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COMPARATIVE STUDY ON DETECTION OF BIOFILM IN STAPHYLOCOCCUS SPECIES FROM CLINICAL SAMPLES BY USING PHENOTYPIC METHODS

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Abstract

Introduction: Staphylococcus aureus is considered to be a pathogen and coagulase Negative Staphylococcus (CoNS) is considered to be normal commensal, but is reported to cause infections, particularly with indwelling medical devices, in neonates and immunocompromised subjects. CoNS is also found to be linked to nosocomial infections. The ability to form biofilms plays an important role in the Staphylococcus species contribution to virulence. These biofilms, once established, make the cells less available to the organism's defense system, thus impairing antibiotic action and in turn, reflecting the basic survival strategies of these microorganisms. In order to detect biofilm development and also to test three phenotypic approaches, the current research has been scheduled.

Objectives: To detect biofilm production from clinical samples using phenotypic methods in Staphylococcus species, using: Tissue culture plate (TCP) process, Tube adherence method, and Congo red agar (CRA) method.

Methods: The current research was conducted from January to June 2020 at a tertiary healthcare centre over a period of six months. 56 Staphylococcus aureus and CoNS isolates were extracted from separate clinical samples in this prospective laboratory-based research. Normal biochemical tests identified all 56 isolates. TCP, CRA and tube adherence techniques were used to detect biofilm for all isolates.

Results: 48 (88 %) of 56 isolates were classified as Staphylococcus aureus and 8(14 percent) as CoNS. Biofilm production was 27(48 percent), 23(41 percent) & 26(46 percent) by TCP, Tube process and Congo red agar method, respectively. The three experiments were all conducted in triplicate. TCP as the gold standard Tube process sensitivity and specificity was 87.50 percent & 100 percent and 92 percent & 100 percent respectively by the CRA method. A statistically significant correlation was found between the red agar system of TCP and Congo. Congo red agar system sensitivity with TCP was 92 %, accuracy was 100 percent, 100 percent positive predictive value and 93.94 percent negative predictive value.

Conclusion: CRA has become more susceptible to phenotypic methods for biofilm detection and can be easily done in routine laboratories.

Keywords: Biofilm detection, Staphylococcus, Phenotypic methods

Introduction

Coagulase negative Staphylococci (CoNS) are a significant component of the cutaneous ecosystem's natural flora and function as commensal or saprophytic organisms[1,2]. However, if they invade the host tissue through cutaneous barrier trauma, needle inoculation, or medical device implantation, they can grow into a pathogen[3,4,5]. In immunocompromised subjects, premature newborns, and subjects with embedded biomaterials, CoNS acts as an essential pathogen and its pathogenicity and bacteremia is mainly due to the ability to form biofilms. Once established, these biofilms make the cells less available to the organism's protection system,

thus impairing the action of antibiotics. Various methods for the identification of biofilm production are currently in use[6,7]. Qualitative approaches can be used in routine laboratories, such as the tube adherence test, Congo red agar method and the quantitative method. Due to the increasing clinical importance of CoNS, accurate CoNS species identification is highly desirable to enable a more accurate determination of CoNS host-pathogen relationship, biofilm-producing strains are often more resistant to antibiotics, so current research has been conducted to classify the CoNS at species level and to look for the antibiotic resistance pattern[8,9,10].

MATERIAL AND METHODS:

This research was conducted at India's rural tertiary care hospital. Coagulase negative Staphylococci isolated from the sterile clinical samples of subjects attending and admitted to tertiary care hospital were collected in this prospective laboratory-based analysis and compared using different phenotypic and genotypic methods to detect biofilm formation in Coagulase negative Staphylococci (CoNS).

Isolation: Both CoNS isolates were included in the analysis from sterile clinical samples during the study period. A total of 88 % therapeutic isolates were recovered from sterile clinical samples. All samples were further incubated at 37 °C for 24 hours after culturing on the agar plates. All growths were processed according to standard guidelines on the next day.

Identification of Coagulase negative Staphylococci: By placing several biochemical checks, CoNS was established as per standard guidelines Antibiotics susceptibility testing was performed by Kirby-Bauer disc diffusion process. The strength of the discs used was as per the CLSI 2013 recommendation.

Biofilm detection: The capacity of the organism to form biofilm was detected by using the growth obtained from the cultures.

Controls: Biofilm-producing reference strain of Staphylococcus epidermidis (ATCC 35984) and nonbiofilm forming reference strains of Staphylococcus aureus (ATCC 25923)

Statistical Analysis: The software used in the analysis were SPSS 17.0 and Graphpad Prism 5.0 version.

RESULTS:

88 (10.46 percent) of the total 841 culture positive isolates were identified as CoNS, 86 (97.73 percent) CoNS were isolated from the blood out of 88 isolates identified as CoNS, and one (1.14 percent) each from pleural fluid and CSF. All 88 CoNS isolates were of the paediatric departments.

TABLE 1: Species distibution of CoNS isolates

Species	Total	%
S. epidermidis	49	55.68%
S. haemolyticus	15	17.05%
S. capitis	12	13.64%
S. xylosus	6	6.82%
S. cohinii	3	3.41%
S. lugdunensis	2	2.27%
S. saprophyticus	1	1.14%
Total	**88**	**100.00%**

49 (55.68 percent) of the total of 88 isolates were classified as S. epidermidis. 15 (17.05 percent) have been classified as S. Haemolyticus, 12 (13.64%) is recognised as S. Capitis, 6 (6.82 percent) was named S. xylosus, 3 (3.41%) as S. Cohinii and so on. Of the total biofilm production

of isolates, 61(69.32 percent) Tissue Culture Plate (TCP) isolates were observed, of which 32 (36.36 percent) were heavy biofilm producers, while 29 (32.95 percent) were moderate biofilm producers. The production of biofilm by the Congo red agar method was 60 (68.18 percent), of which 20 (22.73 percent) were large and 40 (45.45 percent) were moderate producers of biofilm. The production of biofilm in the tube method was in 56 isolates (63.64 percent), of which 18 (20.45 percent) were strong and 38 (43.18 percent) were moderate producers of biofilm.

Table 2: Comparison of biofilm detection methods

Method		TCP		Sensitivity	Specificity	PPV	NPV
		Producer	Non producer				
Tube	Producer	53	3	86.89	88.89	94.64	75
	Non producer	8	24				
CRA	Producer	56	3	91.8	88.89	94.92	82.76
	Non producer	5	24				

TCP-Tissue culture plate method, CRA- Congo red agar method, PPV- Positive predictive value, NPV- Negative predictive value

TABLE 3: Antibiotic resistance pattern of biofilm producing and non-biofilm producing CoNS (by TCP method)

Antibiotics	Biofilm Producing (n= 61)	%	Non biofilm producing (n= 27)	%	א2-value p-value
Erythromycin	29	47.54%	9	33.33%	4.67 p=0.031,S
Penicillin	41	67.21%	12	44.44%	10.7 p=0.001,S
Trimethoprim-sulphamethoxazole	18	29.51%	7	25.93%	0.39 p=0.52,NS
Gentamycin	7	11.48%	3	11.11%	0.000 p=1.000,NS
Ciprofloxacin	11	18.03%	4	14.81%	0.52 p=0.32,NS
Oxacillin	27	44.26%	5	18.52%	14.5 p=0.000,S
Linezolid	0	0.00%	0	0.00%	-

Of the total of 61 TCP-based biofilm isolates, 29 (47.54 percent) were erythromycin-resistant, 41 (67.21 percent) were penicillin-resistant, 18 (29.51 percent) were trimethoprim-sulphamethoxazole-resistant, 7 (11.48 percent) were gentamycin-resistant, 11 (18.03 percent) were ciprofloxacin-resistant, 27 (44.26 percent) were oxacillin-resistant and none were Linezolid-resistant. Of the 27 non-biofilm-producing isolates: 9 (33.33%) were resistant to Erythromycin, 12 (44.44%) were resistant to penicillin, 7 (25.93%) were resistant to trimethoprim-sulphamethoxazole, 3 (11.11%) were resistant to gentamycin, 4 (14.81%) were resistant to ciprofloxacin, 5 (18.52%) were resistant to oxacillin, and no isolate was resistant to linezolid.

DISCUSSION:

In different infections and most commonly isolated organisms in nosocomial infections, Coagulase-negative Staphylococci (CoNS) has been recognised as the etiological agent and very little is known about the virulence factors generated by CoNS that contribute to the pathogenesis of infections caused by these microorganisms. To differentiate between infecting from contaminating isolates and to plan effective therapy, a thorough characterization of isolates of coagulase-negative Staphylococci through speciation, genetics and antibiotic susceptibility may be required[11-16]. Qualitative methods such as the tube adherence test described by Christensen et al, the Congo red agar (CRA) method described by Freeman et al and quantitative methods such as the tissue culture plate (TCP) assay described by Christensen et al are used in routine laboratories for identification of biofilm in clinical samples. In our analysis, the isolation rate of the CoNS was 10.46 percent out of 841 culture positive samples, which was almost close to the isolation rate of the study by Silvia Natoli et al which was 9.24 percent. CoNS isolation from neonates was 49 (55.68 percent) in our study, 30 (34.09 percent) from the paediatric age group, while 9 (10.22 percent) was less in adults. These figures were different from Rajeevan et al 's analysis. The separation of CoNS from adults was 94.12 percent and 5.88 percent in the paediatric age group. This high incidence of overall newborn CoNS isolation may be attributed to the maximum number of samples obtained from the neonatal age group[17-20]. Even if they are found in blood or cerebrospinal fluid, many clinical microbiology laboratories do not recognise CoNS at the level of the species. However, it is important to classify them at the species level because of the increasing importance of CoNS, and for true correlation, to learn more about the epidemiology and pathogenic potential of individual species. With regard to blood culture isolates, this may be especially important as it is often difficult to assess the clinical significance of an individual isolate. The percentage of biofilm production in various studies ranges from 14 percent to 88 percent. The sensitivity of PCR with TCP was 91.80 percent, precision was 100 percent, positive predictive value was 100 percent, negative predictive value was 84.38 percent. The statistically relevant correlation between PCR and TCP was identified. There is very strong agreement between PCR and TCP (k=0.87) in Kappa statistics. The CRA method was more sensitive as compared to the CRA and Tube method, but the specificity was the same. Compared to TCP, the accuracy of the CRA test was more important. A significant association between the development of biofilm and the existence of ica genes was found[21].

CONCLUSION:

In conclusion, CoNS can cause infections in paediatric subjects and may be responsible for medical device infections. Tube and CRA methods are the best way to detect biofilm formation, and it can be easily detected by CRA method as it is cost-effective and can be done in any laboratory.

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