

Phenotypic Characterization and Antibiotic Susceptibility Studies of Coagulase- Negative Staphylococci (Cons) Isolated From Chronic Skin Ulcer of Patients In Kaduna State.

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Abstract: The study was aimed at characterizing the different Coagulase- negative staphylococci (CoNS) isolated from chronic skin ulcer out-patients attending four different hospitals in Kaduna State, Nigeria. A total of 292 swab samples were collected from January 2012 to January 2013. The samples were cultured using Chocolate and Blood agar media. Phenotypic characterization of the CoNS revealed the presence of *Staphylococcus epidermidis* (n=10, 28.5%), *Staphylococcus haemolyticus* (n=8, 22.9%), *Staphylococcus chromogenes* (n=7, 20.0%) *Staphylococcus hyicus* (n=4, 11.4%), *Staphylococcus xylosus* (n=5, 14.3%) and *Staphylococcus intermedius* (n=1, 2.9%). The different sites of chronic skin ulcer from where the CoNS were recovered revealed that the leg harbored the highest number of CoNS isolates of (n= 1, 51.4%), while the breast has the least isolate of (n= 1, 2.9%). The CoNS isolates were found mostly to be susceptible to Gentamicin, Ciprofloxacin, Trimethoprim-sulphamethoxazole, Chloramphenicol, Vancomycin and Erythromycin. However, they showed resistance mostly to Cefoxitin, Amoxicillin-clavulanic acid and tetracycline.

Key words: Phenotypic, CoNS, Antibiotic, Susceptibility, ulcer.

I. Introduction

The skin becomes broken when there is a cut or abrasion on the skin, and there is every likelihood that their protective defense mechanism becomes obstructed. When this happens, the environment becomes conducive for bacteria to contaminate the skin, increase in number and possibly cause an infection. The bacteria contaminating wounds are from the environment, through dust particles, bacteria on hands, clothing and equipment. Different kinds of wounds exist, ranging from superficial burns, bite wounds and surgical wounds (Bowler, 2001). The activities of microorganism in the wound could cause delayed healing in such wounds, thereby making the wound to be chronic. The most common type of chronic wound is an ulcer, which occurs usually in the lower leg of individual having underlying diabetes. The healings of such ulcer would delay, even when less pathogenic microorganisms are present (Williams et al., 2004). Coagulase negative staphylococci (CONS), previously considered as non- pathogenic have been identified as the etiological agents in most hospital acquired infections, and have been frequently isolated from such infections (Cunha et al., 2006). Apart from *Staphylococcus epidermidis* that is commonly isolated in wounds, other species have been implicated to cause infections. *Staphylococcus xylosus*, *Staphylococcus haemolyticus* and *Staphylococcus lugdunensis* have been reported to cause infection ranging from urinary tract infections (UTI), osteomyelitis and sepsis respectively (Venkatesh et al., 2006). *Staphylococcus epidermidis* has emerged in recent years as a pathogen in a growing number of other serious nosocomial infections, such as in neonatal intensive care units (NICUS), most especially bloodstream infections (Hall, 1991; Gaynes et al., 1996). *S. epidermidis* has also been widely recognized as an etiologic agent of bacteremia, prosthetic and natural valvular endocarditis, osteomyelitis and urinary tract infections, however in a frequent association with the colonization of intravascular catheters and orthopaedic devices (Sheagren, 1984; Brumfitt and Hamilton-Miller, 1989). *Staphylococcus saprophyticus* has been documented to be the second most frequently encountered microorganism after *Escherichia coli* in acute UTI (McTaggart et al., 1990; Gupta et al., 1999). Several other species of coagulase-negative Staphylococci have been implicated at low incidence in a variety of infections (Javid et al., 2006). One of the factors that favours the CoNS to wreak havoc in their host is their ability to produce enzymes such as lipases, proteases and other exo- enzymes, which have made them to be persistent, and possibly degrade host tissues (Otto, 2004). Several species of CoNS have been reported to produce haemolysins which made it possible for them to bind to susceptible host cells, and causing lysis of the red blood cells for the release of free iron which is eventually utilized by the bacteria (Azukah, 2013). The major reservoirs of coagulase-negative Staphylococci in hospitals are colonized or infected in-patients and colonized hospitals workers, with carriers at risk for

developing endogenous infection or transmitting infection to health care workers and patients (Harbarth et al., 2001). Transient hand carriage of these bacteria on the hand of health care workers account for the major mechanism for patient to patient transmission (Klimek, et al., 1976). Fourteen species of CoNS are currently recognized as human isolates (Kloos, 1995) Owing to the frequent and indiscriminate use of antibiotics by individuals to cure several infections earlier mentioned caused by the CoNS, they have become resistant to these antibiotics so used. However, some antibiotics are still effective against the CoNS isolates.

II. Materials And Methods

Sampling

Two hundred and ninety-two (292) swab samples were collected from clinically diagnosed chronic skin ulcer patients between January 2012 to January, 2013 from patients attending four hospitals in Kaduna state: Ahmadu Bello University Teaching Hospital, Zaria, Barau Dikko Specialist Hospital, Kaduna, General Hospital, Kafanchan and Hajiya Gambo Sawaba General Hospital, Zaria. The swab samples were collected from Surgical Out- Patients' Department (SOPD) clinics, General Out-patients' Department (GOPD) clinics, Orthopedics and Dental clinics across the four hospitals. Cases were recruited consecutively from the hospitals.

Ethical Approval: Ethical approval was obtained from the authorities of the Ahmadu Bello University Teaching Hospital, Zaria (ABUTH/HREC/TRG/36) and from the Ministry of Health, Kaduna state, (MOH/ADM/744/VOL.1/96) to cover the other three hospitals. Informed consent was however obtained from the patients concerned.

Isolation of Coagulase-negative Staphylococci

The samples were cultured on blood and chocolate agar plates using the streak method. The plates were incubated at 37°C, agar plates were observed after 24 hours, (Cheesbrough, 2002). Sub-culturing was carried from the agar plates in order to obtain pure cultures of the isolates. Thereafter, distinct well-separated and creamy white colonies on chocolate and blood agars were picked aseptically and stored on agar slants, which were used for further characterization.

Biochemical Characterization Coagulase-negative Staphylococci isolate

The isolates were subjected to some preliminary tests, such as, Gram stain, Catalase and Coagulase tests, before further biotyping to specie level using the Microgen Staph ID Kit (UK), following the manufacturer's instructions.

Antibiotic Susceptibility Testing

The isolates were sub-cultured as in the case of the biochemical tests by sub-culturing onto fresh Nutrient agar slants incubated at 37°C for 24 hours, before the antibiotic susceptibility testing was carried out. Suspensions were prepared from the sub-cultured isolates into clean, sterilized tubes according to 0.5 McFarland's standard using *S. aureus* ATCC 25923 as positive control strain.

The isolates were then tested for their susceptibility to 10 different antibiotics: Co-trimoxazole (25µg), Gentamicin (10µg), Cefoxitin (30µg), Tetracycline (30µg), Ciprofloxacin (5µg), Chloramphenicol (30µg), Erythromycin (15µg), Vancomycin (30µg) and Amoxicillin-clavulanic acid (30 µg).

The antibiotic discs (Liofilchem, Italy) were gently pressed to make sure they were in contact with the inoculated Mueller - Hinton agar surface, and the plates were incubated at 37°C for 18-24 hours. The zones of inhibition were measured after incubation to the nearest millimeter. The interpretation of the zones of inhibition was done using the chart adapted from Clinical and Laboratory Standards Institute (CLSI), (2007).

III. Results.

Table 1: Biotyping of Coagulase negative Staphylococci (CoNS) isolated with chronic ulcer in patients in Kaduna state.

ISOLATE	No of Isolate	% of Isolate
<i>Staphylococcus epidermidis</i>	10	28.5
<i>S. haemolyticus</i>	08	22.9
<i>S. chromogenes</i>	07	20.0
<i>S. xylosus</i>	05	14.3
<i>S. hyicus</i>	04	11.4
<i>S. intermedium</i>	01	2.9
Total no/% of isolates	35	100

The outcome of the biotyping of the isolates revealed the dominance of *S. epidermidis* over the other CoNS from the chronic skin ulcer of the patients recruited consecutively from the four hospitals under study. *Staphylococcus intermedius* has the least no of isolates with only one, as presented in table 1 above.

Table 2: Coagulase negative staphylococci (CoNS) isolated from different sites of chronic ulcer in patients.

Isolates	Breast	Leg	Hand	Mouth Region	Abdominal region	Ear	Buttocks
<i>S.epidermidis</i>	1	6	1	0	1	0	1
<i>S.haemolyticus</i>	0	6	0	2	0	0	0
<i>S.chromogens</i>	0	3	1	0	1	0	2
<i>S. xylosus</i>	0	2	1	1	1	0	0
<i>S. hyicus</i>	0	0	3	0	0	1	0
<i>S. ntermedius</i>	0	1	0	0	0	0	0
Total isolates from the sites	1(2.9%)	18(51.4%)	6(17.1%)	3(8.5%)	3(8.5%)	1(2.9%)	3(8.5%)

The different species of coagulase negative staphylococci which colonize the different anatomic sites of infection of the patients is as presented in the table 2 above, i.e., the number of isolates of each specie on the sites of infection. The percentages of isolates of the species of CoNS on the sites of infection are shown in parentheses.

Table 3: Percentage Resistance of Coagulase negative staphylococci (CoNS) to the antibiotics.

ANTIBIOTICS	<i>Staphylococcus epidermidis</i> (n = 10)	<i>S.haemolyticus</i> (n = 8)	<i>S. hyicus</i> (n =4)	<i>S.xylosus</i> (n = 5)	<i>S.chromogens</i> (n = 7)	<i>S.intermedius</i> (n = 1)
Cefoxitin(30µg)	60.0	100.0	100.0	80.0	57.0	0.0
Amoxicillin - Clavulanic acid(30µg).	50.0	87.5	100.0	60.0	57.0	100.0
Vancomycin(30µg)	50.0	37.5	50.0	40.0	28.6	0.0
Gentamicin(10µg)	10.0	0.0	0.0	0.0	14.3	0.0
Erythromycin(15µg)	30.0	12.5	0.0	20.0	28.6	0.0
Tetracycline(30µg)	50.0	50.0	75.0	40.0	28.6	0.0
Ciprofloxacin(5µg)	10.0	0.0	0.0	0.0	0.0	0.0
Trimethoprim - Sulfamethoxazoe(25µg)	30.0	37.5	75.0	40.0	28.6	0.0
Chloramphenicol(30µg).	10.0	25.0	0.0	20.0	14.3	0.0

n = no of isolates tested

From the antibiotic susceptibility testing, most of the isolates of CoNS showed resistance to cefoxitin, amoxicillin –clavulanic acid and tetracycline, however with varying degree of resistance to the said antibiotics as revealed in table 3 above. All the species of CoNS showed resistance to amoxicillin –clavulanic acid (augmentin).

Table 4: Percentage Susceptibility of Coagulase negative Staphylococci to the antibiotics

ANTIBIOTICS	<i>Staphylococcus epidermidis</i> (n = 10)	<i>S.haemolyticus</i> (n = 8)	<i>S. hyicus</i> (n =4)	<i>S.xylosus</i> (n = 5)	<i>S.chromogens</i> (n = 7)	<i>S.intermedius</i> (n = 1)
Cefoxitin(30µg)	40.0	0.0	0.0	20.0	42.9	100.0
Amoxicillin - Clavulanic acid(30µg)	50.0	12.5	0.0	40.0	42.9	0.0
Vancomycin(30µg)	50.0	62.5	50.0	60.0	71.4	100.0
Gentamicin(10µg)	90.0	100.0	100.0	100.0	71.4	100.0
Erythromycin(15µg)	50.0	75.0	100.0	80.0	42.8	0.0
Tetracycline(30µg)	30.0	37.5	25.0	40.0	42.8	100.0
Ciprofloxacin(5µg)	90.0	87.5	100.0	100.0	85.7	100.0
Trimethoprim – Sulfamethoxazoe(25µg)	70.0	62.5	0.0	60.0	71.4	100.0
Chloramphenicol(30µg)	80.0	62.5	50.0	80.0	71.4	100.0

n = no of isolates tested

Table 4 above shows the susceptibility of the isolates of CoNS to the antibiotics used in the study. It was found that most of the isolates were susceptible to gentamicin, ciprofloxacin, trimethoprim-sulphamethoxazole, chloramphenicol, vancomycin and erythromycin. However, the most effective antibiotics against almost all the isolates as revealed in- vitro are ciprofloxacin and gentamicin.

IV. Discussion

Staphylococcus haemolyticus, one of the coagulase negative staphylococci isolated in the present study has been reported to be one of the commonest species of coagulase negative staphylococci (CoNS) isolated from clinical samples in a previous study carried out by Bouchami et al. (2011). This also agreed with the report by Cuevas et al. (2004), Secchi et al.(2008), that *Staphylococcus haemolyticus* was isolated from clinical samples. Akpaka et al. (2006) reported that infection with *Staphylococcus epidermidis*, and less commonly with *S.haemolyticus* usually involves implantation of medical devices such as catheter. In support of this submission, a lot of the patients from whom swab samples were taken in this present study had implanted medical devices, like the catheter. This might have led to the isolation of *Staphylococcus epidermidis* and *S.haemolyticus* in the present study. *S.epidermidis* that was previously regarded as non-pathogenic (Tattevin et al.2006), is now been considered a pathogen, owing to the frequent isolation of this bacterium using bone biopsies (Javier et al., 2010).

In a previous study conducted by Campolini and Harding (2010), *Staphylococcus epidermidis* has been reported to be responsible for the majority of chronic osteomyelitis associated with orthopedic implants. Recently, a study has been published that stresses the importance of *S. epidermidis* as a pathogen in foot ulcers with protruding bone, that suggest underlying osteomyelitis, and this led to the proposal that *S. epidermidis* should be considered as a nosocomial pathogen (Gallowska et al.,2009). A research study conducted by Rahman et al. (2012) on specie distribution of CoNS isolated from different clinical specimens revealed that CoNS appeared to be the third causative agent of nosocomial infections, having collected 240 culture positive single isolates from surgical wound; 80 from pus of skin infections, 30 from burnt ulcer exudates and 20 from diabetic ulcer exudates. Common among the CoNS isolated were *S. epidermidis* *S.haemolyticus*, *S.xylosus*, *S. hyicus*, *S. chromogenes* etc., with *S. epidermidis* the most prevalent (17%).This finding by Rahman et al (2012) agrees with the findings in the present study (Table 4.1), since the swab samples taken, cut across surgical wounds, pus of skin infections ,burnt ulcer and diabetic ulcer exudates. *Staphylococcus .haemolyticus* (38%) and *S.xylosus* (4%) were also isolated from clinical sources ranging from catheter, blood, graft tissue and pus in a study conducted by Bouchami et al. (2011) .Another research study showcased a higher rate of CoNS in bone biopsy samples (Seneville et al., 2006), this finding may also support the idea that CoNS are true pathogens and not mere contaminants.

An important finding among the staphylococci isolated in the present study is *S. intermedius*. The name of this specie reflects the fact that while the organism possesses some phenotypic properties of *S.aureus*, it also exhibits some properties of *S. epidermidis* (Sudha et al., 2004). To justify the isolation of *S. intermedius* in the present study, the organism was previously recognized as an invasive zoonotic pathogen that has been isolated from 18% of canine – inflicted wounds (Lee, 1994). In three cases, it was also isolated in pure growth from non- canine inflicted wounds; two elderly patients with infected varicose ulcers and a 13 year old with an infected suture line, each with a history of exposure to dogs (Lee, 1994).

Sudha et al. (2004) also reported two cases of non- inflicted *S. intermedius* wound infection from two separate hospitals which occurred over an 8- month period.

The CoNS that were isolated in the present study include *S. epidermidis*, *S. haemolyticus*, *S.hyicus*, *S.xylosus*, *S.chromogenes* and *S.intermedius* .The antibiotic that was found to be effective in the present study against all the listed CoNS is gentamicin, of which they are highly susceptible to. Studies on antibiotic susceptibility pattern of CoNS in a tertiary care hospital showcased that the isolates were 77% susceptible to gentamicin (Saxena et al., 2013), corroborating the result of the present study. In agreement with this, Duran et al. (2012) reported 74% susceptibility of CoNS isolates from clinical samples of patients to gentamicin. Contrary opinion to these, however arises, where studies on the prevalence of CoNS isolates revealed 71% resistance to gentamicin (Martins et al., 2012).

Another antibiotic of high activity against CoNS isolates in the present study is ciprofloxacin. In support of this, is the report of Saxena et al. (2013), on the susceptibility of CoNS, it was submitted that ciprofloxacin has high activity of 83% against the isolates. This was however negated by Martins et al. (2012), whose submission stated that the CoNS isolates of venous ulcers showed resistance to ciprofloxacin by 71% .Chloramphenicol also proved to be effective against CoNS isolates as evident in the present study .Bouchami et al .(2011), on the antibiotic sensitivity pattern of CoNS isolated from clinical specimen, revealed 85% susceptibility of the isolates to chloramphenicol, to support the results of

the present study. Most of the CoNS isolates were susceptible to trimethoprim-sulfamethoxazole (co-trimoxazole) and vancomycin as reported in the present study. Antibiotic sensitivity pattern of CoNS from clinical specimens indicated that CoNS isolates were susceptible to co-trimoxazole by 95% (Bouchami et al., 2011; Duran et al., 2012).

This submission agrees with the report of the present study. The efficacy of vancomycin on the CoNS as reported in the present study conform with the findings on the susceptibility pattern of CoNS, where the drugs was found to be 100% active against CoNS (Duran et al.,2012;Saxena et al.,2013).

Methicillin resistant coagulase negative staphylococci (MRCoNS) isolates were also found to be present in chronic skin ulcers of the patients sampled across the hospitals in the study area..MRCoNS were reported on the basis of their resistance to cefoxitin (Oxacillin resistance) (CLSI, 2012). Most of the CoNS isolates were resistant to cefoxitin. This finding is in accordance with the report of Martins et al. (2012). Another antibiotic of interest that was found to be resisted by the CoNS is amoxicillin- clavulanic acid. The reason for this may be due to the fact that CoNS, primarily *S.epidermidis* and *S.heamolyticus* are often resistant to multiple antibiotics, and glycopeptides are the likely drugs to be used in order to manage the infection caused by CoNS (Cunningham, 1992; Menichetti, 1992).

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