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Speciation of Coagulase Negative Staphylococci and Their Antibiogram

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Abstract: *Coagulase Negative Staphylococci (CoNS) have surfaced as important pathogens, preying primarily on patients with some sort of prosthetic or indwelling device. They are currently the most frequent agents of nosocomial bacteraemia. Recovery of these organisms from specimens should always be correlatedwith the clinical condition of the patient before their role in an infectious process can be established.With the increasing number of Staphylococcus species being recognised in human infections and the finding of resistance to multipleantimicrobial agents, it is imperative that the clinical microbiologist be familiar with current methods for characterising these organisms. This study was undertaken to identify the prevalence ofclinical isolates of CoNS, their speciation using a simple schemeof biochemical reactions and to determine their antibiotic sensitivity/resistantpatterns. A total of 100 isolates were collected from different samples and subjected tobiochemical characterization using a simple scheme. Antimicrobial susceptibility testing was done by Kirby-Buer's disc diffusion method. The commonest species identified was S.epidermidis (42%) followed by S.haemolyticus (28%) and S.saprophyticus (18%). S.schleiferi constituted 4%, S.simulans 2%, S.cohnii, S.warneri and S.capitis 1% each. We could not identify 3 isolates using this scheme. Antibiotic susceptibility testing showed maximum resistance to penicillin (90%), Ampicillin (79%), Erythromycin (65%) and Cotrimoxazole (62%).Methicillin resistance was found to be 72% by using Cefoxitin disc. The increased recognition of pathogenic potential in CoNS and emergence of drug resistance among them demonstrates the need to adopt simple laboratory methods to identify the species and determine the antibiotic resistantpatterns. It will help the clinicians in treating the infections caused by CoNS.*

Keywords: *Coagulase negative Staphylococcus, Mannose,Novobiocin resistance, Ornithine decarboxylase, Urease.*

I. Introduction

Coagulase negative Staphylococci (CoNS) historically have been regarded as saprophytes with little pathogenic potential[1].Since 1950, infections with these organisms have been reported with increasing frequency[2]. Over the last two decades, however these organisms have become recognised as important agents of human disease[3].

CoNS are common colonisers of the skin, anterior nares and ear canals of humans[4]. They act as opportunistic pathogens in debilitated, compromised patients often by colonising biomedical devices such as prostheses, implants and intravascular lines[5,6].CoNS has emerged as predominant pathogen in hospital acquired infections and vary in pathogenic potential[7].

More than 30 species of CoNS are recognised but only a few are commonly incriminated in human infections[8].

S.epidemidis is the CoNS species most frequently isolated from infections. In the reports of national survey, this species has been remarkably quoted as primary nosocomial pathogen[9]. It has been implicated as the etiological agent in infections of wounds, urogenital tract, respiratory tract, meninges, conjunctiva and intravenous catheter associated infections[10].

S.haemolyticus has been documented in many studies as a clinically opportunistic pathogen and is usually the second most common CoNS species recovered from documented infection sites[11]. It has been implicated in naïve-valve endocarditis, septicaemia, peritonitis, wound, bone and joint infections.

S.saprophyticus was shown to be an important cause of urinary tract infections in young females[12]. It is second to coliforms as the most common cause of acute urethral syndrome[13].

S. warneri is a well recognised cause of catheter related bacteraemia, naïve-valve endocarditis, haematogenous vertebral osteomyelitis and ventriculo-peritoneal shunt associated meningitis.S.hominis has occasionally been isolated from infections causing catheter related sepsis in immunocompromised hosts.S.simulans has been established as a cause of septicaemia, osteomyelitis, septic arthritis, vertebral osteomyelitis and prosthetic joint infection.S.schleiferi has been isolated from several human infections including brain empyema, wound infections, bacteraemia complicating vertebral osteitis, infection of hip prosthesis and indwelling catheter infections.S.lugdunensis has been isolated from abscesses in the pelvic girdle

region.S.cohnii is an emergent opportunistic agent having been reported as a cause of community acquired pneumonia.

Several commercial kit identification systems and automated instruments are available which can identify a number of Staphylococcus species accurately but are still out of reach of most of the laboratories in developing countries. Hence convenient,reliable and inexpensive identification methods are needed to identify most of the CoNS species, which can be utilised by most of the laboratories where automated methods are not yet available.

In the present study an attempt was made to identify the CoNS species isolated from various samples by using minimum number of testswhich were simple, inexpensive and easy to perform. Antibiogram of the isolates was also done.

II. Materials And Methods

The present study was conducted in the department of microbiology, Rangaraya Medical College, Kakinada for a period of 6 months from March to August 2015. A total of 100 clinically significant CoNS isolates wereidentifiedin different clinical samples (urine, sputum, blood, pus and CSF) and processed usingconventional microbiological methods. The isolates were initially identified bycolony morphology, Gram staining, catalase, slide and tubecoagulase test[3].

The tests which were simple, inexpensive and easy to perform, were selected from the scheme of Kloos and Shleifer to identify CoNS species[14,15]. Speciation of CoNS was done by Novobiocin resistance, urease activity, ornithine decarboxylase & acid production from mannose as noted in TABLE I.

Antibiotic susceptibility of the isolates is done by Kirby Bauer's disc diffusion method following CLSI guidelines. Methicillin resistance was tested by using Cefoxitin disc.

One isolate from each species identified was confirmed on siemen's automated identification system.

III. Results

As per TABLE II, among 100 CoNS isolates the present scheme identified the commonest species as S.epidermidis (42%) followed by S.haemolyticus (28%) and S.saprophyticus (18%) together constituting 88% of the total CoNS species.S.schleiferi constitutes 4%, S.simulans 2%, S.cohnii, S.warneri and S.capitis 1% each. We could not identify 3 isolates may be because of aberrant reactions which were later identified on Siemenn'sautoscan as S.lugdunensis.

As per TABLE III majority showed resistance to penicillin (90%), Ampicillin (79%), Erythromycin (65%) and Cotrimoxazole (62%).Methicillin resistance in CoNS was found to be 72%which was identified by using Cefoxitin disc. Resistance to Vancomycin which is the drug of choice for methicillin resistant strains was also noted in 7% of cases, which is an alarming sign.

IV. Discussion

Infections with CoNS have been reported with increasing frequency. Because there is increasing pathogenecity and resistance of these organisms, CoNS should be identified to the species level by simple, reliable and preferably inexpensive methods possible.

In the present study 100 strains of CoNS were speciated by using a simple scheme and the results are compared with other studies as follows.

In our study the most common species isolated was S.epidermidis (42%) which is correlating with the studies by Shubhra Singh et al[17]where the rate of isolation was 40%.

The next common species in our study was S.haemolyticus seen in 28% . Similar results were seen in other studies, Sheik and Mehdinejad et al[18] ,samanthSharvani et al[19] and Usha M G et al[20].But in other studies, Mohan et al[21], N P Singh et al[16], Shubhra Singh et al[17], Surekha Y Asangi et al[16] and Shubha D S etal[23] S. saprophyticuswas the second most common species.

In the present study antibiotic susceptibility testing showed multidrug resistance among the CoNS species. Methicillin resistance in our study was 72% which was nearly correlating with Surekha Y Asangiet al[22] (67.7%).

Although many studies showed 100% sensitivity to Vancomycin, our study showed 7% resistanceto Vancomycin. Emerging vancomycin resistant CoNS isolates have been reported from India and other countries.

V. Conclusions

CoNSis increasingly being implicated as a significant nosocomial pathogen. Many of the CoNS species are becoming resistant to antibiotics that are being indicated for Staphylococcal infections. Hence there is a need for identification of these isolates to species level by simple, inexpensive methodology. Italso helps in monitoring the reservoir and distribution ofCoNS involved in nosocomial infection. We are able to identify

more than 90% of CoNS species by a simple scheme which can be employed in conventional diagnostic laboratories.

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Table.1: Identification of CoNS by simple scheme[16, 3]

Species	Clumping factor	Tube coagulase	Ornithine decarboxylase	Urease	Mannose	Novobiocin 5µg
S.epidermidis	-	-	+	+	+	S
S.haemolyticus	-	-	-	-	-	S
S.saprophyticus	-	-	-	+	-	R
S.warneri	-	-	-	+	-	S
S.lugdunensis	+	-	+	+	V	S
S.schleiferi	+	-	-	-	+	S
S.simulans	-	-	-	+	+	S
S.capitis	-	-	-	-	+	S
S.cohnii	-	-	-	-	+	R

Table II: Frequency of different CoNS species isolated (n=100)

Species	Number isolated	Percentage
S.epidermidis	42	42
S.haemolyticus	28	28
S.saprophyticus	18	18
S.schleiferi	4	4
S.simulans	2	2
S.cohnii	1	1

S.warneri	1	1
S.capitis	1	1
Unidentified	3	3

Table III: Showing resistance pattern of CoNS to various antibiotics

SPECIES	P	AMP	E	CX	LZ	VA	G	AK	COT
S.epidermidis(42)	36	31	27	33	09	02	06	04	22
S.haemolyticus (28)	26	24	22	21	03	03	05	04	19
S.saprophyticus (18)	16	12	10	11	05	02	03	03	12
S.schleiferi (4)	04	04	03	03	01	00	01	01	03
S.simulans (2)	02	02	00	01	00	00	00	00	01
S.cohnii (1)	01	01	00	00	00	00	01	00	02
S.warneri (1)	01	01	00	00	00	00	00	00	01
S.capitis (1)	01	01	01	01	00	00	00	00	01
S.lugdunensis (3)	03	03	02	02	00	00	01	00	01
TOTAL(100)	**90**	**79**	**65**	**72**	**18**	**7**	**17**	**12**	**62**

P=Penicillin, AMP=Ampicillin, E=Erythromycin, Cx=Cefoxitin, LZ=Linezolid, VA= Vancomycin, G=Gentamicin, AK=Amikacin, COT=Cotrimoxazole