World Journal of Medical Sciences 4 (2): 65-69, 2009
ISSN 1817-3055

Nasal Carriage Rates of Methicillin Resistant *Staphylococcus aureus* in Healthy Individuals from a Rural Community in Southeastern United States

[1]*Lauren Martinez,* [2]*Andrew D.Oncale,* [2]*Melody B.Oncale,* [1]*Angela Corbin and* [1]*Rajkumar Nathaniel*

[1]Department of Biological Sciences, Nicholls State University, Thibodaux, LA 70310, USA
[2] School of Medicine, Tulane University, New Orleans, LA 70112, USA

Abstract: *Staphylococcus aureus*, an opportunistic pathogen that colonizes the external nares and skin of greater than 20% of the healthy population, has emerged in recent years as a major public health concern because of the occurrence of multi-drug resistant strains. Methicillin-resistant *Staphylococcus aureus* associated with nosocomial infections are termed HA-MRSA to differentiate these strains from CA-MRSA, which are isolates obtained from individuals in a community setting. These two strains differ in pathogenesis, antibiotic resistance profiles and expression of virulence genes. In order to investigate the colonization rates of *S.aureus* in the community, nasal swab culture isolates were recovered from 294 healthy volunteers from Thibodaux and Houma in southern Louisiana, USA. Resistance to oxacillin was used to identify MRSA. Polymerase chain reactions were used to detect thermonuclease gene *nuc* to confirm *S.aureus* species, the presence of *mecA* to confirm methicillin resistance, staphylococcal cassette chromosome *mec* typing patterns to distinguish HA-MRSA from CA-MRSA and the presence of Panton Valentine leukocidin genes to confirm CA-MRSA. Our results show an overall 21.4% (63/294) colonization rate of healthy individuals with *Staphylococcus aureus* and 0.68% (2/294) rate of MRSA nasal carriage. Antibiotic resistance profiles were generated for the MRSA isolates. The MRSA isolates belonged to Staphylococcal cassette chromosome type II and IVa indicating that both HA-MRSA and CA-MRSA respectively were found in the community. These results highlight the usefulness of routine surveillance of healthy populations to assess the prevalence of virulent organisms and to aid in the prediction of potential community outbreaks.

Key words: MRSA · *Staphylococcus* · *mecA* · Nasal carriage · PVL · SCC*mec*

INTRODUCTION

Staphylococcus aureus is one of the most prevalent gram positive cocci that are found as transient normal flora of human skin and mucosal surfaces in 20-90% of the population [1]. *Staphylococcus aureus* is an opportunistic pathogen that causes a wide range of infections including skin lesions, abscesses, endocarditis, septicemia and toxic shock syndrome [2]. The acquisition of multiple drug resistance genes has enabled this organism to emerge as a public health problem [3]. Methicillin resistance is conferred by the *mecA* gene that encodes for a modified penicillin binding protein 2a [4]. Since its discovery, methicillin resistant *S.aureus* (MRSA) was recognized as a nosocomial pathogen affecting indwelling catheter users, dialysis patients, nursing home residents and individuals who have been recently hospitalized or have undergone a surgical procedure [5]. Since the 1990s, however, the number of infections of MRSA in individuals lacking any health care associated exposure has increased significantly. Furthermore, it is now apparent that the health care associated type (HA-MRSA) is different from the community associated strain (CA-MRSA) in virulence mechanisms, antibiotic resistance patterns and presentation of infection [5]. Soft tissue and skin infections constitute the majority of the reported cases of CA-MRSA that can easily spread by human to human contact [5].

The *mecA* gene can be located in one of seven types of staphylococcal cassette chromosome (SCC) elements that can be transferred horizontally between Staphylococcal species [6]. Typing SCC*mec* elements has shown that CA-MRSA mainly harbors the smaller types IV and V, while larger types I-III have been found in

Corresponding Author: Rajkumar Nathaniel, 222 Gouaux Hall, Department of Biological Sciences, Nicholls State University, Thibodaux, LA 70310

HA-MRSA strains [6]. In addition, CA-MRSA strains have been shown to produce Panton-Valentine leukocidin (PVL) [7], a bi-component leukotoxin encoded by PVL genes *luk-S-PV* and *luk-F-PV* that possess a strong affinity for leukocytes [8]. Compared to HA-MRSA isolates, which are typically multi drug resistant, CA-MRSA strains are generally susceptible to most antibiotics; for example, clindamycin and trimethoprim-sulfamethoxazole are drugs used to treat CA-MRSA [3]. CA-MRSA has also been reported to have a growth advantage over HA-MRSA, which is thought to be one of the factors that have led to the emergence of community outbreaks worldwide [9]. The aggressiveness of CA-MRSA infections is thought to be due to increased virulence and greater bacterial fitness [7]. With increased numbers of CA-MRSA infections being reported in nosocomial settings, there is now a blurring of distinction between hospital acquired versus community associated strains [7].

In order to assess carriage rates of MRSA within the community, nasal swab culture isolates were recovered from 294 healthy volunteers from Thibodaux and Houma, two small rural towns south west of New Orleans in southern Louisiana, USA. *Staphylococcus aureus* isolates identified by standard culture were genotyped, characterized for the presence of *mecA*, PVL, SCC*mec* type, and tested to determine antibiotic resistance profiles.

MATERIALS AND METHODS

Bacterial Strains: The following control strains were used in this study. Methicillin sensitive *Staphylococcus aureus* (ATCC 25923), *Staphylococcus intermedius* (ATCC 29663), Methicillin resistant *Staphylococcus aureus* (ATCC 43300).

Sample Collection: Culturette swabs (Fisher Scientific, USA) of external nares, were collected from 294 healthy individuals in the general population from southern Louisiana, USA. The swabs were then incubated in 2 mL of Luria-Bertani broth (Becton Dickinson, USA) at 37°C for 24 hours.

Bacterial Culture and Characterization: Twenty-four hour broth cultures were streaked on mannitol salt agar plates (MSA) (Becton Dickinson) for the isolation of putative *S. aureus* colonies. All gram positive cocci cultures were subjected to catalase and tube coagulase test by standard clinical methods. Up to 5 catalase positive colonies were analyzed from each individual. Individual colonization by *S.aureus* was determined by having any one of the five colonies test positive for tube coagulase. Coagulase positive methicillin resistant *Staphylococci* were identified based on resistance to oxacillin (1μg) disk by Kirby Bauer disc diffusion method (Becton Dickinson).

DNA Extraction and PCR: Pure cultures of bacteria were grown in 3 mL Luria Bertani broth (Becton Dickenson) for 18 hours at 37°C, centrifuged at 10,000 x g for 5 minutes. Total DNA was extracted from bacterial pellets using the Fast ID Genomic DNA Extraction Kit (Genetic ID, USA) according to manufacturer's instructions. The final elution volume was 100 μL and 1 μL of DNA was used in a 50 μL polymerase chain reaction (PCR). The primer pairs used to identify, the *S.aureus* species specific thermonuclease *nuc* was according to Baron [10], *mecA*, SCC*mec* typing and PVL was according to previous reports [11,12]. Each PCR was performed in a 50 μL reaction volume containing 0.5 units of *Taq* with Thermopol buffer (NEB, USA), 200 μM of each deoxynucleotide triphosphate (dATP, dTTP, dGTP, dCTP) (Fisher Scientific) and 1 μM of each primer. Amplification was performed in a GeneAmp 2700 thermal cycler (Applied Biosystems, USA) beginning with an initial denaturation step at 94°C for 5 min followed by 30 cycles of 94°C for 30 seconds, appropriate annealing temperature for 30 seconds, 72°C for 30 sec and ending with a final extension step at 72°C for 7 min followed by a hold at 4°C, unless mentioned otherwise in the specific published

Table 1: Characterization of MRSA isolates

Test	Isolate 1126c	Isolate 1233a
mecA	+	+
SCC*mec* type	IVa	II
PVL	+	-
Benzylpenicillin	R	R
Clindamycin	S	S
Erythromycin	R	R
Gentamicin	S	S
Levofloxacin	I	R
Linezolid	S	S
Oxacillin	R	R
Quinupristin-Dalfopristin	S	S
Rifampicin	S	S
Tetracycline	S	R
Trimethoprim-Sulfamethoxazole	S	S
Vancomycin	S	S

protocol. Ten microliters of the PCR was electrophoresied on a 1% Tris-acetate-EDTA agarose gel at 100 volts, stained with ethidium bromide and visualized under UV light.

Antibiotic Sensitivity Testing: Susceptibility patterns to the various antibiotics (Table 1) was performed using the Vitek 2 (Biomerieux, USA) system according to manufacturer's instructions and was a kind courtesy of Department of Clinical Laboratory Medicine, Thibodaux Regional Medical Center, USA.

RESULTS

Nasal swabs were obtained from a total of 294 healthy individuals in southeastern USA. From the initial cultures, 215 individuals were colonized by gram positive-catalase positive cocci. Of these, only 63 individuals harbored coagulase positive strains indicating a 21.4% (63/294) rate of nasal colonization by *S.aureus*. Species identification and confirmation showed that all coagulase positive *Staphylococci* produced a PCR amplicon for thermonuclease *nuc*, migrating at 420 bp as expected [10] and is seen by the representative samples in Figure 1, lanes 1-5, confirming *S.aureus* identification. This experiment ruled out the presence of other coagulase positive species such as *S.intermedius*. Only 2 isolates (1126c and 1233a) were found to be resistant to oxacillin by Kirby Bauer disk diffusion method. Methicillin (oxacillin) resistance was confirmed by PCR amplification of a 310 bp product corresponding to the *mecA* gene as previously described [12]. As seen in Figure 1, lanes 6-10, isolates 1126c, 1233a and the positive control MRSA ATCC 43300 produced amplicons of the expected size.

In order to determine which type of mobile genetic elements were present in the *mecA* containing strains, a series of PCRs were performed on DNA from the two MRSA isolates. A 776 bp product corresponding to SCC*mec* type IVa was observed in reactions involving 1126c (Fig.1, lane 14), whereas the 398 bp product corresponding to SCC*mec* type II was observed in reactions with isolate 1233a (Fig.1, lane 12) as previously described by Zhang [11]. Since SCC*mec* type IVa is typically indicative of CA-MRSA, amplification of the PVL locus was performed. Figure.1, lanes 17-19 show the amplification of the expected 433 bp product corresponding to PVL being found only in isolate 1126c. The presence of PVL in a SCC*mec* type IVa MRSA context is in agreement with other reports [13].

Antibiograms were generated using an automated Vitek 2 system (Table 1). Based on previous reports [13], PVL positive, CA-MRSA isolate 1126c was susceptible to most antibiotics and differed in comparison with HA-MRSA isolate 1233a which was resistant to levofloxacin and tetracylcine. Both MRSA isolates were resistant to penicillin and erythromycin.

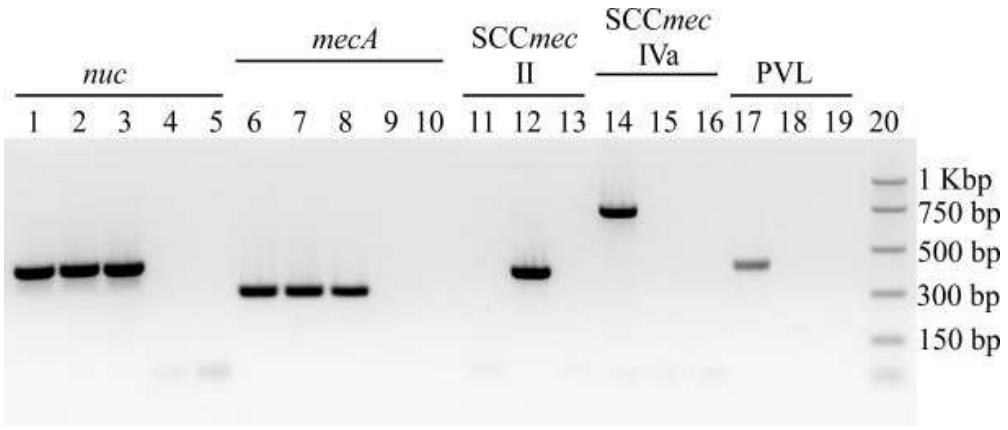

Fig. 1: PCR of various markers from isolates 1126c and 1233a. Confirmation of *Staphylococcus aureus* species by *nuc* PCR: lane 1, isolate 1126c; lane 2, isolate 1233a; lane 3 MRSA ATCC 43300; lane 4, ATCC 29663; lane 5, no template control. Detection of *mecA*: lane 6, isolate 1126c; lane 7, isolate 1233a; lane 8, MRSA ATCC 43300; lane 9, *S.aureus* ATCC 25923; lane 10, no template control. Detection of SCC*mec* II: lane 11, isolate 1126c; lane 12, isolate 1233a; lane 13, no template control. Detection of SCC*mec* IVa: lane 14, isolate 1126c; lane 15, isolate 1233a; lane 16, no template control. Detection of PVL: lane 17, isolate 1126c; lane 18, isolate 1233a; lane 19, no template control; lane 20, molecular weight PCR marker (Promega, USA)

DISCUSSION

The aim of this study was to assess nasal carriage rates of *Staphylococcus aureus* in a rural community in southeastern USA. A recent study compared nasal carriage rates of *S. aureus* from a population across the United States from 2001 to 2004 and found that colonization rates varied from 32.4% (2002) to 28.6% (2004) [14]. Our study shows a lower nasal carriage rate of 21.4% (63/294). The use of PCR for confirmation of the *mecA* gene identified two of the *Staphylococcus aureus* isolates as MRSA. While Gorwitz reported MRSA colonization rates to be 1.5% in the healthy population [14], our data indicate a much lower 0.68% (2/294) rate of MRSA carriage. The overall lower rates of nasal carriage seen in our study could be due to the fact that our study was conducted in a purely rural setting (less dense), reinforcing the idea that human to human contact or population density influences the incidence rates and spread of these organisms.

Of the two MRSA isolates, based on SCC*mec* type, PVL presence and antibiotic susceptibility, it is evident that 1126c is of the CA-MRSA strain type. Isolate 1233a shows all the hall marks of being a HA-MRSA strain. The recovery of both of the strains indicates a concurrent distribution of both hospital associated and community acquired strains in this population. The use of pulse field gel electrophoresis (PFGE) on *S.aureus* isolates has been well established to determine strain relatedness and to identify evolutionary patterns as those seen in the epidemic outbreaks involving emergent types such as the notorious USA300 strain which is a CA-MRSA versus USA100 that is a HA-MRSA type [12-14]. Both 1126c and 1233a isolates could be subjected to PFGE to answer these questions.

In conclusion, these data show that both HA-MRSA and CA-MRSA are present in the healthy population in southern USA. The rates of nasal carriage of *S.aureus* and MRSA are an important indicator of strain prevalence. Collection of such data aid in shaping public health policy, help predict emergent outbreaks and serve as an important tool in preventive medicine.

ACKNOWLEDGEMENTS

This work was made possible by Louisiana Board of Reagents LEQSF (2007-2012) ENH-PKSFI-PES-05 support, National Science Foundation EPSCoR (2008)-PFUND-114 to RN and Nicholls Research Council grant 212278 to AC.

REFERENCES

1. Foster, T.J., 2005. Immune Evasion by *Staphylococci*. Nature Reviews/Microbiology, 3: 948-958.
2. Jarraud, S., C. Mougel, J. Thioulouse, G. Lina, H. Meugnier, F. Forey, X. Nesme, J. Etienne and F. Vandenesch, 2002. Relationships between *Staphylococcus aureus* genetic background, virulence factors, agr groups (alleles), and human disease. Infect Immun Feb., 70(2): 631-41.
3. Jones, R.D., S.A. Kania, B.W. Rohrbach, L.A. Frank and D.A. Bemis, 2007. Prevalence of oxacillin- and multidrug-resistant staphylococci in clinical samples from dogs: 1,772 samples (2001-2005). J. Am. Vet. Med. Assoc. Jan., 15;230(2): 221-7.
4. Bemis, D.A., R.D. Jones, L.E. Hiatt, E.D. Ofori, B.W. Rohrbach, L.A. Frank and S.A. Kania, 2006. Comparison of tests to detect oxacillin resistance in *Staphylococcus intermedius, Staphylococcus schleiferi*, and *Staphylococcus aureus* isolates from canine hosts. J. Clin Microbiol. Sep., 44(9): 3374-6.
5. Bartlett, J.G., 2008. Methicillin-resistant *Staphylococcus aureus* infections. Top HIV Med. Dec., 16(5): 151-5.
6. Deurenberg, R.H. and E.E. Stobberingh, 2008. The evolution of *Staphylococcus aureus*. Infect Genet Evol. Dec., 8(6): 747-63.
7. Gordon, R.J., F.D. Lowy, 2008. Pathogenesis of methicillin-resistant *Staphylococcus aureus* infection. Clin Infect Dis. Jun 1;46 Suppl 5: S350-9.
8. Bocchini, C.E., K.G. Hulten, E.O. Mason, B.E. Gonzalez, W.E. Hammerman, and S.L. Kaplan, 2006. Panton-Valentine Leukocidin Genes Are Associated With Enhanced Inflammatory Response and Local Disease in Acute Hematogenous *Staphylococcus aureus* Osteomyelitis in Children. Pediatrics, 117: 433-440.
9. McAleese, F., E. Murphy, T. Babinchak, G. Singh, B. Said-Salim, B. Kreiswirth, P. Dunman, J. O'Connell, S.J. Projan and P.A. Bradford, 2005. Use of ribotyping to retrospectively identify methicillin-resistant *Staphylococcus aureus* isolates from phase 3 clinical trials for tigecycline that are genotypically related to community-associated isolates. Antimicrob Agents Chemother. Nov., 49(11): 4521-9.
10. Baron, F., M. Cochet, J.L. Pellerin, N. Ben Zakour, A. Lebon, A. Navarro, I. Proudy, Y. Le Loir and M.J. Gautier, 2004. Development of a PCR test to differentiate between *Staphylococcus aureus* and *Staphylococcus intermedius*. J Food Prot Oct., 67(10): 2302-5.

11. Zhang, K., J. McClure, S. Elsayed, T. Louie and J.M. Conly, 2005. Novel multiplex PCR assay for characterization and concomitant subtyping of staphylococcal cassette chromosome *mec* types I to V in methicillin-resistant *Staphylococcus aureus*. J Clin Microbiol., 43(10): 5026-5033.
12. McClure, J.A., J.M. Conly, V. Lau, S. Elsayed, T. Louie, W. Hutchins and K. Zhang, 2006. Novel multiplex PCR assay for detection of the staphylococcal virulence marker Panton-Valentine leukocidin genes and simultaneous discrimination of methicillin-susceptible from –resistant staphylococci. J Clin Microbiol. Mar., 44(3): 1141-4.
13. Tenover, F.C., S. McAllister, G. Fosheim, L.K. McDougal, R.B. Carey, B. Limbago, D. Lonsway, J.B. Patel, M.J. Kuehnert and R. Gorwitz, 2008. Characterization of *Staphylococcus aureus* isolates from nasal cultures collected from individuals in the United States in 2001 to 2004. J Clin Microbiol. Sep., 46(9): 2837-41.
14. Gorwitz, R.J., D. Kruszon-Moran, S.K. McAllister, G. McQuillan, L.K. McDougal, G.E. Fosheim, B.J. Jensen, G. Killgore, F.C. Tenover and M.J. Kuehnert, 2008. Changes in the prevalence of nasal colonization with *Staphylococcus aureus* in the United States, 2001-2004. J Infect Dis. May 1; 197(9): 1226-34.