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Major Article

Sex differences in the risk factors for *Staphylococcus aureus* throat carriage

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Key Words:
Body mass index
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Background: Male gender and adiposity are considered to be risk factors for *Staphylococcus aureus* carriage. We tested whether colonization is related to free testosterone (fT) level and adiposity, measured with body mass index (BMI) and body fat percentage (BFP), in healthy adults.

Methods: Blood sample and throat swabs were taken twice (at 4-week intervals) from healthy men and women aged 18-36 years. fT level, height, weight, and BFP were measured. Participants were classified as persistent carriers, intermittent carriers (excluded from the analyses), and noncarriers. The final sample was 152 participants: 85 men and 67 women.

Results: BFP, but not BMI, correlated positively with *S aureus* colonization ($P = .02$) in men. BMI became a significant predictor of carriage only when comparing groups within and above norms ($P = .04$). There was no relationship for BMI nor BFP in women. Higher fT level was related to persistent carriage ($P = .02$) in women, there was no relationship for fT level in men.

Conclusion: Risk factors for *S aureus* carriage are sex dependent. Within-sex variation in colonization is related to fT level in women, whereas in men it is related to the amount of body fat.

INTRODUCTION

Staphylococcus aureus is both a commensal bacteria and a frequent infectious agent in the community. Due to a broad spectrum of localized and systemic infections that *S aureus* can cause, as well as its increasing resistance to various antimicrobial agents, carriage of this bacteria is a major public health problem. The various *S aureus* virulence factors determine the capacity to easily cause skin, soft tissue, bone, central nervous system, and lung infections.[1] *S aureus* is also the second (after coagulase-negative staphylococci) most common pathogen causing nosocomial bloodstream infections that lead to increased hospital stays, morbidity, and mortality.[2]

The most frequent carry site for *S aureus* in humans are anterior nares, but multiple body sites; for example, the skin, pharynx, and gastrointestinal tract, can also be colonized.[2] Asymptomatic colonization with *S aureus* is common; approximately 20% of the population are persistent carriers and up to 70% are intermittent carriers.[2]

In general, carriage of pathogenic bacteria plays a key role in the epidemiology and pathogenesis of various community-acquired and hospital-acquired infections, due to a risk of autoinfection.[3] Also, *S aureus* carriage is associated with subsequent endogenous infections, due to the fact that the colonized site may be a primary source of the pathogen. It is well documented that *S aureus* infections occurred significantly more frequently among carriers compared with noncarriers—a higher frequency of nosocomial infections was reported in carriers compared with noncarriers in the case of surgical, hemodialysis, or peritoneal dialysis patients.[2,4,5] Data presented by Wertheim et al[6] showed that about 80% of invasive hospital infections had an endogenous source in nasal carriage. Eradication of carriage state effectively reduces incidence of staphylococcal infection in many cases.[4]

Although most *S aureus* screening programs require the obtainment of swab samples from the anterior nares, some data suggest that a swab specimen from the throat should also be considered to be standard. The results of some recent studies show that the throat is the most common carriage site for *S aureus*.[7,8] Some studies confirmed the previous observation that the throat may be selectively colonized and seems to be more sensitive to the detection of the persistence of carriage than the anterior nares.[7]

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Adipose tissue and propensity to S aureus *carriage*

The constant increase in multidrug-resistant strains is now a global problem of microbiology and infectious medicine. In addition to limited alternative methods of treatments, we need to test infection-prevention strategies in health care; for example, look for factors that may predispose to infection and effectively eliminate them, thereby reducing the risk of infection.

Adipose tissue is not only a simple form of energy storage, but also a structural and functional complex of the endocrine and immunologic glands. It produces lots of immunologic mediators that influence the homeostasis of the system as well as reproductive and immune functions.[9] Some of the cytokines secreted by fat tissue—adipokines (such as leptin)—as well as adipose-derived mediators (such as interleukin 6 and tumor necrosis factor α) or free fatty acid released during lipolysis may act as proinflammatory factors and lead to induce a subclinical inflammatory state and cytokine balance dysregulation, which impair immune functions.[10,11]

Due to the fact that obesity can be associated with infectious diseases, during the past few years some researchers have made an attempt to examine the relationship between obesity and the colonization of the upper respiratory tract by *S aureus,* and they showed a higher risk of nasopharyngeal colonization by *S aureus* in obese people. In most of these studies obesity was classified based on body mass index (BMI) value (rather than waist circumference),[12-15] whereas BMI may be a false indicator of obesity because it overestimates fatness in muscular individuals. Besides, a BMI-based definition of obesity misclassifies as nonobese individuals with normal weight but with an excess of body fat.[16] The latter phenomenon is called normal-weight obesity (NWO). Batsis et al[17] demonstrated that NWO (as well as normal obesity) may be a risk factor for cardiovascular mortality and cardiometabolic dysregulation. We hypothesize that individuals with NWO may be also characterized by a high risk of *S aureus* colonization, as well as individuals with BMI-based obesity. Therefore, to evaluate precisely the influence of obesity on the propensity to *S aureus* colonization, a more precise assessment of body fat is needed.

Thus, the first aim of our study was to examine the relationship between body fat and *S aureus* carriage using more precise measurements of obesity—bioimpedance assessing body-fat percentage (BFP). We hypothesize that both BMI and BFP above the norm will be associated with a higher risk of *S aureus* colonization, but BFP will be better associated with the proneness to colonization than BMI.

Testosterone level and propensity to colonization

Sex differences in the frequency of *S aureus* carriage were observed in many studies[18,19] and suggest that men seem to be more prone to *S aureus* colonization than women. Being a man is even mentioned as a risk factor for carriage, next to obesity and age.[20] Some researchers suggested that the risk of *S aureus* carriage in human men should be associated with sex-hormone levels.[12,13] Sex differences in immune system functioning is well documented in many studies and demonstrates a higher susceptibility to bacterial infections in men.[21,22] It is also evident that testosterone, the main sex hormone in men, has strong immunomodulatory properties.[23,24] The immunosuppressive role of testosterone is assigned a key factor in a reduced resistance to infection in men.[25]

To our knowledge, susceptibility to *S aureus* colonization has not yet been examined in the context of sex-hormone levels. Therefore the second aim of this study was to investigate the relationship between colonization and testosterone level.

METHODS

Participants were 116 healthy men aged 19.0-36.1 years (mean, 27.2 ± 4.6 years) and 91 healthy women aged 18.6-36.1 years (mean, 25.7 ± 4.4 years). The study consisted of 2 stages, with an interval of 4 weeks (a similar interval—1 month—was used by Botelho-Nevers et al[14]). Each stage was conducted between 7.30 a.m. and 8.30 a.m., on an empty stomach. During each visit, a blood sample and throat swab were taken. During first visit, anthropometric measurements (height, weight, and BFP) were also performed.

Throat swab samples were taken from the posterior wall of the pharynx. Specimens were collected by medical staff and transported on sterile cotton swabs with Portagerm Amies agar (BioMerieux, Warsaw, Poland) to a microbiology laboratory for further analysis. No more than 4 hours after collection, the swabs were streaked onto Columbia agar with 5% sheep's blood and Chapmann agar (BioMerieux) and incubated aerobically at 37°C for up to 24 hours for Columbia agar with 5% sheep's blood and 48 hours for Chapmann agar. Bacterial strain identification was confirmed by a colony-morphology and Staphyloslide latex test (Becton Dickinson Polska, Warsaw, Poland). Participants were classified as persistent carriers (2 positive culture samples among 39 men and 28 women), intermittent carriers (only 1 positive culture sample among 31 men and 21 women) and noncarriers (no *S aureus* detected in any swabs among 46 men and 39 women).[14]

Intermittent carriers were excluded from further analyses. Such carriers might be colonized temporarily, as a consequence of the recent immunosuppression caused by the patient's last cold.

It is then only the constant colonization state that is adequate to analyze and properly interpret the results on the relationship between body morphology or hormone levels and proneness to be colonized by *S aureus*. Besides, there is the evidence that only persistent carriage, and not intermittent carriage, is a risk factor for acquiring *S aureus* infection.[26]

After each blood donation, blood samples was centrifuged and the separated serum was frozen at −70°C until analyzed. Serum free testosterone (fT) levels were determined by enzyme-linked immunosorbent assays using commercial kits (Demeditec, Kiel, Germany). Serum samples were assayed in duplicate according to the manufacturer's instructions. Hormone concentrations were calculated in relation to the standard curve and expressed in picograms per milliliter. For each participant, testosterone level was averaged from the 2 study stages. Figure 1 shows men and women ordered by testosterone level (after exclusion of intermittent carriers). Three women had extreme outlying values of testosterone level (Grubbs test $P < .001$) (Fig 1) and were excluded. Thus, the final analyses were conducted for 152 participants: 85 men aged 19.0-36.1 years (mean, 27.8 ± 4.5 years) and 67 women aged 19.5-36.1 years (mean, 25.3 ± 4.3 years). Carriers and noncarriers did not differ in terms of age (male carriers: mean, 27.5 ± 4.1 years; male noncarriers: mean, 28.1 ± 4.8 years; $H = 0.52$; $P = .47$; female carriers: mean, 25.0 ± 4.4 years; female noncarriers: mean, 25.5 ± 4.3 years; $H = 0.20$; $P = .66$).

We recruited people declaring no chronic diseases (eg, diabetes, autoimmune disorders, or metabolic or hormone problems), not showing symptoms of infection on the day of swab collection or a few days before, without antibiotics use a few weeks before (the lowest time interval was 3 weeks), and in the case of women not taking hormonal contraceptives. General health status was also assessed using blood morphology and basic biochemical tests such as glucose, liver enzymes, creatinine, C-reactive protein levels, and lipid profiles (eg, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triglyceride). All participants included in this study had morphology and biochemical blood parameters in the normal range (ie, 3.8-10.0 $10^3/\mu L$ for leukocyte count, 70-99 mg/dL for glucose, 0-41 U/L for alanine aminotransferase in men,