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PREVALENCE AND ASSESSMENT OF ANTIBIOTIC SUSCEPTIBILITY PROFILES OF COAGULASE-NEGATIVE STAPHYLOCOCCI ISOLATED FROM CLINICAL SAMPLES.

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ABSTRACT

Background: Persistent increase in Microorganisms' resistance to available antibiotics has become a global concern. The changing role of many microorganism from non-pathogenic normal body flora to pathogenic strains increases the need for regular surveillance of antibiogram profile of clinical isolates. This study was designed to investigate the prevalence and susceptibility pattern of Coagulase-Negative Staphylococci (CoNS) in Sagamu area of Ogun State, Nigeria. **Materials and Methods:** This 5-year retrospective study covered between 2011 and 2015 with 115 coagulase negative staphylococci isolated. The isolation, identification, assessment for methicillin resistance and susceptibility testing of the isolates followed the standard methods of Clinical Laboratory Standard Institute (CLSI). **Results:** CoNS were isolated from 72 females and 43 male patients with 30.4% isolated from Urine samples. The highest number of CoNS (34) was isolated in 2014 while the lowest (14) in 2012. Seventy-three (63.5%) of the CoNS isolates were screened methicillin resistant (MRCoNS). All the MRCoNS were resistant to Penicillin, 84.9% to Cephalexin and 28.8% to Gentamycin. No resistance against Ciprofloxacin among MSCoNS but 71.2% among MRCoNS. Multidrug resistance was observed among the two groups of CoNS. **Conclusion:** The importance of continous monitoring of the antibiogram of CoNS cannot be overemphasized. This study will provide the needed guide for clinicians in antibiotic selection and formulation of antibiotic policy to curb spread of resistant strains.

KEYWORDS: Coagulase-Negative Staphylococci (CoNS), Sagamu, Retrospective study, Methicillin Resistance CoNS, MSCoNS.

INTRODUCTION

Coagulase-negative Staphylococci (CoNS) is a group of opportunistic pathogen causing wide spectrum of diseases in humans. Its association with increased number of hospital acquired infections has been documented (Koksal *et al.*, 2009; Sohn *et al.*, 2001). Skin and mucous membranes are the common habitats of most Coagulase-negative Staphylococci (CoNS) associated with clinical diseases. CoNS's residency on the skin makes them the most frequent contaminant of blood cultures often complicating the interpretation of laboratory results (Favre *et al.*, 2005). The significance of CoNS isolated in culture can be difficult to determine. The increasing number of patients with impaired host defences and the use of invasive devices such as catheter have contributed to CoNS infection rate (Mayhall, 2004). CoNS involvement in bacteremia has been associated with long term usage of indwelling venous catheters, previous antibiotic abuse, nature of illness e.g. burns and non-adherence to infection control practices especially hand washing practices of medical staff (Winn *et al.*, 2006; Mandel *et al.*, 2000). The aetiology of bacteremia in developing countries especially among immunocompromised patients has continue to change, however, in Nigeria, the rate of bacteremia due to CoNS is below 2% (Samuel *et al.*, 2006) Although, there are about 20 CoNS species, *S. epidermidis* is the most prevalent species accounting for approximately 60-70% of all CoNS on the skin. Though often neglected, identification to species level can aid in the recognition of outbreaks and in tracking resistance trends. CoNS have been implicated as the causative agents in urinary tract infections, shunt infections, pneumonia, surgical wound infections, breast abscess, osteomyelitis and endocarditis (Wu *et al.*, 2006; Moazzez *et al.*, 2007). Though many studies have been carried out on CoNS involvement in bacteremia, not much has been done on general clinical specimens especially in Nigeria because most isolations of CoNS are often viewed as contaminants. This study was therefore carried out to determine the prevalence and antibiotic susceptibility pattern of CoNS in Sagamu area of Ogun State. The

result might also be very useful in the formulation of antibiotic policy and institution of control measures in cases of outbreaks.

MATERIALS AND METHODS

This was a five-year retrospective study done in a Government registered Laboratory in Sagamu, Ogun State, Nigeria between January 2011 and October 2015. The patients' biodata were obtained from the laboratory's register. A total of 115 CoNS isolated from clinical specimens which include blood (5) wounds /burns (32) semen (7) urine (35) aspirates (10) ear swab (9) urethral swabs (5) eye swabs (8) and CSF (4) were identified based on colonial morphology, Gram's reaction, positive catalase and negative coagulase tests adopting standard methods (CLSI, 2010).

Methicillin Resistance test

This was performed using the cefoxitin disc diffusion method recommended by CLSI (CLSI, 2010). The isolates were considered methicillin resistant if zone of inhibition was 10mm or less.

Antibiotic Susceptibility testing

Antimicrobial susceptibility of the CoNS strains were determined by the disk diffusion method on Mueller-Hinton agar according to the Clinical and Laboratory Standards Institute (CLSI,2010) to the following antibiotics; gentamycin (10µg) penicillin (60µg) erythromycin (10µg) cotrimoxazole (25µg) ciprofloxacin (5µg) tetracycline (10 µg) cephalexin (30µg) chloramphenicol (32µg) Azithromycin (15µg) cloxacillin (5µg).

RESULTS

A total of 115 CoNS was isolated from 43 males and 72 females with the highest number of isolates (43) recovered among the age group of 31-40 years as shown in Table 1.

Table 1. Demographic information about the patients involved in the study.

(a)	Sex		% occurrence
	Male	43	37.4
	Female	72	62.6
(b)	Distribution within age range (years)		
	≤ 10	8	7.0
	11-20	12	10.4
	21-30	15	13.0
	31-40	43	37.4
	41-50	25	21.7
	≥51	12	10.4

Table 2 showed the breakdown of CoNS among the specimens analysed. Urine was the most infected sample where 35 samples (30.4%) grew CoNS which was closely followed by wound/burns with 32(27.8%). The colonization was low in CSF 4(3.5%), blood 5(4.4%) and Urethral swab 5(4.4%).

Table 2: Distribution of CoNS within clinical specimens analysed.

Specimen	No isolated	% isolated
Blood	5	4.4
Wound/Burns	32	27.8
Semen	7	6.0
Urine	35	30.4
Aspirates	10	8.7
Ear swabs	9	7.8
Urethral swabs	5	4.4
Eye swab	8	7.0
CSF	4	3.5

All the MRCoNS were resistant to Penicillin, 84.9% to Cephalexin but 28.8% to Gentamycin as shown in Table 3. Among the MSCoNS, no resistance was shown against Ciprofloxacin and only 26.2% against Gentamycin.

Table 3. The antibiotic resistance pattern of CoNS isolated from clinical samples.

Antibiotics	MRSCoNS (n=42)	%Resistance	MRCoNS (n=73)	%Resistance
Gentamycin	11	26.2	21	28.8
Penicillin	35	83.3	73	100.0
Erythromycin	29	69.1	65	89.0
Ciprofloxacin	0	00.0	52	71.2
Cephalexin	38	90.5	62	84.9
Tetracycline	20	47.6	39	53.4
Chloramphenicol	27	64.3	27	37.0
Azithromycin	20	47.6	65	89.0

Cloxacillin	0	00.0	73	100.0
Cotrimoxazole	39	92.9	57	78.1

The yearly record of CoNS is shown in Figure 1 with the highest isolation of 34(29%) in 2014 and the lowest 14(12%) in 2012 while the susceptibility pattern to methicillin is shown in Figure 2 where 73 (63.5%) of the species were identified to be Methicillin Resistant CoNS (MRCoNS).

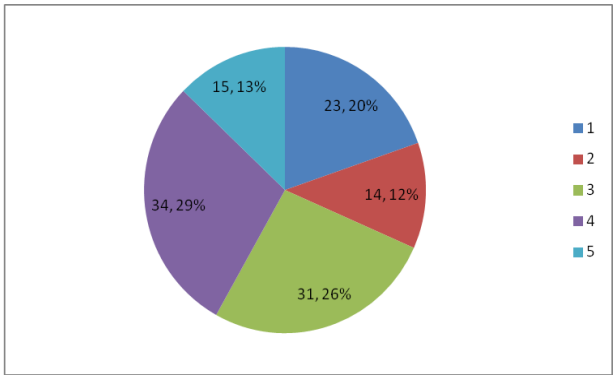

Figure1. Distribution of CoNS according to year of isolation.

Key: **1**- 2011; **2**- 2012; **3**- 2013; **4**- 2014; **5**- 2015

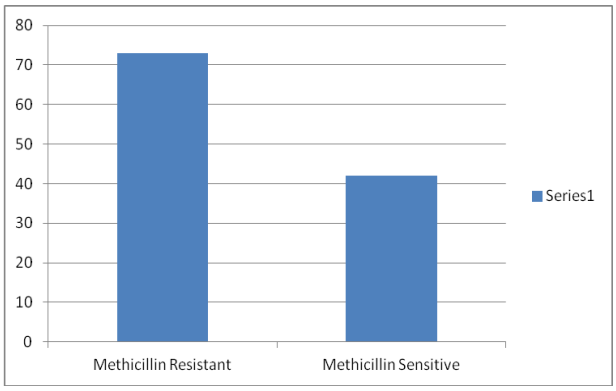

Figure 2. Distribution of CoNS according to Methicillin Susceptibility.

DISCUSSION

As efforts are on to produce novel antibiotic that will break all the resistant mechanisms of bacteria, the organisms too are evolving new mechanisms to evade the destructive ability of the drugs. This calls for regular and critical evaluation of the susceptibility profile of comon pathogens in clinical practice. CoNS are a group of bacteria regarded as normal commensal of the mucous membrane, skin, groin and axillae (Gruer *et al.*, 1984). The isolation and identification of CoNS from normally sterile fluids (CSF, Blood etc) must likely have resulted from the breakdown of normal physical barriers to bacterial entry and hence has been reported as one of the most common agents in nosocomial infections (Nash *et al.*, 2013). In this study, 62.6% of the CoNS isolates were from female patients, though no gender distribution has been reported, this might be in proportion to their visitation to hospitals and diagnostic centers. Bertakis (2000) reported that women had a significantly higher mean number of visits to their primary care clinic and diagnostic services than men. The highest number of CoNS isolated among the young adults (ages 31-40) in this study was in disagreement with their visitation to hospital and the report of Lau *et al*, (2014), who ascerted that young adults had significantly lower rates of overall healthcare utilization. The frequency of MRCoNS in this study was 63.5%. Koksal *et al* (2007) in a similar study reported a prevalence of 67.5%. However,our finding was in contrast with similar studies done by Kahdri and Alzohairy (2010) in which they reported a prevalence of 39.4% and Srikanth and Mir (2013) who also reported 40%. The varied prevalence in these studies might be influenced by many factors ranging from cultural diversity, economic status to degree of antibiotic abuse. All MRCoNS have been shown to contain a mecA gene which can easily spread to all other species of Staphylococci probably through transposons (Mayhall, 2004). MRCoNS have been reported to be a predominant pathogen among hospitalized patients with increasing infection rates (Sohn *et al.*, 2001; Koksal *et al.*, 2009). They have become a major concern with the extra ordinary ability to adapt to antibiotic stress of the hospital environment. necessitates the adoption of a regular screening for methicillin resistance in all isolates from clinical specimens. Prevalence of CoNS in bacteremia from this study was 4.4% Slightly higher than was reported for national average of below 2% (Samuel *et al.*, 2006). This can be better explained as the effect of increased awareness of the role CoNS play in infection, with a resultant attention being given to their isolation and hence increase in prevalence. The isolation of CoNS from various clinical samples as found in this study, has also been reported from previous studies (Srikanth and Mir,2013; Nash *et al.*, 2013; Jagadeeswari *et al.*, 2013). However, Urine and Wound/Burns gave the highest isolation with 30.4% and 27.8% respectively which is closely related to the findings of Srikanth and Mir (2013). There is therefore, the need to critically assess any correctly and carefully collected clinical sample for the presence of CoNS. In this study, a high resistance demonstrated against cotrimoxazole (92.9%) Cephalexin (90.5%) and Penicillin (83.3%) among MSCoNS have also been reported (Jagadeeswari, 2013; Schumacher-Perdreau, 1991). This high level of resistance will make treatment of infections caused by these pathogensdifficult and equally neccessitated the screening of isolates for β-Lactamase production. A very high resistance was demonstrated by both MSCoNS (92.9%) and MRCoNS (78.1%) against cotrimoxazole. The resistance by Cotrimoxazole has been shown to be between 10-50% in Turkey and 47-76% in European countries (Sakarya *et al.*, 2004; Altoparlak *et al.*, 2004). The increasing resistance of CoNS against cotrimoxazole, which is an alternative drug in the treatment of MRCoNS is generating uneasiness and thereby receiving attention. No resistance was shown against Ciprofloxacin among the MSCoNS, though a

common- self-prescribed and used antibiotic, this raises hope in the treatment of infections caused by MSCoNS. However, its use over a long period of time may lead to the development of cross-resistance (CLSI, 2010). On the other hand, high resistance was demonstrated among MRCoNS making its use highly dependent on the methicillin resistance assessment of the incriminated species. Gentamycin is one of the most readily available and cheap antibiotics, but being an injectable drug will greatly reduce its abuse. There was a relatively low resistance of both MRCoNS and MSCoNS to Gentamycin in this current study. This contradicts some earlier studies that reported high resistance to gentamycin (Srikanth and Mir, 2013; Sanyal *et al.*, 1993). Multidrug resistance was observed among the two groups just as it has been reported (Koksal *et al.*, 2009; Khadri and Alzohairy, 2010). Conclusion: A baseline information has been generated by this study about the prevalence and susceptibility pattern of CoNS a deviation from the normal studies generally done on *Staphylococcus aureus*

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