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High prevalence of colonization with *Staphylococcus aureus* clone USA300 at multiple body sites among sexually transmitted disease clinic patients: an unrecognized reservoir

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Abstract

Extranasal colonization is increasingly recognized as an important reservoir for *Staphylococcus aureus* among high-risk populations. We conducted a cross-sectional study of multiple body site colonization among 173 randomly selected STD clinic patients in Baltimore, Maryland. Staphylococcal carriage at extranasal sites, including the oropharynx, groin, rectum, and genitals, was common among study subjects. The USA300 clone was particularly associated with multiple sites of colonization compared with non-USA300 strains ($p = .01$). Given their high burden of multi-site colonization and confluence of established staphylococcal risk factors, STD clinic patients may represent a community-based reservoir for *S. aureus* and be well suited for innovative infection control initiatives.

Keywords

S. aureus; Community-associated MRSA; Molecular epidemiology

1. Introduction

There is limited understanding of how novel strains of *Staphylococcus aureus* spread and become established within communities. Colonization of body sites other than the nares may serve as an important reservoir for community-associated (CA) *S. aureus*, especially for the epidemic USA300 strain [1, 2]. Several reports have noted increased rates of community-associated (CA)-MRSA infection among persons infected with HIV, sexually-active men who have sex with men (MSM), and individuals with high risk sexual behaviors (as

measured by infrequent or incorrect condom use) [1–6]. Although *S. aureus* is not traditionally defined as a sexually-transmitted pathogen, these populations may be united through their increased prevalence of *S. aureus* carriage at multiple body sites, including the genitals, and may consequently be at elevated risk of infection. As patients presenting to sexually transmitted disease (STD) clinics often possess a confluence of established CA-MRSA risk factors (e.g., illicit drug use, prior incarceration) [7], we hypothesize that they may represent a well-defined but unrecognized reservoir for the dissemination of emerging community-associated *S. aureus* strains.

2. Materials and methods

We conducted a cross-sectional study of *S. aureus* multiple body site colonization at the Baltimore City Health Department Druid STD clinic. The clinic provides care for a primarily African American, inner-city population with a high burden of STDs, HIV, and illicit drug use. Individuals >18 years old were eligible to participate and study recruitment occurred at randomly selected clinic sessions between October 2010 and April 2011. All participants completed informed consent. The study protocol was approved by the Institutional Review Boards of Johns Hopkins University and the Baltimore City Health Department.

Trained interviewers administered a questionnaire on patient demographics, medical and sexual history, personal hygiene, and other known and suspected risk factors associated with *S. aureus* colonization and infection (e.g., illicit drug use, animal contact, etc.) [7]. Participants were also asked about recent skin infection using a standardized collection of staphylococcal infection photographs employed in our previous research [8]. During routine physical examination, samples of the nares, oropharynx, inguinal skin folds, rectum, and vagina were collected using pre-moistened culturette rayon-tipped swabs (Becton Dickinson, Franklin Lakes, NJ). Refrigerated specimens were incubated in 6% sodium chloride-supplemented tryptic soy broth (Becton Dickinson) at 37 °C overnight to enrich for *S. aureus* growth [9]. Samples were plated onto mannitol salt agar (Becton Dickinson) and incubated at 35 °C for 48 h. Individual positive colonies were subsequently streaked onto sheep blood agar and incubated for an additional 24 h. Isolates were confirmed as *S. aureus* using the Murex StaphAurex rapid latex agglutination test (Remel, Lenexa, KS) [9]. All positive samples underwent *spa* sequencing for genotyping, as previously described [9]. Methicillin resistance was determined by PCR amplification of the staphylococcal chromosomal cassette *mec* (*SCCmec*) element [9].

Pearson's chi-squared or Fisher's exact tests were used to assess risk relationships for categorical data and the Wilcoxon two sample test was used for continuous data. The kappa test of concordance was utilized to assess the independence of culture results obtained at different body sites. Statistical analysis was performed with SAS version 9.3 (SAS Institute, Cary, NC).

3. Results

173 individuals were enrolled in the study with a mean age of 30.6 years. There were 94 (54%) men; 162 (94%) were African American. The large majority ($n = 161$, 93%) identified themselves as heterosexual. The majority ($n = 120$, 69%) also reported a history of STDs at any time in the past. 46 (27%) subjects reported antibiotic use over the past three months when questioned by the study interviewer. Nearly all patients (99%) were HIV-negative by serology. Of all participants interviewed, 25 (14%) reported a suspected *S. aureus* infection over the past three months.

Culture results identified a high prevalence of staphylococcal carriage; 77 (45%) study participants were colonized with MSSA and 12 (7%) were colonized with MRSA at one or more sites (Table 1). The most common site of colonization was the oropharynx, although the nares, inguinal skin folds, rectum, and vagina were also frequently colonized (Table 1). Overall, 41 (24%) patients were colonized at a pelvic site (inguinal skin folds, rectum, vagina); 16 (9%) were colonized only at a pelvic site. Since the kappa test showed no correlation between culture results at different body sites, each site was analyzed independently. The colonization prevalence at the anterior nares differed significantly by gender with men almost four times more likely to be nasally colonized than women (Table 1, $p < .001$). The etiology of this finding is unclear and is worthy of follow-up evaluation. No other gender differences were noted in the location of sites colonized, the number of sites colonized, and clonal types represented. Sexual practices, number of partners, condom use, history of STIs, current STIs, and antibiotic use were not associated with *S. aureus* colonization. Body shaving was significantly associated with carriage at a pelvic site (RR 2.9 for those who shave their chest, $p = .02$).

Among the 89 colonized individuals, 50 *spa* types were represented. *Spa* t008 (PFGE type USA300) predominated, colonizing 14 (16%) of the 89 positive subjects. Of those colonized with USA300, 64% (9/14) had MRSA compared with 4% (3/75) of those colonized with non-USA300 strains. The second most commonly encountered strain was *spa* t002 (PFGE type USA100), colonizing 7/89 (8%) study subjects. The pattern of colonization of USA300 differed from non-USA300 strains. Although USA300 was more commonly isolated from the anterior nares than non-USA300 strains (7/14 subjects, 50% v. 27/75 subjects, 36%, respectively), this was not statistically significant ($p = .38$). USA300 colonized pelvic sites more frequently than non-USA300 strains. 64.3% (9/14) of the USA300-positive subjects were colonized rectally compared with 17.3% (13/75) of the subjects colonized with non-USA300 strains ($p < .001$). Similarly, 57.1% (4/7) of women carrying USA300 were colonized vaginally compared with 21.9% (7/32) of women carrying non-USA300 strains ($p = .16$). Among the 14 USA300-colonized individuals, 12 (85.7%) were positive at one or more pelvic site compared with 29 (38.7%) of the 75 colonized with non-USA300 strains ($p = .001$).

Conversely, non-USA300 strains appeared more efficient at colonizing the pharyngeal site. 68% (51/75) of study subjects colonized with a non-USA300 strain were positive at the pharyngeal site compared with 35.7% (5/14) of study subjects colonized with USA300 ($p = .033$). The median number of body sites colonized by USA300 compared with non-USA300 isolates was 2 versus 1, respectively ($p = .01$). Further, 71.4% (10/14) of USA300 colonized individuals were positive from more than one body site compared with 36% (27/75) from non-USA300 colonized individuals ($p = .03$). While infections were more frequently reported among those positive for USA300 compared with non-USA300 strains (4/14, 28.6% v. 9/75, 12%, respectively), this did not reach statistical significance ($p = .22$).

4. Discussion

The 7% prevalence of MRSA carriage in our study participants was notably higher than the 1.5% reported in the general population [10]. Our assessment of extranasal sites contributed to a portion of this elevation, underscoring the importance of multiple body site sampling in establishing colonization status. Despite this, our elevated prevalence suggests that persons attending an STD clinic may serve as an important reservoir for MRSA colonization and transmission in the non-outbreak community setting. Previous reports of staphylococcal carriage among other high-risk groups such as sexually-active MSM and HIV-infected individuals have shown MRSA prevalence ranging from 0 to 17% [2]. Other ambulatory populations at elevated risk, such as individuals presenting to emergency departments, have

also been found to have increased prevalence of MRSA carriage [11]. While the variability in reported prevalence of MRSA colonization may relate to differences in body site culturing, it may also reflect diverse intensities of staphylococcal exposure on the community level [2]. Individuals at elevated risk of MRSA carriage and infection, such as those attending STD clinics, may experience a greater magnitude of staphylococcal exposure in their social networks and environment, thereby perpetuating a high burden of staphylococcal colonization and infection. Our data suggest that the STD clinic population may represent such a reservoir of increased transmission and consequently be an ideal target for infection control initiatives.

The most commonly isolated clone among our study participants was USA300, colonizing 16% of those carrying *S. aureus*. Consistent with earlier studies, the strain appeared more efficient than non-USA300 strains in colonizing the pelvic sites [2]. In addition, USA300 colonized multiple sites more frequently than other strains. The relationship between carriage at multiple sites and transmission of colonization and infection remains unclear. While the cross-sectional nature of our study cannot address this question, the contribution of multi-site carriage to USA300's success in transmission is a plausible hypothesis to test in a longitudinal fashion. Future prospective studies involving patient dyads or networks would be best suited to address these issues. If the clone's increased burden of colonization was linked with either transmission or clinical infection, novel strain-specific interventions could be considered in high-risk patient populations.

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Table 1 Prevalence of *S. aureus* colonization by body site.

A. All participants (<i>n</i> = 173)			
Culture site	Men (<i>n</i> = 94)	Women (<i>n</i> = 79)	Total (<i>n</i> = 173)
Nares	28 (29.8)	6 (7.6)	34 (19.7)
Pelvic areas	23 (24.5)	18 (22.8)	41 (23.7)
Vaginal	–	11 (13.9)	11 (–)
Rectal	14 (14.9)	8 (10.1)	22 (12.7)
Inguinal	14 (14.9)	8 (10.1)	22 (12.7)
Oropharyngeal	29 (30.9)	27 (34.2)	56 (32.4)
Total	50 (53.2)	39 (49.4)	89 (51.4)
MRSA+	7 (7.4)	5 (6.3)	12 (6.9)
USA300+	7 (7.4)	7 (8.9)	14 (8.1)
B. <i>S. aureus</i> -colonized participants (<i>n</i> = 89)			
Culture Site	Men (<i>n</i> = 50)	Women (<i>n</i> = 39)	Total (<i>n</i> = 89)
Nares	28 (56)	6 (15.4)	34 (38.2)
Pelvic areas	23 (46)	18 (46.2)	41 (46.1)
Vaginal	–	11 (28.2)	11 (–)
Rectal	14 (28)	8 (20.5)	22 (24.7)
Inguinal	14 (28)	8 (20.5)	22 (24.7)
Oropharyngeal	29 (58)	27 (69.2)	56 (62.9)
C. USA300-colonized participants (<i>n</i> = 14)			
Culture Site	Men (<i>n</i> = 7)	Women (<i>n</i> = 7)	Total (<i>n</i> = 14)
Nares	5 (71.4)	2 (28.6)	7 (50)
Pelvic areas	6 (85.7)	6 (85.7)	12 (85.7)
Vaginal	–	4 (57.1)	4 (–)
Rectal	5 (71.4)	4 (57.1)	9 (64.3)
Inguinal	3 (42.9)	2 (28.6)	5 (35.7)
Oropharyngeal	2 (28.6)	3 (42.9)	5 (35.7)
D. Non-USA300-colonized participants (<i>n</i> = 75)			
Culture Site	Men (<i>n</i> = 43)	Women (<i>n</i> = 32)	Total (<i>n</i> = 75)
Nares	23 (53.5)	4 (12.5)	27 (36)
Pelvic areas	17 (39.5)	12 (37.5)	29 (38.7)
Vaginal	–	7 (21.9)	7 (–)
Rectal	9 (20.9)	4 (12.5)	13 (17.3)
Inguinal	11 (25.6)	6 (18.8)	17 (22.7)
Oropharyngeal	27 (62.8)	24 (75)	51 (68)

Note: Data are no. (%) of study subjects; MRSA, methicillin resistant *S. aureus*.