Role of Biofilm in Bacterial Infection and Antimicrobial Resistance

Khilasa Pokharel, Bishwa Raj Dawadi, Lok Bahadur Shrestha

Department of Microbiology, Kathmandu Medical College and Teaching Hospital, Sinamangal, Kathmandu, Nepal, Department of Emergency Medicine, Grande International Hospital, Dhapasi, Kathmandu, Nepal, School of Medical Sciences and The Kirby Institute, University of New South Wales, Sydney, Australia.

ABSTRACT

Biofilm refers to the complex, sessile communities of microbes found either attached to a surface or buried firmly in an extracellular matrix as aggregates. Microbial flora which produces biofilm manifests an altered growth rate and transcribes genes that provide them resistance to antimicrobial and host immune systems. Biofilms protect the invading bacteria against the immune system of the host via impaired activation of phagocytes and the complement system. Biofilm-producing isolates showed greater multidrug resistance than non-biofilm producers. Biofilm causes antibiotic resistance through processes like chromosomally encoded resistant genes, restriction of antibiotics, reduction of growth rate, and host immunity. Biofilm formation is responsible for the development of superbugs like methicillin-resistant Staphylococcus aureus, vancomycin-resistant Staphylococcus aureus, and metallo-beta-lactamase producing Pseudomonas aeruginosa. Regular monitoring of antimicrobial resistance and maintaining hygiene, especially in hospitalized patients are required to control biofilm-related infections in order to prevent antimicrobial resistance.

Keywords: antibiotic resistance; bacterial infections; biofilm; MRSA.

INTRODUCTION

Biofilm is commonly seen in chronic conditions and microorganisms remain dormant causing the acute infection. Persistence of bacteria depends upon the formation of biofilm that remains intact on the surface. When there is a biofilm formation, the host cell develops a fundamental part that forms a biofilm matrix. This formed matrix inhibits the diffusion of antimicrobials to the cells. This review highlights the structure of biofilm, its effect in gram-positive and negative bacteria, and infections caused by them, and discusses the relation of biofilm with antimicrobial resistance.

ULTRASTRUCTURE OF BIOFILM

Biofilm is the extracellular polymeric substance (EPS) that provides specific properties during the colonization of bacterial cells. Biofilm is an aggregate of microorganisms with sessile cells, biofouling, corrosion, and deterioration of drinking water quality. Human dental plaque, skin, and gut are dominant biofilm present in Eukaryotes. Microbial cells inside the biofilm are resistant to physical factors. EPS accounts for 50% to 90% of the total organic carbon of biofilm. It is primarily composed of polysaccharides. In the case of Staphylococcus chemical composition of EPS is cationic. Biofilms are heterogeneous, with microcolonies of bacterial cells enclosed in an EPS matrix and separated from other microcolonies by interstitial voids (water channel). Biofilm formation can be divided into five stages: initial reversible attachment, irreversible attachment, maturation, and dispersion. The initial contact of the moving planktonic bacteria with the surface is the starting point, which is still reversible at this stage. The bacteria will then start to form a monolayer and will produce an extracellular matrix or slim for protection. The matrix consists of extracellular polysaccharides, structural proteins, cell debris, and nucleic acids; referred to as EPS. The initial steps of the matrix formation are dominated by extracellular DNA (eDNA), whereas polysaccharides and structural proteins take over later. This helps the cells survival, increases the availability of nutrients, and better opportunities for cellular communication and transfer of genetic
material among microorganisms which makes them more drug-resistant.

**EPIDEMIOLOGY**

Microbial flora which produces biofilm manifests an altered growth rate and transcribes genes that provide them resistance to antimicrobial and host immune systems. Those biofilms contribute to causing chronic inflammatory disease. From one of the studies that were conducted in 2015, of total gram-positive bacteria and gram-negative bacteria of the total of 190 isolates, 68.9% of isolates showed biofilm formation. Biofilm formation was common in *Pseudomonas aeruginosa*, *Klebsiella* species, and *Staphylococcus aureus*. It was also detected that biofilm-producing isolates showed greater multidrug resistance than non-biofilm produces. Another study conducted between 2017 to 2018, stated that 40% of the positive isolates from the study were biofilm-producing bacteria of which *Escherichia coli* was found to be the most common organism.

**BIOFILM FORMATION IN GRAM-POSITIVE BACTERIA**

Biofilm-associated antimicrobial resistance starts with attachment and gradually increases as biofilm ages. Effective treatment plans will incorporate antimicrobials and kill biofilm organisms or treatments that disrupt or target specific components of the biofilm matrix. In *Staphylococci*, adhesion to human tissue or indwelling devices is regulated by anchored proteins which bind to a host cell, which is referred to as microbial surface components recognizing adhesive matrix molecules. In *Staphylococci* and *Enterococci*, similar factors contribute to the biofilm matrix composition including polysaccharides, proteins, teichoic acid, lipoteichoic acid, and extracellular DNA. In *Staphylococci*, the polysaccharide intercellular adhesion (PIA) also known as poly-N-acetyl glucosamine (PNAG), according to chemical composition is an important adhesive molecule during biofilm formation. From a study done, among gram-positive organisms antimicrobials with different degrees of activity against biofilm, Dalbavancin seems to provide effective therapy in a significant preparation of cases due to its effectiveness in the setting of MDIs (metered-dose inhalers) with a relatively low number of side effects. From the review it is reflected that biofilm has a clinical impact on the infection and use of potentially therapeutic drugs. The management of several biofilm-related gram-positive infections, it requires prolonged antibiotic therapy.

**BIOFILM FORMATION IN GRAM-NEGATIVE BACTERIA**

An increase in drug resistance at both the levels in the community and hospital levels is shown by multidrug resistance and pan resistance which leads to treatment failure increased mortality and morbidity which causes an impact on the cost of medical treatment and prevention of bacterial infectious disease. Biofilm causes antibiotic resistance through processes like chromosomally encoded resistant gene, restriction of antibiotics, reduction of growth rate, and host immunity. Knowledge of biofilm formation and antibiogram of bacterial isolates are important for empirical antibiotic therapy. From one of the studies association between MBL production and biofilm formation was statistically significant whereas it was insignificant between ESBL and biofilm production. Some studies reveal that biofilm and MBL-producing strains were multi-drug resistant. In another study, antibiotics belonging to the class of Fluoroquinolones, Carbapenems, and Aminoglycosides were found to be more effective for biofilm-producing *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumoniae*. Understanding biofilm will help in the novel treatment and will reduce real threat. Biofilm is also a major virulence factor in the case of Uropathogenic *Escherichia coli*.

**ROLE OF BIOFILM PRODUCTION CAUSING METHICILLIN AND VANCOMYCIN RESISTANCE**

Vancomycin has long been considered the last resort treatment for Methicillin-resistant *Staphylococcus aureus* (MRSA) infection. Its excessive use resulted in the emergence of Vancomycin- intermediate *Staphylococcus aureus* (VISA), Vancomycin-resistant *Staphylococcus aureus* (VRSA), and heterogeneous Vancomycin intermediate *Staphylococcus aureus* (hVISA) strains. MRSA strains are able to form biofilm as a fitness and survival mechanism that is mediated by strong adhesion, increase in drug resistance, and reduction of effectiveness of sanitizers. In presence of biofilm, the resistance of *Staphylococcus aureus* to antimicrobials was reported to increase by 1000 times. Ability of MRSA and VRSA isolates to produce biofilms and the presence of high rates of antimicrobial resistance is quite alarming.

**ROLE OF BIOFILM PRODUCTION CAUSING CARBAPENEM RESISTANCE**

The biofilm-forming ability of most gram-negative bacteria is high. In one of the phenotypic studies, it has
been shown that there is a positive correlation between biofilm formation and carbapenem resistance. From the same study it has been mentioned that strong biofilm reduces the number of days alive for the patient to 3.33 days from poor or negative biofilm-biofilm resistant Pseudomonas aeruginosa isolates with 11.33 days. It demonstrates that the production of biofilm increases the mortality rate. In one of the studies done biofilm formation in Carbapenem susceptible and carbapenem-resistant Pseudomonas aeruginosa isolates is 83.6% and 95.0% respectively.

**KNOWLEDGE GAP ON BIOFILM**

Host tissue-related biofilm infections are often chronic, including chronic lung infections of cystic fibrosis patients, chronic osteomyelitis, chronic prostatitis, chronic rhinosinusitis, chronic otitis media, chronic wounds, recurrent urinary tract infection, endocarditis, periodontitis, and dental caries. The ability of pathogenic biofilms to survive in presence of a high concentration of antibiotics is called recalcitrance leads to treatment failure, infection recurrence, and chronic infections. The exact extent to which antimicrobial resistance contributes to antimicrobial resistance is still not crystal clear. Recently, some research regarding biofilm formation and antimicrobial resistance are being conducted in Nepal. To our knowledge, there is a lack of a good review article summarizing research from Nepal, which we are attempting to address. This review article will be helpful in informing the clinicians and nurses regarding the current scenario of biofilm formation and the importance of good infection control measures.

**INFECTION CAUSED BY BIOFILM**

Biofilm formation of infectious significance is found in implant devices. Pseudomonas aeruginosa is the second most common cause of ventilator-associated pneumonia (VAP) and Catheter-associated urinary tract infection (CAUTI). Carbapenem susceptible and carbapenem-resistant Pseudomonas aeruginosa forms biofilms on endocardial tubes and catheters in CAUTI and VAP patients. Mostly biofilms occur with indwelling medical devices such as Central venous Catheters, peritoneal dialysis catheters, mechanical heart valves, and urinary catheters. Biofilm composed depends on devices and the duration of action of microbial species. Microorganism causing periodontitis, like Pseudomonas aeruginosa and Fusobacterium nucleatum has the ability to form biofilm on the mucosal surface in the oral cavity. Microbial colonization of teeth surface permits them to invade mucosal cells and alter the flow of calcium. In epithelial cells they release toxins. Plaque then develops within 2-3 weeks. Plaque then mineralizes with calcium and phosphate ions which form calculus. Biofilm formation on medical devices affects surgical and instrumental procedures and public health. Biofilm formation also has implications for non-device-related health complications. Therefore, good hygiene conditions and practices are necessary to avoid biofilm formation.

**WAY FORWARD**

This review was made to identify and characterize biofilm, determine the biofilm-forming bacteria, and examine its effect on the pathogenesis of bacterial infection and antimicrobial resistance. In this study, we have summarized the relationship between biofilms and the extent of antimicrobial resistance. So, we need to prevent biofilm formation through strict antimicrobial resistance surveillance, proper hygiene, and proper infection control measures, especially during the implantation of an intravenous catheter, mechanical ventilation, and urinary catheterization. Regular detection of biofilm formation by various phenotypic and molecular methods should be introduced in all health care settings.

**Conflict of Interest:** None.

**REFERENCES**

1. Kiedrowski MR, Kavanaugh JS, Malone CL, Mootz JM, Voyich JM, Smelzer MS, et al. Nuclease modulates biofilm formation in community-associated methicillin-resistant Staphylococcus aureus. PLoS One. 2011;6(11):e26714. [PubMed | Full Text | DOI]

2. Raksha L, Gangashettappa N, Shantala GB, NandanBR, Sinha D. Study of biofilm formation in bacterial isolates from contact lens wearers. Indian J Ophthalmol. 2020 Jan;68(1):23-8. [PubMed | Full Text | DOI]

3. Xu Z, Fang X, Wood TK, Huan ZJ. A systems-level approach for investigating pseudomonas aeruginosa biofilm formation. PLoS One. 2013;8(2):e57050. [PubMed | Full Text | DOI]

4. Lynch AS, Robertson GT. Bacterial and fungal biofilm infections. Annu Rev Med. 2008;59:415-28. [PubMed | Full Text | DOI]

5. ManandharS, SinghA, Varma A, Pandey S, ShrivastavaN. Biofilm producing clinical staphylococcus aureus isolates augmented prevalence of antibiotic resistant cases in tertiary care hospitals of Nepal. Front Microbiol. 2018 Nov 27;9:2749. [PubMed | Full Text | DOI]

6. Characklis WG, Trulear MG, Bryers JD, Zelver N. Dynamics of biofilm processes: methods. Water Research. 1982;16(7):1207-16. [Full Text | DOI]

7. Flemming HC, Wurtz S. Bacteria and archaea on Earth and their abundance in biofilms. Nature Reviews Microbiology. 2019;17:247-60. [PubMed | Full Text | DOI]
8. Flemming HC, Wingender J, Szewzyk U, Steinberg P, Rice SA, Kjelleberg S. Biofilms: an emergent form of bacterial life. Nat Rev Microbiol. 2016 Aug 11;14:563-75. [PubMed | Full Text | DOI]

9. Sharma D, Misba L, Khan AU. Antibiotics versus biofilm: an emerging battleground in microbial communities. Antimicrob Resist Infect Control. 2019 May 16;8:76. [PubMed | Full Text | DOI]

10. Yin W, Wang Y, Liu L, He J. Biofilms: The microbial “Protective Clothing” in extreme environments. Int J Mol Sci. 2019 Jul 12;20(14):3423. [PubMed | Full Text | DOI]

11. Tsuneda S, Aikawa H, Hayashi H, Yuasa A, Hirata A. Extracellular polymeric substances responsible for bacterial adhesion onto solid surface. FEMS Microbiol Lett. 2003 Jun 27;223(2):287-92. [PubMed | Full Text | DOI]

12. Hussain M, Wilcox MH, White PJ. The slime of extracellular polymeric substances: A biofilm matrix. Trends Microbiol. 2020 Aug;28(8):668-81. [PubMed | Full Text | DOI]

13. Flores-Vargas G, Bergsveinson J, Lawrence JR, Korber DR. Environmental biofilms as reservoirs for antimicrobial resistance. Front Microbiol. 2021 Dec 13;12:766242. [PubMed | Full Text | DOI]

14. Wolcott RD, Rhoads DD, Dowd SE. Biofilms and chronic wound inflammation. J Wound Care. 2008 Aug;17(8):333-41. [PubMed | Full Text | DOI]

15. Shrestha LB, Syangtan G, Basnet A, Acharya KP, Chand AB, Pokhrel K. Methicillin-resistant Staphylococcus aureus in Nepal. J Nepal Med Assoc. 2021, 59(237):518-22. [PubMed | Full Text | DOI]

16. Shrestha M, Baral R, Shrestha LB. Metallo-β-lactamase producing non-fermentative gram-negative bacilli from various clinical isolates in a tertiary care hospital: a descriptive cross-sectional study. J Nepal Med Assoc. 2021, 59(241):875-80. [PubMed | Full Text | DOI]

17. Asati S, Chaudhary U. Prevalence of biofilm producing aerobic bacterial isolates in burn wound infections in a tertiary care hospital in northern India. Ann Burns Fire Disasters.2017;30(1):39–42. [PubMed | Full Text | DOI]

18. Shrestha LB, Bhattacharjee N, Khanal B. Comparative evaluation of methods for the detection of biofilm formation in coagulase-negative staphylococci and correlation with antibiogram. Infect Drug Resist. 2018 Apr 24;11:607-13. [PubMed | Full Text | DOI]

19. Shrestha LB, Bhattacharjee N, Rai K, Khanal B. Antibiotic resistance and mecA gene characterization of coagulase-negative Staphylococci isolated from clinical samples in Nepal. Infect Drug Resist. 2020 Sep 14;13:3163-9. [PubMed | Full Text | DOI]

20. Gunardi WD, Karuniaiwati A, Umbas R, Bardoso S, Lydia A, Soebandrio A, et al. Biofilm-Producing Bacteria and Risk Factors (Gender and Duration of Catheterization) Characterized as Catheter-Associated Biofilm Formation. Int J Microbiol. 2021 Feb 22;2021:8869275. [PubMed | Full Text | DOI]

21. Patel R. Biofilms and antimicrobial resistance. Clin Orthop Relat Res. 2005 Aug;(437):41-7. [PubMed | Full Text | DOI]

22. Donlan RM. Role of biofilms in antimicrobial resistance. ASAIO J. 2000 Nov-Dec;46(6):S47-52. [PubMed | Full Text | DOI]

23. Verderosa AD, Totsika M, Fairfull-Smith KE. Bacterial biofilm eradication agents: a current review. Front Chem. 2019 Nov 28;7:824. [PubMed | Full Text | DOI]

24. Karygianni L, Ren Z, Koo H, Thurnheer T. Biofilm matrixome: Extracellular components in structured microbial communities. Trends Microbiol. 2020 Aug;28(8):668-81. [PubMed | Full Text | DOI]

25. Nguyen PTM, Nguyen MTH, Bolhuis A. Inhibition of biofilm formation by alpha-mangostin loaded nanoparticles against Staphylococcus aureus. Saudi J Biol Sci. 2021 Mar;28(3):1615-21. [PubMed | Full Text | DOI]

26. Oliva A, Stefani S, Venditti M, Di Domenico EC. Biofilm-related infections in gram-positive bacteria and the potential role of the long-acting agent Dalbavancin. Front Microbiol. 2021 Oct 22;12:749685. [PubMed | Full Text | DOI]

27. Morshdy AEMA, El-Tahlawy AS, Qari SH, Qumsani AT, Bay DH, Sami R, et al. Anti-biofilms’ activity of garlic and thyme essential oils against Salmonella typhimurium. Molecules. 2022 Mar 28;27(7):2182. [PubMed | Full Text | DOI]

28. Tanwar J, Das S, Fatima Z, Hameed S. Multidrug resistance: an emerging crisis. Interdiscip Perspect Infect Dis. 2014;2014:541340. [PubMed | Full Text | DOI]

29. Dumaru R, Baral R, Shrestha LB. Study of biofilm formation and antibiotic resistance pattern of gram-negative Bacilli among the clinical isolates at BPKIHS, Dhankuta. BMC Res Notes. 2019 Jan 18;12(1):38. [PubMed | Full Text | DOI]

30. Follieri V, Franci G, Dell’Annunziata F, Giugliano R, Foglia F, Sperlengo R, et al. Evaluation of antibiotic resistance and biofilm production among clinical strain isolated from medical devices. Int J Microbiol. 2021 Aug 14;2021:903278. [PubMed | Full Text | DOI]

31. Shah C, Baral R, Bartaula B, Shrestha LB. Virulence factors of uropathogenic Escherichia coli (UPEC) and correlation with antimicrobial resistance. BMC Microbiol. 2019 Sep 2;19(1):204. [PubMed | Full Text | DOI]

32. Saber T, Samir M, El-Mekawy RM, Ariny E, El-Sayed SR, Enan G, et al. Methicillin- and Vancomycin-resistant staphylococcus aureus from humans and ready-to-eat meat: characterization of antimicrobial resistance and biofilm formation ability. Front Microbiol. 2022 Feb 8;12:735494. [PubMed | Full Text | DOI]

33. Craft KM, Nguyen JM, Berg LJ, Townsend SD. Methicillin-resistant Staphylococcus aureus (MRSA): antibiotic-resistance and the biofilm phenotype. Medchemcomm. 2019 Mar 14;10(8):1231-41. [PubMed | Full Text | DOI]

34. Muhammad MH, Idris AL, Fan X, Guo Y, Yu Y, Jin X, et al. Beyond risk: bacterial biofilms and their regulating approaches. Front Microbiol. 2020 May 21;11:928. [PubMed | Full Text | DOI]
35. Tarawneh O, Abu Mahfouz H, Hamadneh L, Deeb AA, Al-Sheikh I, Alwahsh W, et al. Assessment of persistent antimicrobial and anti-biofilm activity of p-HEMA hydrogel loaded with rifampicin and cefixime. Sci Rep. 2022 Mar 10;12(1):3900. [PubMed | Full Text | DOI]

36. Devanga Ragupathi NK, Muthuirulandi Sethuvel DP, Triplicane Dwarakanathan H, Murugan D, Umashankar Y, Monk PN, et al. The influence of biofilms on carbapenem susceptibility and patient outcome in device associated k. pneumoniae infections: insights into phenotype vs genome-wide analysis and correlation. Front Microbiol. 2020 Dec 14;11:591679. [PubMed | Full Text | DOI]

37. Heidari R, Farajzadeh Sheikh A, Hashemzadeh M, Farshadzadeh Z, Salmanzadeh S, Saki M. Antibiotic resistance, biofilm production ability and genetic diversity of carbapenem-resistant Pseudomonas aeruginosa strains isolated from nosocomial infections in southwestern Iran. Mol Biol Rep. 2022 May;49(5):3811-22. [PubMed | Full Text | DOI]

38. Buommino E, Scognamiglio M, Donnarumma G, Fiorentino A, D’Abrosca B. Recent advances in natural product-based anti-biofilm approaches to control infections. Mini Rev Med Chem. 2014;14(14):1169-82. [PubMed | Full Text | DOI]

39. Shrestha LB, Baral R, Khanal B. Comparative study of antimicrobial resistance and biofilm formation among Gram-positive uropathogens isolated from community-acquired urinary tract infections and catheter-associated urinary tract infections. Infect Drug Resist. 2019 Apr 23;12:957-63. [PubMed | Full Text | DOI]

40. McGuffie BA, Vallet-Gely I, Dove SL. σ factor and anti-σ factor that control swarming motility and biofilm formation in Pseudomonas aeruginosa. J Bacteriol. 2015 Nov 30;198(5):755-65. [PubMed | Full Text | DOI]

41. Donlan RM. Biofilms and device-associated infections. Emerg Infect Dis. 2001 Mar-Apr;7(2):277-81. [PubMed | Full Text | DOI]

42. Shrestha LB, Bhattarai NR, Khanal B. Bacteriological profile and antimicrobial susceptibility pattern among isolates obtained from body fluids. J Nepal Health Res Counc. 2019 Aug 4;17(2):173-7. [PubMed | Full Text | DOI]

43. Basnet A, Chand AB, Shrestha LB, Pokhrel N, Karki L, Shrestha SKD, et al. Co-infection of uropathogenic Escherichia coli among covid-19 patients admitted to a tertiary care centre: a descriptive cross-sectional study. JNMA J Nepal Med Assoc. 2022 Mar 11;60(247):294-8. [PubMed | Full Text | DOI]

44. Baral R, Shrestha LB, Ortuno-Gutierrez N, Pyakure P, Rai B, Rimal SP, et al. Low yield but high levels of multidrug resistance in urinary tract infections in a tertiary hospital, Nepal. Public Health Action. 2021 Nov 1;11(Suppl 1):70-6. [PubMed | Full Text | DOI]

45. Lamont RJ, Jenkinson HF. Life below the gum line: pathogenic mechanisms of Porphyromonas gingivalis. Microbiol Mol Biol Rev. 1998 Dec;62(4):1244-63. [PubMed | Full Text | DOI]

46. Overman PR. Biofilm: a new view of plaque. J Contemp Dent Pract. 2000 Aug 15;1(3):18-29. [PubMed]

47. Ingendoh-Tsakmakidis A, Mikolai C, Winkel A, Szafranski SP, Falk CS, Rossi A, et al. Commensal and pathogenic biofilms differently modulate peri-implant oral mucosa in an organotypic model. Cell Microbiol. 2019 Oct;21(10):e13078. [PubMed | Full Text | DOI]

48. Sharma N, Bhatia S, Sodhi AS, Batra N. Oral microbiome and health. AIMS Microbiol. 2018 Jan 12;4(1):42-66. [PubMed | Full Text | DOI]

49. Roy R, Tiwari M, Donelli G, Tiwari V. Strategies for combating bacterial biofilms: A focus on anti-biofilm agents and their mechanisms of action. Virulence. 2018 Jan 1;9(1):522-54. [PubMed | Full Text | DOI]

50. Carrascosa C, Raheem D, Ramos F, Saraiva A, Raposo A. Microbial biofilms in the food industry—a comprehensive review. Int J Environ Res Public Health. 2021 Feb 19;18(4):2014. [PubMed | Full Text | DOI]

51. Li X, Sun L, Zhang P, Wang Y. novel approaches to combat medical device-associated biofilms. Coatings. 2021;11(3):1-31. [Full Text | DOI]

52. Bhardwaj SB, Mehta M, Sood S, Sharma J. Biofilm formation by drug resistant enterococci isolates obtained from chronic periodontitis patients. J Clin Diagn Res. 2017 Jan;11(1):DC01-DC03. [PubMed | Full Text | DOI]