Acta Scientific Microbiology (ISSN: 2581-3226)

Volume 8 Issue 7 July 2025

Review Article

The Role of Biofilm in Antibiotic Resistance and Chronic Wound Infections of Patients

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Received: June 18, 2025

Published: June 28 2025

Abstract

A biofilm is a group of microorganisms that adheres securely to inanimate or living surfaces and is encased in an extracellular polymeric matrix or self-generated substance. Chronic wound infections have a major clinical and financial impact on people all over the world. They also have a big impact on healthcare systems and the lives of those who are impacted. With a focus on important topics like the pathophysiology of biofilm growth and the clinical implications of biofilms in chronic infections, this review aims to provide a thorough examination of biofilm formation in chronic illnesses. Methicillin-resistant Staphylococcus aureus (MRSA), Coagulase-negative staphylococci (CoNS), and multidrug-resistant Gram-negative bacilli, including Metallo Beta-Lactamase (MBL), AmpC Beta-Lactamase, and Extended Spectrum Beta-Lactamase (ESBL), as well as mechanisms like efflux and porin deficiency, are among the multidrug-resistant organisms that are becoming more prevalent in these areas. Biofilms pose a significant challenge to modern healthcare and are a substantial contributor to the chronicity, persistence, and resistance to treatment of wound infections. Their multi layered structure, which is maintained by a protective extracellular matrix, slows down the immune system and antimicrobial penetration, which prolongs healing, increases patient morbidity, and raises health care costs.

Keywords: Biofilm; Chronic Wound Infections; Multidrug-Resistant Organisms and Health Care

Introduction

A biofilm is a collection of microorganisms enclosed in a self-generated substance or extracellular polymeric matrix, firmly adhering to living or inanimate surfaces. This matrix provides a protective barrier, shielding the bacteria from both the penetration of antimicrobial agents and the host immune system [1,2]. This phenomenon serves as a valuable adaptation for microorganisms, allowing them to thrive in specific environments [3].

Biofilms exhibit a remarkable ability to withstand antibiotic treatment, with bacteria residing within biofilms demonstrating resistance levels hundred to thousand times greater than their planktonic counterparts [4]. This elevated resistance is a multifaceted phenomenon arising from several mechanisms, including impaired antibiotic penetration through the biofilm matrix, reduced metabolic activity of bacteria within the biofilm, and the presence of persister cells [5]. The complex architecture of biofilms and the physiological state of the embedded bacteria contribute significantly to the recalcitrance of chronic wound infections [6]. The extracellular matrix acts as a physical barrier, impeding the diffusion of antibiotics and preventing them from reaching the bacteria at effective concentrations. These biofilms may either be linked to medical devices or develop independently from foreign materials through the colonization of host tissue, a phenomenon primarily seen in chronic infections [7].

Biofilm formation and composition

Biofilm formation is a multistep process.

Development of biofilms

The Planktonic bacteria adhere to a surface via physical and chemical interactions. Bacteria produce adhesive structures and begin synthesizing extracellular matrix components. Microcolonies grow and develop into a complex, three-dimensional structure with distinct microenvironments. Cells or clusters are released to colonize new sites [8]. This dynamic process is regulated by environmental signals and bacterial communication mechanisms such as quorum sensing [9,10].

Composition of the Biofilm Matrix The biofilm matrix, also known as extracellular polymeric substance (EPS), comprises it Provides structural integrity and adhesion. Proteins are involved in enzymatic functions and structural support. Extracellular DNA (eDNA) is Contributing to biofilm stability and gene exchange. Lipids and other macromolecules: Enhancing biofilm cohesion and defense. This matrix protects embedded bacteria from antimicrobial agents and immune system attacks [11].

Biofilm in chronic wound infection

Chronic wound infections pose a significant global clinical and economic burden and exert a marked influence on healthcare systems and the quality of life of the affected individuals [11]. These infections are commonly refractory to the usual antibiotic therapies and may result in prolonged hospital stay, increased morbidity, and even amputation in more severe ones [3]. The formation of biofilms is considered a key factor to the persistence and antibiotic resistance of chronic wound infections [12].

The formation of biofilms in wounds is a dynamic process, with research indicating that bacteria can develop a mature biofilm on a wound bed within 24 hours. Chronic wounds are predominantly characterized by a poly-microbial biofilm present in 60% of cases, in contrast to only 6% in acute wounds [13,14]. The process of wound healing is intricate, commencing with an injury and culminating in successful closure. When healing is compromised, it can result in the colonization of microorganisms within the wound bed, leading to the production of exudate and associated pain [15].

Normal wound healing is a complex process that involves specific stages, summarized in the following: Coagulation and haemostasis, Inflammation, Proliferation, Remodelling. A biofilm associated with chronic wounds consists of various groups of bacteria, typically exhibiting distinct genotypes, and is additionally bound together by extracellular polymeric substances (EPS). Numerous pathogenic microorganisms, both aerobic and anaerobic, including bacteria and yeasts, are recognized for their ability to colonize wounds [16,17]. When an injury takes place, the skin barrier's protective function is compromised, leaving the wound area vulnerable to numerous pathogens, which facilitates microbial colonization at the site of the wound.

Patients with chronic ulcers usually harbour a variety of colonizing bacterial species [18]. These pathogens, when embedded in biofilms, create a protective environment that makes them more resistant to the host's immune response and conventional antimicrobial treatments. These bacteria can produce a variety of virulence factors and is known for its resistance to antibiotics, making infections challenging to treat. Principal contributors to delayed wound healing and infection *encompass beta-hemolytic streptococci, Candida albicans, Pseudomonas aeruginosa*, and *Staphylococcus aureus* [8].

This review seeks to deliver an in-depth analysis of biofilm formation in chronic infections, concentrating on key aspects such as the pathogenesis of biofilm development and the clinical consequences of biofilms in chronic infections. By examining the mechanisms that contribute to biofilm formation and persistence, this review underscores the difficulties posed by biofilm-related infections and the shortcomings of existing treatment approaches. Ultimately, the objective of this review is to educate and direct future research and clinical practices in the management of chronic infections associated with biofilms, providing valuable insights into effective prevention and treatment strategies.

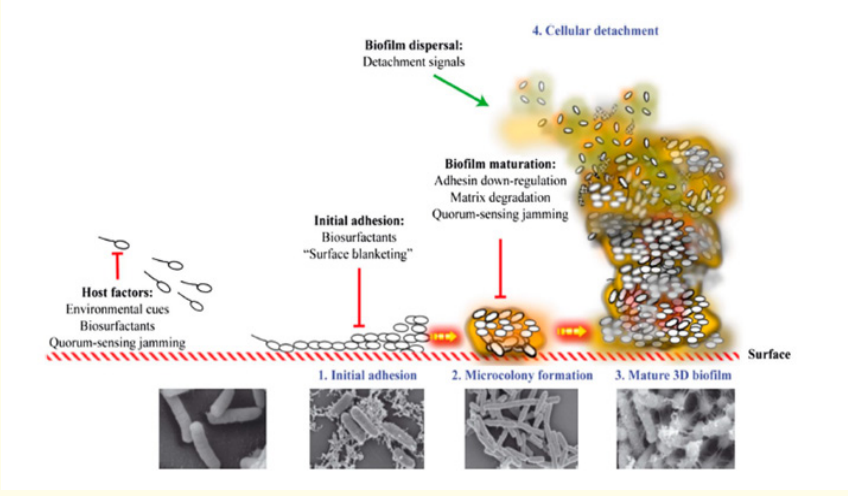

Figure 1: Stages in the biofilm formation process (Hadla and Halabi MA, 2018).

Methodology

A comprehensive review of the literature was conducted using PubMed, Scopus, Web of Science, and Google Scholar, incorporating specific search terms such as 'chronic infections,' 'resistance,' 'biofilm formation,' and 'clinical implications,' focusing on publications in English from the year 2015 to 2025. Studies which had a focus on research in original articles, systematic reviews, or a meta-analysis and dealt with biofilm-related disease and its treatment were eligible for inclusion. Conversely, non-English publications, conference abstracts, and studies deemed to be of low quality were excluded from consideration. Data from the selected studies were extracted and synthesized in a narrative format, emphasizing biofilm mechanisms, clinical implications, and therapeutic approaches. A quality assessment was performed using suitable evaluation tools, and the findings were organized into thematic categories, with a descriptive analysis that underscored significant trends.

Initially, 94 articles were identified from databases including PubMed, Google Scholar, Scopus, ResearchGate, and ScienceDirect. After excluding irrelevant articles [19], removing duplicates [12], and accounting for articles that couldn't be retrieved [12], 51 articles remained. Of these, 14 were excluded due to access issues or lack of relevance, resulting in 37 studies being included in the final review. Ethical approval was not required since the review relied on previously published research, with potential limitations including variability among studies and language constraints.

Identification of studies via databases and registers

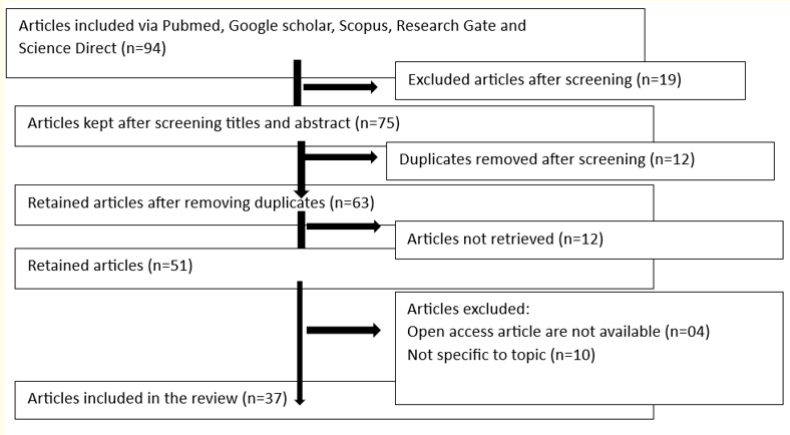

Figure 2: PRISMA flow chart.
n: Number of studies; PRISMA: Preferred Reporting Items for Systematic Reviews.

Lab diagnosis of bacterial biofilms

The diagnosis of infectious bacteria biofilm within chronic wounds includes qualitative, quantitative and molecular methods. Direct evidence for the presence of biofilms in tissues is obtained using visualization techniques such as light, fluorescent, and electron microscopy [19]. A chromogenic Congo red agar for the differentiation of biofilm producing from non-biofilm producing strains of *S. epidermidis* using colony color change. These quantitative techniques, which comprise plate counts, flow cytometry (FCM), and microtiter assays, quantify biofilm-related biomass and bacterial burden.

Detection as part of dressings and devices includes roll plating and sonication. Molecular diagnostics, such as those using PCR as platform resulting in gene-specific amplification, allow selective detection of biofilm forming microorganisms, which is a necessity for proper treatment planning [20]. Understanding these diagnostic methodologies aids in developing targeted interventions to disrupt biofilms and improve chronic wound management.

Biofilms and drug resistance

Bacterial biofilms are the etiological agent of a number of chronic diseases. The enhanced resistance of cells within the biofilms to antibiotics is thought to be the main cause for the chronic nature of these infections. This resistance is likely to be polyfactorial, and different mechanisms of resistance co-interact together to provide a higher level of general resistance to the biofilm [21].

In developing countries inappropriate use of antimicrobials, overcrowded hospitals, poor infection control practices add to the problem in form of multidrug resistance.

The rising incidence of multidrug-resistant organisms, such as Methicillin-resistant Staphylococcus aureus (MRSA), Coagulase-negative staphylococci (CoNS), and multidrug-resistant Gram-negative bacilli, including Extended Spectrum Beta-Lactamase (ESBL), Metallo Beta-Lactamase (MBL), and AmpC Beta-Lactamase, along with mechanisms like efflux and porin deficiency, significantly complicates the situation and poses a serious challenge in these regions [22].

Various factors include patient related factors (old age, nutritional status, comorbid illness, pre-existing infection, the length of preoperative hospital stay) and procedure related factors (poor surgical technique, prolonged duration of surgery, preoperative part preparation, and inadequate sterilization of surgical instruments) can influence the risk of wound infections significant.

In addition to known risk factors, the likelihood of infection is significantly influenced by the virulence and invasiveness of the infecting microorganism, the immune status of the host, and the physiological condition of the wound tissue. These elements collectively contribute to prolonged hospital stays and increased healthcare costs. Therefore, effectively reducing the incidence of chronic wound infections requires a comprehensive and pragmatic strategy, recognizing that multiple interrelated factors impact infection risk. In light of these insights, numerous studies have been conducted to explore chronic wound infections, with particular emphasis on the factors that contribute to the persistence and recurrence of infections at postoperative surgical sites.

Impact on antimicrobial treatments

Resistance associated with biofilm is quite a complication for treatment modalities. Young biofilms (i.e., cells with rapid rates of division) are more susceptible to antimicrobials than older biofilms (i.e., cells with relatively low metabolic activities) [23]. Resistance is also modified under oxygen availability as Escherichia coli biofilm grown at lower oxygen tension were more resistant to antibiotics [24].

The development of novel therapies seeking to ameliorate drug penetration and microbial defences has come from advancements in biofilm research [25,26]. Further, horizontal gene transfer allows greater antibiotic resistance by permitting the assimilation of resistance genes into biofilms from adjacent microorganisms [21].

Mycobacterial biofilms demonstrate extreme resilience against disinfectants and antibiotics like amikacin and clarithromycin *in vitro* [27]. During the later stages of biofilm formation, bacteria are less susceptible to antibiotic intervention, thus Research suggests that these types of antibiotics are best utilized at the early stages of biofilm formation [28].

Impact of Biofilm in chronic wound infections

Study	Country	Bacteria	Biofilm Prevalence	Clinical Impact	References No
Kala Harika., *et al*. 2020	India	*S. aureus, K. pneumoniae*	High	Non-healing ulcers	[29]
Karim., *et al*. 2021	India	*K. pneumoniae*	75%	Resistant diabetic ulcers	[30]
Vadla Shravani., *et al*. 2023	India	*P. aeruginosa*	High	Persistent post-surgical infections	[31]
Sharma S., *et al*. 2023	India	Various	Present	Device-related resistance	[4]
Pai L., *et al*. 2023	India	Various	Not quantified	Genetic resistance mechanisms	[5]
Bhatt., *et al*. 2015	USA	*P. aeruginosa*	76.8% MDR	Delayed healing	[32]
Ahmed., *et al*. 2016	Egypt	*K. pneumoniae*	Gene-positive	High cephalosporin resistance	[33]
Di Domenico., *et al*. 2017	Italy	Mixed	100%	Chronicity of ulcers	[18]
Hera Nirwati., *et al*. 2019	Indonesia	*Klebsiella pneumoniae*	85.7% strong/ moderate	Biofilm formation strongly associated with multidrug resistance	[34]
Bidossi., *et al*. 2020	Italy	*S. aureus, S. lugdunensis*	Majority	Joint infection complications	[35]
Falcone., *et al*. 2021	Italy	Mixed	High	Requires surgical debridement	[15]
Schulze., *et al*. 2021	Germany	Various	Virulence linked	Targeted therapy needs	[36]
Assefa., *et al*. 2022	Ethiopia	*S. aureus, A. baumannii*	Not specified	Prolonged hospitalization	[3]
Mendhe., *et al*. 2023	USA	Various	High	Immune evasion, late diagnosis	[2]
Binzhi Dan., *et al*. 2023	China	*Klebsiella pneumoniae*	High (moderate–strong)	Strong link with multidrug resistance; persistent infections	[37]

Table 1

Biofilm Activity in India: A Growing Concern

Recent studies from India have highlighted the alarming role of bacterial biofilms in chronic wound infections and device-associated complications. These biofilms—communities of microorganisms encased in a protective matrix—are not only difficult to eradicate but are also strongly linked with increased antibiotic resistance and prolonged healing times. For instance, the study by Kala Harika., *et al*. (2020) found that biofilm formation was common among Staphylococcus aureus and Klebsiella pneumoniae isolates from chronic wound cases. These infections, particularly in diabetic patients, often failed to respond to conventional treatment, leading to non-healing ulcers. The persistent nature of these infections can be attributed to the biofilm's ability to shield bacteria from both antibiotics and immune responses. In a similar vein, Karim., *et al*. (2021) reported that a significant proportion—75%—of K. pneumoniae strains isolated from diabetic ulcers were biofilm producers. These strains also showed a high level of resistance to commonly used antibiotics. This reinforces the idea that biofilm formation and multidrug resistance often go hand in hand, making such infections especially difficult to treat.

Vadla Shravani., *et al*. (2023) examined Pseudomonas aeruginosa, a notorious pathogen in post-surgical infections. Their findings revealed strong biofilm-forming tendencies along with the presence of quorum-sensing genes that regulate virulence and resistance mechanisms. These infections often persist despite aggressive therapy, leading to delayed recovery and increased hospital stays.

Shifting focus from wounds to medical devices, Sharma., *et al*. (2023) investigated biofilms in infections associated with catheters, implants, and other hospital equipment. Even without exact measurements of biofilm density, the presence of biofilms was linked to treatment failure and recurrent infections, pointing to the challenges faced in managing device-associated infections.

Lastly, Pai., *et al*. (2023) provided insights into the genetic background of biofilm-related resistance. Their work discussed how certain genes enable bacteria to form biofilms and withstand antibiotics, even though the study did not focus on specific infection

sites. This genetic angle offers a deeper understanding of why traditional treatments often fail against biofilm-forming pathogens.

The link between biofilm formation and antibiotic resistance makes it crucial to revise current treatment strategies. Early identification of biofilm-producing strains, use of combination therapies, and adoption of alternative approaches such as phage therapy or anti-biofilm agents could offer better outcomes. At the same time, routine screening for biofilm activity in clinical laboratories and strict infection control measures can play a key role in managing these stubborn infections effectively.

Global insights into biofilm - Associated infections and resistance

Across the globe, there is growing evidence of the significant role played by biofilms in chronic and drug-resistant infections. A wide range of studies from different countries consistently highlight how biofilm formation contributes to treatment failure, prolonged illness, and increased healthcare burdens.

In the United States, Bhatt., *et al*. (2015) reported a high prevalence of multidrug resistance (MDR) among Pseudomonas aeruginosa isolates—76.8% to be exact. These strains were predominantly recovered from chronic wounds were delayed healing was a major clinical concern. The presence of biofilms in these cases not only protected the bacteria from antibiotics but also impaired tissue repair mechanisms.

Ahmed., *et al*. (2016) from Egypt highlighted the emergence of Klebsiella pneumoniae strains harboring specific resistance genes. These gene-positive isolates showed high resistance to cephalosporins, raising red flags about their limited treatment options. The study emphasized the molecular underpinnings that often go hand-in-hand with biofilm development and antibiotic resistance.

European data further confirm the severity of the issue. Di Domenico., *et al*. (2017) from Italy documented a 100% biofilm formation rate among isolates from chronic ulcer patients. These infections were notably persistent and resistant to standard therapies, reinforcing the link between biofilms and wound chronicity.

From Indonesia, Hera Nirwati., *et al*. (2019) investigated K. pneumoniae and found that 85.7% of strains exhibited moderate to strong biofilm-forming abilities. These isolates were also associated with multidrug resistance, clearly indicating that biofilm strength correlates with resistance patterns.

Similarly, Bidossi., *et al*. (2020) in Italy explored joint infections and reported that both Staphylococcus aureus and *S. lugdunensis* were capable of forming biofilms, leading to post-surgical complications such as inflammation and implant failure.

A subsequent Italian study by Falcone., *et al*. (2021) emphasized the severity of biofilm-mediated infections, which frequently required surgical interventions like debridement due to their resistance to medical treatment alone. These infections often involved mixed microbial communities, further complicating management.

From Germany, Schulze., *et al*. (2021) provided insight into how virulence factors in biofilm-forming bacteria necessitate more targeted therapeutic strategies. Their findings support the idea that not just resistance, but pathogenicity itself is enhanced in the presence of biofilms.

In Ethiopia, Assefa., *et al*. (2022) identified S. aureus and Acinetobacter baumannii as common biofilm producers in hospitalized patients. Though biofilm prevalence was not quantified, the infections were associated with prolonged hospital stays and high treatment costs, underscoring the economic burden of such infections in resource-limited settings.

Mendhe., *et al*. (2023) in the USA addressed another dimension—immune evasion. The biofilm-producing bacteria they studied often went undetected in early stages, leading to late diagnoses and missed treatment windows. This delay worsened clinical outcomes. Finally, Binzhi Dan., *et al*. (2023) from China highlighted the strong association between biofilm strength (moderate to strong) in K. pneumoniae and multidrug resistance. These infections were persistent and frequently relapsed, complicating patient recovery and increasing the likelihood of systemic complications. These international studies underscore the global nature of the biofilm challenge. Despite regional differences in pathogens and healthcare systems, one pattern remains clear: biofilm formation is a major contributor to chronicity, antimicrobial resistance, and poor patient outcomes. There is a pressing need for global cooperation

in developing diagnostic tools for early biofilm detection, novel anti-biofilm agents, and guidelines for personalized treatment strategies that consider both resistance profiles and biofilm-forming ability.

Clinical and public health perspective

The potential issue in treating infection is that it is very difficult to eliminate the biofilm-forming bacteria in the majority of cases from a clinic or public health aspect. Clinically, biofilms are behind the long-lasting, non-closing infections, high antibiotic resistances, and failure of treatments. Healthcare systems are heavily challenged by medical device-associated and biofilm-associated infections. They extend the length of hospital stays, increase healthcare expenditure, and are associated with increased mortality. Moreover, the persistence of such infections often leads to increased antibiotic use, which in turn accelerates the development and spread of antimicrobial resistanc (Sharma, Surbhi., *et al*. 2023).

Addressing these challenges calls for a comprehensive approach that combines clinical vigilance with public health initiatives. Early diagnosis, incorporation of anti-biofilm agents, improved infection control practices, and strong antimicrobial stewardship are essential to curb the impact of biofilm-associated infections and reduce their burden on healthcare systems.

Conclusions

Biofilms are an important cause of the persistence, chronicity and therapeutic resistance of wound infections and represent a major problem for present healthcare. Their multilayered structure supported by a protective extracellular matrix, slows down the penetration of antimicrobials as well as immune response, resulting in prolonged healing, greater morbidity to the patient, and increasing health-care cost. In addition, the ubiquitous presence of biofilm-forming, MDR bacteria, including Pseudomonas aeruginosa, Staphylococcus aureus and Klebsiella pneumoniae has further complicated the treatment of infection, emphasizing the urgent demand for accurate diagnosis and personalized treatment strategies. Developments in laboratory diagnostics such as molecular, quantitative and microscopic techniques, have improved the detection and characterization of biofilm-related infections. However, treatment remains challenging due to biofilms' inherent resistance mechanisms, such as reduced metabolic activity, efflux pumps, genetic adaptations, and horizontal gene transfer. Addressing biofilm-related antibiotic resistance is crucial not only for effective wound management but also in the broader fight against antimicrobial resistance globally.

Acknowledgment

The authors are thankful to the HOI, Nabira Mahavidyalaya, Katol and the Head, Department of molecular biology and genetics to providing necessary facilities for carrying out the research work.

Conflict of Interest

The authors declare no financial or commercial conflict of interest.

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