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STUDY OF BIOFILM FORMATION AND ANTIMICROBIAL RESISTANCE PATTERN AMONG CLINICAL ISOLATES FROM MEDICAL DEVICE RELATED INFECTIONS AT A TERTIARY CARE HOSPITAL

Clinical Microbiology

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ABSTRACT

Background: Biofilms are usually associated with indwelling medical devices. Implementation of efficient diagnostic techniques for the biofilm production in diagnostic laboratories would help in early detection of pathogenic strains producing biofilms and thus minimize the disease burden. **Methods:** This is a prospective study conducted in the Department of Microbiology, Guntur Medical College over a period of 18 months from September 2022 to March 2024. Samples from 100 in-patients suspected of infected indwelling medical devices at Government General Hospital, Guntur were analyzed and phenotypic detection of Biofilm is performed using Congo Red Agar method. **Results:** Out of the total 63 micro-organisms isolated, 39 (61.9%) were detected to be producing biofilms. **Conclusion:** Higher antimicrobial resistance among the isolates is clearly evident to be because of the biofilm producing ability of the organisms. Congo red agar method can be advocated in routine clinical testing for screening of biofilm formation as it is accurate, economical and easy to perform.

KEYWORDS

biofilm, device related infections, antimicrobial resistance, clinical isolates

INTRODUCTION

A Bio-film is an assemblage of surface associated microbial cells enclosed in an extracellular polymeric substance matrix. Microorganisms growing in a biofilm are intrinsically resistant to most of the antimicrobial agents. Biofilms are usually associated with certain health conditions like peritonitis, dental plaque, respiratory tract and urinary tract infections along with indwelling medical devices.

Device related infections like Catheter- associated urinary tract infections, Central line- associated blood stream infections, Implant associated infections, and Ventilator- associated pneumonia etc. are important to understand because of the morbidity and mortality associated with them. Further these infections often remain a diagnostic challenge and are often difficult to treat due to the antimicrobial resistance.

The various methods to detect biofilm production include, Tissue Culture Plate method (TCP) which is considered as the gold standard method, Tube adherence Method (TM), Congo Red Agar method (CRA), bioluminescent assay, piezoelectric sensors, and fluorescent microscopic examination. Various genes involved in the synthesis of biofilm can be detected by RT-PCR.

MATERIALS & METHODS

Study Design

This is a prospective study conducted in Department of Microbiology, Guntur Medical College over a period of 18 months from September 2022 to March 2024. The sample size was 100. Approval of the Institutional Ethics Committee has been obtained while beginning the study. Demographic data and history were obtained from the patient and explained about the study procedure. Informed consent was taken in local language. Patients of all age groups and both sexes suspected of infected indwelling medical devices at Government general hospital, Guntur were included in the study. Patients with infected indwelling medical devices diagnosed to have any other infectious diseases and Patients not willing to be as study participants were excluded.

Figure 1: Congo Red Agar method demonstrating Biofilm formation. (Black colour colonies with crystalline consistency indicating biofilm producers and red colour colonies biofilm non producers.)

Culture And Microscopy

Culture of the samples is done by roll plate method. The samples received in laboratory were streaked on Blood agar and MacConkey agar. The culture plates were incubated at 37° C for 24 hours. Any growth on the plates was identified by colony morphology, pigmentation, odour, gram staining and standard biochemical tests. Antibiotic sensitivity was done on MHA by Kirby bauer disk diffusion method as per CLSI guidelines. Phenotypic detection of Biofilm is performed using Congo Red Agar method.

RESULTS

Out of the total 100 samples, 38 samples didn't have any growth or normal commensals were grown. 62 samples were culture positive with growth of different micro-organisms. 36 were males (58%) & 26 were females (42%). Males outnumbered females in this study. Higher incidence of device related infections among the samples collected & processed in this study is seen among 48-63 years. Total number of organisms isolated are 63. Klebsiella (36.5%), S.aureus (19%), Pseudomonas (13%), CONS (11%) Acinetobacter (8%), E.coli (6%), Enterococci (3%) and Candida (3%). Biofilm production ability was detected by CRA method and 39 micro-organisms (61.9%) were detected to be producing biofilms. Predominantly, Klebsiella species isolates produced biofilm which accounts to 38.4% of the total isolates followed by S.aureus (23.0%), Pseudomonas (12.8%), CONS (10.2%), Acinetobacter (7.6%), E. coli (5.1%) and Enterococci (2.5%). Antibiotic resistance is higher in isolates producing Biofilm when compared with Biofilm non-producing organisms.

DISCUSSION

There are different methods for biofilm detection. Many studies were conducted comparing these detection methods. T A Dhanalakshmi et al. concluded in their study that Congo red agar and tube methods can be considered for detection of biofilms in resource constraint conditions. In our centre we have tried Congo red agar method in detecting biofilm formation for routine diagnostic purpose and the findings were reported in this study. Many other studies detected biofilm formation from all the clinical isolates whereas we focussed on detecting the biofilm formation ability of organisms isolated from medical device related infections. In this study we evaluated 63 isolates from 100 different samples by Congo Red Agar method out of which 39 isolates (61.9%) were detected as biofilm producers.

Table 1. Comparison Of Biofilm Detection By CRA Method With Different Studies

Auhors	Year	Place	Biofilm detection
Allam et al.	2017	Egypt	64.2%
Dhanalakshmi *et al*	2018	Karnataka	46.9%

R Dumaru *et al*	2019	Nepal	62.7%
L Raksha et al	2020	Karnataka	76.7%
Basnet A et al.	2023	Nepal	38.9%
Present study	2024	Guntur	61.9%

In our study the most common organisms associated with DRI are Klebsiella species (38.4%), followed by S. aureus (23.0%) and Pseudomonas (12.8%). Noteworthy finding in our study is even though Klebsiella species is mostly associated with DRI, the organism with highest biofilm producing ability is Staphylococcus aureus. 75% of S. aureus isolates i.e., 9 among 12 isolates are positive for biofilm formation by CRA method whereas 15 out of 23 i.e., 65.2% isolates of Klebsiella species are positive for biofilm formation. The antibiotic resistance for various drugs tested is apparently higher in biofilm producing organisms. Our findings corelated with that of other studies such as Ghadiri H et al. (2014), Heydari S et al. (2015), Punia P et al. (2016), and Shrestha LB et al. (2017) which documented that there is a higher proportion of antibiotic resistance in biofilm producers when compared to non-producers.

CONCLUSION

DRI appear to be the major threats to the safety of patients being admitted in a hospital as they tend to increase patient morbidity and mortality along with excess costs and prolonged hospital stay. Moreover, there is very limited data available on the bacteriological profile and antibiotic resistance pattern of such infections in our country. This study aimed to identify the organisms associated with medical device related infections, the antibiotic resistance pattern of different organisms isolated and to detect biofilm formation capability of the isolated organisms. Congo red agar method used for determining biofilm formation can be advocated in routine clinical testing for screening as it is accurate, economical and very easy to perform. There is a lot of scope in future for research on newer techniques for identifying biofilm producing ability of the micro-organisms and also manufacturing different medical devices that prevent bacterial adherence thus hindering biofilm production by the micro-organisms.

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