Nasal carriage of inducible dormant and community-associated methicillin-resistant Staphylococcus aureus in an ambulatory population of predominantly university students


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ABSTRACT

Background: We studied risk factors for nasal colonization with inducible dormant methicillin-resistant Staphylococcus aureus (ID-MRSA) and community-associated MRSA (CA-MRSA) in a cohort of predominantly university students.

Methods: Nasal surveillance cultures were performed in student health and ambulatory clinics. Molecular features were identified and risk factors for CA-MRSA and ID-MRSA colonization were determined by logistic regression.

Results: Of the 1000 participants, 89% (n = 890) were university students. Sixty-four percent were female, 59% Caucasian. The mean age was 23.5 years; 1.6% (n = 16) were CA-MRSA and 1.4% (n = 14) were ID-MRSA colonized. Fifteen (94%) of the CA-MRSA strains were PFGE type IV, pvl (Panton–Valentine leukocidin gene) positivity was 75% in CA-MRSA and 57% in ID-MRSA. ID-MRSA isolates were pulsed-field gel electrophoresis (PFGE) type I, 7%; type II, 14%; type V, 7%; and type IV, 71%. CA-MRSA SCCmec classification was 94% type IV and 6% type V. Risk factors for carriage of CA-MRSA were older age (OR 1.046, p = 0.040) and dog ownership (OR 1.450, p = 0.019). Single family home (OR 0.040, p = 0.007) was a protective factor. There were no significant variables of association found for ID-MRSA colonization.

Conclusions: ID-MRSA/CA-MRSA colonization was low. Most isolates were PFGE types IV and II, pvl-positive and susceptible to several antibiotics. Older age and dog ownership were risk factors for CA-MRSA. Future studies are needed to assess the impact of ID-MRSA carriage.

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1. Introduction

Staphylococcus aureus is a major human pathogen causing both nosocomial and community-associated infections. In the community, S. aureus is best known as a cause of furuncles and soft tissue infections.1 Up to 30% of healthy people carry S. aureus in their anterior nares or other body areas.2 Community-associated methicillin-resistant S. aureus (CA-MRSA) differs from hospital-acquired MRSA (HA-MRSA), as the former is more likely susceptible to tetracycline, clindamycin, and trimethoprim–sulfamethoxazole (TMP/SMX).3,4 Recently, Kampf et al. reported inducible dormant MRSA (ID-MRSA). These are mecA gene-positive S. aureus isolates that change from initial methicillin-sensitive S. aureus (MSSA) phenotype to CA-MRSA phenotype after β-lactam antibiotic exposure.5 They can be identified by staphylococcal cassette chromosome mec (SCCmec) type.

Risk factors for MRSA colonization and infection include disruptions of the integrity of the skin (insulin injections, allergy therapy, cosmetic body shaving, intravenous (IV) drug use, eczema, and burns), underlying diseases (respiratory infections, HIV infection), prolonged hospitalization, and exposure to other infected or colonized individuals.6

A recent publication suggests that 7% of adults are MRSA nasal carriers.7 Limited data exist on the epidemiology of ID-MRSA, including risk factors for acquisition and cross-transmission within families and households. We studied the epidemiology of ID-MRSA and CA-MRSA in a cohort of ambulatory patients consisting predominantly of university students. We attempted to identify risk factors and prevalence of ID-MRSA and CA-MRSA nasal colonization and secondary transmission within households. We characterized the genotypic and phenotypic features, including antibiotic susceptibility, pvl (Panton–Valentine leukocidin gene) positivity, and the presence of SCCmec gene.

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2. Methods

2.1. Setting

The study was conducted over a 27-month period, in the University’s ambulatory student health, general internal medicine, HIV, and dermatology clinics. The study was approved by the institution’s investigational review board.

2.2. Design

Prospective surveillance was performed to identify the prevalence of MRSA nasal carriage and risk factors for colonization. Molecular features of these strains were compared with the major strains in the USA.

2.3. Recruitment and enrollment

Participants were recruited during scheduled appointments. Participants were excluded if they met any of the following criteria: hospitalization, admission to a nursing home, skilled nursing facility, or hospice; dialysis and/or surgery within the last year. Participants were also excluded if they had permanent indwelling catheters or invasive medical devices, an inability to undergo nasal specimen collection, and/or were pregnant or breastfeeding.

After obtaining informed consent, descriptive data were collected using a standardized data collection form. These included demographic information, medical history, previous antimicrobial therapies, and potential risk factors for MRSA colonization and transmission.

2.4. Nasal swabs

Nasal samples were collected from both nostrils by use of a collection swab (Figure 1 shows the study protocol). The tip of the swab was inserted approximately 1 inch into the anterior vestibule of the nose and rolled five times in each nostril. Collected specimens were transported to the research laboratory.

2.5. Microbiology

Each swab was inoculated into enrichment broths to increase the isolation rate of S. aureus. After incubation, the broths were streaked onto a mannitol salt agar (MSA) plate, were further incubated aerobically for 48 h at 35 °C, and subsequently examined for growth. Identification of S. aureus was based on the basis of Gram stain, production of catalase, and results of Staphaurex latex agglutination test (Remel, Lenexa, KS, USA). Susceptibility testing was performed by disk diffusion susceptibility tests following the method recommended by the Clinical and Laboratory Standards Institute (CLSI). Oxacillin resistance was confirmed by using BBL CHROMagar MRSA (CHROM-MRSA; BD Diagnostics, Sparks, MD, USA). S. aureus antibiotic susceptibility was determined by conventional methods as recommended by the CLSI (2008). Control strains for all assays included MRSA ATCC 43300 and MSSA ATCC 25923. The presence of inducible clindamycin resistance was tested in CA-MRSA and ID-MRSA isolates that were clindamycin-sensitive and erythromycin-resistant by the agar disk diffusion test (D-test) method in accordance with the recommendations of the CLSI.

Chromosomal DNA was prepared using a Qiagen (Valencia, CA, USA) genomic DNA preparation kit according to the manufacturer’s instructions. SCCmec types were determined by the use of specific primers for amplification of the key genetic elements as described by Oliveira and de Lencastre. PCR was performed with a Taq PCR MasterMix kit (Qiagen) with a 50-µl reaction volume in a MiniCycler thermocycler (MJ Research, Boston, MA, USA). As SCCmec standards, we used S. aureus strains COL (SCCmec type I), N315 (SCCmec type II), ANS46 (SCCmec type III), and USA300 and MW2 (SCCmec type IV). pvl genes: (lukSF−/PV and lukFE−/PV) were detected by PCR as previously described. Pulsed-field gel electrophoresis (PFGE) was performed to identify the clonal distribution of MRSA. The preparation of genomic DNA and genomic Smal digests were adapted from the method described by Bannerman et al. The resulting band patterns for MRSA isolates were visually compared and interpreted using standard guidelines.

For methicillin-susceptible mecA-positive S. aureus isolates, induction of phenotypic resistance was performed by using Luria-Bertani broth (LB, USB Corp., Cleveland, OH, USA) either with or without sub-inhibitory 0.5 µg/ml oxacillin (Sigma Scientific, St. Louis, MO, USA) and grown at 37 °C with shaking (180 rpm). S. aureus 130113 was used as a positive control. Selection of inducible MRSA cells was verified by efficiency of plating (EOP) assays as described by Chambers, except that methicillin was substituted for oxacillin.

2.6. Follow-up visit

To differentiate between transient and persistent MRSA colonization, participants initially colonized with MRSA were reevaluated by nasal culture at 3-month follow-up. Household contacts of MRSA-colonized participants were processed similarly. If the first nasal culture from domiciliary contacts was negative, the nasal culture was repeated in 3 months. CA-MRSA colonized individuals were decolonized with Bactroban Nasal (2% mupirocin calcium ointment) at the conclusion of the study protocol. The dosage was 1/2 of the ointment from a single-use tube squeezed into each nostril two times a day (morning and evening) for 5 days. Patients colonized with non-CA-MRSA were also decolonized with intranasal mupirocin. Individuals with non-CA-MRSA were not further evaluated. Only household contacts of patients with CA-MRSA and ID-MRSA were evaluated for colonization and transmission.

2.7. Data analysis

Statistical analyses were performed using SPSS v15.0 (SPSS Inc., Chicago, IL, USA). Comparisons of categorical variables and percentages between groups were analyzed using the Pearson Chi-square test or Fisher’s exact test, as appropriate. Identification of potential risk factors for MRSA colonization was determined by univariate analysis. Variables significantly associated with MRSA colonization in univariate analysis (p < 0.05) were applied to a multivariate conditional logistic regression model. Odds ratios (OR) and 95% confidence intervals (CI) were calculated; the threshold for a significant difference was designated as a p-value of <0.05. All tests were two-tailed.

3. Results

One thousand subjects were enrolled; the majority of the subjects were university students (89% (n = 890)). Sixty-nine percent (n = 691) of study participants were born in the USA. The mean body mass index was 24.81 kg/m^2 (SD 7.39). Seventy-six percent (n = 760) reported sexual activity within the past year. The remaining characteristics of the study population can be found in Table 1.

3.1. Questionnaire responses

Table 2 summarizes risk factors for ID-MRSA and CA-MRSA colonization. These included piercing within the last year (17.4%; n = 174), a new tattoo within the last year (34.4%; n = 344), facial shaving (38%; n = 380), shaving legs (61.4%; n = 614), shaving arm-
pits (63.9%; n = 639), and genital shaving (63.9%; n = 639). Participation in contact sports was 25% (n = 250). Soap types included bar soap (69.3%; n = 693), liquid soap (23%; n = 230), foam (84.4%; n = 844), and hand gel (81.4%; n = 814). Shared household items included utensils (71%; n = 710), cups (70%; n = 700), razors (11.3%; n = 113), bath towels (32.4%; n = 324), toothbrush (5.8%; n = 58), washcloth (19.5%; n = 195), bars of soap (42.4%; n = 424), liquid soap (66.8%; n = 668), bathroom (83.2%; n = 832), handkerchief (1.7%; n = 17), clothes (25.5%; n = 255), undergarments (2.1%; n = 21), shoes (17.2%; n = 172), socks (17.3%; n = 173), bedroom (41.4%; n = 414), and bed (36.4%; n = 364).

3.2. Microbiology specimens

One thousand and fifty-two specimens were collected, of which 95% (n = 997) were first specimens and 5% (n = 55) were second specimens. Of the first specimens, 47% (n = 465) were methicillin-susceptible (Figure 2). Ninety percent (n = 430) of S. aureus specimens were mecA gene-negative (MSSA), while 10% (n = 35) were mecA gene-positive (Figure 3). Of the mecA gene-positive, 46% (n = 16) were CA-MRSA, 40% (n = 14) were ID-MRSA, and 4% (n = 5) were HA-MRSA. Overall CA-MRSA colonization was 1.6% (n = 16); 69% (n = 11) were students. Overall ID-MRSA colonization was 1.4% (n = 14); 79% (n = 11) were students. MSSA colonization was 43% (n = 430).

We screened 28 households, sampling 49 individual subjects; 14 households completed follow-up, five had partial follow-ups (one or more returned for second specimen), and nine households were lost to follow-up. Twenty-two household participants completed both first and second screenings, while 26 household participants completed only one screening episode. There was one CA-MRSA conversion from negative to positive and one from

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**Figure 1.** Community-associated MRSA study algorithm.

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positive to negative. There were six ID-MRSA conversions from positive to negative. We identified two households with secondary transmission of CA-MRSA. There were no household transmissions of ID-MRSA.

3.3. Antimicrobial susceptibility

Details of the antimicrobial susceptibilities for CA-MRSA and ID-MRSA are provided in Table 3. The prevalence of inducible clindamycin resistance among the CA-MRSA isolates was of 37.5% (6/16); it was 21.4% (3/14) among the ID-MRSA isolates.

3.4. Molecular typing

Seventy-five percent (n = 12) of CA-MRSA strains were pvl gene-positive (Table 4). The PFGE typing results for CA-MRSA were: 94% (n = 15) type IV and 6% (n = 1) type V. ID-MRSA isolates also underwent PFGE typing (Table 4).

3.5. Risk factors for colonization

Association variables of significance in the univariate model for CA-MRSA colonization were older age (p = 0.013), living in a single family home (p = 0.002), total household income of $60 000–75 000 annually (p = 0.011), and dog ownership (p = 0.035) (Table 5). In multivariate analysis, the following were statistically significant CA-MRSA risk factors: older age (OR 1.046, p = 0.040) and dog ownership (OR 1.450, p = 0.019); living in a single family home (OR 0.040, p = 0.007) was found to be a significant protective factor. Univariate analysis of ID-MRSA colonization revealed no significant unique association factors for colonization.

Figure 2. Initial screening results.
4. Discussion

Our prospective cohort is the largest study to evaluate ID/CA-MRSA colonization among non-athlete students. The prevalence of CA-MRSA nasal colonization was 1.6% (n = 16), lower than the 3% reported by Morita et al., yet consistent with previous reports of CA-MRSA prevalence in non-student populations. Of the 16 CA-MRSA nasal-colonized participants, 69% (n = 11) were recruited from the University’s student health clinics. Of the nasal-colonized students, 81% (n = 13) lived off campus.

Multivariate analysis of CA-MRSA colonized participants identified older age and dog ownership as significant risk factors for CA-MRSA carriage. Sixty-three percent (n = 629) of participants owned a pet, usually a dog (40.3%). Recent reports identified dog ownership as a risk factor for CA-MRSA. A previous report of CA-MRSA student colonization failed to identify significant associations for gender, receipt of antibiotics, and pet ownership. Our multivariate analysis identified living in a single family home as a statistically significant protective factor for CA-MRSA nasal colonization. This may be a surrogate marker of hygiene and a non-crowded living environment.

There are numerous case reports of CA-MRSA outbreaks in athletic teams. In these, the rates of nasal colonization vary from 2.9% to 31%. Our non-athlete student cohort did not reveal the high degree of nasal colonization observed in previous studies of student athletes.

Despite the low prevalence of CA-MRSA in our study population, pvl gene positivity was high (75%), as previously reported in the literature. The antibiotic susceptibility profile of the CA-MRSA isolates revealed susceptibility to clindamycin (69%), sulfamethoxazole (81%), tetracycline (81%) and vancomycin (100%), as reported earlier. Our findings suggest that the CA-MRSA strains were identical or clonally related to USA300 by PFGE and SCCmec typing.

A unique observation was the detection of ID-MRSA. Of the 1000 participants, the prevalence of ID-MRSA nasal colonization was 1.4% (n = 14) and similar to that of CA-MRSA. The susceptibility and pvl profile of the ID-MRSA isolates was also similar to that of the CA-MRSA isolates. These isolates were similar to those previously reported. Unlike CA-MRSA, ID-MRSA had a different genotypic distribution.

Univariate analysis did not identify unique association factors for ID-MRSA colonization. There are no prior epidemiological reports of ID-MRSA in the non-healthcare setting. Kampf et al. determined the carriage rate of methicillin-susceptible mecA-positive S. aureus (dormant MRSA) among healthcare workers (HCWs). From a cohort of 447 HCWs, seven isolates of dormant MRSA were isolated in six nurses and one doctor (1.6%). After four
days of repetitive antibiotic exposure, six of seven dormant MRSA were highly resistant to oxacillin. Two of the dormant MRSA were clonally related by PFGE. The study did not identify risk factors for ID-MRSA colonization.

In healthcare facilities, transmission of ID-MRSA from a HCW to a patient has been postulated. In one study, seven HCWs were ID-MRSA-colonized. The PFGE pattern of one ID-MRSA isolate was identical to that of a patient cared for by the ID-MRSA colonized HCW, thus raising the possibility of cross-transmission. In an earlier report, a HCW was colonized with MRSA after treatment with cephalaxin. Exposure to the antibiotic was the purported inducer of phenotypic resistance.

The potential impact of ID-MRSA colonization on CA-MRSA colonization and subsequent development of skin and soft tissue infections or invasive disease is not known. As in the hospital setting, ID-MRSA colonization may serve as a reservoir, thereby promoting cross-transmission within a household, dormitory, athletic team, or social unit. Colonized individuals may theoretically cross-transmit ID-MRSA isolates to close contacts. Additionally, ID-MRSA colonization or transmission may result in MRSA phenotypic conversion if the appropriate selective antibiotic pressure is applied. Furthermore, individuals persistently colonized with ID-MRSA may play an important role in households with high rates of CA-MRSA infections. These individuals will likely screen negative for CA-MRSA and thus may not undergo decolonization for CA-MRSA carriage. This would represent missed epidemiologic opportunities for decolonization and may explain the recurrence of CA-MRSA skin and soft tissue infections within a household.

We identified two households with secondary CA-MRSA transmission. The low number of secondary transmissions is likely due to the transient nature of the student population. Many of the CA-MRSA-colonized participants were lost to follow-up due to changes in address or matriculation status. No household transmissions of ID-MRSA were identified. Further, large-scale prospective studies are needed to better define household transmission of both ID-MRSA and CA-MRSA.

Our study has several strengths, including rigorous exclusion criteria and a prospective design. We utilized standardized questionnaires and data collection forms and a laboratory methodology that has been reported elsewhere. Additionally, we utilized logistic regression analysis to determine risk factors for MRSA colonization.

Our study has several limitations. All cultures were obtained at a single university setting. As such, the results may not be generalizable. All participants were volunteers and were not randomly selected. This may have introduced a selection bias with the study participants not being representative of the greater university student body. All data elements of the study were collected by survey and subject to recall bias. Additionally, we screened for MRSA nasal colonization only, with a single culture performed at the time of each screening episode. The reported sensitivity of a single MRSA nasal screening by direct plating with MSA is 85%. Thus, the prevalence of CA-MRSA and ID-MRSA colonization reported in our study may be an underestimate. Lastly, we did not follow participants prospectively beyond 3 months to determine the incidence of infection with CA-MRSA or ID-MRSA.

Our study adds to the body of literature on both CA-MRSA and ID-MRSA. Further prospective research is needed to characterize both the prevalence of ID-MRSA in healthy student populations and the incidence of infection following ID-MRSA colonization. Other unique populations known to harbor CA-MRSA, such as dialysis patients and HIV patients, may additionally be predisposed to ID-MRSA carriage. Given that these populations interface between the community and the hospital setting with great frequency, they may pose as important vectors for ID-MRSA transmission or ID-MRSA/CA-MRSA co-colonization. Future prospective studies are needed to assess the duration and epidemiologic impact of ID-MRSA carriage on healthy individuals, chronically ill patients, and HCWs.

**Conflict of interest:** No conflict of interest to declare.

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