ORIGINAL ARTICLE

Nasal *Staphylococcus aureus* Carriage in Ablution Performers

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ABSTRACT

Background: *Nasal washing is a part of religious ablution (Wodou), which is practiced normally five times a day, as a prerequisite for the Muslims' prayer. However, studies addressing its effect on the nasal colonization state are scarce and conflicting.* ***Objective****: to investigate whether carriage rates of nasal Staphylococcus aureus (S. aureus) would differ between performers and non-performers of ablution.* ***Methodology:*** *A total of 600 first-year students from Zagazig University, Egypt, were randomly selected and classified by their performance of ablution into two groups, performers and non-performers of ablution. A nasal swab was obtained from each participant and investigated for S. aureus with the standard technique. The antibiotic susceptibility of the isolated organisms was determined with the Vitek-2 system.* ***Results:*** *The overall S. aureus carriage rate was 30.2%. The performers of ablution had significantly lower S. aureus and methicillin-resistant S. aureus (MRSA) carriage rates than the non-performers (P<0.001 for both comparisons). In the logistic regression analysis, the most significant independent risk factor for S. aureus nasal carriage was the non-performance of regular ablution. We also found that the strains isolated from the performers, compared to non-performers, were more susceptible to antibiotics.* ***Conclusions:*** *Performers of ablution, compared to non-performers, have reduced carriage rates of nasal S. aureus and MRSA, and higher susceptibility profile to antibiotics. The study advocates regular ablution as an effective and inexpensive method that helps in checking the increasing problem of S. aureus antimicrobial resistance.*

INTRODUCTION

The Gram-positive *Staphylococcus aureus* (*S. aureus*) continues to be one of the most important human pathogens in the world [1]. It causes a broad range of both hospital and community-acquired infections, varying from minor skin infections, such as boils and abscesses, to severe, life-threatening infections, such as necrotizing pneumonia and septicemia [2]. Treatment is getting increasingly problematic because of the increasing number of infections caused by methicillin-resistant *S. aureus* (MRSA) strains [3].

In contrast, however, *S. aureus* is also a commensal organism that preferentially colonizes the anterior nares of about one-third of the human population without causing symptoms[4]. Colonization may be persistent or intermittent[5]. Although the anterior nares are considered the primary colonization site, some studies claimed that *S. aureus* carrier rates of the throat are even higher for certain populations [6]. Less common *sites for S. aureus* colonization *include* skin, perineum, vagina and *gastrointestinal tract*[7]. Yet, anterior nares are usually the key source of inoculation of these sites by means of hand transmission.

While nasal colonization gives *S. aureus* a niche to conceal itself from host defenses, it increases the risk of invasive infections when these defenses are breached, as would occur, for example, when patients undergo surgery[8], patients are on dialysis [9], or patients are in intensive care units (ICUs)[10].

The relation between colonization and infection is further indicated by molecular studies showing that most invasive *S. aureus* infections are caused by the patient's own colonizing flora [11]. Therefore, elimination of nasal carriage of *S. aureus* is considered the most straightforward strategy to prevent infections caused by *S. aureus* [12]. Various decolonization methods have been tried. The most commonly used approach involves a combination of topical mupirocin ointment, oral antibiotics, such as rifampicin or doxycycline, and bath washes with chlorhexidine or diluted bleach, in addition to hygiene education[13].

However, with the growing use of mupirocin, the risk of development of resistance associated with treatment failure is increased [14], and the need for alternatives is accentuated. In this regard, decolonization approaches through personal hygiene measures for the body, and particularly the nose, should not be forgotten. A significant decrease in colonization rate has been reported in association with the nasal wash using water twice daily [15]. This has been confirmed by a more recent study which found that patients' compliance with decolonizing washing procedures was associated with successful decolonization of multidrug-resistant bacteria [16].

Interestingly, hygiene or cleanliness in Islam is so much valued that it is regarded as part of faith. Thus, ablution (*wodou*) is a prerequisite for the prayer, the *second most important pillar of Islam*. Ablution involves twenty-six cleansing actions, which are normally carried out before prayers five times a day. In each time, nasal cleansing is done by sniffing up water into the nostrils three times consecutively. Although ablution is a very common practice among Muslims, the relation between ablution and the nasal colonization (carriage) state has not been sufficiently investigated, and the findings of the few available studies are contradictory. According to some studies, nasal washing in ablution can successfully reduce *S. aureus* nasal colonization [17,18].

By contrast, others have claimed that ablution is associated with increased risk of serious infections[19]. For example, fatal primary amoebic meningoencephalitis due to *Naegleria fowleri*, has been reported in connection with the religious practice of ablution, particularly in developing countries, but actual figures in these countries can be much higher, arguably because these rituals are sensitive topic and infections are very likely to be missed or not reported [20].

Egypt is a developing country with Muslim majority. Religious rituals are well observed by many of its people [21]. In this study, we aimed to investigate whether carriage rates of nasal *S. aureus* would differ between performers and non-performers of daily repeated ablution in a sample of Egyptian university students.

METHODOLOGY

Study design

This cross-sectional study was conducted at the beginning of the academic year 2017/ 2018. The place of the study was the campus of Zagazig University, which is situated within the capital city of Sharkeya governorate but is surrounded by rural villages. A total of 600 subjects were selected from the University students on the condition that they were not included in another study of ours[22]. Every participant had to provide a signed informed consent form before enrollment. They were all healthy and none of them used antibiotics for the last two months before the study. Of these students, 300 were first-year medical students. The rest of participants were randomly selected from Faculties of Arts, Commerce, and Law of the same university, 100 first-year students from each faculty. Approval was obtained from the ethical committee of Faculty of Medicine, Zagazig University. Permissions were also taken from the vice-deans for post-graduate studies and research of the other participating non-medical faculties.

Socio-demographic data and detailed information on religious practices were collected. Participants were classified into two groups by their regularity in performing ablution:

I. Performers, who were regularly practicing ablution and washing their nose, at least once daily.
II. Non-performers, who were not practicing ablution and not washing their nose every day.

Collection and culture of specimens

A nasal swab was obtained from each participant by inserting a sterile moistened cotton swab into both nostrils with a rotating technique. A total of 600 isolates were obtained from 600 participants. The samples were immediately transported to the Microbiology Laboratory of the Clinical Pathology Department. No transport medium was used. The swabs were cultured onto mannitol salt agar (MSA) and incubated aerobically for 48 hours at 37^0 C. Suspected colonies of *S. aureus* were subcultured on nutrient agar (NA) (Oxoid Ltd, UK) and confirmed by being Gram-positive, catalase positive and coagulase positive.

Antibiotic susceptibility testing

The Vitek-2 system with an AST-GP67 card (Biomérieux Inc., Durham, USA) was used for determination of the antibiotic susceptibility of the isolated organisms. The used antibiotics included Ampicillin, Benzylpenicillin, Cefoxitin, Clindamycin, Ciprofloxacin, Erythromycin, Gentamycin, Oxacillin, Linezolid, Levofloxacin, Moxifloxacin, Nitrofurantoin, Rifampicin, Streptomycin, Tetracycline, Tigecycline, Trimethoprim/Sulphamethazo, Quinupristin/Dalfopristin and, Vancomycin. E-test strips (Biomérieux, France) were used for further verification of the sensitivity of MRSA isolates to Vancomycin. A Mueller Hinton (MH) culture broth (Oxoid) of bacterial turbidity equivalent to a 0.5 McFarland standard was used. The suspension was then inoculated on MH agar plates (Oxoid) to which E-test strips were applied, and incubated at $35C^0$ for 24 hours. Organisms that could grow in 4% NaCl agar screening medium, supplemented with 6 µg/ml Oxacillin, and showed a MIC of ≥4 µg/ml were considered Methicillin resistant. While organisms that could grow in an agar screening medium with added 6 µg/ml Vancomycin and showed a MIC of ≥32 µg/ml were regarded as Vancomycin resistant. The reference control of *S. aureus* strain was INCQS 000381 (ATCC 29213).

For oxacillin, a cut off ≥4 ug/ml was considered resistant while for cefoxitin, a positive screen by Vitek-2 was considered resistant.

Statistical analysis:

Categorical data were described as absolute values and percentages (%). Chi-square test was used to compare the significance of the difference in distribution. Odds ratios (OR) and 95% confidence intervals (CI) were calculated. Univariate analysis was used to identify potential risk factors for *Staphylococcus aureus* nasal carriage. Factors that met statistical significance at $P < 0.05$ were then included in a multivariate logistic regression analysis. Collinearity diagnostic statistics were performed to test the multicollinearity. Variance inflation factor (VIF) values ≥2.0 or tolerance < 0.4 may indicate concern for multicollinearity in logistic regression models [23]. We checked the goodness of fit of the model by the Hosmer–Lemeshow test. The *P-value* for statistical *significance* was *set at P < 0.05*. Statistical analyses were conducted with the IBM SPSS Statistics for Windows software, version 21.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Characteristics of the participants, stratified by ablution performance, are summarized in table (1). Performers and non-performers of ablution were similar in all characteristics except for a statistically significant preponderance of male gender and urban residence in the group of non-performers. Overall, *S. aureus* was detected in the nasal swabs of 181 subjects, yielding a carriage rate of 30.2%. The carriage rate was significantly higher among the non-performers of ablution (38.5%) than the performers (24.4%). MRSA carriage rate was also significantly higher among the non-performers (38.7%) than the performers (12.8%) (table 2).

Table 1: Characteristics of performers and non-performers of regular ablution.

Characteristic	**Performers**		**Non-performers**		**P-value**
	N	**(%)**	**N**	**(%)**	
	353	**(100)**	**247**	**(100)**	
Age (years):					0.887
≤ 18	54.4	(54.4)	133	(53.8)	
>18	161	(45.6)	114	(45.6)	
Gender:					0.033
Male	142	(40.2)	121	(49.0)	
Female	211	(59.8)	126	(51.0)	
Education type:					0.454
Medical	172	(48.7)	128	(51.8)	
Non-medical	181	(51.3)	119	(48.2)	
Residence:					<.0001
Rural	178	(50.4)	41	(16.6)	
Urban	175	(49.6)	206	(83.4)	
Smoker:					0.511
Yes	68	(19.3)	53	(21.5)	
No	285	(80.7)	194	(78.5)	
Family member working in a health care facility:					0.560
Yes	51	(14.4)	40	(16.2)	
No	302	(85.6)	207	(83.8)	

Table 2: *Staphylococcus aureus* (*S. aureus*) nasal carriage and methicillin sensitivities in performers and non-performers of ablution.

Culture	**Performers**		**Non-performers**		**p**	**OR (95% CI)**
	N	**(%)**	**N**	**(%)**		
S. aureus nasal carriage					<0.001	0.411 (0.356- 0.745)
Positive	86	(24.4)	95	(38.5)		
Negative	267	(75.6)	152	(61.5)		
Total	353	(100.0)	247	(100.0)		
Methicillin sensitivities					<0.001	4.301(1.939- 9.717)
MSSA	75	(87.2)	65	(61.3)		
MRSA	11	(12.8)	41	(38.7)		
Total	86	(100.0)	106	(100.0)		

Abbreviations: OR, odds ratio; CI, confidence interval; MSSA, methicillin-sensitive *S. aureus*; MRSA, methicillin-resistant *S. aureus*.

In the univariate analysis, the risk for *S. aureus* nasal carriage was significantly associated with male gender, rural residence, smoking and regular ablution (table 3). Out of these factors, as shown by the multivariate logistic regression analysis, non-performance of regular ablution was the most significant independent risk factor for *S. aureus* nasal carriage (table 4). Multicollinearity analysis showed VIF values ≥2.0 and tolerance < 0.4, indicating that there was no evidence of multicollinearity in the logistic regression model.

Table 3: Univariate analysis of potential factors for *Staphylococcus aureus* nasal carriage among total sample (N=600).

Risk factor	OR (95% CI)	P-value
Age (years): ≤ 18/>18	1.126 (0.828- 1.665)	0.368
Gender: Male Female	0.538 (0.400- 0.808)	0.021
Education Medical Non-medical	0.735 (0.31- 1.69)	0.491
Residence: Rural Urban	0.275 (0.186- 0.859)	<.0001
Smoker Yes No	0.503 (0.386- 886)	0.018
Family member working in medical care facilities: Yes No	0.681 (0.427- 1.085)	0.104
Regular ablution: Yes No	2.840 (1.363- 2.763)	<.0001

Abbreviations: OR= odds ratio, CI= confidence interval

Table 4: Multivariate logistic regression analysis of risk factors for Staphylococcus aureus nasal carriage*.

Risk factor	OR (95% CI)	P-value
Male gender	1.136 (0.801- 1.611)	0.048
Rural residence	0.640 (0.464- 0.939)	0.021
Smoker	0.727 (0.512- 1.033)	0.075
Non-performer of regular ablution	4.689 (1.163- 8.543)	<.0001

Abbreviations: OR= odds ratio; CI= confidence interval

*The risk factors which were significant in the univariate analysis were included in multivariate analysis.

Table (5) shows the antibiotic susceptibility patterns of *S. aureus* strains isolated from performers and non-performers of ablution. The highest frequency of susceptibility in both groups was observed with tigecycline, linezolid and vancomycin. The lowest was observed with ampicillin and benzylpenicillin. Isolates from the performers of ablution were significantly more susceptible to clindamycin, gentamycin, oxacillin and tetracycline than isolates from the non-performers.

Table 5: Antibiotic susceptibility profiles of Staphylococcus aureus isolates from performers and non-performers of ablution.

Antibiotic	Performers		Non-performers		P	OR (95% CI)
	N 86	(%) 100	N 95	(%) 100		
Ampicillin	5	5.8	4	4.2	0.738*	0.712 (0.185- 2.743)
Benzylpenicillin	5	5.8	3	3.2	0.480*	0.528 (0.122- 2.280)
Cefoxitin	66	76.7	65	68.4	0.212	0.657 (0.339- 1.272)
Clindamycin	71	82.6	47	49.5	<.001	0.207 (0.104- 0.411)
Ciprofloxacin	69	80.2	68	71.6	0.175	0.621 (0.310- 1.241)
Erythromycin	52	60.5	43	45.3	0.041	0.541 (0.299- 0.977)
Gentamycin	67	77.9	39	41.1	<.001	0.198 (0.103- 380)
Oxacillin	75	87.2	28	29.5	<.001	0.061 (0.028- 0.133)
Linezolid	83	96.5	90	94.7	0.723*	0.651 (0.151- 2.807)
Levofloxacin	59	68.6	61	64.2	0.532	0.821 (0.442- 1.525)
Moxifloxacin	45	52.3	44	46.3	0.420	0.786 (0.438- 1.410)
Nitrofurantoin	21	24.4	21	22.1	0.708	0.878 (0.440- 1.752)
Rifampicin	81	94.2	82	86.3	0.077	0.389 (0.133- 1.142)
Streptomycin	13	15.1	11	11.6	0.484	0.735 (0.311- 1.741)
Tetracycline	28	32.6	17	17.9	0.023	0.452 (0.226- 0.902)
Tigecycline	86	100	95	100.0	1.0*	
Trimethoprim/Sulphamethazol	41	47.7	38	40.0	0.299	0.732 (0.406- 1.319)
Quinupristin/Dalfopristin	82	95.3	86	90.5	0.257*	0.466 (0.138- 1.573)
Vancomycin	86	100	89	93.7	1.0*	

Abbreviations: OR, odds ratio; CI, confidence interval
* Fisher's Exact Probability Test (two tailed)

DISCUSSION

Studies from various parts of the world suggest wide geographical differences in the rates of *S. aureus* nasal carriage[24,25]. This study, which found no difference in the *S. aureus* nasal carriage rates between medical and non-medical students, is consistent with that reported for another random sample of the first-year medical students drawn from the same place of the current study. However, they differed from the results of the sixth year medical students who have more clinical exposure to patients [22].

The present study also showed that participants who were non-performers of regular ablution had higher nasal *S. aureus* and MRSA carriage rates compared to performers of ablution. In regression analysis, we found that the non-performance of regular ablution was the most significant independent risk factor for *S. aureus* nasal carriage. Our data may be interpreted as an indication of the importance of ablution in reducing *S. aureus* nasal colonization. They should also provide support to the few available studies, which found that the microbial density of *S. aureus* was significantly lower among worshipers than non-worshipers [17], and which could not isolate MRSA from any practitioner of ablution [18].

In keeping with previous literature, we found that male gender was an important risk factor for *S. aureus* nasal carriage [26, 27]. An explanation can be based on the repeated observation that males are less likely than females to clean their nasal cavities with regularity [28]. *In the current study, there were* significantly *fewer* males than females, who cleaned the nose through regular performance of ablution.

One more significant independent risk factor for *S. aureus* nasal carriage found in this study was the rural residence. Although this contrasts with some studies, which reported that the *S. aureus* carriage rate was higher in the urban areas [29, 30], there are studies, in support of our finding, which reported that people living in rural areas were more likely to be colonized with *S. aureus* and MRSA than urban dwellers [31]. The strong association of *S. aureus* nasal carriage with rural residence in our study is worrisome as it may be a reflection of a less hygienic environment in rural Egypt. However, it is true that significant progress, regarding direct access to safe water and basic sanitation services, has been already made in the last decades [32], but much more may still be needed. After all, the safety of ablution has been connected with the water used [20], and to have safe and clean water is simply a human right.

In our previous study of *S. aureus* nasal carriage and antibiotic susceptibility in preclinical and clinical medical students at Zagazig University [22], we observed emergence of vancomycin-resistant *S. aureus* (VRSA), but only in some isolates from students who had clinical contact with patients. In the present study, where none of the participants had direct contact with patients, there were no VRSA isolates. Yet, isolates from performers of ablution, compared to those from non-performers, demonstrated higher rates of susceptibility to methicillin and several other antibiotics. This is in agreement with other studies, which found that successful decolonization of multidrug-resistant bacteria could be achieved through compliance with enforced hygiene procedure of daily repetitive decolonizing washing [16].

This study, however, *has* at least two *limitations* that deserve mention. First, the study had a cross-sectional design, which could not differentiate between persistent carriers, intermittent carriers, and non-carriers. Second, participants were students recruited from Zagazig University campus. This may limit the generalizability of our findings to students from other locations and the general population.

CONCLUSION

Performers of ablution, compared to non-performers, have reduced carriage rates of nasal *S. aureus* and MRSA and higher susceptibility profile to antibiotics. The study advocates regular ablution as an effective and inexpensive method that helps in checking the increasing problem of *S. aureus* antimicrobial resistance. However, the finding of a strong association between *S. aureus* nasal carriage and rural residence will need further investigations in relation to the water sanitation.

Conflicts of interest:
The authors declare no conflicts of interest.

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