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Brief Communication

SIMPLE AND ECONOMICAL METHOD FOR SPECIATION AND RESISTOTYPING OF CLINICALLY SIGNIFICANT COAGULASE NEGATIVE STAPHYLOCOCCI

R Goyal, *NP Singh, A Kumar, I Kaur, M Singh, N Sunita, M Mathur

Abstract

An attempt was made to speciate 102 clinically significant isolates of coagulase negative staphylococci (CoNS) by a practical scheme adapted from various references. This scheme utilizes slide and tube coagulase test, urease test ornithine decarboxylase, novobiocin susceptibility and aerobic acid from mannose for assigning species group. Inclusion of one or two additional tests in a species group could identify the isolates to species level. Ninety eight (97%) isolates were conveniently identified as *S. epidermidis* (41%), *S. saprophyticus* (16.6%), *S. haemolyticus* (14.7%), *S. hominis* (14.7%), *S. lugdunensis* (4.9%), *S. schleiferi* (1.9%) and *S. capitis* (1.9%). Only four isolates were not identified to the species level, two of which were probably *S. capitis* subspecies *ureolyticus* /*S. warneri*/ *S. simulans*. Antibiotic susceptibility testing showed maximum resistance to ampicillin (89%) followed by cefotaxime (59%) with no resistance to vancomycin. The increasing recognition of pathogenic potential of CoNS and emergence of drug resistance amongst them denotes the need to adopt simple laboratory procedures to identify and understand the diversity of staphylococci isolated from clinical material.

Key words: *Antibiotic susceptibility, clinical isolates, coagulase negative staphylococci, identification*

Coagulase negative staphylococci (CoNS) are increasingly being recognized as significant nosocomial pathogens, partly due to the growing appreciation of this group of organisms as opportunistic pathogens or due to increase in the use of transient or permanent medical devices in seriously ill and immunocompromised patients.[1] In a routine microbiology laboratory *Staphylococcus* is identified by a rapid screening test (coagulase test) and all non *S. aureus* isolates are reported as CoNS.[2,3]

CoNS are one of the most frequent causes of nosocomial infections and are reservoirs of multiple antimicrobial resistant determinants.[4] Identification of CoNS by reference method of Kloos and Schleifer is cumbersome, time consuming and requires expensive reagents which are not routinely available in most of the clinical laboratories.[5] 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, commonly implicated in majority of infections, which can be utilized by most of the laboratories where automated methods are not yet available.

The present study aimed to identify the most prevalent clinical isolates of CoNS by minimum number of tests necessary and sufficient to discriminate between the 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 tests were included to complete the strain identification wherever necessary. Antimicrobial susceptibility profiles of all the isolates were done by agar disk diffusion method.[7]

Materials and Methods

A total of 102 consecutive non-repeat clinically significant CoNS isolates were collected from July 2004 to January 2005 in the Department of microbiology, University College of Medical Sciences and GTB Hospital, New Delhi. Strains were isolated from deep wounds, blood samples, ear swabs, CSF, ascitic fluid, synovial fluid and urine samples. The isolates were considered clinically significant when isolated in pure culture from infected site or body fluid or if the same strain was isolated twice.[1] The strains collected were initially identified by colony morphology, Gram staining, catalase, slide and tube coagulase (read after 4, 24 hours) and anaerobic acid from mannitol. Bacitracin (0.04 U) and furazolidone (100 µg) susceptibilities were done to exclude *Micrococcus* and *Stomatococcus* spp.[5,8]

Identification

All the strains which were either slide or tube coagulase negative were further identified by a scheme developed in our laboratory after reviewing a number of references.[1,3,6,9-11]

The identification scheme concentrated on species

*Corresponding author (email: <renugoyal_123@yahoo.co.in>)
Department of Microbiology, University College of Medical Sciences and GTB Hospital, New Delhi - 110 095, India
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groups/species commonly encountered in clinical practice: the *S. epidermidis* group (i.e., *S. epidermidis*, *S. capitis* subsp. *ureolyticus* and *S. caprae*), the *S. haemolyticus* group (*S. haemolyticus*, *S. auricularis* and *S. caseolyticus*), the *S. saprophyticus* group (*S. saprophyticus* subsp. *saprophyticus* and *S. hominis* subsp. *novobiosepticus*), the *S. warneri* group (*S. warneri* and *S. hominis* subsp. *hominis*), the *S. cohnii* group (*S. xylosus* and *S. cohnii* subsp. *ureolyticum*), *S. lugdunensis, S. shleiferi* subsp. *schleiferi*, *S. capitis* subsp. *capitis, S. simulans* and *S. cohnii* subsp. *cohnii*.[6] This scheme involved a two step procedure (Table 1), first step aimed to identify species group and combined slide and tube coagulase with novobiocin resistance, test for urease activity, ornithine decarboxylase and aerobic acid from mannose. If identification required 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 the *S. haemolyticus* group, trehalose for the *S. saprophyticus* group, anaerobic thioglycollate broth for the *S. warneri* group and xylose for the *S. cohnii* group.[6] All the tests were performed according to reference method.[5]

Antimicrobial susceptibility

The antibiotic susceptibility testing of all the isolates was performed on Mueller-Hinton agar by the standard disk diffusion method as per national committee of clinical laboratory standards.[7]

Results

Thirty-nine out of 102 strains of CoNS (38.2%) were isolated from wound infections, 29 (28.4%) from urine samples, 15 (14.7%) from blood cultures, 11 (10.7%) from catheters, 4 (3.9%) from cerebrospinal fluid and 2 (1.9%) each from synovial fluid and ascitic fluid. Ninety-five of these strains were negative for both clumping factor and tube coagulase and seven strains were slide coagulase positive and tube coagulase negative. None of the strains was slide coagulase negative and tube coagulase positive. (Table 2) shows the CoNS species group/species by the present scheme from various specimens.

The scheme could identify 97% of CoNS isolated from clinical samples by incorporation of one or two additional tests wherever needed. *S. epidermidis* was the most frequent isolate and was identified if ornithine decarboxylase was positive (37 isolates) while ornithine decarboxylase negative isolates required inclusion of trehalose and mannitol for speciation. This scheme could not delineate two ornithine decarboxylase negative isolates as *S. capitis* subsp. *ureolyticus* / *S. simulans* / *S. warneri*.

S. haemolyticus, *S. hominis* subsp. *hominis* were identified with ease after the inclusion of test for acetoin production and anaerobic growth respectively. Only two isolates were unidentified because of aberrant characteristics.

The antibiotic susceptibility pattern revealed no resistance to vancomycin with 89% resistant to ampicillin followed by cefotaxime (59%), cloxacillin (25%), erythromycin (23%), ciprofloxacin (29%) and gentamicin (20%).

Discussion

Many laboratories do not identify clinical isolates of CoNS to the species level as they are considered normal inhabitants of skin and nares capable of causing only opportunistic infections.[1] Moreover, the conventional identification methods, though accurate, are cumbersome and employ a large battery of biochemical reactions, which often give variable results and all the tests are generally not available in most of the routine diagnostic laboratories.[3] The commercial identification methods including automated systems, are uneconomical to use for all the isolates of CoNS. As CoNS is increasingly being implicated as significant nosocomial pathogen several reviewers have emphasized the need for species identification, which is possible only by a simple, easily adaptable, inexpensive method.[1-6] The species identification is important in monitoring the reservoir and distribution of CoNS involved in nosocomial infections and determining the etiological agent.[4,5]

The present scheme conveniently identified the most frequently encountered clinical isolates in our hospital as *S. epidermidis* (41%), *S. haemolyticus* (14.7%), *S. saprophyticus* (16.6%) and *S. hominis* (14.7%) up to species level by incorporation of one or two additional tests. The scheme directly identified 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 give delayed result.[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 pyroglutamyl β napthylamide (PYR) hydrolysis test, a key component for their identification, being expensive, was not included. Though PYR test has been considered crucial for the differentiation of *S. warneri*, *S. capitis* subsp. *ureolyticus* from *S. simulans* (PYR +), a few studies have reported misidentification of *S. warneri* as *S. simulans* if PYR test comes positive, thereby questioning the reliability of PYR.[6] Various workers from India have reported *S. epidermidis* and *S. saprophyticus* to be the most common isolate similar to our study.[9,10,12,13] None of the reports have shown isolation of *S. warneri/ S. simulans* which are probably not common in India. Though a single report has mentioned isolation of *S. cohnii* which was not isolated in our study, however the scheme described here can identify this isolate.[9] Thus, this scheme identified 97% of CoNS upto species level.

Antimicrobial susceptibility testing showed a variable

Table 1: Identification of CONS by simple scheme and additional tests

Group/species	Clumping factor	Tube coagulase	Ornithine decarbo-xylase	Urease	Novobio-cin (5 µg)	Mannose	Species/ Subspecies	Trehalose growth	Mannitol	Acetoin	Lactose	Anaerobic	Xylose
S. epidermidis group	-	-	+	+	S	+	*S. epidermidis*	-	-				
							S. caprae	+	-				
							S. capitis subsp. *ureolyticus*	-	+				
S. haemolyticus group	-	-	-	-	S	-	*S. haemolyticus*			+			
							S. auricularis			-	-		
							S. caseolyticus			-	+		
S. saprophyticus group	-	-	-	+	R	-	*S. saprophyticus* subsp. *saprophyticus*	+					
							S. hominis subsp. *novobiosepticus*	-					
S. warneri group	-	-	-	+	S	-	*S. warneri*					+	
							S. hominis subsp. *hominis*					-	
S. lugdinensis	-	-	+	±	S	+							
S. schleiferi subsp. *schleiferi*	+	-	-	-	S	+							
S. schleiferi subsp. *coagulans*	-	+	-	+	S	+							
S. simulans	-	-	-	+	S	±							
S. capitiis subsp. *capitis*	-	-	-	-	S	+							
S. cohnii subsp. *cohnii*	-	-	-	-	R	±	*S. xylosus*						+
S. cohnii group	-	-	-	+	R	+	*S. cohnii* subsp. *ureolyticum*						-

Table 2: Frequency of clinically significant CoNS

Species	No. (%)	Blood	Catheter	Wound	Urine	CSF	Synovial fluid	Ascitic fluid
S. epidermidis	42 (41)	10	8	11	9	2	1	1
S. saprophyticus	17 (16.6)	-	-	3	14	-	-	-
S. haemolyticus	15 (14.7)	4	2	7	1	1	-	-
S. hominis	15 (14.7)	-	-	12	2	1	-	-
S. lugdunensis	5 (4.9)	1	1	2	1	-	-	-
S. schleiferi	2 (1.9)	-	-	1	1	-	-	-
S. capitis	2 (1.9)	-	-	1	-	-	1	-
S. capitis subsp.*uroelyticus/*								
S. warneri/S. simulans	2 (1.9)	-	-	1	-	-	-	-
Unidentified	2 (1.9)	-	-	1	-	-	-	1
Total	**102**	**15**	**11**	**39**	**29**	**4**	**2**	**2**

sensitivity and resistance patterns, similar to earlier reports.[2,9,10,12,13] It is therefore recommended to assess the importance of CoNS, speciate the clinically relevant CoNS to whatever level possible and perform the antibiotic susceptibility testing before any typing procedure for epidemiological studies are undertaken. This simple, inexpensive methodology will prove useful in a routine microbiology laboratory for the presumptive identification of CoNS. Further studies are needed with well characterized strains for the evaluation of the present scheme.

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