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Speciation and Antibiotic Susceptibility Testing of Coagulase Negative Staphylococci at a Tertiary Care Teaching Hospital

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A B S T R A C T

The aim of the study was to speciate the CoNS isolates from various clinical samples and to study their antibiotic susceptibility pattern. Over a period of 18 months from December 2013 to May 2015, 142 Coagulase negative staphylococcal isolates grown in pure culture from various clinical samples were isolated and identified by standard procedure. Speciation of CoNS was done using novobiocin resistance test, urease activity, ornithine decarboxylase, pyrrolidonyl arylamidase and aerobic acid production from mannose. Out of 142 isolates, 56.3% were *S. hemolyticus*, 26.8% *S. epidermidis*, 5.6% *S. warneri*, 4.9% *S. saprophyticus* and 3.5% *S. schleiferi* sub sp *schleiferi*. Isolation of CoNS from urine, blood, conjunctival swab and pus were 62%, 12.7%, 7.8%% and 6.3% respectively. Antibiotic susceptibility testing showed maximum resistance to penicillin (91.6%). No resistance was noted against linezolid and vancomycin. Methicillin resistance was noted in 73.9% % of the isolates; 7% of isolates showed erythromycin induced clindamycin resistance. 45% of the total CoNS isolates were found to be multidrug resistant. 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.

Introduction

Coagulase-negative *staphylococci* (CoNS) are normal human flora but as typical opportunists represent one of the major nosocomial pathogens. Describing CoNS is challenging because they represent a heterogeneous group within the genus *Staphylococcus* that is not based on phylogenetic relationships (Becker *et al*., 2014). Often, identification of the *staphylococci* is limited to a rapid screening test for *Staphylococcus aureus*, and non-*S. aureus* isolates are simply reported as coagulase negative *staphylococci* (CoNS) (Kleeman *et al*., 1993). CoNS are the most common cause of nosocomial bloodstream infections, responsible for 30% to 40% of these infections. Coagulase- negative bacteremia occurs as a result of long term usage of indwelling central venous catheters, administration of parenteral nutrition and previous antibiotics, co- morbid conditions in the patient and other predisposing factors like intensive-care unit stay and non adherence to infection control practices and hand washing practices of medical staff (Kleeman *et al*., 2009).

CoNS express resistance to multiple antibiotics which not only pose a serious therapeutic problem but also serve as a hospital reservoir of antibiotic resistance genes (Sharma *et al*., 2010).

Materials and Methods

The present prospective study was conducted over a period of 18 months from December 2013 to May 2015. Clinical samples such as urine, blood, pus and wound swab, conjunctival swab, ear swab, central line tip, endotracheal tube tip, high vaginal swab, umbilical vein catheter tip etc submitted to the diagnostic microbiology over the study period were considered for the study.

CoNS isolates grown in pure culture or in mixed culture (not more than two organisms) from urine samples and isolates grown in pure culture from blood, body fluids and other samples were included in the study**.** CoNS isolates grown in mixed cultures (>2 types of organisms) from urine samples and grown in mixed cultures from blood, body fluids and other samples were excluded.

The initial identification of the isolate was done on the basis of colony morphology, Grams staining and catalase test. The isolates were differentiate from Micrococcus by testing the susceptibility to bacitracin(0.04 units) and furazolidone (100µg) (Winn *et al*., 2006). Speciation of CONS was done by novobiocin resistance test, urease activity, ornithine decarboxylase, pyrrolidonyl arylamidase (PYR) test and aerobic acid production from mannose (Table.1).

One or two additional tests were used to resolve each species group (De Paulis *et al*., 2003).

Antibiotic susceptibility testing was performed by Kirby-Bauer disc diffusion method on Mueller Hinton agar, according to CLSI guidelines. Methicillin resistance was detected by using Cefoxitin disk (30 µg) diffusion method and Inducible clindamycin resistance was detected using D test (Clinical and laboratory standards institute (CLSI), 2015).

Results and Discussion

A total of 142 CoNS isolates were obtained from various clinical samples. CoNS isolation was higher (70%) from female patients. Among the various age groups, the age group of 21- 30 yr showed highest isolation of CoNS (23.2%) followed by 51-60 yr (13.4%) and < 1 yr(13.4%).Urine samples yielded 62% of the total isolates where as the isolation from blood, conjunctival swab and pus were 12.7%, 7.8% and 6.3% respectively. When the isolation of CoNS from various clinical settings was compared, 51.4% of the CoNS isolates were from outpatients. Among inpatients, paediatric patients showed an isolation of 9.2%.

S.hemolyticus (56.3%) and *S.epidermidis* (26.8%) were the commonly isolated species of CoNS, followed by *S.warneri* (5.6%), *S.saprophyticus* (4.9%) and *S.schleiferi* subsp *schleiferi* (3.5%) (Fig.1). From urine and pus the predominant isolate was *S.hemolyticus*, followed by *S.epidermidis* whereas from blood samples *S.hemolyticus* and *S.epidermidis* were isolated in equal numbers.

The overall antibiotic resistance pattern of the CoNS isolates was analysed. Highest resistance was shown against penicillin (91.6%). No resistance was noted to linezolid and vancomycin. Teicoplanin resistance was seen in 6.3% of the isolates.(Fig.2)

Methicillin resistance was noted in 73.9% of the CoNS isolates. Methicillin resistance was higher (54.3%) in isolates from inpatients.

Methicillin resistant CoNS isolates revealed higher resistance to other groups of antibiotics like quinolones, aminoglycosides, macrolides etc than methicillin sensitive CoNS isolates (Fig.3). Erythromycin induced Clindamycin resistance was noted in 7% and constitutive Clindamycin resistance was seen in 16% of the isolates.

The antibiotic resistance pattern of various species was compared and almost all species showed highest resistance to penicillin. Resistance was low to amikacin in all species except *S.capitis* (100%) and *S.schleiferi* (40%). Methicillin resistance was high in all species but comparatively less in *S.saprophyticus* (28.6%) and S.epidermidis (52.6%). Teicoplanin resistance was seen in *S.epidermidis* (7.9%) and *S.hemolyticus* (7.5%).

The antibiotic resistance pattern of the two predominant isolates *S.hemolyticus* and *S.epidermidis* were compared and *S.hemolyticus* was found to be more resistant than *S.epidermidis*.(Fig.4)

Multidrug resistance in CoNS

Isolates resistant to three groups of antibiotics (penicillins, macrolides and quinolones)[9] were taken as multidrug resistant and 45% of the total CoNS isolates were found to be multidrug resistant. In the ICU isolates, 62.5% were found to be multidrug resistant. Only 41.5% of the non ICU ward isolates were MDRs. Among methicillin resistant CoNS isolates, 56.2% were multidrug resistant where as only 13.5% of the methicillin susceptible isolates were multidrug resistant.

Among various species of Coagulase negative *Staphylococcus*, least multidrug resistant were *S.epidermidis* (21.05%) and *S.warneri (*25%).Among *S.hemolyticus* isolates, 60% were multidrug resistant. Though a part of normal cutaneous ecosystem, Coagulase negative staphylococci are being recognized as important agents of nosocomial infection. They are responsible for high morbidity and mortality mainly in hospitalised patients and usually show multidrug resistance. The species identification of CoNS is important in monitoring the reservoir and distribution of CoNS involved in healthcare associated infections and will help to understand the pathogenic potential of individual CoNS species (Khan *et al*., 2014).

In the present study urine samples yielded 62% of the total isolates where as the isolation from blood, conjunctival swab and pus were 12.7%, 7.8% and 6.3% respectively. A study by Sheik *et al.,* (2012) showed a similar isolation rate from urine (51.5%) and blood (25.4%). Sharma *et al.,* (2011) also obtained majority of their study isolates from urine samples (36%) followed by blood samples (27%).

In this study, *S.hemolyticus* (56.3%) and *S.epidermidis* (26.8%) were the commonly isolated species of CoNS followed by *S. warneri* (5.6%), *S.saprophyticus* (4.9%) and *S.schleiferi* (3.5%). Ma *et al.,* (2011) from China had similar results with *S.hemolyticus* constituting 34.1% and *S.epidermidis* 27.4%. Chaudary *et al*., (2007) from Andhra Pradesh in their study also found out *Staphylococcus hemolyticus* as the predominant CoNS isolate which is similar to our study. But a study from New Delhi by Goyal *et al.,* (2006) reported *S.epidermidis* (41%) as the predominant isolate followed by *S.hemolyticus* (14.7%). Sheikh *et al.,* (2012) showed a higher isolation of *S. epidermidis* (19.4%) than *S. hemolyticus* (14.9%) from Africa. A higher isolation of *S.epidermidis* (82.3%) was shown in a study by Mohan *et al.,* (2002). The difference in the predomintly isolated CoNS species may be due to change

in geographical location and patient population, from urine samples, the predominant isolate was *S.hemolyticus*, followed by *S.epidermidis*. Chaudary *et al.*, (2007) found *S.hemolyticus* as the most common *Staphylococcus* species involved in nosocomial urinary tract infection in their hospital. On the contrary Sheik *et al.*, (2012) found out that majority of the urine isolates were *S.epidermidis* closely followed by *S.saprophyticus*. *S.hemolyticus* was the fourth common species from urine in their study.[11] In the study by Goyal *et al.*, (2006) *S.saprophyticus* was the major urinary pathogen followed by *S.epidermidis.* The catheterisation practices and sex preponderance may be the cause for this difference.

Antibiotic resistance study revealed highest resistance to penicillin (91.6%). In the study by Sheikh *et al.*, (2012) the resistance against ampicillin, penicillin and amoxicillin were 88.1%, 83.6% and 64.9% respectively which shows the penicillin resistance is widespread in CoNS isolates. Among aminoglycosides, amikacin showed a lower resistance of 12% which is similar to the studies by Usha *et al.*, (2012) and Mohan *et al.*, (2002).

Drug resistant CoNS infections are most commonly treated with glycopeptides including vancomycin, but there has been increasing concern regarding emerging resistance to these agents (Piette *et al*., 2009). Teicoplanin resistance was seen in 6.3% of the study isolates in the present study.In a study from China comparing the antibiotic resistance pattern of CoNS over a period of 2004 to 2009, there was an increase in the teicoplanin resistance from no resistance noted till 2007 and 6.7% resistance in 2009. Teicoplanin resistance of 2.7% was observed in Begum *et al.*, (2011) study. As for vancomycin universal sensitivity was notedin our study. Similar results were obtained in other studies also. But in a study by Sheikh *et al.*, (2012) vancomycin resistance was noted in 20.9% of the isolates. A very high (65.7%) resistance rate to vancomycin was noted by Manikandan *et al.*, (2005).

There was no resistance noted against linezolid in our study. Chaudary *et al.*, (2007) and Sharma *et al.*, (2010) also noted similar results. So we can resort to linezolid use for treating multidrug resistant CoNS infections.

Of the two predominant species isolates, *S.hemolyticus* had a higher resistant profile than *S.epidermidis* in our study similar to the findings of Asangi *et al.*, (2011) Methicillin resistance was also noted higher in *S.hemolyticus* than *S.epidermidis* in our study.

Methicillin resistance

Methicillin resistant coagulase negative *staphylococci* are emerging nosocomial pathogens and also act as reservoir of drug resistance genes. Methicillin resistance was seen in 73.9% of the isolates in our study which is similar to the observations of Chaudary *et al.*, (2007). In the study by Sheikh *et al.*, (2012) maximum resistance was shown against oxacillin (94%) whereas Usha *et al.*, (2013) noted a comparatively lower methicillin resistance in their study (56%). In the present study the isolates from the inpatients showed higher methicillin resistance than the isolates from outpatients. On addition, the methicillin resistant CoNS isolates revealed a higher resistance to other groups of antibiotics when compared with their methicillin sensitive counterparts. This is similar to the findings of Sharma *et al.*, (2010).

Clindamycin resistance

Clindamycin sensitivity was seen in 77% of the CoNS isolates in the present study similar

to the study by Sharma *et al.*, where it was 70%. Erythromycin induced clindamycin resistance was noted in 7% and constitutive clindamycin resistance was seen in 16% of the isolates in the present study. Ciraj *et al.*, (2009) observed an iMLSB rate of 6.38%. Aghazadeh *et al.*, (2015) from Iran reported inducible clindamycin resistance in 8.9% of the isolates.

Table.1 Presumptive identification of CoNS by simple scheme6

Species	Novobiocin	Urease	Acid from D- Mannose	PYR	Ornithine decarboxylase
S. epidermidis group	S	+	+	-	+/-
S. hemolyticus group	S	-	-	+	-
S. saprophyticus group	R	+	-	-	-
S. lugdunensis	S	+/-	+	+	+
S. warneri	S	+	-	-	-
S. schleiferi subsp. *schleiferi*	S	-	+	+	-
S. simulans	S	+	+/-	+	-
S. capitis subsp. *capitis*	S	-	+	-	-
S. cohnii subsp. *cohnii*	R	-	+/-	-	-
S. cohnii group	R	+	+	+/-	-

Fig.1 Distribution of various species of Coagulase negative staphylococci

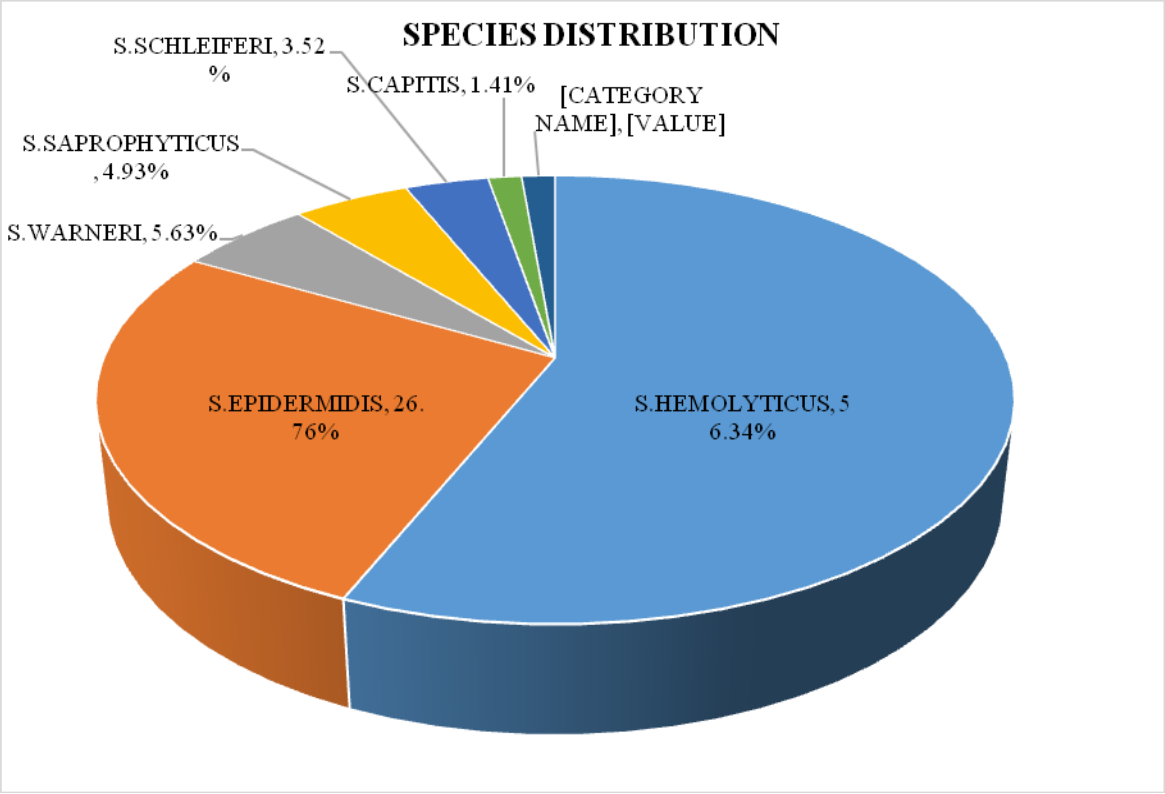


Fig.2 Overall antibiotic resistance pattern of the CoNS isolates

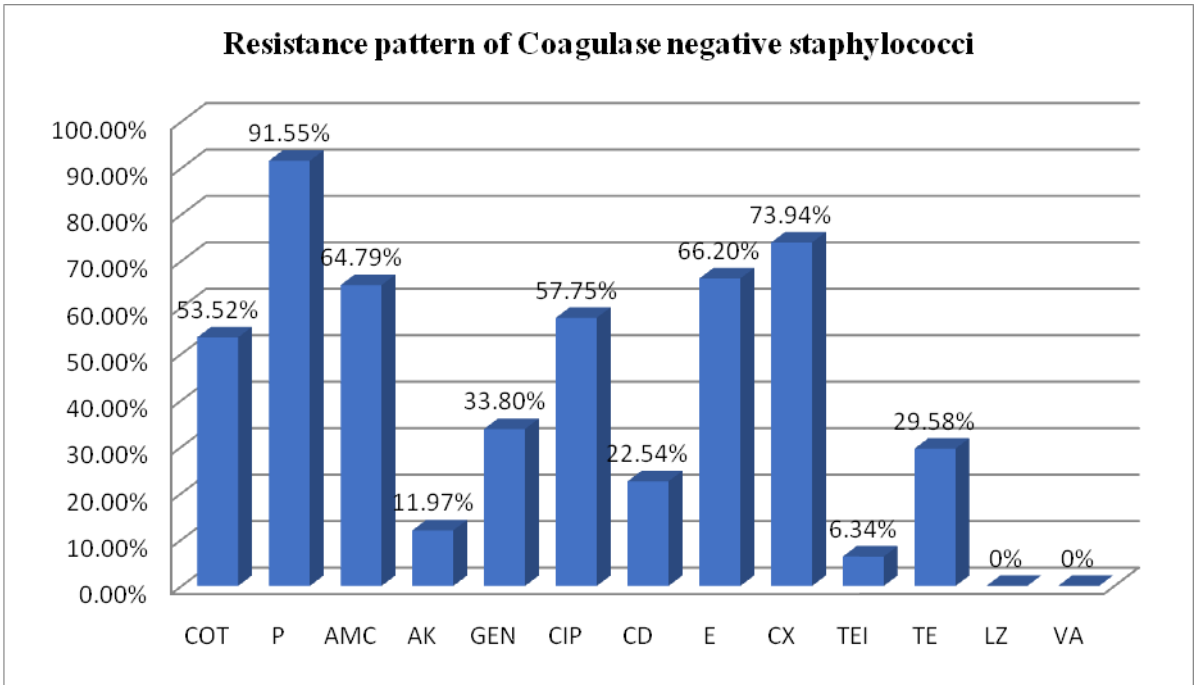


Fig.3 Comparison of antibiotic resistance pattern among methicillin resistant and methicillin sensitive CoNS isolates

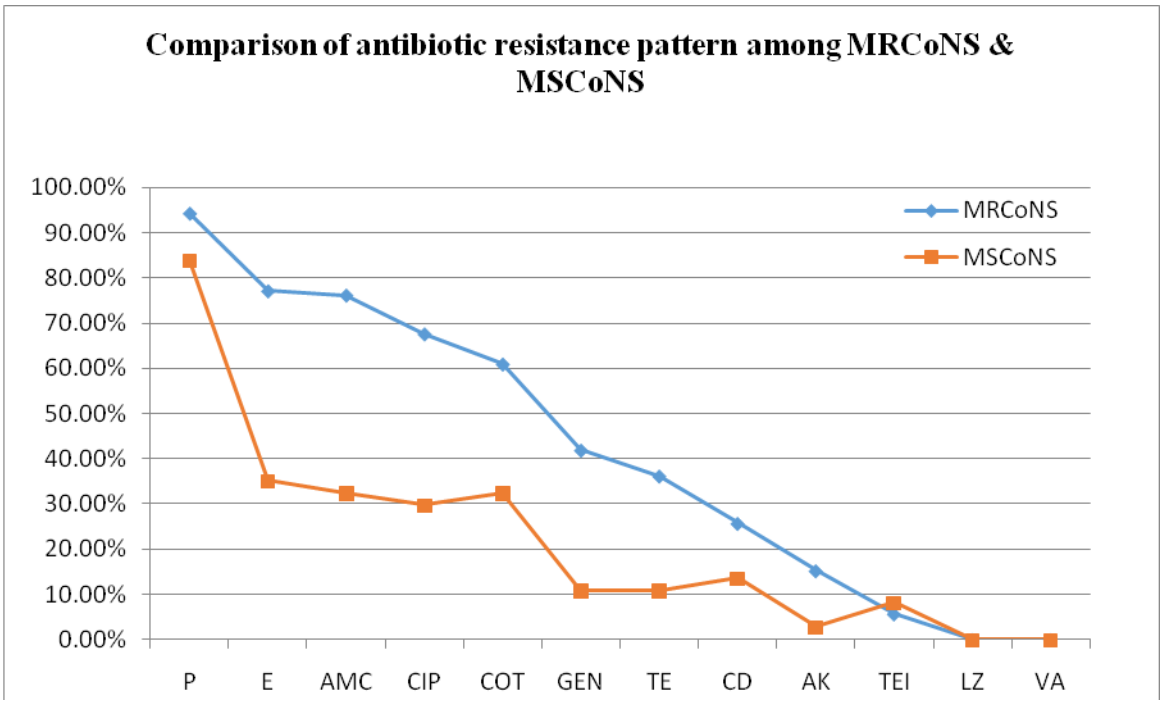
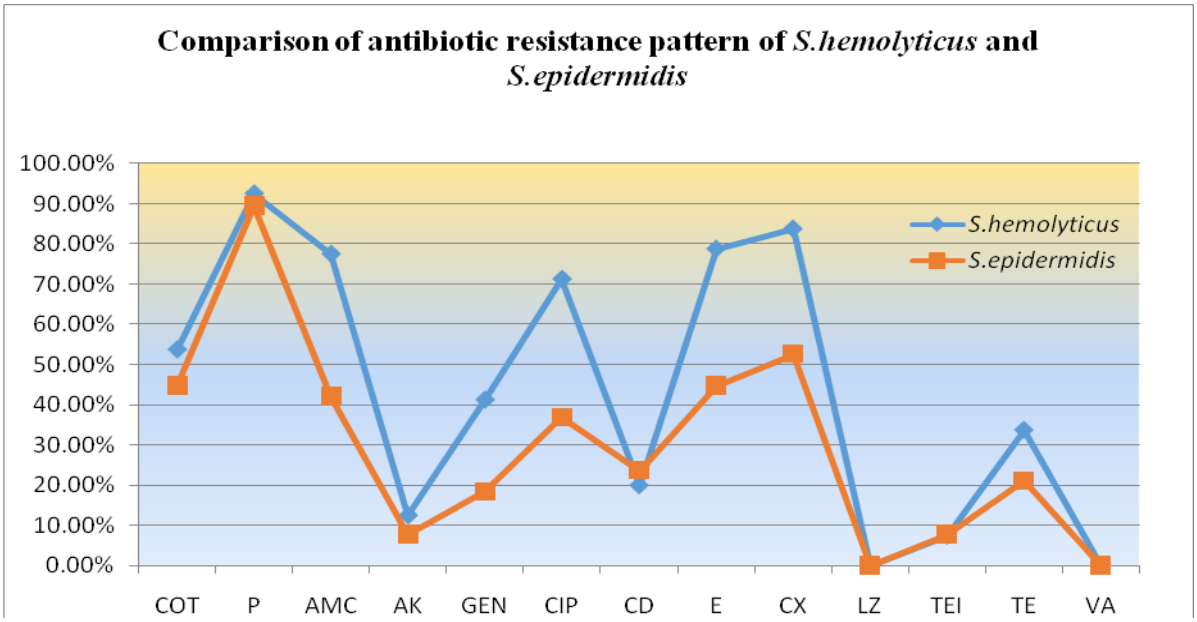


Fig.4 Comparison of antibiotic resistance pattern of S.hemolyticus and S.epidermidis



Multidrug resistant CoNS

Multidrug resistance was noted in 45% of the total CoNS isolates and multidrug resistant isolates were higher in ICU set up. Slightly higher prevalence of multidrug resistance was noted by Begum *et al.*, (2011) in their study. Multidrug resistance was higher among methicillin resistant isolates than methicillin sensitive ones in our study. Among various species of Coagulase negative *staphylococcus*, *S.hemolyticus* was found to be more multidrug resistant than *S.epidermidis*. Similar results were obtained by Begum *et al.*, (2011) and Ma xx *et al.*, (2011).

In conclusion, *S. hemolyticus* and *S.epidermidis* were the commonly isolated CoNS species. *S.hemolyticus* species had a higher antibiotic resistance antibiotic profile than *S.epidermidis*. Methicillin resistance was noted in 73.9% of the isolates. Multidrug resistance was noted in 45% of the isolates. ICU isolates showed higher multidrug resistance. Multidrug resistance was higher among MRCoNS than MSCoNS. Species identification of CoNS can be of use in further understanding of the microbiology of the organisms as well as their role in disease. The simple five test scheme used in the study can be used with ease and can be adopted for routine use in the laboratories. But because of the widespread presence of CoNS as commensal flora distinction of true pathogens from contaminating flora is a serious challenge. The increase in the methicillin resistance and multidrug resistance among CoNS should be viewed with critical importance because the therapeutic options for multidrug resistant CoNS are reducing.

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