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Original Research Paper

Staphylococcus aureus biofilm formation from the isolates of clinical cases: A prospective study

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ABSTRACT:

Background: The aim of the present study is to assess the biofilm production among clinical isolates of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in Tertiary Care Hospital, Central India. This aim were achieved under the following objectives such as antibiotic susceptibility pattern of Methicillin-Sensitive *Staphylococcus aureus* (MSSA) and MRSA among isolate and phenotypic pattern of MRSA and detection and prevalence of biofilm producing MSSA and MRSA isolated obtain from various clinical samples. **Methods:** This is a study that focuses on description and observation. Following receipt of approval on an ethical level from the Institutional Ethical Committee of Index Medical College Hospital & Research Centre in Indore, the research project was carried out. **Results:** We observed highest number of pus isolates that are Biofilm producers in MSSA & MRSA, which accounts to 20 & 24 respectively. The strong Biofilm producers accounts to 7 cases in MSSA and 6 cases in MRSA cases out of 70 and 44 respectively. Whereas weak Biofilm producers accounts to 38 cases in MSSA and 28 cases in MRSA subjects. When compared between the two groups to identify the significance of Biofilm producing capacity, we observed a significant change ($p < 0.05$). **Conclusion:** Biofilm formation detection is a simple and cost-effective laboratory test that can be performed on a regular basis. A better understanding of biofilm will allow clinicians to better treat the infections, resulting in a lower mortality and morbidity rate for patients.

Keywords: Staphylococcus aureus, Methicillin-Sensitive Staphylococcus aureus, biofilm , Multidrug resistance

INTRODUCTION:

Antibiotic resistance is responsible for 700,000 deaths worldwide each year [1,2]. Regrettably, if no immediate and effective action is taken, the death toll could rise to 10 million annually by the year 2050. A biofilm forms when microorganisms settle on a surface and become encased in a film of slime. By producing an extracellular matrix substance, slowing growth rates, and activating or deactivating specific genes, biofilm-associated cells can be distinguished from their liquid-dissolving counterparts [3,4]. Attachment is a multi-step process that is influenced by the growth medium, substrate, and cell surface properties. MRSA ability to form biofilms on both living and nonliving surfaces makes the problem even more difficult to solve. Staphylococci have been known for a long time to be the most common cause of infections that are related to biofilms [2]. Antibiotics were first developed with the intention of targeting individual bacterial cells; however, the majority of research has been conducted on bacteria growing in planktonic cultures [5,6]. It has become abundantly clear that bacteria have a marked preference for sessile communities [1,3]. There is evidence to suggest that biofilms are the cause of nearly 80 percent of all human infections, and one of the most important characteristics of biofilms is their high level of resistance to antibiotics, host immune defenses, disinfectants, and environmental stress [7,8]. Biofilms on medical devices, such as

catheters and mechanical heart valves, joint prostheses and orthopedic devices, can also be a source of endocarditis and osteomyelitis [9,10]. Both conditions are potentially life-threatening infections. Biofilm cells are more resistant to antibiotics than planktonic cell cultures [10]. This is due to the presence of an extracellular matrix (which prevents antibiotics from penetrating the cell), altered metabolic states, and faster cell growth [10]. Even more challenging for chemotherapeutic treatment is the capacity of MRSA strains to form biofilm, as well as the frequently associated multidrug-resistant profile of these strains. Bacteria that are in close quarters with one another are more likely to engage in horizontal genetic transfer, conjugation, and movement of antimicrobial resistance genes within the biofilm [1-3].

The aim of the present study is to assess the biofilm production among clinical isolates of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in Tertiary Care Hospital, Central India. This aim was achieved under the following objectives such as antibiotic susceptibility pattern of Methicillin-Sensitive *Staphylococcus aureus* (MSSA) and MRSA among isolate and phenotypic pattern of MRSA and detection and prevalence of biofilm producing MSSA and MRSA isolated obtain from various clinical samples.

MATERIAL AND METHODS:

This is a study that focuses on description and observation. Following receipt of approval on an ethical level from the Institutional Ethical Committee of Index Medical College Hospital & Research Centre in Indore, the research project was carried out.

Subjects Criteria:

For the purposes of this study, only 170 specimens that had been received at the microbiology laboratory of Index Medical College Hospital & Research Centre in Indore in response to a physician's order for an investigative procedure were considered for inclusion. Standard procedures for microbiological sampling were followed in the collection of the samples [11]. Spotless, leak-proof, screw-capped containers delivered the samples. There were no obvious signs of contamination, and the containers were correctly labeled with patients' demographic information. The study included all S. aureus isolates from HAI patients treated in outpatient and inpatient departments. The study proforma was completed in its entirety. Patients receiving anti-retroviral therapy were excluded from the study. A total of 170 samples were used for the study.

Sample collection:

In the hospital's microbiology laboratory, pus, urine, blood, sputum, urethral swab, CSF, and pleural fluid were processed for culture and antibiotic susceptibility testing [12]. Isolation and characterization of S. aureus involved inoculating all the samples directly onto blood agar and MacConkey agar. Following gram staining, possible colonies of S. aureus were transferred to mannitol salt agar and allowed to grow at 37 degrees Celsius for 24 hours. The characteristics of S. aureus colonies that were grown on mannitol salt agar were used to identify the colonies. S. aureus was identified by its characteristic golden yellow color, round, convex, and opaque colonies. The colonies were then transferred to nutrient agar and incubated at 37 degrees Celsius for twenty-four hours. In addition, confirmation was accomplished through the utilization of biochemical tests such as catalase, oxidase, DNase, and coagulase tests [11].

Examination of S. aureus strains that produce biofilm:

Using tissue culture plates, biofilm-producing S. aureus strains were screened. This quantitative test followed [11]. In a nutshell, a loopful of test organisms were isolated from a freshly prepared agar plate, and they were inoculated in 2 mL of tryptic soy broth (TSB). A 37-degree Celsius overnight incubation was performed on the broth. After that, the culture was diluted with fresh TSB medium to a ratio of 1:100. The individual wells of a 96-well microtiter plate were each given 200 L of diluted culture broths to be placed in them. In a manner not dissimilar to that described above, the organisms serving as controls will also be processed. The plate was kept at 37°C for a day. After the incubation period was complete, the contents of each well were extracted using a tapping motion. In order to get rid of any free-floating bacteria, the wells were washed four times with a total volume of 200 L of phosphate buffer saline with a pH of 7.3. After being fixed with sodium acetate at a concentration of 2 percent, the biofilms produced by bacteria that were adherent to the wells were stained with crystal violet at a concentration of 0.1 percent in a volume of 100 L for 15 minutes at room temperature. After carefully rinsing the plate with deionized water to remove any excess stain, the plates were stored away to dry. After solubilizing the attached biofilm in ethanol at a concentration of 95 percent, biofilm concentration was determined by

measuring the absorbance of the sample at 630 nm using an ELISA reader. The experiment was done three times with three different materials. The interpretation of the production of biofilm was carried out using the criteria that were mentioned [12]. The following standards were utilized as the criteria: Non-producers have an Optical Density (O.D.) value lower than O.D., weak biofilm producers have an O.D. value less than 2 O.D., moderate biofilm producers have an O.D. value between 2 O.D. and 4 O.D., and strong biofilm producers have an OD value greater than 4 O.D. The cut-off O.D. was three standard deviations higher than the average negative control O.D.

a) Antibiotic Susceptibility Test: The Kirby–Bauer disk diffusion method was used to test the antibiotic susceptibility of each of the isolates to the various antibiotics. This method is recommended by the Clinical Laboratory Standard Institute [13]. Using a sterile cotton swab, the Mueller-Hinton agar surface was swabbed uniformly and compared to the McFarland 0.5 turbidity standard. Then, antibiotic discs were placed, including tetracycline, ciprofloxacin, gentamycin, clindamycin, cotrimoxazole, erythromycin, vancomycin, linezolid, and penicillin. The plates were then kept in an incubator at 37 degrees Celsius for the entire night. The zone diameter organism was classified as resistant, intermediate, or sensitive after exposure to the pathogen. According to the susceptibility pattern of the isolates, the bacteria that were multidrug resistant (MDR) were resistant to three or more than three classes of antibiotics. The susceptibility of S. aureus isolates to cefoxitin (30 g) was determined using a modified Kirby–Bauer disc-diffusion method according to CLSI guidelines. These cefoxitin-resistant S. aureus strains were screened to determine if they were MRSA [13].

b) Controlling Quality: The quality of the prepared media was examined by putting one plate from each batch through a sterility test and a performance evaluation. Purity plates were utilized to ensure that the inoculation that was utilized for the biochemical tests was of the highest possible quality and to determine whether or not the biochemical tests were carried out in an aseptic environment. Both S. aureus ATCC 700,699 (MRSA) and S. aureus ATCC 29,213 (MSSA) were utilized as part of the quality control process.

Statistical Analysis:

All the information that was gathered was entered into SPSS (version 22.0), and then it is going to be analyzed. Between MRSA and MSSA, a descriptive analysis as well as a comparison of antibiotic susceptibility, MRSA, and the formation of biofilm was carried out.

RESULTS:

Table 1 shows the Biofilm producers of isolates of MSSA as well as MRSA subjects. We observed highest number of pus isolates that are Biofilm producers in MSSA & MRSA, which accounts to 20 & 24 respectively. Next blood culture isolates were Biofilm producers.

Table 2 shows the comparison between Biofilm producing capacity in MSSA and MRSA subjects in the present study. Th strong Biofilm producers accounts to 7 cases in MSSA and 6 cases in MRSA cases out of 70 and 44 respectively. Whereas weak Biofilm producers accounts to 38 cases in MSSA and 28 cases in MRSA subjects.

Figures 1 & 2 show the percentage of Biofilm producers in MSSA and MRSA group that we observed in the present study. When compared between the two groups to identify the significance of Biofilm producing capacity, we observed a significant change ($p < 0.05$).

DISCUSSION:

Using a high-throughput polystyrene 96-peg plate format, 114 clinical isolates of Staphylococcus aureus were examined to determine whether or not they were capable of forming biofilms. Patients at Index Medical College & Hospital provided the source material for the collection of 44 MRSA and 70 MSSA S. aureus isolates. A measurement of each biofilm's biomass was obtained through the use of crystal violet staining. Isolates were categorized as fully established biofilms, moderately attached biofilms, or weakly attached biofilms with the help of a biofilm-forming strain that was previously identified. The percentage of MRSA and MSSA isolates that formed biofilms that were moderately adherent was 38 and 61.4 percent, respectively, while the percentage of those that formed highly adherent biofilms was 23 and 25 percent, respectively. 14 percent of the MRSA isolates and 12 percent of the MSSA isolates had biofilms that were fully established, while 63 percent of the MRSA isolates and 63 percent of the MSSA isolates had biofilms that were weak. It was discovered that the susceptibility to methicillin and the formation of biofilm have a strong correlation (P 0.05). When compared to S. aureus strains isolated from urine or blood, the ability of

S. aureus strains isolated from pus on patients' skin to form biofilms was significantly higher than that of S. aureus strains isolated from other bodily fluids. More adherent biofilms grew in uniform monolayers but did not mature into a mature three-dimensional structure. Biofilms that adhered poorly and moderately did not colonize the entire peg, while biofilms that adhered more strongly grew in monolayers but did not mature. In comparison to the control strain, one-fourth of the seventy MSSA isolates that were tested formed biofilms with a moderate level of adhesion. Sixty-three percent of the MSSA isolates had weak biofilms, while the other fourteen percent had biofilms that were completely established. Significantly different patterns of biofilm formation were observed between methicillin-resistant S. aureus isolates and those that were susceptible to the antibiotic (P 0.05). Both types of bacteria were capable of forming large biofilm structures, which suggests that susceptibility to methicillin and biofilm formation are connected in some way. Biofilm-associated microorganisms have been shown to be up to 1,000 times more resistant to antibiotics than planktonic bacteria. Multidrug resistance was found in 97.6 percent of the isolates, and 37 percent (46.3 percent) of the MDR isolates formed biofilm. Rajendra et al., 2022 discovered that 80.4 percent of MDR organisms formed biofilms, which is a much higher percentage than the current finding. Close cell-to-cell contact is thought to cause multiple drug resistance in biofilm-forming organisms, allowing plasmids containing MDR genes to be easily transferred between cells [14,15]. Tolerance is a non-heritable and temporary trait shared by organisms that form biofilms. As a result, antibiotics whose action is dependent on cell division are ineffective against dormant and slow-growing biofilm-associated microbes. As previously stated, the polysaccharide matrix of the biofilm impedes drug permeation [16]. The pH and osmotic changes in the biofilm microenvironment affect drug efficacy [17]. Biofilms inhibit host defense mechanisms in addition to interfering with antimicrobial agents. Leukocytes are deactivated by anti-phagocytic properties in the polysaccharide matrix. A component of the matrix also inhibits both complement and host antibodies. It is possible that the capacity of a large proportion of MRSA and MSSA isolates to form biofilms that are both moderately adherent and fully established is the reason why these organisms are able to facilitate infection of the host and survive in the environment of a hospital.

CONCLUSION:

Biofilm formation detection is a simple and cost-effective laboratory test that can be performed on a regular basis. A better understanding of biofilm will allow clinicians to better treat the infections, resulting in a lower mortality and morbidity rate for patients. According to our findings, an infection's susceptibility to reinfection may be linked to the bacterial biofilm. Biofilm-producing bacteria have been shown to be more resistant to antibiotic than non-biofilm-producing bacteria in the treatment of a chronic diabetic foot infection. As a result, additional testing for drug-resistant and non-resistant organisms like MSSA, which is frequently found in biofilms, should be taken into account. Research into the genetic mechanisms of biofilm formation in *S. aureus* will result in improved methods for the management of infections caused by biofilm.

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Table 1: Biofilm producing cases of MSSA & MRSA in study subjects

Specimen	Number of Biofilm producers of MSSA	Number of Biofilm producers of MRSA	Total
Pus	40	24	64
Urine	7	12	19
Blood culture	6	2	8
High vaginal swab	2	2	4
Sputum	6	1	7
Swab culture	6	1	7
Tissue culture	2	1	3
Semen C/S	1	0	1
Pleural fluid	0	1	1
Total	70	44	114

Table 2: Comparison of Biofilm-forming capacity in MSSA & MRSA cases

Type of Biofilm	MSSA (n=70)	MRSA (n=44)
Weak if < 2 O.D	38	28
Moderate if = 2 to ≤ 4 O.D	15	10
Strong if ≥ 4 O.D	7	6

Figure 1: Percentage of Biofilm producers in MSSA cases

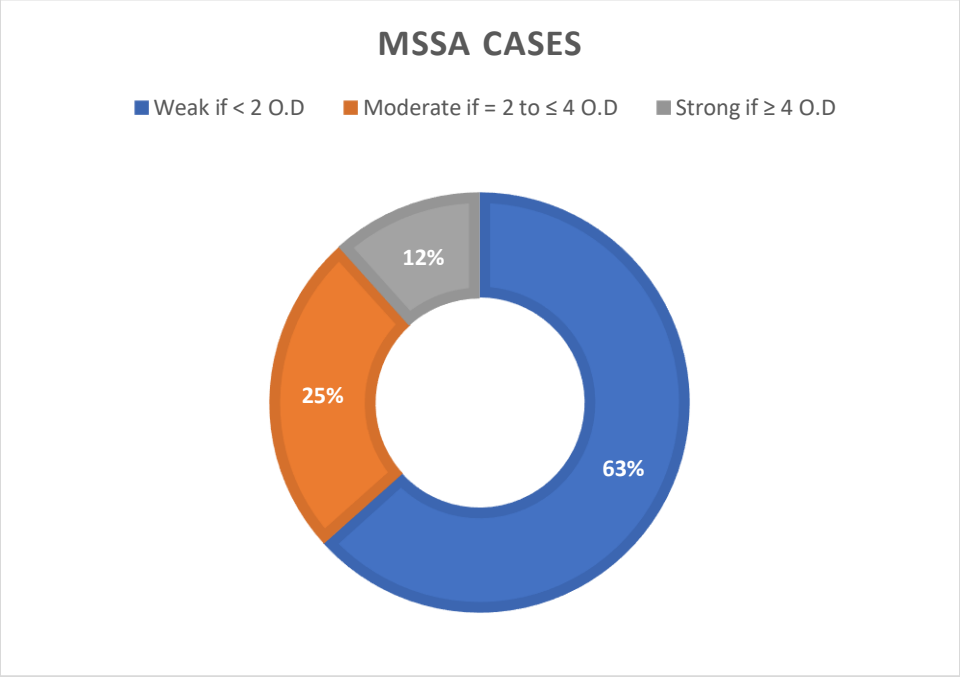

Figure 2: Percentage of Biofilm producers in MRSA cases