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Species Distribution and Virulence Factors of Coagulase Negative Staphylococci Isolated From Clinical Samples From the University of Benin Teaching Hospital, Edo State, Nigeria.

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Abstract
Coagulase negative staphylococci (CoNS) have become a major public health problem due to an increase in invasive procedures and antibiotic resistance. The prevalence and some virulence factors of CoNS species isolated from clinical samples obtained from a hospital in Benin-City, Nigeria was investigated. A total of 79 clinical samples were collected from clinical specimens over a six month period in a non consecutive manner from a teaching hospital in Nigeria. Samples were cultured on MacConkey agar, Cystine –Lysine-Electrolyte-Deficient agar (CLED) and blood agar (Oxoid Ltd Basingstoke UK). Identification of the various CoNS species was done using Microbact staph ID system (Oxoid product). Determination of hemolysin production was done in blood agar plates while slime detection was carried out using the modified congo red agar method. The most commonly isolated species were *S. haemolyticus* (28.3%), *S. epidermidis* (26.7%) and *S. saprophyticus* (18.33%). Others were *S. simulans* (10%), *S. xylosus* (10%), *S. chromogenes* (15%) and *S. schleiferi* (1.67%). Hemolysis on blood agar plates was observed in 34.2% of CoNS with *S. haemolyticus* (58.8%) predominating followed by *S. epidermidis* (25%). Slime production was positive in 75.95% of all CoNS species isolated. All isolates of *S. simulans* (100%) and *S. xylosus* (100%) were positive for slime production followed by *S. epidermidis* (93.7%) and *S. haemolyticus* (70.6%). Due to the increasing medical significance of CoNS, these organisms should not be ignored or classified as mere contaminants.
Keywords: Coagulase negative *Staphylococcus*, virulence factors, infections, slime, hemolysin.

INTRODUCTION

Coagulase negative staphylococci (CoNS) have been identified as the etiological agent in various infections and are among the microorganisms most frequently isolated in nosocomial infections (Cunha *et al*., 2006). This has generated a new research interest in them. CoNS have been implicated in neonatal septiceamia (Ghelbi *et al.*, 2008), bacterial eye infections (Ogbolu *et al.*, 2011) infective endocarditis (Patel *et al.*, 2000), and prosthetic joint infections (Trampuz and Zimmerli, 2005). Although *Staphylococcus epidermidis* is the CoNS most commonly isolated in the clinical microbiology laboratory, other CONs species are also increasingly being associated with infections. Other species known to cause infections includes *Staphylococcus xylosus, Staphylococcus hominis, Staphylococcus schleifieri, Staphylococcus saprophyticus (*urinary tract infections in immunocompetent women), *Staphylococcus lugdunensis* (implicated in sepsis), and *S. haemolyticus* which has been associated with endocarditis and osteomyelitis (Venkatesh *et al.*, 2006). Evidence indicates that pathogencity might be related to the production of an extracellular polysaccharide known as slime that permits these microorganisms to adhere to smooth plastic surfaces, colonizing catheters, prosthetic heart valves, pacemakers and Joint prosthesis (Vogel *et al*., 2000) and tissue surfaces.

Many CoNS also produce several lipases, proteases and other exoenzymes which possibly contribute to the persistence of CoNS in the host and may degrade host tissues (Otto, 2004). Hemolysins were also produced by isolates of *S .epidermidis, S. haemolyticus, S. warneri*. Hemolysin binds to susceptible cells leading to rapid release of vital molecules. Most importantly, they lyse red blood cells for release of free iron taken up by the bacteria. *S. haemolyticus* has also been reported to produce hemolysin and is often multiple antibiotic resistant (Gunn and Davis, 1988). Although *S. epidermidis* is believed to account for most of the infections caused by CoNS, the specific infecting CoNS species remains undetermined in most clinical laboratories.

CoNS species identification which is necessary in order to establish epidemiological trends, confirm treatment failures and determine the cause of specific infections in the community. Understanding the mechanisms of pathogenesis will help in the development of preventive and control measures. This research is aimed at determining the prevalence of CoNS, identifying the various species and some of the virulence factors of CoNS isolated from clinical samples in our community.

MATERIALS AND METHOD

Sample collection

Seventy-nine samples from different clinical conditions were collected from a teaching hospital over a period of

6 months (April to September, 2011). The clinical samples were from infected wound (14), urine (13) exudates from male genital infections (10), swab samples from female genital infections (10), tonsilitis and pharyngitis (8), urinary catheter (2), blood (suspected septiceamia) (4), eye infections (6), otitis media (1), infected burns (6) and other clinical samples (5).

Culture and identification of Coagulase Negative Staphylococci Species

All samples except urine samples were cultured on Blood agar plates, MacConkey agar, Robertson Cooked Meat agar (RCM) and incubated aerobically at 37^{o}C for 24 hours. Urine samples were cultured on Cystine Lysine Electrolyte Deficient (CLED) medium and MacConkey agar plates at 37^{o}C for 24 hours. Suspected staphylococcal colonies were identified by Gram staining, catalase and coagulase test. CoNS were detected by using the slide and tube coagulase tests. Identification of CoNS species was done using the MICROBACT *Staphylococcus* identification system 12S (Oxoid Ltd Basingstoke UK). The system uses a combination of sugar utilization and colorimetric enzyme detection substrates. Each kit contains Staph 12S test strips each consisting of 12 wells containing different dehydrated substrates, suspending media consisting of buffering agents and peptones for preparing the inoculum, a holding tray, an organism report form and a color interpretation chart. The color chart was used as a guide for interpreting reactions and the results entered into a computer aided software to produce probable species.

Hemolysin Production

Production of hemolysin was determined on blood agar plates incubated at 37^{o}C for 24 hours. A positive result was indicated by the formation of hemolytic zones around the colonies.

Slime formation

Assessment of slime formation was determined using the Congo red agar method (Freeman *et al.* 1989). Several colonies of a single isolate were inoculated on plates of the medium and incubated aerobically for 24hours at 37^{o}C. A positive result was indicated by black colonies. Non-slime producers usually remained pink.

RESULTS

A total of 105 CoNS isolates from different clinical samples were obtained following either tube or slide coagulase tests. With both tube and slide coagulase, 79 isolates were coagulase negative and the remaining 26 coagulase positive which was discarded.

CoNS were most commonly isolated from infected wounds (17.7%), followed by urine from cases of urinary tract infections (16.5%) and least isolated from ear infections (1.26%) (Table I). Infected wounds were mainly from surgical wounds, diabetic foot ulcer and prosthetic devices.

S. haemolyticus (28.3%) was the most commonly isolated CoNS closely followed by *S. epidermidis* (26.7%), then *S. saprophyticus* (18.3%). The remaining species were distributed between *S. simulans* (10%), *S. xylosus* (10%), *S. chromogenes* (5%) and *S. schleiferi* (1.67%). (Table II)

S. haemolyticus also had the highest number of heamolytic strains (58.8%) followed by *S. epidermidis* (25%), *S. saprophyticus* (25%), *S. xylosus* (50%), *S. simulans* (50%) and non speciated CoNS (10.53%). (Table III)

Slime formation by CoNS species is shown in Table IV. Most of the isolates were positive for slime production (75.95%). Predominantly, *S. xylosus, S. schleiferi, S. simulans* (100%), *S. epidermidis* (93.7%) and *S. heamolyticus* (70.6%) also had a good number of slime producing strains.

DISCUSSION

CoNS, once considered as members of the normal flora of the human body are now known to be a major cause of opportunistic infections.

The highest number of CoNS isolates were from found wounds samples (17.7%) followed by urine (16.5%). This is in line with the reports of Vuong and Otto. (2002) who reported CoNS as the major aetiologic agent in wound infections and urinary tract infections.

S. haemolyticus (28.33%), *S. epidermidis* (26.67%) and *S. saprophyticus* (18.33%) were the most commonly isolated CoNS species and this result is not significantly different from other reports where *S. epidermidis* was the highest isolated followed by *S. haemolyticus* in clinical infections (Begum *et al.,* 2011). Kloos and Bannermann (1995) also reported that *S. epidermidis* and *S. saprophyticus* as the most isolated CoNS species in clinical infections. Other isolates were *S. simulans* (10%), *S. xylosus* (10%), *S. chromogenes* (5%) and *S. schleiferi* (1.67%). This is similar to a work done by Adeleye *et al.* (2010) and Akinkunmi and Lamikanra, (2010) that isolated similar CoNS species from clinical samples. *S. epidermidis* was the most isolated specie of CoNS implicated in wound infections and this is in accordance with similar works earlier reported (Duran *et al*., 2010). Lark *et al.* (2001) reported that the skin of 80-90% of people is colonized with *S. epidermidis* and that most CoNS infections are acquired from patients own flora. In Nigeria, CoNS is one of the common causes of infections of open fractures in wounds and delay in wound debridement is a major predisposing factor to wound

infection (Ikem *et al.*, 2004). *S. simulans* has been associated with cases of osteomyelitis and prosthetic joint infections. It is common in the normal flora and primarily acquired through contact with domestic animals (Kline, 2010). CoNS were also reported in burns in this study and this can be attributed to the immunocompromising effect of burn wounds (Shahnaz and Mohammadali, 2011).

Prevalence of *S. saprophyticus*, *S. epidermidis* and *S. haemolyticus* has also been reported in urine samples (Kumari *et al.*, 2001) Pathogenicity of *S. saprophyticus* in urinary tract could be attributed to the fact that it has the ability to adhere to uroepithelial cells (Raz *et al.*, 2004). *S. haemolyticus* grows in moderate numbers on human skin and is one of the few CoNS species found in urethra or periurethra of men and women. (Gunn and Davis, 1988) and this could be a source of infection. They've been implicated in UTI in males of all ages leading to epididymitis, urethritis and prostratitis. Isolation of *S. epidermidis* from catheterized patients was not surprising as they have been reported to be the most prominent cause of intravascular associated catheter infection (Rupp and Archer, 1994). *S. epidermidis* also produce slime which enables them adhere to these surfaces leading to biofilm formation. CoNS have also been isolated in catheter associated urinary tract infections in Nigeria (Taiwo and Aderounmu, 2006).

Infections of the female genitals investigated in this study were of pelvic inflammatory disease and cervicitis. *S. haemolyticus* and *S. saprophyticus* were isolated from these sites. The isolation of *S. epidermidis* and *S. simulans* from samples of scrotal aspirate, urethral swab and semen is similar to reports of Barcs *et al* (1989). The implication of *S. saprophyticus*, *S. epidermidis* and *S. simulans* in ear, nose and throat (ENT) infections is in agreement with the reports of Yildirim *et al.*, 2004 who isolated CoNS from 45.8% of chronic sinusitis and Akinjogunla and Enabulele (2010) who implicated CoNS in 42.9% of cases of otitis media. These bacteria may proliferate to cause infections under conditions that defeat the normal mucocillary defense mechanism. *S. simulans* however has not been reported in the past in throat infections. More reports are necessary to ascertain its status as an opportunistic pathogen.

S. xylosus was the only CoNS species isolated from blood and it has earlier been reported by Begum *et al.* (2011) as an etiologic agent of bacteraemia. *S. xylosus* has been reported to produce a delta-hemolysin that gave synergistic, complete hemolysis of washed human, sheep, and ox blood cells in an area of beta-lysin activity from strains of *S. aureus* and *S. intermedius*. *S. chromogenes* which is rarely a human pathogen was also isolated in this study and it has earlier been implicated in blood stream infections of some patients in Nigeria (Adeyemi *et al.*, 2010). Implication of *S. epidermidis* in eye infections have also been reported earlier in patients from south western Nigeria where they were incriminated in endopthalmitis, chronic blepharitis and corneal ulcers (Ogbolu *et al.*, 2011).

Researchers have earlier reported hemolysin production by CoNS (Cunha *et al.*, 2006, Akinjogunla and Enabulele, 2010). It was mostly observed in *S. haemolyticus* (37.6%) and lowest in *S. simulans* (11.1%) and *S. xylosus* (11.1%). Predominance of hemolysin production by *S. haemolyticus* is not surprising as it has been reported to be its major virulence factor (Gunn and Davis, 1988). Hemolysin production has also been earlier reported in *S. saprophyticus* by Raz *et al.* (2005) as well as *S. epidermidis.*

Biofilm formation is thought to play an important role in the pathogenesis of CoNS induced infections. A high percentage (76%) of CoNS species isolated in this report was positive for slime production. Slime production was observed in 93.7% of *S. epidermidis* isolates and this is similar to a work reported by Ishak *et al.* (1985) where slime producing strains of *S. epidermidis* represented 13 of 14 (92.86%) isolates. The virulence factors of *S. saprophyticus* include adherence to uroepithelial cells by means of a surface associated protein and this could be attributed to slime production observed in *S. saprophyticus* (54.5%). Other virulence factors of *S. saprophyticus* are reported to include lipoteichoic acid, hemagglutinin and hemolysin (Raz *et al.*, 2005). Some researchers have also observed adherence in *S. schleiferi* which is presumed to be evidence of slime production (Herbert, 1990).

CONCLUSION

Coagulase negative staphylococci regarded as normal commensals of the human body have also become one of the most important pathogens in clinical infections especially among immunocompromised patients with medical devices (Vuong and Otto, 2002). Because of its increasing medical significance which has also been confirmed in this survey, routine laboratory identification of this group of pathogens is necessary in order to establish epidemiological trends and in infection control.

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TABLE 1: Occurence of CoNS isolated from various clinical samples

Source	**No of Isolates (%)**
Wounds infected	14(17.72%)
Infected burns	6(7.59%)
Exudates from male genitals infections	10(12.66%)
Swab samples from female genitals infections	10(12.66%)
Tonsilitis and pharyngitis	8(10.13%)
Urine from urinary tract infections	13(16.5%)
Urinary catheter	2(2.53%)
Blood (suspected septiceamia)	4(5.06%)
Eye infections	6(7.59%)
Otitis media	1(1.26%)
Other clinical samples	5(6.33%)
Total	79(100%)

TABLE 2: Distribution of CoNS species among the various clinical samples

	Source of samples (n)	*Staphylococcus.epidermidis*	*S.saprophyticus*	*S.heamolyticus*	*S. xylosus*	*S. simulans*	*S.chromogenes*	*S. schleiferi*	Non-speciated CoNS
A	Wounds infected(14)	4	1	4	1	1	-	-	3
B	Burns infected (6)	3	1	1	-	-	-	-	1
C	Urine infected (13)	2	4	3	-	-	1	-	3
D	Urinary catheter (2)	1	-	-	-	-	-	-	1
E	Swab samples from female genital infections (10)	-	3	6	-	-	-	-	1
f	Exudates from male genital infections (10)	3	-	1	1	2	-	-	3
g.	Throat (tonsillitis and pharyngitis) (8)	1	2	-	-	3	-	-	2
H	Eye infections (6)	1	-	-	-	-	2	1	2
I	Suspected septicemia (4)	-	-	-	4	-	-	-	-
J	Otitis media (1)	1	-	-	-	-	-	-	-
k	Other clinical samples (5)	-	-	1	-	-	1	-	3
	Total number of each Isolate (%)	16(26.70%)	11(18.30%)	17(28.3%)	6(10.00 %)	6(10.00 %)	3(5%)	1(1.67 %)	19(24.05 %)

n= number of samples

TABLE 3: Prevalence of hemolysin production in Coagulase negative Staphylococci

Isolates	Total no of isolates	No of hemolytic strains (%)
Staphylococcus epidermidis	16	4 (25%)
S. saprophyticus	11	4 (36.36%)
S. haemolyticus	17	10 (58.82%)
S. simulans	6	3 (50%)
S. xylosus	6	3 (50%)
S. chromogenes	3	-
S. schleiferi	1	-
Non- speciated CoNS	19	2 (10.53%)

TABLE 4: Slime formation by Coagulase negative Staphylococci

CoNS species	No. of isolates	No of slime producing isolates (%)
Staphylococcus haemolyticus	17	12 (70.58 %)
S. epidermidis	16	15(93.75%)
S. saprophyticus	11	6(54.55%)
S. simulans	6	6 (100%)
S. xylosus	6	6 (100%)
S. chromogenes	3	1 (33.33%)
S. schleiferi	1	1 (100%)
Non-speciated CoNS	19	12(63.15%)
Total	79	60(75.95%)