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Methicillin Resistance and Biofilm Formation of Clinically Important Coagulase Negative Staphylococci

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A B S T R A C T

Nowadays Coagulase-negative staphylococci (CoNS) are the most common causes of device related infections, especially in extreme age group of patients such as neonates and old age. The antibiotic resistance and biofilm production of CoNS produce negative impact on clinical outcome of patients, The study is therefore conducted to determine the antimicrobial susceptibility pattern and surface adherence property of CoNS isolated from various clinical specimens.. During the six months study period 46 CoNS were isolated from various clinical specimens Staphylococcus epidermidis (47%) was the common isolate followed by Staphylococcus haemolyticus (24%), and Staphylococcus saprophyticus (24%). CoNS isolates showed maximum resistance to Ampicillin (76%), Clindamycin(74%), Cotrimoxazole (74%) and Erythromycin(72%). CoNS isolates revealed 100% sensitivity to Vancomycin. 72% of CoNS isolates showed Methicillin resistance and 61% were biofilm producers by Congo Red Agar method. Methicillin resistance and Biofilm production of CoNS lead to increased morbidity and mortality in hospitalized patients. Aseptic management should be followed in intravascular devise insertion and other invasive procedures to reduce the spread of CoNS infection.

Introduction

Previously Coagulase-negative staphylococci (CoNS) are considered as non-pathogenic commensals of human skin and mucosa. With the advancement of medical sciences, especially with the increasing use of medical devices, the infections caused by CoNS are rapidly increased [(1, 2)]. CoNS are an important causative agent for Neonatal septicemia worldwide[(2,3)]. Commonly isolated species of CoNS include *S. epidermidis S. haemolyticus, S. saprophytiicus, S. lugdunensis, S. warneri, S. cohinii, and S. hominis.*[(4)] Resistant to multiple antibiotics is commonly reported in CoNS. These strains are not only resistant to

multiple antibiotics, but also act as reservoirs for drug resistance gene. Methicillin resistance (MR) among CONS is particularly important due to resistance to all beta-lactam agents and cross resistance to other anti-microbial classes. The resistance to methicillin and other beta-lactam antibiotics in both MR Staphylococcus aureus and MR-CoNS is primarily caused by the acquisition of the mec A gene, which encodes a modified penicillin-binding protein 2a that has a low binding affinity for all beta-lactam antibiotics. [5, 6, 7]

Biofilm production is considered as a main virulence determinant of CoNS and it protect them from host immune system and antimicrobial therapy. Biofilm consists of multilayered cell clusters embedded in a matrix of extracellular polysaccharide called polysaccharide intracellular adhesion (PIA).The development of the biofilm is begun with bacteria adhering to a biotic or an abiotic surface mediated by microbial surface components recognizing adhesive matrix molecules (MSCRAMMs)[6]. Then, the bacteria multiply to form a multilayered biofilm, associated with production of PIA which mediates cell to cell adhesion. The synthesis of PIA is mediated by the products of the intracellular adhesion (ica) operon. [5,6,7,8, 9]. Detection of biofilm production and Methicillin resistance is very important for the treatment of infections that are caused by CoNS [10,11,12]. The present study is therefore conducted to determine the antimicrobial susceptibility patternand surface adherence property of CoNS isolated from various clinical specimens.

Materials and Methods

This prospective study was conducted at Thoothukudi Medical College over a period of six months. During the study period 46 coagulase negative Staphylococci were isolated from various clinical specimens.

The isolates were initially identified by colony morphology, Gram staining, catalase, slide and tube coagulase test acid formation from mannitol, Novobiocin sensitivity test and urease test.[13, 14]

Susceptibility to antibiotics were performed on Mueller–Hinton agar plates by Kirby-Bauer disc diffusion method. Zone diameter was measured and interpreted as per the Clinical and Laboratory Standards Institute (CLSI) guidelines.

Minimum Inhibitory Concentration determination

MIC of Cefoxitin & Vancomycin was determined by an Epsilometer test (E test). A peptone water suspension of each CoNS isolate was adjusted to a McFarland standard of 0.5 and inoculated over the surface of the Mueller Hinton agar plate. The MIC strip was applied on the agar surface using sterile forceps. Then the plates were incubated for overnight at 37°C and the MIC was the point where the elliptical zone of growth inhibition intersected the MIC scale on the E test strip. The concentration range of antimicrobial on the E test strip corresponds to two fold dilutions in a conventional MIC method[15,16].

Detection of Methicillin resistance

Methicillin resistance was detected by Cefoxitin Disc Diffusion Method and also by Cefoxitin E Test.[15,16]

Phenotypic characterization of biofilm formation by Congo Red Agar Method [17, 18, 19].

Congo red agar method

This test was performed on Congo red agar (CRA) plates as described by Freeman and coworkers and based on the property of Congo

red to stain polysaccharides black. The composition of the medium are Brain Heart Infusion (37 gms/L), sucrose (50 gms/L), agar no.1 (10 gms/L) and congo red stain (0.8 gms/L). CoNS isolates were plated on congo red agar plate and were incubated at 37°C for 24 h. The biofilm forming strains produced black colonies while non-forming strains developed red colonies.

Results and Discussion

During the study period 46 CoNS were isolated from various clinical specimens. Staphylococcus epidermidis (47%) was the common isolate followed by *Staphylococcus haemolyticus* (24%), *Staphylococcus saprophyticus* (24%) and *Staphylococcus lugdunensis* (5%).

CoNS isolates showed significant resistance to Ampicillin (76%), Clindamycin (74%), Cotrimoxazole (74%) Erythromycin (72%), Linezolid(6%). CoNS isolates revealed 100% sensitivity to Vancomycin. 33 (72%) CoNS isolates showed Methicillin resistance.

Out of 46 CoNS isolates 28 (61%) were biofilm producers by Congo Red Agar method

MIC of Vancomycin was <2µg. For MSCoNS MIC of Cefoxitin was <2µg and for MRCoNS MIC of Cefoxitin was >4µg.

CoNS are considered one of the most common causes of the device related infections in the recent two decades, especially in extreme age group of patients such as neonates and old age. The increasing prevalence of CoNS infections is attributable to their increasing antibiotic resistance and their ability to form biofilms on foreign bodies such as intravascular catheters [17,18,19]. In the present study *Staphlococcus epidermidis* (47%) was the common isolate followed by *Staphlococcus haemolyticus* (24%) and *Staphlococcus saprophyticus* (24%). This study correlates well with other studies conducted by Manjushree *et al.*, Singh *et al.*, Saroj Golia *et al.*, and Vijayashri *et al.*, who reported *S.epidermidis* was the most frequent isolate.

In the present study, antibiotic susceptibility testing showed all CoNS isolate were sensitive to Vancomycin and maximum resistance to ampicillin (76%). Similarly Vijayashri *et al.*, reported ampicillin resistance (79%), and no resistance to Vancomycin. Manju shree *et al.*, also reported all the CoNS isolates were susceptible to Vancomycin.

In the present study Methicillin resistance was observed in 72%.This was concordant with Vijayashri *et al.*, who reported Methicillin resistance in72% of CONS and Surekha *et al.*, reported 64.6% of Methicillin resistance. But lower Methicillin resistance 19.2 %was pubhilished by Manju shree *et al.*,

The ability of biofilm formation seems to play an essential role in the virulence of coagulase-negative staphylococci (CoNS). [20]Regarding the important role of genes associated with biofilm biomass production, the icaAD gene was found to be involved in biofilm formation, while the bap, fnbA, and cna genes were found to play a role in attachment to biotic or abiotic surfaces, which represents the first step of the process of biofilm formation. In the present study61% were Biofilm producers by CRA method.

Chart.1

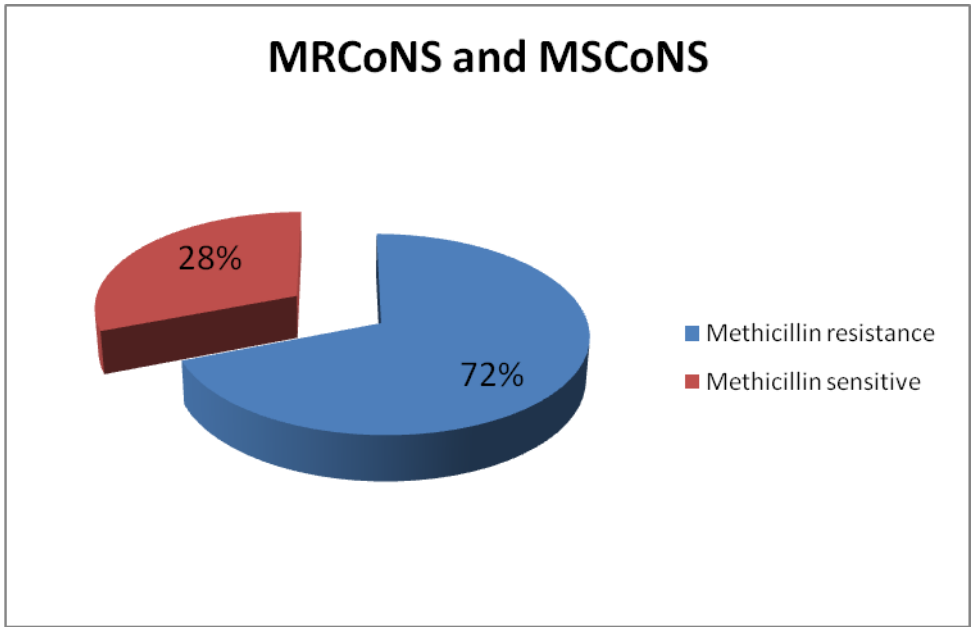

Fig.1 Congo Red Agar Method
Black colored colonies positive for biofilm production
Red colored colonies negative for biofilm production

Fig.2 MIC determination for Vancomycin and Cefoxitin

Similar findings were observed by Vijayashri *et al.,* who reported (69%) were Biofilm producers by CRA method. Mathur *et al.,* and Riyaz Sheriff *et al.,* identified that Tissue culture plate method was very sensitive and very specific method to detect the biofilm production.

Newer molecular methods available to detect genes responsible for biofilm formation. But in our study biofilm production was detected only by CRA method.

The high prevalence of biofilm producing strains and MR-CoNS demonstrated in this study indicates to follow the preventive measures to reduce the spread of MR-CoNS.

Increase in the use of medical devices, such as intravascular catheters and prosthetic devices, in neonates, will increase the prevalence of CoNS in infections. Biofilm-forming CoNS strains limit the immune system to counteract the infection. The Methicillin resistance and biofilm production of CoNS produce negative impact on clinical outcome of infected patients.

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