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Lack of Doxycycline Antimalarial Prophylaxis Impact on *Staphylococcus aureus* Tetracycline Resistance

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Abstract

There is concern that susceptibility of *Staphylococcus aureus* to tetracyclines may decrease due to use of antimalarial prophylaxis (doxycycline). We examined characteristics related to tetracycline resistance, including doxycycline exposure, in *S. aureus* isolates collected via admission surveillance swabs and inpatient clinical cultures from United States military personnel injured during deployment (June 2009-January 2012). Tetracycline class resistance was determined using antimicrobial susceptibility testing. The first *S. aureus* isolate from 168 patients were analyzed, of which 38 (23%) isolates were resistant to tetracyclines (class). Tetracycline-resistant isolates had a higher proportion of resistance to clindamycin ($p=0.019$) compared to susceptible isolates. There was no significant difference in tetracycline resistance between isolates collected from patients with and without antimalarial prophylaxis; however, significantly more isolates had *tet(M)* resistance genes in the doxycycline exposure group ($p=0.031$). Despite 55% of the patients receiving doxycycline as antimalarial prophylaxis, there was no association with resistance to tetracyclines.

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Keywords

Staphylococcus aureus; tetracyclines; antibiotic resistance

1.0 INTRODUCTION

Staphylococcus aureus is a widely prevalent pathogen associated with infections in hospitalized patients, in addition to generally healthy individuals (Boucher et al., 2009; Creech et al., 2010; Ellis et al., 2004; Klevens et al., 2007; Sievert et al., 2013; Tenover et al., 2006). One population that is frequently affected by both healthcare-associated (e.g., strain USA100) and community-associated (CA; e.g., strain USA300) *S. aureus* infections is the military. Specifically, skin and soft-tissue infections (SSTI) related to methicillin-resistant (MRSA) and methicillin-susceptible *S. aureus* (MSSA) are commonly reported among military trainees, non-deployed active-duty personnel, and deployed service members (Ellis et al., 2004, 2009; Landrum et al., 2012; Murray et al., 2009, 2010). An analysis of Army personnel undergoing training in the United States found that 3% and 28% of the soldiers were colonized with CAMRSA and CA-MSSA, respectively (Ellis et al., 2004). In addition, the rate of CA-MRSA and CA-MSSA SSTIs among TRICARE beneficiaries (2005-2010) was estimated at 82.7 and 59.2 per 100,000 person-years, respectively. Regarding hospital-associated SSTIs and bacteremia, the rate of MRSA and MSSA infections was 0.5 and 0.4 per 100,000 person-years, respectively (Landrum et al., 2012).

Along with being resistant to β -lactams, MRSA isolates may develop resistance to other broad-spectrum antimicrobial agents, such as tetracyclines (i.e., tetracycline, minocycline, and doxycycline) (Ardic et al., 2005; Han et al., 2007; Trzcinski et al., 2000). Two main mechanisms of tetracycline resistance through the acquisition of resistance genes (*tet* genes) have been described: active efflux due to energy-dependent efflux pumps associated with acquisition of plasmid-mediated *tet(K)* and *tet(L)* genes; and ribosomal protection involving elongation factor-like proteins, which are encoded by chromosomal or transposonal *tet(M)* and *tet(O)* genes (Leamer et al., 2013). Resistance to tetracycline in *S. aureus* is usually conferred by *tet(K)*, while tetracycline class resistance is associated with *tet(M)* (Schwartz et al., 2009; Trzcinski et al., 2000). Among non-deployed military personnel, tetracycline resistance has been reported in up to 6% of CA-MRSA colonizing or pathogenic (i.e., linked to a SSTI) isolates (Ellis et al., 2009; Leamer et al., 2013). Furthermore, 5-18% of MRSA isolates collected from U.S. military personnel at combat support hospitals in Iraq have been tetracycline-resistant (Co et al., 2011; Huang et al., 2011; Murray et al., 2010).

Although tetracycline resistance among *S. aureus* isolates is presently low, there is the possibility that susceptibility may decrease due to use of doxycycline and minocycline to treat MRSA infections (Han et al., 2007; McDougal et al., 2010; Nurjadi et al., 2015; Ruhe and Menon, 2007; Schwartz et al., 2009). In particular, doxycycline has been shown to induce its own resistance in MRSA isolates carrying *tet(K)* (Schwartz et al., 2009). Furthermore, use of doxycycline as antimalarial prophylaxis is also a concern (Lesens et al., 2007; Vento et al., 2013). In accordance with U.S. Department of Defense (DoD) guidelines (Department of the Army 2013; Department of Defense, 2010, 2013), military personnel

serving in Afghanistan are prescribed doxycycline or atovaquone/proquanil (Malarone®) (mefloquine is used if there are contraindications to primary antimalarials), which is initiated prior to entering the country and continued throughout deployment. Studies have indicated that compliance with primary antimalarial prophylaxis among deployed soldiers ranges from 38 to 61% (Brisson et al., 2012; Kotwal et al., 2005; Newton et al., 1994; Whitman et al., 2010). Moreover, 74% of military personnel wounded in Afghanistan and evacuated to Landstuhl Regional Medical Center (LRMC; Germany) were prescribed doxycycline by clinicians in order to comply with antimalarial prophylaxis guidelines (Rini et al., 2014). While prophylaxis is warranted when serving in malaria-endemic regions, there are unavoidable consequences of extensive use of doxycycline, including potential for increased tetracycline resistance among *S. aureus*.

A recent analysis compared data related to *S. aureus* colonization between active-duty military personnel who were stationed in the United States (non-deployed) and Afghanistan (deployed). The deployed personnel received doxycycline antimalarial prophylaxis, while non-deployed personnel were not administered any antibiotics within 30 days of *S. aureus* isolate collection. Although there was no significant difference in doxycycline susceptibility between the populations, a significantly higher proportion of *S. aureus* isolates collected from deployed personnel were resistant to tetracycline (Vento et al., 2013).

Among clinicians caring for combat wounded personnel, the occurrence of multidrug-resistant organisms presents challenges. Thus, the possibility of MRSA isolates acquiring resistance to tetracyclines is a concern. We examined characteristics related to tetracycline resistance in *S. aureus* isolates collected from military personnel wounded during the recent conflicts, including doxycycline antimalarial prophylaxis.

2.0 MATERIALS AND METHODS

2.1 Study Population and Demographics

The overarching project for this analysis is the Trauma Infectious Disease Outcomes Study (TIDOS), which is an ongoing observational cohort study of short- and long-term infectious complications among military personnel injured during deployment in Iraq and Afghanistan (Tribble et al., 2011). Trauma patients were eligible for inclusion in TIDOS if they were active-duty personnel or DoD beneficiaries, at least 18 years of age, and sustained deployment-related injuries requiring medical evacuation through LRMC before being transferred to a participating clinical facility in the United States, including National Naval Medical Center and Walter Reed Army Medical Center in the National Capital Region (merged to become Walter Reed National Military Medical Center after September 2011) and Brooke Army Medical Center (renamed San Antonio Military Medical Center after September 2011) in San Antonio, TX. A bacterial repository was also established to store isolates collected at LRMC and U.S. clinical facilities as a result of standardized admission screening and from clinical cultures.

Information related to trauma history and inpatient care was obtained through the DoD Trauma Registry (Eastridge et al., 2006) and supplemented by the TIDOS infectious disease module. *S. aureus* isolates were collected from surveillance swabs at admission (i.e., groin

swabs and anterior nares cultures) and inpatient clinical cultures of wounded military personnel admitted between June 2009 and January 2012. Isolates were classified as surveillance, colonizing, or infecting. Surveillance isolates were collected from admission surveillance swabs. Colonizing isolates were collected from cultures that were not associated with an infection. Infecting isolates were collected as part of infection work-ups. While groin swabs and inpatient cultures were performed at all clinical facilities, nares cultures were only collected at the U.S. sites, and not LRMC. If patients had serially positive cultures that grew *S. aureus*, the first *S. aureus* isolate collected was utilized in the analysis. Doxycycline exposure was defined by receipt of the antibiotic after medical evacuation and prior to isolate collection.

2.2 Clinical Microbiology and Antimicrobial Susceptibility Testing

Isolates were defined as MRSA by demonstrating resistance to oxacillin or ceftiofur, along with detection of the *mecA* gene (Division of Healthcare Quality Promotion, 2016). Tetracycline class resistance was defined as resistance to tetracycline, doxycycline, and minocycline. Antimicrobial susceptibility was assessed using the BD Phoenix Automated Microbiology System (Becton, Dickinson and Company, Sparks, MD) per manufacturer guidelines utilizing PMIC/ID-106 and PMIC/ID-107 panels, and interpreted according to the Clinical Laboratory and Standards Institute criteria (Clinical Laboratory and Standards Institute, 2013).

Isolates were screened by PCR for the presence of tetracycline resistance genes [*tet(K)*, *tet(L)*, *tet(M)*, and *tet(O)*]. Genomic DNA was extracted from overnight cultures utilizing the QIAamp DNA Mini Kit (Qiagen, Valencia, CA) following the manufacturers protocol. All PCRs were conducted using 5 PRIME MasterMix (5 PRIME Inc., Gaithersburg, MD) and 400 nM of each primer (**Table 1**). Amplifications of *tet(K)* and *tet(M)* genes were performed with the following parameters: 2 minutes at 94°C, 30 cycles of 94°C for 30 seconds, 50°C for 30 seconds, and 72°C for 50 seconds, followed by a final extension at 72°C for 3 minutes. The *tet(L)* and *tet(O)* genes were amplified using the following conditions: 2 minutes at 94°C; 30 cycles of 94°C for 30 seconds, 50°C for 30 seconds, and 72°C for 100 seconds, followed by a final extension at 72°C for 3 minutes.

Using methods previously published (McDougal et al., 2003), pulsed-field gel electrophoresis was used to evaluate genotypic patterns amongst the *S. aureus* isolates. The resulting pulsed-field types (PFTs) were assessed using the commercial software BioNumerics (Applied Maths, Inc., Austin, TX) and clonality was evaluated per established criteria (McDougal et al., 2003; Tenover et al., 1995).

2.3 Statistical Analysis

Statistical analysis was performed using SPSS software (IBM SPSS Statistics version 22, Chicago, IL) and Cytel Studio Software (version 1.0, Chesterbrook, PA). Univariate analysis included chi-square, Fisher's exact test (Fisher-Freeman-Halton test with Monte Carlo as appropriate), and Mann-Whitney U test. Statistically significant variables related to doxycycline exposure were assessed for inclusion in a logistic regression multivariate

analysis using stepwise selection (significance level of $p=0.2$). A p -value of <0.05 was considered significant.

3.0 RESULTS

3.1 Study Population

Between June 2009 and January 2012, 4051 U.S. wounded personnel were admitted to LRMC, of which half transferred to one of the participating clinical facilities in the United States. *S. aureus* isolates were collected from 168 patients who were utilized as the study population. In general, patients in the study population were young (median age: 24.5 years) men (99%) serving in the U.S. Army and Marines (66% and 27%, respectively) and wounded in military operations in Afghanistan (83%). The patients predominantly sustained a blast injury (69%) and were admitted to the intensive care unit (ICU) in the U.S. hospitals (46%). The median injury severity score, which is an overall measure of injury severity based on anatomical regional values (Linn, 1995), was 14 (moderate injuries).

3.2 Staphylococcus aureus Isolates

A total of 168 isolates were utilized in the analysis, of which 45 (27%), 47 (28%), and 76 (45%) were surveillance, colonizing, and infecting, respectively. Isolates were collected a median of 6 days post-injury (interquartile range [IQR]: 3-80 days). Among the isolates, 45 (27%) were MRSA and 38 (23%) were resistant to the tetracycline antimicrobial class.

3.2.1 Methicillin Resistance—Between the patients with MRSA and MSSA isolates (45 and 123, respectively), there was no significant difference in injury mechanism, injury severity score, ICU admission status, or facility where the isolate was collected, but there was a significant difference in the PFT profile ($p<0.001$; **Table 2**). A significantly higher proportion of MRSA isolates were surveillance and infecting ($p=0.037$). Moreover, while there was a significant difference in the distribution of enlisted versus warrant/officers between patients with MRSA and MSSA isolates ($p=0.026$), the majority of isolates in both groups were collected from enlisted personnel. A greater number of MRSA isolates were also resistant to other antimicrobials (i.e., clindamycin, levofloxacin, moxifloxacin, rifampin, and trimethoprim-sulfamethoxazole; $p<0.001$). There was no significant difference between the groups with regards to tetracycline resistance and doxycycline exposure.

3.2.2 Tetracycline Class Resistance—Thirty-eight (23%) isolates were resistant to the tetracycline antimicrobial class (**Table 3**), of which, 100% were resistant to tetracycline, 66% to doxycycline, and 61% to minocycline. Regarding *tet* genes, *tet(M)* and *tet(K)* contributed the highest proportion among the tetracycline-resistant isolates. Compared to susceptible isolates, a significantly higher proportion of tetracycline-resistant isolates were collected from personnel wounded in Afghanistan ($p=0.032$) when compared directly against all non-Afghanistan theater data. Tetracycline-resistant isolates were collected a median of 5.5 days post-injury (IQR: 4-22 days), while it was a median of 9.5 days (IQR: 3-109 days) for tetracycline susceptible isolates. When methicillin resistance was considered, there was no significant difference between the tetracycline-resistant and susceptible groups regarding the overall PFT profile. Nonetheless, a higher proportion of

tetracycline-resistant isolates were also resistant to clindamycin and trimethoprim-sulfamethoxazole ($p=0.019$). Among the 25 isolates resistant to doxycycline, 17 (68%) were from patients with antimalarial prophylaxis. There was no significant difference in doxycycline exposure between the groups. The limited number of isolates with tetracycline resistance did not allow for a multivariate risk factor analysis.

3.2.3 Doxycycline Exposure—Ninety-two isolates were from patients exposed to doxycycline prior to isolate collection (**Table 4**), of which 98% were injured in Afghanistan compared to 66% among patients who did not receive doxycycline ($p<0.001$). Among the 92 patients, doxycycline exposure was initiated a median of 2 days post-injury (IQR: 2-3 days). Isolates were collected a median of 50 (IQR: 6-138.5 days) and 3 days post-injury (IQR: 2-5 days) from patients with and without doxycycline exposure, respectively. A greater proportion of patients who received doxycycline also sustained blast injuries (79% versus 55%; $p<0.001$); however, less were admitted to a U.S. hospital ICU (41% versus 51%; $p=0.171$). In addition, significantly more isolates from patients with doxycycline exposure were collected at hospitals in the National Capital Region (56% versus 25%; $p<0.001$) and as the result of infection work-ups (61% versus 26%; $p<0.001$). There was no significant difference regarding methicillin and tetracycline resistance and PFT profiles between the groups of isolates from doxycycline-exposed and unexposed patients. Although the profile of *tet* genes was not significantly different between the groups, a statistically higher proportion of *tet*(M) genes were found in doxycycline-exposed patients ($p=0.031$). Although there was no statistically significant difference related to the overall profile of resistance to other antimicrobials between the two groups, there was a significantly greater proportion of isolates from patients exposed to doxycycline that were resistant to levofloxacin (25% versus 10%; $p=0.016$), and moxifloxacin (25% versus 10%; $p=0.016$). In a logistic regression multivariate model, sustaining an injury in Afghanistan was significantly associated with doxycycline exposure (odds ratio: 29.9; 95% Wald confidence interval: 6.3-141.2). Isolate type (e.g., infecting) and occurrence of *tet*(M) gene were not significantly associated with doxycycline antimalarial prophylaxis.

3.2.4 Isolates Associated with Infection—Seventy-six patients had isolates classified as infecting (**Table 5**), of which a greater proportion were collected at hospitals in the National Capital Region (54%) compared to non-infecting isolates (33%; $p=0.012$). Infecting isolates were collected a median of 69.5 days post-injury (IQR: 7-154 days). In addition, infecting isolates were predominantly collected from wound specimens (61% versus 10% of non-infecting isolates; $p<0.001$), while non-infecting isolates were primarily from respiratory specimens (37% versus 29% of infecting isolates). Among the infecting isolates, 15 (20%) were tetracycline-resistant. Nonetheless, there was no significant difference regarding methicillin and tetracycline resistance, profile of PFTs, and *tet* genes between the patients with infecting and non-infecting isolates. There was a significantly greater ($p<0.001$) proportion of infecting isolates from patients with doxycycline exposure (74%) compared to the group that had non-infecting isolates (39%). Infecting isolates from patients with doxycycline exposure were collected a median of 97.5 days post-injury (IQR: 16.5-187.5 days).

Thirty-three patients had serially positive infection work-ups with *S. aureus* growth. When characteristics of first isolates were compared to the last isolates collected from the same patients, there were no significant differences related to methicillin resistance (36% and 39% of first and last isolates, respectively; $p=0.80$), tetracycline resistance (24% and 21%, respectively; $p=0.77$), PFT profiles (21% USA300 in both first and last isolates; $p=0.999$), *tet* genes (21% and 18% with *tet*(M), respectively; $p=0.77$), and other antimicrobial resistance (30% and 33%, respectively, resistant to clindamycin, levofloxacin, and moxifloxacin; $p=0.81$).

4.0 DISCUSSION

Increasing multidrug resistance among bacterial pathogens presents a continuing challenge for clinicians. During the recent wars, there was concern that use of doxycycline antimalarial prophylaxis among military personnel deployed to Afghanistan would have the unintended consequence of increased *S. aureus* tetracycline resistance (Lesens et al., 2007; Vento et al., 2013). We examined 168 *S. aureus* isolates collected from wounded military personnel with regards to tetracycline resistance, MRSA, and doxycycline exposure. Overall, we found no statistically significant association between doxycycline exposure and resistance to the tetracycline antimicrobial class nor to the profile of *tet* resistance genes; however, when *tet*(M) was assessed separately, there was a significantly higher proportion among patients exposed to doxycycline.

Among the *S. aureus* isolates in our analysis 27% and 73% were MRSA and MSSA, respectively, with USA300, USA400 and USA800 contributing the majority of the PFTs. In addition, isolates were predominantly obtained through infection work-ups (45%), of which 33% were MRSA and 20% were resistant to tetracyclines. It is noteworthy that there was no significant difference regarding tetracycline resistance and occurrence of *tet* genes between the MRSA and MSSA groups; however, MRSA isolates had a significantly higher proportion of resistance to other antimicrobials, including clindamycin and fluoroquinolones.

Overall, 23% of the *S. aureus* isolates were resistant to tetracyclines. The majority of isolates with *tet* genes were resistant to tetracycline with only two isolates (1%) carrying the *tet*(K) gene classified as susceptible. Tetracycline-resistant isolates were also significantly more resistant to other antimicrobials. Furthermore, a significantly higher proportion of tetracycline-resistant isolates were collected from military personnel wounded in Afghanistan; however, there was no significant association with doxycycline antimalarial prophylaxis. A possible explanation could be that the larger proportion of wounded in our study were from the Afghanistan theater (83%). Nevertheless, our findings are comparable with a prior analysis that showed that 26% of *S. aureus* isolates (MRSA and MSSA) collected from personnel deployed to Afghanistan were resistant to tetracycline, while 5% were resistant to doxycycline or minocycline. Isolates from non-deployed personnel (no doxycycline exposure) were also considered in the analysis and they showed 98-100% susceptibility to tetracyclines (Vento et al., 2013). In another analysis, nasal cultures were collected from 273 French soldiers and 98 with *S. aureus* colonization were identified, of which 43% were carriers of doxycycline-resistant MSSA/MRSA. Approximately 90% of the

soldiers had previously been prescribed doxycycline for malarial prophylaxis (Lesens et al., 2007).

Tetracycline resistance has been reported among *S. aureus* isolates collected from military personnel wounded in Iraq as well as Afghanistan. In one analysis, isolates obtained from U.S. and non-U.S. patients in a combat support hospital (50% and 34% MRSA, respectively) were found to be 82% and 63% susceptible to tetracycline, respectively (Co et al., 2011). Additional studies have reported tetracycline susceptibility ranging from 90 to 95% among MRSA isolates (79-80% PFT USA300) collected from combat support hospitals in Iraq (Huang et al., 2011; Murray et al., 2010). High tetracycline resistance has also been observed in isolates from civilian populations. In particular, 72% tetracycline resistance was reported in one MRSA strain (USA300 variant) collected from a Boston health center over a 19-month period (Han et al., 2007). Following the Wenchuan earthquake in China, pathogenic *S. aureus* was isolated from 12% of the trauma patients, of which approximately 25% and 65% was resistant to minocycline and tetracycline, respectively (Zhang et al., 2012). Resistance to tetracycline was also reported among 63% of *S. aureus* isolates from volunteers in Haiti. Prior usage of doxycycline was examined, but no association was identified (Rosenthal et al., 2014).

As expected, 98% of patients with doxycycline antimalarial prophylaxis sustained injuries in Afghanistan. The association between the Afghanistan theater and doxycycline use was confirmed in the multivariate model. A significantly higher proportion of blast-related injuries was also observed among patients with doxycycline exposure. Although there was no correlation between blast injuries with combat theater in the multivariate model, it has been well-documented that the rate of traumatic amputations substantially increased after transition of personnel to Afghanistan due to dismounted patrols encountering improvised explosive devices, which may explain the higher proportion of blast-injured patients with doxycycline exposure (Krueger et al., 2012). An increase in isolates classified as infecting was also observed in the doxycycline exposure group, but the association was not significant in the multivariate model and may be due to restricting the analysis to the first isolate collected from each patient. Although doxycycline usage was not associated with tetracycline resistance, there were significantly more isolates with the *tet(M)* gene ($p=0.031$). One prior analysis reported the potential for doxycycline to induce its own resistance in MRSA PFT USA300 that carried the *tet(K)* gene and not *tet(M)* (Schwartz et al., 2009). Our findings did not find any evidence of doxycycline use as antimalarial prophylaxis inducing doxycycline resistance; however, only 9% of isolates within the doxycycline-exposed group carried the *tet(K)* gene and 20% had *tet(M)*.

Our retrospective analysis has limitations which should be considered. In particular, the patients that comprise our study population generally sustained more severe injuries than those who transferred to non-TIDOS-participating U.S. clinical sites (Tribble et al., 2011). Therefore, data reported herein may not be applicable to all wounded military personnel who were medically evacuated to LRMC before returning to the United States. In addition, the limited number of isolates with tetracycline resistance did not allow for a multivariate risk factor analysis. Furthermore, the limited number of isolates with tetracycline class resistance may have reduced the statistical power of the analysis of doxycycline exposure in

relation to resistance. Lastly, DoD guidance recommends use of doxycycline throughout duration of service in support of operations in Afghanistan; however, information on antimalarial prophylaxis self-compliance by military personnel while in theater was not collected by our study. Nonetheless, these data may have broader applicability due to the occurrence of infections involving *S. aureus* following natural disasters, such as the Wenchuan earthquake (Zhang et al., 2012).

5.0 CONCLUSION

Overall, our findings highlight a low proportion of MRSA collected from wounded military personnel during the recent wars; however, over half of the MRSA isolates were classified as infecting. There was also a low level of resistance to tetracycline among *S. aureus* isolates (MSSA and MRSA). Although there was no association between the utilization of doxycycline and resistance to tetracyclines, including doxycycline, further analysis is needed before a definite conclusion can be reached.

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Highlights

- Twenty-three percent of isolates were resistant to tetracycline antibiotic class
- Clindamycin resistance was higher among tetracycline-resistant isolates
- Tetracycline class resistance was not associated with antimalarial prophylaxis

Table 1PCR Oligonucleotide Primers for the Amplification of Tetracycline Resistance (*tet*) Genes

Gene	Primer Sequence (5'-3')	Product Size (bp)
<i>tet</i> (K)	F: TAGGGGGAATAATAGCACATT R: AATCCGCCCATAACAAATA	613
<i>tet</i> (L)	F: ATAAATTGTTTCGGGTCGGTAAT R: AACCAGCCAACATAATGACAATGAT	1077
<i>tet</i> (M)	F: GAACTCGAACAAGAGGAAAGC R: ATGGAAGCCCAGAAAGGAT	740
<i>tet</i> (O)	F: AGCGTCAAAGGGGAATCACTATCC R: CGGCGGGGTTGGCAAATA	1723

F – phenylalanine; R – arginine

Table 2

Characteristics of United States Military Personnel Subjects with Methicillin-Resistant or Methicillin-Susceptible *Staphylococcus aureus* Isolates

Characteristics, No (%) ^a	Total (N=168)	Patients with MRSA Isolates (N=45)	Patients with MSSA Isolates (N=123)	p-value
Male	167 (99.4)	44 (97.8)	123 (100)	
Age, median year (IQR)	24.5 (22.2-29.0)	25.1 (22.1-30.4)	24.0 (22.2-28.3)	0.397
<i>Branch of Service</i>				0.929
Army	111 (66.1)	29 (64.4)	82 (66.7)	
Marine	46 (27.4)	13 (28.9)	33 (26.8)	
Navy	5 (3.0)	1 (2.2)	4 (3.3)	
Air Force	4 (2.4)	1 (2.2)	3 (2.4)	
Other	2 (1.2)	1 (2.2)	1 (0.8)	
<i>Rank</i>				0.026
Enlisted	147 (87.5)	37 (82.2)	110 (89.4)	
Officer	16 (9.5)	3 (6.7)	13 (10.6)	
Warrant	3 (1.8)	3 (6.7)	0	
<i>Theater of Operation</i>				0.473
Iraq	24 (14.3)	5 (11.1)	19 (15.4)	
Afghanistan	140 (83.3)	39 (86.7)	101 (82.1)	
<i>Mechanism of Injury</i>				0.535
Blast	115 (68.5)	33 (73.3)	82 (66.7)	
Gunshot wound	23 (13.7)	4 (8.9)	19 (15.4)	
Other ^b	30 (17.9)	8 (17.8)	22 (17.9)	
<i>Initial Medical Facility</i>				0.834
Mobile medical unit within combat zone	51 (30.4)	15 (33.3)	36 (29.3)	
Hospital within combat zone	107 (63.7)	27 (60.0)	80 (65.0)	
LRMC	10 (5.9)	3 (6.7)	7 (5.7)	
Injury Severity Score ^c , median (IQR)	14 (9-24)	18 (10-26)	14 (9-24)	0.158
<i>Intensive Care Unit admission^d</i>				0.470
LRMC only	24 (14.3)	8 (17.8)	16 (13.0)	
U.S. hospital ± LRMC	77 (45.8)	23 (51.1)	54 (43.9)	
Non-ICU	59 (35.1)	13 (28.9)	46 (37.4)	
<i>Facility of Isolate Collection</i>				0.103
LRMC	54 (32.1)	9 (20.0)	45 (36.6)	
BAMC	43 (25.6)	15 (33.3)	28 (22.8)	
National Capital Region ^e	71 (42.3)	21 (46.7)	50 (40.7)	
<i>Isolate Type^f</i>				0.037
Surveillance	45 (26.8)	14 (31.1)	31 (25.2)	
Colonizing	47 (28.0)	6 (13.3)	41 (33.3)	
Infecting	76 (45.2)	25 (55.6)	51 (41.5)	

Characteristics, No (%) ^a	Total (N=168)	Patients with MRSA Isolates (N=45)	Patients with MSSA Isolates (N=123)	p-value
<i>PFTs</i>				<0.001
USA100	9 (5.4)	9 (20.0)	0	
USA200	17 (10.1)	0	17 (13.8)	
USA300	28 (16.7)	15 (33.3)	13 (10.6)	
USA400	22 (13.1)	4 (8.9)	18 (14.6)	
USA500	8 (4.8)	2 (4.4)	6 (4.9)	
USA600	10 (6.0)	0	10 (8.1)	
USA800	20 (11.9)	6 (13.3)	14 (11.4)	
USA900	9 (5.4)	0	9 (7.3)	
USA1000	9 (5.4)	3 (6.7)	6 (4.9)	
MRSA other types	6 (3.6)	6 (13.3)	0	
MSSA other types	30 (17.9)	0	30 (16.3)	
<i>Specimen Site</i>				0.077
Nares	30 (17.9)	10 (22.2)	30 (16.3)	
Groin	15 (8.9)	4 (8.9)	11 (8.9)	
Respiratory	56 (33.3)	7 (15.6)	49 (39.8)	
Blood	5 (3.0)	2 (4.4)	3 (2.4)	
Urine	3 (1.8)	1 (2.2)	2 (1.6)	
Wound	57 (33.9)	19 (42.2)	36 (29.3)	
Other	4 (2.4)	2 (4.4)	2 (1.6)	
<i>Tetracycline Resistance Genes</i>				1.00
<i>tet</i> (K)	15 (8.9)	4 (8.9)	11 (8.9)	
<i>tet</i> (L)	2 (1.2)	0	2 (1.6)	
<i>tet</i> (M)	24 (14.3)	6 (13.3)	18 (14.6)	
<i>tet</i> (O)	0	0	0	
None	130 (77.4)	36 (80.0)	94 (76.4)	
<i>Tetracycline Resistance</i>				0.941
Doxycycline	25 (14.9)	5 (11.1)	20 (16.3)	
Minocycline	23 (13.7)	5 (11.1)	18 (14.6)	
Tetracycline	38 (22.6)	9 (20.0)	29 (23.6)	
<i>Other Antimicrobial Resistance</i>				<0.001
Clindamycin	45 (26.8)	21 (46.7)	24 (19.5)	
Levofloxacin	31 (18.5)	27 (60.0)	4 (3.3)	
Moxifloxacin	31 (18.5)	27 (60.0)	4 (3.3)	
Rifampin	2 (1.2)	2 (4.4)	0	
Trimethoprim-Sulfamethoxazole	9 (5.4)	4 (8.9)	5 (4.1)	
Doxycycline Exposure	91 (54.2)	28 (62.0)	64 (52.0)	0.240

BAMC – Brooke Army Medical Center; IQR – interquartile range; LRMC – Landstuhl Regional Medical Center; MRSA – methicillin-resistant *Staphylococcus aureus*; MSSA – methicillin-susceptible *Staphylococcus aureus*

^aData are missing for some variables: rank is missing from two patients, intensive care unit admission from eight patients, and theater of operation is missing from four patients

^bOther mechanisms include motor vehicle crashes, burns, and falls

^cCalculated at LRMC admission

^dAdmission is recorded within the first week of care at each facility

^eNational Capital Region includes National Naval Medical Center and Walter Reed Army Medical Center (June 2009 through August 2011) and Walter Reed National Military Medical Center following the merging of the two facilities in September 2011.

^fIsolates classified as surveillance were collected through admission surveillance cultures; colonizing isolates were defined as those collected from cultures but not associated with a specific infection; infecting isolates were collected as part of infection workups

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Table 3

Characteristics of Tetracycline Resistance among *Staphylococcus aureus* Isolates Collected from Wounded United States Military Personnel

Characteristics, No (%) ^a	Patients with Tetracycline-Resistant Isolates (N=38)	Patients with Tetracycline Susceptible Isolates (N=130)	p-value
Male	38 (100)	129 (99.2)	~1.0
Age, median year (IQR)	25.1 (22.4-28.3)	24.5 (22.2-29.7)	0.759
<i>Branch of Service</i>			0.047
Army	25 (65.8)	86 (66.2)	
Marine	9 (23.7)	37 (28.5)	
Navy	4 (10.5)	1 (0.8)	
Air Force	0	4 (3.1)	
Other	0	2 (1.5)	
<i>Rank</i>			0.695
Enlisted	33 (86.8)	114 (87.7)	
Officer	4 (10.5)	12 (9.2)	
Warrant	1 (2.6)	2 (1.5)	
<i>Theater of Operation</i>			0.094
Iraq	2 (5.3)	22 (16.9)	
Afghanistan	36 (94.7)	104 (80.0)	0.032 ^b
<i>Mechanism of Injury</i>			0.666
Blast	28 (73.7)	87 (66.9)	
Gunshot wound	5 (13.2)	18 (13.8)	
Other ^c	5 (13.2)	25 (19.2)	
<i>Initial Medical Facility</i>			0.190
Mobile medical unit within combat zone	16 (42.1)	35 (26.9)	
Hospital within combat zone	21 (55.3)	86 (66.2)	
LRMC	1 (2.6)	9 (6.9)	
Injury Severity Score ^d , median (IQR)	10 (6-24)	15 (10-25)	0.090
<i>Intensive Care Unit admission^e</i>			0.445
LRMC only	4 (10.5)	20 (15.4)	
U.S. hospital ± LRMC	17 (44.7)	60 (46.2)	
Non-ICU	17 (44.7)	42 (32.3)	
<i>Facility of Isolate Collection</i>			0.766
LRMC	13 (34.2)	41 (31.5)	
BAMC	8 (21.1)	35 (26.9)	
National Capital Region ^f	17 (44.7)	54 (41.5)	
<i>Isolate Type^g</i>			0.125
Surveillance	15 (39.5)	30 (23.1)	0.045 ^b
Colonizing	8 (21.1)	39 (30.0)	

Characteristics, No (%) ^a	Patients with Tetracycline-Resistant Isolates (N=38)	Patients with Tetracycline Susceptible Isolates (N=130)	p-value
Infecting	15 (39.5)	61 (46.9)	
<i>Methicillin Resistance</i>			0.624
MRSA	9 (23.7)	36 (27.7)	
MSSA	29 (76.3)	94 (72.3)	
<i>PFTs</i>			0.050
USA100	0	9 (6.9)	
USA200	2 (5.3)	15 (11.5)	
USA300	6 (15.8)	22 (16.9)	
USA400	4 (10.5)	18 (13.8)	
USA500	1 (2.6)	7 (5.4)	
USA600	2 (5.3)	8 (6.2)	
USA800	7 (18.4)	13 (10.0)	
USA900	1 (2.6)	8 (6.2)	
USA1000	0	9 (6.9)	
MRSA other types	4 (10.5)	2 (1.5)	
MSSA other types	11 (28.9)	19 (14.6)	
<i>Tetracycline Resistance Genes</i>			0.229
<i>tet</i> (K)	13 (34.2)	2 (1.5)	
<i>tet</i> (L)	2 (5.3)	0	
<i>tet</i> (M)	24 (63.2)	0	
<i>tet</i> (O)	0	0	
<i>Other Antimicrobial Resistance</i>			0.019
Clindamycin	15 (39.5)	30 (23.1)	
Levofloxacin	7 (18.4)	24 (18.5)	
Moxifloxacin	7 (18.4)	24 (18.5)	
Rifampin	2 (5.3)	0	
Trimethoprim-Sulfamethoxazole	6 (15.8)	3 (2.3)	
Doxycycline Exposure	24 (63.2)	68 (52.0)	0.237

BAMC – Brooke Army Medical Center; CA – community-associated; IQR – interquartile range; LRMC – Landstuhl Regional Medical Center; HA – healthcare-associated; MRSA – methicillin-resistant *Staphylococcus aureus*; MSSA – methicillin-susceptible *Staphylococcus aureus*

^aData are missing for some variables: rank is missing from two patients, intensive care unit admission from eight patients, and theater of operation is missing from four patients

^bChi-square p-value for comparison of Afghanistan theater against all non-Afghanistan theater data (sum of Iraq theater and missing [4 patients])

^cOther mechanisms include motor vehicle crashes, burns, and falls

^dCalculated at LRMC admission

^eAdmission is recorded within the first week of care at each facility

^fNational Capital Region includes National Naval Medical Center and Walter Reed Army Medical Center (June 2009 through August 2011) and Walter Reed National Military Medical Center following the merging of the two facilities in September 2011.

^gIsolates classified as surveillance were collected through admission surveillance cultures; colonizing isolates were defined as those collected from cultures but not associated with a specific infection; infecting isolates were collected as part of infection workups

Table 4

Characteristics of Doxycycline Exposure Related to *Staphylococcus aureus* Isolates Collected from Wounded United States Military Personnel

Characteristics, No (%) ^a	Patients with Doxycycline Exposure ^b (N=92)	Patients without Doxycycline Exposure (N=76)	p-value
Male	92 (100)	75 (98.7)	0.452
Age, median year (IQR)	24.3 (24.3-29.5)	24.5 (22.1-28.8)	0.971
<i>Branch of Service</i>			0.046
Army	68 (73.9)	43 (56.6)	
Marine	19 (20.7)	27 (35.5)	
Navy	2 (2.2)	3 (3.9)	
Air Force	3 (3.3)	1 (1.3)	
Other	0	2 (2.6)	
<i>Rank</i>			0.766
Enlisted	79 (85.9)	68 (89.5)	
Officer	10 (10.9)	6 (7.9)	
Warrant	2 (2.2)	1 (1.3)	
<i>Theater of Operation</i>			<0.001
Iraq	2 (2.2)	22 (28.9)	
Afghanistan	90 (97.8)	50 (65.8)	
<i>Mechanism of Injury</i>			<0.001
Blast	73 (79.3)	42 (55.3)	
Gunshot wound	13 (14.1)	10 (13.2)	
Other ^c	6 (6.5)	24 (31.6)	
<i>Initial Medical Facility</i>			0.001
Mobile medical unit within combat zone	36 (39.1)	15 (19.7)	
Hospital within combat zone	55 (59.8)	52 (68.4)	
LRMC	1 (11)	9 (11.8)	
Injury Severity Score ^d , median (IQR)	14 (10-23)	14 (6-25)	0.661
<i>Intensive Care Unit admission^e</i>			0.171
LRMC only	16 (17.4)	8 (10.5)	
U.S. hospital ± LRMC	38 (41.3)	39 (51.3)	
Non-ICU	37 (40.2)	22 (28.9)	
<i>Facility of Isolate Collection</i>			<0.001
LRMC	8 (8.7)	46 (60.5)	
BAMC	32 (34.8)	11 (14.5)	
National Capital Region ^f	52 (56.5)	19 (25.0)	
<i>Isolate Type^g</i>			<0.001
Surveillance	24 (26.1)	21 (27.6)	
Colonizing	12 (13.0)	35 (46.1)	
Infecting	56 (60.9)	20 (26.3)	

Characteristics, No (%) ^a	Patients with Doxycycline Exposure ^b (N=92)	Patients without Doxycycline Exposure (N=76)	p-value
<i>Methicillin Resistance</i>			0.240
MRSA	28 (30.4)	17 (22.4)	
MSSA	64 (69.6)	59 (77.6)	
<i>PFTs</i>			0.079
USA100	7 (7.6)	2 (2.6)	
USA200	12 (13.0)	5 (6.6)	
USA300	18 (19.6)	10 (13.2)	
USA400	9 (9.8)	13 (18.2)	
USA500	5 (5.4)	3 (3.9)	
USA600	7 (7.6)	3 (3.9)	
USA800	10 (10.9)	10 (13.2)	
USA900	7 (7.6)	2 (2.6)	
USA1000	3 (3.3)	6 (7.9)	
MRSA other types	4 (4.3)	2 (2.6)	
MSSA other types	10 (10.9)	20 (26.3)	
<i>Tetracycline Resistance Genes</i>			0.252
<i>tet</i> (K)	8 (8.7)	7 (9.2)	
<i>tet</i> (L)	2 (2.2)	0	
<i>tet</i> (M) ^h	18 (19.6)	6 (7.9)	
<i>tet</i> (O)	0	0	
<i>Tetracycline Resistance</i>			0.237
Doxycycline	17 (18.5)	8 (10.5)	
Minocycline	15 (16.3)	8 (10.5)	
Tetracycline	24 (26.1)	14 (18.4)	
<i>Other Antimicrobial Resistance</i>			0.658
Clindamycin	30 (32.6)	15 (19.7)	
Levofloxacin ⁱ	23 (25.0)	8 (10.5)	
Moxifloxacin ⁱ	23 (25.0)	8 (10.5)	
Rifampin	1 (11)	1 (13)	
Trimethoprim-Sulfamethoxazole	5 (5.4)	4 (5.3)	

BAMC – Brooke Army Medical Center; CA – community-associated; IQR – interquartile range; LPMC – Landstuhl Regional Medical Center; HA – healthcare-associated; MRSA – methicillin-resistant *Staphylococcus aureus*; MSSA – methicillin-susceptible *Staphylococcus aureus*

^aData are missing for some variables: rank is missing from two patients, intensive care unit admission from eight patients, and theater of operation is missing from four patients

^bDoxycycline exposure occurred prior to isolate collection

^cOther mechanisms include motor vehicle crashes, burns, and falls

^dCalculated at LPMC admission

^eAdmission is recorded within the first week of care at each facility

^fNational Capital Region includes National Naval Medical Center and Walter Reed Army Medical Center (June 2009 through August 2011) and Walter Reed National Military Medical Center following the merging of the two facilities in September 2011.

^gIsolates classified as surveillance were collected through admission surveillance cultures; colonizing isolates were defined as those collected from cultures but not associated with a specific infection; infecting isolates were collected as part of infection workups

^hGroups are statistically significantly different; p=0.031.

ⁱGroups are statistically significantly different; p=0.016.

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Table 5

Characteristics of Infection Status among *Staphylococcus aureus* Isolates Collected from Wounded United States Military Personnel

Characteristics, No (%) ^a	Patients with Infecting Isolates ^b (N=76)	Patients with non-Infecting Isolates ^b (N=92)	p-value
<i>Theater of Operation</i>			0.062
Iraq	6 (7.9)	18 (19.6)	
Afghanistan	69 (90.8)	71 (77.2)	
<i>Mechanism of Injury</i>			0.094
Blast	50 (65.8)	65 (70.7)	
Gunshot wound	15 (19.7)	8 (8.7)	
Other ^c	11 (14.5)	19 (20.7)	
<i>Initial Medical Facility</i>			0.192
Mobile medical unit within combat zone	28 (36.8)	23 (25.0)	
Hospital within combat zone	45 (59.2)	62 (67.4)	
LRMC	3 (3.9)	7 (7.6)	
Injury Severity Score ^d , median (IQR)	17 (10-24)	14 (6-24)	0.179
<i>Intensive Care Unit admission^e</i>			0.350
LRMC only	9 (11.8)	15 (16.3)	
U.S. hospital ± LRMC	40 (52.6)	37 (40.2)	
Non-ICU	25 (32.9)	34 (37.0)	
<i>Facility of Isolate Collection</i>			0.012
LRMC	17 (22.4)	37 (40.2)	
BAMC	18 (23.7)	25 (27.2)	
National Capital Region ^f	41 (53.9)	30 (32.6)	
<i>Specimen Site</i>			<0.001
Nares	0	30 (32.6)	
Groin	0	15 (16.3)	
Respiratory	22 (28.9)	34 (37.0)	
Blood	5 (6.6)	0	
Urine	0	3 (3.3)	
Wound	46 (60.5)	9 (9.8)	
Other	3 (3.9)	1 (1.1)	
<i>Methicillin Resistance</i>			0.104
MRSA	25 (32.9)	20 (21.7)	
MSSA	51 (67.1)	72 (78.3)	
<i>PFTs</i>			0.740
USA100	5 (6.6)	4 (4.3)	
USA200	7 (9.2)	10 (10.9)	
USA300	14 (18.4)	14 (15.2)	
USA400	10 (13.2)	12 (13.0)	

Characteristics, No (%) ^a	Patients with Infecting Isolates ^b (N=76)	Patients with non-Infecting Isolates ^b (N=92)	p-value
USA500	2 (2.6)	6 (6.5)	0.755
USA600	6 (7.9)	4 (4.3)	
USA800	8 (10.5)	12 (13.0)	
USA900	5 (6.6)	4 (4.3)	
USA1000	6 (7.9)	3 (3.3)	
MRSA other types	3 (3.9)	3 (3.3)	
MSSA other types	10 (13.2)	20 (21.7)	
<i>Tetracycline Resistance Genes</i>			0.417
<i>tet</i> (K)	5 (6.6)	10 (10.9)	
<i>tet</i> (L)	1 (1.3)	1 (1.1)	
<i>tet</i> (M)	10 (13.2)	14 (15.2)	
<i>tet</i> (O)	0	0	
None	60 (78.9)	67 (72.8)	
<i>Tetracycline Resistance</i>			0.269
Doxycycline	11 (14.5)	14 (15.2)	
Minocycline	10 (13.2)	13 (14.1)	
Tetracycline	15 (19.7)	23 (25.0)	
<i>Other Antimicrobial Resistance</i>			<0.001
Clindamycin	20 (26.3)	25 (27.2)	
Levofloxacin	20 (26.3)	11 (12.0)	
Moxifloxacin	20 (26.3)	11 (12.0)	
Rifampin	1 (1.3)	1 (1.1)	
Trimethoprim-Sulfamethoxazole	4 (5.3)	5 (5.4)	
Doxycycline Exposure	56 (73.7)	36 (39.1)	

BAMC – Brooke Army Medical Center; IQR – interquartile range; LRMC – Landstuhl Regional Medical Center; MRSA – methicillin-resistant *Staphylococcus aureus*; MSSA – methicillin-susceptible *Staphylococcus aureus*

^aData are missing for some variables: rank is missing from two patients, intensive care unit admission from eight patients, and theater of operation is missing from four patients

^bIsolates classified as infecting were collected as part of infection workups while non-infecting isolates were collected through admission surveillance cultures and from cultures not associated with a specific infection and so determined to be colonizing

^cOther mechanisms include motor vehicle crashes, burns, and falls

^dCalculated at LRMC admission

^eAdmission is recorded within the first week of care at each facility

^fNational Capital Region includes National Naval Medical Center and Walter Reed Army Medical Center (June 2009 through August 2011) and Walter Reed National Military Medical Center following the merging of the two facilities in September 2011.