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SPECIATION OF COAGULASE NEGATIVE STAHYLOCOCCI FROM CLINICAL SAMPLES

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Aabstract–The main objective of the study to speciate Coagulase negative staphylococci and to determine antibiotic susceptibility from various clinical specimens. A total of 175 clinically significant Coagulase negative Staphylococci were isolated from clinical specimens. CoNS were defined based on colony morphology, Gram staining, positive catalase reaction, and the absence of tube coagulase test. Isolated CoNS were subjected to biochemical tests to speciate. Antimicrobial susceptibility of isolates was performed by Kirby-Bauer's Disk diffusion method. Pus was the specimen yielded higher number of CONS and accounted for 47 (26%) followed by blood 41 (23%). Among isolated CONS, *Staphylococcus epidermidis* was found to be more and accounted for 44.58%. Methicillin resistant coagulase negative Staphylococci (MRCONS) were often isolated from pus (44%) and blood (43%) specimens followed by sputum (36%). The present study indicated that, Coagulase negative Staphylococci were found to be pathogenic and produced signs and symptoms of infection. Appropriate antimicrobial therapy should be instituted early to prevent further complications of infection.

INTRODUCTION

Coagulase negative staphylococci (CoNS) are unlike group of Gram-positive cocci, usually exist as normal flora of skin (Gautam *et al*., 2017). Previously , when isolated from clinical specimens, CoNS were often discarded as contaminants. However, this has changed over the last decade with CoNS emerging as etiological agents especially in causing hospital acquired infections. (Singh *et al*., 2016)

More than 30 species of CoNS have been recognized, but only a few have been recognized as pathogens. Infections caused by CoNS, can be caused by either community or hospital acquired isolates (Melzer *et al*., *1999). The predominant Coagulase negative Staphylococci of humans are S. epidermidis, widely distributed over the body surface. At the same time, S. epidermidis is by far the most frequently recovered organism in bacteremia cases, accounting for 50% to over 80% of isolates. Other commonly implicated species include S. haemolyticus, S. lugdunensis, S. capitis, S. warneri, S. hominis, S. simulans, and S. saprophyticus* (Winn *et al*., 2006). These organisms express resistance to multiple antibiotics which not only pose a serious therapeutic problem but also serve as a hospital reservoir of antibiotic resistance genes. An increase in incidence of nosocomial infections caused by CNS which are resistant to methicillin (MRCNS) and other antibiotics has been reported in some of the studies (Gahrn-Hansen *et al*., 1987; Kloss,1994).

Hence, the present study was undertaken to speciate Coagulase negative staphylococci and to determine antibiotic susceptibility from various clinical specimens in a tertiary care teaching hospital.

MATERIALS AND METHODS

The present descriptive study was conducted in the department of microbiology , Vinayaka missions medical college and hospital during January to October 2017. Clinical specimens were processed as per standard protocol A total of 175 clinically significant Coagulase negative Staphylococci were isolated from clinical specimens. CoNS were defined based on colony morphology, Gram staining, positive catalase reaction, and the absence of tube coagulase test.

Speciation: Isolated CoNS were subjected to different tests such as, phosphatase, arginine hydrolysis, sugar (trehalose and maltose) fermentation and novobiocin susceptibility, to identify them till species level. For phosphatase test, one loopful of the 24 hours isolates obtained from the phosphate agar was touched onto a filter paper which was saturated with 1N NaOH. Formation of a pink colour indicated a positive result. For arginine hydrolysis test, colour change of inoculated arginine broth from yellow to purple, after 24 to 48 hours, indicated a positive result. For sugar fermentation test, sterile peptone broth which contained phenol red and respective fermentable sugars *viz*., trehalose and maltose was prepared. The tubes were inoculated with the isolates, they were incubated at 37 °C for 24–28 hours, and observed for acid production. Novobiocin susceptibility of the isolates was done on sterile Muller Hinton agar plates by using standard procedures (Priya *et al*., 2014).

Antibiotic susceptibility testing

To evaluate antimicrobial susceptibility of isolates Kirby-Bauer's Disk diffusion method was done according Clinical Laboratory and Standards Institute (CLSI; formerly National Committee for Clinical Laboratory Standards) criteria (CLSI, 2007).

Detection of Methicillin resistant Coagulase negative Staphylococci: The Cefoxitin disc diffusion method was carried out on Mueller-Hinton agar by using a 30 µg cefoxitin disc. An inhibition zone diameter of ≤ 21 mm was reported as methicillin resistant and a diameter of ≥ 22 mm was considered as methicillin sensitive 8 (CLSI, 2007).

Inclusion criteria: Urine- ≥ 10^5 CFU/mL and absence of other recognized bacterial pathogens

Non urinary-Growth 1+ and single bacterial morphotype of CoNS and absence of other recognized bacterial pathogens. Growth greater than 2 + and absence of other recognized bacterial pathogens (York, 2004).

RESULTS

A total of 175 CONS were isolated from various specimens. Pus was the specimen yielded higher number of CONS and accounted for 47(26%) followed by blood 41(23%). Among isolated CONS, Staphylococcus epidermidis was found to be more and accounted for 44.58%.

Percentage of CONS from clinical samples (Table 1)

Specimen	No. of CONS (Percentage)
Pus	47(26%)
Blood	41 (23%)
Urine	29 (16%)
Sputum	19 (10%)
Eye	18 (10%)
Body fluids	12 (6%)
Ear	9 (5%)
Total	175

S.epidermidis was frequently isolated from pus and blood specimens and accounted for 29% and 26% respectively. *S.hemolyticus* found to the most common species from blood (44%). Only two species recovered from urine samples *S.saprophyticus* (77%). was frequently isolated from urine specimens. Other species was *S.epdermidis* (22%). *S.ludgunensis* (50%) found to second common pathogen from pus. Methicillin resistant coagulase negative Staphylococci (MRCONS) were often isolated from pus (44%) and blood (43%) specimens followed by sputum(36%).

In the present study, methicillin resistance was observed frequently in *S. hemolyticus* (10/18) and accounted for 55%. No methicillin resistance was observed in *S.warneri*. All isolated species were found to be susceptible to vancomycin(100%) and teicoplanin(100%).

DISCUSSION

In the present study, seven distinct species of coagulase negative Stahylococci were isolated. Among isolated CONS, Staphylococcus epidermidis was found to be common and accounted for 44.58%. *S. ludgunensis* and *S.saprohyticus* were second most common CONS isolated and accounted for 12.57 % each species. S.hemolyticus was found to be 10.29%. But as per the study conducted by Sharma et al , five distinct species were isolated, *S. epidermidis, S.haemolyticus, S. saprophyticus, S. warneri* and *S. cohnii. S. epidermidis* was the commonest (54%) followed by *S. haemolyticus* (40%) (Sharma *et al*., 2010).

In our study *S.epidermidis* (23/78, 29%) was frequently isolated from pus samples. According to the study conducted by Chavan *et al*., (2017), abscesses and wound infections were most commonly caused due to *S. lugdunensis* (36.6%) followed by *S. epidermidis* (32.7%) and *S.*

Table 2. Speciation and percentage of Coagulase negative Staphylococci from clinical samples

CONS	Pus	Blood	Urine	Sputum	Eye	Body fluid	Ear
S.epidermidis (78)	23 (29%)	21 (26%)	5(6%)	9(11%)	5(6%)	10 (12%)	5 (6%)
S.hemolyticus (18)	3 (16%)	8 (44%)	-	1(5%)	6 (33%)	-	-
S.saprophyticus (22)	5(22%)	-	17 (77%)	-	-	-	-
S.ludgunensis (22)	11(50%)	3 (13%)	1(4%)	5(22%)	-	-	2 (9%)
S.hominis (14)	1(7%)	4 (28%)	-	1 (7%)	7 (50%)	1(7%)	-
S.capitis (15)	2 (13%)	5 (33%)	5 (33%)	1(6%)	-	-	2 (13%)
S.warneri (6)	2(33%)	-	1(16%)	2 (33%)	-	1 (16%)	-
Total	47	41	29	19	18	12	9
MRCONS	21(44%)	18(43%)	7(24%)	7(36%)	5(27%)	1(8%)	-

CONS: Coagulase negative Staphylococci
MRCONS: Methicillin resistant coagulase negative Staphylococci

Table 3. Antibiotic susceptibility pattern of Coagulase negative staphylococci

CONS	Va	Te	Nit	Cip	Ak	E	Amp	Gen	Cot
S.epdermidis (78)	78(100%)	78(100%)	65(83%)	41(52%)	67(85%)	35(44%)	12(15%)	43(55%)	55(70%)
S.hemolyticus(18)	18(100%)	18(100%)	9(50%)	7(38%)	12(66%)	7(38%)	2(11%)	11(61%)	11(61%)
S. saprohyticus (22)	22(100%)	22(100%)	17(77%)	9(40%)	13(59%)	4(18%)	3(13%)	17(77%)	11(50%)
S. ludgunensis (22)	22(100%)	22(100%)	14(63%)	8(36%)	11(50%)	4(18%)	2(9%)	10(45%)	9(40%)
S.hominis (14)	14(100%)	14(100%)	5(35%)	2(14%)	7(50%)	6(42%)	1(7%)	10(71%)	8(57%)
S.capitis (15)	15(100%)	15(100%)	7(46%)	3(20%)	7(46%)	3(20%)	2(13%)	11(73%)	11(73%)
S.warneri (6)	6(100%)	6(100%)	5(83%)	3(50%)	6(100%)	4(66%)	2(33%)	4(66%)	6(100%)

haemolyticus (25%) (Chavan *et al*., 2017) As per Tan *et al*., (2006) *S. lugdunensis* (46.8%) to be the commonest isolate from the abscesses and wound infections followed by S. epidermidis (43.8%). S. lugdunensis has the virulence factors that resemble those of S. aureus and can cause similar aggressive infection (Tan *et al*., 2006).As it is rarely found as a contaminant, the identification of *S. lugdunensis* raises the index of suspicion for aggressive disease (Shin *et al*., 2007)

In our study, *S.hemolyticus* (44%) and *S.epidermidis* (26%) were found to be commonest species from blood. CoNS are the most common cause of nosocomial BSI, responsible for 30% to 40% of these infections (Rupp, 2007). As per Yalaz *et al*., (2012) CoNS of the *S. epidermidis* group are the most frequent agents of central venous catheter (CVC)- and umbilical catheter associated BSIs in neonatal ICUs (Yalaz *et al*., 2012).

In the present study, *S.saprophyticus* (17/22, 77%) was the common isolate from urine. This is in accordance to the study conducted by Chavan *et al*., (2017) 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 (Chavan *et al*., 2017). But, study by Chaudary *et al*., (2007), showed *S.hemolyticus* as predominant CONS from urine (84.1%) (Chaudhury *et al*., 2007).

S.hominis was the common CONS isolated from specimens of infected ophthalmic sites. S.epidermidis and S.hemolyticus were also isolated from ophthalmic specimens. But, as per Singh and Banerjee ophthalmic specimens yielded S. epidermidis (40%) as the most frequently obtained clinical isolate, followed by *S. saprophyticus* (14%), *S. haemolyticus* (12%), *S. hominis* (6%) and *S. lugdunensis*. (Singh and Banerjee, 2008)

In the present study, overall methicillin resistant coagulase negative staphylococci were found to be 33%. Methicillin resistance among CONS in India reported in a range of 18.44% to 67.7%.(Singh et al 2016) Compared to previous studies by Sinhal *et al*., (2007) (62%) and Singh *et al*., (2008) (38%) from India, in our study prevalence of methicillin resistance is low (Singhal *et al*., 2001; Singh *et al*., 2008)

Eventhough *S.epidermidis* isolated predominantly, only 18 out of 78 isolates exhibited methicillin resistantance (23%). In our study, no methicillin resistance was reported in *S.warneri*. Methicillin resistance was predominantly observed in CONS isolated from pus and blood. Previous studies reported *S. haemolyticus* strains show highest resistance followed by *S. epidermidis, S. lugdunensis* and *S. hominis* strains (Joseph and Alexander, 2007)

A comparison of antibiotics susceptibility patterns of MRCNS and MSCNS of our study showed that MRCNS had higher level of resistance to various classes of antibiotics as compared to MSCNS. This is in concurrence with the findings of previous authors (Singhal *et al.*, 2007). Most commonly MRCONS showed coresistance to certain classes of antibiotics such as aminoglycosides, quinolones and nitrofurantoin. This is in accordance to the studies conducted earlier (Mohan *et al*., 2002; Jain *et al*., 2004)

In the present study, all isolates were susceptible to vancomycin and teicoplanin which is similar to the findings of previous authors (Singh *et al*., 2008; Udo *et al*., 2009) . *S. haemolyticus* is the first CoNS species in which vancomycin and teicoplanin resistance was identified (Schwalbe *et al*., 1987) MDR strains of CoNS can serve as a reservoir of resistance genes and can spread to the other microorganisms. Therefore, in order to prevent further spread of multi-drug resistant CoNS, the use of antibiotics should be monitored and implementation of infection control. In the other hand, continued use of antibiotic for treatment of infections associated with CoNS isolates should be supported by monitoring of antimicrobial susceptibility to prevent the spread of resistant isolates and also eliminate the use of antibiotics for a prolonged period. (Mehdi *et al*., 2014).

CONCLUSION

The present study indicated that, Coagulase negative Staphylococci were found to be pathogenic and produced signs and symptoms of infection. Appropriate antimicrobial therapy should be instituted early to prevent further complications of infection. It was observed that CONS was isolated predominantly from pus, blood and urine(26%-16%). It was also noted that, specimens collected from ocular sites, body fluids and ear varied from 9-18%.

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