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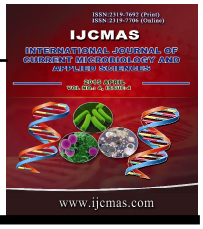

Original Research Article

Phenotypic Characterization of Clinically Significant Coagulase Negative Staphylococci and Their Susceptibility Pattern in a Tertiary Care Hospital

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A B S T R A C T

Coagulase negative staphylococci (CoNS) are a major component of the normal flora of the cutaneous ecosystem including the skin and the mucous membrane. However, if the cutaneous organ system has been damaged by trauma, inoculation by needles or direct implantation of needles or foreign bodies, these organisms can gain entry into the host and they may become pathogenic. Coagulase negative staphylococcus (CoNS) is increasingly recognized as significant nosocomial and opportunistic pathogen. This study aimed to phenotypically characterize CoNS isolated from various clinical samples. A total of 165 clinically significant CoNS isolates were collected in department of microbiology. Coagulase negative staphylococci were identified by Gram staining, catalase test, slide coagulase test and tube coagulase test and were further speciated by various biochemical and phenotypic tests. Antibiotic susceptibility testing was performed on all isolates by Kirby Bauer disk diffusion method. Out of the 490 Staphylococcus isolates, 165 were identified as CoNS. Among the CoNS species, *S.epidermidis* (56%) was the most commonly encountered species, followed by *S.haemolyticus* 30%, *S.saprophyticus* 7%, *S.lugdunensis* 5% and *S.schleifer* 2%. All strains were susceptible to vancomycin, teicoplanin and linezolid. Isolates were most resistant to penicillin. Speciation of CoNS is increasingly become important as they are established etiological agents of nosocomial infection especially where there is breach of sterile sites.

Introduction

Coagulase negative staphylococci are most abundant bacteria colonizing the human skin and mucous membrane, infrequently encountered as primary pathogen but because of changing practice of medicine and host populations, CoNS have become formidable pathogens. Staphylococcus epidermidis is most frequent cause of bacteraemia and encountered commonly in indwelling devices (Mark E Rupp,Paul D Fey.2010) and account for 9% of nosocomial infections (Kloos WE, Bannerman TL ,1994).

Speciation of CoNS to species level ia difficult by conventional methods, as many

biochemical tests have to be performed for correct identification (Kloos WE, Musselwhite MS,1975). Various identification systems and automated systems are available in the market but these technologies are quiet expensive. Therefore, in many diagnostic laboratories operating in medical colleges or in small centers these are not being used.

In a routine microbiology laboratory once an isolate is identified as Staphylococcus ,coagulase test is performed to differentiate *S.aureus* from other CoNS (Bailey,Scott,2007). The CoNS isolates show multiple drug resistance including resistance to methicillin and of late even resistance to glycopeptides has been reported (Xiao Xue et al 2011).

This present study aimed to identify and speciate clinically significant isolates of CoNS. In addition, to also study their antibiotic susceptibility pattern of CoNS isolated from clinical samples received in the department of Microbiology at Chettinad Hospital and Research Institute, Chennai.

Materials and Methods

A total of 165 clinically significant CoNS isolates were included in the study . Strains were isolated from blood, urine, pus, wound swabs, respiratory samples, CSF, and other body fluids.

Repeated isolation, or pure growth of isolate from sterile or infected site was considered clinically relevant.The strains isolated were first identified by colony morphology, Gram staining, catalase test, slide coagulase and tube coagulase test. Bacitracin sensitivity was done to exclude Micrococcus (Shubra Singh et al 2009, Koneman et al 1997) .

Identification

All the strains which are either slide coagulase or tube coagulase negative were speciated based on various biochemical tests.

Identification scheme used to identify CoNS groups/species commonly encountered in clinical practice, includes detection of heat stable nuclease, ornithine decarboxylase test, pyrollidone, urease production, Voges-Proskauer test, O-Nitrophenyl-β-D-galactopyaranoside alkaline phosphatase, polymyxin B susceptibility, novobiocin sensitivity, nitrate reduction, fermentation of glucose, maltose, sucrose, mannitol, lactose and mannose (Koneman et al 1997,Goyal R et al 2006).

Antibiotic sensitivity test was done by Kirby Bauer disc diffusion testing on Mueller-Hinton agar. The antibiotics discs used were Penicillin(10U), Cefoxitin(30µg), Oxacillin (1 µg), Cefazolin(30 µg), Erythromycin (15 µg), Clindamycin(2 µg), Cotrimoxazole (30 µg), Netilmycin (30 µg), Amikacin (30 µg), Linezolid(30 µg), Vancomycin(30 µg), Tetracycline(30 µg), Rifamppicin(5 µg), Ofloxacin(5 µg), Gentamicin(10 µg), Teicoplanin(30 µg), ciprofloxacin(5 µg), Novobiocin (30 µg), Polymixin B (300u). Kirby Bauer's disc diffusion method done as per clinical and laboratory standards institute (CLSI) guidelines (CLSI,2010)

Result and Discussion

Out of the 490 Staphylococcal species, 325 were identified as *Staphylococcus aureus* and 165 as CoNS. Out of the 165 isolates, 55 were from post operative wound infection (30%), 40% blood (23%), 30 urine (18%), 30 pus (23%), 5 CVP tips (3%) and 5 body fluids (3%) Fig 1. 28% of the isolates were obtained from medical ward, 25%

from ICU, 15% from gynaecology, 15% from surgical ward, 9% from pediatric ward and 8% from other wards like dermatology, orthopaedics ward etc (Table I).

Among the CoNS speciated, *S.epidermidis* was the most commonly encountered species i.e., 56% of the isolates, 30% were *S.haemolyticus*, 7% *S.saprophyticus*, 5% *S.lugdunensis* and 2% *S.schleiferi* (Table II). Table III shows species wise distribution of various CoNS isolates.

The antibiotic sensitivity revealed 81% resistance to penicillin, 55% resistance to erythromycin, 32% resistance to cefoxitin, 27% resistance to cefazolin, 22% to clindamycin, and 35% to cotrimoxazole and with no resistance to vancomycin, linezolid and ciprofloxacin (Figure2). Number of methicillin resistant CoNS is 55(32%) among the 165 isolated strains.

Coagulase negative staphylococcus are normal commensals of skin and mucous membrane and are indigenous to many mammalian hosts. Though they are considered less virulent than *S.aureus* they are the most common cause of prosthetic device infections. Approximately half of the identified CoNS species have been associated with human infection.

As CoNS is increasingly being identified as significant nosocomial pathogens several reviewers have emphasized the need for species idenification, by simple, easy and inexpensive method. Differentiation between culture contamination and true infection is another problem encountered when CoNS is grown in culture..

In our study the most frequently encountered clinical isolates of CoNS in our hospital was *Staphylococcus epidermidis* (56%), *Staphylococcus haemolyticus*(30%), *Staphylococcus saprophyticus*(7%), *Staphylococcus lugdunensis*(5%) and *Staphylococcus schleiferi*(2%).

This correlates with study by Shubra Singh et al (2009), where *Staphylococcus epidermidis* was isolated in 40% of their clinical isolates followed by *Staphylococcus haemolyticus* (14%), *Staphylococcus saprophyticus* (12%), *Staphylococcus lugdunensis* (6%) and *Staphylococcus hominis* (6%) (10) Manikandan et al (2005) showed *S. epidermidis* as the most predominant CoNS [57%] follwed by *Staphylococcus hominis* (22.8%) and Larry et al (1986) reported *S. epidermidis* as most often identified species for both clinical saprophytic strains .

In our study, maximum resistance was seen in penicillin followed by cefoxitin, erythromycin, cotrimoxazole, cefazolin, clindamycin and with no resistance to vancomycin,linezolid and ciprofloxacin. Among the CoNS speciated 67% were found to be Methicillin resistant CoNS. Diekema et al (2001) showed more than 80% of coagulase negative Staphylococcus isolates were resistant to methicillin and semisynthetic penicillin .

Despite the recent introduction of antimicrobial agents and medical improvements in controlling the frequency and morbidity of staphylococci infections, they are persistent as important hospital and community pathogen. Methicillin resistance among CoNS has very common occurrence now.

All methicillin-resistant CoNS have been displayed to contain a mecA gene or its gene product, PBP-2a, and it may easily spread to all methicillin-resistant CoNS, probably through transposons (Mayhall C G ,2004) .

Table.1 Ward wise distribution

S.No	Ward	No.	%
1.	Medical ward	47	28%
2.	ICU	42	25%
3.	Pediatrics	15	9%
4.	Gynaecology	25	15%
5.	Surgical ward	25	15%
6.	Others	14	8%

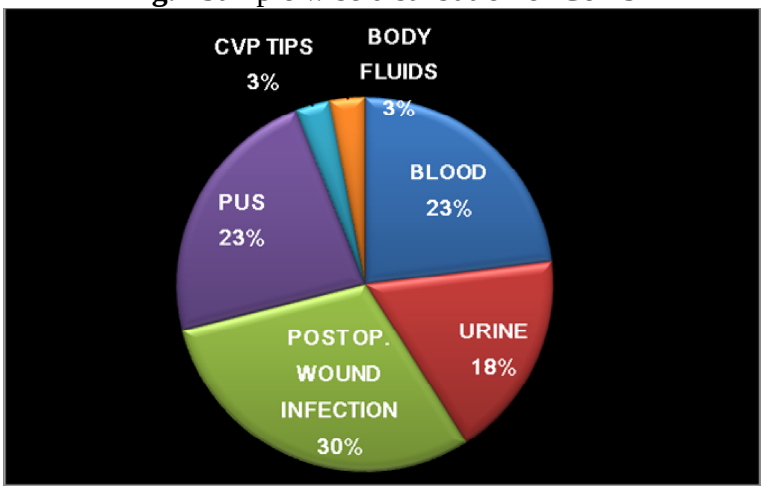
Table.2 Split up of species of CoNS

S.No	CoNS isolated	No.	%
1.	*S.epidermidis*	93	56%
2.	*S.haemolyticus*	50	30%
3.	*S.saprophyticus*	12	7%
4.	*S.lugdunensis*	9	5%
5.	*S.schleiferi*	4	2%

Table.3 Species wise distribution of CoNS in various specimen

	Post operative wound	Blood	Pus	Urine	CVP	Body
S.epidermidis	28	21	28	12	0	3
S.haemolyticus	21	13	7	0	1	0
S.saprophyticus	1	0	0	6	0	1
S.lugdunesis	0	3	1	0	3	1
S.schleferi	0	1	2	0	1	0
Total	50	38	38	18	5	5

Fig.1 Sample wise distribution of CoNS



The only choice for these would be vancomycin but recently CoNS have also shown resistance to vancomycin and even other glycopeptides (Xiao Xue et al 2011) .

CoNS may adhere to medical devices and surfaces through slime, and the slime allows multi-resistant CoNS to colonize within hospital environment..Thus, they may serve as a reservoir of antimicrobial resistance determinants in hospital(Livermore DM, 2000) .

Tolerance to antibiotics and persister cells is a common with CoNS and other bacteria growing within a biofilm. Studies show that Staphylococci growing in a biofilm have significantly less effective. In view of the number of species of CoNS which cause nosocomial infection and the with increasing prevalence of methicillin resistant CoNS , speciation and study of antibiogram of CoNS assumes greater importance.

Coagulase negative staphylococci were previously disregarded as non virulent commensals have now become recognized as true pathogens. They cause a variety of clinical infections ,many related to foreign bodies and prosthetic medical devices. CoNS are a major cause of nosocomial bacteraemia and septicemia, especially for the patients who have immune deficiency and malignancy, which can lead to morbidity and even mortality.

Easy phenotypic methods as well as commercial systems are available to speciate CoNS now. Coagulase –negative staphylococci isolated from nosocomial environments are almost always resistant to multiple antimicrobial agents. But fortunately several newer antibiotics that have good antibacterial activity including daptomycin, linezolid, tigecycline, dalbavancin, televancin and ceftaroline are now available in market.

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