One-Year Surveillance of Methicillin-Resistant
Staphylococcus aureus Nasal Colonization and Skin
and Soft Tissue Infections in Collegiate Athletes

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Objective: To determine the frequency and clinical importance of methicillin-resistant Staphylococcus aureus (MRSA) colonization in student athletes.

Design: Prospective observational cohort study.

Setting: A major university in the southeastern United States.

Participants: Student athletes participating in the men’s football and women’s lacrosse programs (N = 126).

Main Exposure: Monthly assessment of S. aureus nasal colonization.

Main Outcome Measures: Trends in S. aureus colonization over time and the occurrence of skin and soft tissue infections.

Results: Methicillin-resistant S. aureus nasal colonization varied significantly through the athletic season (4%-23%), peaking during times of highest athletic activity. This increase in colonization was not associated with the development of an outbreak of skin and soft tissue infections, and no single MRSA clone emerged as a dominant isolate.

Conclusions: During the athletic season, there is a considerable burden of MRSA colonization in student athletes; however, colonization alone appears to be insufficient to trigger an outbreak of staphylococcal infections. A combination of distinct molecular characteristics in the organism and specific host factors may govern the development of staphylococcal disease.

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Each year, infections caused by methicillin-resistant Staphylococcus aureus (MRSA) are responsible for nearly 20,000 deaths in the United States alone. At the root of the problem is that S. aureus is a ubiquitous microorganism, colonizing the anterior nares of nearly one-third of the entire human population at any given time. However, S. aureus does not remain merely a commensal; rather, it is the leading cause of skin, soft tissue, and bone infections and has a spectrum of illness ranging from boils and cellulitis to infective endocarditis, necrotizing pneumonia, sepsis, and death. The challenge of S. aureus (and therefore MRSA) is generated by 2 distinctive properties, an assortment of potent virulence factors responsible for each of these disease phenotypes and the ability to rapidly develop antimicrobial resistance, providing the organism with the tools to prosper as a commensal, thrive as a pathogen, and elude nearly all antistaphylococcal agents. These characteristics make S. aureus one of the most important bacterial pathogens in the United States.

Since the widespread emergence of community-associated MRSA (CA-MRSA), numerous reports have suggested that sports team participants, particularly those involved in contact sports, may be at increased risk for infections with CA-MRSA. In a recent survey of the National Athletic Trainers Association, more than half of all athletic trainers (53%) reported treating MRSA infections in their athletes, the vast majority of which (92%) were skin and soft tissue infections. In 2003, the Centers for Disease Control and Prevention highlighted the problem of MRSA in wrestlers, fencers, and football players. While each of these reports provided important information regarding the characteristics of infected athletes, they did not provide longitudinal data characterizing the frequency of nasal colonization, the rate of CA-MRSA during times of highest athletic activity. This increase in colonization was not associated with the development of an outbreak of skin and soft tissue infections, and no single MRSA clone emerged as a dominant isolate.

To provide such a longitudinal assessment, we systematically sampled colle-
giate athletes for *S. aureus* nasal colonization (including MRSA) and prospectively assessed them for the development of skin and soft tissue infections. We present the molecular features of the MRSA isolates recovered from the participants and the risk factors associated with colonization and disease.

**METHODS**

Student athletes participating in the Vanderbilt University men’s football and women’s lacrosse teams were invited to participate in the study. While the entire varsity athletic program at Vanderbilt comprises nearly 400 individuals aged 18 to 24 years, the football and lacrosse teams number 125 students and compete in Division I of the National Collegiate Athletic Association’s Southeastern Conference (football) and the American Lacrosse Conference (women’s lacrosse). Subjects were eligible to participate in the study on entrance to training camps, occurring in both the spring (March) and fall (August), and they were asked to remain in the study for the academic year, until completion of undergraduate education or cessation of involvement in the varsity athletic program. The Vanderbilt University Medical Center institutional review board and university counsel approved the study.

After informed consent was obtained, a questionnaire was administered to the athletes to assess potential risk factors for nasal colonization. The questionnaire included items regarding position on the team, history of skin or soft tissue infections, history of staphylococcal infections, recent antibiotic use, recent hospitalization or surgery, history of chronic skin disorders, and exposure to individuals with confirmed MRSA disease. Nasal swabs were collected each month by study personnel by moistening Culturette swabs (BD, Franklin Lakes, New Jersey) with sterile media, rotating the swab in the anterior nares, placing the swabs in liquid Amies medium (BD Culturette Plus), and promptly delivering them to the laboratory.

In addition to nasal screening visits, all subjects were repeatedly instructed to alert their athletic trainer or sports medicine physician if signs or symptoms of skin or soft tissue infections developed. These symptoms included redness of the skin, development of a pustule or other fluid collection, and poorly healing, tender, or unusually erythematous abrasions. When these symptoms were reported, study personnel were contacted and all skin and soft tissue infections, as diagnosed by team physicians and study personnel, were cultured, where possible, and antibiotics were prescribed at the discretion of the team physicians. Nasal decolonization strategies were not performed.

Once in the laboratory, swab samples were placed in tryptic soy broth with 6.5% sodium chloride. After incubation at 37°C for 18 hours, an aliquot was plated onto paired mannitol salt agar plates, with and without 4 µg/mL of oxacillin (Hardy Diagnostics, Santa Maria, California), and incubated at 37°C for 48 hours. After an additional 18 hours at room temperature, plates were inspected for yellow colonies indicative of mannitol fermentation, characteristic of *S. aureus*. After subculturing onto blood agar plates, rapid latex agglutination testing for clumping factor and protein A was performed on all isolates (Staphaurex Plus; Remel, Lenexa, Kansas) and positive isolates were stored at -80°C for further characterization.

Any colonies growing in the presence of oxacillin were considered to be putative MRSA isolates and underwent confirmation of the presence of the mecA gene by polymerase chain reaction.1 Isolates confirmed to be MRSA underwent staphylococcal cassette chromosome mec (SCCmec) typing using the multiplex strategy of Oliveira and de Lencastre3; however, for those strains unable to be characterized by the multiplex strategy, concerted and mec complex typing were performed as previously described.15 Genomic DNA was used as a template for polymerase chain reaction detection of the nuclease gene (specific to *S. aureus*), the staphylococcal-specific cytolytic toxin Panton-Valentine leukocidin (PVL), and the arginine catabolic element, using previously validated primers.17,18 Repetitive-element, sequence-based polymerase chain reaction (Diversilab System; BioMerieux, Durham, North Carolina) was used to determine genetic relatedness between strains and classification of genotype (eg, USA100, USA300).20-22

Using a conservative baseline MRSA colonization rate of 6%, and estimating that the frequency of MRSA colonization would increase 3-fold during the course of the season, 114 subjects were needed (from football and lacrosse teams combined) to adequately detect a difference between colonization rates (α = 0.05; β = 0.8; Pearson χ² method). Pearson χ² method was used to measure differences in colonization rates between the off-season (limited sports-related activities), preseason (intense practice sessions but no competitive activities), regular season (the time of highest activity), and postseason (returning to limited sports-related activities). Multivariate analysis for potential risk factors for MRSA colonization at any time was performed by logistic regression. All analyses were performed using Stata 10.0 (StataCorp, College Station, Texas).

**RESULTS**

**MEN’S FOOTBALL.**

To determine the frequency of *S. aureus* colonization over time, nasal swabs were collected from 100 subjects, representing 98% of the entire football team, over 8 sampling periods. At any one time, *S. aureus* nasal colonization rates ranged from 12% to 30%, with the lowest colonization rates observed in the summer off-season and the highest observed during the football season (Figure 1). Overall, MRSA nasal colonization was detected in as few as 4% of participants, during the summer off-season, and as many as 19% at the end of the regular football season. The MRSA colonization rates during the regular football season were significantly higher than in spring training (16.5% vs 8.4%; *P* = .003), the off-season (16.5% vs 4.4%; *P* = .004), or post-season (16.5% vs 7.7%; *P* = .04).

To define risk factors associated with staphylococcal nasal carriage, data from each subject’s baseline questionnaire and monthly updates were linked to his carriage pattern over time. A majority of subjects (54%) had at least 1 positive nasal culture for *S. aureus* during the study period; 37% had at least 1 positive culture for MRSA. There were no differences in the rates of *S. aureus* nasal colonization, either methicillin-susceptible *S. aureus* or MRSA, by race (*P* = .97), college year (*P* = .61), football position (*P* = .97), history of antibiotic use within 6 months (*P* = .85), or hospitalization within 12 months (*P* = .06) (Table). Multivariate logistic regression, including tests for interaction, revealed no significant differences based on these characteristics. Seventy-nine players (79%) had 2 or fewer positive cultures for *S. aureus* (of 8 sample periods), while 10% of players had 5 or more positive cultures. There were no differences in the number of positive cultures based on race, college year, football position, history of antibiotic use, or history of hospitalization.
WOMEN’S LACROSSE

To determine if the colonization characteristics observed in the football cohort were generalizeable to other student athletes, we enrolled 26 women from the women’s lacrosse team, representing 100% of the group. Nasal swabs were obtained during both the winter and spring lacrosse seasons, but the team members did not remain on campus during the summer; therefore, colonization was assessed over only 6 sample periods. S aureus nasal colonization rates ranged from 28% to 39% of subjects at any one time, with the lowest frequency of colonization observed during the winter postseason and the highest, during the fall season (Figure 2). The MRSA nasal colonization rates ranged from 11% to 23% of subjects, with 2 relative peaks, 1 during the spring season and 1 during the fall season. Despite the relative increase in colonization rates during these 2 sample periods, overall differences in nasal colonization across all points and between peak and nadir did not achieve statistical significance (23.1% [fall season] vs 11.5% [preseason]; \(P=0.04\)). In addition, there were no differences in the number of positive methicillin-susceptible S aureus or MRSA cultures based on race, college year, position, history of antibiotic use, or history of hospitalization.

SKIN AND SOFT TISSUE INFECTIONS

To identify whether staphylococcal colonization portends a higher risk for infection, we assessed all skin and soft tissue infections that were consistent with staphylococcal disease. Five individuals experienced infections, 4 from the football cohort and 1 from the lacrosse cohort. Two of these subjects (1 from the football team, 1 from the lacrosse team) had pustular lesions that spontaneously drained prior to culturing. Surface swab cultures of the lesions did not reveal a pathogen (1 of these subjects had MRSA nasal colonization at the sample time prior to the infection). Two
football players had carbuncles drained within days of each other and both grew Proteus mirabilis. Neither of the subjects had evidence of staphylococcal nasal colonization immediately before or after the infection occurrence. The last of these 5 individuals with skin infections developed recurrent MRSA furunculosis during football spring training. Nasal cultures were negative for MRSA before and after furuncles developed; however, during the football season, he developed asymptomatic MRSA nasal colonization.

**MOLECULAR CHARACTERISTICS OF MRSA ISOLATES**

To study the discrepancy between high colonization rates and the infrequent incidence of skin and soft tissue infections, we characterized the molecular feature of the nasal and infecting MRSA strains. Each of the 73 MRSA colonizing isolates possessed the mecA gene, characteristic of MRSA, and the S aureus–specific nuc gene. None of the carriage isolates possessed genes encoding PVL or the arginine catabolic element. Further analysis of 53 viable strains revealed that 43 (81.1%) possessed a type IV SCCmec, 8 possessed a type II SCCmec (15.1%), 1 possessed a type III cassette (1.8%), and 1 possessed a type V cassette (1.8%). There was considerable heterogeneity between strains, with the most common genetic lineages being USA200 (20.8%), USA900 (18.9%), USA300 (9.4%), USA400 (9.4%), USA600 (9.4%), and USA800 (9.4%). The single MRSA isolate recovered from an active infection was characterized as a PVL⁺, SCCmec type IV, USA300 CA-MRSA.

In this study of healthy collegiate athletes, we demonstrate that MRSA nasal colonization rates increased significantly during the course of the athletic season. This increase was not due to the emergence of a single MRSA strain; rather, there was significant heterogeneity among nasal MRSA isolates. Despite the frequency of MRSA colonization in these student athletes, MRSA infections were uncommon, suggesting that colonization alone may be insufficient to initiate a staphylococcal outbreak. Of particular interest is that the frequency of MRSA nasal colonization during the off-season and preseason was very similar to the frequency of MRSA carriage in other groups that we have reported in our region. Yet, in both the football and lacrosse teams, colonization significantly increased during the regular season, implying that factors unique to this time—whether exposure related (encountering other teams with their own MRSA colonization rates, shared equipment, or uniforms) or host related (more frequent abrasions)—play an important role in the dynamics of staphylococcal colonization. It seems unlikely that this observation is due to calendar seasonality alone since the lacrosse team experienced 2 MRSA carriage peaks, during both their fall and spring seasons.

To our knowledge, this is the first longitudinal study to assess nasal MRSA colonization in competitive sports teams in a nonoutbreak scenario. Kazakova et al investigated a CA-MRSA outbreak in a professional football team, detected methicillin-resistant Staphylococcus aureus (MRSA) nasal colonization was highest in the fall season (23.1%) and lowest in the preseason (11.5%), though differences between seasons were not statistically significant (23.1% vs 11.5%; P = .36).
of effective infection control, improved hygiene measures, and the use of antimicrobial agents prior to sampling could have confounded their assessment. More recently, Romano et al. reported 3 years’ experience with CA-MRSA skin and soft tissue infections in a collegiate football team. During a year in which skin and soft tissue infections were occurring frequently, nasal cultures were performed 1 month into the regular football season. Methicillin-resistant *S. aureus* nasal carriage was detected in 6.6% of the team, a higher rate than was observed at the beginning of the next year’s season (2.9%), suggesting an effect from either timing in the season or the nature of the outbreak itself. Molecular characterization of the isolates was not performed in their study; thus, it is unclear whether nasal and skin and soft tissue infection strains were similar.

We propose that the disparity between high colonization rates and the low incidence of CA-MRSA skin and soft tissue infection in our cohort is due primarily to the relatively low frequency of PVL+ *S. aureus* strains of USA300 CA-MRSA among carriage isolates. While the precise role of PVL in the pathogenesis of CA-MRSA infections remains unclear, strains that possess PVL are strongly associated with a variety of clinical phenotypes, such as osteomyelitis, necrotizing pneumonia, and furunculosis. Previous reports suggest that PVL is nearly ubiquitous in *S. aureus* strains of MRSA; however, more recent studies suggest that a much larger pool of *S. aureus* type IV, PVL+ strains exist, particularly in asymptomatic carriage. Wang et al. studying colonization in patients with end-stage renal disease, found that only 38.5% of *S. aureus* type IV strains were positive for PVL, a finding consistent with previous studies. Regardless of the role of PVL in pathogenesis, strains that possess this cytolytic toxin may possess other characteristics, such as the agrinine catalytic element or phenol-soluble modulins that enable them to more effectively cause disease than PVL- strains.

A potential caveat to our study is that the monthly frequency of nasal swabs does not allow us to determine which subjects are persistent carriers, intermittent carriers, or noncarriers; a characterization typically made by weekly cultures over a 12-week period. Persistent staphylococcal carriers might be a source for continued transmission of *S. aureus* in groups such as athletic teams; therefore, future studies should consider whether more frequent cultures (eg, weekly) provide a more precise assessment of colonization dynamics. In addition, we only assessed colonization in the anterior nares rather than sampling the axilla, perineum, or oropharynx; as a result, we may have underestimated the true frequency of colonization in our cohort. For example, Nilsson and Ripa described preferential throat carriage of MRSA in a cohort of health care workers and hospitalized patients. Of 125 patients screened for both throat and nasal carriage in their study, 80 of 125 (64%) were identified by nasal cultures alone; however, an additional 24 subjects were identified (104 of 125 [83%]) with throat cultures alone. Whether the dynamics of oropharyngeal colonization over time are similar to that in the anterior nares remains unknown.

In summary, in this cohort of collegiate athletes, there were surprisingly high MRSA nasal colonization rates that reached their peak during highest athletic activity within the season. However, these high rates of MRSA nasal colonization alone were insufficient to trigger an outbreak of skin and soft tissue infections in this cohort. The potential exists for other unknown factors, including the molecular characteristics of carriage strains, to govern the development of a disease outbreak. Additional longitudinal studies of staphylococcal colonization and disease are critically needed to determine the most effective means of primary prevention of this potentially devastating pathogen.

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**REFERENCES**


Announcement

Submissions. The Editors welcome contributions to Picture of the Month. Submissions should describe common problems presenting uncommonly, rather than total zebras. Cases should be of interest to practicing pediatricians, highlighting problems that they are likely to at least occasionally encounter in the office or hospital setting. High-quality clinical images (in either 35-mm slide or electronic format) along with parent or patient permission to use these images must accompany the submission. The entire discussion should comprise no more than 750 words. Articles and photographs accepted for publication will bear the contributor’s name. There is no charge for reproduction and printing of color illustrations. For details regarding electronic submission, please see: http://archpedi.ama-assn.org.