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Isolation and characterization of coagulase–negative Staphylococci from clinical specimens in Lagos, Nigeria

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ABSTRACT: Coagulase negative staphylococci (CoNS) live naturally on the skin and mucous membranes of humans. In many cases these are picked up from the skin during specimen collection and are not involved in any disease process, while in some cases they have been shown to cause infection. The purpose of this study was to determine the occurrence and possible involvement of CoNS in infections from various clinical specimens in Lagos and characterize the CoNS isolates obtained using both conventional biochemical methods and API staph-Ident Systems (ID32 STAPH). A total of 745 gram-positive catalase positive clustering cocci were obtained over 18-month period from Microbiology laboratories of Lagos University Teaching Hospital (LUTH) and General Hospital Ikeja (GHI) Lagos. A total of 244 isolates of CoNS were obtained of which 241 isolates were characterized to species level, while 3 were unclassified *Staphylococcus epidermidis* was the most commonly isolated species from all the specimens. It accounted for (44.7%) of all the CoNS isolates. Other CoNS were identified as follows: *S. saprophyticus* (14.8%), *S. capitis* (11.1%), *S. haemolyticus* (9.8%), *S simulans* (9.8%) *S. warneri* (2.9%), *S. xylosus* (2.4%), *S. hominis* (2.1%) *S. lugdunensis* (0.8%) and *S. cohnii* (0.4%). Comparing the conventional methods with the rapid commercial API kit (ID 32 STAPH), there was 98.8% specificity for the kit and 95.9% specificity for the former while there was no significant difference at p=1. From this study, strains of coagulase-negative staphylococci have been associated with a number of human infections in the environment and as such are no longer to be regarded as commensals or contaminants.

Key Words: Coagulase-negative staphylococci, isolation, characterization, Lagos.

Introduction

Coagulase-negative staphylococci (CoNS) are a major component or common inhabitants of the normal skin and mucous membrane (Kloos,1990; Kloos and Bannerman 1994). These organisms are frequently isolated in cultures from a variety of clinical specimens. In many cases these staphylococci are "picked up" from the skin during specimen collection and are not involved in any disease process, while in some cases they have been shown to cause infection. In recent times, the CoNS have been studied extensively because of their pathogenicity and involvement in some human and animal diseases. Thus, the emergence of coagulase-negative staphylococci as one of the major nosocomial pathogens implicated in a variety of infections has been reported (Kloos and Bannerman, 1994; Pfaller and Herwaldt, 1988; Otto, 2004).

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Infections caused by coagulase-negative staphylococci can be life-threatening in seriously ill and immuno-compromised patients, for example patients in intensive care units, premature newborns, human Immuno-deficiency virus patients, cancer and transplant patients with the increase in the use of transient or permanent medical devices. These include intravascular catheters and prosthetic devices (Nicastri *et al*, 2001; Miele *et al*., 2001; Liljedahl *et al*., 2004). The need to rapidly identify this group of bacteria became essential and proper characterization of these organisms is important as culture results may be misinterpreted resulting in confusion. This has also been facilitated by the use of different classification schemes and other rapid commercial identification systems (micromethod) with either manual or automated instrumentations (Kloos and Bannerman, 1995; Kloos *et al*., 1992). Most of these methods have been able to identify the coagulase-negative staphylococcus species and sub-species.

In Nigeria, coagulase-negative staphylococci have been implicated in some infections (Udo *et al*., 1997). Only very few reports on the involvement of CoNS in bacterial infections in our hospitals are available. The aim of this study was to determine the occurrence and possible involvement of CoNS in infections from various clinical specimens in Lagos. The species pattern of CoNS isolated was determined. This study also compared the conventional methods of identification of staphylococci to the commercial rapid identification systems.

Materials and Methods

Study Population and Bacterial Strains

The study was carried out in Lagos, Nigeria. Seven hundred and forty-five isolates of gram-positive, catalase-positive clustering cocci were obtained from various clinical specimens from the Clinical Microbiology Laboratories of Lagos University Teaching Hospital (LUTH) Idi-Araba, Lagos and General Hospital Ikeja (GHI) Lagos over 18-months period. These were clinical specimens collected from in-patients and out-patients comprising of neonates, children and adults of both sexes. Specimens included high vaginal swabs (HVS); endocervical swabs; pus from drainages; swabs from infections of the skin, ear, eye, surgical sites, burns, septic wounds, bones, abscesses/boils, urethra, mid-stream urine from urinary tract infections, pyelonephritis etc, cerebrospinal fluid, seminal fluid derived from infections of the urinary tracts and infertility patients, blood cultures in cases of blood stream infections (BSI), peritonitis and endocarditis. The clinical significance was based on the review of Kloos and Bannerman, (1994) and (1995).

Control Strains

Staphylococcus aureus ATCC 25923, *Staphylococcus epidermidis* ATCC 14990 and *S. saprophyticus* ATCC 15305 were used.

Statistical Analysis

Comparison of analytical methods were done using Analysis of Variance (ANOVA).

Isolation Procedure

Primary isolation of the microorganisms associated with the diseases was performed on the following media, Nutrient agar (Oxoid), MacConkey agar (Oxoid) and Blood agar in the Laboratories of LUTH and GHI. Processing of the isolates obtained was carried out in the Microbiology Laboratory of the Nigerian Institute of Medical Research, Yaba, Lagos, Nigeria. The gram-positive clustering cocci were inoculated onto Nutrient agar, Purple agar(Naylor and Burg, 1956) and Mannitol salt agar (Oxoid). Each plate was subsequently incubated aerobically at 37^{o}C for 18-24 hours and at times for 48-72 hours in the case of Purple agar (P agar) plates.

Colonial characteristics including size and pigmentation were observed on P agar while strains capable of fermentation and growth on mannitol were distinguished within 24 hours. Grams reaction, coagulase production (slide and tube methods) and catalase activity were also carried out. Differentiation of isolates (CoNS) from Micrococci was carried out based on tests recommended by Schleifer and Kloos, (1975)

Determination of CoNS species by Conventional Biochemical Tests

All gram-positive, coagulase-negative staphylococci were further characterized using the method of Kloos and Schleifer (1975). This method uses 13 key characteristics including coagulase activity, haemolysis on 5% sheep blood agar, nitrate reduction, phosphatase activity, lysostaphin susceptibility, novobiocin susceptibility, aerobic acid production from various carbohydrates. Other tests used in the classification of the isolates included acetoin and oxidase productions.

Determination of CoNS species by API STAPH-IDENT SYSTEMS (ID 32 STAPH).

Each isolate was grown on P agar and several identical colonies were picked for the preparation of an inoculum suspension having a turbidity equal to 0.5 Mcfarland standard. This was used to inoculate each cupule of ID 32 STAPH strip. Each ID 32 STAPH permits the determination of 25 characteristics which includes arginine dihydrolase, ornithine decarboxylase, esculin hydrolysis, nitrate reduction, acetoin production, β-galactosidase, arginine arylamidase, alkaline phosphatase, pyrrolidonyl arylamidase, β-glucuronidase, novobioin resistance and fermentation of sugars.

Results

A total of 745 gram-positive clustering cocci were obtained from various clinical specimens from LUTH and GHI microbiology laboratories. Tests for Gram's reaction, production of the enzyme coagulase (free, bound or both) and catalase activity yielded 488 gram-positive, catalase-positive, coagulase-positive, clustering cocci and 257 gram-positive, catalase-positive coagulase-negative clustering cocci. The coagulase-positive staphylococci were excluded from the study.

Table 1 shows the sources of CoNS isolates. Using the conventional biochemical tests of Kloos and Schleifer scheme alone, 236 isolates of CoNS isolates were characterized to species level while 10 isolates were unclassified. The frequency and distribution of CoNS isolates from clinical specimens are shown in Table 2.

Using the API kit (ID 32 STAPH), two of the isolates previously identified as S. *warneri* were now reclassified as *S. lugdunensis* while 5 out of the 10 unclassified group were identified as *S. haemolyticus*. Two isolates out of the unclassified group were found to be stomatococcus species by API kit (Table 3).

By combining both conventional biochemical method and Rapid API commercial kit (ID 32 Staph), 241 coagulase-negative staphylococci were characterized to species level (Table 4). *S. epidermidis* was the most commonly isolated CoNS. It accounted for 109(44.3%) CoNS isolates. Thirty-six (14.6%) strains were *S. saprophyticus*, while 27 (11.0%) strains were identified as *S. capitis*. Twenty-four(9.8%) strains each were identified as *S. simulans* and *S. haemolyticus* respectively. Other CoNS species S. *hominis* (5)(2.0%) *S. lugdunensis* (2)(0.8%), *S. cohnii*(1)(0.4%), *S. warneri*(7)(2.8%) and *S. xylosus*(6)(2.4%) were isolated. Three CoNS isolates were unclassified. Comparison between the conventional biochemical method and ID 32 staph is shown in Table 5.

Discussion

In this study, a total of 244 isolates of CoNS were isolated in pure cultures from various infections and 241 isolates were characterized to species level. Species-to-species differences have emerged. *Staphylococcus epidermidis* accounted for 44.3% of all clinical isolates and the most prevalent in almost all groups of clinical specimens. This prevalence of *S. epidermidis* is consistent with other studies on species characterization of CoNS that relate *S. epidermidis* to be the most prevalent and major species of clinical relevance and the species colonizing human skin ((Kloos,1990; Kloos and Bannerman 1994).

Table 1: Sources of isolates of coagulase-negative Staphylococci in various specimens from patients with different diseases (No. of isolates).

Clinical diagnosis	Wound	Blood	Urine	Urethra	Catheter Tips	Ear	Vagina/ Cervix	Male Secretions	Tissue Biosy	Bone secretions	Eye	Fluids	Total
Burn/Skin sepsis	7	-	-	-	-	-	-	-	-	-	-	-	7
Multiple Boils	5	-	-	-	-	-	-	-	-	-	-	-	5
Post op. Wound Inf.	23	-	-	-	-	-	-	-	-	-	-	-	23
Ulcer (Leg. Penile) etc	29	-	-	-	-	-	-	-	-	-	-	-	29
Urinary tract inf	-	-	32	8	-	-	11	-	-	-	-	-	51
Otitis media	-	-	-	-	-	7	-	-	-	-	-	-	7
Osteomylitis	-	-	-	-	-	-	-	-	-	6	-	-	6
Urethritis	-	-	-	18	-	-	-	5	-	-	-	-	23
Prostatis -	-	2	-	-	-	-	3	-	-	-	-	5	
Peurperal sepsis	-	-	-	-	-	-	4	-	-	-	-	-	4
Endocarditis	-	2	-	-	-	-	-	-	-	-	-	-	2
Infertility	-	-	-	-	-	-	-	16	-	-	-	-	16
Pyleonephritis	-	-	7	-	-	-	-	-	-	-	-	-	7
Neonatal sepsis	-	23	-	-	2	-	-	-	-	-	-	-	25
Neonatal tetanus	-	2	-	-	-	-	-	-	-	-	-	-	2
Broncho Pneumonia	-	4	-	-	-	-	-	-	-	-	-	-	4
PUO	-	8	-	-	4	-	-	-	-	-	-	-	12
Thyroid septicaemia	-	-	-	-	2	-	-	-	-	-	-	-	2
Conjunctivitis	-	-	-	-	-	-	-	-	-	-	8	-	8
Pelvic infl. Dis.	-	-	-	-	-	-	3	-	-	-	-	-	3
Conjestive Heart Failure	-	-	-	-	-	-	-	-	-	-	-	1	1
Gastritis	-	-	-	-	-	-	-	-	4	-	-	-	4
Total	64	39	41	26	8	7	18	24	4	6	8	1	246

Key:

PUO – Pyrexia of unknown origin.
Post op. wound inf. – Post-operative wound infection.
Pelvic infl. Dis – Pelvic inflammatory disease
inf – infection

Table 2: Frequency and distribution of coagulase-negative staphylococci isolated from clinical specimens by conventional method. No. (%) of isolates

Species	Wound	Blood	Urine	Urethra	Catheter Tips	Ear	Vagina/ Cervix	Male secretions	Bone secretions	Eye	Tissue Biopsy	Sterile Fluids	Total
S. capitis	12(8.8)	4(10.2)	2(4.9)	2(7.7)	1(12.5)	4(57.1)	-	2(8.3)	-	-	-	-	27(11.0)
S. cohnii	-	-	1(2.4)	-	-	-	-	-	-	-	-	-	1(0.4)
S. epidermidis	24(37.5)	30(76.9)	15(36.5)	12(46.2)	6(75.0)	-	4(22.2)	5(20.8)	3(50.0)	6(75.0)	4(100)	-	109(44.3)
S. haemolyticus	7(10.9)	1(2.6)	2(4.9)	-	-	2(28.6)	3(16.7)	2(8.3)	-	2(25.0)	-	-	19(7.7)
S. hominis	1(1.6)	1(2.6)	2(4.9)	-	1(12.5)	-	-	-	-	-	-	-	5(2.0)
S. saprophyticus	3(4.7)	-	12(29.3)	9(34.6)	-	-	7(38.9)	5(20.8)	-	-	-	-	36(14.6)
S. simulans	7(10.9)	1(2.6)	3(7.3)	2(7.7)	-	-	4(22.2)	6(25.0)	-	-	-	1(100)	24(9.8)
S. warneri	5(7.8)	-	2(4.9)	-	-	-	-	-	2(33.3)	-	-	-	9(3.7)
S. xylosus	2(3.1)	-	2(4.9)	1(3.8)	-	1(14.3)	-	-	-	-	-	-	6(2.4)
Unidentified	3(4.7)	2(5.1)	-	-	-	-	-	4(16.7)	1(16.7)	-	-	-	10(4.1)
Total	64(100)	39(100)	41(100)	26(100)	8(100)	7(100)	18(100)	24(100)	6(100)	8(100)	4(100)	1(100)	246(100)

Table 3: Frequency and distribution of coagulase-negative staphylococci isolated from clinical specimens by API (ID 32 STAPH) Kit. No. (%) of isolates

Species	Wound	Blood	Urine	Urethra	Catheter Tips	Ear	Vagina/ Cervix	Male secretions	Bone secretions	Eye	Tissue Biopsy	Sterile Fluids	Total
S. capitis	12(8.8)	4(10.2)	2(4.9)	2(7.7)	1(12.5)	4(57.1)	-	2(8.3)	-	-	-	-	27(11.0)
S. cohnii	-	-	1(2.4)	-	-	-	-	-	-	-	-	-	1(0.4)
S. epidermidis	24(37.5)	30(76.9)	15(36.5)	12(46.2)	6(75.0)	-	4(22.2)	5(20.8)	3(50.0)	6(75.0)	4(100)	-	109(44.3)
S. haemolyticus	9(14.0)	2(5.1)	2(4.9)	-	-	2(28.6)	3(16.7)	4(16.7)	-	2(25.0)	-	-	24(9.8)
S. hominis	1(1.6)	1(2.6)	2(4.9)	-	1(12.5)	-	-	-	-	-	-	-	5(2.0)
S. lugdunesis	2(3.2)	-	-	-	-	-	-	-	-	-	-	-	2(0.8)
S. saprophyticus	3(4.6)	-	12(29.3)	9(34.6)	-	-	7(38.9)	5(20.8)	-	-	-	-	36(14.6)
S. simulans	7(10.9)	1(2.6)	3(7.3)	2(7.7)	-	-	4(22.2)	6(25.0)	-	-	-	1(100)	24(9.8)
S. warneri	3(4.6)	-	2(4.9)	-	-	-	-	-	2(33.3)	-	-	-	7(2.8)
S. xylosus	2(3.2)	-	2(4.9)	1(3.8)	-	1(14.3)	-	-	-	-	-	-	6(2.4)
Unidentified	-	2(5.1)	-	-	-	-	-	1(4.16)	1(16.7)	-	-	-	3(1.2)
Stomatococcus	1(1.6)	-	-	-	-	-	-	1(4.16)	-	-	-	-	2(0.8)
Total	64(100)	39(100)	41(100)	26(100)	8(100)	7(100)	18(100)	24(100)	6(100)	8(100)	4(100)	1(100)	246(100)

Table 4: Frequency and distribution of coagulase-negative staphylococci isolated from clinical specimens by conventional method and API Kit. No. (%) of isolates

Species	Wound	Blood	Urine	Urethra	Catheter Tips	Ear	Vagina/ Cervix	Male secretions	Bone secretions	Eye	Tissue Biopsy	Sterile Fluids	Total
S. capitis	12(19.0)	4(10.2)	2(4.9)	2(7.7)	1(12.5)	4(57.1)	-	2(8.7)	-	-	-	-	27(11.0)
S. cohnii	-	-	1(2.4)	-	-	-	-	-	-	-	-	-	1(0.4)
S. epidermidis	24(38.1)	30(76.9)	15(36.5)	12(46.2)	6(75.0)	-	4(22.2)	5(21.7)	3(50.0)	6(75.0)	4(100)	-	109(44.3)
S. haemolyticus	9(14.0)	2(5.1)	2(4.9)	-	-	2(28.6)	3(16.7)	4(17.4)	-	2(25.0)	-	-	24(9.8)
S. hominis	1(1.6)	1(2.6)	2(4.9)	-	1(12.5)	-	-	-	-	-	-	-	5(2.0)
S. lugdunesis	2(3.2)	-	-	-	-	-	-	-	-	-	-	-	2(0.8)
S. saprophyticus	3(4.8)	-	12(29.3)	9(34.6)	-	-	7(38.9)	5(21.7)	-	-	-	-	36(14.6)
S. simulans	7(11.1)	1(2.6)	3(7.3)	2(7.7)	-	-	4(22.2)	6(26.1)	-	-	-	1(100)	24(9.8)
S. warneri	3(4.8)	-	2(4.9)	-	-	-	-	-	2(33.3)	-	-	-	7(2.8)
S. xylosus	2(3.2)	-	2(4.9)	1(3.8)	-	1(14.3)	-	-	-	-	-	-	6(2.4)
Unidentified	-	1(2.6)	-	-	-	-	-	1(4.36)	1(16.7)	-	-	-	3(1.2)
Total	63(100)	39(100)	41(100)	26(100)	8(100)	7(100)	18(100)	23(100)	6(100)	8(100)	4(100)	1(100)	244(100)

Table 5 Comparison and distribution of coagulase-negative staphylococci from clinical specimens using conventional method and ID32 STAPH kit.

SPECIES	CONVENTIONAL METHOD	ID 32 STAPH KIT
S. capitis	27	27
S. cohnii	1	1
S. epidermidis	109	109
S. haemolyticus	19	24
S. hominis	5	5
S. lugdunesis	-	2
S. saprophyticus	36	36
S. simulans	24	24
S. warneri	9	7
S. xylosus	6	6
Stomatococcus spp	-	2
Unidentified	10	3
Total	246	246

The predominance of this group of CoNS in isolates from blood may also be attributed to earlier reports of the special ability of this species to infect in-dwelling artificial devices and to cause bacteraemias in immuno compromised patients (Nicastri *et al*, 2001; Miele *et al*., 2001; Liljedahl *et al*., 2004; Otto, 2004). Thus CoNS infections often can be life threathening in these patients. Other reports elsewhere in Nigeria have implicated *S*. epidermidis in septicaemia especially in neonatal septicaemia (Udo *et al*., 1997 and Olusanya *et al*., 1991). These reports are also in agreement with the findings in this study. *In-vitro* studies using rat model of endocarditis and *in–vivo* studies of phagocytic killing indicate greater virulence of *S.epidermidis* when compared with other CoNS (Baddour *et al*., 1984). It is not surprising that *S. epidermidis* accounted for 44.3% of all the isolates of CoNS and the most prevalent in this study.

S. saprophyticus accounted for 14.6% of the total isolates and 38.9% of all the isolates from high vaginal swabs (HVS), endocervical swabs and pelvic inflammatory diseases. It is also the most prevalent isolates in cases of urinary tract infection (UTI). They were also isolated in good numbers in cases of urethritis and prostatis in males. These are consistent with other studies (Marrie *et al*.,1982 Bhalla and Agarwal 1986) implicating *S. saprophyticus* as an important opportunistic pathogens in human urinary tract infections. *S. saprophyticus* is usually found in small transient-populations on a variety of body sites but this species possesses surface properties that allow it to adhere readily to urogenital cells unlike *S. epidermidis* which is present in anatomic site that contains indigenous flora. This might explain its presence in such areas as opportunistic pathogen. While Kloos and Bannerman, 1995 reported *Staphylococcus haemolyticus* as the second most frequently encountered CoNS species after *S. epidermidis* this study shows this species as the third most isolated species. The isolation rates being reported for *S. capitis, S. warneri*, *S. xylosus*, *S. hominis* and *S. cohnii* are expected since *S. epidermidis* is the most commonly isolated species of clinical importance. *S.capitis* which initially was not a common occurrence in many studies(Pfaller and Herwaldt, 1988) was isolated from various clinical specimens though in rather low figures except in wound infection samples where it accounted for 19.0% of all wound isolates after *S. epidermidis* (39.0%). The findings might be due to the habitat of *S. capitis* as was reported in a study (Kloos and Bannerman 1994) where moderate-sized to large population of these organisms were found on the face, eyebrow and external auditory meatus.

Data on the clinical significance of the other four species mentioned are limited. Two isolates of *S. lugdunensis* were obtained. This species is one of the relatively new species of CoNS. It was reported that this species accounted for 10% of all staphylococcus species in human clinical specimens if *S. aureus* and *S. epidermidis* are excluded (Herchline and Ayer,1991). In this study 0.8% was obtained for *S. lugdunensis.* The reason for this percentage might be that the population of *S. lugdunensis* in human clinical specimens seems not to be very significant in Lagos. The isolation of this species was only made possible by the use of API kit (ID 32 STAPH) which reclassified this species from *S.warneri* .

This Kit was able to characterize 7 out of the 10 CoNS isolates that were unclassified by the conventional method. Strains of *stomatococcus* species which were misidentified as CoNS were re-classified. The reason for the misidentification might be because these strains showed a catalase positive reaction almost identical to those of ordinary staphylococci strain. The API kit was able to identify these stomatococcus species which conventional methods could not do. The API kit (ID 32 STAPH) used in this study has an overall accuracy of 98.%. When the API kit method of characterization was compared to the conventional methods there was no significant difference at *p=1* but there was 98.8% specificity for the kit and 95.8% for the conventional method. But the kit provided a rapid and accurate method for identifying the various CoNS examined in this study. The main limitation to the use of this rapid commercial kit is the availability of the kit and its cost in our environment.

Thus CoNS once regarded as contaminants and not pathogens are now subject of growing interest owing to their ability to cause infection under certain conditions and their emergence as an important pathogen in nosocomial blood stream infections. It is clear from this study that one should expect to isolate different species and strains of CoNS in laboratories here in Lagos. The clinical relevance and significance of CoNS continue to increase in our environment.

References

1. Baddour LM, Christensen GD, Hester MG, Bisno AL. Production of experimental endocarditis by coagulase-negative staphylococci: variability in species virulence *J. infect Dis* 1984;150: 721-727.
2 Bhalla p, Agarwal DS. Incidence of urinary tract infections to S*taphylococcus saprophyticus. Ind. J. Med. Microbiol.* 1986; 4: 169-175.

3. Herchline TE, Ayer LW. Occurrence of *Staphylococcus lugdunensis* in consecutive clinical cultures and relationship of isolation to infection *J. Clin. Microbiol* 1991: 29:419-421.
4. Kloos W E. Systematics and natural history of staphylococci. *J. Appl. Bacteriol Symp. Suppl.* 1990; 69: 295-375.
5. Kloos W E. Bannerman, T. L. Update on clinical significance of coagulase-negative staphylococci *Clin. Microbiol. Rev.* 1994; 7:117-140.
6. Kloos WE, Bannerman TL. Staphylococcus and Micrococcus. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, eds Manual of Clinical Microbiology 6th ed. *American Society for Microbiology,* Washington D. C. 1995; 282-298.
7. Kloos WE, Berkhoff HA, Muller E, Bannerman TL, Ballard DN. Relationship between cutaneous persistence in natural populations of coagulase-negative staphylococci and their ability to produce catheter infections, biofilm and Lipopolysaccharide adhesin. 92nd Gen. Meeting. *American Society for Microbiology,* Washington D.C. Abstra. B. 1992; 240:66.
8 Kloos WE, Schleifer KH. Simplified scheme for routine identification of human S*taphylococcus* species. *J. Clin. Microbial* 1975, 1:82-88.
9. Liljedahl M, Bodin L, Schollin J. Coagulase-negative Staphylococcal sepsis as a predictor of bronchopulmonary dysplasia. *Acta Paediatr.* 2004; 93(2): 211-
10. Marrie, TJ, Kwan C, Noble MA, West A, Duffield L. *Staphylococcus saprophyticus* as a cause of urinary tract infections. *J.Clin Microbiol* 1982; 16.427-431
11. Miele PS, Kogulan PK, Levy CS, *et al*. Seven cases of Surgical native valve endocarditis caused by coagulase-negative staphylococci. An under appreciated disease.. *Am. Heart. J.* 2001; 142(4) 571-576.
12. Naylor HB, Burg E. Observations on abortive infection of *Micrococcus lysodeikticus* with bacteriophage. *Virology.* 1956;2:577-593.
13 Nicastri E., Petrosiillo N, Viale P, Ippolito G. Catheter-related bloodstream infections in HIV-infected patients. *Ann. Ny Acad. Sci.* 2001; 946:274-290.
14. Olusanya D, Olanrewaju DM, Ogunforowa OB, Laditan M. Neonatal septicaemia at the Ogun State University Teaching Hospital, Shagamu *Nig. Med Pract.* 1991; 22: 39-42.
15. Otto M. Virulence Factors of the coagulase-negative Staphylococci. *Front- Biosci.* 2004; 9:841 – 863.
16. Pfaller MA, Herwaldt, LA. Laboratory, Clinical and epidemiological aspects of coagulase-negative staphylococci. *Clin. Microbiol. Rev.* 1988; 1:281-299.
17 Schleifer Kh, Kloos WE. A simple test system for the separation of staphylococci from micrococci. *J. Clin. Microbiol*. 1975; 1:337-338.
18. Udo SM, Ita FI, Dom Egba R. Slime production, antimicrobial resistance and Beta Lactamase activities of clinically significant coagulase-negative staphylococci *Nig. Qt J. Hosp. Med.* 1997; 7(4):342-344.