Species distribution, Antibiotic sensitivity pattern and methicillin resistance of coagulase negative staphylococci isolated from various clinical samples at a tertiary care hospital

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Abstract
Introduction: Coagulase negative staphylococci (CoNS) which were formerly regarded as contaminants of clinical samples, these undoubtedly need more recognition as their pathogenic potential is being increasingly understood and can cause serious human infections. They are causing problems to clinicians because of their drug resistance. Susceptibility testing should be done an isolate considered to be a cause of infection because of the resistance of these organisms to a wide spectrum of antimicrobial agents.
Materials and Methods: Total One hundred strains of CoNS were isolated from 1486 clinical Samples processed (blood – 320, urine – 518, exudates – 522 and body fluids-126.Out of 100 CoNS were isolated from blood (35), urine (33), exudates (29), and body fluids (3) samples collected from both outpatients and inpatients of our Hospital. The organisms were identified and speciation was done by standard biochemical reactions. Antibiotic susceptibility testing was done by Kirby-Bauer disk diffusion method.
Results: Out of total 100 strains of CoNS isolated, majority were in the age group of 15- 45 years (43%). Maximum isolates were from females 54(54%) than males 46 (46%). Maximum CoNS were isolated from blood (35%), followed by urine (33%), exudates (29%) and body fluids (3%). Most common species isolated was S. epidermidis (45%), followed by S. saprophyticus (22%), S. haemolyticus (13%), S. xylosus (5%), S. lugdunensis (4%), S. hominis (4%), S. capitis (4%) and S. cohnii (3%). Methicillin resistance was found in (61%) strains. Linezolid, Amikacin, Doxycycline, Gentamicin, Erythromycin, Norfloxacin and Ciprofloxacin were found to be the most effective antibiotics.
Conclusion: S. epidermidis was the predominant species isolated. The most effective antibiotics were Linezolid and Amikacin.

Keywords: S. epidermidis, Linezolid and Amikacin.

Introduction
Coagulase negative staphylococci (CoNS) were generally considered to be contaminants in the past having little clinical significance. Over the past two decades, however, these organisms have become recognized as important agents of human disease.[1] CoNS are opportunistic pathogens that cause infection in debilitated patients such as premature neonates, burn patients and end stage renal disease.[2] Literature available from the Western World has established CoNS as the most common organisms associated with late onset nosocomial septicemia in neonates, responsible for more than 50% of cases.[3-6] The two most frequently isolated CoNS species in clinical samples are *S. epidermidis* and *S. saprophyticus*. Overall *S. epidermidis* is the predominant agent in nosocomial infection, bacteremia, UTI and surgical wound infection.[7] In the reports of national survey, *S. epidermidis* has been remarkably quoted as primary nosocomial pathogen *S. epidermidis* has been implicated as the aetiological agent in infections of wound, urogenital tract, respiratory tract, meninges, conjunctiva and skin.[8,9] *S. saprophyticus*, a CoNS species, has been identified as a common cause of primary urinary tract infections, particularly in young women of child bearing age.[10] Clinical studies, have indicated *S. epidermidis, S. haemolyticus, S. warneri* and *S. hominis* as the most prevalent CoNS in hospital infections.[11,12] The majority of infections assumed to be caused by CoNS are a significant consequence of hospitalization.[13] Nosocomial bacteraemia is most commonly caused by CoNS, so it is important to explore the sources of CoNS for prevention and management of infections.[14] Multiresistant CoNS commonly colonizing the skin of hospitalized patients and hospital personnel, serves as a potential reservoir for antibiotic resistance genes that can transfer among CoNS and be acquired by *S. aureus.*[15] Multiple antibiotic resistance is a common finding among clinical CoNS isolates indicating its potential pathogenicity.[9] Resistance of these organisms to wide range of antimicrobial agents is well documented.[16] Methicillin resistance among CoNS is particularly important due to cross resistance to virtually all betalactam agents and other anti microbial classes. Susceptibility testing should be done on any isolate considered to be a cause of infection because of the resistance of these organisms to a wide spectrum of antimicrobial agents.[17] This type of study was not conducted so far in this Institute, an attempt was made to isolate and speciate CoNS from various clinical samples with their antibiogram.

Objectives
Speciation of CoNS, their antibiogram and methicillin resistance.

Materials and Methods
This was an observational study and conducted at Department of Microbiology, S Nijalingappa Medical College and Hospital, Bagalkot from December 2014 to August 2015 after obtaining the Institutional Ethical

Committee clearance. All clinical samples were collected under aseptic precautions and following standard clinical laboratory guidelines. The isolates were identified as CoNS by colony morphology, Gram stain, catalase test and coagulase test (slide and tube coagulase). Bacitracin (0.04 U) susceptibility was performed to exclude Micrococci and *Stomatococcus species*.[13]

The isolates which were clinically significant, slide and tube coagulase negative were selected for further speciation. Speciation was done after reviewing the scheme of Kloos and Schleifer and Koneman, et al.[12-14] The various biochemical tests used for speciation are as follows: Ornithine decarboxylase test, Phosphatase test, Urease test, Nitrate reduction test and Carbohydrate fermentation test (Mannose, Mannitol and Xylose).

The antibiotic sensitivity testing was performed on Mueller-Hinton agar by the Kirby-Bauer disc diffusion method, The antibiotics included Amikacin (AK), Amoxicillin-Clavulanate (AMC), Cotrimoxazole (COT), Ciprofloxacin (CIP), Doxycycline (DO), Erythromycin (E), Gentamicin (GEN), Linezolid (LZ) Norfloxacin (NX), Nitrofurantoin (NIT), Novobiocin (NV) and Cefoxitin15 (CX). Four to five colonies from 16 to 24 hours grown culture from an agar plate was suspended in peptone water. This is compared with 0.5 McFarland turbidity standards and inoculated onto Mueller Hinton Agar to get a confluent growth. Plates were incubated at 37oC for 18 to 24 hours. Zone of growth inhibition was measured.[18]

Detection of Methicillin resistance: Cefoxitin (CX-30µg) was used to identify methicillin resistant coagulase negative Staphylococci (MR-CoNS) 15and *Staphyloccus aureus* ATCC 25923 was used as control strain. A 0.5 Mc Farland suspension of the isolate was made and lawn culture done on MHA plate. Plates were incubated at 30ºC for 18 h and zone diameters were measured. An inhibition zone of ≥ 22 mm was considered as susceptible and ≤ 21 mm resistant for cefoxitin. The results of the test are interpreted as sensitive and resistant as per CLSI Guidelines (2015).[15]

Results

The maximum isolates were from female patients (54%) than male patients (46%). Most common age group affected was 15-45 years (43%) followed by 0-14years (38%); 46-60 years (12%) and >60years (7%). Highest percentage among females were in the age group of 15-45 years 24(44.44%) and highest percentage among males were in the age group of 15-45 years 19(41.30%). The important clinically significant samples were blood, urine followed pus in our study. The majority of CoNS were isolated from blood 35(35%), followed by urine 33(33%), exudates 29(29%) [pus 28(28%) and ear discharge 1(1%)] and body fluids 3(3%) [ascitic fluid 2(2%) and pleural fluid1(1%)].The majority of CoNS species isolated were *S. epidermidis* 45(45%), followed by *S. saprophyticus* 22(22%), *S. haemolyticus* 13(13%), *S. xylosus* 5(5%), *S. lugdunensis* 4(4%), *S. hominis* 4(4%), *S.capitis* 4(4%) and *S. cohnii* 3(3%).Maximum number of *S. epidermidis* were isolated from urine 18(54.55%) followed by blood 15(42.86%) and exudates 10(34.48%). 30(30%) were novobiocin resistant. Majority of novobiocin resistant strains were isolated from urine 14(42.42%) followed by exudates 10(34.48%) and blood 5(14.29%). 61(61%) were methicillin resistant and 39(39%) were methicillin sensitive.The methicillin resistance in CoNS isolates was found as follows: *S. epidermidis* 26(42.62%), *S. saprophyticus* 15(24.59%), *S. haemolyticus* 8(13.11%) and *S. xylosus, S. hominis* each 3(4.92%); *S. lugdunensis*, *S. capitis* and *S. cohnii* each 2(3.28%). All the strains of CoNS were sensitive to Linezolid (100%), Amikacin (78%) and Doxycycline (77%). However they were resistant to Amoxycillin-Clavulanic acid 65(65%), Cotrimoxazole 55(55%) and Ciprofloxacin 45(45%).

Discussion

Coagulase Negative Staphylococci form a part of normal flora, more over if CoNS isolated along with another organism, its pathogenic potential may be totally neglected. Hence it is necessary to speciate CoNS and understand the pathogenic potential of individual CoNS.[19] Repeated isolation or pure growth of isolate from sterile or infected site was considered clinically relevant.[20] CoNS are the most important cause of life threatening blood stream infection in some European countries and mucocutaneous commensals can cause serious invasive infections in NICU patients.[8] CoNS species in causing nosocomial infections attention has now been focused on them because of their apparently changing status from non pathogens to opportunist pathogens.[21] Simplicity and speed are very important in certain circumstances, e.g., for the identification of CoNS isolates from normally sterile body sites such as blood cultures, in which these isolates are the most common cause of nosocomial bacteremia, as well as the most common blood culture contaminants. Repeat CoNS isolates from patients with invasive diseases should be identified to allow a comparison of the strains. On the other hand, species identification is a prerequisite before typing procedures for epidemiological studies are undertaken.[22]

In the present study, out of 100 CoNS isolated, majority was from females 54(54%) than males 46(46 %). Most common age group affected was 15-45 years 43(43%) followed by 0-14 years 38 (38%); 46-60 years 12(12%) and >60 years 7(7%). Highest percentage among females were in the age group of 15-45years 24(44.44%) and highest percentage among males were in the age group of 15-45 years 19(41.3%) The above findings correlated with the study of Kumari N et al. (2001)[23] who reported majority of CoNS isolates from females 32(54.1%) than males 27(45.9%).15-45 years age group had highest percentage among females 20(62.4%) while 46-60 years age group had highest percentage among males 8(29.60%).[23]

In the present study out of 100 CoNS isolated majority were from blood 35(35%), followed by urine 33(33%), exudates 29(29%) and body fluids 3(3%). The below mentioned workers, Sewell CM et al. (1982) -43%24 Shrikhande S et al. (1996) -43%25 and Fule RP et al. (1996)- 40.68%26have reported majority of isolates from

exudates and Deighton MA et al.(1988),[27] Ieven M et al. (1995)[28], Usha MG et al., (2013)[25], Murad Ehsan et al(2013),[14] Bhamare S et al.,(2014)[29] have reported majority of isolates from blood and exudates.

In the present study, majority of isolates from urine were *S. epidermidis* 18(54.55%) and *S. saprophyticus* 14 (42.42%) similarly from blood *S. epidermidis* 15(42.86%) and *S. haemolyticus* 10(28.57%). [Table 3] This is in correlation with Sarathbabu R et al, (2013).[30] Out of all the CoNS isolated, *S. epidermidis* was the common species from all samples and from urine where *S. saprophyticus* was most common in the present study. Other species isolated were *S. haemolyticus*, *S. xylosus, S. lugdunensis, S. hominis, S. capitis,* and *S. cohnii* in the present study.

Methicillin resistant CoNS (MR-CoNS) most notably *S. epidermidis, S. haemolyticus, S. hominis* are major MR-CoNS and the main colonizers of the anterior nares and human skin. Methicillin resistant staphylococcal strains have acquired and integrated into their genome the staphylococcal cassette chromosome *mec* (SCC*mec*), which carries the methicillin resistance *(mec*A) gene, and other antibiotic resistance determinants.[31] The methicillin resistant strains are heterogeneous; each population contains both methicillin susceptible and methicillin resistant organisms. The methicillin resistant organisms grow more slowly and prefer lower temperatures and a more hypertonic environment, which necessitates the use of special procedures to enhance detection in susceptibility tests.[17] The percentage of MR-CoNS isolated from clinical specimens by different workers varies from 13.8% to 82.8%.

In the present study, out of 100 CoNS isolated, 61(61%) were methicillin resistant This is in correlation with other workers such as Singh M et al 52% (2015),[32] Marsik FJ et al. 64.1% (1982),[33] Golia S et al 66.4% (2015)[19] and Koksal F et al. 67.5% (2009).[34] In the present study, highest number of isolates were sensitive to Linezolid (100%) and Amikacin (78%) and Doxycycline (77%) [Table 6]. This is similar to above mentioned workers.

Variability in the antibiotic susceptibility pattern of CoNS has been observed by various workers which positively reflect the different protocols and panels of antibiotics being used in different hospitals and differences in the geographical locations from where these isolates have been obtained.[35]

Conclusion

However, the increase in the implication of CoNS as significant nosocomial pathogens with a high rate of resistance to antimicrobial agents has underlined the need for species identification which is important in monitoring the reservoir and distribution of CoNS involved in infections and determining the aetiological agent.[36] Despite the introduction of various antimicrobial agents, antibiotic resistance is increasing day by day. It is more prevalent in developing countries due to their misuse.[14] It is important to monitor antibiotic consumption and resistance trends of nosocomial staphylococci, especially with infection control measures to prevent emergence and spread of multi-resistant bacteria within the hospital environment.[34]

Conflict of Interest: None.

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