

Full Length Research Paper

Antibiotic resistant *Staphylococcus aureus* in Abia State of Nigeria

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A total of 70 ear and nasal swab samples collected from 35 persons, 16-hospital population and 19 non-hospital population was examined for presence of *Staphylococcus aureus*. Eighty percent of the population studied were found to be carriers of *S. aureus*. Of the 28 positive cases, 35.7% were carriers of *S. aureus* in both the ear and nostrils, while 14.3% and 50.0% had it only in their ear and nostrils, respectively. The *S. aureus* isolates varied in their antibiotic susceptibility pattern when tested for their sensitivity to 16 antibiotics. Eighty percent of the isolates were resistant to more than one antimicrobial agent. All the isolates showed resistance to nalidixic acid and 100% sensitivity to rifampicin.

Key words: *Staphylococcus aureus*, antibiotic resistance, inhibition zone diameter.

INTRODUCTION

Staphylococcus aureus is a Gram-positive, catalase positive, coagulase positive, non-motile coccus bacterium that causes a variety of human infection in all age groups (Boyce, 1981). It is the major causative agent in surgical wound infections and epidermal skin diseases in newborn infants (Baldwin et al., 1957). *S. aureus* infection may also be superimposed on superficial dermatological diseases such as eczema, pediculosis and mycosis (Kloos and Bannerman, 1995). They live as commensals in anterior nares of over half the population of humans (Doig, 1981). The cocci are spread from these sites into the environment by the hands, handkerchief, clothing and

dust. *S. aureus* is an opportunistic pathogen in the sense that it causes infection most commonly in tissues and sites with lowered host resistance such as in individuals with diabetes, old malnourished persons and other chronic cases (Burnett et al., 1996).

S. aureus causes folliculitis, boil, furunculosis, scalded skin syndrome, conjunctivitis, paronychia, mastitis, and toxic shock syndrome for menstruating women who use tampons. Staphylococcal pneumonia can occur if staphylococcal infection spreads to the lungs (Klodkowska-Farner, et al., 1995). Hospital acquired Staphylococcal infections are common in newborn babies, surgical patients and hospital staff. Patients develop sepsis in operation wounds, which take place in the theatre during operation, and others post-operations in the ward (Tuo et al., 1995). Staphylococcal food poisoning can also occur in which a toxin produced by

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the bacteria is ingested with food. Food with a high salt or sugar content favours the growth of *S. aureus* (Tuo et al., 1995). Many outbreaks of staphylococcal food poisoning result from hand contacts (Bryant et al., 1998).

Attempts to control these diseases by chemotherapy through the use of antimicrobial agents particularly antibiotics have resulted in increased prevalence of resistance to these agents (Levy, 1998). Several investigations have been conducted to study the antimicrobial resistance pattern of *S. aureus* and it has been shown that the organism is resistant to β -lactam antibiotics, amino glycoside and macrolides (Atkinson and Lorian, 1984; Maple et al., 1989). *S. aureus* strains carry a wide variety of multi-drug resistant genes on plasmids, which can be exchanged and spread among different species of Staphylococci (Neihart et al., 1988).

The multi-resistance determinants can be transferred to new bacterial hosts. The situation is made more difficult in developing countries such as Nigeria where antimicrobial drugs are readily available to consumers across the counter with or without prescription from a medical practitioner. Such a practice has led to misuse of antimicrobial drugs with the associated high prevalence of drug resistance among the Staphylococci (Nnochiri, 1973; Adekeye, 1979; Paul et al., 1982). Hospital strains of *S. aureus* are usually resistant to a variety of different antibiotics. Few strains are resistant to all clinically useful antibiotics except vancomycin. Some workers have reported however the presence of vancomycin resistant strains (Aubry-Damon et al., 1998; Shakibaie et al., 2002). This work was undertaken to determine the prevalence of antibiotic resistant *S. aureus* in hospital and non-hospital populations in Abia State of Nigeria.

MATERIALS AND METHOD

Antibiotics and media

Antibiotic discs used and their concentrations were as follows: penicillin (30 μ g/disc), rifampicin (10 μ g/disc), peflacin (10 μ g/disc), streptomycin (30 μ g/disc), gentamycin (10 μ g/disc), lincomycin (30 μ g/disc), ciprofloxacin (10 μ g/disc), nalidixic acid (30 μ g/disc), chloramphenicol (25 μ g/disc), septrin (cotrimoxazole) (25 μ g/disc), erythromycin (25 μ g/disc), tetracycline (30 μ g/disc), ampicillin (25 μ g/disc), ampiclox (30 μ g/disc), amoxil (Amoxycillin) (10 μ g/disc), cloxacillin (12.5 μ g /disc). Mannitol salt agar and Nutrient agar are the media used.

Sample collection

Ear and nasal swabs were collected from hospitalized patients and staff of Priscilla Hospital and New Era Hospital in Umuahia (referred to as Hospital population) and students of Michael Okpara University of Agriculture Umudike, Abia State, Nigeria (referred to as non-hospital population) using sterile swab sticks (EVEPON). All specimens were transported to the laboratory and cultured within 3 to 4 hours of collection. A total of 70 samples (32 hospital and 38 non-hospital population) were collected.

Isolation and characterization of bacteria

The swab specimens were inoculated on mannitol salt agar (Difco) and streaked with sterilized wire loop so as to obtain discrete colonies. The plates were incubated at 37°C for 24 h under aerobic conditions. After 24 h of incubation, the culture plates were examined recording the appearance, size, colour, and morphology of the colonies. Gram stain reaction, catalase test and coagulase test were carried out. Isolates that were gram-positive cocci, catalase positive, and coagulated human plasma were considered *S. aureus* in this study.

Susceptibility of Isolates to various antibiotics

Antibiotic sensitivity test was carried out on all isolates using paper disc diffusion technique. A total of 16 antibiotics shown above were tested. A 0.2 ml of 12-h peptone water culture of the test organism was used to inoculate on a dry sterile nutrient agar plate. This was spread over the entire surface of the nutrient agar using a sterile glass spreader and allowed to dry for about 15 to 30 min. The antibiotic discs were placed on the agar using sterile forceps. Each disc was placed far from each other to avoid their zones of inhibition from coalescing into the other. The plates with the antibiotic discs were then incubated at 37°C for 24 h to observe the zones of growth inhibition produced by the antibiotics.

Table 1. Frequency of isolation of *S. aureus* from hospital and non-hospital population.

Source	Number sampled	<i>S. aureus</i> positive (%)
Hospital	16	14 (87.5%)
Non-hospital	19	14 (73.7%)
Total	35	28 (80%)

Table 2. Frequency of isolation of *S. aureus* from nostril and ear.

Source	Pattern of <i>S. aureus</i> colonization (%)		
	Ear and nostril	Ear only	Nostril only
Hospital	35.7	14.3	50
Non-hospital	35.7	14.3	50

RESULTS

Of 35 persons screened, 80.0% were positive for *S. aureus*. Table 1 shows that out of 16 persons from the hospital population (Hp) screened, only 2 did not harbour any *S. aureus* and 14 (87.5%) were colonized either in the ear or nostril or both. Out of nineteen persons from non-hospital population (NHp), 5 did not harbor any *S. aureus*, while 14 (73.7%) were colonized. In both populations, 14 persons each were colonized in ear and nostril (Table 2).

Table 3. Inhibition zone diameter (IZD) of test antibiotics against different isolates of *S. aureus* from non-hospital sources.

Code of Strains	Antibiogram																
	NA	CO	CP	TE	PF	ST	SP	LN	AP	PN	GN	ER	CL	RF	AX	AC	PR
NHP-1E	R	S+++	S+++	R	S++	S+	R	S+++	R	R	R	S++	R	S+++	R	S++	50
NHP-1N	R	R	R	R	R	S+	R	R	S++	R	R	R	R	R	R	R	87.5
NHP-2E	R	S++	S+++	R	S++	S+	S+	S++	R	S+	R	S++	R	S+++	R	S+	37.5
NHP-2N	R	R	S++	R	R	S+	R	S+	S+	S+	S+	R	S+	S+	R	S+	43.8
NHP-3E	R	S+++	S++	R	S++	S+	R	S++	R	S+	S+	S+	S+	S+	R	S+	31.3
NHP-4E	R	S+++	S++	S+	S++	R	R	S+	S+	S+	R	S++	R	S+	R	R	43.8
NHP-4N	R	S++	S++	S+	S++	R	S+	S+	R	S+	S++	S++	R	S++	R	S+	31.3
NHP-5E	R	S++	S+	R	S+	S++	S++	S++	R	R	S++	R	S+	S+	R	R	43.8
NHP-6N	R	S++	S+	R	R	S+	R	R	R	R	S++	S++	R	S+++	R	S++	56.3
NHP-7N	R	S+++	S+	S+	S++	S+	R	R	S+	S+	S+	S+	R	S+	R	S++	31.3
NHP-8N	R	S++	S+++	S++	S++	S++	S++	S+	R	R	R	S++	S+	S+	R	S++	25
NHP-11N	R	S++	S++	R	S++	S+	R	S+	R	R	S++	S++	R	S+++	R	S+	43.8
NHP-12N	R	S++	S+	S+	R	S++	R	S++	R	R	S+	R	R	S++	R	R	56.3
NHP-15E	R	S++	S+++	R	S++	S++	R	S++	S++	S+	S++	S++	R	S++	R	S++	31.3
NHP-15N	R	S++	S+	S++	S++	S++	R	S+++	S+	R	S+++	S+	R	S++	R	R	37.5
NHP-16E	R	S++	S+	S+	S++	S++	R	R	S+	S++	R	R	R	S+	S+	R	43.8
NHP-16N	R	R	R	R	R	R	S+	R	R	R	R	R	R	S++	R	R	87.5
NHP-17N	R	S++	S+	R	R	S+	S++	S++	S+	R	S++	S+	R	S+	R	S++	37.5
NHP-18N	R	R	S++	R	S+++	R	S++	R	R	R	S+	S+	R	S+	R	R	62.5

NA, nalidixic acid; CO, cloxacillin; CP, ciprofloxacin; TE, tetracycline; PF, peflacin; SP, septrin; LN, lincomycin; AP, Ampicillin; PN, penicillin; GN, gentamycin; ER, erythromycin; ST, streptomycin; CL, chloramphenical; RF, rifampicin; AX, amoxil; AC, ampiclox; PR, percent resistance (%).

0 to 5 mm Resistance (R)
 5 to 15 mm Sensitive (S)
 15 to 25 mm Sensitive (S+)
 25 to 35 mm Sensitive (S++)

Table 4. Inhibition zone diameter (IZD) of test antibiotics against different isolates of *S. aureus* from hospital sources.

Code of Strains	Antibiogram																
	NA	CO	CP	TE	PF	ST	SP	LN	AP	PN	GN	ER	CL	RF	AX	AC	PR
HP-1E	R	S++	S++	S+	S+++	S+	S++	S+	R	R	R	S+++	R	S+++	R	S++	37.5
HP-1N	R	S+++	S+++	S+	S++	S++	S++	S+	S++	R	S++	S++	S+	S+++	R	S++	18.8
HP-2N	R	S+++	S+++	S+	S+++	S++	S+	S++	S+	R	S+++	S++	S+	S+++	S++	S++	12.5
HP-3N	R	S+	R	R	R	S+	R	R	R	S++	S++	R	S+	S+	R	S+	56.3
HP-4N	R	R	R	R	S+	R	R	S+	R	R	S++	R	S+	S+++	R	R	68.8
HP-5E	R	S++	S++	S+	S++	S+	S++	S+	R	S+	R	R	R	S++	S++	S++	25
HP-5N	R	R	R	R	S++	S++	R	S++	S+	S+	R	R	R	S++	R	R	62.5
HP-7E	R	R	S+	R	R	S+	R	R	R	R	R	R	R	S+	R	R	81.3
HP-7N	R	S+	R	R	S+	R	R	R	S+	R	S++	R	S+	S+	R	R	62.5
HP-8N	R	S+	R	R	S+	R	R	S+	R	R	S+	S++	R	S++	R	R	62.5
HP-9N	R	R	R	R	S++	R	R	R	R	R	R	R	R	S+++	R	R	87.5
HP-11E	R	S++	S++	S+	S++	S++	R	S++	S++	S++	S++	S+	R	S+++	R	S+++	25
HP-11N	R	R	S++	R	S++	R	R	S++	R	R	S+	R	R	S++	R	S+	62.5
HP-12N	R	R	R	R	S+	R	R	S++	R	R	R	R	R	S++	R	R	81.3
HP-13E	R	S+	S++	S+	S+	S+	S+	R	R	R	S++	S+++	S++	S+++	R	S+	31.3
HP-13N	R	R	S+	R	R	R	R	S+++	R	R	S+	S+	R	S+++	R	R	68.8
HP-14E	R	S+++	S+	S++	S+++	R	S++	R	S+	S+	S++	S+	R	S++	R	R	37.5
HP-15N	R	S++	S+	S++	S++	S++	R	S+++	S+	R	S+++	S+	R	S++	R	R	37.5
HP-16E	R	S++	S+	S+	S++	S++	R	R	S+	S++	R	R	R	S+	S+	R	43.7

NA, nalidixic acid; CO, cloxacillin; CP, ciprofloxacin; TE, tetracycline; PF, peflacin; SP, septrin; LN, lincomycin; AP, Ampicillin; PN, penicillin; GN, gentamycin; ER, erythromycin; ST, streptomycin; CL, chloramphenical; RF, rifampicin; AX, amoxil; AC, ampiclox; PR, percent resistance (%).

0 to 5 mm Resistance (R)
 5 to 15 mm Sensitive (S)
 15 to 25 mm Sensitive (S+)
 25 to 35 mm Sensitive (S++)

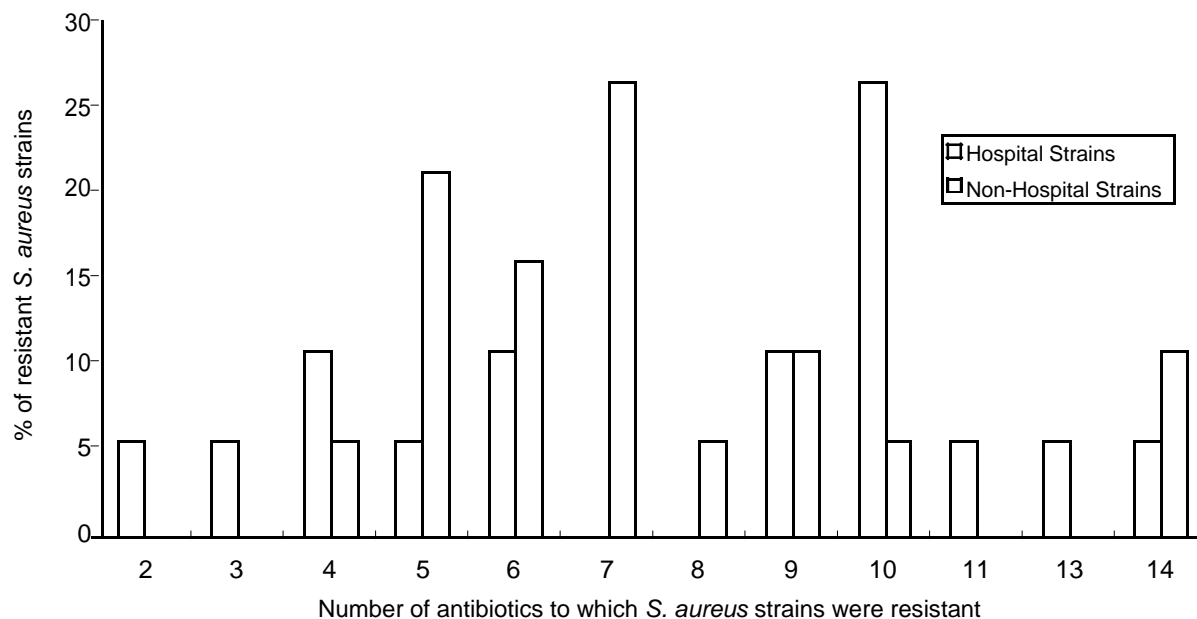


Figure 1. Distribution of antibiotics resistant (*S. aureus*) according to source of isolates.

The inhibition zone diameter (IZD) range of test antibiotics against the different isolates of *S. aureus* from hospital and non-hospital sources are shown in Tables 3 and 4, respectively. No isolate showed susceptibility to nalidixic acid (Na) whereas rifampicin was active against all strains. The results are summarized as antibiotic sensitivity (S) and resistance (R) pattern for both ear and nostril isolates. No strain was sensitive to all 16 antibiotics; rather there was multiple drug resistance ranging from resistance of 2 antibiotics to 14 antibiotics (87.5%). Figure 1 represents a summary of the multiple drug resistance patterns among hospital and non-hospital strains. About 5.26% of hospital strains and 10.53% of non-hospital were resistance to 14 antibiotics.

DISCUSSION

The carrier rate of *S. aureus* in this study was 35.7% and 14.3% for ear and nostril, respectively. There were more colonization in nostrils alone (50.0%) followed by both ear and nostril (37.5%) and then ear alone (14.3%). The nasal carrier rate in this study was higher than what earlier workers reported in normal population (Osuide et al., 1996). This may be attributed to its function as the air passage, making it more prone to dust carrying *S. aureus*. Individuals also touch their nose more frequently thereby transferring bacteria from the hand and skin to nostril. The ear is colonized by *S. aureus* less because of constant cleaning of the ear with cotton buds, soap and water thereby reducing the microbial load. Individuals

also touch their ear less often than their nose. The carrier rate in the male and female were comparable (data not shown) indicating that sex is not a notable factor in carriage and there is no activity or behavior of any of the sexes which predisposes them to *S. aureus* infection.

All the isolates of *Staph aureus* from both hospital and non-hospital populations were found to be resistant to nalidixic acid. Nalidixic acid is an antibiotic specifically use for gram-negative organisms and all strains tested were gram-positive, which explains why there seems to be a high resistance in all the isolates tested. Rifampicin recorded the highest inhibition zone diameter and all the isolates were sensitive to it. This high rate of sensitivity may be because rifampicin is not in common use and is normally used in the treatment of tuberculosis caused by *Mycobacterium tuberculosis*. Cloxacillin, ciprofloxacin, peflacin and lincomycin also recorded high sensitivity. This may be because they are relatively new and not in common use by the population as compared to penicillin, chloramphenicol, ampicillin, ampiclox and septrin.

In this study, the isolates from hospital population showed resistance to many antibiotics than isolates from non-hospital population, which is similar to previous reports (Osuide et al., 1996; Shakibaie et al., 2002). The higher prevalence of resistance to anti-microbial agents in this environment could be due to widespread, indiscriminate use of antibiotics. The formulation and implementation of a national drug policy by governments are fundamental to ensure rational drug use. Proper use of drugs has to be promoted by providing objective information and training.

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