Distribution and antibiotic resistance profiles of Staphylococcus aureus strains in hospital and non-hospital environments in Nsukka

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Abstract

A total of 196 samples, made up of 130 clinical (nosocomial) samples and 66 non-clinical (community) samples were examined for Staphylococcus aureus. Antibiotic resistance profiles of the S. aureus isolates were determined. Also homogeneity/heterogeneity of the clinical and community strains was determined by comparing their plasmid profiles. Out of the 196 samples screened, 53 (27%) yielded Staphylococcus aureus. Of the 130 clinical samples, 41 (32%) were positive for S. aureus. Of these, 21 (51%) were from formites, while 20 (49%) were from patients. There was a significantly (p<0.05) lower incidence of S. aureus in the non-clinical samples with only 12 out of 66 samples (18%) yielding the organism. Also the clinical S. aureus isolates were significantly (p<0.05) more resistant to antibiotics, particularly the older antibiotics, than the community isolates. Plasmid profiling showed, however, that there was a great degree of homogeneity between the clinical and community S. aureus isolates suggesting that it is mostly the same strain of S. aureus that is circulating in both the nosocomial and community environments in Nsukka metropolis.

Keywords: Staphylococcus aureus; nosocomial; community; antibiotic resistance; plasmid profiling

INTRODUCTION

Staphylococcus aureus is a major human pathogen, which causes infections ranging from food poisoning or minor skin infections to severe life threatening infections. The organism lives as a commensal on the anterior nasal mucosa of 30 – 50% of the general population with higher carrier rates among hospital staff and patients [1, 2, 3]. S. aureus has been identified as a major cause of infections in many healthcare institutions, especially since the emergence of methicillin resistant Staphylococcus aureus, which is said to account for more than 50% of all S. aureus isolated in hospitals [4, 5]. Nosocomial occurrence of S. aureus has been attributed to the admission, to hospital, of a colonized or infected patient who serves as a reservoir or to colonized or infected health care workers who disseminate the organism directly to patients. The principal mode of transmission is via transiently colonized hands of health care workers who acquire the organism after close contact with colonized patients, contaminated equipment or their own flora [6].

In addition to those reported in hospitals, infections and outbreaks due to S. aureus are also common in nursing homes and among outpatient populations [7, 8, 9]. There has been some controversy as to the origin of the strains (clinical or community), or rather, homogeneity or heterogeneity of the strains. Some researchers have suggested that the hospital and community strains are the same and that it is the selective pressure of antibiotic therapy that makes the clinical strains recalcitrant [6]. Other researchers have suggested, however, that community-associated and hospital-associated strains of S. aureus are microbiologically distinct, possessing different gene profiles [4]. In view of all these, studies on the occurrence of the organism in hospital environments, which includes both human carriers and formites, is an important key to containment of the organism.

The aim of this study was to investigate the distribution and antibiotic resistance profiles of S. aureus strains in hospital and non-hospital environments within Nsukka metropolis and also determine homogeneity or heterogeneity of the strains by comparing their plasmid profiles.
MATERIALS AND METHODS

Collection of samples
A total of 196 samples, made up of 130 clinical samples and 66 non-clinical samples, were used in this study. The clinical samples comprised 62 nasal swab specimens from patients in three different hospitals (Hospital A, 30; Hospital B, 16; and Hospital C, 16) and 68 swab specimens from formites in the three hospitals (Hospital A, 20; Hospital B, 34 and Hospital C, 14). The non-clinical samples comprised nasal swabs from non-hospitalized individuals residing around the hospitals and away from the hospitals. To collect the nasal swab of each subject (patient or healthy), a sterile swab was carefully removed from its container, gently rolled over both anterior nares of the individual and replaced in its container. To collect the specimens from formites, sterile swabs were removed from their containers, rolled over the surfaces to be sampled and then replaced in the containers. Surfaces sampled included tables, chairs, beds, windows and floors. All samples were returned to the laboratory for culture within three hours.

Isolation and identification of organisms
All samples were inoculated onto Nutrient agar (Oxoid), containing 7.5% sodium chloride and incubated at 37°C for 24 h under aerobic conditions. Following incubation, resulting colonies were characterized in terms of Gram reaction, catalase and coagulase production. Colonies testing Gram positive, catalase and coagulase positive were considered to be Staphylococcus aureus. The S. aureus isolates were purified by subculturing and stock cultures were prepared on Nutrient agar slants and stored at 4°C.

Antibiotic susceptibility testing
Antibiotic sensitivity tests were carried out on all isolates considered to be S. aureus. Susceptibility testing was done by the disc diffusion method in accordance with CLSI [10] and Swedish Reference group for antibiotics standards [11]. Isolates were tested against a panel of ten antibiotics: ciprofloxacin (CPX), 10 µg; norfloxacin (NB), 10 µg; gentamycin (CN), 10 µg; lincomycin (LC), 20 µg; streptomycin (S), 30 µg; rifampicin (RD), 20 µg; erythromycin (E), 30 µg; chloramphenicol (CH), 30 µg; ampiclox (APX), 20 µg and floxapen (FLX), 20 µg. Inhibition zone diameters were measured in millimeters and susceptibility scored as resistant, intermediate or sensitive, according to CLSI guidelines.

Plasmid DNA profiling
Twenty three clinical and ten non-clinical S. aureus isolates were randomly selected for plasmid profiling. Also, a reference S. aureus strain (ATCC 12600) served as a standard. Plasmid DNA was extracted from both the test isolates and the reference strain by a modified alkaline lysis method of Zhou et al. [12]. The strains were inoculated into different sterile test tubes containing nutrient broth and incubated for 72 h at 37°C. Thereafter, the cells were spunned at 10,000 rpm for 5 min in a microcentrifuge to pellet cells. The supernatants were gently decanted leaving the cell pellets. Lysis buffer (300 µl) comprising 10 mM Tris-HCL, 1 mM EDTA, 0.1 N NaOH and 0.5% sodium dodecyl sulphate (SDS) was added to the cell pellets and vortexed at high speed for 2 – 5 seconds until the mixtures became sticky. Then, 150 µl of 3.0 M sodium acetate (pH 5.2) was added. The mixtures were again centrifuged for 5 min in the microcentrifuge to pellet cell debris and chromosomal DNA. The supernatants were then transferred to fresh tubes and mixed well with 0.9 ml of 100% ethanol, which was pre-cooled to -20°C. The tubes were again spunned for 5 min to pellet plasmid DNA. The supernatants were discarded and pellets were rinsed twice with 1 ml of 70% ethanol and dried by evaporation. The pellets were then resuspended in 20 – 40 µl of TE buffer for further use.

Plasmids were separated by electrophoresis on a 1.3% agarose gel at 60 V. The samples were loaded into the gel wells along with Hind III digest of lambda DNA (Sigma chemicals) used as molecular weight standard. Molecular weights of the plasmids were determined from a standard plot of molecular weights of the Hind III DNA fragments against their mobilities.

Statistical analysis
Differences between the occurrences of S. aureus in hospital and non-hospital environments and in the different hospitals were analysed by the Chi-square ($\chi^2$) test. Significance was determined at the 95% confidence level.

RESULTS

Out of a total of 196 samples screened, 53 (27%) yielded Staphylococcus aureus. Out of 130 clinical samples, 41 (32%) were positive for S. aureus. Of these, 21 (51%) were from formites, while 20 (49%) were from patients. Of the 66 non-clinical samples, on the other hand, only 12 (18%) were positive for S. aureus. These results are summarized in Table 1. The difference between occurrence of S. aureus in the hospital and non-hospital samples was significant.
Table 1. Occurrence of \textit{S. aureus} in Clinical and Non-Clinical Samples

<table>
<thead>
<tr>
<th>Source of sample</th>
<th>Number of samples</th>
<th>Number yielding \textit{S. aureus} (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospital</td>
<td>130</td>
<td>41 (32)</td>
</tr>
<tr>
<td>Non-hospital</td>
<td>66</td>
<td>12 (18)</td>
</tr>
<tr>
<td>Total</td>
<td>196</td>
<td>53 (27)</td>
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</tbody>
</table>

A consideration of individual hospitals used in the study, showed that 36%, 34% and 20% of samples from Hospitals A, B and C, respectively, yielded \textit{S. aureus} as shown in Table 2. These differences in occurrence were, however, not statistically significant.

Table 2. Occurrence of \textit{S. aureus} in different hospitals

<table>
<thead>
<tr>
<th>Hospital</th>
<th>Number of samples</th>
<th>Number yielding \textit{S. aureus} (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>50</td>
<td>18 (36)</td>
</tr>
<tr>
<td>B</td>
<td>50</td>
<td>17 (34)</td>
</tr>
<tr>
<td>C</td>
<td>30</td>
<td>6 (20)</td>
</tr>
<tr>
<td>Total</td>
<td>130</td>
<td>41 (32)</td>
</tr>
</tbody>
</table>

The antibiotic susceptibility assay showed that the \textit{S. aureus} isolates in this study had percentage resistance to different antibiotics ranging from zero to 22%. Resistance was higher among clinical isolates than in non-clinical isolates (Figure 1). Whereas resistance was recorded for every antibiotic amongst clinical isolates, resistance was recorded for only three antibiotics amongst the community isolates. Resistance was highest to floxapen, followed by lincomycin and ampiclox, particularly among clinical isolates with recorded values of 22%, 20% and 17% for the three antibiotics respectively. The least resistance was to ciprofloxacin with 7% resistance.

Multidrug resistance (resistance to three or more antibiotics) was recorded in some (20%) of the clinical isolates, while none of the community isolates was multidrug resistant. Four of the clinical isolates were resistant to all ten antibiotics.

![Figure 1](image_url)

**Figure 1. Comparison of resistance of clinical and non-clinical \textit{S. aureus} isolates to individual antibiotics.**

\(FLX = \) floxapen; \(S = \) streptomycin; \(NB = \) norfloxacin; \(CH = \) chloramphenicol; \(CPX = \) ciprofloxacin; \(E = \) erythromycin; \(LC = \) lincomycin; \(CN = \) gentamycin; \(APX = \) ampiclox and \(RD = \) rifampicin.

Experiments to determine homogeneity or heterogeneity of the \textit{S. aureus} isolates by plasmid profiling, showed that for the most part, there was homogeneity among the isolates, particularly the clinical isolates. Most of the isolates showed two plasmid bands, with molecular weights of 24 and 32 kb, on agarose gel (Figures 2 and 3). The same plasmids were also present in the reference \textit{S. aureus} strain, included as control, as well as in some of the non-clinical isolates (Figure 4). Some of the non-clinical isolates, however, had different profiles as shown in Figure 4. Some had no plasmids (Fig. 4, lane 3) while some had only one plasmid band (Fig. 4, lanes 10 and 11).
Figure 2. Plasmid profile of *Staphylococcus aureus* isolates from Hospital A. Lane 1 contained molecular weight standard; No sample was loaded in lane 2; Lanes 3 – 15 were loaded with plasmid DNA from *S. aureus* isolates. Only one isolate did not contain plasmids (lane 3). All other isolates contained two plasmids of molecular weights approximating 24 kb and 32 kb, respectively.

Figure 3. Plasmid profile of *Staphylococcus aureus* isolates from Hospital B. Lane 1 contained molecular weight standard; No sample was loaded in lane 2; Lanes 3 – 12 were loaded with plasmid DNA from *S. aureus* isolates. All isolates contained two plasmids of molecular weights approximating 24 kb and 32 kb, respectively.

Figure 4. Plasmid profile of non-clinical (community) *Staphylococcus aureus* isolates. Lane 1 contained molecular weight standard; No sample was loaded in lane 2; Lanes 3 – 12 were loaded with plasmid DNA from *S. aureus* isolates; Lanes 13 and 14 contained plasmid DNA from a reference *S. aureus* strain (ATCC 12600). One isolate (lane 3) did not contain plasmids. Two isolates (lanes 10 and 11) contained only one plasmid of molecular weight 32 kb. All other isolates contained two plasmids of molecular weights approximating 24 kb and 32 kb, respectively.

**DISCUSSION**

*Staphylococcus aureus* is a human pathogen which lives as a commensal on the anterior nasal mucosa of 30 – 50% of the general population [3, 13]. Between 20 and 35% of the population are persistent carriers, about 60% are intermittent carriers, while others never carry the organism [13, 14]. Studies have suggested that nasal carriage of the organism is significantly higher in hospitalized subjects, ranging from 20 to 45%, than in non-hospitalized individuals with 15 – 30% carriage rate [1, 2, 3]. The results from this study are consistent with these reports, showing a 32% incidence of *S. aureus* carriage (20 out of 62 samples) among hospital patients and a significantly lower incidence of 18% carriage in the community.
The principal mode of transmission of *S. aureus* in the hospital is suggested to be via transiently colonized hands of health care workers who acquire the organism after close contact with colonized patients, contaminated equipment or their own flora [6]. There is need, therefore, to consider both human carriers and formites, as important keys to containment of the organism. This study has certainly shown a high rate of occurrence of *S. aureus* (20 to 36%) among individuals as well as formites in all the hospitals sampled. This presents a serious concern in terms of nosocomial infections.

Compounding the problem of nosocomial *S. aureus* infections is the problem of antibiotic resistance. Most nosocomial infections including those of *S. aureus*, are caused by bacteria resistant to multiple antibiotics. Studies suggest that multidrug resistance rates remain highest among nosocomial strains as compared to community-acquired strains [6, 15]. Results from this study tend to agree with this trend, showing that the *S. aureus* isolates in this study had percentage resistance to different antibiotics ranging from zero to 22%. Resistance was higher among clinical isolates than in non-clinical isolates as shown in Figure 1.

There has been some debate concerning whether the nosocomial strains originate from the community and then become resistant in the hospital environment or whether the community strains originate from the hospital, and opinions on this vary. What is clear, however, is that resistant strains are increasingly found in the community even among individuals who have never been hospitalized [5, 16, 17]. Various studies suggest that whether community or hospital-acquired, some resistant bacteria have evolved locally, whereas for others, there is evidence for the international spread of specific clones, particularly for methicillin-resistant *Staphylococcus aureus* [MRSA] [6]. The findings in this study make a stronger case for local evolution of resistance because the results showed higher resistance amongst isolates to older and more commonly administered antibiotics (floxapen, lincomycin and ampiclox) than to ciprofloxacin, chloramphenicol or gentamicin. Hence suggesting drug overuse or misuse as a possible cause of resistance.

Homogeneity/heterogeneity of the nosocomial and community isolates was investigated in this study by comparing their plasmid profiles. The results showed that to a great extent, there was homogeneity between the nosocomial and community isolates. However, a few of the community isolates were different. Reports from other investigators have suggested that community-acquired strains are genetically distinct, at least in relation to meticillin resistance [6, 18]. So, the results from this study can only be said to partially agree with this. For the most part, the results suggest that the nosocomial and community *S. aureus*, in this study, are the same and that there is a common index in their occurrence.

**REFERENCES**


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