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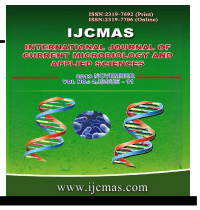

Original Research Article

Antibiotic susceptibility and multiplex PCR analysis of Coagulase Negative Staphylococci isolated from laboratory workers

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A B S T R A C T

Health care personnel have high risk of acquiring infections through various routes. As their microflora are continuously exposed to pathogenic organisms, the chance for these microflora to acquire the antibiotic resistance and virulence factors from pathogens and also their role as carriers for these factors is comparatively high and is often difficult to understand. As some of the commensal microflora like coagulase negative staphylococci (CoNS) are increasingly considered as emerging pathogens, studies on presence and distribution of antibiotic resistance and virulence properties among commensal CoNS from health care workers are important. The present study was conducted to analyse species diversity, prevalence of antibiotic resistance and biofilm production in commensal CoNS isolated from skin of laboratory workers. In the present study, 30 skin commensals of laboratory workers were isolated and identified by conventional biochemical method. Also susceptibility of the CoNS isolates to 15 antibiotics were analysed by disc diffusion assay. MPCR using primers specific to *mecA* (methicillin resistance gene) and *icaAB* (biofilm specific gene) was also conducted. The various CoNS species identified in the study were *S. epidermidis* (60%), *S. hominis* (33.3%), *S. hemolyticus* and *S. saprophyticus* (both 3.3%). Antibiotic susceptibility analysis revealed all the isolates to have resistance towards ampicillin and 72% isolates with methicillin resistance. Out of this, 41% of the isolates showed presence of *mecA* gene. The study identified various species of commensal CoNS from skin of laboratory workers with comparatively high multi drug resistance and methicillin resistance. Considering the recent understanding on the role of CoNS as reservoir and carrier of antibiotic resistance, the observed multiple resistance is highly significant.

K e y w o r d s

Laboratory workers; coagulase negative staphylococci; methicillin resistance; multiplex PCR.

Introduction

As coagulase negative staphylococci (CoNS) have been reported to have highly adapted mechanisms to cause device related infections, they are considered as emerging pathogens. The hospital environment, hospital personal or the

patient itself can act as source of CoNS causing device infection. Eventhough they are present on various parts of the body as commensal, the multiple antibiotic resistance present in these organisms is highly remarkable. Also acquisition of genetic elements from other pathogenic organisms might have favored CoNS to cause the difficult to treat infections upon accidental introduction in to the body especially associated with device insertion. As the health care workers such as doctors, nurses and laboratory staffs are on continuous exposure to different clinical samples, the probability of their skin flora especially CoNS to become altered by the acquisition of antibiotic resistance are high. By considering the potential of CoNS to act as source and reservoir of resistance determinants and virulence factors, studies on such properties of CoNS from healthcare personals are very important.

Since frequent isolation of CoNS from clinical samples has changed its status from harmless commensal to opportunistic pathogens, studies on antibiotic resistance associated with them is very important. The major CoNS species associated with humans include *S. epidermidis*, *S. capitis*, *S. hominis*, *S. haemolyticus*, *S. saccharolyticus*, *S. warneri*, *S. lugdunensis*, *S. saprophyticus* and *S.cohnii*. Among these, the most frequently isolated species is *S. epidermidis* (Otto, 2008). CoNS infections associated with indwelling medical devices may lead to life threatening conditions in immunocompromised patients. The diverse infections caused by CoNS include central nervous system shunt infections, endophthalmitis, surgical site infections, bacterimia, wound and joint infections, endocarditis etc (Piette and Verschraegen, 2009; Upadhyayula *et al.,* 2012).

Pathogenic potential of CoNS is mainly due to biofilm formation and multiple antibiotic resistances. CoNS have been reported to have methicillin resistance mediated by an altered penicillin-binding protein (PBP2a), encoded by the *mecA* gene. The mecA gene is located on a genetically mobile chromosomal element known as staphylococcal cassette chromosome (SCC) which also harbors multiple antibiotic determinants (Zhang *et al*., 2005). Very importantly, CoNS are increasingly considered as source for the assembly and evolution of novel SCC mec elements. Biofilm formation facilitates CoNS to form highly resistant cell mat on indwelling medical devices and also their dissemination to other parts of the body (Valle *et al.,* 2012). Interestingly CoNS have been reported to have very diverse and yet to know mechanisms of biofilm formation mediated by an array of factors. Considering these emerging knowledge, screening of CoNS for biofilm formation and antibiotic resistance from health care personal is very important.

Hospital personal forms an important source for the evolution of multidrug resistant CoNS and is often least investigated. An understanding on such potential CoNS will facilitate methods to develop strategies to resist nosocomial infections due to CoNS. Thus the current study was conducted on species diversity, antibiotic resistance and biofilm formation of CoNS isolated from lab workers.

Materials and Methods

Sample Collection

Skin commensals (n=30) of healthy laboratory workers were collected from MOSC Medical College Hospital, Kolenchery, a tertiary care hospital in

Ernakulam district of Kerala, India. Sampling was done from the upper part of the arm above the wrist using sterile cotton swabs moistened with sterile saline. The inoculum was swabbed on the blood agar plate and was incubated for 24- 48 hours at 37ºC. The colonies which were confirmed as coagulase negative staphylococci were subcultured on to TSA slants and maintained as pure stocks. From this, inoculation was done onto nutrient broth tubes for further staining and biochemical studies.

Biochemical Characterization of the isolates

Gram staining was performed and gram positive cocci in clustures were only selected for further biochemical identification. Preliminary confirmation for the genus Staphylococcus was achieved by conducting catalase test, oxidase test, Hugh and Leifson's oxidation- fermentation test and bacitracin susceptibility test. Species level identification of the isolates was done according to the methods described by Kloos and Schleifer (Iorio *et al.,* 2007). These includes series of biochemical tests; tube coagulase test, MSA test, Urease test, Ornithine Decarboxylase test, Oxidation Fermentation test, Nitrate Reduction test, Phosphatase test, Hemolysis, acid production from sucrose, trehalose, maltose, lactose, mannose, arabinose and xylose, and susceptibility to novobiocin (5mcg).

Multiplex Polymerase Chain Reaction

Multiplex PCR was conducted as per the procedures explained by Iorio *et al* (2011) for the simultaneous detection of *mecA* (specific to methicillin-resistance) and *icaAB* (specific to biofilm) genes. For conducting the PCR, rapid DNA extraction was done using the method explained by Zhang *et al* (2005). Primers used for multiplex PCR were: *MRS1* (5′-TAG AAATGA CTGAAC GTC CG-3′) and *MRS2* (5′-TTG CGA TCA ATG TTA CCG TAG-3′) to detect a 154-bp fragment of *mecA* (methicillin-resistance) and *icaAB-F* (5′-TTA TCA ATG CCG CAG TTG TC-3′) and *icaAB-R* (5′-GTT TAA CGC GAG TGC GCT AT-3′) to detect a 546-bp of *icaAB* genes. The 25 µl PCR mix contained, 2.5 µl of 10X PCR buffer, 200 µM of each dNTPs, 0.625 units of *Taq* DNA polymerase, 5 pmoles of each of the above primers and 2 µl of the extracted DNA as template. The volume was made upto 25µl with MilliQ water. The PCR conditions used were : Initial denaturation for 3 min at 94^{o}C, followed by 35 cycles of denaturation at 94ºC for 1 min, annealing at 58ºC for 1 min and elongation at 72ºC for 1min, with a final extension at 72ºC for 5 min. The amplified products were analyzed on 1.5% of agarose gel and were visualized on UV transilluminator.

Antibiotic susceptibility test

Antibiotic sensitivity test of the commensals was done by agar disk diffusion on Mueller-Hinton agar (Himedia, Mumbai) using method described by Kirby and Bauer (1966). The antibiotic discs used in this study were Ampicillin (25 mcg), Bacitracin (8 mcg), Chloramphenicol(30mcg), Ciprofloxacin (30 mcg), Erythromycin (15 mcg), Furazolidone (50 mcg), Fusidic acid (30 mcg), Gentamycin (10 mcg), Levofloxacin (5 mcg), Methicillin (5 mcg), Nalidixic acid (30 mcg), Novobiocin (5 mcg), Oxacillin (5 mcg) , Penicillin (10 mcg), Rifampicin (15 mcg) , Tetracycline (30 mcg) and Vancomycin (15 mcg).

Result and Discussion

Biochemical identification of commensals

30 commensals isolated from the hands of laboratory workers were classified under four different groups based on biochemical properties. CoNS species observed in the study were *S. epidermidis* group (60%) followed by *S. hominis* (33.3%), *S. hemolyticus* and *S. saprophyticus* (3.3%). [Table.1]

Multiplex polymerase chain reaction

On multiplex PCR amplification, out of the 22 CoNS isolates identified as methicillin resistant by the disk diffusion test, 9 were found to have *mecA* gene. Interestingly no amplification was observed in any of the isolate for *ica*AB genes responsible for biofilm formation.

Antibiotic susceptibility analysis

Among the 30 skin commensals studied, one isolate showed resistance to 15 antibiotics tested, two isolates were resistant to 13 antibiotics, six were resistance to 12 antibiotics and four were resistant towards 11 antibiotics [Table.2]. All the 30 CoNS skin commensal were found to be resistant to ampicillin followed by resistance to tetracycline (96%). Among *S. epidermidis* all were resistant to tetracycline, 53.3% were resistant to ciprofloxacin and furazolidone, and 38.88% showed penicillin resistance. All of the *S.hominis* isolates were resistant for ciprofloxacin and 60% showed resistance towards pencillin. The CoNS commensal isolates showed highest sensitivity towards levofloxacin (99%) followed by susceptibility towards vancomycin (90%). Very interestingly two *S. epidermidis* isolates and one *S. hominis* were found to be resistant for vancomycin. Approximately 73% of skin CoNS commensals showed resistance to methicillin, 17% showed borderline resistance and 10% were sensitive. For *S. epidermidis* group and *S. hominis*, methicillin resistance was observed as 72% and 70% respectively. *S. saprophyticus* isolate also showed resistance but the single isolate of *S. hemolyticus* were found to be sensitive to methicillin.

Emergence of CoNS as highly potential nosocomial pathogen is because of their heterogenic speciation and increased drug resistance. Hence, species identification and antibiotic susceptibility studies of CoNS are very important. In the present study, the small white colonies formed on blood agar plate which were observed as gram positive cocci in clusters were confirmed as CoNS by performing tube coagulase test. *S. epidermidis* (60%) was the major CoNS species isolated in this study, followed by *S. hominis* with a prevalence of 33.3% and *S. hemolyticus* and *S. saprophyticus* with a prevalence of 3.3%. Species distribution of CoNS flora on human skin and the low isolation frequency of *S. saprophyticus* and *S. hemolyticus* as observed in the study were in accordance with the previous results (Larson *et al.,* 1986). *S. epidermidis* usually form predominant isolates from both commensal and clinical samples because of their greater numbers and increased pathogenic potential. *S. hemolyticus* and *S. saprophyticus* are also common isolates from clinical samples whereas a small percentage of *S. hominis* is only associated with clinical conditions (Bouchami *et al.,* 2011).

Table.1 Species prevalence of coagulase negative staphylococci isolated from skin of lab workers.

CoNS Species	Percentage of isolation
S. epidermidis	60%
S. hominis	33.3%
S. hemolyticus	3.3%
S. saprophyticus	3.3%

Table.2 Resistance pattern of coagulase negative staphylococcal commensals.

Antibiotics	Number of CoNS isolates resistant to each antibiotic			
	S. epidermidis **(n=18)**	***S.hominis*** **(n=10)**	***S. hemolyticus*** **(n=1)**	***S. saprophyticus*** **(n=1)**
Ampicillin	18	10	1	1
Erythromycin	2	5	0	0
Ciprofloxacin	15	10	0	1
Chloramphenicol	7	3	0	1
Fusidic acid	14	6	0	1
Furazolidone	15	9	0	1
Gentamycin	3	3	0	0
Levofloxacin	0	0	0	0
Methicillin	13	7	0	1
Nalidixic acid	10	4	0	0
Oxacilin	15	8	0	1
Penicillin	7	6	0	0
Rifampicin	15	4	1	1
Tetracyclin	18	9	1	1
Vancomycin	2	1	0	0

The antibiotic resistance was higher for CoNS species isolated and all the 30 commensal were found to be resistant for ampicillin. All of the *S. epidermidis* isolates were resistant towards tetracycline and all of the *S. hominis* isolates showed resistance towards ciprofloxacin. Also penicillin resistance was higher for the commensals. Health care personnel associated CoNS isolates with increased level of multidrug resistance were also reported in previous studies (De Mattos *et al.*, 2003). Previous studies of Zhang *et al* (2011) showed a high MRCoNS carriage in nurses than in the general population. Very high prevalence of nasal carriage of CoNS and MRCoNS in health care workers are also reported (Mahesh *et al.*, 2012). Health care workers can expect to have high methicillin resistance than the general population as observed in the study. But only limited studies were conducted in this direction which indicates the significance of this study.

All the CoNS commensal isolates were susceptible towards levofloxacin and 90% of the isolates were susceptible for vancomycin. But 10% of vancomycin resistance in commensals observed in the study is very important. The exposure to clinical samples must have greatly favored

the commensal CoNS of health care personnel to acquire the multidrug resistance as observed in the study. This result especially vancomycin resistance indicates the requirement of periodic analysis of samples. Knowledge about the susceptibility pattern of CoNS to antibiotics can be effectively used for treatment stratergies. Various reports favouring the use of oral levofloxacin for decolonization of staphylococcal nasal carriage in health care workers are there (Akhtar, 2010). Multidrug combinational therapy developed against staphylococci found to be effective in treating severe infections which include the use of rifampicin, vancomycin and fusidic acid and new drugs such as linezolid, daptomycin combinations (Howden *et al.*, 2010).

Methicillin resistant CoNS isolates (72%) as selected by disc diffusion test were further subjected to PCR based detection of *mecA* gene. Very interestingly only 41% of the selected isolates were found to have *mecA* gene.

MecA-negative methicillin resistance in CoNS may be due to non-penicillin-binding protein-dependent mechanism of hyperproduction of β-lactamase, or due to low affinity penicillin-binding proteins (Suzuki *et al.*, 1992; Geha *et al.*, 1994). Multiplex PCR is used for rapid and reliable diagnosis of infections (Pimenta *et al.*, 2008). By including the primers for methicillin resistance and also for virulence factor, the potential of multiplex PCR was effectively applied in the study for the rapid screening of methicillin resistant biofilm forming CoNS. Eventhough some reports are there on isolation of biofilm forming CoNS from commensal, in the present study none of the isolate was found to have *icaAD* biofilm genes.

Persons working in laboratories are at risk of becoming infected with bacteria majorily through accidental exposures. There is high chance for the asymptomatic carriage and dissemination of multidrug bacteria through these health care workers to the community. Isolation and identification of 30 CoNS skin commensal of lab workers showed their species prevalence as *S. epidermidis* (60%) followed by *S. hominis* with 33.3% and 3.3% of both *S. hemolyticus* and *S. saprophyticus*. High antibitiotic resistance was observed for the commensal isolates and also 41% of the isolates showed *mecA* gene presence.

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