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Original Research Article

Clinical and antimicrobial profile of Coagulase Negative *staphylococci* in a tertiary care hospital

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ABSTRACT

Background: Coagulase negative staphylococci (CoNS) are gaining importance because of their role as pathogens in certain clinical conditions and their marked resistance to antibiotics. Their species distribution and slime production has important correlation with the antimicrobial susceptibility pattern. Aim of this study was to determine clinically significant CoNS, their species distribution, slime production and antimicrobial susceptibility pattern in a tertiary care hospital.
Methods: Identification, speciation and antimicrobial sensitivity testing were performed using standard microbiological techniques. Slime production was also tested by microtiter plate. Antimicrobial susceptibility testing was performed by modified Kirby Bauer method as per the CLSI guidelines.
Results: A total 204 (49.88%) CoNS were found to be clinically significant. Percentage of clinical significance was high in urine isolates (88.88%) followed by pus (47.78%) and blood (45.56%). The most common CoNS infection was septicaemia (54.9%) followed by abscesses and wound infections (26.5%) and urinary tract infection (15.8%). S. epidermidis (46.1%) was the commonest species in CoNS infection followed by *S. haemolyticus* (22.1%), *S. lugdunensis* (11.8%) and *S. saprophyticus* (8.3%). Slime production was seen in 56.4% isolates by microtiter plate method. Maximum resistance was seen to penicillin (92.25%), followed by cotrimoxazole (73.03%), norfloxacin (73.03%), tetracycline (71.07%), gentamicin (69.6%) and cefoxitin (63.2%).
Conclusions: The role of CoNS as pathogen, particularly nosocomial and opportunistic is increasing. Identification of species, slime production and antimicrobial susceptibility of CoNS is highly desirable to permit a more precise determination of host-pathogen relationship and knowledge of pathogenicity.

Keywords: Antimicrobial susceptibility, CoNS, Clinically significant, Speciation, Slime

INTRODUCTION

Coagulase negative Staphylococci (CoNS) are one of those amongst the commonly isolated organisms in the clinical microbiology laboratory. Interest in CoNS is increasing because of their role as pathogens in certain clinical conditions and their marked resistance to antibiotics. At present CoNS includes 41 recognized taxons of which 21 types represent etiological agents in the human diseases. CoNS are reported to be the third common causative agent of nosocomial infections and the most common cause of nosocomial blood stream infections.[2,3]

The clinical significance of CoNS as a pathogen was determined by Ishak et al[4] and Singhal et al.[5] Their role as significant pathogens following various surgeries, neonatal septicemia, immunocompromised patient,

prosthetic devices, bacteremia, urinary tract infections, shunt and graft infections, peritoneal dialysates infections, osteomyelitis, ocular and cutaneous infections are also documented.[6].

Clinically significant CoNS should be identified to the species level, because of their increasing importance. In CoNS, loosely bound exopolysaccharide layer slime has been found in addition to capsule which has been associated with infections and reduced antibiotic susceptibility.[4,7]

Full and accurate identification of CoNS isolates in clinical samples is therefore of immense importance for epidemiological purposes and infection control measures[8]. However scant data are available on CoNS responsible for infections in developing countries.[5] With the above facts in mind, the study was carried out to speciate clinically significant (pathogenic) CoNS and to determine their antimicrobial susceptibility.

METHODS

The study was carried out in department of microbiology for one year. Various samples like blood, pus, urine, CSF, wound swab, stitch abscess, body fluids like pleural/peritoneal/pericardial/synovial/drain, devices such as intravenous, urinary and suction catheters, peritoneal dialysates, plastic shunts, and orthopedic, cardiac and other implants were collected from clinically diagnosed infected patients. Sputum, throat swab, conjunctival swab, stool, cervical and vaginal swabs were excluded. Specimens were processed by standard microbiological techniques.[9]

Wet preparation of uncentrifuged urine and centrifuged deposits of other body fluids were examined for the presence of pus cells and microorganisms. Smears of specimens were fixed and stained using gram's method and examined for pus cells and different morphological forms of microorganisms. *Staphylococci* appeared as gram positive cocci, arranged as single cells, short chains or in grape like clusters. All the specimens were inoculated on 10 % sheep blood agar and MacConkey agar and incubated at 37 ^{0}C for 18-24 hours. On blood agar, colonies of *staphylococci* were 1-3 mm in diameter, smooth, low convex with entire edges, glistening, densely opaque and of butyrous consistency. Pigmentation and beta hemolysis if present were specially noted.

Confirmation of *Staphylococcus* was done by catalase positivity, furazolidone susceptibility and bacitracin resistance. Coagulase negative Staphylococcus (CoNS) was identified by negative slide and tube coagulase test respectively. The CoNS isolates were further differentiated into the commensals and the clinically significant isolates on the basis of the Criteria by Ishak et al for blood and Singhal et al for another specimen.[4,5] Criteria by Ishak et al included ten points viz.[4] Organisms growing in more than one blood culture bottle or in more than one blood culture set, blood culture positive within the first 3 days, another site (i.e. catheter) growing the same organism, as determined by species, biotype and the slime test result, inflammatory signs at site of intravascular catheter, fever (temperature of >38°C for more than 24 hours), significant decrease in blood pressure (systolic pressure, less than 100 mm Hg), increase in creatinine value by at least 20% or significant decrease in urine output, or both, significant increase in leukocyte count over base-line level, belief of physician that patient is septic, rapid improvement after specific therapy or follow-up consistent with true infection. A clinical index score of >50% holds true infection. Criteria by Singhal et al specified repeated isolation of tube coagulase negative staphylococcus from; normally sterile body fluids and tissues, urine samples with significant colony count (>105 organism/ml), IV catheter tips (>15 colonies), predominant or the only organism in moderate to heavy growth from deep pus.[5]

Species identification of CoNS was done by ornithine decarboxylase, phosphatase, nitrate reduction, urease, carbohydrate fermentation of xylose, sucrose, trehalose, lactose, fructose, maltose, mannose and novobiocin resistance test.[6] All the clinically significant CoNS were tested for slime production by microtiter plate method where optical density (OD) of stained adherent bacteria was determined at 570 nm wavelength. If OD value was >0.240 then it was strong slime producer.[10]

Antimicrobial susceptibility testing was performed by modified Kirby Bauer method as per the CLSI guidelines.[11,12] Antibiotics tested were Amikacin (Ak), Cefoxitin (Cn), Chloramphenicol(C), Ciprofloxacin (CIP), Clindamycin (Cd), Erythromycin (Er), Gentamicin (G), Linezolid (Lz), Nitrofurantoin (Nf), Penicillin G(P), Tetracycline (T), Trimethoprim-Sulfamethoxazole (Co), Vancomycin (Va).

RESULTS

A total 409 CoNS were isolated from the processed clinical specimens. Out of them, 204 (49.88%) isolates were found to be clinically significant. Percentage of clinical significance was high in urine isolates (88.88%) followed by pus (47.78%) and blood (45.56%).

Table 1: Infections caused by clinically significant CoNS (n=204).

CoNS infections	Number of patients (%)
Septicaemia	112 (54.9)
Abscesses and wound infections	54 (26.5)
Urinary tract infections	32 (15.7)
Ascitis	2 (0.98)
Osteomyelitis	2 (0.98)
Meningitis	1 (0.49)
Keratitis	1 (0.49)

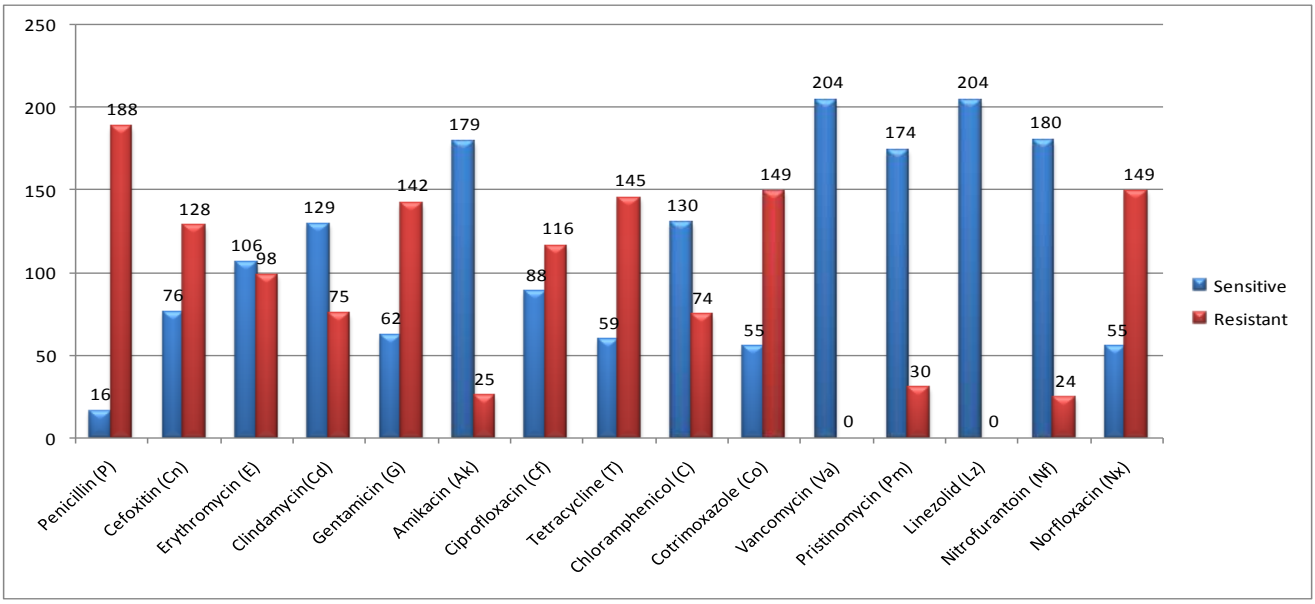
The most common CoNS infection was septicaemia (54.9%) followed by abscesses and wound infections (26.5%). Urinary tract infection was found in 15.8 % cases. There were two cases of ascitis (1 %), two cases of osteomyelitis (1%) and a case of meningitis (0.4%) and keratitis each (Table 1).

S. epidermidis (46.1%) was the commonest species in CoNS infection followed by *S. haemolyticus* (22.1%), *S. lugdunensis* (11.8%), *S. saprophyticus* (8.3%) and others. *S. epidermidis* (51.8%) was the commonest species to cause septicaemia, followed by *S. haemolyticus* (26.8%). Abscesses and wound infections were mainly due to *S. lugdunensis* (36.6%) followed by *S. epidermidis* (32.7%) and *S. haemolyticus* (25%). Urinary tract infections were most commonly due to *S. saprophyticus* (53.1%) and *S. epidermidis* (34.4%). Ascitis, osteomyelitis, meningitis and keratitis were caused specifically due to *S. epidermidis*. Slime production was seen maximally in *S. saprophyticus* (70.5%), *S. epidermidis* (70.2%), *S. haemolyticus* (45%) and *S. lugdunensis* (42%) by microtiter plate method (Table 2).

Table 2: Distribution of clinically significant CoNS species in various samples and their slime production.

Species	Blood (%)	Pus (%)	Urine (%)	Asc fluid (%)	Syn fluid (%)	CSF (%)	Corn Scrap (%)	Slime production (%)
S. epidermidis (94)	58 (51.8)	19 (35.2)	11 (34.4)	2 (100)	2 (100)	1 (100)	1 (100)	66 (70.2)
S. hemolyticus (45)	30 (26.8)	13 (24.1)	2 (6.25)	-	-	-	-	20 (45)
S. lugdunensis (24)	4 (3.6)	19 (35.2)	1 (3.12)	-	-	-	-	10 (42)
S. saprophyticus (17)	-	-	17 (53.1)	-	-	-	-	12 (70.5)
S. cohnii (6)	6 (5.4)	-	-	-	-	-	-	2 (33.3)
S. xylosus (5)	4 (3.6)	-	1 (3.1)	-	-	-	-	2 (40)
S. capitis (6)	4 (3.6)	2 (3.7)	-	-	-	-	-	1 (16.7)
S. hominis (5)	5 (4.6)	-	-	-	-	-	-	1 (20)
S. simulans (1)	-	1 (1.9)	-	-	-	-	-	0 (0)
S. warneri (1)	1 (0.9)	-	-	-	-	-	-	1 (100)
Total (209)	112	54	32	2	2	1	1	115 (56.4)

Figure 1: Antimicrobial sensitivity pattern of clinically significant CoNS (n = 204).



Out of 204 clinically significant CoNS, slime production was seen in 56.4% isolates by microtiter plate method (Table 2). Maximum number of the clinically significant CoNS were sensitive to linezolid (100%) followed by vancomycin (100%), nitrofurantoin (88.23%), amikacin (87.74%), pristinomycin (85.29%). Maximum resistance of the clinically significant CoNS was seen to penicillin (92.25%), followed by cotrimoxazole (73.03%),

norfloxacin (73.03%), tetracycline (71.07%), gentamicin (69.6%) and cefoxitin (63.2%) (Figure 1).

DISCUSSION

Coagulase Negative *Staphylococci* (CoNS) were generally regarded to be the contaminants, having little clinical significance in the past. In the period 1992 to 1998, surveillance conducted in medical-surgical units revealed that CoNS were the most common cause of primary bloodstream infections and the second commonest cause of post-surgical infections.[13] The predisposing risk factors identified for CoNS infections are rheumatic fever with infective endocarditis, intravascular catheters, prosthetic devices, prolonged antibiotics, immunocompromised status, surgery etc.[6] Most developed countries have reported an increase in colonization and infection in hospitalized patients by CoNS, which are resistant to methicillin and other antibiotics.[5]

In the present study, most common infection caused by CoNS was septicaemia i.e. 112 cases (54.9%) cases followed by abscesses and wound infections (26.5%) and urinary tract infection15.7%). Current study matches with Singhal et al who reported 54.2% cases of CoNS septicaemia, 30.1% cases of abscesses and wound infection and 3.6% cases of urinary tract infections due to CoNS.[56] Data by Emori and Gaynes shows that CoNS accounted for 31% of the nosocomial septicaemia 14 % of surgical wound infection and 4% of UTIs in United states.[14] It is widely believed that CoNS on the skin adjacent to catheter entry sites or surgical incisions are often the source of catheter-related infections, post-surgical infections and infected implanted prostheses.[13]

In the present study, CoNS were found clinically significant in 2 (1%) cases of osteomyelitis and ascitis each. Goyal et al also reported 2 (1.9%) cases of ascites and 2 (1.9%) cases of osteomyelitis.[15] In this study, a single (0.4%) case of neonatal meningitis was reported. An Australian study reported 5 (0.4%) cases of CoNS meningitis.[16] The role of CoNS in meningitis is equally controversial. When cases of neonatal CoNS meningitis are described in the absence of foreign material, clinical picture consistent with meningitis, and either a positive CSF culture or elevated CSF white cell count (> 100/µl) in association with a positive blood culture holds true infection.[16] In the study, single case (0.4%) of keratitis was found to be associated with CoNS. Tan et al also reported a single case of CoNS keratitis.[17]

Particular species of CoNS are associated with distinct types of infections and patterns of antimicrobial susceptibility.[7] Because of increasing clinical significance of CoNS, accurate species identification of CoNS is highly desirable to permit a more precise determination of host-pathogen relationship of CoNS.[18]

In the present study, the commonest species isolated in clinically significant CoNS was *S. epidermidis (*46.1%) followed by *S. haemolyticus* (22.1%) followed by *S. lugdunensis* (11.8%), S. saprophyticus (8.3%), *S. cohnii* (2.9%), *S. capitis* (2.9%), *S. hominis* (2.5%), *S. xylosus* (2.5%), *S. simulans* (0.5%) and *S. warneri* (0.5%). Singhal et al reported *S. epidermidis* (33.7%) followed by *S. haemolyticus* (13.3%), *S. lugdunensis* (13.3%), *S. capitis* (9.6%), *S. xylosus* (6%), *S. hominis* (2.4%) and *S. warneri* (1.2%).[5] The slight variation in the number of species isolated may be due to variation of specimens, schemes applied for the identification and the species predominating in various setup.

In current study, *S. epidermidis* (51.8%) was the commonest species to cause septicaemia, followed by *S. haemolyticus* (26.8%), *S. cohnii* (5.4%), *S. hominis* (4.6%) and others (Table 2). Kim et al also found *S. epidermidis* (52.94%) to be the commonest species causing septicaemia, followed *by S hemolyticus* (11.8%), *S. hominis* (11.8%), *S. warneri* (11.8%).[19] In the present study, abscesses and wound infections were most commonly caused due to *S. lugdunensis* (36.6%) followed by *S. epidermidis* (32.7%) and *S. haemolyticus* (25%). Tan et al found *S. lugdunensis* (46.8%) to be the commonest isolate from the abscesses and wound infections followed by *S. epidermidis* (43.8%).[17] *S. lugdunensis* has the virulence factors that resemble those of *S. aureus* and can cause similar aggressive infection. As it is rarely found as a contaminant, the identification of *S. lugdunensis* raises the index of suspicion for aggressive disease.[20] Urinary tract infections were most commonly due to *S. saprophyticus* (53.1%) and *S. epidermidis* (34.4%) in this study. *S. saprophyticus* is determined to be the true urinary tract pathogen, which is found to be the second most common cause of urinary tract infection after *E. coli* in females.[6] The reasons for the association of *S. saprophyticus* with urinary tract infections in young women remain unclear, but may relate to carriage of the organism in the rectum or introitus.[21] Other CoNS are rare cause of urinary tract infections, and about 80-90% of these infections are caused by *S. epidermidis*, which are usually catheter associated or associated with urological abnormalities.[6] In the present study, ascites, osteomyelitis, meningitis, and keratitis were all caused specifically due to *S. epidermidis* (Table 2). Goyal et al also reported *S. epidermidis* to be the commonest cause of infections like ascitis (50%), osteomyelitis (50%) and meningitis (50%) due to CoNS.[15]

Previous reports have suggested that slime has a role in the pathogenesis of CoNS infections. Both slime production and the species of the organism appeared to be crucial factors in the determination of pathogenicity.[4] Out of 204 clinically significant CoNS, slime production was seen in 56.4% isolates by microtitre plate method. Mathur et al found 53.8% clinically significant CoNS to be slime producers by microtitre plate.[22]

Present study found variable amount of slime production in various species. Slime production was seen maximally in S. *saprophyticus* (70.5%), *S. epidermidis* (70.2%), *S. haemolyticus* (45%) and *S. lugdunensis* (42%) by microtitre plate method. Alcaraz et al found slime production maximally in *S. saprophyticus* (83.3%), *S. epidermidis* (57.7%), *S. hominis* (42.9%) and *S. haemolyticus* (37.5%).[23]

In the present study, the maximum number of the clinically significant CoNS were sensitive to linezolid (100%), vancomycin (100%) followed by amikacin (87.74%) and pristinomycin (85.29%). Urinary antibiotic nitrofurantoin showed 88.23% sensitivity. Maximum resistance of the clinically significant CoNS was seen to penicillin (92.25%), followed by tetracycline (71.07%), gentamicin (69.6%) and cefoxitin (63.2%). Urinary antibiotics cotrimoxazole and norfloxacin both showed similar resistance (73%). Mohan et al showed maximum sensitivity of CoNS isolates to vancomycin (100%), methicillin (71.7%), amikacin (71.3%) and nitrofurantoin (66.6%) and maximum resistance to penicillin (90.6%), norfloxacin (77.7%), gentamicin (66.2%), ciprofloxacin (66.2%), erythromycin (64.6%) and chloramphenicol (54.6%).[24] Similar to this study, Singhal et al reported maximum sensitivity for linezolid (100%), vancomycin (100%), teicoplanin (100%) and amikacin (77.2%) and maximum resistance to cotrimoxazole (74.7%) followed by penicillin (72.3%), methicillin (62.7%), ciprofloxacin (62.7%) and erythromycin (60.2%).[5]

CONCLUSION

To conclude, Coagulase Negative *Staphylococci* (CoNS) have always been considered as either commensal or contaminant in the clinical specimen. Their role as pathogen, particularly nosocomial and opportunistic pathogen has been increasingly recognized in recent years. Species of CoNS are associated with distinct types of infections, slime production and patterns of antimicrobial susceptibility. Hence, accurate species identification, slime detection and antimicrobial susceptibility of CoNS is highly desirable to permit a more precise determination of host-pathogen relationship, knowledge of pathogenicity and epidemiologic information. Thus, CoNS today should not be neglected as unimportant organisms isolated from precious clinical samples for the ultimate benefit to the patient.

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