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Isolation, Identification and Determination of Antibiotic Susceptibility Pattern of *Staphylococcus epidermidis* Isolated from Infected Skin

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Abstract

Today, *Staphylococcus epidermidis* is a major nosocomial pathogen posing significant medical and economic burdens. The study was aimed to isolate, identify and determine the antibiotic susceptibility pattern of *S. epidermidis* isolated from infected skin in Kano, Northern Nigeria. A total of 75 wound swab were collected from patients (53 males and 22 females) attending three different hospitals within Kano State metropolis (Murtala Muhammad Specialist Hospital, Muhammad Abdullahi Wase Specialist Hospital and Aminu Kano Teaching Hospital) using sterile swab sticks. The wound swab samples were inoculated onto the surface of freshly prepared Nutrient agar for isolation. The bacteria

isolated were identified by conventional microbiological methods namely; Gram staining and biochemical tests (such as catalase, coagulase, DNase and haemolysis test). The isolates were subjected to antibiotic susceptibility testing using the agar disc diffusion method. The result showed that *S. epidermidis* was highly susceptible to Gentamicin 13 (61.90%), Levofloxacin 12 (57.14%) and Nitrofurantoin 11 (52.38%). On the other hand, the isolate was highly resistant to Ceftazidime 20 (95.24%), Cloxacillin 19 (90.48%) then Cefoxitin, Erythromycin and Amoxicillin/clavulanic acid with 15 (71.43%) each. It is concluded that *S. epidermidis* develop resistance to some classes of antibiotics.

Keywords: Antibiotic, Identification, Isolation, Resistance, *Staphylococcus epidermidis*

1. Introduction

Staphylococcus spp. are commensal bacteria of human skin and have been isolated from diverse clinical sources such as urinary tract infections (UTIs), respiratory tract infections (RTIs), wound infections (WIs), soft tissue infections, blood infections, and endocarditis (Dehkordi *et al.*, 2017) ^[6]. *S. epidermidis* is a common commensal bacterium, which belongs to coagulase-negative Staphylococci (CoNS). It has been suggested that *S. epidermidis* is one of the most important species of this group. It is a Gram-positive, non-spore forming, non-motile, facultative anaerobic, and catalase-positive and coagulase negative bacterium responsible for different types of hospital and nosocomial infections (Namvar *et al.*, 2014; Oliveira *et al.*, 2017) ^[13, 16]. Risk factors for coagulase negative *Staphylococcus* (CoNS) infection include the presence of indwelling medical implants, such as intravascular catheters, or immunosuppression due to cancer treatment or HIV/AIDS. Treatment of CoNS infections is complicated by the emergence of antibiotic-resistant strains (such as particularly MRSE, methicillin-resistant *S. epidermidis*) (Rogers *et al.*, 2009) ^[18]. CoNS are an integral part of the normal flora on the human skin and mucous membranes, and preferentially colonize moist areas (Grice *et al.*, 2009) ^[8].

S. epidermidis results in approximately 13% of prosthetic valve endocarditis infections, with a high rate of intra-cardiac abscess formation (38%) and mortality (24%) (Chu *et al.*, 2009) ^[5]. *S. epidermidis* strains usually resist against several types of antibiotic classes such as tetracyclines, aminoglycosides, cephalosporins, fluoroquinolones, penicillins, and macrolides (Klevens *et al.*, 2007) ^[11]. Nowadays, resistant *S. epidermidis* has become a serious problem in hospitals (Cabrera-Contreras *et al.*, 2013) ^[3]. Resistant staphylococcal strains are responsible for about 100,000 cases of infections with around 10% mortality rate each year in the USA (Klevens *et al.*, 2007) ^[11]. The presence of certain antibiotic resistance genes is responsible for the occurrence of antibiotic resistance (Duran *et al.*, 2012) ^[7]. *MecA*, *aacA-D*, *tetK* and *tetM*, *ermA* and *ermC*, *msrA* and *msrB*, *linA* and *vatA*, and *vatB* and *vatC* antibiotic resistance genes are responsible for the occurrence of resistance against methicillin, aminoglycosides, tetracyclines, macrolide-lincosamide-streptogramin B, macrolides, lincosamides, and streptogramin A groups of antibiotics, respectively (Duran *et al.*, 2012) ^[7]. Limited information is available on *S. epidermidis* clinical isolates in Nigeria.

The study was aimed to isolate, identify and determine the prevalence and antibiotic susceptibility pattern of *S. epidermidis* isolated from infected skin in Kano, Northern Nigeria.

Materials and Materials

Study Area

Kano State is a state located in North-Western Nigeria and one of the largest State of the Nigerian Federation, Created on May 27, 1967 from part of the Northern Region. Kano state is bordered by Katsina state to the North, Jigawa state to the North-east, Bauchi state to the South-east and Kaduna state to the West. Kano is located on 12° N and 8°30' E. It has a total area of 20,131 km². The urban area covers 137 km² and comprises of six LGAs - Kano municipal, Fagge, Dala, Gwale, Tarauni and Nassarawa with population of 2,163,25 as at 2006 (NPC, 2006) [15]. Climate of the study areas have been described as 'AW' type as identified by Koppen's climatic classification. The vegetation is a Savanna type simply described as closed grass or other predominantly herbaceous vegetation with scattered or widely spaced woody plants. Vegetation types in the state are the northern Guinea savanna and Sudan savanna. Northern Guinea Savanna is open woodland with grasses shorter than in the southern guinea where grasses are 1.5 to 3m tall. The Sudan Savanna has scattered trees in open grassland with grasses under 1.2m tall. The vegetation has been largely cleared for cultivation to form cultivated parkland. Parkland has scattered protected trees at some distance apart in open cultivated land (Nas *et al.*, 2018) [14].

Ethical Clearance

Ethical approval was obtained from Kano State Hospital Management Board based on the consent of Murtala Muhammad Specialist Hospital, Muhammad Abdullahi Wase Specialist Hospital and Aminu Kano Teaching Hospital ethical committees.

Sample Collection

A total of 75 samples were collected, a standard epidemiological formula (Fisher's formula) was used to calculate the sample size. Seventy five (75) wound swab were collected from patients (53 males accounted for 70.7% and 22 females accounted for 29.3%) attending three different hospitals within Kano State metropolis (Murtala Muhammad Specialist Hospital, Muhammad Abdullahi Wase Specialist Hospital and Aminu Kano Teaching Hospital) using sterile swab sticks over a period of eight months (October, 2016 to May, 2017)

Isolation and Identification of *S. epidermidis*

The wound swabs collected were inoculated onto the surface of freshly prepared Nutrient agar (Biomark). The plates were incubated at 37°C for 24 hours for colony formation. Each colony was isolated in a pure form by sub culturing for further studies and identification. Discrete colonies of each isolate were kept in peptone water. The bacterial strains were then stored at 4°C for further experiments. The isolated organisms were subjected to Gram staining, Mannitol fermentation and Biochemical tests (catalase, coagulase, DNase and haemolysis tests) as described by Cheesbrough (2010) [4] for identification

Antimicrobial Susceptibility Testing

The bacteria isolates were subjected to antibiotic susceptibility testing using the disc diffusion method as described by Bauer *et al.* (1966) [2]. Mueller Hinton agar (Oxoid, UK) plates were inoculated with an overnight culture of each isolate by streak plating. The standard antibiotic sensitivity discs were then aseptically placed at equidistance on the plates and allowed to stand for 1 hour. The plates were then incubated at 37°C for 24 hours. Sensitivity pattern of the isolates to levofloxacin 5µg, amikacin 30µg, cefepime 30µg, ceftazidime 10µg, imipenem 10µg, gentamicin 10µg, nitrofurantoin 300µg, ceftriaxone 30µg, ciprofloxacin 5µg, ceftiofur 30µg, Amoxicillin/clavulanic acid 30µg, ofloxacin 30µg, cloxacillin 5µg, erythromycin 5µg and cefuroxime 30µg. The result of each antibiotic was reported as susceptible, intermediate or resistant using the standard given by the Clinical and Laboratory Standards Institute (CLSI) as adopted by Ali *et al.* (2018) [1].

Statistical Analysis

The data generated were subjected to descriptive statistical analysis using percentages and Chi-square analysis was used in determining the prevalence rates. $p < 0.05$ was considered indicative of a statistically significant difference.

Results

Age Distribution of the Patients

The Table 1 presented the age distribution of the patients diagnosed with skin infection. The highest infection was observed in the age group 0-15 in all the three hospitals where the research was conducted namely Murtala Muhammad Specialist Hospital (MMSH), Muhammad Abdullahi Wase Specialist Hospital (MAWSH) and Aminu Kano Teaching Hospital (AKTH) with 23 subjects (30.7%), followed by the age group 16-25 with 21 subjects (28%) while the infection was lower in the age group above 60 years old patients with 6 subjects (8%). However, the result is not significant at $p < 0.05$

Table 1: Age Distribution of the Patients among the Hospitals

Age (years)	MMSH	MAWSH	AKTH	Total (%)	P-Value
0 – 15	11	7	5	23 (30.7)	.988905
16 – 30	12	6	3	21 (28.0)	
31 – 45	6	5	3	14 (18.7)	
46 – 60	4	4	3	11 (14.6)	
60 – above	3	2	1	06 (8.0)	
Total	36	24	15	75 (100)	

Key: *The X^2 value is 1.6984, and the result is not significant at $p < 0.05$. MMSH: Murtala Muhammad Specialist Hospital; MAWSH: Muhammad Abdullahi Wase Specialist Hospital; AKTH: Aminu Kano Teaching Hospital.

Identification of *S. epidermidis*

The identification of *S. epidermidis* from the samples obtained from the Hospitals is presented in the Table 2. The *S. epidermidis* was identified using Gram staining, biochemical test and Mannitol fermentation test. Result showed that the isolates were positive for Gram staining, catalase, coagulase, and DNase and Mannitol fermentation test. The isolates showed β -haemolysis on blood agar plates.

Table 2: Morphological and Biochemical Identification for *S. epidermidis*

S/N	Test	Inference
1	Gram staining	Positive
2	Catalase	Positive
3	Coagulase	Negative
4	DNase	Negative
6	Mannitol salt agar	White grey
7	Haemolysis	Negative
8	Novobiocin test	Sensitive

Antibiotic sensitivity profile of *S. epidermidis*

The average zones of inhibition and the standard deviation of the isolates against standard antibiotics used are presented in Tables 3. The isolates were classified into resistance,

susceptible or intermediate depending on the figure obtained according to standard guidelines of Clinical and Laboratory Standards Institute (CLSI).

Table 3: Mean Diameter of Zone of Inhibition with standard deviation for the *S. epidermidis* isolated (n = 21) against some antibiotics

Antibiotics	Concentration	ZOI
LEV	5µg	14.60 ±4.08
AK	30µg	19.68 ±1.90
FEP	30µg	13.23 ±2.44
CAZ	10µg	7.87 ±1.91
IMI	10µg	8.86 ±1.69
CN	10µg	13.17 ±3.53
F	30µg	18.82±4.01
CRO	30µg	12.84 ±4.39
CIP	5µg	17.65 ±1.97
FOX	30µg	16.38 ±2.99
ERY	5µg	12.12±3.99
CXC	5µg	9.09±3.40
CRX	30µg	9.38±2.73
AUG	30µg	7.91±2.95

Key: CAZ-Ceftazidime, FEP-Cefemine, FOX-Cefoxitin IMI-Imipenem, CRO-Ceftriaxone, CIP-Ciprofloxacin, F-Nitrofurantoin, AK-Amikacin, CN-Gentamicin, LEV-Levofloxacin, AUG-Amoxicillin/clavulanic acid, CXC-Cloxacillin, CRX-Cefuroxime and ERY-Erythromycin, ZOI-Zone of Inhibition

Number and percentage of Susceptible, Intermediate and Resistant *S. epidermidis*

Table 4 represent the number and percentage of *S.*

epidermidis species susceptible, intermediate and Resistant *S. epidermidis* to selected antibiotics. *S. epidermidis* was highly susceptible to Gentamicin 13 (61.90%), Levofloxacin 12 (57.14%) and Nitrofurantoin 11 (52.38%). On the other hand, the isolate was highly resistant to Ceftazidime 20 (95.24%), Cloxacillin 19 (90.48%) then Cefoxitin, Erythromycin and Amoxicillin/clavulanic acid with 15 (71.43%) each.

Table 4: Number and percentage of Susceptible, Intermediate and Resistant *S. epidermidis* to selected antibiotics

Antibiotics	Concentration	Susceptible	Intermediate	Resistant
LEV	5µg	12 (57.14%)	8(38.09%)	1 (4.76%)
AK	30µg	5 (23.80%)	4(19.05%)	12 (57.14%)
FEP	30µg	6 (28.57%)	1(4.76%)	14 (66.67%)
CAZ	10µg	0 (0.00%)	1(4.76%)	20 (95.24%)
IMI	10µg	4 (19.05%)	5(23.81%)	12 (57.14%)
CN	10µg	13 (61.90%)	1(4.76%)	7 (33.33%)
F	30µg	11 (52.38%)	5(23.81%)	5 (23.81%)
CRO	30µg	6 (28.57%)	2(9.52%)	13 (61.90%)
CIP	5µg	7 (33.33%)	3(14.89%)	11 (52.38%)
FOX	30µg	5 (23.81%)	1(4.76%)	15 (71.43%)
ERY	5µg	3 (14.29%)	3(14.89%)	15 (71.43%)
CXC	5µg	0 (0.00%)	2(9.52%)	19 (90.48%)
CRX	30µg	6 (28.57%)	1 (4.76%)	14 (66.67%)
AUG	30µg	5 (23.81%)	1 (4.76%)	15 (71.43%)

Key: CAZ-Ceftazidime, FEP-Cefemine, FOX-Cefoxitin IMI-Imipenem, CRO-Ceftriaxone, CIP-Ciprofloxacin, F-Nitrofurantoin, AK-Amikacin, CN-Gentamicin, LEV-Levofloxacin, AUG-Amoxicillin/clavulanic acid, CXC-Cloxacillin, CRX-Cefuroxime and ERY-Erythromycin

Discussion

It has been suggested that *S. epidermidis* is one of the most

important species of this group (Oliveira *et al.*, 2017) [16]. It is a Gram-positive, non-spore forming, non-motile, facultative anaerobic, and catalase-positive and coagulase negative bacterium responsible for different types of hospital and nosocomial infections (Oliveira *et al.*, 2017) [16]. The antimicrobial susceptibility pattern of *S. epidermidis* isolated shows that *S. epidermidis* was sensitive to Gentamicin

(61.90%), Levofloxacin (57.14%), and Nitrofurantoin (52.38%) while resistant to Ceftazidime (95.24%), Cloxacillin (90.48%) and Amoxicillin/clavulanic acid (71.43). In this study, it has been found that both the cloxacillin and Amoxicillin/clavulanic acid were highly resistant to *S. epidermidis*, indicating increasing resistance of the organism against beta-lactam drugs. Many beta-lactam antibiotics such as penicillins and cephalosporins have hydrolytically susceptible chemical bonds (e.g. esters and amides) and several enzymes (including β -lactamases) in *Staphylococcus* are known to destroy the antibiotic activity of such drugs by targeting and cleaving these bonds (Poole, 2004) [4]. The β -lactamases can often be excreted by the bacteria, inactivating antibiotics before they reach their target within the bacteria

Staphylococcus sp. developed differing mechanisms and means to resist against different antibiotics depending on their mechanism of action. Enzymatic degradation of drugs, structural modification of target and antibiotic efflux are mere strategies of resistance followed by the bacteria (Lowy 2003) [12]. Drug-resistant genes are either chromosomally located or plasmid lodged. However, conjugative plasmid is majorly accused of emerging resistance as it successfully transfers genetic material in both inter and intra-species (Grohmann *et al.*, 2003) [9]. This confers a basis for spreading resistance genes among bacterial population quicker than native mutation and vertical evolution (Kalpana and Harish, 2015) [10].

Conclusion

Based on the findings of the present study, *S. epidermidis* has demonstrated significant resistance against multiple antibiotics. The multiple antimicrobial-resistant indexes show an increase in the rates of resistance among these organisms due to enzymatic degradation of drug, structural modification of target and antibiotic efflux by the organisms thus making antimicrobial susceptibility surveillance and testing more crucial in selecting empiric regimen or definitive treatment. It is recommended that the widespread use of antibiotics should be controlled. The development of inhibitors of resistance enzymes to be administered as co-drugs with the antibiotics is also an important strategy to overcome resistance.

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