ISOLATION AND IDENTIFICATION OF *Staphylococci* FROM BODY SURFACE OF HUMANS AND THEIR SENSITIVITY TO COMMONLY USED ANTIBIOTICS

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ABSTRACT
Isolation and identification of Staphylococci was intended to isolate Staphylococcus species from body surfaces and determine their sensitivity to the commonly used antibiotics. A total of seventy three (73) skin swab samples were collected from student's resident at the hostels of Usmanu Danfodiyo University, Sokoto, Nigeria. Using bacteriological and biochemical identification methods, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus warneri and streptococcus species were isolated. Out of the isolates obtained on manitol salt agar, 29 (30.21%) were identified as S. aureus, 44 (45.83%) were identified as S. epidermidis, 10 (10.42%) were identified as S. warneri, 13 (13.54) were identified as Streptococcus species. Antibiotics used for sensitivity test Amoxyclav, Cefalaxin, Ciprofloxacin, Clindamycin, Co-trimoxazole, Erythromycin and Tetracycline using disc diffusion method, reveal that S. aureus had the highest resistance (84.48%), followed by S. warneri (20.00%) and then S. epidermidis (15.34%). S. aureus have now continued to become resistant to most of the common antibiotics used locally.

1. INTRODUCTION

Staphylococcus is a genus of gram positive bacteria. Under the microscope they appear round (cocci), and form grape-like clusters (Ryan and Ray 2004). The *Staphylococcus* genus includes atleast40 species (Harries *et al.*, 2002). Of these, nine have two subspecies and one have three subspecies (Harries *et al.*, 2002). Most are harmless and reside normally on the skin and mucous membranes of humans and other organisms (Madigan and Martinko, 2005). Found worldwide, they are small components of soil microbial flora (Madigan and Martinko, 2005).

Infections caused by *Staphylococcus aureus* pose serious threat in health care institutions (NNIS, 2004). It is one of the most widely spread and virulent nosocomial pathogen and is usually resistant to multiple antibiotics making infections difficult to treat (Cooper *et al*. 2004). It appears to add to the total burden of *Staphylococcus* infections in the hospitals, rather than replacing sensitive *S. aureus*, and is associated with sharp risk in mortality attributable to *Staphylococcal* infection worldwide (Crowcroft & Catchpole 2002). *S. aureus* strains continue to be a major problem in many healthcare institutions especially with emergence of Methicillin resistant *Staphylococcus aureus (MRSA)* and now account for more than 50% of *S. aureus* recovered from patients in intensive care units and about 40% of *S. aureus* isolated from non-intensive care unit (Boyce 2003).

MRSA is one of the numbers of greatly feared strains of *Staphylococci* that develop resistance to most commonly used antibiotics today. MRSA include those strains that have acquired a gene conferring resistance to methicillin and other beta-lactam antibiotics (Palavecino, 2007). MRSA strains are most often found associated with institutions such as hospitals but are becoming increasingly prevalent in community acquired (CA) infection (Duguette and Nutall, 2004). CA-MRSA first appeared in high risk populations such as intravenous drug users, people in nursing homes and people who were chronically ill, but they have been reported even in healthy children (Duguette and Nutall, 2004). People with weak immune systems, diabetics, young children, college students living in dormitories, people living or staying in health care facility for an extended period of time, people who spent time in confined spaces with other people; including prison inmate, military recruit in basic training are some of the population at risk (Duguette and Nutall, 2004).

Therefore, this research will broaden the knowledge of the risk associated with living in confined places thereby reduce increase transmission of resistant strain of *Staphylococci* among humans. Hence the purpose of this study is to isolate *Staphylococcus* species and determine the antibiogram of the isolates against some selected commercial antibiotics.

2. MATERIALS AND METHODOLOGY

2.1 Specimen collection

Samples were collected from 73 individuals within the sampling site. All the people present at the time of sampling and consented to participate were included. Samples were taken from different locations of the skin namely armpit, neck region, chest region, e.t.c., as follows: A swab stick was gently rotated on

the skin surface of consenting individuals. The specimens were immediately sent to the Microbiology Laboratory of Usmanu Danfodiyo University, Sokoto for processing, within one hour of collection.

2.2 Isolation and identification of the organism

The swab samples were directly plated onto plates of Manitol Salt Agar (MSA) using streak plate's method. The plates were then incubated at 37^0C for 24 hours. Discrete colonies were then isolated to obtain a pure culture, and then subcultured onto plates of nutrient agar (NA). The isolates were kept at 4^0C for further research work (Benson, 2002).

A smear of the isolates was then made on a clean grease free slide and allowed to air dry. The smears were covered with crystal violet and allowed it to stain for 60 seconds; it was then washed with distilled water. The slides were then covered with iodine and allowed to stain for 30 seconds, then washed with distilled water. They were then decolourized with acetone and then washed immediately with distilled water. The slides were then counter stained with safranine which was also allowed to stain for 60 seconds and then washed with distilled water. They were then air dried and viewed under X100 objective lens (Cheesbrough, 2006).

The isolates which were considered to be *Staphylococcus* from their shape under the microscope were selected for further studies.

2.3 Biochemical Tests

Coagulase Test

A drop of serum was placed onto a clean grease free slide and a loopful of the growth was inoculated and emulsified thoroughly. The mixture was then rotated for 5 minutes and then coagulation was observed (Benson, 2002).

Catalase Test

A drop of hydrogen peroxide was placed on a clean grease free slide and a loopful of growth of the test organism was placed and then emulsified. Effervescence caused by the liberation of oxygen as gas bubbles indicate the presence of catalase (Benson, 2002).

Sugar fermentation Test

The sugars used were glucose, sucrose and lactose. Triple sugar iron (TSI) agar was used to carry out this test. The agar was prepared and formed slant in the test tubes. The isolates were then inoculated and incubated at 37^0C for 24 hours. Fermentation of sugar was indicated by the change of colour in the agar from red to yellow (Oyeleke and Manga, 2008).

Urease Test

Christesen's urea media were prepared in universal bottles and three drops of urease enzyme were added into each bottle. The bottles were then slanted and allowed to solidify. A speck of the inoculums each from the isolates was inoculated and then incubated at 37^0C for 24 hours. The reactions of the slant bottles were observed. A positive result was indicated by the appearance of red colour (Cheesbrough, 2006).

Sensitivity Test

A speck of growth of each isolates was spread onto a plate of nutrient agar. Sensitivity disc containing conventional antibiotics Amoxyclav, Cefalaxin, Ciprofloxacin, Clindamycin, Co-trimoxazole, Erythromycin and Tetracycline (Mastering Laboratory Limited, India) was placed on the surface of nutrient agar plates containing the streaked test isolates and incubated at 37^0C for 24 hours. The antibiogram was then read and recorded (diameter zone of inhibition). In this study, zone of inhibition less than 10mm is considered as resistance as used by Cheesbrough, (2006).

3. RESULTS

From the 73 swab samples analyzed, 96 bacterial colonies were isolated. Microscopy of the stain isolates show that 20 (20.83%) of the isolates were gram positive rod and 76 (79.17%) were gram positive cocci.

The biochemical test show four different organisms namely *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus warneri* and *Streptococcus species*. The frequency of

occurrence of the organisms isolated (Table 1) show that *S. epidermidis* has the highest number of isolates (45.83%), followed by *S. aureus* (30.21%), *Streptococcus species* (13.54%) and then *S. warneri* with (10.42%).

Table 1: Species Isolated from Human Body Part Surfaces

Serial no	Organisms	No. of isolates	Frequency of occurrence
1	*Staphylococcus aureus*	29	30.21%
2	*Staphylococcus epidermidis*	44	45.83%
3	*Staphylococcus warneri*	10	10.42%
4	*Streptococcus species*	13	13.54%

The result for sensitivity test of the Staphylococcal isolates (Table 2) shows that *S. aureus* has the highest level of resistance to the antibiotics used in this research. All the *Staphylococcus aureus* isolates were resistant to cloxacillin and tetracycline. Twenty four (24) out of the *Staphylococcus aureus* isolates were resistant to Clindamycin and Cefalaxin, while twenty three (23) were resistant to Amoxyclav, Ciprofloxacin and Co-trimoxazole, and twenty one (21) isolates show resistance to Erythromycin. All *Staphylococcus epidermidis* are sensitive to Ciprofloxacin and Clindamycin but resistant to other antibiotics tested. *S. warneri* shows resistance to Amoxyclav, Cefalaxin, Cloxacilin, Erythromycin and Tetracycline but were sensitive to only Ciprofloxacin and Co-trimoxazole.

Table 2: Summary of Sensitivity Test of the Isolates Showing the Number of Resistance Isolates

Isolates	AMC	CN	CIP	CD	COX	COT	E	TE	FREQUENCY
S. aureus	23	24	23	24	29	23	21	29	84.48%
S. epidermidis	9	6	0	0	11	11	9	8	15.34%
S. warneri	5	4	0	0	4	0	1	2	20.00%

Key; AMC-Amoxyclav, CN-Cefalaxin, CIP-Ciprofloxacin, CD-Clindamycin, COX-Co-trimoxazole, E-Erythromycin, TE-Tetracycline

4. DISCUSSION

The most isolated specie in this study was *Staphylococcus epidermidis*, accounting for 45.83% of all the isolates. The prominence of *S. epidermidis* on the skin was reported by Khashu, *et al.*, (2006) in Columbia. In their findings, *S. epidermidis* accounted for 43% of bacteria on the human skin. Mohan, *et al,* (2007) however, reported that *S. epidermidis* was the most commonly isolated specie accounting for 82.3% of their isolates while *S. saprophyticus* was next with an incidence of 15.6% in India. The highest isolation of *S. epidermidis* on skin may be as a result of the salty environment it produces in order to inhibit the growth of pathogenic bacteria such as *Staphylococcus aureus.*

Staphylococcus aureus show resistant to most of the antibiotics used in this research; this was in comformity with the work of Obasola, *et al,* (2010) who reported that multi-drug resistance was increasingly encountered among *S. aureus* isolates in Nigeria. The resistance in *S. aureus* may be as a result of the acquisition of *MecA* gene, the gene for penicillin binding protein (PBP); *mecl* and *mecR1*, regulatory genes controlling *mecA* expression and numerous other elements and resistant determinants (Diep, *et al,* 2006). Multi drug resistant was not observed in *S. epidermidis* though other workers have reported it in their findings (Mohan, *et al,* 2007).

Isolation and identification of *Staphylococci* was intended to isolate *Staphylococcus species* from body surfaces and determine their sensitivity to the commonly used antibiotics. The isolate were identified and confirmed 83 isolates of *Staphylococci* from the samples. The sensitivity test show resistance to certain antibiotics used which might be difficult to treat with most commonly used antibiotics when those organisms turn to opportunistic pathogens.

5. CONCLUSION

The present study shows that *S. aureus* isolated from students of Usmanu Danfodiyo University Sokoto, Nigeria is mostly resistant to the antibiotics used in this research. This requires the decongestion of the student's hostel to reduce the possible transfer of this strain. But coagulase negative *Staphylococci* (*S. epidermis* and *S. warneri*) are mostly sensitive to the antibiotics used. Due

to this increased resistance of *Staphylococci*, there is the need to promote antibiotics for treatment of this strain in humans and laboratory test should be conducted before the use of antibiotics for treatment.

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