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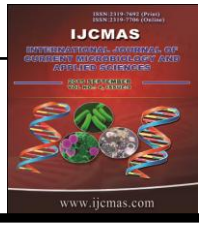

Original Research Article

Incidence and Speciation of Coagulase Negative *Staphylococcus* Isolates from Clinically Relevant Specimens with their Antibiotic Susceptibility Patterns

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Coagulase Negative *Staphylococcus*, (CoNS) Antibiotic susceptibility patterns, MRCoNS

A B S T R A C T

Coagulase-negative *Staphylococci* (CoNS), which are the normal skin flora, have emerged as predominant pathogens in hospital-acquired infections. CoNS are significant and commonly encountered pathogens in hospitals and they are occurring as the most preponderant isolates of all nosocomial infections. A total of 112 CoNS strains were isolated from 723 clinically relevant specimens. The strains were identified as CoNS by colony morphology, Gram stain, catalase test and coagulase test. Speciation was done based on various biochemical tests. Susceptibility to novobiocin and polymyxin B was performed as per the regular procedure for speciation. The antibiotic sensitivity testing was performed on Muller-Hinton agar by the Kirby-Bauer's disc diffusion method using Ampicillin, amoxyclav, ceftriaxone, cotrimoxazole, cefotaxime, cefoxitin, gentamicin, amikacin and vancomycin. Out of the total of 723 relevant clinical samples collected, 112 (15.4%) samples were identified as Coagulase negative *Staphylococcus*. The highest number of CoNS isolates were *Staphylococcus epidermidis* (50.8%) followed by Staphylococcus haemolyticus (26.7%). The other species isolated were *Staphylococcus lugdanensis* (10.7%), *Staphylococcus schleiferi* (7.1%) and *Staphylococcus saprophyticus* (4.46%). The maximum number of isolates was resistant to ampicillin (55.35%). All isolates were sensitive to Gentamicin and vancomycin. The incidence of MRCoNS (Methicillin resistant CoNS) in this study was found to be 33%. The antibiotic resistance pattern of CoNS shows resistance to multiple antibiotics like ampicillin, cotrimoxazole and cefoxitin. These emerging resistance patterns and increased prevalence of CoNS is a result of frequent use of intravascular devices and raise in the number of immunocompromised patients in hospitals. This scenario emphasizes the need for rapid identification and speciation of CoNS with their antibiotic susceptibility for improved management of such cases and prevent emergence of drug resistance.

Introduction

Gram positive bacteria are widespread in nature and can be isolated from the environment or as commensal inhabitants of the skin, mucous membrane and other body sites in humans and animals. The ubiquity of the Gram positive bacteria in nature makes the interpretation and their recovery from patient specimens occasionally difficult unless clinical manifestations of an infectious disease are apparent.

Coagulase-negative Staphylococci (CoNS), which are the normal skin flora, have emerged as predominant pathogens in hospital-acquired infections. CoNS are significant and commonly encountered pathogens in hospitals and they are occurring as the most preponderant isolates of all nosocomial infections (Mulu *et al.*, 20121). Recovery of these organisms from specimens should always be correlated with the clinical condition of the patient and with their role to cause infections. Risk factors include patients with intravascular catheters, or other foreign bodies in place, prosthetic devices and postoperative wound infections. These bacteria usually infect immunocompromised hosts, such as premature newborns and patients with leukemia or other malignant diseases (Sheikh and Mehdinejad, 2012).

Among the CoNS, *Staphylococcus epidermidis* is the principal cause of infection, chiefly in hospitalized patients with indwelling foreign bodies (Sheikh and Mehdinejad, 2012). The clinical significance of species other than *S. epidermidis* has been increasingly recognized in the recent years. The coagulase-negative species *S. saprophyticus* is a recognized pathogen causing primarily acute urinary tract infections in young healthy, sexually active women (Sheikh and Mehdinejad, 2012). Infections with many of the other species are acquired in the hospital setting. The more commonly implicated species include *S. haemolyticus, S. lugdanensis, S. schleiferi, S. warneri, S. hominis, S. simulans* and *S. saccharolyticus* (Usha *et al.,* 2013). These species have been documented as a cause of nosocomial bacteremia, wound infections, urinary tract infections, and pediatric and neonatal infections. These infections are not easy to treat because of the risk factors and the multiple drug resistance displayed by these organisms (Asangi *et al.,* 2011).

The drug resistance is highest in nosocomial infections because in hospitals, repeated contact with antibiotics leads to elimination of sensitive organisms from the flora and their substitution with resistant strains acquired by cross infection, especially CoNS. CoNS will persist to be an infective agent in the future and hence studies on CoNS will facilitate in formulating and implementing particular antibiotic policies for treating CoNS infections and to control further emergence of drug resistant strains, in future. This study was carried out to identify the incidence and speciation of coagulase negative Staph with their antibiogram from clinically relevant specimens coming to microbiology laboratory in a tertiary care hospital in North Karnataka.

Materials and Methods

A total of 112 CoNS strains were isolated from 723 clinically relevant specimens collected from Jan-2013 to Dec-2013 in our hospital. CoNS were isolated from pus and wound swabs, sputum, blood, urine, ear swabs, throat swabs and other body fluids. The strains were identified as CoNS by colony morphology, Gram stain, catalase test and coagulase test. Bacitracin sensitivity was done to exclude *Micrococci* and

Stomatococcus species (Hebert *et al.,* 1988). Speciation was done based on various biochemical tests. The conventional tests that are simple, easy to perform and non-expensive were chosen from the scheme of Kloos and Schleifer and Koneman. These include the ornithine decarboxylase test, nitrate reduction test, Voges-Proskauer test, urease test and fermentation of sucrose, lactose, maltose, mannose, mannitol, xylose and trehalose. Susceptibility to novobiocin and polymyxin B was performed as per the regular procedure for speciation (De paulis *et al.,* 2003; Winn *et al.,* 2006). The antibiotic sensitivity testing was performed on Muller-Hinton agar by the Kirby-Bauer's disc diffusion method using ampicillin, amoxyclav, ceftriaxone, cotrimoxazole, cefotaxime, cefoxitin, gentamicin, amikacin and vancomycin.

Results and Discussion

Out of the total of 723 relevant clinical samples collected, 112 (15.4%) samples were identified as Coagulase negative *Staphylococcus*. Out of the 112 isolates, 87(77.6%) were from wounds, 7(6.2%) from sputum, 6(5.3%) from blood, 6(5.3%) from urine, 2(1.7%) from body fluids, 2(1.7%) from throat swabs, 2(1.7%) from ear swabs.

Of the 112 relevant clinical samples which were positive for CoNS in our hospital, 65(58%) samples were collected from surgical ward, 12(10.7%) were from medical ICU, 10(8.9%) were from orthopedic ward, 10(8.9%) were from gynecology ward, 6(5.3%) were collected from ENT ward and 3(2.6%) samples each from surgical ICU, neonatal ICU (2.6%) and dermatology ward (2.6%). The highest number of CoNS isolates were *Staphylococcus epidermidis* (50.8%) followed by *Staphylococcus haemolyticus* (26.7%). The other species isolated were *Staphylococcus lugdanensis* (10.7%), *Staphylococcus schleiferi* (7.1%) and *Staphylococcus saprophyticus* (4.46%). (Table 1).

The species wise distribution of CoNS in various clinical samples displayed *Staphylococcus epidermidis* as the predominant species isolated from pus & wound swabs in our study. The other common species of CoNS in various clinical samples is displayed in table 2.

There was no specific gender predisposition for isolation of CoNS in our study. However, the maximum number of isolates was from the age group of 61–70 years, followed by the age group of 41–50 years, 31–40 years and 11–20 years. The remaining isolates were from the age group of 21–30 years, 0–10 years and 51–60 years. The least number of isolates were from the age group of 71–80 years. The age distribution with percentage is demonstrated in Table 3.

The antibiotic sensitivity testing was performed on Muller-Hinton agar by the Kirby-Bauer disc diffusion method using a panel of nine antibiotics, which included Ampicillin (A), Amoxyclav (Amc), Ceftriaxone (Ci), Cotrimoxazole (Co), Cefotaxime (Ce), Cefoxitin (Cn), Gentamicin (G), Amikacin (Ak) and Vancomycin (Va). The maximum number of isolates were resistant to ampicillin (55.35%). All isolates were sensitive to gentamicin and vancomycin. The incidence of MRCoNS (Methicillin resistant CoNS) in this study was found to be 33%. The resistance pattern of CoNS isolates is displayed in Table 4 and methicillin resistance pattern of CoNS species is displayed in Table 5.

In the present study, a total of 723 samples were collected, out of which 112 samples were identified as Coagulase negative

Staphylococcus. Maximum number of samples was pus samples and the distribution of individual species of CoNS varied in different samples depending on the type of specimen. From pus, *Staphylococcus epidermidis* was the predominant species, followed by *Staphylococcus haemolyticus*, *Staphylococcus lugdanensis* and *Staphylococcus schleiferi*. *Staphylococcus epidermidis* was also the most frequently isolated species from sputum and blood. In urine samples in our study, *Staphylococcus saprophyticus* was the common species isolated. These findings are similar to the findings by various studies done on CoNS (Sheikh and Mehdinejad, 2012; Usha *et al.*, 2013).

Table.1 Incidence of different species of CoNS in the study

Species of CoNS	**Number of isolates**	**Percentage**
Staphylococcus epidermidis	57	50.8%
Staphylococcus haemolyticus	30	26.7%
Staphylococcus lugdanensis	12	10.7%
Staphylococcus schleiferi	8	7.1%
Staphylococcus saprophyticus	5	4.46%

Table.2 Species of CoNS in various clinical samples

CoNS Species	Pus And Wound Swabs	Sputum	Blood	Urine	Throat Swabs	Ear Swabs	Body Fluids	Total
Staphylococcus epidermidis	47	3	3	1	1	1	1	57
Staphylococcus haemolyticus	23	2	3	1	-	-	1	30
Staphylococcus lugdanensis	11	-	-	-	1	-	-	12
Staphylococcus schleiferi	5	2	-	-	-	1	-	8
Staphylococcus saprophyticus	1	-	-	4	-	-	-	5

Table.3 Age distribution of CoNS in the study

Age	Number of CoNS	Percentage
0-10 years	8	7.14%
11-20 years	17	15.17%
21-30 years	14	12.5%
31-40 years	17	15.17%
41-50 years	19	16.96%
51-60 years	8	7.14%
61-70 years	23	20.53%
71-80 years	6	5.35%

Table.4 Antibiotic sensitivity pattern of CoNS isolates in the study

Antibiotic	**Resistance pattern (%)**	**Number of isolates**
Ampicillin	55.35%	62
Amoxyclav	27.6%	31
Ceftriaxone	30.35%	34
Co-trimoxazole	37.5%	42
Cefotaxime	27.6%	31
Gentamicin	Nil	0
Amikacin	17.8%	20
Cefoxitin	33%	37 (MRCoNS)
Ciprofloxacin	12.5%	14
Vancomycin	Nil	0

Table.5 Methicillin resistance pattern of CoNS species

CoNS Species	Number of strains tested	Number of MRCoNS
Staphylococcus epidermidis	57	21
Staphylococcus haemolyticus	30	10
Staphylococcus lugdanensis	12	2
Staphylococcus schleiferi	8	0
Staphylococcus saprophyticus	5	4
Total	112	37

The antibiotic sensitivity pattern of the isolates revealed 55.35% resistance to ampicillin, 37.5% resistance to Co-trimoxazole and 33% resistance to cefoxitin (MRCoNS). There was no resistance to vancomycin and gentamicin. These findings have been noted in similar studies done on CoNS (Sheikh and Mehdinejad, 2012; Usha *et al.*, 2013).

Clinical isolates of CoNS to the species level are usually not identified by many laboratories as they are considered normal inhabitants of skin and anterior nares. They are known to be causing only opportunistic infections. Moreover the methods used for speciation of CoNS utilize a series of biochemical reactions which are burdensome and often give inconsistent results. CoNS is being implicated as a significant nosocomial pathogen in current years, therefore emphasizing the need for speciation of these strains. Speciation assists in monitoring the reservoir of particular species of CoNS and its distribution. Moreover it also helps in determining the etiological agent of particular nosocomial infection based on the clinical sample received.

The present study revealed maximum number of CoNS was isolated from the surgical ward and from pus samples. The maximum number of isolates was from the age group of 61–70 years with no particular gender predominance. The frequency of drug-resistant strains of CoNS has increased in the recent years, hence significant CoNS isolates should be tested for antimicrobial susceptibility to help in the choice of an appropriate drug for successful management. Early detection of MRCoNS

and reduction in indiscriminate use of antibiotics like vancomycin are the only suitable approaches to reduce the emergence of these drug resistant strains.

It will also be necessary for the hospital authorities to adapt specific antibiotic policies for treatment of all staphylococcal infections in accordance with their antibiogram reports and avoid using drugs in inadequate doses and for inappropriate duration.

In conclusion, CoNS have become one of the major causes of nosocomial infections with *Staphylococcus epidermidis* being the most common species. The antibiotic resistance pattern of CoNS in our study shows resistance to multiple antibiotics like ampicillin, cotrimoxazole and cefoxitin. These emerging resistance patterns and increased prevalence of CoNS is a result of frequent use of devices and rise in the number of immunocompromised patients in hospitals. This scenario emphasizes the need for rapid identification and speciation of CoNS with their antibiotic susceptibility for improved management of such cases and to prevent emergence of drug resistance.

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