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Simple method for speciation of clinically significant *coagulase negative Staphylococci* and its antibiotic sensitivity/resistant pattern in NICU of tertiary care centre.

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Abstract

An attempt was made to speciate and antimicrobialy differentiate 150 clinically significant isolates of CoNS by practical scheme adapted from various references.

Total 150 isolates were collected from NICU, Pediatrics Department, CSMMU, Lucknow from different sites and subjected to antimicrobial screening and biochemical characterization using conventional microbiological methods. Antibiotic sensitivity test was performed for all clinically significant isolates.

90% isolates were conveniently identified. S.*epidermidis* (40%), S.*saprophyticus* (14%), S.*haemolyticus* (12%), S.*hominis* (6%), S.*lugdunensis* (6%), S.*schleiferi* (2%), S.*capitis* (4%), S.*warneri* (6%), 15 isolates were not identified to the species level. Antibiotic susceptibility testing showed maximum resistance to ampicillin and penicillin with 80% and 38% strains showed the resistance to oxacillin.

The increasing recognition of pathogen potential *CoNS* and emergence of drug resistance among them demonstrates the need to adopt simple laboratory procedure to identify and determine the prevalence and antibiotic sensitivity of *CoNS*. It will help in treating clinicians for first line treatment in hospital.

Introduction

Co-agulase negative staphylococci have become common cause of nosocomial infections, particularly blood stream infection [1] and infections related to prosthesis [2]. They account for 9% of nosocomial infection [3]. The natural habitats of *staphylococcus epidermidis* and several other species of CoNS include skin and nares [4]; a clinical specimen could therefore, contain both contaminants and pathogens of these species. If contaminant is mistakenly identified as a pathogen i.e. source of isolate was the skin and not an infective process, the patient will probably receive unneeded antimicrobial therapy. It is now possible to identify the different species of *staphylococcus* by methods practical for diagnostic laboratories. Because of this more laboratories can access what potential clinical or epidemiological benefits this addition information might have.

The present study was aimed to identify the most prevalent clinical isolates of *CoNS* by minimum number of tests necessary and sufficiently to discriminate between species. The tests, which were simple, inexpensive and easy to perform, were selected from the scheme of Kloos and Shleifer to identify *CoNS* species group (group approach) or species [5,6]. Further one or two additional test was included to complete the strain identification wherever necessary. Antimicrobial susceptibility profiles of all isolates were done by Disk Diffusion Method.

Material and Methods

A total of 150 consecutive non-repeat clinically significant *CoNS* isolates were collected from January 2006 to December 2006 from Pediatrics Department and proc-

essed in the Department of Microbiology, Chhatrapati Shahuji Maharaj Medical University, Lucknow. Strains were isolated from blood sample, pus, urinary catheter tip and urine. The isolates were considered clinically significant when isolated in pure culture from infected site. The strains collected were initially identified by colony morphology, Gram staining, catalase, slide and tube coagulase (read after 4-24 hr) and anaerobic acid from mannitol. Bacitracin (0.04U) susceptibility was done to exclude micrococcus and stomatococcus spp [5,7].

Identification

All the strains, which were either, slide or tube coagulase negative were further identified by a scheme [3, 8, 9, 10, 11, 12]. The identification scheme concentrated on species groups/ species commonly encountered in clinical practice the *S.* epidermidis group (i.e. *S.epidermidis, S. capitis* subspecies *urolyticus* and *caprae*), the *S. haemolyticus* group (*S. haemolyticus*, S. *auricularis* and *S.caseolyticus*), the *S.saprophyticus* group (*S .saprophyticus* subspecies *saprophyticus* and S.*hominis* subspecies *novobiosepticus*), the S.warneri group (*S.warneri* and *S.hominis* subspecies *hominis*), the *S.cohnii* group (*S.xylosus* and *S. cohnii* subspecies *urolyticum*), *S. lugdunensis* , *S.shleiferi* subspecies *schleiferi*, *S.capitis* subspecies *capitis*, *S.simulans* and *S.cohnii* subspecies *cohnii* [6]. This scheme involves a two-step procedure (Table-1), first step aimed to identify species group and combined slide and tube coagulase with novobiosin resistance test for urease activity, ornithine decarboxylase & aerobic acid production from mannose. If identification requires additional tests, a maximum of two tests were selected from Table 1: trehalose and mannitol for the *S. epidermidis* group, acetoin production and lactose for *S.haemolyticus* group, trehalose for the *S.saprophyticus* group, anaerobic thioglycollate both for *S. warneri* group and xylose for *S. cohnii* group [6]. The entire tests were performed according to reference method [5].

Antimicrobial susceptibility test

The susceptibility of the isolates to antimicrobial agents, including oxacillin was determined by the disk diffusion method with Mueller-Hinton agar plates (Hi-Media, Mumbai) according to the guidelines NCCLS [13, 14]. Disk contain following antibiotic at the specific absolute concentrations penicillin 10µg, ampicillin 10µg, chloramphenicol 30µg, erythromycin 15µg, rifampicin 5µg, gentamycin 10µg, vancomycin 30µg, oxacillin 1µg, staphylococcus aureus ATCC 43300 was used as control.

Table-1 Frequency of Clinically significant CoNS

SPECIES	No. (%)	BLOOD (%)	PUS (%)	CATHETER TIP (%)	URINE (%)
s. epidermidis	60 (40)	30(50)	12 (20)	9(15)	9 (15)
s. saprophyticus	21 (14)	12(57.14)	6 (28.57)	3(14.28)	0
s. haemolyticus	18 (12)	12(66.66)	6(33.33)	0	0
s.hominis	9 (6)	6(66.66)	0	0	3 (14.28)
s.lugdunensis	9 (6)	3(33.33)	6 (66.66)	0	0
s.shleiferi	3 (2)	3(100)	0	0	0
s. capitis	6 (4)	6(100)	0	0	0
s.warneii	9 (6)	0	6 (66.66)	3 (33.33)	0

Table-2 Showing resistance pattern of CoNS to different antibiotics

	R	C	G	Va	P	A	E	Cf	Ox
S	102 (68%)	102 (68%)	102 (68%)	150 (100%)	30 (20%)	30 (20%)	33 (22%)	48 (32%)	93 (62%)
R	48 (32%)	48 (32%)	42 (28%)		120 (80%)	120 (80%)	81 (54%)	81 (54%)	57 (38%)
I	0	0	6 (4%)		0	0	36 (24%)	21 (14%)	0

R= rifampicin, C=chloramphenicol, G=gentamycin , Va= vancomycin, P=penicillin A=ampicillin E=erythromycin , Cf=ciprofloxacin , Ox=oxacillin

Table-3: identification of CoNS by simple schemes and additional tests

Ornithin e ecarboxylase	Urease	Novobiocin (5µg)	Mannose	Species/ subspecies	Trehlose growth	Mannitol	Acetonin	Lactose	Anaerobic	Xylose
+	+	S	+	S.epidermidis	-	-				
				S.caprae	+	-				
				S.capitis Subsp. Ureolyticus	-	+				
-	-	S	-	S. haemolyticus			+			
				S.auricularis			-	-		
				S.caseolyticus			-	+		
-	+	R	-	S.saprophyticus Subsp. Saprophyticus	+					
				S. hominis Subsp. Novobiosepti-cus	-					
-	+	S	-	S.warneri					+	
				S.hominis Subsp.hominis					-	
+	±	S	+							
-	-	S	+							
-	+	S	+							
-	+	S	±							
-	-	S	+							
-	-	R	±	S.xylosus						+
-	+	R	+	S. cohnii Subsp. ureolyticum						-

β Lactamase detection

Acidometric determination of β Lactamase production was performed by using growth from around the oxacillin disk.

Result

Seventy two out of 150 strains of CoNS (60%) were isolated from blood samples, 36 from pus samples, 15 from urinary catheter tip and 12 from the urine samples. All 150 strains were negative for blood clumping factor and tube coagulase negative. Table 3 shows the CoNS species group /species by the scheme [15].

The scheme could identify 90% of CoNS isolated from clinical samples incorporation of one or two additional test whenever needed. *S. epidermidis* was the most frequent isolate and was identified if ornithine decarboxylase was positive (60 isolates), while ornithine decarboxylase negative isolates required inclusion of trehlose and mannitol for speciation. 15 isolates were unidentified because of aberrant characteristics.

Antibiotic susceptibility testing showed maximum resistance to penicillin (80%) and ampicillin (80%) followed by erythromycin (54%), ciprofloxacin (54%), oxacillin (38%), rifampicin (32%), chloramphenicol (32%), gentamycin (28%), no resistance to vancomycin was seen.

Resistance to oxacillin was highest among isolates of *S.haemolyticus* (76%) followed by *S.epidermidis* (33%). *S.haemolyticus* was most resistance species for both erythromycin (80%) and rifampicin (42%). *S.epidermidis* and *S.hominis* also showed a significant percentage of isoltes resistance to both of these agents. Resistance to gentamycin (76%) and ciprofloxacin (42%) was greatest for s.haemolyticus followed by *S.saprophyticus*. Resistance to chloramphenicol, ampicillin and penicillin was greatest for *S.epidermidis*.

Discussion

Many laboratories do not identify clinical isolates of CoNS to the species level as they are considered normal

inhibitant of skin and nares capable of causing only opportunistic infections [9]. Moreover, the conventional identification methods, though accurate, are cumbersome and employ a large battery of biochemical reactions, which often gives variable results and all the tests are generally not available in most of the routine diagnostic laboratories [16]. As *CoNS* is increasingly being implicated as significant nosocomial pathogen, several reviewers have emphasized the need for species identification, which is possible only by simple, easily adaptable, inexpensive method [3,19,18,15,5,6]. The species identification is important in monitoring the reservoir and distribution of *CoNS* involved in nosocomial infections and determining the etiological agent [15,5]. The present scheme conveniently identified the most frequently encountered clinical isolates in our hospital as *S.epidermidis* (40%), *S.haemolyticus* (12%), *S. saprophyticus* (14%), *S. hominis* (6%), *S.lugdunensis* (6%) upto species level by incorporation of one or two additional tests. This scheme could directly identify even the newly described species *S.schleiferi* subsp. *Schleiferi* and *S. lugdunensis* using slide agglutination test for detection of clumping factor or fibrinogen affinity factor provided no slide is discarded before 10 seconds as *S.lugdunensis* sometimes gives delayed results [5]. Consequently, the laboratories performing only slide agglutination test without tube coagulase test may misidentify *S.schleiferi* subsp. *Schleiferi* and *S. lugdunensis* as *S.aureus*. This scheme could not differentiate *S.capitis* subsp. *Ureolyticus*, *S.warneri, S.simulans* as pyroglutamyle β napthylamide (PYR) hydrolysis test, a key component for their identification, being expensive was not included. Various workers from India have reported *S.epidermidis* and *S.saprophyticus* to be the most common isolate similar to our study [9, 10, 17, 18]. Though a single report has mentioned isolation of *S.cohnii* which was not isolated in our study, however, the scheme described can identify this isolate [9].

This scheme identified 90% of CoNS upto the species level which is almost similar to earlier report [19, 9, 10,17,18]. The most obvious finding was observed in smaller numbers by others [21]. Out of all species *S. haemolyticus* has the most antibiotic resistant profile. Approximately 76% of our isolates of S. *haemolyticus* showed resistance to oxacillin (76%), gentamycin (76%), erythromycin (80%). Although, *S.epidermidis* can also show significant multiple resistance patterns. It is therefore recommended to assess the importance of *CoNS* speciate whatever level possible and perform the antibiotic susceptibility testing before any typing procedure for epidemiological studies are being undertaken.
This simple, inexpensive methodology will prove useful in routine microbiology laboratory for the presumptive identification of *CoNS*.

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