Nasal Carriage of *Staphylococcus aureus* and Methicillin Resistant *Staphylococcus aureus* (MRSA) in Students at the University of Central Oklahoma

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**Abstract:** Nasal carriage of *Staphylococcus aureus* has been identified as a significant risk factor for subsequent infections and is a target for decolonization approaches. The efficacy of the decolonization methods may be dependent on the load and type of *S. aureus* present in the nose of individuals in the community. The objectives of this study were to determine the rates of carriage for *S. aureus* and MRSA, quantify the level of *S. aureus* and MRSA carriage, and to determine the relatedness of the *S. aureus* and MRSA isolates recovered from a healthy student population. Nasal swab specimens were collected from 247 healthy University of Central Oklahoma students, serially diluted, and cultured onto blood agar plates containing 4% NaCl for qualitative and quantitative analysis. Methicillin resistance was determined with cefoxitin disk diffusion and PCR for meca. Relatedness was determined by spa sequence typing. *S. aureus* prevalence was 21.5% (MRSA 2.4%), with a geometric mean of 1,820 CFU/swab (MRSA 412 CFU/swab). Twenty-two different spa types were identified among the 42 spa positive *S. aureus*/MRSA positive samples. *S. aureus*/MRSA carriage rates were similar to other studies. spa typing revealed a high degree of carriage diversity.

**Introduction**

In the past few decades, the rate of infections caused by *S. aureus* has increased. *S. aureus* has an incredible ability to become resistant to antibiotics (DeLeo et al. 2010). Two years after methicillin was introduced in 1961, resistant species were found (DeLeo et al. 2010). Methicillin-resistant *S. aureus* (MRSA) has become a major cause of nosocomial infections worldwide (Hallin et al. 2007) and the National Nosocomial Infection Surveillance (NNIS) System showed an increase in infections caused by MRSA in ICU patients over time (Boucher and Corey 2008). MRSA kills approximately 19,000 hospitalized patients every year (Boucher and Corey 2008). The rise in MRSA infections has increased health care costs due to its multidrug resistance (Buehlmann et al. 2008).
S. aureus and MRSA nasal carriage

2008); these isolates are resistant to all β-lactam drugs (cephalosporins and penicillins) (Gorwitz et al. 2008). Some of the more serious diseases commonly caused by MRSA are bacteremia, endocarditis, and pneumonia (Hallin et al. 2008; Boucher and Corey 2008; Gorwitz et al. 2008).

S. aureus is considered to be part of the normal flora because it is present in one in three people without causing an associated disease (DeLeo et al. 2010). The anterior nares are the most frequently colonized site (Wertheim et al. 2005). Colonization is a high risk factor for subsequent infections despite most colonized individuals not developing a disease (Gorwitz et al. 2008). An association was first found between S. aureus nasal carriage and staphylococcal disease in the early 1930’s (Wertheim et al. 2005).

In the past 20 years, concern has shifted to healthy individuals with MRSA infections who have had no contact with a health care facility (Klevens et al. 2006). The strains causing disease in the community are distinctly different from those found in a hospital setting and tend to be less resistant to non-β-lactam drugs (Klevens et al. 2006). The conclusion drawn from healthy individuals getting community-associated MRSA (CA-MRSA) is that these strains have greater virulence than hospital-associated MRSA (HA-MRSA) (DeLeo et al. 2010). In the USA, MRSA has become one of the leading causes of death by a single infectious agent (DeLeo et al. 2010; Hallin et al. 2008); it has also become a problem in almost all industrialized countries, although not all are at the same level (DeLeo et al. 2010). MRSA strains acquire resistance through the acquisition of a mobile exogenous element called staphylococcal chromosomal cassette mec (SCCmec) which contains the mecA gene that encodes for resistance to β-lactam antibiotics (DeLeo et al. 2010; Hallin et al. 2008). While there are no risk factors for CA-MRSA it seems to be linked to person-to-person contact with a colonized individual and occurs more frequently among prison inmates, athletes, individuals using intravenous drugs, and children in day cares (DeLeo et al. 2010; Boucher and Corey 2008; Buehlmann et al. 2008).

Within the Staphylococcus aureus species there are numerous strains, most of which can be characterized by their spa gene. One study found that MRSA isolates with spa type t041 were more difficult to decolonize than other spa types, although the difference was not statistically significant (Beuhlmann et al. 2008).

The goal of this study was to quantify and characterize S. aureus isolates found colonizing the anterior nares in a healthy population of college students. In order to develop safe and effective decolonization strategies to reduce the potential for post-surgical S. aureus and MRSA infections, we first need to understand the epidemiology of those found in healthy individuals to allow development of the most effective strategies. The efficacy of these methods may be dependent on the load and type of S. aureus present. Not all strains of S. aureus or MRSA react to antibiotics or decolonization efforts in the same manner. Our general hypothesis is that carriage levels and spa types of MSSA and MRSA are highly variable in a healthy student population. To test this hypothesis, we determined the rates of carriage, quantified the level of carriage, and determined the spa types of the S. aureus and MRSA isolates obtained.
Materials and Methods

Specimen collection, isolation, identification and characterization: Nasal swab samples were collected from students at the University of Central Oklahoma from July 2010 to July 2012. Students were only allowed to participate after signing the informed consent as approved by the Institutional Review Board (UCO IRB# 10104). Samples were collected from the external nares using the Copan Eswab. The swabs were rotated 3 times in each nostril and then placed into the Copan liquid and transport tube for elution of the sample from the swab. Samples were serially diluted 10-fold and plated onto 5% sheep blood agar plates with 4% NaCl and incubated at 35°C for 24-48 hours. Colonies morphologically consistent with *S. aureus* were counted as colony forming units (CFU). Gram staining, catalase, and tube FRDJXODVHWHVWVZHUHSHUIRUPHGWRFRQ¿UPWKH colonies were *S. aureus*. Methicillin resistance ZDVGHWHUPLQHGZLWKFHIR[ZLVH XVLRQ 3&5IRU mec $DQG3%3DDJJOXWLQDWLRQ

Molecular analysis: A previously described PXOWLSOH[3&5ZDVXVHGIRUWKHGHWHFWLRQRIWKH 3DQWRQ9DOHQWLQHOHXNRFLGLQ pvl gene, the *spa* gene and the mecA gene (Larsen et al. 2008).

*spa* typing: Samples confirmed to be positive for *S. aureus* or MRSA were subjected to *spa* typing. Amplification of the *spa* repeat region for typing was done according to Ridom GmbH protocol for DNA sequencing of the *spa* gene. DNA for amplification was obtained by washing a loopful of bacterial cells with distilled H₂O and incubated with 200 μl of 6% InstaGene matrix solution (BIO-RAD, Hercules, CA.) for 20 minutes at 56 °C. The suspension was vortexed and heated for 8 minutes at 100 °C and centrifuged at 8,000 x g for 3 minutes. Twenty microliters of the supernatants was used for PCR amplification. PCR analysis was performed using spa-1113f (5'-TAAAGACGATCCTCCGTTGAG-3'), and spa-1514r (5'-CAGCAGTAGTGCTGCCGTTGCT-3') primers and Qiagen Taq PCR master mix. Amplification of the *spa* repeat region was initiated at 80°C for 5 minutes, followed by 35 cycles of 94°C for 45 sec, 60°C for 45 sec, 72°C for 90 sec, and a final extension at 72°C for 10 minutes. To ascertain the presence of the *spa* gene, the amplicons were run through a 2% agarose gel (UltraPure™ LMP Agarose). PCR products were sequenced by Eton Bioscience. The *spa* sequence types were assigned using the BioNumerics Software version 6.0 (Applied Math, Austin, TX, USA) through the Ridom Spa Server.

Statistical analysis: Significant differences between male and female carriage rates were determined using a two-tailed student's t-test.

Results and Discussion

Nasal swab samples were collected from 247 subjects (100 male, 147 female). Of these, 21.5% (53/247; 29 male, 24 female) were positive for *S. aureus* nasal colonization with a mean (Log_{10}) CFU per nares culture of 2.98±1.17 (2.96±1.04 for males, 3.00±1.33 for females). The geometric mean per nares culture for all subjects was 1,820 (1,505 male, 2,282 female) (Table 1).

11% of the *S. aureus* isolates were methicillin resistant. The carriage rate among males was higher than females although not significantly different (p = 0.27). Carriage levels among males were lower than females although not significantly different. (p = 0.12).

Figure 1 shows multiplex PCR results for representative MRSA and MSSA isolates.

Only two out of 53 *S. aureus* isolates were *pvl* positive, both of which were also mecA positive. One of the *pvl* positive isolates (N063A) is shown in figure 1. MRSA isolates in lanes 5 and 7 were PCR negative for *spa*, but were confirmed to be *S. aureus* through positive catalase and coagulase tests. ATCC BAA1707 was included as a *pvl* positive MRSA control. The *spa* sequencing resulted in 22 different *spa* types (figure 2A).

As it is shown in the clustering dendrogram of figure 2A, four of the isolates were *spa* type t3297, four isolates were *spa* type t012, and...
three were spa type t216. The other 19 spa types identified were found in only one or two isolates. A minimal spanning tree was constructed using the BioNumerics software version 6.0 (Applied Math, Austin, TX, USA) grouped the 22 spa types into 19 distinct clusters (figure 2B). The distribution of these clusters highlights the variability of the spa types detected in this study. Eleven of our 53 S. aureus/MRSA isolates were spa negative, but were confirmed to be S. aureus through positive catalase and coagulase tests.

In this study, the nasal carriage rates of S. aureus (21.5%) and MRSA (2.4%) were within the ranges of previous reports (Kluytmans et al. 1997; Chatterjee et al. 2009; Askarian et al. 2009; Chen et al. 2012; Sharma et al. 2014). A small number of similar studies of S. aureus and MRSA nasal carriage among the general population of healthy college students have been conducted. A study published in 2009 from Texas State University reported nasal carriage rates of 29.6% for S. aureus and 7.4% for MRSA (Rohde et al. 2009). In a study published in 2013 from a historically black college in Virginia, the nasal carriage rate of MRSA was only 0.65% (Shen et al. 2013). Among collegiate student athletes, MRSA carriage rates have been reported to be

<table>
<thead>
<tr>
<th>Subject Population</th>
<th>% (+) for nasal colonization</th>
<th>Mean Log$_{10}$±SD, CFU per nares culture</th>
<th>Geometric mean per nares culture</th>
</tr>
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<tbody>
<tr>
<td><strong>S. aureus</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>All subjects</td>
<td>21.5% (53/247)</td>
<td>2.98 ± 1.17</td>
<td>1,820</td>
</tr>
<tr>
<td>Male subjects</td>
<td>29.0% (29/100)</td>
<td>2.96 ± 1.04</td>
<td>1,505</td>
</tr>
<tr>
<td>Female subjects</td>
<td>16.3% (24/147)</td>
<td>3.00 ± 1.33</td>
<td>2,282</td>
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<tr>
<td><strong>MRSA</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>All subjects</td>
<td>2.4% (6/247)</td>
<td>2.04 ± 1.65</td>
<td>412</td>
</tr>
<tr>
<td>Male subjects</td>
<td>4.0% (4/100)</td>
<td>2.15 ± 1.33</td>
<td>359</td>
</tr>
<tr>
<td>Female subjects</td>
<td>1.4% (2/147)</td>
<td>1.82 ± 2.88</td>
<td>545</td>
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</tbody>
</table>

Table 1: *Staphylococcus aureus* and MRSA nasal carriage among healthy university students.

Figure 1. Examples of multiplex PCR results for *Staphylococcus* isolates. Lanes: 1, 100-bp DNA ladder; 2, isolate N063A; 3, isolate N064; 4, isolate N068; 5, isolate N071; 6, isolate N077A; 7, N091A; 8, ATCC BAA1707.
anywhere from 0.65% to 34.9% (Rackham et al. 2013; Champion et al. 2014). Carriage rates between studies are often difficult to compare due to differences in sample sizes, culturing techniques, and demographics. In this study, we were interested in comparing the carriage rates between males and females. Our results showed that the carriage rate was not significantly different (p=0.27), which is in agreement with studies conducted by Askarian et al. and Sharma et al. in both healthcare and community settings (Askarian et al. 2009; Sharma et al. 2014). However, Chen and colleagues found carriage rates of S. aureus to be significantly higher in males than in females in a study carried out among Taiwanese university medical students. Based on our findings and the findings of others, whether or not gender is a factor associated with S. aureus and MRSA nasal carriage remains unclear. There are a number of other factors such as age, healthcare exposure, occupation, and health status that alone or in combination likely play more of role in nasal carriage than just gender. In our study the average carriage levels were slightly higher in females than in males for both MSSA and MRSA. A study published by Mermel et al. in 2010 found that in hospitalized patients the average MRSA carriage levels were slightly higher in males than in females (Mermal et al. 2010). Interestingly the average

Figure 2. A) A representative dendrogram showing spa typing of the recovered Staphylococcus aureus strains including MRSA. B) Minimum spanning tree showing the clustering of the recovered Staphylococcus aureus strains including MRSA. Each circle represents one of the 19 spa clusters of the 22 different spa types detected in this study. The letter inside each circle represents a cluster/group. The darker the color of the circle is proportional to the number of strains represented in each cluster. Thick lines denote closer association between the types and thin lines denote less while the dashed lines denote the least association.
MRSA carriage levels in the Mermel study were approximately two times higher than what we found in our study. Additional studies would be needed to determine if carriage levels in MSSA or MRSA positive patients are consistently higher than MSSA and MRSA positive healthy individuals in the community.

Panton-valentine leukocidin (PVL) is a pore-forming cytotoxin and pvl positive S. aureus and methicillin resistant S. aureus strains have been associated with more severe skin and soft tissue infections, bone and joint infections, and necrotizing pneumonias (Lina et al. 1999; Badiou et al. 2010). Historically, pvl rates have been reported to be much higher in S. aureus isolates associated with primary necrotic infections than those not associated with these types of infections. (Lina et al. 1999; Couppié et al. 1994; Prévost et al. 1995). However, very little is known about the prevalence of pvl in S. aureus isolates not associated with infection at all. A study by Harbarth and colleagues reported a 4:1 ratio between colonization and infection with community associated MRSA possessing pvl and suggests that surveillance of S. aureus carriers is important to understanding the true prevalence of PVL-producing strains (Harbarth et al. 2005). In our study none of the methicillin sensitive S. aureus (MSSA) isolates were pvl positive but 33% (2/6) of the MRSA isolates were pvl positive, which is in contrast to several reports of pvl positive rates in community-associated MRSA being greater than 75% (Naimi et al. 2003; Shukla et al. 2004; Naas et al. 2005). It may indeed be the case that the overall prevalence of pvl in MRSA is well below 75% but additional studies including many more healthy subjects is needed determine this. Since PVL producing S. aureus infections can be cause for altering therapeutic approaches, some countries such as England and France are now testing for PVL production by clinical isolates of S. aureus (Gillett et al. 2007; Health Protection Agency 2008; Etienne and Dumitrescu 2009). With this being the case, it is entirely possible that decolonization efforts could also be impacted by the presence of pvl positive MSSA or MRSA in carriers. As pvl positive strains can be clinically challenging when associated with infections, it would be beneficial to understand the efficacy of decolonization strategies to minimize the potential for infection by these strains and knowing the true prevalence of these strains as Harbarth et al. suggested may be critical to successful decolonization efforts.

spa typing is a technique used to distinguish strains of S. aureus with a resolution that is comparable to MLST and PFGE (Badiou et al. 2010; Koreen et al. 2004). The spa typing of our MSSA and MRSA isolates revealed a wide variety of S. aureus strains present in this university setting. Interestingly, spa type t3297, which was one of our most common spa types (4/37) was also found to be the most common spa type among the MSSA isolates (31/38) 81.6% from burn centers in the southeast of China (Chen et al. 2012). spa type t012, which was equally represented in our study, was shown to be the most common spa type to be found among healthy nasal carriers in a study conducted in Norway (Sangvik et al. 2011). The authors of that study also showed that the prevalence of t012 decreased significantly with age and that males had a lower risk of t012 carriage than females. In our study, all of the subjects were college age students, therefore we are not able to perform age associated analysis. With the relatively small number of t012 isolates (4), we found them to be equally distributed between males and females.

In conclusion, our study found carriage rates in a healthy UCO college student population to be consistent with earlier reports and we found no difference between the carriage rates among men and women. The diversity of the isolates obtained in this study as indicated by the variety of spa types identified does suggest that any decolonization method developed should show efficacy against a broad range of isolates.

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References


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