Research Article

Phenotypic characterization of coagulase negative staphylococci from various clinical isolates

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Abstract

Background: Despite their frequency as contaminants, coagulase-negative staphylococci (CNS) have become important nosocomial pathogens, accounting for 9% of all nosocomial infections. These infections are difficult to treat because of the risk factors and the multiple drug resistance shown by these organisms. **Material and Methods:** One hundred and forty CNS were isolated from various clinical samples like blood, pus, urine body fluids, urine, catheter tip, gastric lavage and wound swab. After confirming the isolates as CNS, species level identification was performed by simple, nonexpensive conventional methods and antibiotic sensitivity testing was also carried out. **Result:** 140 isolates could be identified to species level. Among these 140 identified CNS isolates, *S. saprophyticus* was the most frequently isolated 58 (41.43%) from various clinical sample, followed by *S. epidermidis* 45 (32.14%), *S. lugdunensis* 23 (16.43%), *S. hemolyticus* 11 (7.86%), *S. schleiferi* 3 (2.14%). In the present study, the susceptibility pattern of CNS species against antimicrobial agents showed that 65.71 % of the isolates were Methicillin Sensitive (MSCNS). Majority of the CNS species were sensitive to Amikacin, (94.29%), Nitrofurantoin (85.71%), Tetracycline (75.71%), and clindamycin (75%). None of the CNS species showed resistance to vancomycin and Linezolid. **Conclusion:** The increased pathogenic potential and multiple-drug resistance demonstrates the need to adopt simple, reliable and non-expensive methods for identifying and determining the antibiotic sensitivity of CNS.
Keywords: Antibiotic sensitivity test, CNS, *S. saprophyticus, S. epidermidis,* MRCNS

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INTRODUCTION

Coagulase-negative Staphylococci (CNS), which are the normal skin flora, have emerged as predominant pathogens in hospital-acquired infections[1]. The clinical significance of species other than *S. epidermidis* has been increasingly recognized in the recent years[2]. They are rarely significant when isolated from skin, sputum and nasal swabs but may well be significant when isolated from wound swabs, pus, body fluids or blood cultures especially if foreign material is present[3]. Distinguishing clinically significant pathogenic strains from contaminant strains are one of the major challenges faced by clinical microbiologists[3]. It is important to study the clinical significance of the CNS isolate in a given clinical situation in view of deciding its pathogenic role. The present study was carried out to identify the prevalent species of CNS and their antibiogram.

MATERIALS AND METHODS

The present study was performed after obtaining the Institutional Ethics Committee clearance. A total of 140 non-repeat CNS were isolated and studied over a period of 2 year 1 month from November 2010 to December 2012 from different clinically significant specimens such as blood, pus, urine, body fluids, urine catheter tip and gastric lavage, wound swab, received from patients of all ages and any sex .The isolates were considered clinically significant when isolated in pure culture repeatedly. The isolates were identified by colony morphology, Gram stain, catalase test and coagulase test (slide and tube coagulase), susceptible to Furazolidone (100µg) and resistant to Bacitracin. Bacitracin susceptibility was performed to exclude Micrococcus and Stomatococcus species[4]. The conventional tests that are simple, easy to perform and non-expensive were chosen from the scheme of Koneman *et al*, 6TH edition. These include the heat stable nucleases, ornithin decarboxylase test, PYR, acetoin, urease test, beta-galactosidase test, alkaline phosphatase test, and fermentation of glucose, sucrose, maltose, mannose, mannitol, and trehalose. Susceptibility to novobiocin and polymyxin B was performed as per the standard procedure[5]. The antibiotic sensitivity testing was performed on Muller-Hinton agar by the Kirby-Bauer disc diffusion method[6] using a panel of antibiotics, which includes ampicillin (A), amikacin(AK), cotrimoxazole (Co), cefotaxime (Ce), cefazolin, cefoxitin, cephelexin, chloramphenicol, ciprofloxacin, clindmycin, erythromycin, linezolid, nitrofurantoin, tetracycline, gentamicin (G), and vancomycin (Va). Cefoxitin was used to detect methicillin-resistance.

OBSERVATION AND RESULTS

Of the 140 CNS isolates, 77 (55%) were from urine samples, 43 (30.71%) from pus samples, 7 (05%) from blood culture, 6 (4.29%) from wound swab, 3 (2.14%) from ear swab 1 (0.71%) from pleural fluid and one each from the tip of central line, fluid from blister, vaginal swab. **(Table 1)**

Table 1: Distribution of 140 isolates in different clinical sample

Sr. No.	Specimen	CNS (%)
1	Urine	77(55%)
2	Pus	43(30.71%)
3	Blood culture	7(05%)
5	Pleural fluid	1(0.71%)
6	Ear swab	3(2.14%)
7	Wound swab	6(4.29%)
8	Tip of Central Line	1(0.71%)
9	Fluid from blister	1(0.71%)
10	Vaginal swab	1(0.71%)
	Total	**140**

The simple conventional methods identified 140 CNS isolates to species level. Among these 140 identified CNS isolates, *S. saprophyticus* was the most frequently isolated 58 (41.43%) from various clinical samples, followed by *S. epidermidis 45* (32.14%), *S. lugdunensis 23* (16.43%), *S. hemolyticus* 11 (7.86%), *S. schleiferi* 3 (2.14%), Table 2 shows the species-wise distribution of CNS in the different clinical specimens. Urinary tract infection were most commonly due to *S. saprophyticus* (74.03%), followed by *S. epidermidis* (10.39%), *S hemolyticus* (10.39%) *S. lugdunensis* (2.60%), *S. schleiferi* (2.60%) Abscesses were mainly due to *s.epidermidis* (55.81%) *followed by s. lugdunensis* (39.53%), *s.hemolyticus* (2.33%), and *s, schleiferi* (2.33%), Bacteremia was caused most commonly by *s. epidermidis* (85.71%) followed by *s. hemolyticus* (14.29%). **(Table 2)**

Table 2: Frequency of species of CNS in various clinical specimens

Sr. No.	CONS Species	Urine (%)	Pus (%)	Blood culture (%)	Pleural fluid (%)	Ear swab (%)	Wound swab (%)	Tip of Central Line (%)	Fluid from blister (%)	Vaginal swab	Total
1	S.Sapro.	57 (74.03)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1(100)	58
2	S.Epider.	8 (10.39)	24 (55.81)	6 (85.71)	1 (100)	1 (33.33)	3 (50)	1 (100)	1 (100)	0(00)	45
3	S.Lugdunensis	2 (2.60)	17(39.53)	0 (0)	0 (0)	2 (66.67)	2 (33.33)	0 (0)	0 (0)	0 (0)	23
4	S.Hemolyticus	8 (10.39)	1 (2.33)	1 (14.29)	0 (0)	0 (0)	1 (16.67)	0 (0)	0 (0)	0 (0)	11
5	S.Schleiferi	2 (2.60)	1 (2.33)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3
	Total	**77**	**43**	**7**	**1**	**3**	**6**	**1**	**1**	**1**	**140**

The incidence of CNS was high among females (60%) compared with males (40%). In the present study, the usceptibility pattern of CNS species against antimicrobial agents showed that 65.71 % of the isolates were MSCNS. Majority of the CNS species were sensitive to Amikacin, (94.29%), Nitrofurantoin (85.71%), Tetracycline (75.71%), and clindamycin (75%). Majority of the CNS species were resistant to penicillin (95.83%), erythromycin (77.08%), ampicillin (72.92%), cotrimoxazole (62.50%), clindamycin (56.25%). None of the CNS species showed resistance to vancomycin. and linezolid.

DISCUSSION

Coagulase negative staphylococci (CNS) were generally regarded to be the contaminants, having little clinical significance in the past[7]. CNS are now recognized as a major cause of nosocomial infections in critically ill patients especially in intensive care units, that leads to morbidity and even mortality[8]. Because there is increasing pathogenecity of these organisms, CNS should be identified to the species level by simple, reliable and preferably inexpensive methods[9]. The overall incidence of clinically significant CNS among all the isolates from various clinical samples received in the study period was found to be 6.9 % in our study. The majority of CNS isolates were from urine (55%) and pus (30.71%).The CNS infection was more common in females (60%) than in males (40%) in the present study, which is shown in another study[14] as well as our study. In present study, the commonest species isolated in clinically significant CNS was *S. saprophyticus* (41.43%) followed by *S. epidermidis* (32.14%) isolates amongst CNS. The other species isolated were *S. lugdunensis* (16.43%), followed by *S.hemolyticus* (7.86%) and *S. shleiferi* (2.14%). In our study, the most commonly isolated species was *S. Saprophyticus* (41.43%) followed by S. Epidermidis

(32.14%). Similar results were seen in other studies as shown by Nord *et al*[10] and John J F *et al*[11]. As majority of samples included in our study were urine specimens and *S. saprophyticus* is the commonest cause of UTI. *S. saprophyticus* is determined to be the true urinary tract pathogen, which is found to be the second most common cause of urinary tract infection after *E. coli* in females[12]. The reasons for the association of *S. saprophyticus* with urinary tract infections in young women remain unclear, but may relate to carriage of the organism in the rectum or introitus[13]. The present study revealed that, urinary tract infections by CNS were most commonly due to *S. saprophyticus* (74.03%) followed by *S. epidermidis* (10.39%), *S. hemolyticus* (10.39%), *S. lugdunensis* (2.60%) and *S. schleiferi* (2.60%), **(Table 2).** The results are similar to study of Nord *et al*. The incidence of methicillin resistance was 56% in this study, and many other studies have documented a still higher resistance[14]. None of the isolates showed resistance to vancomycin in our study. However, others have noted a reduced susceptibility to vancomycin[15].

CONCLUSIONS

Recently, CNS have emerged as a potential pathogen, especially CNS are now recognized as a major cause of nosocomial infections in critically ill patients especially in intensive care units[16]. There is also an increased resistance among these isolates and glycopeptides have become the drug of choice in the treatment of these infections. Hence, there is a need for accurate identification of these isolates to a species level and their antibiotic sensitivity pattern to avoid decreased susceptibility to glycopeptides. In this study, the most common species identified was *S. saprophyticus.* Resistance to ampicillin and amoxyclav was high and none of the isolates showed resistance to vancomycin. All the methicillin resistant and methicillin sensitive strains of CNS were susceptible to Linezolid and vancomycin.

REFERENCES

1. Badwi JA, Memon AH, Soomro AA. Coagulase Negative Staphylococci (CONS) is the contaminant in the clinical specimen. Med Channel 2012; 19:23-7.
2. Makki AR, Sharma S, Duggirala A, Prashanth K, Garg P, Das T. Phenotypic and Genotypic Characterization of Coagulase Negative Staphylococci (CoNS) Other than Staphylococcus *epidermidis* Isolated from Ocular Infections. Invest Ophthalmol and VisSci 2011; 52:9018-22.
3. Alcaraz LE, Satoress SE, Lucero RM, Puig de Centorbi ON. Species identification, slime production and oxacillin susceptibility in coagulase–negative staphylococci isolated from nosocomial specimens. Braz J Microbiol; 2003, 34; 45-51
4. Singh S, Banerjee G, Agarwal SK, Kumar M, Singh RK. Simple method for speciation of clinically significant Coagulase Negative Staphylococci and its antibiotic sensitivity/resistant pattern in NICU of tertiary care centre. Biomed Res 2008; 19:97-101.
5. Koneman EW, AllenSD, Janda WM, Schreckenberger PC, Winn WC. The Gram Positive Cocci: Part 1: Staphylococci and related organisms. In: Colour atlas and textbook of Diagnostic Microbiology. 6th edition. Lippincott, Philadelphia, New York, 1997: 539— 576.
6. Miles RS, Amyes SG.Laboratory control of antimicrobial therapy. In:Collee JG, Fraser AG, Marmion BP, Simmons A, editors. Mackie and Mc Cartney practical Medical Microbiology. 14th ed. New York:Churchill Livingston;1996. p. 151-78.
7. *Winn WC, Allen SD, Janda WM, Koneman EW, Procop GW, Schreckenberger PC, Woods GL. Gram positive cocci: Staphylococci and related gram positive cocci. In Koneman's colour atlas and textbook of diagnostic microbiology. 6th ed, Lippincott Williams and Wilkins, 2006; p 624-73.*
8. Huang SY, Tang RB, Chen SJ, Chung RL. Coagulase negative staphylococcal bacteremia in critically ill children: risk factors and antimicrobial susceptibility. J Microbiol Immunol. Infect, 2003; 36: 51-55.
9. Ieven M, Verhoeven J, Pattyn SR, Goossens H. Rapid and Economical Method for Species Identification of Clinically Significant Coagulase-Negative Staphylococci. J Clin Microbiol 1995;33:1060-3
10. Nord CA,S Holta-Oie, Characterisation of coagulase-negative staphylococcal species from human infections;Zenalbi,Bacteriol,Parisit,Enkd,indektionskz,Hyg.Abt;I, suppl.5;105-111
11. Mohan U, Jindal N, Aggarwal P. Species distribution and antibiotic sensitivity pattern of coagulase negative staphylococci isolated from various clinical specimens. Indian J Med Microbiol 2002;20:45-6
12. Winn WC, Allen SD, Janda WM, Koneman EW, Procop GW, Schreckenberger PC, Woods GL. Gram positive cocci: Staphylococci and related gram positive cocci. In Koneman's colour atlas and textbook of diagnostic microbiology. 6th ed, Lippincott Williams and Wilkins, 2006; p 624-73
13. Sewell CM. Coagulase-Negative Staphylococci and the Clinical Microbiology Laboratory, Eur. J. Clin. Microbiol, 1984; 3: 94-95.
14. Kleeman KT, Bannerman TL, Kloos WE. Species distribution of coagulase negative staphylococcal isolates at a community hospital and implications for selection of staphylococcal identification procedures. J Clin Microbiol, 1993; 31: 1318-21.
15. Diekema DJ, Pfaller MA, Schmitz FJ, Smayevsky J, Bell J, Jones RN*, et al*. Survey of Infections Due to StaphylococcusSpecies: Frequency of Occurrenceand Antimicrobial Susceptibility of IsolatesCollected in the United States, Canada, LatinAmerica, Europe, and the Western Pacific Region for the SENTRY Antimicrobial Surveillance Program,1997-1999. Clin Infect Dis 2001;32(suppl2):S114-32
16. Huang SY, Tang RB, Chen SJ, Chung RL. Coagulase negative staphylococcal bacteremia in critically ill children: risk factors and antimicrobial susceptibility. J Microbiol Immunol. Infect, 2003; 36: 51-55.