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

Rachael Baalman
Department of Biology, University of Central Oklahoma, Edmond, OK 73034

Yuhang Shang
Department of Biology, University of Central Oklahoma, Edmond, OK 73034

Jonathon Ryan Johnston
Department of Biology, University of Central Oklahoma, Edmond, OK 73034

Mohamed K. Fakhr
Department of Biology, University of Tulsa, Tulsa, OK 74104

Robert Brennan
Department of Biology, University of Central Oklahoma, Edmond, OK 73034

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); 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* (SCC*mec*) 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).

In order to reduce the numbers of staphylococcal infections in hospitalized patients, many hospitals administer MRSA pre-admission screening in order to control high risk patients. Guidelines have recently been put in place for anti-staphylococcal drugs to be used for temporary decolonization (Wertheim et al. 2005; Rohr et al. 2003). These treatments include mupirocin, which is a topical intranasal cream, and bathing in an antiseptic-detergent (Rohr et al. 2003). It has been shown that these methods significantly decrease nasal and whole-body, though not to the same extent, MRSA colonization (Rohr et al. 2003). The decrease in colonization led to fewer incidences of subsequent infections in MSSA and MRSA patients (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 coagulase tests were performed to confirm the colonies were *S. aureus*. Methicillin resistance was determined with cefoxitin disk diffusion, PCR for *mec*A, and PBP2a agglutination.

Molecular analysis: A previously described multiplex PCR was used for the detection of the Panton-Valentine leukocidin (*pvl*) gene, the *spa* gene and the *mec*A 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 H_2O 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'-TAAAGACGATCCTTCGGTGAG-3'), and spa-1514r (5'-CAGCAGTAGTGCTGCCGTTGCTT-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° 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 *mec*A 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

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

Subject Population	% (+) for nasal colonization	Mean Log$_{10}$±SD, CFU per nares culture	Geometric mean per nares culture
S. aureus			
All subjects	21.5.0% (53/247)	2.98 ± 1.17	1,820
Male subjects	29.0% (29/100)	2.96 ± 1.04	1,505
Female subjects	16.3% (24/147)	3.00 ± 1.33	2,282
MRSA			
All subjects	2.4% (6/247)	2.04 ± 1.65	412
Male subjects	4.0% (4/100)	2.15 ± 1.33	359
Female subjects	1.4% (2/147)	1.82 ± 2.88	545

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
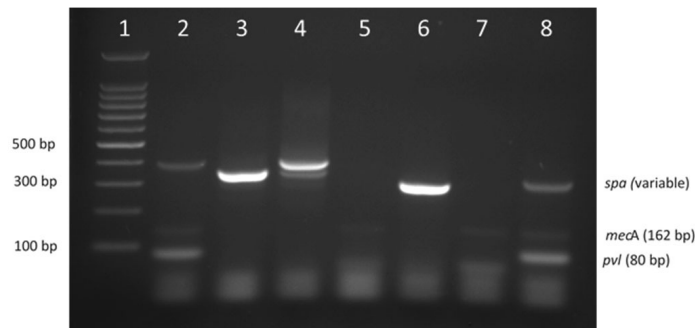

Figure 1. Examples of multiplex PCR results for *Staphylococcus* isolates. Lanes: 1, 100-bp DNA ladder; 2, isolate N063A; 3, isolate N064; 4, isolate N068; 5, isolate N071; 6, isolate N077A; 7, N091A; 8, ATCC BAA1707.

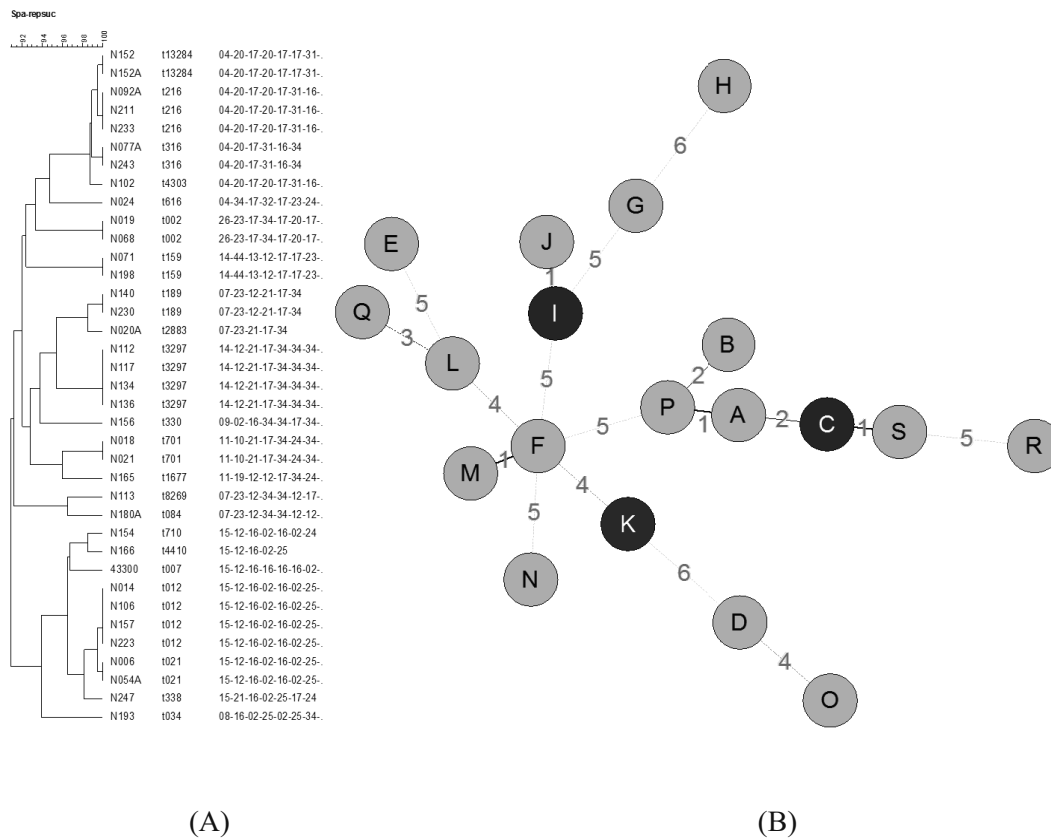(A)   (B)

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.

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 (Mermel et al. 2010). Interestingly the average

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* (Gillet 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.

Acknowledgments

References

Askarian M., Zeinalzadeh A., Japoni A., Alborzi A., Memish Z.A. 2009. Prevalence of nasal carriage of methicillin-resistant *Staphylococcus aureus* and its antibiotic susceptibility pattern in healthcare workers at Namazi hospital, Shiraz, Iran. J Infect Dis. 13:e241-e247.

Badiou C., Dumitrescu O., George N., Forbes A.R.N., Drougka E., Chan K. S., Ramdani-Bouguessa N., Meugnier H., Bes M, Tazir M., Siliopoulou I., Nimmo G. R., Hulten K. G., and Lina G. 2010. Rapid detection of *Staphylococcus aureus* Panton-Valentine Leukocidin in clinical specimens by Enzyme-Linked Immunosorbent Assay and Immunochromatographic tests. J Clin Microbiol. 48(4):1384-90.

Boucher H.W., and Corery G.R. 2008. Epidemiology of methicillin-resistant *Staphylococcus aureus*. Clin Infect Dis. 46(Suppl 5):S344-9.

Buehlmann M., Frei R., Fenner L., Dangel M., Fluckiger U., and Widmer A.F. 2008. Highly effective regimen for decolonization of methicillin-resistant *Staphylococcus aureus* carriers. Infect Control Hosp Epidemiol. 29:510-16.

Champion A.E., Goodwin T.A., Brolinson P.G., Were S.R., Prater M.R., and Inzana T.J. 2014. Prevalence and characterization of methicillin-resistant *Staphylococcus aureus* isolates from healthy university student athletes. Annals of Clinical Microbiology and Antimicrobials 13:33. doi:10.1186/s12941-014-0033-5.

Chatterjee S.S., Ray P., Aggarwal A., Das A., and Sharma M. 2009. A community-based study on nasal carriage of *Staphylococcus aureus*. Ind J Med Res. Vol 130. No. 6:742-48.

Chen C.S., Chen C.Y., and Huang Y.C. 2012. Nasal carriage rate and molecular epidemiology of methicillin-resistant *Staphylococcus aureus* among medical students at a Taiwanese university. Int J Infect Dis. 16:e799-e803.

Couppié P.B., Cribier, Prévost, Grosshans E., Piémont Y. 1994. Leukocidin from *Staphylococcus aureus* and cutaneous infections: an epidemiological study. Arch Dematol. 130:1208-09.

DeLeo F.R., Otto M., Kreiswirth B.N., and Chambers H.F. 2010. Community-associated methicillin-resistant *Staphylococcus aureus*. Lancet. 375:1557-68.

Etienne J., and Dumitrescu O. 2009. Panton-Valentine leucocidin associated *Staphylococcus aureus* infections. BMJ. 339:b4083.

Gillet Y., Vanhems P., Lina G., Bes M., Vandernesch F., Floret D., and Etienne J. 2007. Factors associated with the high fatality rate of Panton-Valentine leucocidin associated *Staphylococcus aureus* necrotizing pneumonia. Clin Infect Dis. 45:315-321.

Gorwitz R.J., Kruszon-Moran D., McAllister S. K., McQuillan G., McDougal L.K., forsheim G.E., Jensen B.J., Killgore G., Tenover F.C., and Kuehnert M.J. 2008. Changes in the prevalence of nasal colonization with *Staphylococcus aureus* in the United States, 2001-2004. J Infect Dis. 197:1226-34.

Hallin M., Denis O., Deplano A., De Mendonca R., De Ryck R., Rottiers S., and Struelens M.J. 2007. Genetic relatedness between methicillin-susceptible and methicillin-resistant *Staphylococcus aureus*: results of a national survey. J Antimicrob Chemother. 59:465-72.

Harbarth S., Francois P., Shrenzel J., Frankhauser-Rodriguez C., Hugonnet S., Koessler T., Huyghe A, and Pittet D. 2005. Community-associated methicillin-resistant *Staphylococcus aureus*. Emerg Infect Dis. 11:962-965.

Harmsen D., Claus H., Witte W., Rothgänger J., Claus H., Trunwald D., and Vogel U. 2003. Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for spa repeat determination and database management. J Clin Microbiol. 41(12):5442-48.

Health Protection Agency. 2008. Guidance on the diagnosis and management of PVL-associated *Staphylococcus aureus* infections (PVL-SA) in England. Health Protection Agency, London, United Kingdom. www.hpa.org.uk/web/HPAwebFile/HPAweb_C/1226478464166.

Klevens R.M., Edwards J.R., Tenover F.C., McDonald L.C., Horan T., Gaynes R., and the National Nosocomial Infections Surveillance System. 2006. Changes in the epidemiology of methicillin-resistant *Staphylococcus aureus* in intensive care units in US hospitals, 1992-2003. Clin Infect Dis. 42:389-91.

Kluytmans J., van Belkum A., and Verbrugh H. 1997. Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks. Clin Microbiol Rev. 10:505-20.

Koreen L., Ramaswamy S.V., Graviss E.A., Naidich S., Musser J.M., and Kreiswirth B.N. 2004. *spa* typing method for discriminating among *Staphylococcus aureus* isolates: implications for use of a single marker to detect genetic micro- and macrovariation. J Clin Microbiol. 42(2):792-799.

Larsen A.R., Stegger M., and Sørum M. 2008. *spa* typing directly from a *mec*A, *spa* and *pvl* multiplex PCR assay – a cost effective improvement for methicillin-resistant *Staphylococcus aureus* surveillance. Clin Microbiol Infect. 14:611-614.

Lina G., Piémont Y., Godail-Gamot F., Ges M., Peter M.O., Gaudocon V., Vandernesch F., and Etienne J. 1999. Involvement of Panton-Valentine Leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. Clin Infect Dis. 29:1128-32.

Mermal L.A., Eells S.J., Acharya M.K., Cartony J.M., Dacus D., Fadem S., Gay E.A., Gordon S., Lonks J.R., Perl T.M., McDougal L.K., McGowan J.E., Maxey G., Morse D., and Tenover F.C. 2010. Quantitative analysis and molecular fingerprinting of methicillin-resistant *Staphylococcus aureus* nasal colonization in different patient populations: a prospective, multicenter study. Infect Control Hosp Epidemiol. 31(6):592-597.

Nass T., Forineau N., Spicq C., Robert J., Jarlier V., and Nordmann P. 2005. Three year survey of community-acquired methicillin-resistant *Staphylococcus aureus* producing Panton-Valentine leucocidin in a French university hospital. J Hosp Infect. 61:321-329.

Naimi T.S., LeDell K.H., Como-Sabetti K., Borchardt S. M., Boxrud D.J., Etienne J., Johnson S.K., Vandenesch F., Fridkin S., O'Boyle C., Danila R.N., and Lynfield R. 2003. Comparison of community and health care-associated methicillin-resistant *Staphylococcus aureus* infection. JAMA. 290(22):2976-84.

Prévost G., Couppié P., PrévostP., et al. 1995. Epidemiological data on *Staphylococcus aureus* strains producing synergohymenotropic toxins. J Med Microbiol. 42:237-245.

Rackham D., Ray S., Franks A., Bielak K., and Pinn T. 2010. Community-associated methicillin-resistant *Staphylococcus aureus* nasal carriage in a college student athlete population. Clin J Sport Med. 20(3):185-188.

Rohde R.E., Denham R., and Brannon A. 2009. Methicillin resistant *Staphylococcus aureus*: Carriage rates and characterization of students in a Texas university. Clin Lab Sci. 22(2)176-184.

Rohr U., Mueller C., Wilhelm M., Muhr G., and Gatermann S. 2003. Methicillin-resistant *Staphylococcus aureus* whole-body decolonization among hospitalized patients with variable site colonization by using mupirocin in combination with octenidine dihydrochloride. J Hosp Infect. 54:305-309.

Sangvik M., Olsen R.S., Olsen K., Simonsen G.S., Furberg A.S., and Ericson Sollid J.U. 2011. Age and gender-associated *Staphylococcus aureus spa* types found among nasal carriers in a general population: the Tromsø staph and skin study. J Clin Microbiol. 49(12):4213-18.

Sharma Y., Jain S., Signh H., and Govil V. 2014. *Staphylococcus aureus*: Screening for nasal carriers in a community setting with special reference to MRSA. Scientifica vol. 2014. doi. org/10.1155/2014/479048.

Shen H., Akoda E., and Zhang K. 2013. Methicillin-resistant *Staphylococcus aureus* carriage among students at a historically black university: A case study. Int J Microbiology vol 2013. doi.org/10.1155/2013/989834.

Shukla S.K., Stemper M.E., Ramaswamy S.V., Conradt J.M., Reich R., Graviss E.A., and Reed K.D. 2004. Molecular characteristics of nosocomial and Native American community-associated methicillin-resistant *Staphylococcus aureus* clones from rural Wisconsin. J Clin Microbiol. 42(8):3752-57.

Wertheim H.F.L., Melles D.C., Vos M.C., van Leeuwen W., van Belkum A., Verbrugh H.A., and Nouwn J.L. 2005. The role of nasal carriage in *Staphylococcus aureus* infections. Lancet Infect Dis. 5:751-62.