Risk Factors for Infection of *Staphylococcus aureus*: Nasal Carriage, Skin Carriage and Multi-antibiotic Resistance in Healthy Individuals

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The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

**Aims:** The study investigated the nasal and skin carriage of *Staphylococcus aureus* in healthy individuals and the antibiotic resistance profile.

**Study Design:** A descriptive laboratory based surveillance study.

**Place and Duration of Study:** Department of Biological Sciences, Ondo State University of Science and Technology, Okitipupa, between May and November 2016.

**Methodology:** Eighty samples were obtained from anterior nares and skin of 40 healthy volunteers aged 19 to 35 years. Isolates were identified by cultural characteristics on Mannitol Salt Agar, biochemical tests. Percentage carriage of *S. aureus* was calculated separately for nasal and skin samples. Antibiotic susceptibility testing was performed by the disk diffusion method to determine the multi-antibiotic resistance (MAR) profile.

**Results:** From 40 nasal samples, 17 (42.5%) yielded *S. aureus* from 12 (30.0%) female and 5 (12.5%) male volunteers. Out of 40 skin samples, 17 (42.5%) samples yielded *S. aureus* from 8 (20.0%) female and 9 (22.5%) male volunteers. There were no differences in the
Keywords: Staphylococcus aureus; colonization; opportunistic pathogen; antibiotic resistance; MAR Index; risk factor; infection.

1. INTRODUCTION

*Staphylococcus aureus* is one of the most important and versatile bacterial pathogens. It is a normal human microbiota that can turn into a potentially lethal opportunistic pathogen. The anterior nares are the primary reservoir in humans [1,2]. The bacterium can also colonize other body sites including skin [3] throat [4], perineum [5], vagina [6,7], and gastrointestinal tract [8]. Being part of the normal microbiota, combined with its immuno-invasive strategies, make *S. aureus* successful as a human pathogen, and colonized persons are the chief source of *S. aureus* in hospitals [9]. *S. aureus* is one of the leading causes of a variety of community-acquired and hospital-acquired bacterial infections. It is unusual for its propensity to cause primary bacteremia among young, otherwise healthy people, as well as in those with risk factors, and has resulted to a recent mortality rate of 20-40% despite appropriate treatment [10]. *S. aureus* causes skin and soft tissue infections, as well as invasive and systemic diseases including post-surgical infections, toxic shock syndrome, pneumonia, endocarditis, osteomyelitis, septic arthritis, and device-related infections [11,12,13]. While invasive disease is by far the most acute and severe, the greatest burden of morbidity is due to skin and soft tissue infections, which are extremely common, often chronic, and frequently recurrent. Invasive disease continues to occur despite improved adherence to infection prevention practices, while the organism has steadily evolved resistance to every licensed anti-staphylococcal agent to date [14,15].

The nasal site is the primary reservoir of *S. aureus* in humans, and it is reported to colonize the anterior nares of 20-80% of the human population [15]. It is often the source of inoculation of other sites via hand transfer, and the greater the bacterial load in the nares, the higher the likelihood that other body sites are colonized and that the colonization is persistent [1,16]. Three patterns of carriage have been distinguished in healthy carriers: about 20% of people are persistent carriers, 60% are intermittent carriers, and approximately 20% almost never carry *S. aureus*. Nasal carriage of *S. aureus* is strongly associated with infection. Clinical studies consistently describe a significantly greater risk of bacteremia among carriers, quoting relative risks from 1.2 to 21.7 in cohorts with regular healthcare contact, especially in the presence of indwelling devices [17,18]. Endogenous colonizing strains of *S. aureus* are responsible for over 80% of nosocomial bacteremias and also increase the incidence of non-bacteremic *S. aureus* healthcare-associated infections [19,20,21,22].

The dominant *Staphylococcus* species on skin is *S. epidermidis* [23]. Other coagulase-negative staphylococci (CoNS) are also found as part of the microbial community across different body sites. [24]. Interestingly, coagulate-positive *S. aureus* is not considered to be part of the natural skin microbiota [22], but is usually transferred to the skin from the nares via hands [1,16]. Humans are frequently exposed to *S. aureus* and it colonizes most people for long or short periods at various stages throughout their lives. *Staphylococcus aureus* colonization of the human skin becomes a risk factor for infections when the integrity of the skin or mucous membrane is breached, or when the immune system of the carrier gets compromised by any other means. Superficial skin infections occur, often leading to bacteremia, and a range of systemic diseases.
S. aureus strains resistant to multiple antibiotics have emerged over the years as a response to their exposure to antibiotics as well as antibiotic abuse. In Nigeria, the multiple-antibiotic resistance (MAR) of S. aureus has been on the increase for decades and a report from a recent study revealed MAR Indices above 0.5 for 70.6% of S. aureus isolated from human sources, which indicates an unusually high level of resistance, and reveals that the isolates originate from an area where antibiotics are commonly used, or abused [14]. Another study in the Niger Delta region revealed 52.5% of S. aureus isolated from human nares were multiple-drug resistant [25].

Colonization of body parts by S. aureus is the greatest risk factor for infection, and presence of MAR strains in the nasal cavity will compound the problem of infection with limited therapeutic options. In view of this, routinely assessment of nasal carriage rate, as well as resistance profile in apparently healthy individuals will provide information that can aid in tackling the challenges of combating the many infections of S. aureus.

The present study therefore aims to determine the carriage rate of S. aureus, and the antibiotic resistance profile among apparently healthy students at Ondo State University of Science and Technology (OSUSTECH), Okitipupa, Nigeria.

2. MATERIALS AND METHODS

2.1 Sampling

Simple random sampling techniques was employed to collect nasal and skin samples from forty (40) apparently healthy student volunteers of OSUSTECH. The volunteers were 20 male and 20 female students between the ages of 19 and 35 years. Sterile swab sticks moistened with sterile water were used to collect nasal and skin swabs from each student by means of circular frictions. A total of eighty (40 nasal and 40 skin) samples were aseptically collected.

2.2 Culture and Identification

Each swab stick was immediately inoculated onto Mannitol salt agar (Oxoid UK) plates and incubated at 37°C for 24 hrs. Colonies that gave golden-yellow color (Plate 1) were isolated and subjected to Gram staining, catalase test and coagulase test according to established microbiological methods. Isolates that yielded Gram positive cocci in clusters, and gave catalase- positive and coagulase-positive tests were considered as S. aureus [26,27]. The S. aureus isolates were sub-cultured on Nutrient agar plates where they grew profusely as large, white colonies.

2.3 Determination of Percentage carriage of S. aureus

The percentage carriage of S. aureus in the nasal and skin samples of the student volunteers was calculated using the formula a/b × 100; where ‘a’ is the number of isolates from nasal cavity or skin, and ‘b’ is the number of samples collected (which is 40 for both nasal and skin samples).

2.4 Antibiotic Susceptibility Testing

The S. aureus isolates were screened using the disk diffusion method of Bauer et al, [28]. Gram positive antibiotic discs (Rapidflex) included ceftazidime 30 µg, cefuroxime 30 µg, ceftriaxone 30 µg, cloxicillin 5 µg, amoxicillin/clavulanic acid 30 µg, gentamicin 10 µg, erythromycin 30 µg, ofloxacin 5 µg. Oxacillin (1 µg) obtained from Oxoid Ltd, Britain was included in the screening.

Antimicrobial susceptibility tests were performed for all isolates according to the criteria of the Clinical and Laboratory Standards Institute [29].

2.5 Determination of Percentage Resistance and Multiple Antibiotic-resistance (MAR) Index

The percentage resistance of each S. aureus isolate to antibiotics screened was calculated using the formula a/b × 100; where ‘a’ is the number of antibiotics the isolate is resistant to; and ‘b’ is the number of antibiotics tested. MAR index of each S. aureus isolate was calculated using the formula: MAR Index = a/b, where ‘a’ is number of antibiotics the isolate is resistant to; and ‘b’ is number of antibiotics tested.

3. RESULTS AND DISCUSSION

3.1 Isolation of S. aureus

A total of eighty (80) samples were collected from the nasal and skin sites of 40 student volunteers (20 females and 20 males).
Table 1. Isolation of *S. aureus* from nasal and skin samples from healthy volunteers among student volunteers of OSUSTECH

| Sex of volunteer | Age of volunteer | Nasal sample code | *S. aureus* nasal isolate | Skin sample code | *S. aureus* skin isolate |
|------------------|------------------|-------------------|--------------------------|-----------------|--------------------------|
| F                | 35               | N1                | +                        | S1              | +                        |
| M                | 19               | N2                | -                        | S2              | -                        |
| F                | 22               | N3                | +                        | S3              | +                        |
| F                | 19               | N4                | +                        | S4              | -                        |
| M                | 24               | N5                | -                        | S5              | +                        |
| M                | 22               | N6                | -                        | S6              | +                        |
| F                | 21               | N7                | +                        | S7              | -                        |
| M                | 23               | N8                | +                        | S8              | -                        |
| M                | 22               | N9                | -                        | S9              | -                        |
| M                | 23               | N10               | -                        | S10             | -                        |
| F                | 20               | N11               | -                        | S11             | +                        |
| M                | 27               | N12               | -                        | S12             | -                        |
| M                | 25               | N13               | -                        | S13             | -                        |
| M                | 23               | N14               | -                        | S14             | +                        |
| F                | 21               | N15               | +                        | S15             | -                        |
| F                | 20               | N16               | +                        | S16             | -                        |
| F                | 28               | N17               | +                        | S17             | -                        |
| F                | 24               | N18               | +                        | S18             | +                        |
| M                | 22               | N19               | +                        | S19             | -                        |
| M                | 21               | N20               | -                        | S20             | -                        |
| M                | 27               | N21               | +                        | S21             | +                        |
| F                | 21               | N22               | +                        | S22             | +                        |
| F                | 24               | N23               | -                        | S23             | +                        |
| M                | 28               | N24               | -                        | S24             | -                        |
| F                | 23               | N25               | +                        | S25             | -                        |
| F                | 26               | N26               | -                        | S26             | -                        |
| F                | 23               | N27               | -                        | S27             | -                        |
| F                | 21               | N28               | +                        | S28             | -                        |
| F                | 22               | N29               | -                        | S29             | -                        |
| F                | 26               | N30               | -                        | S30             | -                        |
| M                | 28               | N31               | +                        | S31             | -                        |
| M                | 21               | N32               | -                        | S32             | +                        |
| M                | 26               | N33               | -                        | S33             | +                        |
| M                | 23               | N34               | -                        | S34             | +                        |
| F                | 21               | N35               | -                        | S35             | -                        |
| M                | 26               | N36               | -                        | S36             | +                        |
| M                | 25               | N37               | +                        | S37             | +                        |
| F                | 21               | N38               | +                        | S38             | +                        |
| M                | 23               | N39               | -                        | S39             | -                        |
| F                | 24               | N40               | -                        | S40             | +                        |

Nasal isolates 5(12.5%) from Males. Nasal isolates 12(30%) from Females.

Skin isolates 9(22.5%) from Males. Skin isolates 8(20.0%) from Females.
3.2 Determination of Percentage Carriage of S. aureus

3.2.1 Percentage carriage of S. aureus in nasal and skin samples

Out of a 40 nasal samples collected, 17 (42.5%) samples yielded S. aureus isolates, obtained from 12 (30.0%) female volunteers and 5 (12.5%) male volunteers. Of a 40 skin samples collected, 17 (42.5%) samples yielded S. aureus isolates, obtained from 8 (20.0%) female volunteers and 9 (22.5%) male volunteers (Table 1).

There was no difference in the number and percentage of nasal and skin samples yielding S. aureus, but there were large differences in the number/percentage of nasal samples of male and female volunteers colonized by S. aureus, while little difference was found in the number/percentage of skin samples of male and female volunteers colonized by S. aureus.

A total of 7 (17.5%) volunteers (5 females and 2 males) yielded S. aureus from both the nasal and skin samples. Ten (25.0%) volunteers yielded S. aureus from nasal samples and none from skin samples. Ten (25.0%) volunteers yielded S. aureus from skin samples and none from nasal samples (Table 1).

Table 2. Percentage (%) of S. aureus isolates from nasal cavity and skin resistant to the antibiotics

| Antibiotic | Percentage (%) of resistant isolates from nares | Percentage (%) of resistant isolates from skin |
|------------|-----------------------------------------------|-----------------------------------------------|
| CAZ        | 100%                                          | 100%                                          |
| CRX        | 100%                                          | 100%                                          |
| CTR        | 100%                                          | 100%                                          |
| CXC        | 100%                                          | 100%                                          |
| AUG        | 100%                                          | 100%                                          |
| GEN        | 0                                             | 9.1%                                          |
| ERY        | 23.1%                                         | 54.5%                                         |
| OFL        | 0                                             | 0                                             |
| OX         | 100%                                          | 100%                                          |

Key: CAZ- ceftazidime, CRX- cefuroxime, CTR- ceftriaxone, CXC- cloxacillin, AUG- amoxicillin/clavulanic acid, GEN- gentamicin, ERY- erythromycin, OFL- ofloxacin, OX- oxacillin

Carriage rates of S. aureus in the present study were found to be 42.5% respectively for both the nasal and skin sites. This supports the findings of Pant and Rai which revealed nasal colonization rates of 43.8% among staff of a teaching hospital in Nepal [30]. Contrarily, Onanuga and Temedie’s findings reveal lower S. aureus colonization of 33.3% of in the nasal cavity of volunteers in the Niger Delta region [25]. However, another study revealed a much lower prevalence of 21.6% for S. aureus in the anterior nares of healthy Europeans [31]. These findings support the report that S. aureus colonizes the anterior nares of 20-80% of the human population [15]. The high carrier rate of S. aureus in developing countries compared to developed countries in Europe may arise from lack of effective nasal decolonization strategies, lack of hygiene, and neglecting the use of hand gloves,
which is often the case in developing countries. The carriage rates of 42.5% respectively for both nasal and skin sites in this study highlight the connection between the primary nasal reservoir and colonization of skin of an individual, usually through hand transfer reported by previous authors [1,16].

This study reports a high MAR ranging from 66.7% to 88.9% for nasal and skin isolates, giving a MAR index of 0.67- 0.89. Again this is higher than MAR rates of 52.5% reported by previous workers in Nigeria [25]. MAR index shows the level of multiple antibiotic resistance attained by a particular bacterial strain. MAR indices vary from 0.00 (not resistant to any antibiotic tested) to 1 (resistant to all antibiotics tested) [14]. The high resistance rates of colonizing strains of S. aureus poses a high risk factor since previous studies have shown that nasal carriage of S. aureus is strongly associated with infection, and carries a significantly greater risk of bacteremia among carriers. Endogenous colonizing strains S. aureus are responsible for over 80% of nosocomial bacteremias and also increase the incidence of non-bacteremic S. aureus healthcare-associated infections [19,20,21,22]. Opportunistic infections with colonizing strains occur when any part of the immune system is compromised. Superficial skin infections, bacteremia and invasive/systemic diseases often result from such infections, with high morbity and significant mortality rates [12,13]. All the S. aureus isolates in this study showed 100% resistance to six antibiotics including ceftazidime, cefuroxime, ceftriaxone, cloxacillin, amoxicillin/clavulanic and oxacillin, placing a severe limitation on the choice of therapeutic agents when it comes to treating S. aureus infections. However, ofloxac, gentamicin and erythromycin recorded high susceptibilities, and are likely drugs of choice for S. aureus infections in Nigeria.

The findings of the study expose the risk factors in the likelihood of transferring MAR S. aureus strains via hands from a healthy carriers to other persons. This could occur in many instances at home or in hospitals, especially if the carriers are care-givers who nurse HIV patients and other immuno-compromised patients. Infection of HIV patients with MAR strains of S. aureus will lead to treatment failures which inevitably result in high mortality. The present study focused on the percentage nasal carriage of S. aureus in apparently healthy individuals, and did not attempt further studies on eradication and its effects, due to time constraint and lack of relevant facilities.

4. CONCLUSION

The high rate of S. aureus colonization of the nasal and skin sites of healthy young persons, combined with high MAR index of colonizing strains, highlight a potential health hazard and calls for a multi-pronged approach in achieving decolonization of the human nasal cavity. Effective nasal decolonization strategies, use of hand gloves and appropriate use of antibiotics will all work together to reduce the risk of S. aureus colonization, transfer and infection, and the consequent high morbidity and mortality. Various authors have discussed the eradication of S. aureus from the nasal cavity of carriers, using mupirocin nasal ointment in view of its epidemiology, associated risks, and the effects. Studied reveal that effects of eradication have been inconclusive in most subgroups, and only in patients on hemodialysis or chronic ambulatory peritoneal dialysis was a significant reduction of infection rate found [22]. Further studies should focus on other treatment regimens.

CONSENT

As per international standard or university standard, written consent has been collected and preserved by the author.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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