Original Article

Journal of Microbiology and Related Research
Volume 3 Number 2, July - December 2017
DOI: http://dx.doi.org/10.21088/jmrr.2395.6623.3217.2

Identification of Coagulase Negative Staphylococci and Their Antibiogram Isolated from Various Clinical Specimen

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Received on 20.06.2017,
Accepted on 28.06.2017

Abstract

Introduction: Coagulase negative staphylococci (CoNS), which are the normal skin flora, have emerged as predominant pathogens in hospital-acquired infections. More than 30 species of CoNS are recognized but only a few are commonly incriminated in human infections. CoNS species identification, which is still difficult for most clinical laboratories, is necessary in order to establish epidemiological trends, confirm treatment failures and to determine the cause of specific infections. Recently use of broad-spectrum antibiotics for treatment of CoNS leads to increase in the development of antibiotic resistance. Therefore the present study was conducted to identify frequency of CoNS species isolated from various clinical specimens and determination of their antimicrobial susceptibility pattern. *Material and Method:* Prospective study included a total of 238 non duplicate coagulase negative staphylococci from various clinical specimens. The specimens were collected using strict aseptic precautions and transported immediately to the laboratory. All the specimens received were processed further for identification by standard microbiological procedures [17]. Qualitative data were compared with the Chi-Square or Z test as appropriate. *Results:* Predominant species isolated in our study was *S. epidermidis* (42.02%) followed by *S. haemolyticus* (21.43%) and *S. saprophyticus* (15.13%). Majority of CoNS species were isolated from pus sample with the exception of *S. saprophyticus* which was more commonly isolated from urine. Majority of CoNS isolates were resistant to penicillin followed trimethoprim/ sulfamethaxazole and tetracycline and all isolates were sensitive to vancomycin, teicoplanin and linezolid. In our study, 58.82% species of CoNS were methicillin resistant. Antimicrobial resistance to most of the commonly used antibiotics was more among methicillin resistant CoNS as compared to methicillin sensitive and the difference was statistically significant. *Conclusion:* The increased recognition of pathogenic potential of CoNS and emergence of drug resistance among them warrants the need to identify various species of CoNS and determine their antibiotic resistance pattern.

Keywords: CoNS; *S. Epidermidis; S. Saprophyticus;* MRCoNS.

Introduction

Coagulase negative staphylococci (CoNS), which are the normal skin flora, have emerged as predominant pathogens in hospital-acquired infections [1]. They have been previously dismissed as contaminants are now emerging as important potential pathogens with the increase in number of severely debilitated patients and increased use of implants in hospitals. More than 30 species of CoNS are recognized but only a few are commonly incriminated in human infections [2]. *S. epidermidis* is the species most frequently isolated from human infections. CoNS species identification, which is still difficult for most clinical laboratories, is necessary in order to establish epidemiological trends, confirm treatment failures and to determine the cause of specific infections [3].

Recently use of broad-spectrum antibiotics for treatment of CoNS leads to increase in the development of antibiotic resistance [4]. Methicillin resistance among CoNS is particularly important due to cross resistance to all other beta-lactam agents and agents of other anti-microbial classes like macrolides and fluoroquinolones [3].

The increased recognition of pathogenic potential of CoNS and emergence of drug resistance among them warrants the need to identify various species of CoNS and determine their antibiotic resistance pattern. Therefore the present study was conducted to identify frequency of CoNS species isolated from various clinical specimens and determination of their antimicrobial susceptibility pattern.

Aims and Objectives

1. To identify Coagulase negative staphylococci (CoNS) from various clinical specimens.
2. To study the species distribution of CoNS.
3. To study antimicrobial susceptibility pattern of CoNS.

Material and Methods

The present study was carried out in the department of Microbiology, at a tertiary care hospital. Prospective study included a total of 238 non duplicate coagulase negative staphylococci from various clinical specimens during period of Nov 2013 to Oct 2015. Ethical clearance from the institutional ethical committee and animal ethical Committee was obtained.

A history was taken with reference to name, age and sex. Clinical history was recorded on a pre designed proforma. The specimens were collected using strict aseptic precautions and transported immediately to the laboratory. All the specimens received were processed further for identification by standard microbiological procedures [5].

The specimens were inoculated onto nutrient agar, blood agar and MacConkey agar plates. All plates were incubated aerobically at 37^0C and observed for growth after 18-24 hours of incubation.

Isolates of staphylococci were identified on the basis of colony characteristics on nutrient agar and blood agar. Fermentative, catalase positive and bacitracin resistant cluster forming Gram positive cocci were further tested by performing coagulase test (slide and tube test) [5]. Isolates which were negative by slide or tube method were labeled as coagulase negative staphylococci and further identified to species level by battery of biochemical tests.

Antimicrobial susceptibility of all the isolates was done on Muller Hinton agar (MHA) plate by using Kirby Bauer's disc diffusion method according to CLSI guidelines [6]. All the antibiotic discs were procured commercially from Hi- Media Laboratories Pvt. Ltd, Mumbai. Erythromycin and clindamycin were not tested for urinary isolates and nitrofurantoin was tested only for urinary isolates. Methicillin resistance was detected by using cefoxitin disc. Vancomycin resistance was detected by vancomycin screen agar method.

Data were presented as proportions and percentages, unless otherwise stated. Qualitative data were compared with the Chi-Square or Z test (Standard error of difference between two proportions), as appropriate. Probability (p) less than 0.05 was considered as significant.

Result and Observations

A total of 238 isolates of coagulase negative staphylococci were obtained from various clinical specimens amongst whom males were more (64.29%) as compared to females (35.71%).

Infection rate was more common in age group of 46-60 years followed by 31-45 yrs. Minimum rate was found in patients below 15 years of age group. Majority of isolates were obtained from patients admitted in various wards (65.97%) than the patients who were attending outpatient department (34.03%). CoNS isolates were more commonly obtained in patients from Surgery department followed by

Orthopedics and Obstetrics and Gynecology department. Maximum number of CoNS were isolated from pus (44.12%) followed by urine (27.73%).

Predominant species isolated in our study was *S. epidermidis* (42.02%) followed by *S. haemolyticus* (21.43%) and *S. saprophyticus* (15.13 %). *S. colnii* (0.84) was least commonly isolated species. Majority of CoNS species were isolated from pus sample with the exception of *S. saprophyticus* which was more commonly isolated from urine.

Majority of CoNS isolates were resistant to penicillin followed trimethoprim/sulfamethaxazole and tetracycline and all isolates were sensitive to vancomycin, teicoplanin and linezolid. In our study, 58.82% species of CoNS were methicillin resistant. *S. colnii* was isolated from two samples and both of them were resistant to methicillin. *S. haemolyticus* showed 62.75% methicillin resistance while *S. xylosus* showed only 28.57% methicillin resistance.

Antimicrobial resistance to most of the commonly used antibiotics was more among methicillin resistant CoNS as compared to methicillin sensitive and the difference was statistically significant, while resistance to gentamicin, amikacin, moxifloxacin and nitrofurantoin was somewhat higher among MRCoNS than MSCoNS and the difference was not statistically significant.

Table 1: Age wise distribution of patients with CoNS infection

Age Group (in years)	No. of Cases	Percentage (%)
< 15 years	21	08.82
16-30	52	21.85
31-45	60	25.21
46-60	78	32.77
>60	27	11.35
Total	**238**	**100**

Table 2: Department wise distribution of patients with CoNS infection

Department	No. of Cases	Percentage (%)
Surgery	75	31.51
Orthopedics	47	19.75
Obstetrics- Gynecology	37	15.55
ICU	25	10.51
Paediatrics	21	08.82
Medicine	18	07.56
ENT	15	06.30
Total	**238**	**100.00**

Table 3: Sample wise distribution of CoNS isolates

Type of Specimen	No. of Samples	Percentage (%)
Pus	105	44.12
Urine	66	27.73
Blood	24	10.09
Foley's catheter tip	12	05.04
Endotracheal Aspirate	10	04.20
Body fluids*	09	03.78
Central Line	08	03.36
Sputum	04	01.68
Total	**238**	**100**

Table 4: Species distribution of CoNS isolates

Species of CoNS	Percentage%
S. epidermidis	100(42.02)
S. haemolyticus	51(21.43)
S. saprophyticus	36(15.13)
S. hominis	15(6.30)
S. lugdunensis	14(05.88)
S. capitis	08(3.36)
S. xylosus	07(2.94)
S. warneri	05(2.10)
S .colnii	02(0.84)
Total	**238**

Table 5: Antibiogram of CoNS isolates

Antibiotics	CoNS isolates (N=238)	
	Sensitive	Resistant
Penicillin G	59 (24.79%)	179 (75.21%)
Cefoxitin	98 (41.18%)	140 (58.82%)
Gentamicin	172 (72.27%)	66 (27.73%)
Amikacin	194 (81.51%)	44 (18.49%)
Erythromycin	101 (63.12%)	59 (36.88%)
Clindamycin	117 (73.12%)	43 (26.88%)
Ciprofloxacin	99 (41.60%)	139 (58.40%)
Moxifloxacin	190 (79.83%)	48 (20.17%)
Nitrofurantoin	60 (76.92%)	18 (23.08%)
Tetracycline	85 (35.71%)	153 (64.29%)
Trimethoprim/Sulfamethoxazole	83 (34.87%)	155 (65.13%)
Teicoplanin	238 (100%)	00 (00%)
Linezolid	238 (100%)	00 (00%)
Vancomycin	238 (100%)	00 (00%)

Erythromycin and clindamycin were tested against isolates from samples other than urine (N=160) and nitrofurantoin was tested only against urinary isolates (N=78)

Table 6: Methicillin resistance among CoNS species

Species	No. of MRCoNS (%)	No. of MSCoNS (%)
S. epidermidis (100)	60 (60%)	40 (40%)
S. haemolyticus (51)	32 (62.75%)	19 (37.25%)
S. hominis (36)	21 (58.33%)	15 (41.67%)
S. saprophyticus (15)	08 (53.33%)	07 (46.67%)
S. lugdunensis (14)	08 (57.14%)	06 (42.86%)
S. capitis (08)	04 (50 %)	04 (50%)
S. xylosus (07)	02 (28.57%)	05 (71.43%)
S. warneri (05)	03 (60%)	02 (40%)
S. cohnii (02)	02 (100%)	00 (00%)
Total (238)	**140 (58.82%)**	**98 (41.18%)**

Table 7: Antimicrobial resistance pattern among methicillin resistant and methicillin sensitive CoNS

Antibiotics	MRCoNS (N=140)	MSCoNS (N=98)	Total (N=238)	Z value	P value
Penicillin	140 (100)	39 (39.80)	179 (75.21)	12.35	<0.05
Gentamicin	41 (29.29)	25 (25.51)	66 (27.73)	0.65	>0.05
Amikacin	27 (19.29)	17 (17.35)	44 (18.49)	0.38	>0.05
Erythromycin	46 (47.42)	13 (20.63)	59 (36.88)	4.56	<0.05
Clindamycin	35 (36.08)	8 (12.70)	43 (26.88)	4.44	<0.05
Ciprofloxacin	94 (67.14)	45 (45.92)	139 (58.40)	3.31	<0.05
Moxifloxacin	30 (21.43)	18 (18.37)	48 (20.17)	0.59	>0.05
Nitrofurantoin	11 (25.58)	7 (20.00)	18 (23.08)	1.02	>0.05
Tetracycline	98 (70.00)	55 (56.12)	153 (64.29)	2.19	<0.05
Trimethoprim/sulfamethoxazole	102 (72.86)	53 (54.08)	155 (65.13)	2.99	<0.05

Figures in parentheses show parentages

Discussion

CoNS are increasingly being incriminated as a significant pathogen associated with healthcare infections and therefore, there is a need for identification of CoNS up to species level as has been emphasized by many investigators. The species identification 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 [7]. CoNS are a major cause of nosocomial bacteremia and septicemia, especially for the patients who have immune deficiency and malignancy, which can lead to morbidity and even mortality. Despite the recent introduction of antimicrobial agents and medical improvements in controlling the frequency and morbidity of staphylococcal infections, they are persistent as an important hospital and community pathogen [8].

In our study, a total of 238 isolates of coagulase negative staphylococci were obtained from various clinical samples.

Age and sex wise distribution of patients in the present study has followed natural epidemiological patterns. Male to female ratio among the included cases was 1.8:1. Exactly similar ratio was shown by Ravindran et al [9] (2014) while Ahmad et al [4] (2012), and S A Sardar et al [3] (2015) documented male to female ratio as 1.3:1 and 1.2:1 respectively.

In the present study, out of 238 patients, majority belonged to age group 46-60 years (32.77%) followed by 31-45 years (25.21%). S A Sardar et al [3] (2015) and Usha et al [1] (2015) has found the most affected age group in their study as 31 to 50 years and 31 to 40 years respectively. Surekha et al [10] (2011) has observed the most affected age group in her study was >40 years (39.05%).

In the present study majority of CoNS isolates were obtained from patients admitted in various wards (65.97%) than OPD patients (34.03%). Similarly Muhammad et al [11] (2013) reported that isolation rate of CoNS was higher from inpatient department (69%) than from patients attending OPDs (31%). Many workers have expressed their view that the duration of hospital stay is directly proportional to the higher prevalence of the infection since the rate of isolation of organisms is higher in indoor than outdoor patients. This could be attributed to infection in wards from patient to patient via hands of nursing staff. Thus frequent hand washing to prevent spread of organisms should be encouraged.

In the current study, rate of isolation of CoNS was higher from surgery department (31.51%) followed by orthopedic (19.75%) and OBGY department (15.55%). Similar observation was made by Tanusri Biswas et al [12] (2008) who showed more isolation from surgery department (59.67%) followed by orthopedic department (19.35%). While in a study done by F Koksal et al [8] (2006), maximum isolation was from intensive care unit (30%), followed by surgery department (17.50 %).

In the present study, maximum number of CoNS were isolated from pus (44.12%) followed by urine samples (27.73%). This finding is comparable with study done by R Goyal et al [13] (2006), who obtained 38.2% of CoNS isolates from pus followed by urine samples (28.4%). Surekha et al [10] (2011), in her study reported 33.3 % of CoNS isolates were from pus followed by urine samples (27.1%) while Usha et al [1] (2015) isolated CoNS species more frequently in blood (52%) than pus (32 %). U Mohan et al [2] (2002) showed maximum isolation of CoNS from urine (48.43%), followed by pus (17.70%).

In our study, as many as nine CoNS species were isolated with varying frequencies from different clinical specimens. Amongst which *S. epidermidis* was the most predominant isolate (42.02%) followed by *S .haemolyticus* (21.43%) and *S. saprophyticus* (15.13%). This finding very well correlates with various studies like F. Koksal et al [8] (2009), Ahmad et al [4] (2012) and Mohammad Mubashir et al [7] (2014) who also reported *S. epidermidis* as most frequently encountered clinical isolates followed by *S. haemolyticus.* while Surekha et al [10](2011), Shubhra singh et al [14] (2008), U Mohan et al [2] (2002) and KS Seetha et al [15] (2000) obtained *S. epidermidis* as the most frequent isolate followed by *S. saprophyticus* and *S. haemolyticus.*

In our study, the specimens from which the CoNS species were isolated showed propensity of certain species for specific culture type. *S epidermidis* which was the predominant isolate was most commonly isolated from pus (49%). Similarly Shrikande et al [16] (1996) had shown maximum isolation of *S. epidermidis* from pus (38.46%), while Usha et al [1] (2015) reported maximum isolation of *S. epidermidis* from blood (59.37%) and Ahmad et al [4] (2012) reported maximum isolation from urine (51.5%). A total of 69.44% isolates of *S. saprophyticus* were recovered from urine where its pathogenic potential is well documented. S A Sardar et al [3] (2015) also reported that *S. saprophyticus* was predominantly isolated from urine (75%).

In the present study, antibiotic susceptibility testing showed maximum resistance to penicillin (75.21%). Somewhat similar penicillin resistance was reported by Shrikande et al [16] (1996) (63.10%). Mohammad et al [7] (2014) and Surekha et al [10] (2014) exhibited higher penicillin resistance of 99.5% and 94.79% respectively in their study. In our study, trimethoprim/sulfamethoxazole resistance was 65.13% which correlates with the findings by S A Sardar et al [3] (2015) and Mayhall CG et al [17] (2004) who showed 59% and 76% resistance to same antibiotic respectively. Koksal et al [8] (2009) reported lower resistance of 47%. Resistance for tetracycline was 64.29% in our study which was similar to the resistance shown by A. Sheikh et al [4] (2012) (66%) while E. Akinkumni et al [18] (2010) showed only 34% resistance.

In the present study, lower gentamicin resistance was found (27.73%) which was corresponding to the results shown by Ravindran et al [19](2014) (20%). S A Sardar et al [3] (2015) and Mohammad Mubashir et al [11] (2014) reported higher gentamicin resistance of 53% and 82% respectively. While Akinkumni et al [18] (2010) reported lower resistance as compared to our study (8%).

All the isolates in the present study were sensitive to vancomycin, teicoplanin and linezolid. Similar findings were shown by S A Sardar et al [3](2015), Ravindran et al [19] (2014) and Koksal et al [8] (2009) hence it may be prudent to use these agents for empirical treatment of serious infections in hospitalized patients [18]. But Manikandan et al [20] (2005) in his study reported vancomycin resistance of 65.7%. Vancomycin has long been considered as an antibiotic of last resort but extensive use of glycopeptide in hospital has been related to decreased susceptibility to these agents. In comparison to our study, Samant et al [21] (2012) reported 4.1% and 0.6% resistance to linezolid and Teicoplanin respectively

In our study, 58.82% CoNS isolates were methicillin resistant by cefoxitin disc diffusion method. Similar to our study, Shubhra et al [14] (2008) and F. Koksal et al [8] (2009) showed 62% and 67.5% MRCoNS respectively in their study. Higher prevalence rate of 78.84% of MRCoNS was given by Artee et al [22] (2015). In our study, maximum resistance to methicillin was shown by *S .haemolyticus* (62.75%) while Shubhra et al [14] (2008) and Surekha et al [10] (2011) showed higher methicillin resistance of 76% and 90% in *S. haemolyticus* respectively. In our study, *S. epidermidis* also showed significant percentage of resistance to methicillin (60%). Similar finding were shown by Surekha et al [10] (2011) and Kumari et al [23] (2001) who showed methicillin resistance of 66% and 65% in *S. epidermidis* respectively. Lowest methicillin resistance was shown by *S. xylosus* in our study.

This increasing prevalence and isolation rate of MRCoNS is alarming because of its self-involvement in the diseased condition and possibility of transferring the mecA gene to *S. aureus*. Therefore continuous monitoring, strict antibiotics policies and resistance program is mandatory, which will contribute in implementation of infectious measures [22].

In the present study, antimicrobial resistance to most of the commonly used antibiotics was more among methicillin resistant CoNS as compared to methicillin sensitive and the difference was statistically significant. Similar results were obtained by Akinkumni et al [18] (2010) who showed that there was reduced susceptibility to most of the antibiotics by MRCoNS as compared to MSCoNS isolates.

Summary and Conclusion

CoNS are a major cause of nosocomial bacteremia and septicemia, especially for the patients who have immune deficiency and malignancy, which can lead to morbidity and even mortality. Despite the recent introduction of antimicrobial agents and medical improvements in controlling the frequency and morbidity of staphylococcal infections, they are persistent as an important hospital and community pathogen. The increased recognition of pathogenic potential of CoNS and emergence of drug resistance among them warrants the need to identify various species of CoNS and determine their antibiotic resistance pattern.

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