

Evaluation of Biofilm Formation in *Staphylococcus aureus* Clinical Isolates

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ABSTRACT

Background and Objectives: *Staphylococcus aureus* is a common cause of nosocomial infections. The ability of *S. aureus* to form biofilm and acquire antimicrobial resistance has made this organism a major health problem. In this study, we investigate the biofilm-forming ability of *S. aureus* isolates from clinical samples.

Methods: Sixty *S. aureus* isolates from clinical specimens were collected from the 5th Azar Hospital of Gorgan (Iran) in 2018. The isolates were identified using conventional methods including Gram staining and biochemical tests (catalase and coagulase). Biofilm formation by *S. aureus* isolates was evaluated using a microplate-based method.

Results: Of 60 *S. aureus* isolates, 47 (78.3%) strains were identified as biofilm-forming and 13 (21.7%) strains were non-biofilm-forming.

Conclusion: The high prevalence of biofilm-producing *S. aureus* isolates in the 5th Azar hospital of Gorgan could pose a major health challenge with serious consequences for hospitalized patients. Therefore, it is crucial to disinfect and sterilize hospital surfaces and equipment effectively to minimize the risk of contamination and spread of bacteria in the hospital settings.

Keywords: Biofilms, *Staphylococcus aureus*, sample.

INTRODUCTION

Hospital infections are a global health problem associated with a variety of factors. Since the 1980s, Gram-positive bacteria, especially *Staphylococcus aureus*, have been considered as the main cause of hospital infections (1). The ability of *S. aureus* to form biofilm and acquire antimicrobial resistance has made this organism a major health problem (2). The bacterium causes a variety of infections, including bacteremia, septicemia, pneumonia, as well as skin, soft tissue and bone infections (3-4). These bacteria are catalase-positive, non-spore forming and often lack capsule. They can grow in different environmental conditions and are the second most common cause of nosocomial infections (5).

Biofilm formation is associated with increased resistance to antibiotics and host defense system (6). Bacteria within the biofilm are surrounded by extracellular polymeric substances (7). Given the importance of *S. aureus* infections in hospitals and the associated risk of mortality (8), we aimed to study biofilm formation ability in *S. aureus* isolates from clinical specimens.

MATERIALS AND METHODS

The study was carried out on 60 *S. aureus* clinical isolates that were collected from the 5th Azar Hospital of Gorgan (Iran) in 2018. The isolates were identified using conventional methods such as Gram staining, biochemical tests (catalase and coagulase) and mannitol

fermentation or DNase test if necessary (9). The biofilm-forming ability of *S. aureus* isolates was studied using a microtiter plate based crystal violet assay. First, 24-hour culture of each bacterial isolate was inoculated into Mueller-Hinton agar and Tryptic soy broth containing 1% glucose. After incubation at 37 °C and obtaining a turbidity equivalent to the McFarland 0.5 standard (or 0.1-0.8% absorbance at 570 nm), 200 µl of bacterial suspension were inoculated into wells of a polystyrene 96-well plate. Wells containing the medium alone were considered as negative controls. *S. aureus* ATCC 35556 was used as the positive control (10). To visualize biofilms, 200 µl of crystalline violet (2%) were added to each well. After five minutes, excess dye was discarded and the well was washed with 200 µl of phosphate buffered saline (PBS) three times. The dye incorporated by the biofilm-forming cells was dissolved by treatment with 200 µl of ethanol-acetone (80: 20%) for 30 minutes. Absorbance of each well at 570 nm was measured by an ELISA plate reader. A semi-quantitative study of biofilm formation was carried out using cut-off (OD₅₇₀) values according to a method described previously (11, 12). The cut-off OD (OD_c) was defined as three standard deviations above the mean OD of the negative control. All absorbance measurements were carried out in triplicate. Table 1 presents classification of the isolates in terms of biofilm formation based on the obtained OD_c.

Table 1- Classification of the isolates in terms of biofilm-forming ability based on the microtiter-plate method

Biofilm formation ability	Cut-off rate	Mean (maximum OD absorption)
Strong	OD > 4×OD _c	OD > 1.2
Moderate	2×OD _c < OD ≤ 4×OD	0.7 < OD ≤ 1.2
Weak	OD _c < OD ≤ 2×OD _c	0.3 < OD ≤ 0.6
Non-biofilm-forming	OD < cut off	OD ≤ 0.3

Cut off = mean OD of negative control + (3×standard deviation of the negative control)

RESULTS

Of 60 *S. aureus* isolates, 27 (47%) were isolated from men and 33 (53%) were isolated from women. In addition, 47 (78.3%) strains were identified as biofilm-forming and 13 (21.7%) strains were non-biofilm-forming. The biofilm-forming strains were isolated from urine (n=12), blood (n=5), cerebrospinal fluid (n=8), wound (n=17) and abscess (n=5) specimens. Furthermore, 30 (50%) isolates were strong biofilm producer and 17 (28.3%) isolates were weak biofilm producer.

DISCUSSION

Biofilm acts as an important virulence factor for *S. aureus* by facilitating adhesion of the bacteria to various surfaces and increasing resistance to antimicrobial agents. Hence, infections caused by biofilm-forming *S. aureus* strains in hospitals are recognized as a major health challenge (13). The increased antibiotic resistance in these bacteria is mainly due to the presence of large quantity of extracellular polysaccharides in the biofilm that provides a favorable condition for the slow growth of

bacteria (14). In our study, 78.3% of the isolates were identified as biofilm forming, which is alarming. In a study by Wang et al. (2012) in China, 66% of the isolates were considered biofilm producers (15). In Ireland, the prevalence of biofilm-forming bacteria was 61.4% among clinical isolates (9). In Germany, 67% of isolates from blood samples and 47% of isolates from urinary tract infections were biofilm producers (8). In a study in the United States, 57% of the strains isolated from blood samples were able to adhere to polystyrene surfaces (16).

CONCLUSION

The high prevalence of biofilm-producing *S. aureus* isolates in the 5th Azar hospital of Gorgan could pose a major health

challenge with serious consequences for hospitalized patients. Therefore, it is crucial to disinfect and sterilize hospital surfaces and equipment effectively to minimize the risk of contamination and spread of bacteria in the hospital settings.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

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