *S. aureus* isolate from a subsequent infection. In addition, retapamulin-resistant isolates were more likely to have coresistance to mupirocin (8/38 [21.1%] compared to 31/344 [9%], *P* = 0.05) and linezolid (2/38 [5.6%] compared to 0/344, *P* = 0.01; both resistant isolates had a linezolid MIC of 8 µg/ml) compared to retapamulin-susceptible isolates. Both isolates with coresistance to linezolid occurred in patients with a history of multiple previous infections; however, neither had previously received linezolid.

A history of multiple SSTIs, recent hospitalization, and recent antibiotic exposure was included in a multivariate logistic regression analysis. Having ≥3 SSTIs (*P* = 0.002, odds ratio [OR] of 3.52, 95% confidence interval [CI] of 1.58 to 7.81) and antibiotic exposure in the previous 3 months (*P* < 0.001, OR of 9.75, 95% CI of 3.32 to 28.57) remained independently associated with retapamulin resistance.

Among retapamulin-nonsusceptible isolates, PFGE type USA300 accounted for 42/56 isolates (75%). The prevalence of the USA300 pulsotype was not significantly different between retapamulin-nonsusceptible and -susceptible isolates. Twenty-four MRSA isolates nonsusceptible to retapamulin underwent SCCmec typing; 23/24 (95.8%) were SCCmec type IV. Four retapamulin-resistant isolates (10.5%) were found to possess the *vgaA/Av* gene conferring pleuromutilin resistance; no isolates carried the *cfr*, *vgaC*, or *vgaE* genes. Three *vgaA/Av*-positive retapamulin-resistant isolates were clindamycin resistant (75%) compared to 6/34 (17.6%) *vgaA/Av*-negative isolates (*P* = 0.03). All *vgaA/Av*-positive strains occurred in patients with multiple previous infections. Three representative retapamulin-resistant isolates as well as both isolates with coresistance to linezolid were chosen for sequencing of the coding region of *rplC* and the 23S rRNA gene. Four of the isolates showed no deviations from published sequences (GenBank accession no. [HE681097](#)); one linezolid-resistant isolate (TCH22824) had a single nucleotide substitution at position 420 of *rplC* that did not result in an amino acid change.

Mupirocin susceptibility. Thirty-nine isolates (9.8%) were resistant to mupirocin, and 31/39 (79.5%) exhibited high-level resistance. There was no difference in total antibiotic use among patients with mupirocin-resistant and -susceptible isolates (23/39 [58.9%] resistant isolates compared to 167/361 [46.3%] susceptible isolates, *P* = 0.1; Table 3). Mupirocin resistance, however, was specifically associated with previous mupirocin use (9/23 [39.1%] compared to 19/167 [11.3%], *P* = 0.002). There was a trend for patients with underlying conditions to more often have mupirocin-resistant isolates; specifically, mupirocin resistance was more often seen in the setting of eczema (11/39 [28.2%] compared to 34/361 [9.4%], *P* = 0.002). Mupirocin resistance was more often seen in patients with a higher number of SSTIs; mupirocin resistance occurred in 5/12 (41.7%) patients with ≥4 SSTIs compared to 24/188 (12.7%) patients with 3 SSTIs (*P* = 0.02). Thirty-five mupirocin-resistant isolates possessed the *mupA* gene; 31 *mupA*-positive isolates had an HMR phenotype, and four isolates had an LMR phenotype. No isolates possessed *mupB*. One *mupA/B*-negative isolate exhibited HMR. One isolate carried both *vgaA/Av* and *mupA*.

smr. Fifty-six of the total isolates (14%) were found to be *smr* positive by PCR. The proportion of *smr*-positive *S. aureus* isolates

TABLE 3 Mupirocin-resistant compared to mupirocin-susceptible infections

Variable	No. of patients (%) ^a		P value
	Mupirocin resistant (n = 39)	Mupirocin susceptible (n = 361)	
Median age, mo (IQR)	36 (20–83)	26 (16–75)	0.1
Female gender	20 (51.3)	212 (58.7)	0.3
African American race	9 (23.1)	91 (25.2)	0.7
Hispanic ethnicity	24 (61.5)	137 (38)	0.005
History of ≥3 SSTIs	29 (74.3)	171 (47.3)	0.001
History of chronic medical conditions	17 (43.6)	104 (28.8)	0.06
Eczema	11 (28.2)	34 (9.4)	0.002
Hospitalization in prior 3 mo	3 (7.6)	33 (9.1)	0.8
Antibiotic use in prior 3 mo	23 (58.9)	167 (46.3)	0.1
Mupirocin	9 (39.1)	19 (11.3)	0.002
Antibiogram			
Methicillin resistant	24 (61.5)	245 (67.8)	0.4
Retapamulin resistant	8 (20.5)	30 (8.3)	0.02
Clindamycin resistant	9 (23.1)	48 (13.3)	0.1
smr positive	7 (17.9)	49 (13.6)	0.4
Treatment failure	2 (5.1)	13 (3.6)	0.6

^a Values are numbers and percentages of patients unless stated otherwise.

increased during the study period from 6/128 (4.6%) in 2010 to 26/94 (27.6%) in 2012 (*P* value for trend = 0.005). *smr* was detected in 16/200 (8%) first-time *S. aureus* SSTI isolates and in 40/200 (20%) recurrent isolates (*P* < 0.001). *smr*-positive *S. aureus* isolates were found more often in children with a history of underlying conditions as well (23/56 [41.1%] compared to 98/344 [28.5%], *P* = 0.06) (Table 4). When examining specific underlying conditions, more patients with an *smr*-positive *S. aureus* isolate had a diagnosis of eczema (12/56, 21.4%) compared to patients with *smr*-negative *S. aureus* (33/344 [9.5%], *P* = 0.01). In addition, *smr*-positive isolates were also more likely to be clindamycin resistant. Of the nine patients who received a topical antiseptic in the previous 3 months, none had an *smr*-positive organism isolated.

Ten *smr*-positive and 10 *smr*-negative isolates were chosen at random for chlorhexidine susceptibility testing. *smr*-positive isolates had higher chlorhexidine MICs and MBCs than *smr*-negative isolates (MIC₅₀ of 0.25 compared to 0.125 µg/ml; MIC₉₀ of 0.5 compared to 0.25 µg/ml; MBC₅₀ of 16 compared to 2 µg/ml; MBC₉₀ of 64 compared to 4 µg/ml; *P* < 0.001).

Treatment. The overwhelming majority of patients were prescribed an antimicrobial agent (378/400 [94.5%]) following presentation at TCH. A systemic agent was used in 370 cases (97.8%), a topical agent in 44 cases (11.6%), and both in 35 cases (9.3%). No patient received retapamulin or chlorhexidine; four patients were prescribed bleach baths by the treating physician. Thirty patients were prescribed mupirocin, of which five (16.7%) had mupirocin-resistant isolates; none of these patients experienced treatment failure.

DISCUSSION

SSTIs represent one of the most common infections in pediatrics, with *S. aureus* being the most common cause. Retapamulin topical preparations are approved for treating minor skin infections due

TABLE 4 *smr*-positive compared to *smr*-negative *S. aureus* isolates

Variable	No. of patients (%) ^a		P value
	<i>smr</i> positive (n = 56)	<i>smr</i> negative (n = 344)	
Age, mo	26.5	27	0.3
Female gender	26 (46.4)	206 (59.8)	0.08
African American race	11 (19.6)	89 (25.9)	0.3
Hispanic	23 (41.1)	138 (40.1)	0.8
History of ≥3 SSTIs	40 (71.4)	160 (46.5)	<0.0001
History of chronic medical conditions	23 (41.1)	98 (28.5)	0.06
History of eczema	12 (21.4)	33 (9.6)	0.01
Hospitalization in prior 3 mo	3 (5.3)	33 (9.5)	0.3
Antibiotic use in prior 3 mo	29 (51.8)	161 (46.8)	0.4
Antibiogram			
Methicillin resistant	37 (66.1)	232 (67.4)	0.8
Clindamycin resistant	14 (25)	43 (12.5)	0.02
Mupirocin resistant	7 (12.5)	32 (9.3)	0.3
Chlorhexidine susceptibility (µg/ml) ^b			
MIC ₅₀	0.25	0.125	0.001
MIC ₉₀	0.5	0.25	
MBC ₅₀	16	2	<0.001
MBC ₉₀	64	4	
Treatment failure	4 (7.1)	11 (3.2)	0.1

^a Values are numbers and percentages of patients unless stated otherwise.

^b Ten *smr*-positive and 10 *smr*-negative isolates underwent susceptibility testing for chlorhexidine MICs/MBCs.

to methicillin-susceptible *S. aureus* (MSSA) or group A streptococcus. There is very little published data on retapamulin resistance in *S. aureus*. Retapamulin resistance among mupirocin-resistant *S. aureus* has been previously shown to be <1% in the United Kingdom (15) and 2.6% among a general sample of MRSA in the United States (16). This is the first study in a pediatric population to investigate retapamulin resistance, finding a rate of 9.5% among *S. aureus* isolates causing SSTI, and it was more common among patients with recurrent infections.

The reasons for the relatively high prevalence of retapamulin resistance in this population are unclear. Given that retapamulin is a semisynthetic agent and *in vitro* studies have previously illustrated that the potential is low for spontaneous resistance to develop (28), one would expect isolates to be uniformly susceptible. It is particularly notable that very few patients experienced previous retapamulin exposure. This may be an artifact of the retrospective design of the study in that previous retapamulin use may not have been documented or incorrectly documented in the medical record or not reported by the patients' families. Alternatively, it is clear from the data that patients with retapamulin-resistant isolates had a greater degree of overall antibiotic exposure; this may have provided the selective pressure necessary for the development of resistant organisms. It is notable, however, that no *S. aureus* isolate in this study had a retapamulin MIC of >64 µg/ml, much lower than the concentration in the commercially available 1% ointment (10,000 µg/ml). Thus, while the percentage of isolates with *in vitro* resistance to retapamulin is alarming, the clinical relevance of these findings for the efficacy of retapamulin is unclear.

While the clinical significance of resistance to topical antimicrobial agents is still being evaluated, it is particularly concerning that there is an associated higher rate of resistance to other systemic antimicrobial agents. Previous studies found that, while the overall prevalence of *S. aureus* mupirocin resistance was low, these organisms were associated with a multidrug-resistant phenotype (29). In the current study, retapamulin-resistant isolates exhibited a higher incidence of concomitant linezolid resistance. Previous studies have documented cross-resistance between linezolid and pleuromutilins (14). In a survey of MRSA clinical isolates, Saravolatz et al. (16) noted that retapamulin resistance was found only in isolates with concomitant linezolid resistance. In addition, the current study revealed a trend for retapamulin-resistant isolates to exhibit cross-resistance with mupirocin. This is surprising given that these agents have very distinct mechanisms of action, with mupirocin acting upstream of retapamulin at the isoleucyl-tRNA synthetase (30). In contrast, retapamulin inhibits peptidyl-transferase activity in addition to 50S ribosomal subunit formation (31, 32). Our observation is likely a consequence of the overall greater degree of antibiotic exposure in patients with retapamulin-resistant isolates rather than true coresistance.

A number of mechanisms have previously been described conferring pleuromutilin resistance in *S. aureus*. Among these include the acquisition of the *cfr* methyltransferase leading to alterations in the drug target and conferring coresistance to oxazolidinones, phenicols, and lincosamides (13). Other mechanisms include the acquisition of genes encoding efflux pumps, such as *vgaA/Av*, which also result in resistance to lincosamides and streptogramins (33, 34). While none of the isolates in this study were found to carry *cfr*, four harbored the *vgaA/Av* efflux pump and were strongly associated with clindamycin resistance. The mechanisms of resistance in the remainder of the isolates are unclear and warrant further investigations.

The use of chlorhexidine gluconate and other antiseptics has been previously demonstrated to decrease rates of health care-associated infections in critically ill populations (5). While there is the potential for benefit from these agents, there exist plasmid-borne genes in *S. aureus* which confer a higher MIC and MBC to these agents. *S. aureus* isolates carrying these genes have previously been identified among pediatric oncology and cardiovascular (CV) surgery patients at TCH following an escalation in chlorhexidine use (6, 7), reaching as high as 45% of health care-associated *S. aureus* isolates. Among community-onset *S. aureus* SSTIs in the current study, 14% harbored *smr*. While this prevalence is lower than in previous studies of health care-associated infections, we observed a significant temporal increase in the fraction of *smr*-positive isolates during the study period. In addition, *smr*-positive isolates were more often found in patients with a history of multiple SSTIs. Although not specifically approved for this indication, many physicians and the Infectious Diseases Society of America recommend the use of chlorhexidine baths/scrubs to decrease the recurrence of MRSA SSTIs (2, 10). While it is notable that the chlorhexidine MICs/MBCs in the *smr*-positive isolates are considerably lower than the concentrations in most commercial preparations (ranging from 1,200 µg/ml for mouthwash to 40,000 µg/ml for hand/body scrub), the increasing prevalence of these isolates hints at a possible survival advantage. This trend should be monitored as antiseptic use is continued in both the hospital and community settings.

There is very little known regarding the prevalence of antiseptic

tolerance genes from community-acquired pediatric staphylococcal isolates. A multicenter study of *S. aureus* isolates from adult patients showed that the prevalence of *smr* exhibits tremendous geographic variability, ranging from 2 to 22% (9). Fritz et al. found that <1% of pediatric *S. aureus* isolates harbored the related *qacA/B* gene also associated with higher MICs/MBCs to chlorhexidine (4). The reasons for the relatively high prevalence of *smr*-positive *S. aureus* in our population are unclear and may be reflective of the dissemination of drug-resistant strains into the community from the health care setting; such a mechanism is supported in that antiseptics were previously used by the minority of patients in this study.

There are a number of shortcomings of this work. The retrospective design and reliance on review of medical records may have underestimated the use of certain antimicrobials, particularly retapamulin and chlorhexidine preparations. As the majority of patients were presenting to the emergency center, documentation of previous antimicrobials that may have been prescribed by their primary care physician was likely limited. In addition, our study design cannot take into account the impact of exposure to family members with staphylococcal infection had on the prevalence of resistant isolates. Also, given that this a single-center study, it is difficult to extrapolate these findings to other regions. Finally, aside from the patients who returned to our hospital for treatment failure or relapse, little can be said about the clinical impact of these isolates exhibiting resistance to topical antimicrobial agents.

In summary, *S. aureus* *in vitro* resistance to topical antimicrobial preparations is common among pediatric SSTIs. The prevalence of retapamulin and mupirocin resistance was 9.5 and 9.8%, respectively, and each was associated with coresistance to systemic antimicrobial agents. *smr* was detected in 14% of isolates and increased throughout the study period. Future work should focus on further understanding the clinical implications of these organisms.

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