



## Major article

## Reduction of nasal *Staphylococcus aureus* carriage in health care professionals by treatment with a nonantibiotic, alcohol-based nasal antiseptic



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## Key Words:

Ethanol  
Nasal colonization  
Bacterial burden  
Infection control

**Background:** Antibiotics used to reduce nasal colonization by *Staphylococcus aureus* in patients before admission are inappropriate for carriage reduction on a regular basis within a hospital community. Effective nonantibiotic alternatives for daily use in the nares will allow reduction of this bacterial source to be addressed.

**Methods:** Our study tested the effectiveness of a nonantibiotic, alcohol-based antiseptic in reducing nasal bacterial carriage in health care professionals (HCPs) at an urban hospital center. HCPs testing positive for vestibular *S aureus* colonization were treated 3 times during the day with topical antiseptic or control preparations. Nasal *S aureus* and total bacterial colonization levels were determined before and at the end of a 10-hour workday.

**Results:** Seventy-eight of 387 HCPs screened (20.2%) tested positive for *S aureus* infection. Of 39 subjects who tested positive for *S aureus* infection who completed the study, 20 received antiseptic and 19 received placebo treatment. Antiseptic treatment reduced *S aureus* colony forming units from baseline by 99% (median) and 82% (mean) ( $P < .001$ ). Total bacterial colony forming units were reduced by 91% (median) and 71% (mean) ( $P < .001$ ).

**Conclusions:** Nasal application of a nonantibiotic, alcohol-based antiseptic was effective in reducing *S aureus* and total bacterial carriage, suggesting the usefulness of this approach as a safe, effective, and convenient alternative to antibiotic treatment.

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Although estimates vary, studies indicate that between 20% and 40% of healthy individuals within the continental United States exhibit nasal vestibular carriage of *Staphylococcus aureus*.<sup>1</sup> All individuals within health care environments in whom subclinical nasal carriage of *S aureus* and other potentially pathogenic bacteria is present contribute to the burden of infection risk to themselves and others. Nasal colonization is known to be predominantly localized in the anterior, vestibular region of the nasal anatomy.<sup>2</sup>

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Global Life Technologies Corp, Bethesda, Maryland, funded this study and provided the antiseptic. The sponsor had no role in data collection, analysis, or manuscript preparation.

Conflicts of interest: None to report.

Data support the premise that individuals exhibiting subclinical nasal colonization by *S aureus* can be grouped into persistent, intermittent, and noncarrier types.<sup>3-5</sup> In a study using artificial inoculation, carriage characteristics were similar in intermittent carrier and noncarriers (comprising 76% of the total), but distinct from those of the persistent carrier group.<sup>6</sup> These findings are consistent with the concept that either most individuals are actually intermittent carriers or are noncarriers who exhibit carriage only under environmental pressure (eg, recurring exposure).<sup>6</sup> In either instance, the demonstrated ability of these 2 groups to sustain transient subclinical carriage for 4-14 days on average would put either of them in a position to increase risk for *S aureus* infection in themselves or others with whom they come in contact during that period.

Within the health care community, there are several categories of individuals who maintain a long-term presence within that

environment who are not screened or treated for *S aureus* carriage, but who may contribute to its presence. These include longer-term patients as well as health care professionals (HCPs) and support staff who regularly come in contact with patients. In a study of 256 HCPs at a large urban tertiary care teaching hospital,<sup>7</sup> including paramedics, nurses, clerical workers, and physicians, *S aureus* was isolated from the anterior nares of 43.8% of those screened with 6.6% exhibiting methicillin-resistant *S aureus* (MRSA) carriage. The authors point to 3 potential consequences of this carriage: self-infection of these workers by their own strains, cross-transmission to patients, and introduction of the pathogen into their communities.<sup>7</sup> Furthermore, a study using whole-genome sequencing to investigate MRSA transmission within a neonatal intensive care unit provided evidence of the complexity of transmission that can occur in both directions involving patients and caregivers.<sup>8</sup>

Strategies to reduce colonization in patients preadmission have been shown to be very successful. These have primarily focused on regimens that include nasal treatment with the antibiotic mupirocin.<sup>9-12</sup> Recently, a multicenter study suggested the potential utility of universal antibiotic treatment of patients at admission without screening for specific carriage to be an effective approach to reduce intensive care unit MRSA infections.<sup>13</sup> Although effective, strategies that incorporate wide use of antibiotics lead to increased opportunities for the development of resistant bacteria. For this reason, nasal antibiotics are typically not used on a regular basis to reduce subclinical colonization in individuals within the health care environment in whom prophylactic treatment might be beneficial. These could include patients with immune deficiency or who are otherwise at higher-risk for infection and longer-term patients, as well as HCPs and other staff who work in the patient environment.

The development of effective and convenient nonantibiotic nasal preparations for daily use could provide appropriate additional methods for infection control, addressing the well-known hand-to-nose-to-hand cycle of inoculation and contamination involving the nasal bacterial repository.<sup>14</sup> A nonselective antiseptic agent, such as ethanol, would be expected to reduce colonization by all strains of bacteria, similar to a broad-spectrum antibiotic, but with both gram-positive and gram-negative bacteria being affected and without the risk of resistance being developed. In patients, daily application of such an agent could reduce the risk of self-inoculation and contamination of the hospital room environment. The regular use of a nasal antiseptic by HCPs could reduce their involvement in the process of bacterial transference, as well as contribute to their own preventive and protective hygiene in the work environment.

The goal of our study was to determine the magnitude and breadth of bacterial reduction by a nonantibiotic, alcohol-based nasal antiseptic applied during a typical workday in primary HCPs. Study subjects included nurses and surgical technicians directly participating in patient treatment and care on the day of testing. Data were collected on the treatment effects on both *S aureus* and total bacterial carriage in the nasal vestibule during a single day of application.

## METHODS

### *Selection and enrollment of study subjects*

Volunteers were recruited from the nursing and technical staff working in the main and ambulatory operating rooms and patient care floors of the Medical University of South Carolina Hospital. This randomized double-blind, placebo-controlled study was approved by the Medical University of South Carolina Institutional Review

Board (Pro000018198). This study is registered on [clinicaltrials.gov](http://clinicaltrials.gov) (Identifier: NCT01861457).

Eligible to participate were healthy HCPs between ages 18 and 70 years who were able and agreed to refrain from using all nasal spray preparations or washes from the time of their screening through their scheduled study day. Exclusionary criteria included symptoms of upper respiratory disease, including chronic rhinitis/sinusitis, seasonal allergies, upper respiratory infection during the previous 4 weeks; the use of antibiotics in the 2 weeks before or during the study; a known allergy to citrus oil; or being a cigarette smoker. Nonsmokers were defined as those individuals who had abstained from smoking for at least 1 year before the study.

After obtaining informed consent, eligible subjects were screened by nasal swab for vestibular carriage of *S aureus* as described below. Subjects who screened positive and who accepted enrollment in the study were scheduled for participation within 10 days to minimize loss of carriage status by the study date.

### *Study protocol*

The study period consisted of a single 10-hour workday, during which nasal swab samples from the right and left nasal vestibules of each subject were obtained and pooled (Fig 1). The first combined sample from each subject was collected by the medical study staff at the start of the workday (hour 0), immediately followed by application of the randomly assigned placebo or test preparation with a saturated swab to both nasal vestibules. Application of the placebo preparation was used to control for the potential mechanical effects of the application process, itself. Reapplications of the placebo and treatment preparations were made at hours 4 and 8. At hour 10, the subjects returned to enable collection of the posttreatment nasal sample by the medical study staff.

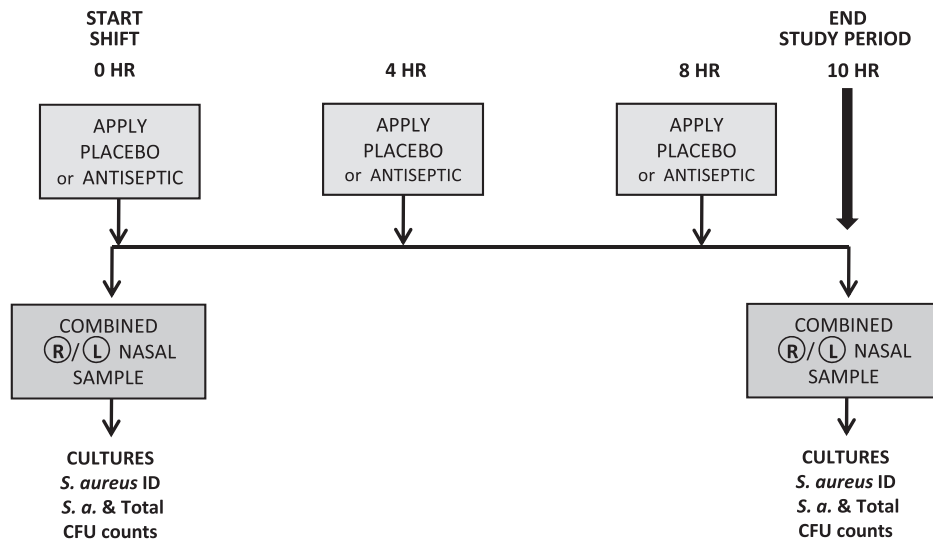
### *Preparation and application of the test and control agents*

A commercially available, nonprescription product, Nozin Nasal Sanitizer antiseptic (Global Life Technologies Corp, Chevy Chase, Md) was used as the test agent in our study. The safety-tested formulation is composed of 70% ethanol active combined with a mixture of natural oil emollients and the preservative benzalkonium chloride. Sterile phosphate-buffered saline with 0.017% peppermint oil as a masking agent was used as placebo control.

Application of the antiseptic or placebo control preparation was made by saturating a sterile swab with 5 drops (~200  $\mu$ L) of solution and rotating the swab around the inside of the vestibular surfaces of both nostrils.

### *Sample collection and analysis*

Nasal samples from both screening and study sample collection were obtained using sterile BD ESwab Collection Kits (Becton, Dickinson & Co, Franklin Lakes, NJ). For screening, neat samples were inoculated onto BBL CHROMagar *Staph aureus* medium (Becton, Dickinson & Co) and incubated at 35°C for 24 hours; mauve colonies were identified as *S aureus* by Gram's stain, catalase, and latex agglutination testing. For evaluation of treatment samples, duplicate 75  $\mu$ L aliquots of neat and 1:10 dilutions of each sample were inoculated onto plates of BBL CHROMagar *Staph aureus* medium and tryptic soy agar with 5% sheep blood to assess *S aureus* and total bacterial colony forming units (CFU) counts, respectively. At 24 hours of incubation at 35°C, CHROMagar plates were photographed and mauve colonies were counted and identified as *S aureus* as described above. At 48 hours of incubation at 35°C, tryptic soy agar plates were photographed for determination of total bacterial CFU. All data from subjects whose baseline inocula



**Fig 1.** Schematic of the study protocol. Three applications of placebo or antiseptic preparation were made at 4-hour intervals during the workday to both nasal vestibules of subjects randomly assigned to 1 of the 2 treatment groups. Combined nasal swab samples from both vestibules were collected before and after treatment, at 0 hours and 10 hours, to assess changes in nasal vestibular *Staphylococcus aureus* (*S aureus*) colonization and total bacterial colony forming units (CFU) counts.

developed fewer than 5 mauve colonies on the CHROMagar plates were excluded from analysis.

#### Data handling and analysis

*S aureus* and total bacterial CFU counts for analysis were derived from pairs of plates on which identical 75  $\mu$  aliquots of neat or 1:10 dilutions of each nasal sample were cultured. Whenever colony densities allowed, CFU counts from the pair of neat pre- and posttreatment plates were used. In the event that neat colony densities were too high to allow accurate counting, the pair of 1:10 dilution plates for that subject was used and the CFU counts were multiplied by 10 to arrive at a "neat" equivalent value. In no instance was a comparison made between neat and diluted samples from a subject in the analysis of pre- to posttreatment values. CFU counts were made by 2 independent, blinded readers using photographs of the cultures and the average values were used.

All data analyses were performed with Medcalc 12.6.1.0 (MedCalc Software, Ostend, Belgium) and Sigma Stat 3.5 and SigmaPlot 11.2 (Systat Software, Inc, Chicago, Ill). Comparisons of baseline subject characteristics and clinical outcomes and their odds ratios were based on  $\chi^2$  analyses with Yates continuity correction applied. Comparisons of the nonnormally distributed median values from different groups and from study treatment effects were analyzed by the Mann-Whitney rank sum test and the Wilcoxon signed rank test, respectively. The Pearson correlation was used to determine the relationship of carriage reduction by the antiseptic on *S aureus* and total bacterial strains. A  $\kappa$  analysis was used to confirm that there was adequate strength of agreement between the 2 independent CFU counts for each cultured sample that were averaged for use as outcome variables (mean weighted  $\kappa = 0.951$ ). A *P* value < .05 was considered indicative of statistical significance.

## RESULTS

#### Demographic factors in *S aureus* carriage

Table 1 summarizes the characteristics of the 387 HCPs who were screened for the presence of nasal *S aureus* colonization. The

**Table 1**  
Characteristics of the screened health care provider study participants

Characteristic	n (%)	<i>Staphylococcus aureus</i> positive		
		n (%)	OR (95% CI)*	<i>P</i> <sup>†</sup>
Mean age, y	40.8			
Total screened	387 (100)	78 (20.2)		
Female	329 (85.0)	65 (19.8)	Ref	-
Male	58 (15.0)	13 (22.4)	1.17 (0.60-2.3)	.77
Position title				
RN	179 (46.3)	47 (26.3)	Ref	-
PCT/ORAs	50 (12.9)	8 (16.0)	0.54 (0.23-1.22)	.19
MD/PA/NP/CRNA	35 (9.0)	7 (20.0)	0.70 (0.29-1.71)	.57
Other	123 (31.8)	16 (13.0)	0.42 (0.23-0.78)	.01
Work location				
Floors	212 (54.8)	41 (19.3)	Ref	-
Operating room	112 (28.9)	24 (21.4)	1.14 (0.65-2.00)	.76
Other	62 (16.0)	13 (21.0)	1.11 (0.55-2.23)	.92
Children aged 6 mo-11 y	102 (26.4)	20 (19.6)	0.96 (0.54-1.68)	.99
Dog(s)	180 (46.5)	37 (20.6)	1.05 (0.64-1.72)	.96
Cat(s)	67 (17.3)	15 (22.4)	1.18 (0.62-2.23)	.74

CI, confidence interval; CRNA, certified registered nurse anesthetist; MD, physician; NP, nurse practitioner; OR, odds ratio; ORA, operating room assistant; PA, physician assistant; PCT, patient care technician; Ref, reference group; RN, registered nurse.

\*Calculated using the indicated reference group and comparing the *S aureus*-positive population with the *S aureus*-negative population.

<sup>†</sup>Calculated using the indicated reference group or the corresponding negative characteristic group and comparing the *S aureus*-positive population to the *S aureus*-negative population. The  $\chi^2$  test was used.

population was predominantly female (85%) and composed of 66% white, 27% African American, 4% Asian, and 3% "other" subjects. Of the screened population, 20.2% tested positive for *S aureus* carriage. Workers were classified by their position title and worksite location. The presence of 1 or more children between ages 6 months and 11 years and dogs and cats at home were determined as potential contributors to colonization risk. Registered nurses tended to exhibit higher carriage prevalence than staff in other positions (26.3%). A peripheral finding was that African American women who were screened were significantly less likely to be colonized than white women (12.6% vs 23.7%, respectively; odds ratio, 0.466 [95% confidence interval, 0.24-0.91]; *P* = .03). There were no other factors observed to be associated with the prevalence of *S aureus* carriage in the screened population.

**Table 2**  
Comparison of characteristics of treatment groups in health care providers testing positive for *Staphylococcus aureus* infection

Characteristic	Placebo	Antiseptic
Total subjects	19	20
Mean age	35.4 (26-67)	34.5 (26-64)
Baseline colonization		
<i>S aureus</i>	562 ± 1,113	756 ± 1,512
All strains*	1,289 ± 1718	1,200 ± 1,694
Female	14	20
Male	5	0
Occupation		
RN	10	15
PCT/ORA	1	2
MD/PA/NP/CRNA	5	0
Other	3	3
Location		
Floors	14	14
Operating room	1	4
Other	4	2

NOTE. Values are presented as n, y (range), or colony forming units ± standard deviation.

CRNA, certified registered nurse anesthetist; MD, physician; NP, nurse practitioner; ORA, operating room assistant; PA, physician assistant; PCT, patient care technician; RN, registered nurse.

\*As determined by growth on tryptic soy agar plates (5% sheep blood).

### Composition of the treatment groups

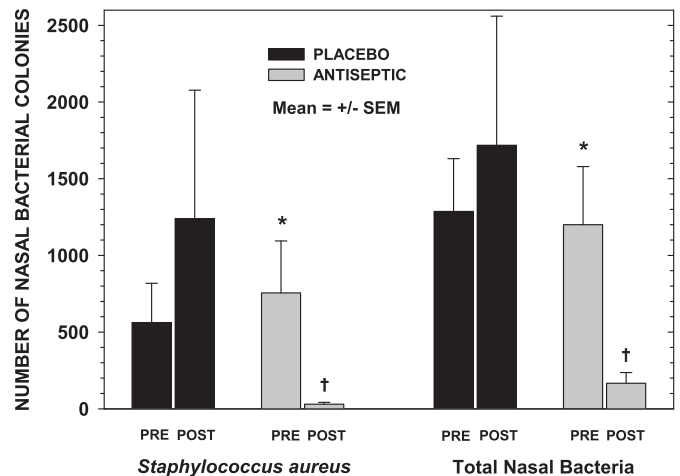
The characteristics of the 39 subjects who tested positive for *S aureus* infection who were randomly assigned to the placebo and antiseptic-treatment groups are shown in Table 2. The 2 groups were statistically indistinguishable with regard to participant average ages or their baseline levels of colonization by either *S aureus* ( $P = .633$ ) or total bacterial strains ( $P = .790$ ). Consistent with the characteristics of the total screened pool from which the *S aureus*-colonized subjects were drawn, the treatment study groups were predominantly women and registered nurses.

### Reduction in *S aureus* and total bacterial carriage

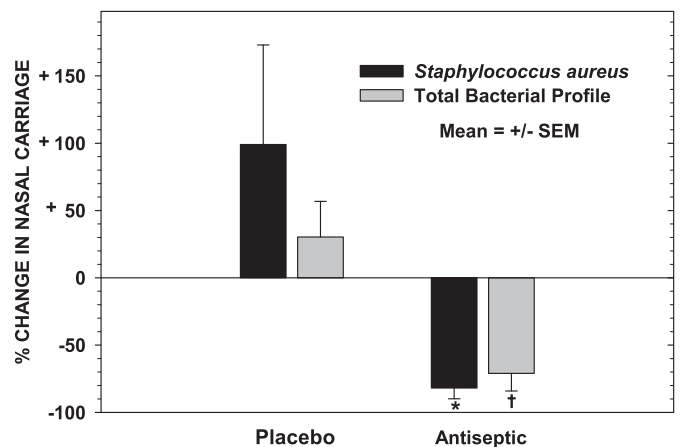
As shown in Figure 2, there was substantial between-subject variation in baseline levels of both *S aureus*-specific and total bacterial strain colonization. This variation was similar in both control (placebo) and test (antiseptic) groups. Comparison of the baseline level of *S aureus* to total bacterial carriage in both groups indicates that, on average, *S aureus* accounted for approximately half of the total vestibular bacterial load in this study population.

Three applications of the antiseptic at 4-hour intervals during the course of the workday significantly reduced the mean ± standard error number of *S aureus* colonies from  $756 \pm 338$  at baseline to  $30 \pm 12$  at the end of the 10-hour study period (Fig 2). Although 19 of 20 subjects demonstrated a reduction in carriage, 10 subjects showed a 100% decrease with an additional 3 subjects who showed >95% decrease. A similarly pronounced and significantly significant response was seen in total bacterial CFUs, with a reduction of  $1,200 \pm 379$  CFU to  $167 \pm 70$  CFU (Fig 2). The observed reduction in carriage was >90% in 12 subjects, including 4 subjects at 100%.

In marked contrast, the placebo treatment application process resulted in a substantial mean increase in *S aureus* colony counts from  $562 \pm 255$  CFU to  $1,239 \pm 838$  CFU. This increase was not statistically significant due to the >3-fold increase in variability of the colonization response (increase in standard deviation of the mean from 1,113 to 3,651 CFU) that accompanied the effect. A similar placebo-induced change from baseline in total bacterial



**Fig 2.** Nasal vestibular bacterial colonization in baseline (PRE) and posttreatment (POST) samples. Numbers of colony forming units in identical samples plated onto CHROMagar SA (Becton, Dickinson & Co, Franklin Lakes, NJ) and tryptic soy agar blood agar plates for assessment of *Staphylococcus aureus* and total bacterial colonization before and after treatment, as described in detail in the Methods section. \*No significant difference from the baseline value in the corresponding placebo-treated group. †Significantly different ( $P < .001$ ) from the PRE value.



**Fig 3.** Comparison of the effects of placebo and antiseptic application procedures on bacterial colonization during the 10-h study period. Mean percent changes in bacterial carriage resulting from the process of applying phosphate buffered saline versus an active antiseptic. \*Significantly different ( $P < .001$ ) from *Staphylococcus aureus* in the placebo-treated subjects. †Significantly different ( $P < .001$ ) from total bacterial profile in the placebo-treated subjects.

CFUs was also not statistically significant. These data are consistent with the notion that the disruption of the relatively stable vestibular colonization caused by the mechanical disturbance caused by the application process itself may stimulate bacterial growth in the anterior nares in certain individuals if an effective antimicrobial agent is not present to inhibit it.

The effects of placebo and antiseptic treatments on bacterial nasal carriage expressed as percent change from baseline are shown in Figure 3. Antiseptic treatment produced a uniform reduction in CFUs at a level of 82% (mean) and 99% (median) for *S aureus* and 71% (mean) and 91% (median) for total bacteria. These reductions were significantly different from corresponding placebo-related effects, despite the wide variance displayed in the latter data. In contrast, the near 100% mean increase observed in *S aureus* colonization in the placebo-treated group was not statistically significant from baseline due to that variance.

### Nonselectivity of antiseptic action in the nasal vestibule

The broad-spectrum, nonselective characteristics of the ethanol-based antiseptic preparation were assessed by evaluating the correlation between its antibacterial effectiveness in reducing *S aureus* colonization and total bacterial colonization in individual subjects. Comparison of individual values for the pair was based on CFU counts made on the same sample. The mean and median percent reductions in colonization of the 2 bacterial target groups fell within 11% and 8% of each other, respectively, and the statistically significant Pearson correlation coefficient value for these data from all antiseptic-treated subjects was 0.827 ( $P < .001$ ). These data are consistent with a comparable effectiveness of the antiseptic preparation in reducing nasal carriage of these 2 bacterial target groups.

### DISCUSSION

The need for a screening process to identify subjects who were *S aureus*-colonized enabled the characterization of the population of 387 HCPs from which the antiseptic treatment study participants were drawn. The 20.2% prevalence of *S aureus* carriage in our study population fell at the lower end of the wide spectrum reported for HCPs, which may range from 20%-40% or higher depending on the facility and nature of the workforce.<sup>1,7,15</sup> Some data suggest that carriage in female HCPs can be lower than that in male HCPs and that may have been a contributing factor to low carriage in our predominantly female screening population.<sup>15,16</sup> The small proportion of men in our screened population (58 men, 15% of those screened) likely provided insufficient power to identify a significant effect of sex on carriage prevalence.

One potential concern for the use of alcohol- or other antiseptic agent-based preparations in the nasal vestibule is dryness, irritation, or other discomfort associated with its repeated use. A formulation designed to address this concern by incorporating moisturizing constituents, such as the natural oil mix of the test preparation used in our study, is essential. There were no reports of these or other adverse consequences made during the exit interview by the participants in the multitreatment protocol. Although the preparation used in our study has been available as an over-the-counter product for the past 6 years, the short-term nature of our study does not address the question of potential adverse effects over time, nor does it provide insight into the potential positive cumulative effectiveness of the preparation in reducing carriage when used in a multiday regimen.

Three applications of the alcohol-based antiseptic preparation during the course of a single workday resulted in a pronounced reduction in nasal vestibular carriage of both *S aureus* and other cultivable bacteria. In general, reductions were very consistent across subjects, with a median decrease in the antiseptic-treated group of 98.8% at the end of the 10-hour workday. The design of our study did not allow assessment of the rate of bacterial killing during the study period. However, a surgical scrub study showed that the bactericidal activity of 70% ethanol in the presence of emollients, as was the case in the test preparation, resulted in near-maximum activity within minutes of application.<sup>17</sup> If applicable to our study, nasal decolonization may also have been demonstrable in the study subjects at times before collection of the end-of-day sample.

There is no obvious explanation for the 1 of 20 subjects in the treatment group who did not demonstrate decolonization. Because that response was unique to that subject and was seen in both *S aureus* and total bacterial CFU counts, it might be that the outcome resulted from a procedural error in application or

sampling or that there was an unreported modification to the nasal environment by the subject during the study day.

Application of the phosphate-buffered saline placebo unexpectedly led to dramatic shifts in colonization. Because the application process was identical in control and antiseptic-treated subjects, it is reasonable to assume that the same effect was produced in both treatment groups. An interesting possibility is that the mechanical process of swabbing can lead to disruption of a stable bacterial environment in some individuals leading to stimulated growth in the absence of a deterring antibacterial agent. Whatever its cause, this effect was completely eliminated in the antiseptic-treated subjects, further supporting the effectiveness of the alcohol-based preparation in achieving carriage reduction.

The observation of highly correlated and statistically significant reductions in *S aureus* and total bacterial CFU counts is consistent with the notion that the antiseptic exhibited broad efficacy against the range of bacteria colonizing the nasal vestibule. Further studies following a similar design and using 16S ribosomal RNA analysis to determine effects on the range of bacterial taxa within a given individual<sup>18</sup> will be needed to verify the consistency of antibacterial reduction across the constituent bacterial flora.

### CONCLUSIONS

The results of our unique study demonstrate the effectiveness of single-day, alcohol-based nasal antiseptic treatment in reducing vestibular colonization by *S aureus* and other potentially pathogenic bacteria in HCPs. The HCPs selected for our study represent 1 of several populations within the health care environment, along with chronic-care patients and support staff, whose long-term presence contributes to the bacterial burden and associated risk of hospital-acquired infection in themselves and others. It is likely that a large, multicenter study will be necessary to establish the evidential basis for the effect of nasal antiseptic use in contributing to reduction of infection outcomes in health care settings. Given the multifaceted nature of the sources likely contributing to the overall bacterial burden and residual infection rates within most health care environments, such multicenter studies could be designed to address carriage by patients, HCPs, or both in combination. Data preliminary to larger-scale studies might be provided by investigations focused on currently nondecolonized patients and HCPs at facilities, perhaps long-term, where prevalence of staph infections are especially high. It may also be possible that data in support of the effectiveness of nonantibiotic nasal antiseptic use will result as a consequence of its adoption by proactive health care facilities, as was the case with the initial adoption of alcohol-based hand sanitizer use by HCPs.<sup>19</sup> Within the patient population, incorporating the use of this antiseptic approach, in conjunction with the use of hand sanitizers and body wipes, may provide an important adjunct or alternative to preventive antibiotic therapy surrounding surgical and medical procedures. Further, because it will not contribute to bacterial resistance, the ethanol-based antiseptic provides a unique opportunity for regular daily use over prolonged periods by patients and staff in long-term care environments.

### Acknowledgments

The authors thank the Medical University Hospital HCPs who agreed to participate in the study and the nursing supervisors and the hospital administrators who enabled their participation.

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