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Characterization and Evaluation of Antibiotic Susceptibility Pattern of Coagulase Negative Staphylococci Isolated from Common Clinical Specimens in a Central Hospital in Delta State, Nigeria

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ABSTRACT: The emergence of coagulase negative Stapylococci as major pathogens reflects the increased use of implants in hospitals. Fifty-four coagulase-negative staphylococci were isolated from a total of 242 clinical specimens at the Central Hospital, Kwale. All isolates were identified by standard bacteriological methods. Antibiotic sensitivity test was performed on all coagulase- negative staphylococci (CoNS) isolates using the modified Kirby-Bauer method with 10 different antibiotics. *Staphylococcus epidermidis* was the commonest species (83.3%), while *Staphylococcus saprophyticus* was 16.7%, mainly from urine samples. Highest sensitivity was found with ofloxacin (81.5%), followed by ciprofloxacin (77.8%) and, highest resistance occurred with cotrimoxazole to which no isolate was sensitive.

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Coagulase-negative Staphylococci (CoNS) are morphologically similar to *Staphylococcus aureus*. They are Gram-positive, non-spurring, non-motile cocci. Colonies are usually non-pigmented (Humphreys, 2002). They are found among the normal flora of human skin and mucous membranes. They have been long regarded as harmless skin commensals and dismissed as culture contaminants. Previously, their potentially important role as pathogens and their increasing incidence have been recognised (Kloos and Bannerman, 1994; Von Eiff *et al.,* 1998).The emergence of CoNS as major pathogens reflects the increased use of implants such as cerebrospinal shunts, intravascular lines and cannulae, cardiac valves, pace-makers, artificial joints, vascular grafts and urinary catheters, and the increasing number of severely debilitated patients in hospitals (Humphreys, 2002). *Staphylococcus epidermidis* accounts for about 75% of all clinical isolates, and together with other novobiocin-susceptible CoNS, has emerged as a major cause of nosocomial infections, particularly, in bacteremia (Von Eiff *et al.,* 2001). *Stphylococcus haemolyticus* is ranked second after *Stphylococcus epidermidis* and has been associated with septicaemia in new-borns and various infections in persons with compromised host defences and implanted foreign bodies (Shittu and Kolawole, 2005). *Staphylococcus saprophyticus* is an important cause of urinary tract infections in young, sexually active men and women (Von Eiff *et al.,* 2001). Other species include *Staphylococcus hominis, Staphylococcus schleiferi, Staphylococcus capitis* and *Staphylococcus lugdunensis,* amongst others (Leven *et al.,* 1995). Previous reports have indicated that *Staphylococcus epidermidis* has caused some cases of osteomyelitis, wound infection, otitis media, endophthalmitis, urinary tract infection and even meningitis and pneumonia, but this has not been proven (Peters *et al.,* 1995; Heilmann and Peters, 2000).Multiple antibiotic resistances, in particular, methicillin resistance, is frequent among CoNS hospital strains on a global scale (Jarlov, 1999). The resistance genes found in multiple resistant CoNS are the same as those in *Stapyylococcus aureus* (Archer and Climo, 1994).

This study is to characterize and evaluate the antibiotic susceptibility pattern of CoNS in common clinical specimens in a General Hospital Delta State, Nigeria.

MATERIALS AND METHODS

Study area: This study was carried out in a Central Hospital Delta State, Nigeria from May to October 2018. Kwale has a population of 114,117l people, located in the South-South geopolitical zone of Nigeria. It is a semi-urban town with the major

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occupation of farming, trading, civil servants and students (World Gazetter, 2007).

Sample collection: A total of two hundred and forty-two (242) samples which include conjunctival swabs; 62, blood samples; 50, wound swabs/biopsies; 47, urine; 56, and ear swabs; 27 were collected from patients by random sampling technique at the Medical Laboratory Department of the Central Hospital Kwale. All Samples were analysed immediately after collection.

Preparation of media: All media used (nutrient agar, MacConkey agar, blood agar, Glucose broth and Sodium thioglycolate broth) were weighed appropriately and prepared according to manufacturers instruction. They were autoclaved at 121°C for 15min and allowed to cool before use (Cheesbrough, 2000).

Isolation of bacteria: Specimens were inoculated on blood agar, MacConkey agar, and chocolate agar with the exception of blood samples. The blood samples were first inoculated in Brain heart infusion broth and Sodium thioglycolate broth and incubated at 37ºC for 10 days. Signs of growth, turbidity, haemolysis, and bubbles were checked for within this period before being sub-cultured into the above-named plates. The plates were incubated at 37ºC for 24hours.

Identification of bacterial isolates: All the CoNS isolates were identified using the bacteriological techniques and parameters, which include the biochemical characteristics, morphological, and cultural appearance as described by Cheesbrough (2000).

Antibiotic sensitivity pattern of CoNS: Antibiotic sensitivity test was carried out using the modified Kirby-Bauer disc diffusion method as described by Cheesbrough (2000).

Ethical permission: Ethical permission/approval was obtained from the ethical committee of Central Hospital Kwale.

RESULTS AND DISCUSSION

Out of the 54 CoNS isolates obtained from this study, *Staphylococcus epidermidis* was the commonest species (83.3%), followed by *Staphylococcus saprophyticus* (16.7%). This is similar to the findings of Mohan *et al* (2002) who in their study of CoNS reported *Staphylococcus epidermidis* as the most common species (82.3%), followed by *Staphylococcus saprophyitcus* (15.6%); and of Monsen *et al* (2005), who recorded that all CoNS isolates were *Staphylococcus epidermidis* except one (Table 1).

Table1: Distribution of 54 CoNS isolates in clinical specimens

Body site	***S. epidermidis***		***S. saprophyticus***	
	Number	Percentage	Number	Percentage
Conjunctival	14	25.9	2	3.7
Blood	14	25.9	-	-
Wound swab/biopsy	12	22.2	1	1.9
Urine	2	3.7	6	11.1
Ear swab/discharge	3	5.6	-	-
Total isolates	45	83.3	9	16.7

However, this result does not agree with that of Monsen *et al* (2005), who reported that all species isolated were *Staphylococcus epidermidis* except only one species of *Staphylococcus haemolyticus;* also Shittu and Kolawole (2005) observed that *Staphylococcus haemolyticus* ranked second after *Staphylococcus epidermidis* (Table 2). The highest frequency of 11.1%was observed in urine samples for *Staphylococcus saprophyticus*. This agrees with the work of Mohan *et al* (2002) who reported that *Staphylocccus saprophyticus* strains were isolated mainly from urine; but disagreed with the findings of Uesugi *et al* (1996) who reported *Staphylocccus epidermidis* as more prevalent (36.0%) than *Staphylocccus saprophyticus* (26.0%) in urine samples. This indicates that *Staphylocccus saprophyticus*is becoming more common in urine samples than in other clinical specimens. This further implies that the clinical importance of CoNS in clinical specimens including urine cannot be overemphasized. Resistance to contrimoxazole was 100%. The report of Zakaria (2005), however, showed a resistance of 63.1% to contrimoxazole tested on CoNS isolates in Palestine as shown on the comparative disc sensitivity (Table 2).All the strains of CoNs were resistant to one or more of the 10 antibiotics used for the study. Highest sensitivity was observed in ofloxacin (81.5%), followed by ciprofloxacine (77.8%), both of which fluoroquinolones and lowest sensitivity occurred in tetracycline (20.4%). No isolate was sensitive to cotrimoxazole (Table 3).

Table 2a: Disc sensitivity pattern of CoNS isolates

Number of strain	OFL		CIP		AUG		AMX		
N	S	R	S	R	S	R	S	R	S
	38	7	38	7	35	10	29	16	61
S. epidermidis N=45					-				
	(84)	(16)	(84)	(16)	(78)	(22)	(64)	(36)	(64)
	6	3	4	5	6	3	4	5	4
S. saprohyticus N=9	(67)	(33)	(44)	(56)	(67)	(33)	(44)	(56)	(44)

Table 2b: Disc sensitivity pattern of CoNS isolates

Number of strain	ERY		CRO		GEN		MET		TET		COT	
N	R	S	R	S	R	S	R	S	R	S		
	16	27	18	27	18	17	28	-	35	-		4 5
S. epidermidis N=45												
	(36)	(60)	(40)	(60)	(40)	(38)	(62)	(22)	(78)	-		
	5	4	5	2	7	-	9	1	8	-		
S. saprohyticus N=9	(56)	(44)	(56)	(22)	(78)	-	(100)	(11)	(89)	-	10 0	

L=Percentage of number; OFL=Ofloxacin; ERY=Erythromycin; N=Number of strains; CIP=Ciprofloxacin; GEN=Gentamicin; S=Sensitivity; CRO=Ceftriaxone; TET=Tetracycline; R=Resistance; AUG=Augmentin; COT=Cotimoxazole; AMX=Amoxycillin; MET=Methicillin

This is similar to the report of Manikandan *et al* (2005) who recorded 60% sensitivity of CoNS isolates to ciprofloxacin, although, it reflects an increased sensitivity. This study was done in India, the difference in geographical area may be responsible for the seemingly higher sensitivity. Olayinka *et al* (2005) reported antibiotic resistance of 16.1% to ciprofloxacin, which is relatively lower than 22.2% resistance obtained in this study. This indicates an increase resistance. Also, the result of this study does not agree with that of Obi *et al* (1996) who found that all CoNS isolates were sensitive to ofloxacin; and of Shobha *et al* (2005) who reported 100% sensitivity to ciprofloxacin in a study of CoNS in India. This antibiotic sensitivity pattern may imply that CoNS isolates are becoming increasingly resistant to fluoroquinolones which may be due to uncontrolled use of antibiotics and, the resistance patterns of CoNS which may vary from one geographical area to another.

Resistance of methicillin was 68.5%, being the third highest resistance, after contrimoxazole and tetracycline. This is relatively high when compared with the result of Mohan *et al* (2000) which showed more than 20% resistance to methicillin, and with that of Olayinka *et al* (2005) who reported that resistance pattern of CoNS to methicillin was 49.1% when studying CoNS in Zaria, Nigeria. This reflects an increasing resistance to methicillin and may correlate with increased indiscriminate use of antibiotic in hospitals, and increased cross-reflection with hospital-acquired CoNS strains.

Table3: Antibiotic sensitivity pattern of CoNS strains

Antibiotic	number of strains	% sensitive strains	% resistant strains
Ofloxacin	44	81.5	18.5
Ciprofloxacin	42	77.8	22.2
Augmentin	41	75.9	24.1
Amoxicillin	36	66.7	33.1
Erythromycin	33	61.1	38.9
Ceftriaxone	31	57.4	42.6
Gentamicin	28	51.9	48.1
Methicillin	17	31.5	68.5
Tetracycline	11	20.4	79.6
Cotrimoxazole	-	-	100.0

Conclusion: Staphylococcus epidermidis is the commonest CoNS species obtained from clinical specimens, followed by *Staphylococcus saprophyticus*, which appears to be prevalent in urine samples. This study has shown that fluoroquinolones such as ofloxacin and ciprofloxacin appear to be the most potent antibiotics against CoNS strains.

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