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Efficacy of a novel ophthalmic antimicrobial drug combination towards a large panel of *Staphylococcus aureus* clinical ocular isolates from around the world

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Abstract

Purpose: *Staphylococcus aureus* is a leading cause of keratitis requiring urgent antimicrobial treatment. However, rising antibiotic resistance has rendered current ophthalmic antibiotics increasingly ineffective. First, a diverse, ocular *S. aureus* strain set was evaluated for resistance to six commonly used ophthalmic antibiotics. Next, a recently discovered antimicrobial drug combination containing polymyxin B/trimethoprim + rifampin that displayed impressive efficacy towards *S. aureus* in both *in vitro* and *in vivo* studies was evaluated as a potential novel keratitis therapeutic through testing this combination's efficacy against the clinical strain set.

Methods: 163 *S. aureus* isolates were collected commercially or from the University of Rochester, Flaum Eye Institute. The minimum inhibitory concentrations (MICs) of moxifloxacin, levofloxacin, vancomycin, erythromycin, tobramycin, rifampin and polymyxin B/trimethoprim (PT) were determined for the entire strain set to establish the incidence of resistance to current treatment options among a contemporary clinical isolate set and compared to the performance of PT + rifampin.

Results: Among all 163 isolates tested, high rates of antibiotic resistance were found toward erythromycin (69% resistance), moxifloxacin (33%), levofloxacin (40%) and tobramycin (17%). Conversely, the entire strain set, including multi-drug resistant (MDR) isolates, was sensitive to PT + rifampin, demonstrating the potency of this combination.

Conclusions: We establish that antibiotic resistance is pervasive among clinical *S. aureus* isolates, underscoring the concern for the effectiveness of current ophthalmic antibiotics. The drug combination of PT + rifampin, however, eradicated 100% of isolates tested, demonstrating the

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ability to overcome existing circulating resistance factors, and as such, may represent a promising therapeutic for *S. aureus* keratitis

Keywords

Staphylococcus aureus; antibiotic resistance; keratitis; clinical isolates

Introduction

Bacterial keratitis (corneal infection) is a vision-threatening disease that can result in devastating consequences such as corneal scarring, perforation, or endophthalmitis[1]. *Staphylococcus aureus* is the leading Gram-positive pathogen responsible for bacterial keratitis[2,3], requiring urgent topical antimicrobial treatment to mitigate corneal damage and preserve vision. Currently, fluoroquinolones are widely used in the treatment of *S. aureus* bacterial keratitis, however, resistance to this important class of antibiotics is rapidly on the rise. Indeed, resistance rates as high as 71–94% have been reported among ocular *S. aureus* isolates to levofloxacin, notably the latest-generation of the three Food and Drug Administration (FDA)-approved fluoroquinolones for the treatment of bacterial keratitis[4,5]. Other ophthalmic antibiotics such as moxifloxacin, a widely-used (off-label) fourth-generation fluoroquinolone, erythromycin, tobramycin, and trimethoprim are also under threat with *S. aureus* rates of resistance ranging upwards to 40%[3,4]. Unfortunately, the clinical consequences of resistant infections are significant and include increased disease severity and worse visual outcomes[6]. Despite this, the arsenal of commercially available ophthalmic antibiotics has remained static with no new ophthalmic antibiotics entering the commercial market in over a decade.

In response to the critical need for novel therapeutics to treat keratitis, we recently described the synergistic antimicrobial activity of a novel drug combination, polymyxin B/trimethoprim (PT) + rifampin toward *S. aureus* and *Pseudomonas aeruginosa*, another leading cause of keratitis, in both *in vitro* and *in vivo* studies[7,8]. While PT alone is commonly used for the treatment of mild bacterial conjunctivitis, its use in more serious corneal infections is limited due to poor antimicrobial potency and inadequate tissue penetration compared to fluoroquinolones[9–11]. However, we have demonstrated that the combination of PT + rifampin overcomes these liabilities and displays antimicrobial efficacy in a murine model of bacterial keratitis that equals or exceeds that of moxifloxacin[8]. Furthermore, PT + rifampin exhibits *in vivo* efficacy toward fluoroquinolone-resistant strains, suggesting that it may be an effective treatment option for strains that otherwise might fail currently available options[7,8].

In order to further investigate the incidence of antibiotic resistance to current treatment options and expand understanding of the potential therapeutic value of PT + rifampin for bacterial keratitis, we performed antimicrobial activity assays on a collection of 163 clinical ocular *S. aureus* isolates collected from across the globe. We first measured the incidence of resistance in a diverse, contemporary ocular strain set. Next, we evaluated the antimicrobial efficacy of PT + rifampin toward the isolate collection. Collectively our results establish that high resistance rates (17–69%) occur to currently available ophthalmic antibiotics,

expanding our understanding of the efficacy of current antibiotic options and accentuating the need for novel therapeutics. Moreover, we establish that 100% of the isolate collection displayed susceptibility to PT + rifampin suggesting that it may represent a potential new therapeutic for bacterial keratitis.

Material and Methods

Bacterial strains and growth conditions.

163 clinical *S. aureus* isolates were obtained either from the University of Rochester, Flaum Eye Institute (n=14) or commercially from International Health Management Associates (IHMA), Schaumburg, Il (n=149). The antibiotic susceptible laboratory strain UAMS-1[12] was used as a control for antibiotic resistance studies. For all experiments, individual isolates were grown at 37°C overnight in Muller Hinton broth (MH), diluted 1:100 in fresh media, grown to early exponential phase (OD_{600nm} of 0.18) and processed.

Minimum Inhibitory Concentration.

The antibiotic susceptibility profiles of each isolate toward moxifloxacin, levofloxacin, vancomycin, erythromycin, tobramycin, polymyxin B/trimethoprim, trimethoprim (alone), rifampin (alone), and the combination of PT + rifampin were evaluated in duplicate following minimum inhibitory concentration (MIC) guidelines[13]. Individual wells of a 96-well microtiter plate containing 88 μl of fresh media were inoculated with 10 μl containing 10^4 colony forming units (CFU) of exponential-phase *S. aureus* strains. Next, across each row, 2 μl of test agent was added to wells in 2-fold increasing concentrations. Following an incubation period of 16 hr at 37°C, each well was assessed visually to determine the concentration of drug that inhibited bacterial growth. *S. aureus* UAMS-1 was used to establish baseline MICs (Supplemental Table 1) to which each clinical isolate was subsequently compared. Since there are no standard susceptibility benchmarks for topical ophthalmic antibiotics, for each antibiotic tested, bacterial strains with MIC values 4-fold higher than the MIC values established for UAMS-1 were considered resistant.

Statistics.

Comparisons of resistance rates between individual groups were calculated using the Fisher's Exact test. Bonferroni correction was used to account for multiple testing in post-hoc analysis. Additionally, further analysis using logistic regression was used to investigate the association of resistance rates with age, gender, and geography. P-values less than or equal to 0.05 were considered statistically significant.

Results

Strain set characteristics.

163 ocular *S. aureus* isolates were obtained from either the University of Rochester Medical Center Flaum Eye Institute (n=14) or the International Health Management Associates (IHMA) (n=149) between 2008 and 2017. Table 1 provides characteristics of the strain set. Forty-six percent (n=75) of isolates were collected from male patients, 48% (n=79) from female patients and 6% (n=9) the gender was unknown. Ages of patients at the time of

isolate collection ranged from 0–97 with the majority of patients aged between 0–19 (29%) and 60–79 years old (29%). Seventy-four of the isolates were isolated from corneal scrapings, 26 from conjunctival swabs, and 63 were broadly categorized as from eyes which could include corneal, conjunctival, intracameral, and/or intravitreal samples. There was broad geographic representation with 110 (67%) of isolates from North America, 30 (18%) from Europe, 9 (6%) from Latin America, 2 (1%) from Africa, 3 (2%) from Asia, and 9 (6%) from the Middle East. Among isolates collected from North America, 3 were from Canada and 107 from 24 states across the United States (Supplemental Table 2). One hundred and thirteen of the strains (69%) were classified as methicillin-sensitive (MSSA) while 50 strains (31%) were classified as methicillin-resistant *S. aureus* (MRSA) based on MIC values to oxacillin performed either by IHMA or in our laboratory.

Antibiotic resistance profiles of isolates toward commercially available ophthalmic antibiotics.

Minimum inhibitory concentration (MIC) testing was performed on the entire strain set in duplicate to measure the effectiveness of 6 commonly used ophthalmic antibiotics: moxifloxacin, levofloxacin, vancomycin, erythromycin, tobramycin, and polymyxin B/trimethoprim (PT). Additionally, MIC testing was completed for rifampin, a current systemic antibiotic with excellent activity towards *S. aureus*, and a component of the novel drug combination PT + rifampin. The pan-sensitive, laboratory strain UAMS-1[12] was used as a control to establish a baseline MIC for each antibiotic and as there are no defined MIC breakpoints for topical ophthalmic antibiotics, resistance was defined as an MIC value 4x greater than that obtained for UAMS-1.

MIC testing revealed that 131 (80%) of isolates tested were resistant to at least one antibiotic (Supplemental Table 1, Table 2). Thirty-seven strains (23%) were resistant to 3 or more classes of antibiotics and thus considered multi-drug resistant[14]. Among MRSA isolates, 46% were considered multidrug resistant compared to 12% of MSSA strains. More specifically, as shown in Table 2, 112 of the 163 isolates were resistant to erythromycin (69%), 53 were resistant to moxifloxacin (33%), 65 to levofloxacin (40%), 30 to trimethoprim (18%), and 27 to tobramycin (17%). Lower rates of resistance were identified toward polymyxin B/trimethoprim (6.7% resistant, n=11,) and rifampin (1.8% resistance, n=3). As shown in Table 3, resistance rates were statistically significantly higher among MRSA isolates compared to MSSA strains for moxifloxacin ($p = 2.2e^{-12}$), levofloxacin ($p = 5.9e^{-11}$) and tobramycin ($p = 2.6e^{-09}$).

Considering geography, after controlling for both age and gender, interestingly there were no statistically significant regional differences in resistance rates among all the antibiotics tested (Supplemental Table 3). However, after controlling for both age and geography, logistic regression analysis did reveal significantly increased resistance rates among female patients for moxifloxacin (43% females vs 17% males, p=0.00231), levofloxacin (52% females vs 24% males, p=0.000866) and tobramycin (24% females vs 6.7% males, p=0.00898) (Table 4). Resistance rates stratified by ophthalmic anatomic source and age are considered below.

Antibiotic resistance profiles by ocular anatomic source.

Given that clinical practice patterns of antibiotic prescribing may vary based on the anatomic site of infection (conjunctivitis vs keratitis vs endophthalmitis), resistance rates were further stratified by isolates collected from the cornea, conjunctiva, or broadly categorized as from eyes (Supplemental Table 4). Similar to the entire strain set, when considered individually, resistance to at least one antibiotic was high with 82% (61/74), 92% (24/26), 73% (46/63) resistance found among corneal, conjunctival and eye isolates, respectively. Multidrug resistance was found among 32% (n=24) of corneal isolates, 12% (n=3) of conjunctival isolates and 19% (n=12) of strains broadly categorized as eye isolates.

Conjunctival isolates demonstrated the highest rates of resistance to erythromycin compared to corneal or ocular strains with 85%, 70%, and 60% resistance, respectively. In contrast, while 9.5% of corneal and 6.3% of broadly categorized eye isolates demonstrated resistance to polymyxin B/trimethoprim, all conjunctival isolates were sensitive to this drug. Of note, corneal isolates demonstrated higher rates of resistance towards moxifloxacin, levofloxacin, trimethoprim, and tobramycin compared to conjunctival or ocular isolates. While statistical analysis did not reveal significant differences in resistance rates comparing anatomical sources, after correcting for multiple testing, there were some trends noted. For example, 43% of corneal isolates were found to be resistant to moxifloxacin, compared to 23% of conjunctival, and 24% of ocular isolates. Similarly, levofloxacin, the latest-generation FDA-approved drug for keratitis, demonstrated 49% resistance among corneal isolates compared to 32% among eye and 35% among conjunctival strains. Moreover, 23% of corneal isolates were resistant to tobramycin compared to 8% of conjunctival strains and 13% of ocular strains. Rates of resistance to trimethoprim also trended higher in corneal isolates (23%) compared to conjunctiva (12%) or ocular strains (16%).

Antibiotic resistance profiles among different patient age groups.

The incidence and clinical outcomes of bacterial keratitis have been shown to vary across age groups[15–18]. Thus, isolates were categorized by the age of the patient at the time of collection into 5 groups: age 0–19 (n=47), 20–39 (n=15), 40–59 (n=31), 60–79 (n=47), and 80–100 (n=23) (Supplemental Table 5). There were no differences in resistance rates between these groups for erythromycin, PT, tobramycin, rifampin, and trimethoprim. However, higher rates of resistance for moxifloxacin and levofloxacin were consistently found in the 60–79 age group. For example, after controlling for both gender and geography, levofloxacin demonstrated statistically significant higher resistance rates in the 60–79 age group (p=0.0077)). Similarly, rates of moxifloxacin resistance were also statistically significantly higher among 60–79 year-old patients compared to all other age groups (p=0.0037).

Synergistic activity of polymyxin B/trimethoprim + rifampin.

To arrive at a ratio/concentration of a PT + rifampin mixture for MIC testing, we first established that the MIC values of PT (alone) and rifampin (alone) were 2.1 ug/ml and 0.01 ug/ml, respectively, toward the antibiotic susceptible strain UAMS-1, yet in combination, decreased to 0.67ug/ml for PT and 0.0015ug/ml for rifampin, confirming the previously described synergistic activity of the combination. Thus, as a basis for further MIC testing of

the entire clinical strain set, a fixed ratio of 0.67ug/ml PT + 0.0015 ug/ml rifampin was used to create 2-fold dilutions both in increasing and decreasing concentrations. 152 (93%) of isolates were susceptible to this low concentration combination, demonstrating the synergistic potency of PT + rifampin. Of the remaining 11 isolates, all were noted to have resistance to PT (alone) with MICs ranging from 10–20 μg ml^{-1} (Table 5). Importantly, however, despite the fact that the low concentration of 0.67ug/ml PT + 0.0015 ug/ml rifampin did not eradicate these strains, all 11 isolates *were* susceptible to the combination at MIC levels below those found for each drug in isolation (Table 5). For example, the MIC of PT (alone) and rifampin (alone) for strain 1308117 was 10μg ml^{-1} and 0.01 μg ml^{-1}, respectively. However, this strain was susceptible to the combination of PT + rifampin with MICs of 5.6 μg ml^{-1} (PT) and 0.0088 μg ml^{-1} (rifampin). In fact, 8/11 of these strains demonstrated a similar phenotype of susceptibility to the combination with decreased MIC values for both PT and rifampin. In the remaining 3 isolates, a decrease in MIC was noted for either PT or rifampin alone.

In order to further highlight the potential of PT + rifampin as an effective drug combination, a formulation containing commercially available ophthalmic PT (1000 u polymyxin B/1mg ml^{-1} trimethoprim) combined with 0.5 mg ml^{-1} of rifampin was evaluated toward the 11 isolates shown in Table 5. Previously, this 2:1 combination has demonstrated *in vivo* synergistic antimicrobial activity and effectively treated both *S. aureus* and *P. aeruginosa* corneal infections in a murine model[7,8]. All 11 strains tested were susceptible to this 2:1 combination of PT + rifampin at MICs well below the concentrations used in our *in vivo* models.

Taken together, this data demonstrates the ability of the combination of PT + rifampin to effectively eradicate 100% of this diverse strain set with significantly lower MIC values than found for each drug in isolation. Additionally, the combination of PT + rifampin can successfully contend with circulating antibiotic resistance among *S. aureus* isolates, including those strains with resistance to the individual components.

Discussion

Antibiotic resistance is currently one of the most pressing concerns in modern medicine resulting in significant clinical adverse outcomes due to treatment failures in addition to escalating healthcare costs[19–21]. The rates of antibiotic resistance in ophthalmologic practice have also been steadily rising, particularly for *S. aureus*, one of the most common causes of ocular infections[3,4,22,23].

Fluoroquinolone resistance among *S. aureus* ocular isolates remains particularly concerning given the ubiquitous use of this class of drugs for both treating infections as well as surgical prophylaxis[24,25]. In the United States *S. aureus* resistance among ocular isolates to moxifloxacin, a late generation fluoroquinolone, ranges from 8–18% for MSSA strains yet are as high as 35–91% among MRSA isolates[4,5,22]. Similar trends are found abroad in India with moxifloxacin resistance rates ranging from 27–55% and 74–87% for ocular MSSA and MRSA isolates, respectively[23,26] underscoring concern for the continued clinical effectiveness of this class of drug.

Moxifloxacin resistance among our diverse, contemporary strain set was also high overall (33%), with 15% resistance among MSSA strains and 72% among MRSA isolates. Similar results were also found for levofloxacin in our strain set (23% MSSA, 78% MRSA). While earlier generation fluoroquinolones such as ofloxacin and ciprofloxacin were not explicitly evaluated in this study, it can be assumed that resistance rates are comparable or higher than moxifloxacin or levofloxacin among our clinical isolate set due to the weaker targeting of identical cellular processes (bacterial DNA synthesis) among earlier generation fluoroquinolones. In fact, resistance to ciprofloxacin among ocular *S. aureus* isolates has been previously shown to be as high as 94%[5]. It is important to note that while MRSA keratitis remains a less common cause of keratitis compared to MSSA, it has been widely reported that the incidence of MRSA associated corneal infections is steadily increasing[2,22,23], underscoring the desperate need for new antibiotics that can effectively treat these highly-resistant *S. aureus* isolates.

In the treatment of *S. aureus* ocular infections alternatives to fluoroquinolones include erythromycin, tobramycin, polymyxin B/trimethoprim, and vancomycin. However, high rates of antibiotic resistance among *S. aureus* ocular isolates have also been identified toward erythromycin and tobramycin. For example, erythromycin resistance among North American *S. aureus* isolates has been reported ranging between 45–73%[3,4,27]. Similarly, rates of tobramycin resistance in North American and Europe have been documented between 7–60%[22,28]. Our current clinical isolate set further bolsters these reports with overall erythromycin resistance found among 65% and 76% of MSSA and MRSA isolates, respectively. Tobramycin resistance was low among our collection of MSSA isolates (4.4%) but 44% among MRSA strains. Of note, while erythromycin resistance was found highest among conjunctival isolates, fluoroquinolone and tobramycin resistance was highest among corneal isolates, likely reflecting current clinical prescribing patterns. Additionally, fluoroquinolone resistance was found to be highest among patients aged 60–79, an age group that has been shown to have a high incidence of keratitis as well as increased likelihood of poor visual prognosis compared to younger patients[18,29]. The fact that the highest rates of resistance are found among isolates collected from keratitis as well as patients with the highest risk for poor outcomes further underscores the need for alternative therapeutics for keratitis.

While resistance to polymyxin B/trimethoprim has remained low, this likely reflects its narrow use in treating serious ocular infections given its slow bactericidal activity and relatively poor corneal tissue penetration compared to fluoroquinolones[9–11]. Unfortunately, the arsenal of commercially available agents effective enough to treat bacterial keratitis is limited and thus clinicians often turn to compounding pharmacies to formulate high concentration drug preparations such as vancomycin[25]. However, these pharmacies are typically confined to large academic centers, leaving the vast majority of patients and clinicians without access to these medications. As a result, patients are faced with seeking care outside of their community or suffer the consequences of inadequate treatment. Moreover, of grave concern is the current lack of novel antimicrobials in clinical development. A search of active or ongoing clinical trials for the treatment of any ocular bacterial infection revealed only a single study testing the efficacy of a new ophthalmic antimicrobial agent for bacterial conjunctivitis, pazufloxacin, which in fact, is another

fourth-generation fluoroquinolone previously used in the systemic treatment of respiratory infections[30].

To address this critical treatment gap, we have recently developed a novel drug combination containing polymyxin B/trimethoprim + rifampin that may represent a promising new treatment modality for bacterial keratitis. In *in vitro* studies, PT + rifampin displayed synergistic, broad spectrum antimicrobial activity that was equal or superior to moxifloxacin[7,8]. Importantly, the potent activity of PT + rifampin extended to an *in vivo* keratitis model where it was shown to eradicate both *S. aureus* and *P. aeruginosa* corneal infections, even those caused by fluoroquinolone-resistant clinical isolates[7,8]. Given the propensity for bacteria to develop antibiotic resistance under selection pressure, we also previously compared how readily both *S. aureus* and *P. aeruginosa* developed resistance to moxifloxacin, PT (alone), rifampin (alone) or the combination of PT + rifampin while exposed to each drug in liquid culture over 40 hours. While resistance to the individual components and moxifloxacin developed rapidly (within 24 hours), resistance to PT + rifampin was not detected[8]. Furthermore, following this prolonged drug exposure, the combination of PT + rifampin retained its synergistic activity, overcoming any increased resistance to the individual components (unpublished data). However, despite the difficulty in selecting for antibiotic resistance to the combination of PT + rifampin, as demonstrated above, there are low rates of circulating resistance to trimethoprim (18%), PT (6.7%) and rifampin (1.8%). Thus, in this study we sought to advance our understanding of the therapeutic potential of PT + rifampin by evaluating the combination's activity towards a diverse panel of clinically relevant ocular isolates, particularly those with existing resistance to currently available ophthalmic antibiotics.

Our results indicate that the combination of PT + rifampin is likely to be effective toward contemporary circulating *S. aureus* strains, including those that are resistant to one or more currently available antibiotics. Moreover, the combination of PT + rifampin appears to be synergistic toward the majority of these isolates, as reflected in the decreased concentrations of PT and rifampin required for efficacy when in combination compared to each agent individually. Even in the setting of PT or rifampin resistance, the MICs for PT and rifampin also showed a corresponding decrease when in combination. Importantly, utilizing a formulation of 2:1 PT:rifampin, 100% of all isolates, regardless of their antibiotic resistance profile, were eradicated. In consideration of further clinical development, the synergy between PT and rifampin results in a potent drug combination which may suggest that clinical efficacy could be achieved quickly with fewer doses, a particularly advantageous feature in the setting of a rapidly progressive corneal infection. Moreover, a clinical formulation could be comprised of low concentrations of each drug component to achieve efficacy which in turn could limit any potential ocular toxicity.

The potency of this combination may be due, in part, to its multiple mechanisms of action. While polymyxin B acts as a detergent to disrupt bacterial cell membranes, trimethoprim inhibits bacterial DNA synthesis through the inhibition of dihydrofolate reductase, and rifampin inhibits DNA transcription through binding to bacterial RNA polymerase[31–33]. The multiple mechanisms of action may also explain how the combination of PT + rifampin can overcome widespread existing circulating antibiotic resistance. In fact utilizing a "cocktail"

of drugs is a common approach to overcome resistance in treating infections such as tuberculosis or HIV[34,35].

In summary, through the characterization of a large clinical ocular strain set, we have demonstrated wide-spread antimicrobial resistance to commonly prescribed ophthalmic antibiotics, including fluoroquinolones, arguably the most widely used class of drugs used for the treatment and prevention of corneal infections. In an era of rising antibiotic resistance, the need for antibiotics that can overcome this high level of resistance has never been greater. With the ability to eradicate ocular clinical isolates of *S. aureus* with varying resistance profiles, our data suggests that the combination of PT + rifampin may represent a promising new therapeutic option to fill this critical need.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1.

Strain characteristics of a 163-member ocular *S. aureus* isolate collection.

Characteristic	n (%)
Gender	
Male	75 (46)
Female	79 (48)
Unknown	9 (5.5)
Age	
0–19	47 (29)
20–39	15 (9)
40–59	31 (19)
60–79	47 (29)
80–100	23 (14)
Source	
Cornea	74 (45)
Conjunctiva	26 (16)
Eye	63 (39)
Geography	
North America	110 (67)
Europe	30 (18)
Latin America	9 (6)
Africa	2 (1)
Asia	3 (2)
Middle East	9 (6)
MSSA	113 (69)
MRSA	50 (31)

Table 2.

Antibiotic resistance among a 163-member clinical isolate strain set.

Antibiotic	n (%)
Vancomycin	0 (0)
Erythromycin	112 (69)
Polymyxin B/trimethoprim	11 (6.7)
Rifampin	3 (1.8)
Moxifloxacin	53 (33)
Levofloxacin	65 (40)
Trimethoprim	30 (18)
Tobramycin	27 (17)
Resistant to 1 antibiotic	131 (80)
Resistance to ≥ 3 antibiotics	37 (23)

Table 3.

Percent antibiotic resistance among MSSA and MRSA isolates

Antibiotic	MSSA	MRSA	P-value
Vancomycin	0	0	1
Erythromycin	65	76	0.204
Polymyxin B/trimethoprim	7.1	6	1
Rifampin	0.8	4	.223
Moxifloxacin	14	72	$2.2\ e^{-12}$
Levofloxacin	23	78	$5.9\ e^{-11}$
Trimethoprim	19	18	1
Tobramycin	4.4	44	$2.6\ e^{-09}$

Table 4.

Percent antibiotic resistance among males and females.

Antibiotic	Male	Female	P-value
Vancomycin	0	0	1
Erythromycin	67	70	0.738
Polymyxin B/trimethoprim	5.3	8.9	0.289
Rifampin	2.7	1.3	0.862
Moxifloxacin	17	43	0.00231
Levofloxacin	24	52	0.000866
Trimethoprim	15	20	0.233
Tobramycin	6.7	24	0.00898

Table 5.

Minimum inhibitory concentrations of polymyxin B/trimethoprim (PT) and rifampin alone and in combination toward *S. aureus* isolates demonstrating PT resistance.

Strain	*Alone* μg ml^{-1}		*Combination* μg ml^{-1}		2:1 μg ml^{-1}	
	PT	Rifampin	PT	Rifampin	PT	Rifampin
UAMS-1	2.1	0.01	0.67	0.0015		
RW53	20	0.005	11.2	0.016	0.078	0.039
1181991	10	0.02	6.09	0.0088	0.078	0.039
1308117	10	0.01	5.6	0.0088	0.078	0.039
1352268	10	0.01	5.13	0.0073	0.078	0.039
1519248	>20	0.02	11.2	0.016	0.079	0.039
1502286	>20	0.02	11.2	0.016	0.078	0.039
1550501	20	0.02	11.2	0.016	0.078	0.039
1510590	20	0.01	11.2	0.016	0.078	0.039
1510591	20	0.02	11.2	0.016	0.078	0.039
1566351	10	0.01	5.6	0.008	0.078	0.039
1581830	10	0.02	11.2	0.016	0.078	0.039