

Biofilm-Associated Multi-Drug Resistance in Hospital-Acquired Infections: A Review

Mulneh Assefa , Azanaw Amare

Department of Medical Microbiology, School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, University of Gondar, Gondar, Ethiopia

Correspondence: Mulneh Assefa, Tel +251945572632, Email mulnehassefa2010@gmail.com

Abstract: Biofilm-related multi-drug resistance (MDR) is a major problem in hospital-acquired infections (HAIs) that increase patient morbidity and mortality rates and economic burdens such as high healthcare costs and prolonged hospital stay. This review focuses on the burden of bacterial biofilm in the hospital settings, their impact on the emergence of MDR in the HAIs, biofilm detection methods, recent approaches against biofilms, and future perspectives. The prevalence of biofilm-associated MDR among HAIs ranges from 17.9% to 100.0% worldwide. The predominant bacterial isolates causing HAIs in recently published studies were *S. aureus*, *A. baumannii*, *K. pneumoniae*, and *P. aeruginosa*. In addition to the use of qualitative and quantitative methods to detect biofilm formation, advanced PCR-based techniques have been performed for detecting biofilm-associated genes. Although there are suggested therapeutic strategies against biofilms, further confirmation of their efficacy for in vivo application and antibiotics targeting biofilm-associated genes/proteins to minimize treatment failure is required for the future.

Keywords: biofilm, multi-drug resistance, hospital-acquired infections

Introduction

Biofilm is an aggregate of microorganisms embedded within a self-produced substance or extracellular polymeric matrix and is irreversibly attached to living or non-living surfaces, and it is a useful adaptation of microorganisms, enabling them to survive in certain environments.^{1,2} Biofilm formation is a sequential and multi-stage phenomenon that includes the attachment of free-floating microorganisms to a surface, microcolony formation or cell growth, maturation or development, and dispersion that enables biofilms to spread and colonize new surfaces.³ Globally, biofilm-associated infections are more prevalent in the hospital settings owing to their recalcitrant nature and difficulty in treatment. Biofilms are estimated to be responsible for more than 65% of nosocomial infections, approximately 80% of chronic infections, and 60% of all human bacterial infections.^{4,5}

Biofilm-related diseases increase the morbidity and mortality rates of patients and economic burdens such as high healthcare costs and prolonged patient stays.⁶ Biofilms play an important role in facilitating the emergence of antimicrobial resistance, generating chronic infections, modulation of host immune response, contamination of medical devices, and existence in the hospital effluents, which are frequent reservoirs of drug-resistant genes.⁷ The formation of biofilms is associated with factors such as the microbial density and type of organisms to which the device is exposed, the duration of device use, presence of essential nutrients, nature of the device, the concentration of drug, environment, and expression of biofilm-associated genes.^{8,9}

Bacterial biofilms in hospitals can be formed in the hospital wastewater, high-touch solid surfaces, left-over drugs, and medical instruments, causing the common hospital-acquired infections (HAIs) such as ventilator-associated pneumonia (VAP), surgical site infections (SSIs), catheter-associated urinary tract infections (CA-UTI), and bloodstream infections (BSIs).¹⁰ Both Gram-positive and Gram-negative bacteria can cause HAIs, with the most prevalent biofilm-forming bacteria being methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis*, Viridans Streptococci,

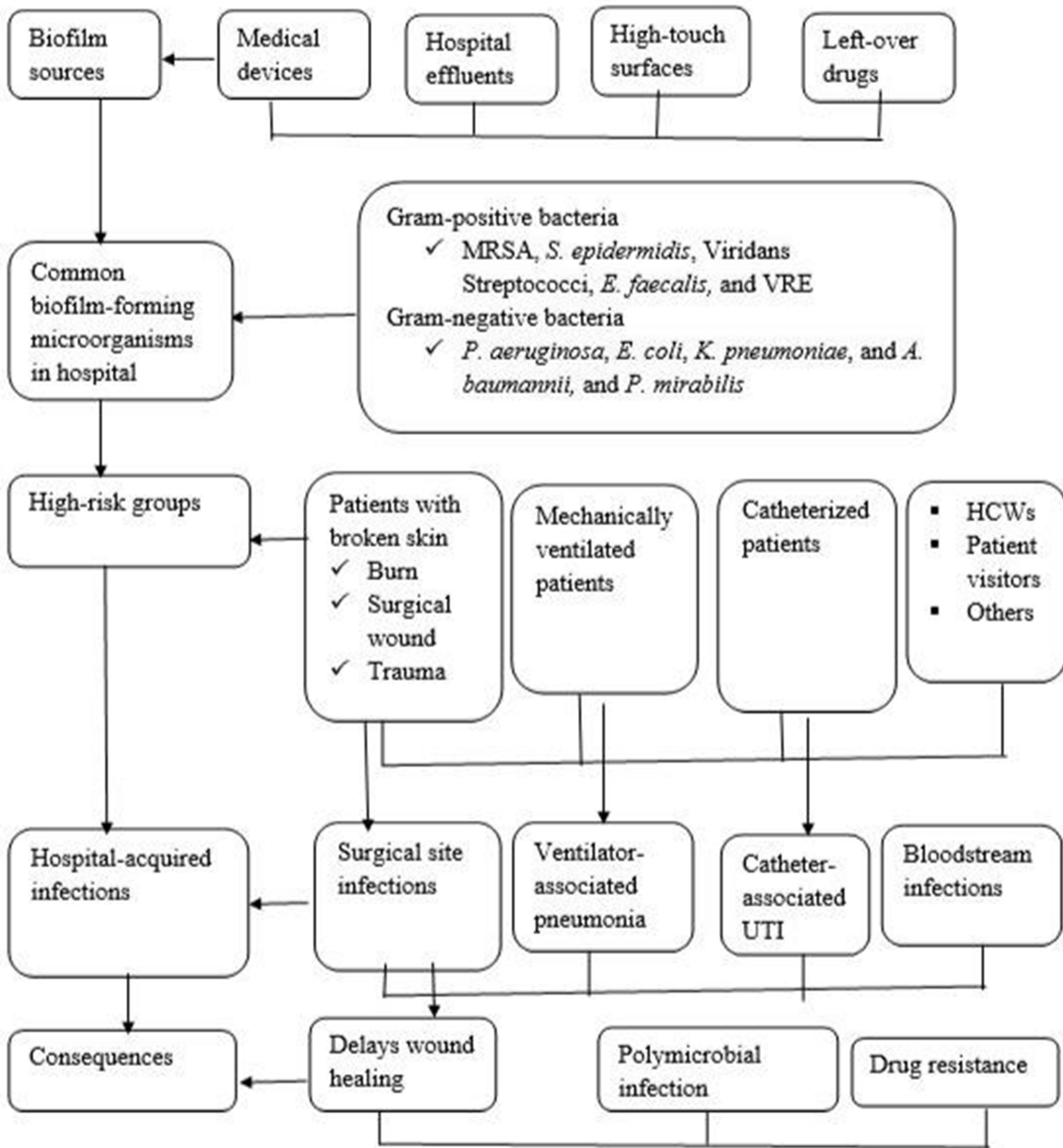


Figure 1 The link between biofilm-hospital-infection.

Abbreviations: HCWs, healthcare workers; UTI, urinary tract infection; MRSA, methicillin-resistant *Staphylococcus aureus*; VRE, vancomycin-resistant Enterococci.

Enterococcus faecalis, Vancomycin-resistant Enterococci (VRE), *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Proteus mirabilis*, and *Klebsiella pneumoniae* (Figure 1).^{11,12}

The Burden of Biofilm in the Hospital Settings

Biofilms in Medical Devices

Biofilms aid microbes to easily adhere to indwelling medical devices (IMDs) such as contact lenses, central venous catheters, mechanical heart valves, peritoneal dialysis catheters, prosthetic joints, pacemakers, urinary catheters, voice

prostheses, intravascular catheters, dental inserts, breast implants, and orthopedic inserts are a potential risk of drug-resistant pathogens for patients following these devices.^{13,14} Biofilms in the endotracheal tubes of mechanically ventilated patients act as a protective shield against host immunity and can induce chronic and recurrent infections that resist commonly prescribed drugs.¹⁵

S. epidermidis has emerged as an important cause of nosocomial bloodstream infections, which can mainly occur through colonization and adherence to medical devices such as central venous catheters because of their inherent capacity to form biofilms.¹⁶ A study on IMDs that included the central venous plexus tip, endotracheal tube, Foley's catheter tip, femoral tip, and ventriculoperitoneal shunt tube detected 46.3% biofilm-producing microbes. The most frequent biofilm-producing Gram-negative bacteria were *K. pneumoniae* (30%), *Burkholderia cepacia* complex (30%), and *P. aeruginosa* (15%), whereas biofilm-producing Gram-positive bacteria were *E. faecalis* (54.5%), *S. aureus* (27.3%) and *S. epidermidis* (18.2%). Strong biofilm production has been observed in *P. aeruginosa* using the quantitative biofilm detection method.¹⁷

Johani et al¹⁸ reported 70% biofilm production on the commonly isolated bacteria associated with intensive care unit (ICU) surfaces, such as *Staphylococcus*, *Propionibacterium*, *Pseudomonas*, *Bacillus*, *Enterococcus*, *Streptococcus*, and *Acinetobacter* species. Evidence for biofilm formation on urinary catheters showed that 40.2% of bacterial isolates were considered biofilm producers, of which 57.1% of them were *E. coli*.¹⁹ Another study reported that catheter-associated biofilm-producing bacteria were found in 36% of isolates with a significant biofilm producer of *E. coli* (24%), extended-spectrum beta-lactamase-producing *E. coli* (2%), *Klebsiella* spp. (19%), *E. faecalis* (8%), *S. aureus* (3%), *P. mirabilis* (18%), *P. aeruginosa* (17%), and *Citrobacter* spp. (9%).²⁰

Biofilms on Hospital Surfaces

Biofilms can also be formed in a hospital dry environment or on high-touch surfaces, such as ward rooms and departments, folders, clipboards, the chair, commode, sanitizing bottles, the trolley, and others. A recent study by Bhatta et al,¹¹ demonstrated using specimens from high-touch hospital sites such as the surface of biometric attendance devices, elevator buttons, door handles, staircase railings, telephone sets, and water taps. *S. aureus* was predominantly identified, followed by *Acinetobacter* spp., *E. coli*, and *Pseudomonas* species. Among the *S. aureus* isolates, 31.8% were biofilm producers and 62.5% of the MRSA isolates were biofilm producers. A study that investigated the occurrence, prevalence, and diversity of dry biofilms on hospital surfaces reported that complex dry biofilms containing bacterial pathogens are virtually universal on hospital surfaces such as MRSA and *Bacillus* spp., even if there was a regular cleaning and disinfection of the hospital surroundings.²¹

A study evaluated the biofilm formation capacity of *Staphylococcus* spp. isolated from surfaces and medicotechnical materials such as beds, soils, carriages, baby vanity tables, weighs babies, mattresses, cupboards, and Cesarean boxes at the university hospital center revealed that 37% of the isolated strains were biofilm formers observed that all *S. lugdunensis* and *S. warneri* isolates were biofilm formers followed by *S. epidermidis* (60%).²² Another study conducted on ICU surface swab samples from bed rails, sinks, food tables, trolley handles, sheets, ventilator inlets, blankets, sheets, door handles, light switches, bedside tables, bedside table drawers, drapes, normal saline stands, and neonatal incubators reported 95.8% biofilm producer *A. baumannii* isolates, with 45.83%, 29.16%, and 20.83% isolates showed strong, moderate, and weak ability for biofilm formation, respectively. The ability of bacterial isolates recovered from the bed rail to form biofilms poses a high risk of infection to hospitalized patients and healthcare workers, and patient visitors since these isolates can form long-lasting surface biofilms that persist and transmit pathogenic microorganisms.²³

Biofilms in the Hospital Effluents

Hospital wastewater is harmful to the environment and human health due to its complex chemical composition and high potency toward becoming a source of biofilm-forming drug-resistant microorganisms and resistant genes to the community.²⁴ In a study conducted using a wastewater sample in a burn center, Iran, biofilm formation was observed in 70% of the *P. aeruginosa* isolates. The potential formation of biofilm was related to gentamicin, imipenem, tobramycin, and piperacillin resistance. Additionally, the *pslA* gene is involved in biofilm-producers with a frequency

of 42.9%.²⁵ A study determined the presence of imipenem-resistant bacteria in the effluent of a teaching hospital, France showed 22.1% imipenem-resistant bacterial isolates from 14-day-old biofilm growth of bacteria formed in the pipe sewer.²⁶ Therefore, the occurrence of biofilm in the hospital settings is the main reason for an increasing multi-drug resistance (MDR) challenging hospitalized patients.

Laboratory Diagnosis of Bacterial Biofilms

The formation of biofilm can be detected *in vitro* via several methods, such as qualitative and quantitative methods. The qualitative detection of biofilm involves the use of microscopy (such as light, fluorescent, scanning electron, and scanning electrochemical), Infrared and Raman spectroscopic analysis of biofilm, small-angle X-ray scattering, and surface plasmon resonance imaging.^{27–31} Additionally, biofilm formation and virulence can be detected by colorimetric means using the Congo red agar method by observing the colony color change, which is commonly used in polysaccharide-rich, slime-producing gram-positive or gram-negative bacteria.³²

In the quantitative detection of biofilms, various direct and indirect methods have been used to quantify the biofilm population. The direct counting methods primarily enumerate cells that can be cultured, including plate counts, microscopic-cell counts, Coulter cell counting, flow cytometry, and fluorescence microscopy. Indirect measurement methods include the determination of dry mass, total organic carbon, ATP bioluminescence, total protein, quartz crystal microbalance, and microtiter plate assay, which is the detection of biofilm using a microtiter reader.^{31,33,34}

The growth of biofilm-embedded bacteria on medical devices can also be detected using the roll plate method, acridine orange staining, streak plating of arginine swab, and sonication, vortex, and plate counting.³⁵ Advances in microbiology have developed molecular detection of biofilm-associated genes using polymerase chain reaction (PCR)-based techniques such as qualitative real-time PCR, multiplex PCR, conventional PCR, and *in-silico* PCR are used for the identification of pathogens by amplifying species-specific nucleic acid sequences and virulence factors by amplifying target virulence genes such as biofilm genes with the usage of gene-specific primers.^{36–38}

Biofilm-Mediated MDR in HAIs

Biofilms are problematic in hospitals due to their risk of drug resistance in hospitalized patients as such individuals are prone to medical devices, have higher exposure to antibiotics, and are harboring colonizers of biofilm-forming bacteria in the surrounding environment that prolongs the course of treatment.³⁹ The microbial biofilm is the main mechanism of drug resistance that contributes to the emergence of MDR microorganisms because of the restricted penetration of antibiotics into the biofilm matrix, high cellular density, quorum sensing abilities, the decreased growth rate of bacteria in the biofilm, an elevated expression of efflux pumps, high mutation frequency to develop new strain, the presence of persistent cells, and overexpression and exchange of resistance genes among bacteria within a biofilm.^{40,41}

Nowadays, studies have reported the burden of MDR related to biofilms, particularly in the hospital settings. In this review, studies reported on the study period, country, specimen source, the type and number of bacterial isolates, the percentage of biofilm production, and MDR related to biofilm have been included and summarized. Among the available studies, biofilm production in the bacterial isolates was found between 13.5% and 100.0%, with up to 36.3%, 50.0%, and 68.4% of strong, moderate, and weak biofilm former isolates, respectively. Biofilm-associated MDR ranged from 17.9% to 100.0%, with MRSA and *E. coli* isolates showing 100.0% MDR (Table 1).

The high prevalence of biofilm formation and biofilm-associated MDR in certain studies is related to differences in the sample size, geographic location, study period, study population, method of biofilm detection, and type of bacterial isolate.

Current Approaches Targeting Bacterial Biofilms and Future Perspectives

Biofilm treatment is very challenging because it requires a combination of antibiotics and a long time. Currently, many researchers have attempted solutions to target microbial biofilms to minimize their effect on patient outcomes, and the inadequate response of conventional antimicrobial strategies to counteract biofilm development demands urgent alternatives. A recent systematic review summarized certain evidence supporting the effects of probiotic cells such as *Lactobacillus*, *Lactococcus*, and *Streptococcus* have a higher capability to hinder biofilm formation, causing

Table I Summary of Studies Reported the Isolate, Biofilm Production, and MDR

Reference	Country	Study Period	Specimen	Bacterial Isolate, Number	Biofilm Production (%)	Biofilm Phenotype	Biofilm-Associated MDR (%)
[9]	Nepal	-	Wound/pus swab	<i>S. aureus</i> , 43	58.1	Strong: 4.65 Moderate: 23.25 Weak: 30.23	86.7
[50]	India	2011	Urine	<i>E. coli</i> , 135	13.5	-	100.0
[51]	China	2010–13	-	<i>A. baumannii</i> , 272	91.0	Strong: 23.0 Weak: 68.4	71.5
[52]	Tehran	2012–13	-	MRSA, 36	100.0	-	100.0
[53]	Thailand	2013–14	Sputum, urine, pus, blood, pleural fluid, ascetic fluid and wound	<i>A. baumannii</i> , 225	76.9	Strong: 23.6 Moderate: 32.9 Weak: 20.4	73.3
[54]	Nepal	2015	Wound/pus swab	<i>S. aureus</i> , 76	46.1	-	68.6
[55]	Iran	2016–17	Urine, wound exudates, intratracheal tube, blood, and sputum	<i>K. pneumoniae</i> , 100	75.0	Strong: 25.0 Moderate: 19.0 Weak: 31.0	51.0
[56]	Iran	2017–18	-	<i>P. aeruginosa</i> , 80	83.75	-	17.91
[57]	Indonesia	2017–18	Sputum, bronchial washing, tracheal aspiration, pus, urine, blood, stool, wound swab, peritoneal fluid, and others	<i>K. pneumoniae</i> , 167	85.63	Strong: 26.95 Moderate: 28.74 Weak: 29.94	49.1
[58]	Iraq	2017–18	Sputum, urine, bronchial wash, swabs (burn, wound, and ear)	<i>P. aeruginosa</i> , 100	98.0	Strong: 22.0 Moderate: 50.0 Weak: 26.0	58.2
[59]	Uganda	2018	-	<i>E. coli</i> , 200	62.5	-	64.0
[60]	Pakistan	2018	Wound swab, sputum, urine, and blood	<i>P. aeruginosa</i> , 52	100.0	Strong: 36.3 Moderate: 40.3 Weak: 17.3	38.4
[61]	Egypt	2018–19	Sputum, endotracheal aspirate, blood, and wound swab	<i>A. baumannii</i> , 94	70.1	Strong: 20.2 Moderate: 34.0 Weak: 16.0	34.8
[62]	Gujranwala	2020	Urine catheter tips	<i>K. pneumoniae</i> , 170	66.66	Strong: 22.66 Weak: 44.0	82.0

Abbreviations: MDR, multi-drug resistance; MRSA, methicillin-resistant *Staphylococcus aureus*; CoNS, coagulase negative *Staphylococcus aureus*.

reductions of up to 99.9%.⁴² Additionally, bacteriophages equipped together with a wide panel of enzyme-degrading extracellular polymeric macromolecules are powerful weapons to combat biofilms.⁴³ Phages can also control carbapenemase-producing organisms associated with microbial biofilms in the healthcare environment.⁴⁴ Moreover, a catheter model suggested that lytic and temperate bacteriophages isolated from sewage samples can be used to treat antibiotic-resistant *Providencia* spp. infections in catheter-associated urinary tract infections.⁴⁵

Multi-drug resistant bacteria consume iron from the hosts for their biofilm formation and are life-threatening for hospitalized patients, particularly ICU patients with long-time hospitalization, severe complications, and lower immunity.⁴⁶ In vitro evidence suggests that the combination of iron chelator deferiprone with the respective antibiotics resulted in a significant decline in bacterial numbers by two to three logs compared to the effect of antibiotics alone against coagulase-negative staphylococci.⁴⁷ A synthetic hexadentate iron chelator, N, N'-bis

(2-hydroxybenzyl) ethylenediamine-N, N'-diacetic acid combined with colistin is highly effective in vitro against biofilms formed by the clinical strains of *P. aeruginosa*.⁴⁸ A study revealed that Fe (II) chelating Terpyridine-micelles are a promising addition to Fe (III) chelating strategies to inhibit biofilm formation in cystic fibrosis lung infections.⁴⁹

Conclusion

Biofilms frequently occur on medical devices, hospital surfaces, and effluents are at high risk for inpatients, outpatients, and even the community. Globally, the prevalence of biofilm-associated MDR among HAIs ranges from 17.9% to 100.0%. The predominant bacterial isolates causing HAIs in recently published studies were *S. aureus*, *A. baumannii*, *K. pneumoniae*, and *P. aeruginosa*. Currently, different qualitative and quantitative methods have been applied to detect biofilm formation and PCR-based techniques for biofilm-associated genes. Biofilm challenged the patient's treatment and resulted in drug resistance and/or treatment failure, particularly in HAIs. Therefore, infection control, more advanced diagnosis of biofilms, effective combination therapy by further confirmation of in vitro studies, and antibiotics targeting genes and proteins essential for biofilm formation are needed.

Abbreviations

BSIs: bloodstream infections, CA-UTI: catheter-associated urinary tract infections, HAIs: hospital-acquired infections, ICU: intensive care unit, IMDs: indwelling medical devices, MDR: multi-drug resistance/t, MRSA: methicillin-resistant *Staphylococcus aureus*, PCR: polymerase chain reaction, SSIs: surgical site infections, VAP: ventilator-associated pneumonia, VRE: vancomycin-resistant Enterococci.

Data Sharing Statement

All the data are freely available online.

Author Contributions

All authors made a significant contribution to this manuscript; took part in the conception, drafting, revising or critically reviewing the article; have agreed on the journal to which the article has been submitted; gave final approval for the version to be published; and agreed to be accountable for all aspects of the work.

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