International Research Journal of Natural and Applied Sciences
ISSN: (2349-4077)
Impact Factor 7.032 Volume 9, Issue 08, August 2022
Website- www.aarf.asia, Email : editor@aarf.asia , editoraarf@gmail.com

CHARACTERIZATION OF BIOFILM POSITIVE ISOLATES FROM MICROBIAL INFECTIONS AT SURGICAL SITE INFECTIONS

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Abstract
Surgical site infections are more unlikely to occur when surgical equipment is managed with care, and those that implement best practice standards may decrease SSI-related morbidity and mortality. The cause of more than 80% of people's SSIs, includes persistent infection of wounds, is biofilm-forming microbes. The objective of the research is to characterise the biofimpositive isolates.Methodology used in the present study of biofilm screening of clinical staphylococcal strains was done by Test tube method and Microtiter plate method. Once Staphylococcus colonize, they develop recalcitrant biofilms and pose a challenge to the treatment. Among the various pathogens, Staphylococcus spp., are predominantly observed in device associated infections. Advancement in medical technology has promoted the use of various indwelling medical devices in patients' treatment. Use of these devices have certainly improved the health status of patients, but in the meantime, they are also causing infections through biofilm production.

Keywords:*Surgical site infection, biofilm, isolates, microbes*

Introduction

Surgical site infection (SSI) as an illness which appears within 30 to 90 days following surgery. 30 to 90 days upon surgical is considered the development period for deep and organ/space SSIs (CDC, 2022). A surface incisional SSI is regarded as having started within 30 days following surgery. Beneath incisional SSIs affect the incision's surrounding tissues that are soft, such as the connective tissues and tendon layers. In surface incisional SSIs, an incision's epithelium and tissue underneath are implicated (Mirzaei et al., 2020). The most hazardous SSIs are organ/space SSIs. Any area of the body that extends beyond the fascial or muscle levels that are worked on throughout anaesthesia is affected. Insignificant incisional SSIs resulting in the individual's additional incision after various puncture of a surgical procedure. Similar criteria may also be used to classify deep incisional SSIs (CDC, 2022).

The formations of bio film are the recent discoveries about the microorganisms triggering biofilm-related implanted infections (Emaneini et al., 2015). Medical implant failure is most often caused by biofilm-related infection, which is still challenging to identify and treat (Mirzaei et al., 2020). Our knowledge of the make-up and intricate operations of the microbiota inhabiting distinct body site niches has been improved by recent technologies(Hrynyshyn et al., 2022). The function of the microbiomes in the surgical sites in implants-related biofilm production, and prophylactic methods to reduce implant enrolment of skin, nasopharynx, and neighbouring mucosal microorganisms on biofilm creation and illnessSerbanescu et al., (2023).In this study, aim is to investigate microbial biofilm positive isolates at surgical sitesinfections.

Material and method

The present study was conducted in the period of September 2020 to December 2022.
The biopsy specimens were taken from several general surgical facilities in Rajasthan with the following geographic characteristics: The high temperature was 41°C, while the low was 15°C.

Laboratory analysis

Within two hours, swab samples from recuperating diseases had been handled and evaluated. Different techniques were used to analyse the samples, including enriching them, microscopy inspection, isolation, and bacterium identification of pathogenic microbes.
Every individual had a positive Staphylococcus examination. On the day of hospital stay, the patient's nasal, pharyngeal, and femoral regions were swabbed, and any kinds of staph aureus that were recovered from the sample cultured were evaluated. Surgery was postponed for patients with positive tests with unresolved angina while they had olfactory mupirocin treatment, daily chlorhexidine treatment downpours, in addition to for individuals with MRSA-positive swabs of their throats, applied the chlorhexidine 0.2% administration for three days.
In present study, biofilm screening of clinical staphylococcal strains was done by Test tube method and Microtiter plate method.

Test tube method

Formation of biofilm will be observed based on the adherence of crystal violet on the walls and bottom of the test tubes. Depending on the adherence of crystal violet to the biofilm, strength was determined by mentioning Strong as "+++", Moderate as "++" and Weak as "+". All the staphylococcal strains were initially screened using this method.

Microtiter Plate Method

Biofilm screening by crystal violet method was performed using a modified protocol. The re-solubilized dye was transferred to a new microtiter plate and OD was measured at 570 nm in a spectrophotometer.

Data analysis

Considering the average, the meaning deviation, samples deviation, standard deviation, and coefficient of deviations, these numbers were investigated using statistics using Excel spreadsheet.

Result and Discussion

Source of isolation and clinical details of biofilm positive isolates

Details of biofilm positive clinical isolates with their clinical symptoms and source of isolation, and infection-wise distribution of biofilm positive isolates is shown in Figure1. Sample numbers given by hospitals has been changed and patients' data is not revealed.

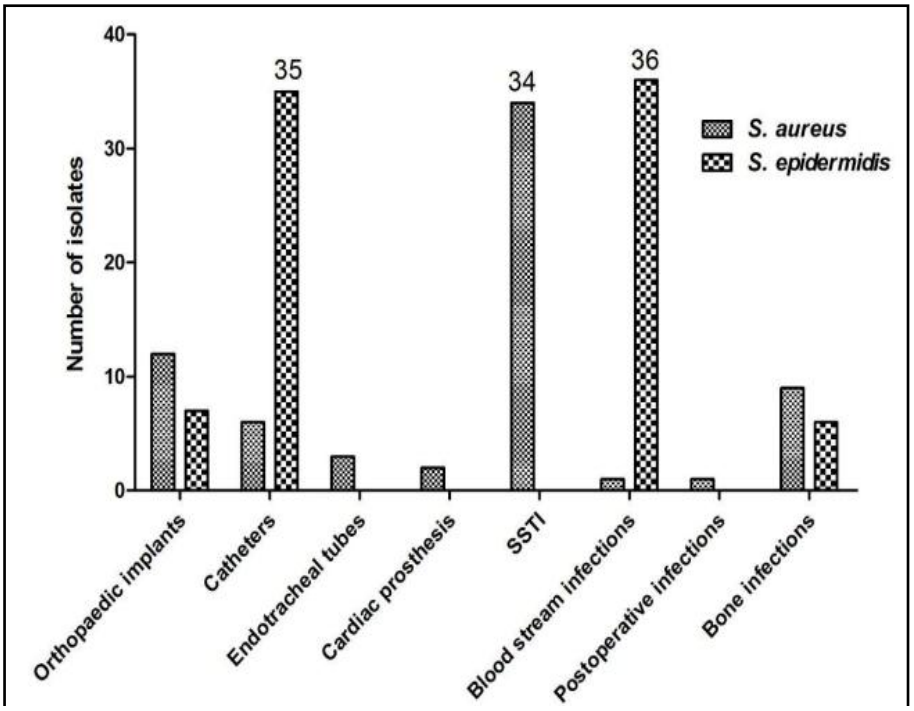

Figure 1: Number of S. aureus and S. epidermidis positive isolates from hospitals

The prevalence of S. aureus and S. epidermidis was studied by collecting the clinical isolates obtained from hospitals. After the calculation of sample size, total of 384 clinical isolates were collected. Some of these hospitals did not confirm Staphylococcal species using molecular tests like PCR. Hence, after collecting the isolates from hospitals, they were subjected to biochemical tests and species were confirmed through PCR. Based on the phenotypic and molecular screening, out of 384 isolates, 299 (67%) were confirmed as Staphylococcal strains. This shows that 86 (33%) of the collected isolates were non-Staphylococcal strains and the hospital staff has misinterpreted these strains as Staphylococcal species. Thus, two-tier confirmation of Staphylococcal strains using phenotypic tests and PCR helped us to rule-out non-staphylococcal strains. Among the confirmed Staphylococcal strains, 210 isolates (70%) were S. aureus and 89 (30%) were identified as S. epidermidis.

Prevalence of staphylococcal strains from respective hospitals is shown in Figure 1. Hospital showed highest prevalence for S. aureus and St. John's hospital showed highest prevalence for S. epidermidis. All the 299 Staphylococcal strains were screened for biofilm production using test tube method and microtiter plate test. Test tube method displayed higher sensitivity, but it failed to identify most of the biofilm positive isolates (Figure 1). Test tube method was able to identify 123 biofilm positive isolates. Whereas microtiter plate method identified 158 biofilm positive isolates. There was a difference of 35 isolates which is unacceptable in prevalence studies. Also, microtiter plate method showed higher positive predictive value and lower negative predictive value. This clearly shows that the modified microtiter plate method which we used in this study is very effective in screening the strength of biofilms. Previous studies have also reported the increased 56 reliability of microtiter plate test (Serbanescu et al., 2023; Walker et al., 2017). Thus, the prevalence of biofilm associated strains were finalized based on the readings obtained from the modified microtiter plate method.

The prevalence of device associated, and non-device associated biofilm positive isolates were further studied using the results obtained from microtiter plate method. Among the biofilm positive isolates 87 (55%) were non-device associated isolates and 65 (41%) were device-associated isolates. Voluminous research has shown increased prevalence of Staphylococcal strains in device-associated infections. But in the past ten years there are considerable reports

on non-device associated infections caused by staphylococcal strains. A recent study from Agartala Medical College at Tripura, Assam has shown 55% of biofilm producing S. aureus strains out of 100 collected isolates (Bhattacharya, Bir, & Majumdar, 2015). In this study, strains were obtained from non-device associated infections like pus, urine, blood, sputum, vaginal secretions etc. Majority of non-device associated isolates in our study were found to be S. aureus associated with SSTI.

Conclusion

It is imperative from these observations that S. aureus and S. epidermidis are predominant in device and non-device associated infections. Interestingly, biofilm producing S. epidermidis is mainly reported in device-associated infections.

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