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(RESEARCH ARTICLE)

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Antibiotic susceptibility pattern and plasmid profiles of nasal staphylococci from apparently healthy Nigerians

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Abstract

A total of 480 nasal samples from apparently healthy Nigerian students were collected aseptically and analyzed bacteriologically. Staphylococci were recovered from 432 (90%) of the subjects, constituting 288 (66.7%) and 144 (33.3%) of *S. aureus* and *S. epidermidis* respectively. The *in-vitro* antibiotic susceptibility testing using the disc diffusion technique showed high multiple resistance to the most commonly used antibiotics by *Staphylococcus aureus* such as penicillin (98.6%), ampicillin (97.2%), tetracycline (95.8%) and streptomycin (84.7%), but less resistance to all the antibiotics tested. Sixty percent of *S. aureus* harbored plasmids which molecular sizes ranged from 0.1 to 12.0 kilobases. The high prevalence of multiple antibiotic resistance appear to be plasmid mediated as plasmid profile analysis showed that about 90% of *S. aureus* harbored plasmids

Keywords: Staphylococci; S. aureus; S. epidermidis; Antibiotic susceptibility pattern; Plasmid Profile analysis

1. Introduction

Staphylococci are ubiquitous colonizers of human epithelial and mucous membrane [1]. The anterior nares are the primary ecological reservoir of *staphylococcus aureus* in humans and *S. aureus* nasal carriage is a major risk factor for a variety of infections [2]. Frequent carriage of *S. aureus* in the anterior nares have been consistently documented [3,4,5]. *Staphylococcus aureus* is the causative agent of many opportunistic infections in humans and animals [6]. *S. aureus* causes superficial deep-skin and soft tissue infections, endocarditis and bacteremia as well as toxin- mediated diseases including gastroenteritis, staphylococcal scalded skin syndrome and toxic shock syndrome [7]. Many pathogenic aerobic and anaerobic microorganisms from the environment often colonize different sites of the human body such as the nasopharynx, on the skin clothing or more rarely in the vagina [8,9].

The nasal cavity is colonized by different pathways such as through hospital equipment, direct contact through lesions discharging pathogenic microorganisms [8,10]. According to Kluytmans [9], *S. aureus* has long been recognized as an important human pathogen and staphylococcal infections occur regularly in hospitalized patients with severe consequences despite antibiotic therapy. Almost everybody will have some type of staphylococcal infection during a lifetime which range in severity from food poisoning or minor skin infection to severe life-threatening infections [11].

Three human nasal *S. aureus* carriage patterns can be distinguished: persistent carriage, intermittent carriage and noncarriage. *S. aureus* density in the anterior nares is highest in persistent carriers, which will explain their increased risk of developing *S. aureus* infections [12].

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S. aureus often possess the capacity to counter almost all the antimicrobial agents available to the clinicians for the treatment of severe staphylococcal infections [6,12]. Resistance to such antimicrobial compounds occur either through spontaneous mutation or are acquired through transmission from other related resistant bacteria [13,14]. Transfer of resistance through plasmids which contains genes for single of multiple antimicrobial agents have been reported in several bacterial pathogens including *S. aureus* [11,15]. However, resistance of staphylococci to a wide array of clinically useful antibiotics including streptomycin, rifampicin, fusidic acid and novobiocin has been attributed to chromosomal mutation [11]. Plasmid profiles have been of significance in epidemiological surveillance of disease outbreaks as well as in tracing sources of antibiotic resistance [12]. The possibility of transfer of bacteria harboring such plasmids from patients to apparently healthy individuals is not unlikely. The aim of the study is to isolate and identify Staphylococci from noses of apparently healthy undergraduates, assess the antibiotic susceptibility pattern, screen *S. aureus* for plasmids and determine any relationship between antimicrobial resistance of staphylococci and carriage of plasmids.

2. Material and methods

2.1. Sample Population

Four hundred and eight apparently healthy students of the Faculty of Science, Lagos State University, Ojo Campus were randomly screened for nasal carriage of staphylococci after obtained consent from students of each of the three Departments: Biochemistry, Botany and Microbiology.

2.2. Biological Analysis of Samples

Four hundred and eighty nasal swabs from the students were examined bacteriologically between January 2017 and December, 2018. Each swab stick (Even pore) was moistened with a sterile distilled water prior to introduction into the nose and was streaked – inoculated directly into sterile blood agar and Staphylococcus 110 agars (Oxoi, Basingoke, U.K). The plates which were in duplicates were then incubated aerobically and at 5% CO₂ for 24 hours respectively. Staphylococci were identified on the basis of colonial, morphological and biochemical tests such as Gram staining, presence of coagulase positive cocci in clumps, the detection of coagulase production with fresh rabbit plasma and fermentation of mannitol [16].

2.3. Antibiotic Susceptibility Testing

The *S. aureus* were tested for the susceptibility to ten different antimicrobial agents using the disc diffusion technique [17]. Bacterial strains inoculated into Mueller-Hinton broth (Oxoid, Basingstoke, U.K) and incubated at 37°C for 18-24hrs. the inoculum was standardized by adjusting the turbidity of an actively growing broth culture to match the barium sulphate (0.5 McFarland) standard. Mueller-Hinton agar plates (Oxoid, Basingoke, U.K) were then inoculated with bacterial suspensions and spread as evenly as possible. Standard sensitivity discs with the following concentration were introduced with a sterile forceps: Penicillin (10 μ g), Amoxicillin (10 μ g), chloramphenicol (50 μ g), erythromycin (15 μ g), gentamicin (10 μ g), tetracycline (30 μ g), streptomycin (10 μ g), cloxacillin (10 μ g), Rocephin (10 μ g) and peflacin (10 μ g) and incubated at 37°C for 18-24hrs. After incubation, the diameter of the zone of inhibition was measured to the nearest millimeter and interpreted [17,18]. *Staphylococcus aureus* strain ATCC 25923 was used as control.

3. Plasmid profile analysis

Plasmid DNA was isolated by the technique of Birnboim and Doly [19] modified by the use of lysostaphin for lysing the cells. Each *S. aureus* isolate was inoculated into 3ml tryptic soy broth and incubated overnight on a roller drum at 37°C. About 1.5ml of each overnight broth culture was transferred into Eppendorf tubes and centrifuged for 1 min at 5,000 rpm at room temperature. The supernatant was discarded and 2μ l of lysostaphin solution (1.0 µg/ml in distilled water) added to the pellet. Tubes were capped, vortexed and placed in ice for 30min. 200µl of alkaline detergent (0.2NaOH; 1% SDS) was added and the tubes were inverted several times and kept in the water bath for 5min. 150µl of 3M sodium acetate (pH 4.8) was added and the tubes inverted several times to mix and kept iced for at least 10 mins. The tubes were centrifuged at room temperature at 15,000 rpm for 5 min and the supernatant transferred into new Eppendorf tubes. 1ml of 95% ice cold ethanol was added to the test tubes which were kept at -20°C for 5min. After 5min, they were centrifuged at 1500 rpm for 3mins; supernatant discarded and the sediment resuspended in 50 µl of Tris/EDTA (10Mm Tris HCL and 1Mm EDTA pH 8.0)

3.1. Agarose gel electrophoresis of plasmid DNA.

Plasmid DNA electrophoresis was carried out in a 0.7% agarose gel (sigma) with Tris-borate-EDTA buffer pH 8.3 [19] at 45Ma for 2h at room temperature using an LKB vertical electrophoresis apparatus (LKB, Bromma). The gels were

stained with ethidium bromide (sigma) for 20min and then photographed with Polaroid 665 film under short wave UV light through a red filter.

4. Results

A total of 432 staphylococci were identified comprising 288 (66.7%) *S. aureus* and 144 (33.3%), *S. epidermis.* A high frequently of resistance by *S. aureus* to penicillin (98.6%), ampicillin (97.22%), tetracycline (95.8%), streptomycin (84.7%) and chloramphenicol (55.6%) was observed. The staphylococci however showed susceptibility to cloxacillin, gentamicin, erythromycin, Rocephin and peflacin (Table 1).

The *S. aureus* isolates were grouped into eleven plasmid profile groups on the basis of the number and molecular weights of the plasmid they harbor. The number of plasmids per isolate ranged between 3 to 6 but most isolates had four plasmids each. The molecular sizes of the plasmids (0.7, 1.4, 1.6kb) most commonly encountered (Table 2). The *S. aureus* which showed very high multiple resistance to many antibiotics had more plasmid with high molecular sizes. Multiple resistance decreased with decreasing plasmid size.

Antimicrobial agent	<i>S. aureus</i> N=288(%)	S. epidermis N=144(%)
Ampicillin	280 (97.2)	66.7
Penicillin	282 (95.8)	104 (72.2)
Tetracycline	276 (95.8)	96 (66.7)
Streptomycin	244 (84.7)	76 (52.8)
Chloramphenicol	160 (55.6)	56 (38.9)
Cloxacillin	44 (15.3)	16 (11.1)
Gentamycin	36 (12.5)	16 (11.1)
Erythromycin	28 (9.7)	12 (8.3)
Rocephin	24 (8.3)	16 (11.1)
Peflacin	12 (4.2)	8 (5.6)

Table 1 Antibiotic Resistance pattern of staphylococci from Nigerian students

Table 2 Antibiotic Resistance Pattern and Plasmid Profile of S. aureus

Antibiotic resistance pattern	No (no=60)	Plasmid size
A C Cl E G Pf Pn R S T	13	1.0, 1.5, 6.0, 11.0, 12.5
A C Cl E G Pn R S T	8	1.0, 1.4, 3.5, 10.0, 12.0
A C Cl E G Pn S T	7	1.0, 1.5, 3.5, 6.0
A C Cl G Pn S T	6	1.0, 1.5, 1.8, 2.8, 5.5
A C Cl Pn S T	6	0.7, 1.4, 1.6, 4.0
A C Pn S T	4	0.7, 1.6, 4.0
A PnST	2	1.0, 1.6, 2.0, 2.5
A Pn T	3	1.0, 1.8, 2.5, 2.8
A Pn	5	0.7, 1.2, 1.6, 1.8
0	6	None

A – Ampicillin, C – chloramphenicol, Cl – cloxacillin, E – erythromycin, G – Gentamycin,

Pf – Peflacin, Pn - Penicillin R – Rocephin, S – streptomycin, T – Tetracycline.

5. Discussion

Several microorganisms have been reported to contaminate some sites on the skin or mucous membrane and other part by interpersonal transfer by air or by direct contact [8]. However, the nasal cavity can be contaminated through handling of specimens, direct contact with a carrier or contact with unsterilized hospital equipment or contact with infected animals [20].

This study revealed a high nasal carriage of *S. aureus* constituting 66.7% of the 432 bacterial isolates from apparently healthy undergraduates studied. The high prevalence of *S. aureus* observed in this study underscores its significance as this organism has been implicated in several human diseases such as skin abscess, endocarditis, osteomyelitis, meningitis etc. [21]. This finding agrees with an earlier report by Kluytmans [9], that a large proportion of apparently healthy population harbor *S. aureus* intermittently. Routine assessment of the carrier rate of *S. aureus* on patient particularly those with skin puncture such as hemodialysis, continuous ambulatory dialysis (CAPD), intravenous drug users, and even in human immunodeficiency virus patients are therefore important [9]. This finding also conforms *with that of* Lamikanra [22], *who reported* a higher prevalence of *S. aureus* from apparently healthy Nigerian students. However, the findings in this study are significantly higher than other studies cited by Rodoguez et al. [24] wherein rates ranged from 7.6 to 53.8%. the substantial proportion of staphylococci isolates in a non-nosocomial setting as identified in this study are of great concern.

The recovery of *S. epidermidis* from 33.3% of the undergraduates is of serious health concern as *S. epidermidis* has been reported to possess virulent factors often similar to that of *S. aureus*, thus may be more intrinsically virulent than previously thought [23]. A similar finding with higher rate of *S. aureus* as against *S. epidermis* was reported [22].

There were no significant differences in age and sex distribution of students sampled, *majority* of female and male who harbor *S. aureus* fell within the 20 to 25 age range with frequencies of 52.8% and 47.2% in females and males respectively.

Wang [26], reported no significant differences in the age and certain behaviors as well as the environment where the person work or live. Nawa's and Fakhoury [15] however, reported a high carrier rate of *S. aureus* among hospital staff.

The high frequency of *in-vitro* resistance demonstrated by *S. aureus* against the commonly used antibiotics like penicillin, chloramphenicol, tetracycline and streptomycin is quite astonishing. A high carrier rate of *S. aureus* with a much as 90% resistance to penicillin has been reported [8,25].

Since different strains of *S. aureus* differ in sensitivity to different antibiotics, the choice of antibiotic in the treatment of patients should be based on sensitivity tests made on isolates from patients [22]. Resistant bacteria may transfer resistance genes to other bacteria hence become important in the spread of antibiotic resistance [12]. Indiscriminate use of antimicrobial agents and antibiotics sale such as sale of antibiotics without prescription, sale of under-dose and substituting brands enhances development of drug resistance [25]. The high occurrence of multiple antibiotics resistance observed in the staphylococci studied may be due to abuse of such drugs in the community and previous exposure of the subject to the antibiotics as earlier reported by Merhase [29]. The dominant role played by *S. aureus* in post-operative wound infections, which source is usually endogenous is also likely among our subjects. These findings agree with earlier reports by several authors such as Kluytmans *et al.*, [9], from 20% upwards, Perez-Fortan *et al.*, [30] with 30 - 7-%, 40% by Joklik *et al.*, [31], and 40 - 50% carrier rate of *S. aureus* by Jawetz [22]. This underscores the need to screen individuals for nasal carriage of *S. aureus* routinely during medical examination. A compulsory preoperative procedure of nasal carriage evaluation of patients is hereby strongly advocated.

Nasal culture has been acknowledged as being a suitable source for the detection of *S. aureus* as the anterior important site of colonization and source of dissemination of *S. aureus* thus associated with a higher rate of staphylococcal infection [14].

The frequency of sampling has been reported to affect the rate of colonization especially among medical staff caring for possible multiple antibiotic resistant *S. aureus* (MARSA), patients which may be transient, short-term or persistent as a single random sampling of subjects may not give the precise rate of colonization with *S. aureus* [30]. Several reports however suggested that colonization may occur at different sites of the body, so culture of multiple sites has been recommended in order to ensure detection of carriage of MARSA particularly methicillin resistant *S. aureus* (MARSA) [14]. In addition, carriage of MARSA has been further compounded by the economic downturn leading to low patients' turnout in hospitals who often patronize traditional medical practitioners alternatively [31].

The lowest prevalence of *S. aureus* observed among the microbiology students can be attributed to the aseptic conditions as well as consciousness of contaminants while working in the laboratory as against other students.

Plasmid profile have been reported to be useful in tracing the epidemiology of antibiotic resistance [12]. It was observed that 85% of the 60 *S. aureus* studied which were multiple antibiotic resistant harbored plasmids. In addition, the *S. aureus* isolates which harbored large molecular size plasmids exhibited high multiple antibiotic resistance thus suggests that plasmid is likely to play a major role in the antibiotic resistance of *S. aureus*. However, it was observed in this study that resistance to various antibiotics was not associated with the presence of specific plasmid or genes. Resistance to antibiotics by *S. aureus* may be likely located either on the chromosomes, plasmids, transposons or integron [11]. *S. aureus* which have plasmids ranging from 5.0 to 12.5Kb *were* resistant to between 5 to 10 different antibiotics. We were however, unable to isolate and relate specific plasmid to specific antibiotic.

6. Conclusion

The high nasal carriage of staphylococci was observed among apparently healthy Nigerians. More of the staphylococci were *S. aureus* and less of S. epidermis. *S. aureus* were multiply resistant to commonly used antibiotics and susceptible to the less commonly used and more expensive antibiotics. Multiple resistance in *S. aureus* is likely to be plasmid mediated as *S. aureus* resistant to 5 to 10 different antibiotics had plasmids which ranged from 5.0 to 12.5 kb. Further studies on nasal carriage of methicillin-resistant staphylococci (MRSA) is strongly recommended.

Compliance with ethical standards

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Disclosure of conflict of interest

Not applicable.

Disclosure of informed of consent

The subjects were informed of the purpose and significance of the study, so only volunteers who gave informed consent were recruited for the study while those who declined were excluded.

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