



Antimicrobial Susceptibility Profile Of *Staphylococcus Aureus* Isolated From Ear And Nostrils Of Primary School Pupils In Umidike, Abia State

NWANKWO, I.U., ONWUAKO, C.E., APPEH, O.G., and ONWUBUARIRI, A.C.

Department of Microbiology, College Of Natural Sciences

Michael Okpara University Of Agriculture, P.M.B 7267,

Umidike, Abia State, Nigeria.

Correspondences Authors email address: immaugo@yahoo.com

ABSTRACT

This study examines the antimicrobial susceptibility profile of *Staphylococcus aureus* isolated from primary school pupils in Umidike. One hundred samples for culture of ear and nose swabs (50 from nose and 50 from ear) collected from primary school pupils in Umidike were examined using standard microbiological procedure to determine the incidence of *Staphylococcus aureus*. Antimicrobial susceptibility profile was determined by modified Kirby-Bauer diffusion method. The susceptibility of *Staphylococcus aureus* to antimicrobial agents allowed for human therapy were examined. The result of this study shows that the nostrils of the case study had the highest frequency of occurrence (100%) of the isolate, while the ear demonstrated 40% case occurrence. The isolates demonstrated high level of susceptibility to ciprofloxacin (228%), Gentamycin (200%), Ampicillin (179%) and erythromycin (168%). The test organism was considerably susceptible to tetramycins (140%) and amikacin (104%), while the isolates was highly resistant to antibiotics such as streptomycin (1325), augmentin (128%). Changing patterns of *Staphylococcus aureus* resistance to drugs continues to pose a problem to health providers globally despite the development of new antibiotics. These high rates of resistance have been attributed to factors such as misuse of these drugs by health professionals and unskilled practitioners among others. Thus, there is need for continuous and regular antimicrobial resistance surveillance in the country in order to guide empirical therapy and to provide adequate control strategies to combat this public health problem.

Keyword: Susceptibility Profile, *Staphylococcus Aureus*, Ear and Nostrils, Primary School Pupils

INTRODUCTION

Staphylococcus aureus are Gram positive, catalase positive cocci belonging to the Staphylococcaceae family (Becker *et al.*, 2004). They are approximately 0.5-1.5µm in diameter, non-motile, non-spore forming, facultative anaerobes (with the exception of *Staphylococcus aureus* anaerobium) that usually occurs in clusters. Many strains produce Staphylococcal enterotoxins, the super antigen shock syndrome toxin (TSST-1), and exfoliate toxins. The main three species of clinical importance are *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Staphylococcus saprophyticus*. Of all these species, *Staphylococcus aureus* is the major human pathogen. Its pathogenic effect is characterized by its ability to haemolyse blood, coagulate plasma (a biochemical test that differentiates it from other non-pathogenic *Staphylococcus*, and produces a variety of extra cellular enzymes and toxins (Jawetz *et al.*, 2004). *Staphylococcus aureus* colonizes skin glands and mucous membrane causing infections both in humans and animals such as rashes, inflammation of bones and meninges as well as Septicaemia (Aklilu *et al.*, 2010). In addition, *Staphylococcus aureus* cause inflammation of the mammary gland in bovine and the lower part of the foot in the poultry (Quinn *et al.*, 2000). Unfortunately, *Staphylococcus aureus* have a

record of developing resistance quickly and successfully to antibiotics via a mechanism that involves the acquisition and transfer of antibiotics resistance plasmids (Tenover *et al.*, 2004), as well as the possession of intrinsic mechanisms (Kloos, 2011). *Staphylococcus aureus* are the principal cause of abscess, respiratory infections such as sinusitis and food poisoning. Some strains of *Staphylococcus aureus* are capable of producing staphyloxanthin; a golden-coloured carotenoid pigment. This pigment serves as the virulence factor, primarily by being a bacterial antioxidant which helps the microbe evade the reactive oxygen species which the host immune systems uses to kill pathogens (Liu *et al.*, 2005). Mutants' strains of *Staphylococcus aureus* modified to lack staphyloxanthin are less likely to survive incubation with an oxidizing chemical such as hydrogen peroxide than pigmented strains. *Staphylococcus aureus* also occur commonly in the environment and is transmitted via aerosols or air droplets. *Staphylococcus* is also the leading cause of blood stream infections throughout (Rasmussen *et al.*, 2011).

MATERIALS AND METHODS

Sample Collection

A total of one hundred specimens consisting of fifty nasal swabs and fifty ear swabs were collected from primary school students in Umudike, Abia State. The sample population comprised of 50 male pupils and 50 female pupils, twenty five samples each were collected for each gender from the two sample source. The primary schools from which the samples were collected are National Root Crops Research Institute Primary School Umudike and Umuariaga Primary School.

Microbiological Examination of Sample

Processing Samples

This was carried out as described by Cheesbrough (2006) and Prescott *et al.*, (2008). A 10-fold serial dilution of the samples were made using physiological saline. 0.1ml aliquot was inoculated on the prepared media and incubated for 24 to 48 hours at 37⁰C. After incubation, plates with growths were isolated using an inoculating loop and subsequently sub-cultured on agar slants for further test.

Identification of Isolates

Distinct colonies from the positive plates were sub-cultured and purified using nutrient agar slants incubated at 37⁰C. The isolates were identified by Bergeys Manual for Determinative Bacteriology and only Coagulase Positive Strains were further tested in accordance with standard procedures. The identification of the isolates were done using gram staining, catalase test and coagulase test (Slide method).

Antimicrobial Susceptibility Testing

The disc diffusion method for in-vitro antibiotics susceptibility tests described by Bauer *et al.*, (1996) was adopted for the study. The concentrations of the antimicrobial sensitivity and the interpretation of zones of inhibition were performed using Mueller-Hinton agar according to The National Committee for Clinical Laboratory Standards (2002). The Antibiotics tested for included Augmentin (30µg), Streptomycin (10 µg), Gentamycin (10 µg), Cloxacillin (5 µg), Amikacin (30 µg), Tetracycline (25 µg), Erythromycin (5 µg), Ciprofloxacin (10 µg), Ampicillin (20 µg) and Chloramphenicol (10 µg). Using sterile forceps, the antibiotics disc were placed on the plates that have already been streaked with each of the bacterial isolates respectively and then incubated at 37⁰C for 24 hours. The presence of zone of inhibition around each of the disc after the period of incubation was regarded as the presence of antibacterial action while the absence was regarded as lack of measurable antimicrobial action. The zone of inhibition around each of the disc was measured to the nearest millimetre using sliding callipers and the inhibition zones were scored as sensitive, intermediate or resistant, according to The National Committee for Clinical Laboratory Standards (2002) recommendations.

RESULTS

A total of hundred samples from primary school pupils in Umudike were investigated for the presence of *Staphylococcus aureus* strains. Table 1 shows the frequency of isolation of *Staphylococcus aureus* strains with respect to the site of sample collection. The results obtained show that out of one hundred primary school pupils sampled, 70% of the initial populace had *Staphylococci* colonization. 50% was from the nostrils while 29% was from the ear.

Table 2 shows the zone of inhibition of the drugs against each isolates. From the table, isolates from samples 2, 42, 13 and 39 were susceptible to all the drugs. Isolates from sample 1, 8, 9, 3, 19 and 13 were susceptible to ciprofloxacin, gentamycin, ampicillin, amikacin and erythromycin only. Isolates from samples 20, 5, 46 and 26 were resistant to all drugs tested.

The percentage rate of sensitivity of *S. aureus* to the individual drugs is as shown in table 3. From the table, ciprofloxacin had the highest level of activity (228%). This was followed closely by gentamycin (200%) while the least inhibitory rate was observed with Cloxacillin (120%), Augmentin (128%) and Streptomycin (132%).

Table 1: Frequency of occurrence of *S. aureus* in the primary school pupils nostrils and ear

Sex	No. of Samples examined		Positive Samples of isolation	
	Nostril	Ear	Nostril	Ear
Male	25	25	25(50%)	13 (26%)
Female	25	25	25(50%)	7 (14%)
Total	50	50	50(100%)	20(40%)

Table 2a: Antimicrobial susceptibility of *Staphylococcus aureus* strains isolated from male pupil.

SOURCE	SAMPLE CODE	CPX	ERY	TET	AMI	STP	GEN	CLX	AUG	CHL	AMP
NOSTRIL	sm 1	18mm	18mm	16mm	17mm	16mm	19mm	15mm	18mm	18mm	18mm
	sm 6	14mm	18mm	15mm	17mm	16mm	17mm	15mm	15mm	18mm	18mm
	sm 49	17mm	15mm	14mm	10mm	12mm	15mm	18mm	16mm	18mm	12mm
	sm 2	16mm	11mm	14mm	11mm	14mm	15mm	18mm	12mm	11mm	18mm
	sm 13	18mm	12mm	11mm	10mm	12mm	16mm	14mm	15mm	11mm	18mm
	sm 12	16mm	16mm	16mm	17mm	15mm	15mm	13mm	12mm	13mm	14mm
	sm 39	14mm	17mm	17mm	17mm	19mm	16mm	11mm	11mm	15mm	16mm
	sm25	15mm	15mm	16mm	16mm	14mm	17mm	16mm	14mm	17mm	15mm
	sm7	14mm	12mm	12mm	12mm	11mm	12mm	15mm	15mm	14mm	12mm
	sm8	11mm	18mm	15mm	10mm	13mm	10mm	11mm	13mm	10mm	14mm
	sm20	10mm	15mm	16mm	12mm	14mm	18mm	14mm	14mm	14mm	15mm
	sm30	16mm	17mm	10mm	13mm	16mm	18mm	18mm	11mm	10mm	13mm
	sm38	15mm	14mm	13mm	10mm	17mm	14mm	12mm	16mm	14mm	15mm
	sm23	17mm	10mm	14mm	17mm	12mm	18mm	10mm	11mm	14mm	18mm
	sm41	14mm	12mm	15mm	12mm	13mm	12mm	15mm	15mm	13mm	13mm
	sm44	14mm	16mm	5mm	11mm	14mm	16mm	14mm	12mm	14mm	17mm
	sm29	12mm	15mm	16mm	14mm	12mm	14mm	13mm	17mm	13mm	11mm
	sm5	13mm	13mm	12mm	12mm	10mm	11mm	14mm	13mm	10mm	14mm
	sm35	11mm	13mm	10mm	10mm	16mm	12mm	18mm	10mm	10mm	16mm
	sm11	16mm	13mm	12mm	17mm	11mm	18mm	10mm	14mm	11mm	12mm
sm26	15mm	14mm	10mm	12mm	14mm	16mm	12mm	11mm	12mm	17mm	
sm42	17mm	14mm	13mm	12mm	15mm	12mm	10mm	15mm	11mm	11mm	
sm10	11mm	17mm	11mm	17mm	17mm	13mm	12mm	11mm	10mm	10mm	
sm14	10mm	10mm	12mm	14mm	10mm	11mm	11mm	10mm	11mm	17mm	
sm9	12mm	12mm	16mm	14mm	12mm	10mm	13mm	11mm	10mm	11mm	
EAR	Sm3	19mm	16mm	18mm	14mm	16mm	19mm	13mm	14mm	16mm	18mm
	Sm19	17mm	15mm	17mm	11mm	15mm	18mm	12mm	12mm	10mm	16mm
	Sm50	11mm	16mm	15mm	10mm	13mm	17mm	10mm	10mm	12mm	17mm
	Sm18	15mm	16mm	14mm	11mm	11mm	16mm	11mm	13mm	11mm	18mm
	Sm32	13mm	9mm	12mm	12mm	10mm	17mm	9mm	14mm	14mm	15mm
	Sm47	18mm	12mm	15mm	13mm	12mm	15mm	12mm	11mm	16mm	14mm
	Sm39	16mm	13mm	17mm	14mm	16mm	14mm	13mm	12mm	14mm	10mm
	Sm27	16mm	14mm	17mm	15mm	16mm	19mm	10mm	13mm	11mm	12mm
	Sm46	11mm	16mm	14mm	9mm	16mm	13mm	14mm	10mm	10mm	13mm
	Sm15	14mm	15mm	13mm	10mm	15mm	12mm	11mm	12mm	14mm	11mm
	Sm17	9mm	16mm	16mm	11mm	10mm	14mm	14mm	11mm	11mm	12mm
	Sm 22	12mm	12mm	12mm	14mm	11mm	10mm	10mm	14mm	13mm	10mm
	Sm 24	15mm	14mm	11mm	11mm	12mm	11mm	11mm	13mm	10mm	11mm

Table 2b: Antimicrobial susceptibility of *Staphylococcus aureus* strains isolated from female pupil.

SOURCE	SAMPLE CODE	CPX	ERY	TET	AMI	STP	GEN	CLX	AUG	CHL	AMP
NOSTRIL	Sf1	16mm	10mm	10mm	12mm	11mm	18mm	14mm	15mm	12mm	16mm
	Sf8	18mm	12mm	11mm	16mm	16mm	20mm	16mm	12mm	19mm	18mm
	Sf15	22mm	18mm	16mm	19mm	12mm	18mm	11mm	18mm	12mm	20mm
	Sf6	19mm	16mm	12mm	18mm	20mm	16mm	12mm	20mm	16mm	18mm
	Sf25	20mm	22mm	18mm	20mm	12mm	18mm	11mm	16mm	11mm	22mm
	Sf9	16mm	18mm	10mm	11mm	10mm	22mm	10mm	18mm	15mm	16mm
	Sf20	19mm	12mm	11mm	16mm	18mm	21mm	12mm	20mm	18mm	20mm
	Sf11	25mm	10mm	16mm	17mm	10mm	25mm	16mm	20mm	17mm	12mm
	Sf29	20mm	18mm	19mm	18mm	16mm	22mm	20mm	16mm	11mm	16mm
	Sf35	21mm	16mm	18mm	12mm	18mm	20mm	16mm	20mm	12mm	11mm
	Sf24	19mm	10mm	16mm	11mm	16mm	25mm	11mm	21mm	10mm	18mm
	Sf19	20mm	16mm	17mm	10mm	17mm	20mm	12mm	11mm	11mm	12mm
	Sf18	19mm	10mm	11mm	10mm	12mm	12mm	18mm	19mm	12mm	11mm
	Sf30	18mm	11mm	18mm	19mm	12mm	16mm	11mm	10mm	18mm	11mm
	Sf39	20mm	15mm	18mm	11mm	15mm	12mm	20mm	18mm	10mm	20mm
	Sf45	19mm	11mm	12mm	16mm	10mm	18mm	19mm	12mm	11mm	10mm
	Sf23	25mm	12mm	10mm	18mm	11mm	20mm	12mm	12mm	20mm	19mm
	Sf40	21mm	22mm	10mm	11mm	18mm	11mm	11mm	11mm	16mm	12mm
	Sf48	19mm	21mm	16mm	12mm	17mm	12mm	16mm	13mm	20mm	18mm
	Sf32	20mm	18mm	19mm	16mm	12mm	16mm	20mm	13mm	11mm	16mm
Sf28	22mm	11mm	12mm	10mm	18mm	17mm	17mm	12mm	11mm	12mm	
Sf49	19mm	12mm	16mm	12mm	10mm	18mm	18mm	12mm	10mm	16mm	
Sf7	20mm	16mm	11mm	12mm	11mm	12mm	16mm	10mm	11mm	17mm	
Sf26	16mm	10mm	11mm	11mm	11mm	10mm	11mm	11mm	10mm	18mm	
Sf16	20mm	16mm	17mm	12mm	12mm	12mm	11mm	10mm	11mm	11mm	

EAR	Sf2	16mm	8mm	10mm	11mm	10mm	18mm	10mm	15mm	18mm	16mm
	Sf10	20mm	12mm	14mm	10mm	11mm	20mm	11mm	19mm	11mm	18mm
	Sf12	18mm	16mm	16mm	12mm	12mm	22mm	12mm	20mm	10mm	19mm
	Sf20	19mm	10mm	11mm	15mm	18mm	18mm	10mm	11mm	12mm	20mm
	Sf38	16mm	11mm	19mm	16mm	12mm	20mm	16mm	18mm	11mm	18mm
	Sf25	15mm	18mm	10mm	12mm	10mm	18mm	18mm	12mm	12mm	11mm
	Sf17	22mm	15mm	12mm	11mm	11mm	19mm	12mm	14mm	19mm	12mm

≥ 13mm = sensitivity

< 13mm =resistant

KEY:

CPX = Ciprofloxacin, CLOX = Cloxacillin, ERY = Erythromycin , AUG = Augmentin, TET = Tetracycline, CHL = Chloramphenicol, AMI = Amikacin, AMP = Ampicillin, STP = Streptomycin, GEN = Gentamicin

Table 3 Rate of sensitivity of *s. aureus* to the individual drugs

Sex	CPX	ERY	TET	Ami	STP	Gen	CLX	AUG	CHL	AMP
M	25	24	22	15	20	25	16	15	15	23
F	32	18	13	11	13	25	14	17	11	21
Total	228%	168%	140%	104%	132%	200%	120%	128%	104%	176%

$$\frac{\text{Number positive}}{\text{Number examined}} \times \frac{100}{1}$$

DISCUSSION

Staphylococcus aureus is a non-motile Gram-positive coccus frequently found as part of the normal skin flora, on the skin and nasal passages. It is well established that at any given time, approximately 30 % of all persons are colonized with *Staphylococcus aureus*, with the anterior nares serving as its critical niche (Kluytmans *et al.*, 2005). *Staphylococcus aureus*, a common colonizer of the skin and nose has become one of the most successful adaptable human pathogens. The bacterium has been reported by David *et al.*, (2006) to have remarkable ability to acquire antibiotic resistance contributing to its emergence as an important pathogen in a variety of setting. In the present study, an overall prevalence of 70% colonization with *Staphylococcus aureus* was obtained from the nasal and ear samples and most of the isolates were from the nasal region. This high level of colonization as seen in the present study could be because nasal regions have been reported to be the major reservoir of *S. aureus* (Appelbaum, 2007).

A high percentage of *Staphylococcus aureus* nasal colonization among male and female primary school pupils in Umudike as shown in table 2 from the present study were seen not to have been affected by gender and therefore did not differ in terms of sex of the participants. This therefore implies that *Staphylococcus aureus* colonises healthy individuals irrespective of the sex or level of education. Similar isolation of *Staphylococcus aureus* from students in a health institution has been reported by Marques *et al.*, (2010). Nasal colonization did not mean that the ears would be colonized as was seen in the present study, agreeing with the fact that the nasal regions are the major reservoirs for the bacterium.

In the present study, the antimicrobial susceptibility test shows that the isolates showed a high level of susceptibility to antibiotics such as Ciprofloxacin (228%), Gentamicin (200%), Ampicillin (179%) and Erythromycin (168%). Antibiotics such as Tetracycline and Amikacin showed intermediate resistance to the test organism. However, the test organism showed the no zone of inhibition and therefore was highly resistant to antibiotics such as Streptomycin (132%) and Augmentin (128%). This could be as a result of antibiotic abuse as suggested by other workers who reported that penicillins are the most misused antibiotics amongst Nigerian communities (Olayemi *et al.*, 2010). The high resistance of isolates from the present study to commonly prescribed antibiotics demonstrate the urgent need for proper management of antibiotics use. Nigeria has a high rate of antibiotic misuse as well as high prevalence of self-medication use. Studies have shown that misuse of antibiotics is a main cause of antimicrobial resistance (Okeke *et al.*, 2007). Therefore misuse and self-medication are factors identified as being responsible for the emergence of antibiotic resistant bacterial strains.

The findings in this study therefore indicate the importance of investigating staphylococcal colonization of the nasal mucosa and ear in order to ascertain the level of antibiotic susceptibility of *Staphylococcus aureus* and thus develop intervention strategies measures as well as treatment for staphylococcal infections.

CONCLUSION

The present study detected alarming levels of *Staphylococcus aureus* isolates, at the same time presence of high level of resistance to variety of antibiotics. Although, staphylococcal disease is most often associated with skin and soft tissue infections, its manifestations are myriad and include syndromes with low morbidity and mortality such as folliculitis, food poisoning and fatal systemic illness such as endocarditis and toxic shock. The carriage of the bacterium has long been known to be one of the most strongly associated risk factors for subsequent infections as reported by various workers. Ear and nasal colonization by *S. aureus* can provide an indication of a higher risk for subsequent infection, especially when they have developed resistance to wide spectrum of antibiotics as a result of frequent usage and misuse of antibiotics

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