

PREVALENCE, SPECIATION AND ANTIBIOTIC RESISTANCE PROFILES OF COAGULASE NEGATIVE STAPHYLOCOCCI ISOLATES FROM CLINICAL SAMPLES

Geetanjali Tupakula¹, R. Kondal Rao², G. Ravindranadh³, GVK Varshith⁴

¹Assistant Professor, Department of Microbiology, ACSR Government Medical College, Nellore.

²Professor and HOD, Department of Microbiology, Kakatiya Medical College, Warangal.

³Professor and HOD, Department of Anatomy, NRIIMS, Visakhapatnam.

⁴Student, Oakridge International School.

ABSTRACT

BACKGROUND

Coagulase-Negative Staphylococci (CONS) are opportunistic pathogens and have become major cause of nosocomial infections. The present study was undertaken to speciate the clinical isolates of CONS and study their antibiogram.

MATERIALS AND METHODS

Samples were collected from various sources under aseptic conditions based on standard guidelines from patients of surgical, medical, orthopaedic wards, MGM Hospital, Warangal and its satellite hospitals. Total 210 samples were collected and the prevalence of CONS by cultural and biochemical methods was studied. Antibiotic susceptibility patterns were determined by Kirby-Bauer disc diffusion method as per CLSI guidelines. Speciation of CONS was done by slide using tube coagulase test, ornithine decarboxylase test, urease activity, trehalose fermentation, mannitol fermentation and novobiocin resistance test.

RESULTS

Out of 210 samples tested, 100 (47.61%) were CONS and majority of the CONS isolates were hospital acquired infections (68%; n = 68). Highest number of CONS isolates were observed in catheters (32/42) followed by wound (38/68), blood samples (14/39), urine (15/55) and CSF (1/6). Among the 100 CONS isolates, *S. epidermidis* was the commonest species isolated found in 48% (n = 48), followed by *S. saprophyticus* (20%; n = 20), *S. haemolyticus* (15%; n = 15), *S. hominis* (14%; n = 14) and *S. caprae* (3%; n = 3). Antibiotic susceptibility testing showed the maximum number of isolates was resistant to amoxicillin (70%), cotrimoxazole (65%), ciprofloxacin (61%) and penicillin (53%), while majority of the CONS isolates were methicillin sensitive (81%) and vancomycin sensitive (69%).

CONCLUSION

The results of the present study reiterate the need for proper species identification and antibiotic susceptibility patterns for proper management of nosocomial infections caused by CONS and also for epidemiological purpose.

KEYWORDS

Coagulase-Negative Staphylococci, Speciation, Antibiogram, Antibiotic Susceptibility.

HOW TO CITE THIS ARTICLE: Tupakula G, Rao RK, Ravindranadh G, et al. Prevalence, speciation and antibiotic resistance profiles of coagulase negative staphylococci isolates from clinical samples. J. Evolution Med. Dent. Sci. 2017;6(26):2134-2136, DOI: 10.14260/Jemds/2017/463

BACKGROUND

The importance of Coagulase Negative Staphylococci (CONS) in causing human infection is well documented. Coagulase negative staphylococci are important causative agents of 10% of pyogenic infections in hospitals which include a range of infections like surgical wound sepsis, bacteraemia, Native Valve Endocarditis (NVE) and prosthetic valve endocarditis, osteomyelitis, pyoarthritis, peritonitis, mediastinitis, prostatitis, infection of vascular grafts and pacemakers, infective intravascular catheters, cerebrospinal fluid shunts, orthopaedic devices and urinary tract infections. Normally commensals on the skin and mucous membrane, coagulase negative staphylococci become opportunistic pathogens in

conditions of lowered resistance in the local area. As this organism occupies more than 10% of hospital infections, antibiotic sensitivity pattern also showed a change and increased degree of antibiotic resistance is being documented. As there are a number of species of coagulase negative staphylococci after their isolation from various specimens received, they were speciated and their antibiogram was studied. Since most of the infections caused by coagulase negative staphylococci are hospital associated, it is extremely relevant to study the resistance pattern so that it would serve as a useful guide to the healthcare providers. Hence, the study attempted to speciate the clinical isolates of coagulase negative staphylococci and to study their antibiogram.

MATERIALS AND METHODS

The samples were collected from outpatients and inpatients of surgical, medical, orthopaedic wards, MGM Hospital, Warangal and its satellite hospitals were included in the present study; 210 samples were collected from infected postoperative cases, infected burns, infected wounds from traumatology unit, diabetic foot, gangrene, non-healing

Financial or Other, Competing Interest: None.

Submission 07-02-2017, Peer Review 18-03-2017,

Acceptance 24-03-2017, Published 30-03-2017.

Corresponding Author:

Dr. Geetanjali Tupakula,

H. No. 24-3-357, Sujathamma Colony,

Main Road, A. K. Nagar, Nellore-524004.

E-mail: drgeeta1129@gmail.com

DOI: 10.14260/jemds/2017/463



ulcers, pyoderma and impetigo, patients with intravenous cannulae were chosen as subjects of study after obtaining approval from the Institutional Ethics Committee and written informed consent was taken from the patients. The comorbid, immunosuppressed patients, patients with Diabetes mellitus, patients with Malnutrition and on Steroid therapy, patients with Hypoproteinaemia, patients with Malignancies and on Antimalignancy drugs was completely excluded.

Serous, serosanguinous or purulent discharge from the ulcers or wounds was collected with sterile swabs from the base of the lesions without touching the surrounding area of skin. In case of spreading lesions of skin and subcutaneous tissue (such as progressive gangrene), the material was collected from the active margins of the lesions rather than from central portion. When the exudate was minimal, gentle pressure was applied at the base of the lesion and the expressed discharge was collected with the swab. Care was taken to avoid topical application of any antibiotics at least 24 hrs. before collection of the sample. Two swabs were collected from each patient, one for making smears and another for culture. Swabs sterilised by autoclaving were used to collect specimens in preference to swabs sterilised by hot air oven.

The samples were processed as per the standard reference procedures (Bailey and Scott 2007).¹ Gram positive cluster forming staphylococci which are catalase positive, oxidase negative, bacitracin resistant, furazolidone sensitive and fermentative by the oxidation fermentation test were identified as staphylococcal. The staphylococci strains were subjected to slide and tube coagulase test and those strains which were negative by both methods were identified as Coagulase Negative Staphylococci (CONS). The identification of all the species of CONS isolated was done by using various standard biochemical tests. Antibigram of isolates was performed using Kirby-Bauer method (Clinical and Laboratory Standards Institute (CLSI) guidelines).²

RESULTS

Total Number of Test Samples 210	Coagulase Negative Staphylococci (CONS)	
	Number	Percentage
	100	47.61

Table 1. Number and Percentage of Coagulase Negative Staphylococci Isolates in the Present Study

Table 1 shows number and percentage of CONS isolates in the present study; out of 210 samples tested, 100 (47.61%) of CONS are isolated.

Sl. No.	Name of the Species	Number	Percentage
1.	S. epidermidis	48	48
2.	S. saprophyticus	20	20
3.	S. haemolyticus	15	15
4.	S. hominis	14	14
5.	S. caprae	3	3

Table 2. Number and Percentage of Coagulase Negative Staphylococci Species found in the 100 Isolates

Table 2 shows speciation of Coagulase negative staphylococci isolates. Out of 100 isolates of Coagulase negative staphylococci, 48 (48%) isolates were identified as

S. epidermidis; 20 (20%) were identified as S. saprophyticus; 15 (15%) were identified as S. haemolyticus; 14 (14%) were identified as S. hominis; 3(3%) were identified as S. caprae.

Sl. No.	Antibiotic	Resistant		Sensitive	
		No.	%	No.	%
1.	Penicillin	42	61.76	26	38.23
2.	Oxacillin	23	33.82	45	66.17
3.	Ciprofloxacin	48	70.58	20	29.41
4.	Vancomycin	16	23.52	52	76.47
5.	Cotrimoxazole	59	86.76	9	13.23
6.	Amoxicillin	56	82.35	12	17.64

Table 3. Antibigram of Coagulase negative Staphylococci under Study

DISCUSSION

In the present study (Table 1), 100 CONS isolates were obtained by processing 210 clinical samples (47.61%). These results indicate the prominent place occupied by CONS in infection and more so in the hospital infections.

The percentage isolation of CONS from clinical samples is not available in similar studies; Shoba et al 2005 collected 205 swabs from the various places in the hospital and from healthy hospital staff.³ They obtained a percentage of 31.7%; surprisingly, all the staphylococcal isolates in their study were CONS. This study represents the magnitude of the hospital source, from which infection can be transmitted to vulnerable patients through various invasive procedures if proper care and aseptic precautions are not taken.

The predominant species of CONS in present study was S. epidermidis (Table 2); 48 strains of S. epidermidis were isolated. S. epidermidis was the most commonly encountered species among the CONS. Its prevalence as nosocomial pathogen was very much related to medical procedures and practices than the capacity of the organism to establish infection (Kloos WE and Bannerman TL).⁴ Carlos et al identified an endemic strain of S. epidermidis in the hospital producing bacteraemia in the neonatal intensive care unit.⁵ S. epidermidis is a notorious slime producer and easily establishes biofilm on polymers within which the organism can limit the effectiveness of antibiotic therapy and multiplies further as reported by Shoba et al 2005. Staphylococcus epidermidis is prevalent in 49.23% of hospital sites including the skin of the healthcare providers; 14% of these strains were oxacillin resistant, thus S. epidermidis can be termed as an important hospital pathogen and hospital infection control programs should include eradication of this organism from the hospital sites.

20% of CONS isolates were identified as S. saprophyticus (Table 2). This organism unlike S. epidermidis was more a member of the community. It is a common organism isolated from urine from community acquired urinary infections in young sexually active females. Establishment of S. saprophyticus as pathogen requires repeated careful processing and quantitative urine cultures as the organism inhabits the normal urethra and perineal skin, it is likely to contaminate urine samples during collection. S. saprophyticus has high capacity to adhere and colonise on surfaces, but unlike S. epidermidis it is a poor producer of slime (Kleeman KT et al).⁶

In the present study, 15% of isolates were identified as S. haemolyticus (Table 2). S. haemolyticus is also part of the

human normal skin flora. It has been documented as a cause of nosocomial bacteremias. Vancomycin resistance has been reported in this organism as well as multidrug resistance. The presence of multiple antibiotic resistant *S. haemolyticus* in the hospital environment and transmission of resistant clones through the hands of healthcare workers have been documented by several investigators using molecular methods (Kloos WE and George CG).⁷ Compared to *S. epidermidis* this organism colonises in much fewer numbers and less commonly incorporated in clinical illness.

S. hominis isolates were 14% among the total CONS isolated in the present study. Among the 14 strains 9 were from wounds, 2 strains each from blood and urine and 1 from catheter. This species is a commensal of the skin of humans and has occasionally been isolated from infections as a low-grade pathogen; however, under antibiotic pressure it is known to develop resistance more easily and readily (Kloos WE and Musselwhite MS).⁸

S. caprae strains (3%) occupied least importance among the total CONS isolates in the present study (Table 2); among them 2 are from wound and 1 from urine. *S. caprae* is rarely cultured from clinical specimen when compared to *S. epidermidis*. It has been reported in association with bone and joint infection (Banerjee SN et al and Richards MJ et al).^{9,10} Strains of *S. caprae* isolated from humans are known to contain a 5 gene *ica* operon that code for the gene products in biofilm formation. The gene products exhibit subtotal amino acid identity with those of *S. epidermidis*.

The antibiogram of Coagulase negative staphylococci (Table 3) under study shows that out of the 68 isolates of CONS 26 (38.23%) were sensitive to Penicillin and 42 (61.76%) were resistant; 45 (66.17%) were sensitive to Oxacillin and 23 (33.82%) were resistant; 20 (29.41%) were sensitive to Ciprofloxacin and 48 (70.58%) were resistant; 52 (76.47%) were sensitive to Vancomycin and 16 (23.52%) were resistant; 9 (13.23%) were sensitive to Cotrimoxazole and 59 (86.76%) were resistant; 12 (17.64%) were sensitive to Amoxicillin and 56 (82.35%) were resistant.

CONCLUSION

In the present study, Coagulase negative Staphylococci were the most predominant organisms (210/100; 47.61%) in various clinical samples and thus Coagulase Negative Staphylococci have been increasingly gaining importance in hospital infections compared to *E. coli*, *Klebsiella*, MRSA and *Pseudomonas*. Majority of the Isolates were from IV catheters (76.19%) stressing the need for more aseptic precautions in ICU settings. Most predominant species of CONS in this study was *S. epidermidis* followed by *S. saprophyticus*. Among the 68 isolates of CONS Penicillin resistance is 61.76%, Oxacillin

resistance is 33.82%, Ciprofloxacin resistance is 70.58%, Vancomycin resistance is 23.52%, Cotrimoxazole resistance is 86.72% and Amoxicillin resistance is 82.35%. Hence, Vancomycin is the drug of choice for the treatment of nosocomial infections caused by CONS and restricted and choicest use of this antibiotic can greatly reduce the risk of acquisition of resistance by the organism.

REFERENCES

- [1] Forbes BAA, Sahm DF, Weissfeld AS. Bailey & Scott's diagnostic microbiology. 12th edn. Elsevier 2007:217-34.
- [2] Bauer AW, Kirby WM, Sherris JC, et al. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol* 1966;45(4):493-6.
- [3] Shobha KL, Rao PS, Thomas J. Survey of staphylococcus isolates among hospital personnel, environment and their antibiogram with special emphasis on methicillin resistance. *Indian J Med Microbiol* 2005;23(3):186-8.
- [4] Kloos WE, Bannerman TL. Update on clinical significance of coagulase-negative staphylococci. *Clin Microbiol Rev* 1994;7(1):117-40.
- [5] Carlos CC, Ringertz S, Rylander M, et al. Nosocomial staphylococcus epidermidis septicaemia among very low birth weight neonates in an intensive care unit. *J Hosp Infect* 1991;19(3):201-7.
- [6] 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(5):1318-21.
- [7] Kloos WE, George CG. Identification of staphylococcus species and subspecies with the MicroScan Pos ID and rapid Pos ID panel systems. *J Clin Microbiol* 1991;29(4):738-44.
- [8] Kloos WE, Musselwhite MS. Distribution and persistence of staphylococcus and micrococcus species and other aerobic bacteria on human skin. *Applied Microbiology* 1975;30(3):381-95.
- [9] Banerjee SN, Emori TG, Culver DH, et al. Secular trends in nosocomial primary bloodstream infections in United States, 1980-1989. National nosocomial infection surveillance system. *Am J Med* 1991;91(3B):86S-9S.
- [10] Richards MJ, Edwards JR, Culver DH, et al. Nosocomial infections in combined medical-surgical intensive care units in the United States. *Infect Control Hosp Epidemiol* 2000;21(8):510-5.