Original Article

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Biofilm Production and Antibiotic Susceptibility Pattern of Coagulase Negative Staphylococci from Various Clinical Specimens in a Tertiary Care Hospital

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Abstract

Introduction: Coagulase-negative staphylococci (CoNS) have become a common cause of nosocomial infections, particularly bloodstream infections and infections related to the prosthesis. They account for about 9% of the nosocomial infections. They cause infections in debilitated or immunocompromised patients and in patients fitted with urinary catheters, cardiac valves, pacemakers, and artificial joints. CoNS are becoming a problem in treating such infections by showing a considerable amount of antibiotic resistance. Biofilms play an important role in the pathogenesis and drug resistance of CoNS infections.

Materials and Methods: The present study was undertaken to study the biofilm production (slime production) and antibiotic susceptibility pattern of CoNS isolated from various clinical specimens.

Results: The distribution of CoNS from various clinical specimens was as follows: Blood 44 (42.72%), urine 29 (28.16%), pus 16 (15.53%), catheter tip 6 (5.83%), endotracheal tube 5(4.85%) and 1 (0.97%) each from peritoneal fluid, sputum and pleural fluid. *Staphylococcus epidermidis* 63 (55.34%) was the most commonly isolated species followed by *Staphylococcus haemolyticus* 22 (21.36%) and *Staphylococcus saprophyticus* 13 (12.62%). Congo red agar was used for studying biofilm production. Biofilm production was seen in 51 (49.51%) of isolates of CoNS. *S. epidermidis* was the predominant species showing biofilm production 27 (52.94%).

Conclusion: Antibiotic susceptibility testing showed multidrug resistance to commonly used antibiotics. All the isolates were sensitive to vancomycin, but all were resistant to penicillin.

Key words: Biofilm production (slime production), Coagulase-negative staphylococci, Nosocomial infections

INTRODUCTION

Historically coagulase-negative staphylococci (CoNS) have been considered as saprophytes with little pathogenic potential. However, under appropriate conditions they can produce serious human infections. They cause infections in debilitated or immunocompromised patients, also in patients fitted with urinary catheters, cardiac valves and artificial joints.[1]

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Some strains of CoNS produce a viscous extracellular material or slime (biofilm). These strains adhere to various biotic and abiotic surfaces. The test for biofilm production helps in deciding the pathogenicity of CoNS and is routinely used in diagnostic laboratories. Biofilm is defined as multicellular communities of bacteria, surrounded by extracellular polymeric matrix produced by the bacteria, which helps it to attach to various biotic and abiotic surfaces.[2,3] This three-dimensional biofilm structure is made up of extracellular matrix which comprises polysaccharides, proteins, enzymes, DNA, bacterial glycolipids, water and

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aggregates of microorganisms.[4] Biofilm development depends on many physical, chemical and biological factors.[5] In staphylococci polysaccharide intercellular adhesin (PIA), also known as a poly-N-acetylglucosamine is responsible for intercellular adhesion.[6] It is a partially deacylated polymer of β-1, 6-N-acetylglucosamine, which with the other polymers such as teichoic acids and proteins can form a major part of the extracellular matrix. Recently, PIA homologs are identified in many pathogens with biofilm formation ability, which shows that the three-dimensional matrix formation plays an important role in bacterial virulence.[7-9] Biofilm protects CoNS, against both; antibiotics used to treat infections and host immune system responses.

Hence, the present study is undertaken to study the biofilm production and antibiogram of CoNS.

MATERIALS AND METHODS

The present study is conducted on various clinical specimens obtained from the patients attending tertiary care hospital. Clinical sample is processed according to standard laboratory procedures. Speciation is done by the following tests-carbohydrate fermentation, phosphatase production, nitrate reduction, ornithine decarboxylation, urease production, novobiocin disc-test, and Polymyxin B resistance.

Biofilm production is studied using Congo red agar. Congo red dye was prepared as concentrated aqueous solution and autoclaved at 121°C for 15 min and added when the agar is cooled to 55°C. Plates are inoculated and incubated aerobically for 24-48 h at 37°C.

A positive result is indicated by black colonies with dry crystalline consistency. Non slime producers remained pink. Antibiotic susceptibility testing was done by Kirby-Bauer disc diffusion method following the Clinical and Laboratory Standards Institute (CLSI) guidelines.[10] Antibiotics tested vancomycin (30 mcg) cefoxitin (30 mcg), netilmicin (30 mcg), co-trimoxazole (25 mcg), Cefotaxime (30 mcg), norfloxacin (10 mcg), ciprofloxacin (5 mcg), chloramphenicol (30 mcg), penicillin-G (10 units), erythromycin (15 mcg), amikacin (10 mcg), gentamycin (10 mcg), and ampicillin (10 mcg).

RESULTS

A total of 103 strains of CoNS isolated in pure form from various clinical specimens are included in the study. The distribution of CoNS is as follows: Blood 44 (42.72%), urine 29 (28.16%), pus 16 (15.53%), catheter tip 6 (5.83%), endotracheal tube 5 (4.85%) and 1 (0.97%) from peritoneal fluid, sputum and pleural fluid, respectively. Maximum numbers of CoNS were isolated from blood specimen (Table 1 and Figure 1). *Staphylococcus epidermidis* 63 (55.34%) was the most commonly isolated species followed by *Staphylococcus haemolyticus* 22 (21.36%) and *Staphylococcus saprophyticus* 13 (12.62%). Slime production was seen in 51 (49.51%) of isolates of CoNS. *S. epidermidis* was the predominant species showing slime production 27 (52.94%) (Table 2 and Figure 2).

Antibiotic susceptibility testing is performed by Kirby-Bauer disk diffusion method following the CLSI guidelines. Multidrug resistance was seen to commonly used antibiotics. All the isolates were sensitive to vancomycin, but all were resistant to penicillin. Maximum sensitivity was seen to netilmicin 71 (68.93%). Cefoxitin is used as a marker of methicillin resistance. (Table 3 and Figure 3).

Table 1: Distribution of CoNS in various clinical specimens

Specimen	Total number of CoNS	Percentage
Blood	44	42.72
Urine	29	28.16
Pus	16	15.53
Catheter tip	6	5.83
Peritoneal fluid	1	0.97
ETT	5	4.85
Sputum	1	0.97
Pleural fluid	1	0.97
Total	103	100

CoNS: Coagulase negative staphylococci, ETT: Endotracheal tube

Table 2: Slime production in different species

Species	Total	Percentage
S. epidermidis	27	52.94
S. haemolyticus	19	37.25
S. Saprophyticus	3	5.88
S. Schleiferi	1	1.96
S. lugdunensis	1	1.96
S. cohnii	0	0
Total	51	100

S. epidermidis: Staphylococcus epidermidis, S. haemolyticus: Staphylococcus haemolyticu, S. Saprophyticus: Staphylococcus saprophyticus, S. Schleiferi: Staphylococcus schleiferi, S. lugdunensis: Staphylococcus lugdunensis, S. cohnii: Staphylococcus cohnii

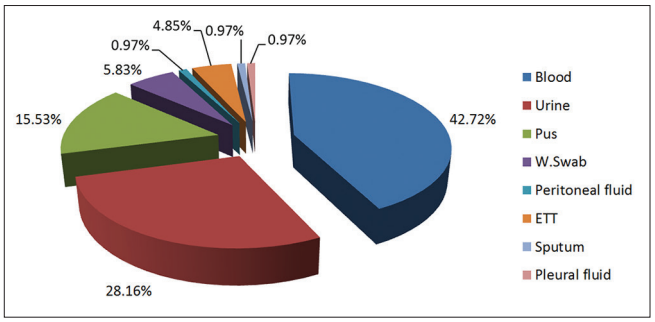

Figure 1: Distribution of coagulase negative staphylococci in various clinical specimens

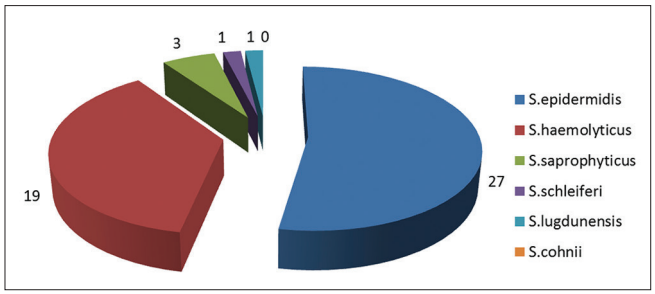

Figure 2: Slime production in different species

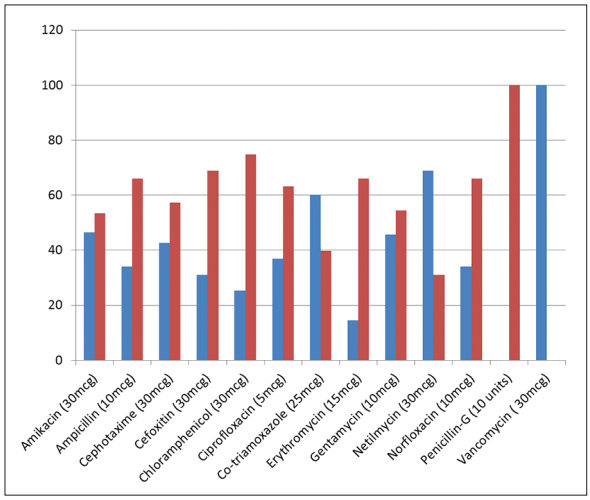

Figure 3: Antibiotic sensitivity pattern of coagulase negative staphylococci

DISCUSSION

CoNS cause hospital infections. An important step in the development of catheter or implant-associated infections caused by CoNS is the adhesion and attachment of these bacteria to biomaterial surfaces.

Among the various mechanisms involved in bacterial adhesion, the production of an extracellular polysaccharide substance called slime plays a relevant role. It strengthens the surface permitting the agglomeration of bacterial cells into biofilms. These biofilms render the cells less accessible to the defense system, thus impairing the action of antibiotics and in term represents the basic survival strategies of these micro-organisms.[11]

The slime matrix serves as a barrier to the diffusion inward of the antibiotics and may thus protect the enclosed staphylococci. Furthermore, there is evidence that ESS may specifically inhibit the action of some antibiotics.[12]

Table 3: Antibiotic susceptibility pattern in CoNS

Antibiotic (mcg)	Sensitive	Percentage	Resistant	Percentage
Amikacin (30)	48	46.60	55	53.39
Ampicillin (10)	35	33.98	68	66.02
Cephotaxime (30)	44	42.72	59	57.28
Cefoxitin (30)	32	31.07	71	68.93
Chloramphenicol (30)	26	25.24	77	74.76
Ciprofloxacin (5)	38	36.89	65	63.11
Co-triamoxazole (25)	62	60.19	41	39.81
Erythromycin (15)	35	14.56	68	66.02
Gentamycin (10)	47	45.63	56	54.37
Netilmycin (30)	71	68.93	32	31.07
Norfloxacin (10)	35	33.98	68	66.02
Penicillin-G (10 units)	0	0	100	100
Vancomycin (30)	103	100	0	0

Coagulase-negative staphylococci

CONCLUSION

CoNS are emerging as potential pathogens in the hospital settings. Biofilms have a role in the pathogenesis and drug resistance of CoNS infections. Various infection control measures are required to be taken to decrease transmission and reduce infections caused due to CoNS.

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