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Nostrils of Healthy Volunteers Are Independent with Regard to *Staphylococcus aureus* Carriage

Beau J. Kildow,[a] Johan P. Conradie,[b] and Rachel L. Robson[b]

University of Nebraska College of Medicine, Omaha, Nebraska, USA,[a] and Department of Biology and Chemistry, Morningside College, Sioux City, Iowa, USA[b]

The right and left nares of healthy adults ($n = 251$) were swabbed separately to determine carriage of *Staphylococcus aureus* in each nostril. Carriers were significantly more likely to carry *S. aureus* in one nostril than in both. Of those carrying *S. aureus* in both nostrils, 20% carried genetically distinct strains in each. Nostrils belonging to a single individual should not be assumed to be homogenous with respect to carriage of *S. aureus*.

Staphylococcus aureus is the leading cause of nosocomial infections in the United States (8) and is increasingly associated with community-acquired infections as well (4, 15). Between 25% and 40% of healthy adults asymptomatically carry *S. aureus* (5, 11). A common site of *S. aureus* carriage in asymptomatic carriers is the anterior nares (9), and most screening protocols involve swabbing the nostrils. However, both hospital and surveillance protocols vary in their recommendations as to whether both nostrils should be sampled, and those that do recommend the sampling of both nostrils typically recommend the use of a single swab to do so. (See reference 19 for an example of a clinical protocol that swabs both nostrils using a single swab; see references 1, 2, and 6 for examples of surveillance studies doing so.) Implicit in such protocols is the idea that nostrils are homogenous with respect to the bacterial populations they carry and that it is therefore legitimate to treat two separate nostrils as if they were a single body site. This study tested that hypothesis by sampling right and left nostrils separately for *S. aureus* carriage.

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In this study, the right and left nostrils of healthy adult volunteers ($n = 251$) were swabbed separately with sterile polyester swabs (Fisher Scientific, Pittsburgh, PA), and swabs were immediately plated on mannitol salt agar (Becton, Dickinson Diagnostic Systems, Sparks, MD). Volunteers ranged in age from 18 years old to 70 years old, and 58% ($n = 146$) were women. Volunteers were recruited from the community via advertising in northwestern Iowa and northeastern Nebraska. Volunteers were not compensated for participation. Volunteers were excluded from this study if they reported ever having been diagnosed with a methicillin-resistant *S. aureus* (MRSA) infection, had been treated for any infection in the last 2 months, or were immunocompromised in any way. Sample collection began in November 2007 and continued until May 2010. Samples were defined as culture positive for *S. aureus* if the bacteria were mannitol-fermenting, salt-tolerant, catalase- and coagulase-positive, DNase-positive, Gram-positive staphylococci. Methicillin resistance was determined using the Kirby-Bauer technique utilizing oxacillin disks (Remel, Lenexa, KS) and Clinical and Laboratory Standards Institute (9th edition) zone of inhibition breakpoints (3). At least two isolates per study participant were tested for methicillin resistance. *S. aureus* cultures were preserved in 10% glycerol-90% tryptic soy broth at −70°C. This sample collection protocol and study was approved by the Human Subjects Committee of the Institutional Research Review Board at Morningside College.

In this population, 123 isolates were recovered from 88 (35%) healthy adult volunteers ($n = 251$) who carried *S. aureus* in their nostrils, broadly consistent with the findings of previous studies that have treated both nostrils as a single body site. However, 60% ($n = 53$) of these volunteers were culture positive for *S. aureus* in only one nostril. Proportions of *S. aureus* carriers carrying bacteria in both nostrils versus only one nostril, and proportions of single-nostril carriers carrying *S. aureus* in right versus left nostrils were compared using a test of population proportions, testing the hypotheses that the probability of carrying *S. aureus* in both nostrils was less than the probability of carrying *S. aureus* in one nostril and that the probability of carrying *S. aureus* in the left nostril was equal to the probability of carrying *S. aureus* in the right nostril. Nasal carriers of *S. aureus* were significantly more likely to carry in one nostril than in both (test of population proportions, $P = 0.0015$). For single-nostril carriers, neither the right nostril nor the left nostril was more likely to be an individual's site of *S. aureus* colonization (test of population proportions, $P = 0.39$). All MRSA carriers ($n = 8$; 3.2% of study population) carried MRSA in only one nostril, although three of these individuals carried a methicillin-susceptible *S. aureus* (MSSA) strain in their other nostril.

Agarose plugs containing *S. aureus* isolates were prepared using standard pulsed-field gel electrophoresis (PFGE) techniques (12). The plugs were digested with SmaI for 12 to 18 h at 24°C. PFGE was run for 21 h at 6.0 V/centimeter with an initial switch time of 5 min and a final switch time of 40 min on a contour-clamped homogenous electric field (CHEF) apparatus (Bio-Rad, Hercules, CA). PFGE plugs were prepared in this way for all 123 isolates in this study, and PFGE running conditions were identical for all isolates across all gels run. *S. aureus* NCTC 8325 was used as an internal control. Gels were stained with ethidium bromide and

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Address correspondence to Rachel L. Robson, robson@morningside.edu.
B.J.K. and J.P.C. contributed equally to this work.

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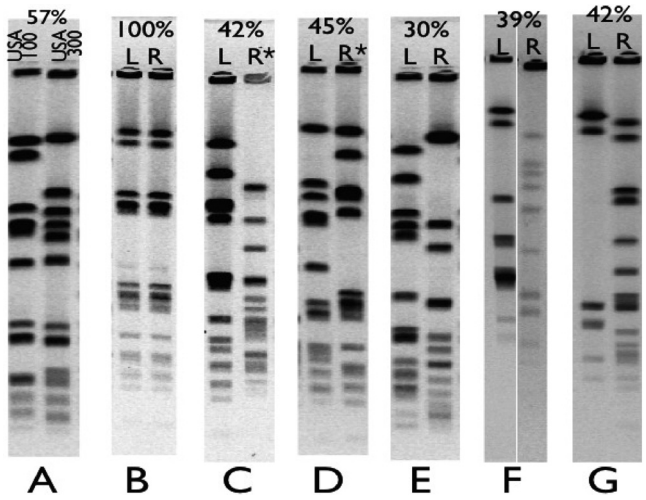

FIG 1 Comparison of PFGE patterns of *S. aureus* found in separate nostrils of the same individual. Five individuals (C through G) carried unrelated *S. aureus* strains in their right and left nostrils. For each pair of lanes, the percentage shows the relatedness per BioNumerics. L and R refer to whether the sample was found in the left (L) or right (R) nostril; an asterisk indicates that the isolate is methicillin resistant. (A) Reference strains USA100 and USA300 with 57% PFGE identity; (B) paired nostril isolates with 100% PFGE identity; (C) paired nostril isolates with 42% PFGE identity, where the left isolate is susceptible to methicillin and the right isolate is MRSA; (D) paired nostril isolates with 45% PFGE identity where the left isolate is susceptible to methicillin and the right isolate is MRSA; (E) paired nostril isolates with 30% PFGE identity where both isolates are MSSA; (F) paired nostril isolates with 39% PFGE identity where both isolates are MSSA; (G) paired nostril isolates with 42% PFGE identity where both isolates are MSSA.

visualized with a GelDoc system (Bio-Rad, Hercules, CA). PFGE banding patterns were analyzed using BioNumerics software.

Of 35 dual-nostril carriers, 28 had *S. aureus* strains with 100% identical PFGE patterns in each nostril, and two more dual-nostril carriers had at least 90% PFGE identity (i.e., isolates varying by only one band) between the isolates in each nostril. Five dual-nostril carriers harbored strains that had less than 50% PFGE identity between the isolates in each nostril. Thus, for these five dual-nostril carriers, isolates in a single individual's right and left nostrils were more distantly related to each other than hospital-associated MRSA strain USA100 is related to community-associated MRSA strain USA300, two distinctly different lineages (18) (Fig. 1 and Table 1).

The prevalence of the Panton-Valentine leukocidin (*pvl*) and methicillin resistance cassette A (*mecA*) genes in this population was determined using the PCR technique using previously described primers (see references 10 and 14, respectively). DNA was extracted from overnight cultures of *S. aureus* using the Puregene yeast/bacterial kit (Qiagen, Valencia, CA). All amplifications used a Phusion PCR kit (New England BioLabs, Ipswich, MA). Analysis of the short-sequence repeat region of the protein A gene (*spa* typing) of the isolates was conducted as previously described using Ridom *spa*-type software (16, 17). For *spa* typing, PCR products were purified using a QiaQuick kit (Qiagen, Valencia, CA) and sequenced by the University of Iowa Carver College of Medicine DNA Core Facility.

The prevalence of *pvl* in this population of isolates was 8%. The prevalence of *mecA* in this population of isolates was 6.5%. One dual-nostril carrier harbored a *pvl*-positive strain in one nostril and a *pvl*-negative strain in the other nostril, and three dual-nostril carriers harbored a *mecA*-positive strain in one nostril and a *mecA*-negative strain in the other nostril. All MRSA isolates carried the *mecA* gene (Table 1). Both isolates from a single individual with identical PFGE patterns also had matching *spa* types, with *spa* type 012 being the most common (15% of isolates).

On the basis of these data, nostrils belonging to a single individual should not be assumed to be homogenous with respect to carriage of *S. aureus*.

This study used culture positivity to define *S. aureus* carriage, rather than the PCR-based techniques that are becoming more common in surveillance studies. PCR detection of *S. aureus* is more sensitive than culture, though less specific (7). For this reason, this study may underestimate the rate of *S. aureus* nasal carriage, although the overall rates of *S. aureus* carriage reported in this study are in agreement with those reported by others (5, 11). It is unlikely that this potential to underestimate *S. aureus* carriage rates in general explains the disparity in proportions of single-nostril versus dual-nostril carriers shown here or the genetic differences observed among isolates from separate nostrils in the same individual.

This study sampled each volunteer's nares only once, rather than repeatedly over time. This study therefore cannot distinguish between intermittent and persistent carriers of *S. aureus* (13). Future studies could examine whether dual-nostril carriers are more likely to be persistent carriers than are single-nostril carriers and whether intermittent carriers are more likely to harbor genetically distinct *S. aureus* strains in each nostril, as well as to help confirm the results reported here.

Researchers conducting surveillance studies and clinicians

TABLE 1 Colonization of separate nostrils of healthy volunteers by distinct *S. aureus* strains[a]

S. aureus and colonization of nostrils	No. of volunteers (no. of isolates with characteristic) unless specified otherwise
Total *S. aureus*	
Colonized in both nostrils	
100% PFGE identity between strains	28 (56)
<95% PFGE identity between strains	7 (14)
Colonized in only one nostril	
Only the right nostril colonized	26 (26)
Only the left nostril colonized	27 (27)
Total no. of volunteers colonized (total no. of isolates)	88 (123)
MRSA (*mecA* positive)	
Colonized in both nostrils	
MRSA in both nostrils	0 (0)
MRSA in one nostril and MSSA in the other nostril	3 (3)
Colonized in only one nostril	
Only the right nostril colonized	1 (1)
Only the left nostril colonized	4 (4)
Total no. of volunteers colonized (total no. of MRSA isolates)	8 (8)
pvl-positive *S. aureus*	
Colonized in both nostrils	
pvl-positive strain in both nostrils	2 (4)
pvl-positive strain in one nostril and *pvl*-negative strain in the other nostril	1 (1)
Colonized in only one nostril	
Only the right nostril colonized	2 (2)
Only the left nostril colonized	3 (3)
Total no. of volunteers colonized (total no. of *pvl*-positive isolates)	8 (10)

[a] There were a total of 251 healthy volunteers in this study.

screening patients for *S. aureus* should be aware of the potential for a single individual's nostrils not to be homogenous in terms of bacterial carriage and should consider swabbing nostrils separately when testing for these bacteria.

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