Original Research Report

BIOFILM FORMATION IN *Staphylococcus aureus* AND COAGULASE-NEGATIVE *Staphylococcus*

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ABSTRACT

Staphylococcus spp. is typically a commensal microorganism that can exist in the human body without causing illness. However, such a bacterium has virulence factors, e.g., biofilm formation, which are important to note. Because biofilms shield bacteria from opsonophagocytosis and antimicrobial agents, they can cause persistent or chronic infections. Once they form biofilms, both *Staphylococcus aureus* and coagulase-negative *Staphylococcus* (CoNS) can potentially cause incurable infections. This study aimed to compare biofilm formation in *Staphylococcus aureus* and coagulase-negative *Staphylococcus* as a guide for the prevention and management of infection, thus maintaining and improving public health. This current study was analytic research with a cross-sectional design. It began by collecting the samples, identifying the species, and testing the biofilm production with a microtiter plate, which was then analyzed with an enzyme-linked immunosorbent assay (ELISA). Data analysis was conducted using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, N.Y., USA). Comparison tests were conducted using an independent t-test. A value of $p<0.05$ was used as the cut-off to indicate significance. The total samples were 36 clinical isolates, consisting of 18 *Staphylococcus aureus* and 18 coagulase-negative *Staphylococcus*. The specimens consisted of 20 blood samples (55.6%) and 7 wound swabs (19.4%). The biofilm test on the samples showed that 83.3% of the samples produced biofilms. The data revealed that the isolates formed biofilms, with 14 isolates (38.9%) in the strong category, 10 isolates (27.8%) in the moderate category, and 6 isolates (16.7%) in each of the weak and non-existent categories. Both *Staphylococcus spp.* appeared to have a biofilm-forming activity, but coagulase-negative *Staphylococcus* appeared to be significantly more dominant ($p=0.008$) than *Staphylococus aureus*. Strong biofilm was produced by 61.1% of coagulase-negative *Staphylococcus* isolates. In conclusion, coagulase-negative *Staphylococcus* formed a stronger biofilm than *Staphylococcus aureus*. Its presence as an infection-causing bacteria, particularly in immunocompromised patients, should not be underestimated.

Keywords: Biofilm; *Staphylococcus*; human and health; immunocompromised patients

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Highlights:
1. The significance of *Staphylococcus aureus* and coagulase-negative *Staphylococcus*, which are more likely to infect immunocompromised patients, needed to be researched in greater depth.
2. Coagulase-negative *Staphylococcus* was found to form significantly more biofilm than *Staphylococcus aureus*.
3. Wound care and changing medical devices in immunocompromised patients on a regular basis may provide benefits to prevent biofilm formation by *Staphylococcus spp.*

INTRODUCTION

Staphylococcus is a genus of Gram-positive bacteria that commonly inhabits the skin and mucous membranes of humans and animals. Gram-positive cocci are also common isolates in the microbiology laboratory (Mahon & Lehman 2022). *Staphylococcus* can be classified into coagulase-

positive and coagulase-negative species based on their ability to produce the enzyme coagulase. The enzyme coagulase produced by coagulase-positive *Staphylococcus* causes blood to clot. The most clinically relevant coagulase-positive species is *Staphylococcus aureus* (*S. aureus*). This strain of bacteria is a major human pathogen that can cause a wide range of infections, including skin and soft tissue infections, pneumonia, endocarditis, and sepsis (Riedel et al. 2019, Mahon & Lehman 2022). *Staphylococcus aureus* inhabits several areas of human body and has the ability to cause infections in humans under certain conditions. Approximately 20–30% of the human population has *Staphylococcus aureus* colonization in the nose, throat, folds, and gastrointestinal tract (Tong et al. 2015).

Coagulase-negative *Staphylococcus* (CoNS), on the other hand, does not produce coagulase. Some of the clinically important coagulase-negative species include *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, and *Staphylococcus haemolyticus*. These species are commonly found on the skin and mucous membranes and are typically considered to be less virulent than *Staphylococcus aureus*. However, they can still cause a range of infections, particularly in individuals with compromised immune systems or who have indwelling medical devices (e.g., catheters or prosthetic devices), due to their ability to colonize (Águila-Arcos et al. 2017, Zheng et al. 2018). It is worth noting that coagulase-negative staphylococci are a common cause of nosocomial (hospital-acquired) infections. In many cases, these bacteria demonstrate resistance to multiple antibiotics (Águila-Arcos et al. 2017).

Biofilms are defined as a community of microbial cells permanently attached to each other on a surface (either inanimate or living organisms) by a matrix of extracellular polymeric substances (EPSs). The term biofilm was coined by John William Costerton in 1978 (Sagar et al. 2016). Biofilms are formed because microorganisms tend to aggregate and create a safe and comfortable environment for their community. As a survival mechanism, birofilms can lead to severe and chronic infections (Rasamiravaka et al. 2015). Biofilms on medical implants are one of the most troubling issues for medical practitioners due to the significant ability of bacteria to evade the body's immune system and antimicrobial agents, or antibiotics. Biofilms, which are an important virulence factor of *Staphylococcus spp.*, have an impact on the outcome of patients infected with *Staphylococcus spp.* (Nourbakhsh & Namvar 2016). Infectious conditions caused by biofilms that are not properly treated will result in antibiotic overuse, which will lead to resistance and increased mortality and morbidity due to unresolved sepsis.

The presence of coagulase-negative *Staphylococcus* does not receive the appropriate concern for its potential impact since *Staphylococcus aureus* infection is generally of more concern to clinicians. Unquestionably, the virulence factors of the two pathogens are distinct, but the process of treating an infection becomes equally challenging when biofilm is present (Águila-Arcos et al. 2017, Riedel et al. 2019). This study aimed to compare the biofilm formation of *Staphylococcus aureus* and coagulase-negative *Staphylococcus* as a guide for the prevention and treatment of infections, as well as for the maintenance and improvement of good health in the community. It is anticipated that this study will encourage clinicians to be more vigilant, allowing the process of infection management to be more effective for both patients and doctors. In addition, unnecessary antibiotic use should be avoided so that resistance does not develop further.

MATERIALS AND METHODS

This was an analytical study with a cross-sectional design to compare biofilm formation in *Staphylococcus aureus* and coagulase-negative *Staphylococcus* (Kesmodel 2018). The study was conducted from July 2022 to March 2023. A total of 36 samples were obtained from patients with staphylococcal infections at Sanjiwani Gianyar Regional General Hospital, Gianyar, Indonesia. The research process continued at the Faculty of Medicine and Health Sciences, Universitas Warmadewa, Denpasar, Indonesia. Patients who participated in this study were those who had a *Staphylococcus spp.* infection or colonization and were hospitalized between October 2022 and January 2023.

The research process began with the identification of *Staphylococcus aureus* and coagulase-negative *Staphylococcus* by culturing the samples on blood agar (BA) media. It was then followed by an incubation process for 24 hours at 37°C (Mahon & Lehman 2022). *Staphylococcus* isolates with positive catalase test results underwent further identification using a VITEK® 2 machine (BioMérieux, USA). This process was carried out in the microbiology laboratory of Sanjiwani Gianyar Regional General Hospital. Methicillin resistance was also determined using the disk diffusion method with a cefoxitin disc according to the Clinical and Laboratory Standards Institute guidelines (Clinical and Laboratory Standards Institute 2022).

The biofilm assay was performed in several stages using a microtiter plate and a crystal violet staining assay in the microbiology laboratory of the Faculty of Medicine and Health Sciences, Universitas Warmadewa. The bacteria from the culture were

inoculated in 1% liquid glucose and 3 mL of phosphate-buffered saline (PBS) at pH 7 (Torlak et al. 2017, Omidi et al. 2020). The turbidity was measured with DENSICHEK® (BioMérieux, USA), and the range for a McFarland standard was 0.50–0.63. A total of 200 µL of suspension was transferred to a microtiter plate and incubated at 37°C for 48 hours without shaking. In each well of the microtiter plate, the remaining solution was discarded and rinsed with distilled water. This process was repeated in every step. Biofilm attached to the wells was stained with 200 µL of 0.1% crystal violet for 5 minutes. The microtiter plate was then rinsed, and 200 µL of 30% acetic acid was added to each well and allowed to dissolve for 5–15 minutes. The fluorescence intensity of crystal violet was measured with a microplate in an enzyme-linked immunosorbent assay (ELISA) reader using a wavelength of 620–670 nm. The methods used in this study referred to previous research with some modifications for method optimization (Samadi et al. 2017).

The collected data were then analyzed using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, N.Y., USA) in two stages. The first step was a descriptive statistical analysis, which was conducted to describe the characteristics of each variable in this study. Data variables were expressed in the form of relative frequency (number and percent). The second stage was a bivariate analysis to determine whether there were significant differences in the ability to form biofilms on both types of *Staphylococcus aureus* and coagulase-negative *Staphylococcus*. The optical density cutoff (ODc) value was determined according to the quantitative value of biofilm. This was defined as the mean OD of the negative control +3 × standard deviation (SD) of the negative control. Biofilm formation by isolates was analyzed and categorized based on the absorbance of attached cells by crystal violet staining. The biofilm formation ability was then divided into 4 categories, i.e., none (OD≤ODc), weak (ODc<OD≤2xODc), moderate (2xODc<OD ≤4xODc), and strong (4xODc<OD) (Pompilio et al. 2020, Kasperski et al. 2023). A comparison test was performed using an independent t-test. The cutoff for statistical significance was $p<0.05$, and the precision value was determined by a 95% confidence interval (CI) (Banerjee 2014).

This study had received ethical approval from the Ethics Committee of the Faculty of Medicine and Health Sciences, Universitas Warmadewa, Denpasar, Indonesia, with registration No. 305/ Unwar/FKIK/EC-KEPK/II/2023 on 23/2/2023.

RESULTS

A total of 36 samples were obtained, comprising 18 isolates of *Staphylococcus aureus* and 18 isolates of coagulase-negative *Staphylococcus*. Thirteen samples (36.1%) were collected from patients in the age range of 46–65 years. The total sample showed a balanced representation of both genders. The largest specimen type utilized in this study was blood, accounting for a total of 20 samples (55.6%). This was followed by 7 wound swabs (19.4%) and 5 sputum samples (13.9%). The prevalent diagnoses seen among the patient population consisted of pneumonia (12 cases, 33.3%), skin and soft tissue infection (8 cases, 22.2%), and chronic kidney disease (CKD) (6 cases, 16.7%). The complete results are visually presented in Figures 1 and 2. Out of the total number of samples tested for sensitivity to cefoxitin, 19 samples (52.8%) exhibited positive results on the cefoxitin test. These positive results were indicative of the presence of meticillin-sensitive *Staphylococcus aureus* (MSSA) and meticillin-resistant *Staphylococcus aureus* (MRSA).

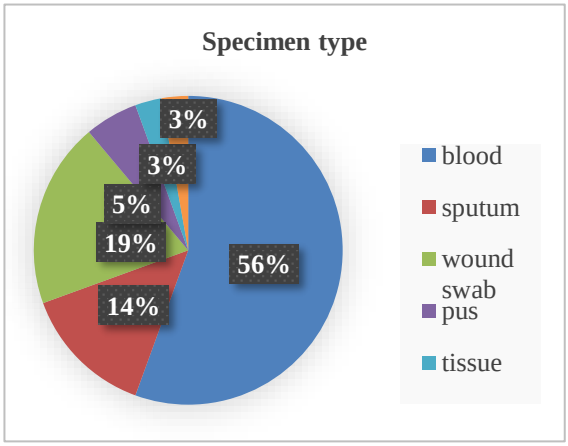

Figure 1. The distribution of specimen types, expressed in percentage (n=36).

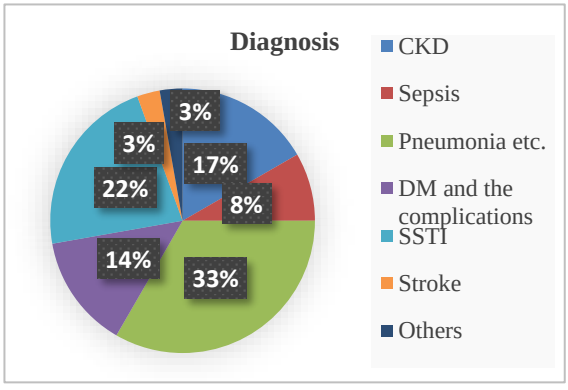

Figure 2. The distribution of diagnoses among the isolates, expressed in percentage (n=36).

The results of the biofilm assay conducted on the samples indicated that 83.3% of the 36 isolates exhibited biofilm formation following incubation for 48 hours at a temperature of 37°C in a 1% glucose solution. The mean optical density of the biofilm produced was determined to be 0.427 using an ELISA reader. The lowest value observed was 0.02, while the highest value recorded was 1.055.

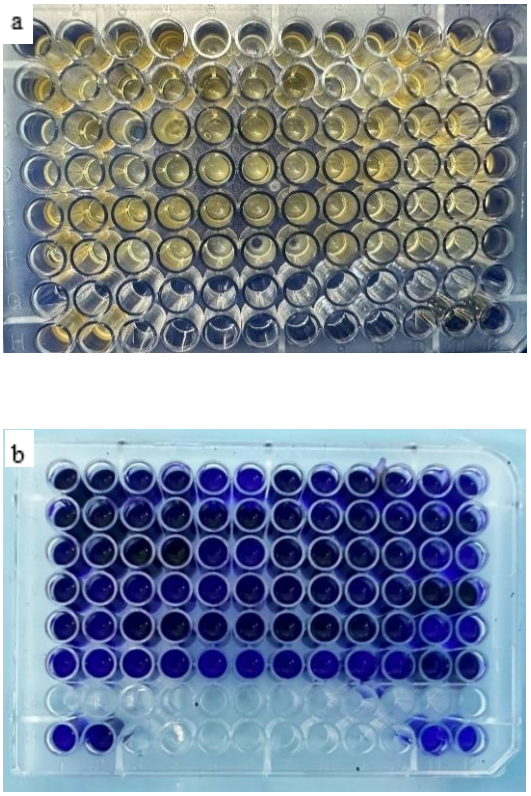

Figure 3. Biofilms appeared as white layers on the bottom of a microtiter plate, both before (a) and after (b) staining with crystal violet.

The data relating to the ability of biofilm formation indicated that a majority of the isolates exhibited strong biofilm formation with 14 isolates (38.9%). This was followed by 10 isolates (27.8%) displaying moderate biofilm formation. Additionally, 6 isolates (16.7%) exhibited weak biofilm formation, while an equal number of isolates showed no biofilm formation. The mean optical density of *Staphylococcus aureus* isolates was found to be 0.277, while the average optical density of coagulase-negative staphylococci isolates was determined to be 0.579. Table 1 presents more details on the classification of biofilms generated by the two distinct groups of *Staphylococcus*.

Table 1. Comparison of the strength of biofilms that were produced.

		Staphylococcus spp.	
		S. aureus	CoNS
Biofilm	Strong	3 (16.7%)	11 (61.11%)
	Moderate	3 (16.7%)	7 (38.8%)
	Weak	6 (33%)	0
	None	6 (33%)	0
Total		18 (100%)	18 (100%)

Note: CoNS = Coagulase-negative *Staphylococcus*.

The ability for biofilm formation was observed in both types of *Staphylococci*. However, it was significantly more evident in coagulase-negative *Staphylococcus* (p=0.008).

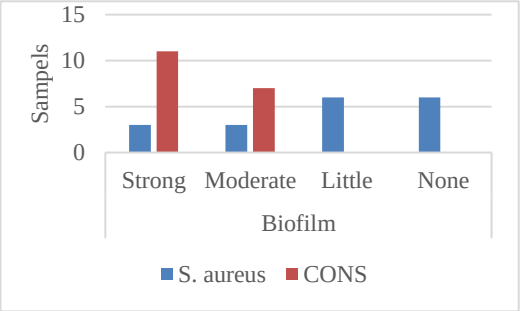

Figure 4. Overview of the ability of biofilm formation.

DISCUSSION

Both *Staphylococcus aureus* and coagulase-negative *Staphylococcus* strains are known for their ability to form biofilms, which are one of the virulence factors. This feature contributes to treatment difficulties because these biofilms are resistant to both the immune system and antibiotics. This resistance is especially prevalent among patients who use medical devices such as infusion catheters, central venous catheters, implanted devices (e.g., pacemakers), and urinary catheters (Silva-Santana et al. 2016, de Oliveira et al. 2021). Furthermore, the formation of biofilm on inadequately cleansed wound surfaces impedes wound healing.

The most common specimen type observed in this study was blood, followed by wound swabs and sputum. Meanwhile, the most prevalent diagnoses were pneumonia, skin and soft tissue infections, and CKD. Patients diagnosed with CKD who undergo hemodialysis commonly possess double lumen access, which makes them prone to bacteremia due to the nature of the procedure. Under pathogenic conditions, *Staphylococcus spp.* emerges as a leading cause of skin and soft tissue infections, including abscesses, furuncles, and cellulitis. *Staphylococcus aureus* is a prominent cause of

bacteremia, sepsis, and infective endocarditis. Additionally, it is responsible for osteoarticular infections, pneumonia, pleurisy, and device-associated infections, including those associated with double-lumen catheters and central venous catheters (Namvar et al. 2013, Tong et al. 2015).

Biofilm was formed in 83.3% of the samples in this study. Biofilm formation was observed in 66% of *Staphylococcus aureus* isolates, which was relevant to previous research. The majority of *Staphylococcus spp.* isolated from blood, urine, pus, and sputum samples were biofilm-forming isolates. In most studies, more than 50% of *Staphylococcus aureus* samples produced biofilm (Nourbakhsh & Namvar 2016, Neopane et al. 2018, Omidi et al. 2020). In a study conducted at Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, a positive biofilm assay was seen in all (100%) MRSA carrier isolates. The study also revealed that 57.9% of clinical isolates produced biofilms according to the positive assay (Suryanditha et al. 2018). A study conducted at an Iranian hospital reported different findings. In the study, 46% of MRSA isolates produced strong biofilms on microtiter plates (Mirzaee et al. 2014).

In this study, it was found that 100% of coagulase-negative *Staphylococcus* isolates formed biofilms in vitro. This was in line with previous studies, which found that more than 90% of coagulase-negative *Staphylococcus* formed biofilms (Seng et al. 2017). Its ability to form biofilms was significantly stronger and more dominant than that of *Staphylococcus aureus* ($p<0.05$). Additionally, a previous study found that *Staphylococcus epidermidis* isolates showed a higher biofilm-forming capacity than *Staphylococcus aureus* isolates (Águila-Arcos et al. 2017).

The presence of methicillin-resistant coagulase-negative *Staphylococcus* (MR-ConS), which accounted for 83% of all coagulase-negative *Staphylococcus* samples, raised serious concern. This may escalate into a bigger problem if the person is immunocompromised, has a chronic illness such as diabetes, or uses medical devices. As the samples were obtained from patients, there was a high likelihood that the patients were infected with these bacteria. Moreover, the presence of biofilm would complicate treatment, exacerbating the bacterial resistance factor (Seng et al. 2017, Águila-Arcos et al. 2017, Piechota et al. 2018). Patients who use medical devices are more likely to develop biofilms, which lead to increased morbidity and mortality. Coagulase-negative *Staphylococcus* is also known as a contributing factor to sepsis in newborns and the elderly in developing countries (Kwiecinski et al. 2019, Ielapi et al. 2020, Singh et al. 2023). The formation of biofilms is unquestionably a contributing factor that complicates treatment for patients, often without proper notice. Through this simple study, it is hoped that clinicians can be aware of this virulence factor when treating patients.

Strength and limitations

This study used clinical isolates obtained from a hospital, where samples were taken from patients admitted with various diagnoses. Therefore, the findings were quite applicable in a clinical setting. However, a limitation of this study was the formation of in vitro biofilms, which may differ from in vivo biofilms due to differences in environmental conditions and host factors. Another limitation of this study was its limited scale.

CONCLUSION

The majority of *Staphylococcus aureus* and all coagulase-negative *Staphylococcus* isolates can produce biofilms in vitro. When compared, coagulase-negative *Staphylococcus* forms significantly more biofilm than *Staphylococcus aureus*. Clinicians are advised to incorporate practices such as using proper wound dressings and regularly replacing medical devices for patients. This approach aims to prevent the formation of biofilms and the unnecessary prolongation of antibiotic use. Furthermore, if there is a suspicion of infection, it is recommended to replace the medical devices as soon as possible, even before the scheduled treatment. Furthermore, commensal bacteria with low virulence factors should not be underestimated, especially when working with people who have compromised immune systems.

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Conflict of interest

None.

Ethical consideration

This study has received ethical approval from the Ethics Committee of the Faculty of Medicine and Health Sciences, Universitas Warmadewa, Denpasar, Indonesia, with registration No.

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Author contribution

MS contributed to the conception and design of this study, the analysis and interpretation of the data, and the drafting of the article. DAPSM contributed to the critical revision of the article for important intellectual content and final approval of the article. AAGI contributed to the analysis and interpretation of the data and provided statistical expertise. KS contributed to the provision of study materials as well as the collection and assembly of data. IKAIA contributed administrative, technical, and logistical support. MAbAR contributed technical advice.

REFERENCES

Águila-Arcos S, Álvarez-Rodríguez I, Garaiyurrebaso O, et al (2017). Biofilm-forming clinical staphylococcus isolates harbor horizontal transfer and antibiotic resistance genes. Frontiers in Microbiology. doi: 10.3389/fmicb.2017.02018.

Banerjee G (2014). Comparative study of biofilm formation in Pseudomonas aeruginosa isolates from patients of lower respiratory tract infection. Journal of Clinical and Diagnostic Research. doi: 10.7860/JCDR/2014/7808.4330.

Clinical and Laboratory Standards Institute (2022). Performance standards for antimicrobial susceptibility testing, 32nd edn. Clinical and Laboratory Standards Institute, USA. Available at: https://www.nih.org.pk/wp-content/uploads/2021/02/CLSI-2020.pdf.

Ielapi N, Nicoletti E, Lorè C, et al (2020). The role of biofilm in central venous catheter related bloodstream infections: Evidence-based nursing and review of the literature. Reviews on Recent Clinical Trials 15**,** 22–27. doi: 10.2174/1574887114666191018144739.

Kasperski T, Romaniszyn D, Jachowicz-Matczak E, et al (2023). Extensive drug resistance of strong biofilm-producing acinetobacter baumannii strains isolated from infections and colonization hospitalized patients in Southern Poland. Pathogens 12**,** 975. doi: 10.3390/pathogens12080975.

Kesmodel US (2018). Cross-sectional studies - what are they good for? Acta Obstet Gynecol Scand 97**,** 388–393. doi: 10.1111/aogs.13331.

Kwiecinski JM, Jacobsson G, Horswill AR, et al (2019). Biofilm formation by *Staphylococcus aureus* clinical isolates correlates with the infection type. Infectious Diseases (Auckl) 51**,** 446–451. doi: 10.1080/23744235.2019.1593499.

Mahon CR, Lehman DC (2022). Textbook of diagnostic microbiology. Elsevier.

Mirzaee M, Peerayeh SN, Ghasemian A-M (2014). Detection of icaABCD genes and biofilm formation in clinical isolates of methicillin resistant *Staphylococcus aureus*. Iranian Journal of Pathology 9**,** 257–262. Available at: https://ijp.iran path.org/article_7049.html.

Namvar AE, Asghari B, Ezzatifar F, et al (2013). Detection of the intercellular adhesion gene cluster (ica) in clinical *Staphylococcus aureus* isolates. GMS hygiene and infection control 8**,** Doc03. doi: 10.3205/dgkh000203.

Neopane P, Nepal HP, Shrestha R, et al. (2018). In vitro biofilm formation by *Staphylococcus aureus* isolated from wounds of hospital-admitted patients and their association with antimicrobial resistance. International Journal of General Medicine 11**,** 25–32. doi: 10.2147/IJGM.S153268.

Nourbakhsh F, Namvar AE (2016). Detection of genes involved in biofilm formation in *Staphylococcus aureus* isolates. GMS Hygiene and Infection Control 11**,** Doc07. doi: 10.3205/dgkh000267.

de Oliveira A, Pinheiro-Hubinger L, Pereira VC, et al (2021). Staphylococcal biofilm on the surface of catheters: Electron microscopy evaluation of the inhibition of biofilm growth by RNAIII inhibiting peptide. Antibiotics 10**,** 879. doi: 10.3390/antibiotics10070879.

Omidi M, Firoozeh F, Saffari M, et al (2020). Ability of biofilm production and molecular analysis of spa and ica genes among clinical isolates of methicillin-resistant *Staphylococcus aureus*. BMC Research Notes 13**,** 19. doi: 10.1186/s13104-020-4885-9.

Piechota M, Kot B, Frankowska-Maciejewska A, et al (2018). Biofilm formation by methicillin-resistant and methicillin-sensitive *Staphylococcus aureus* strains from hospitalized patients in Poland. BioMed Research International 2018**,** 1–7. doi: 10.1155/2018/4657396.

Pompilio A, Ranalli M, Piccirilli A, et al (2020). Biofilm formation among stenotrophomonas maltophilia isolates has clinical relevance: The ANSELM prospective multicenter study. Microorganisms 9**,** 49. doi: 10.3390/microorganisms9010049.

Rasamiravaka T, Labtani Q, Duez P, et al (2015). The formation of biofilms by Pseudomonas aeruginosa : A review of the natural and synthetic compounds interfering with control mechanisms. BioMed Research International 2015**,** 1–17. doi: 10.1155/2015/759348.

Riedel S, Hobden JA, Miller S, et al (2019). Jawetz, Melnick, & Adelberg's medical microbiology,

28e. McGraw Hill. Available at: https://accessmedicine.mhmedical.com/content.aspx?bookid=2629&sectionid=217768737.

Sagar SS, Kumar R, Kaistha SD (2016). Biofilm-an eternalchronicle of bacteria. Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases 37**,** 45. doi: 10.5958/0974-0147.2016.00010.6.

Samadi R, Ghalavand Z, Nikmanesh B, et al (2017). Investigation of biofilm formation among methicillin-resistant *Staphylococcus aureus* isolated from children. Archives of Pediatric Infectious Diseases. doi: 10.5812/pedinfect.61635.

Seng R, Kitti T, Thummeepak R, et al (2017). Biofilm formation of methicillin-resistant coagulase negative staphylococci (MR-CoNS) isolated from community and hospital environments ed. de Lencastre H. PLoS One 12**,** e0184172. doi: 10.1371/journal.pone.0184172.

Silva-Santana G, Lenzi-Almeida KC, Lopes VGS, et al (2016). Biofilm formation in catheter-related infections by Panton-Valentine leukocidin-producing *Staphylococcus aureus*. International Microbiology: The Official Journal of The Spanish Society for Microbiology 19**,** 199–207. doi: 10.2436/20.1501.01.278.

Singh M, Alsaleem M, Gray CP (2023). Neonatal Sepsis. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24974250.

Suryanditha PA, Rasita YD, Debora K, et al (2018). icaA/D genes and biofilm formation of methicillin-resistant *Staphylococcus aureus* in Dr. Soetomo Hospital, Surabaya. Folia Medica Indonesiana 54**,** 263. doi: 10.20473/fmi.v54i4.10709.

Tong SYC, Davis JS, Eichenberger E, et al (2015). *Staphylococcus aureus* infections: Epidemiology, pathophysiology, clinical manifestations, and management. Clinical Microbiology Reviews 28**,** 603–661. doi: 10.1128/CMR.00134-14.

Torlak E, Korkut E, Uncu AT, et al (2017). Biofilm formation by *Staphylococcus aureus* isolates from a dental clinic in Konya, Turkey. Journal of Infection and Public Health 10**,** 809–813. doi: 10.1016/j.jiph.2017.01.004.

Zheng Y, He L, Asiamah TK, et al (2018). Colonization of medical devices by staphylococci. Environmental Microbiology 20**,** 3141–3153. doi: 10.1111/1462-2920.14129.