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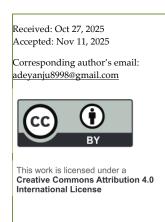


Review Article

# Biofilms in Resource-Limited Settings: Challenges, Opportunities, and Innovative Solutions in Nigeria

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#### Abstract:

Microbial biofilms represent a growing yet often overlooked public health concern, particularly in resource-limited settings where they exacerbate the burden of persistent infections and antimicrobial resistance (AMR). In Nigeria, fragile healthcare infrastructure, poor funding, and a continuous loss of skilled medical professionals compound the difficulty of managing biofilm-associated infections. These microbial communities, embedded in self-produced extracellular matrices, exhibit remarkable resistance to antimicrobials and host immune defenses, leading to chronic and recurrent infections that further strain an already overstretched health system. This review synthesizes evidence published between 2010 and 2025 to examine the burden, challenges, and opportunities surrounding biofilm control in Nigeria. Literature was systematically retrieved from PubMed, Scopus, and Google Scholar, complemented by reports from WHO and the Nigerian Centre for Disease Control. Findings indicate that biofilms not only complicate clinical treatment outcomes but also persist in environmental reservoirs, particularly water systems, serving as hidden amplifiers of resistance and infection transmission. To address these challenges, the review explores low-cost and contextappropriate strategies such as harnessing Nigeria's biodiversity for the discovery of plantderived antibiofilm compounds, implementing decentralized engineering solutions for water treatment, and promoting community-based infection prevention initiatives. It further emphasizes the importance of local innovation, interdisciplinary collaboration, and policy support within a One Health framework that integrates human, animal, and environmental health. By spotlighting the Nigerian experience, this review calls for urgent investment and global attention to biofilm-related infections in Low- and Middle-Income Countries (LMICs).

**Keywords:** Biofilms; Antimicrobial Resistance; Nigeria; One Health; LMICs; Water Systems; Public Health

### Introduction

Biofilms represent a pervasive and complex challenge in global health, impacting various sectors from clinical medicine to environmental management. These intricate microbial communities, characterized by their adherence to surfaces and encapsulation within a self-produced extracellular polymeric substance (EPS) matrix, confer remarkable protection and adaptability to microorganisms (1). This structural organization enhances microbial survival, allowing them to thrive in diverse environments (1, 2). While biofilms can exist in neutral or even beneficial contexts, their role in disease is profound, accounting for an estimated 70% of all microorganism-induced infections globally, with specific national estimates for Nigeria remaining scarce (3).

These infections, often nosocomial and chronic, contribute significantly to patient morbidity and mortality. The protective EPS matrix and altered physiological states within biofilms render the embedded microorganisms highly resistant to conventional antimicrobial agents, often exhibiting resistance levels up to 1000-fold higher than their planktonic counterparts, and effectively evading host immune responses (3, 4).

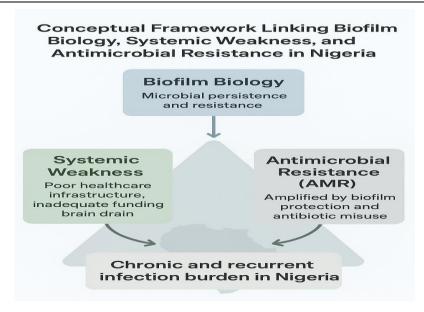
The challenges posed by biofilms particularly acute in Low- and Middle-Income Countries (LMICs), where existing healthcare system vulnerabilities amplify their detrimental effects (3). Nigeria, with its large population and significant health disparities, serves as a critical example of this amplified burden. The nation grapples with a high prevalence of including malaria, infectious diseases, respiratory infections, diarrheal diseases, and tuberculosis, which remain among the leading causes of death, with communicable conditions accounting for a substantial majority of fatalities (5).

The Nigerian healthcare system is severely constrained by multiple systemic challenges. Infrastructure remains inadequate, marked by outdated facilities, overcrowding, limited modern equipment, frequent drug shortages, unreliable power supply, and insufficient access to clean water (6, 7). Financial investment is notably low, with less than 5% of the national budget allocated to healthcare, far below the 15% target recommended by the African Union's Abuja Declaration (7). Consequently, most Nigerians rely on out-of-pocket payments, leaving fewer than 10%

covered by health insurance (6, 7). A critical shortage of medical professionals, worsened by a persistent "brain drain" as skilled personnel migrate abroad for better opportunities, further weakens service delivery (6, 8). The doctor-to-patient ratio in some states is as high as 1:3,500, compared with the World Health Organization's recommended 1:600 (7). These resource gaps undermine infection prevention and control (IPC) practices, with inadequate personal protective equipment (PPE) and IPC materials frequently cited as barriers to compliance (8, 9); overcrowding and a lack of isolation rooms in healthcare facilities further facilitate the spread of healthcare-associated infections (10).

The inherent complexity and resistance of biofilms make them formidable adversaries even in well-resourced healthcare systems. When combined with the systemic deficiencies prevalent in LMICs like Nigeria, the burden of biofilm-associated infections often becomes an unacknowledged or "hidden epidemic" (6). The underlying biofilm nature of persistent infections frequently goes undiagnosed or mismanaged, resulting in prolonged illness, increased morbidity and mortality, and additional strain on already overwhelmed healthcare systems. The true scale of the problem is likely underestimated due to these compounding factors.

Given the convergence of a high infectious disease burden, weak healthcare infrastructure, and the documented presence of antibiotic-resistant biofilms in both clinical and environmental settings, Nigeria represents a critical yet understudied case. While other LMICs, such as India, Kenya, and Bangladesh, have begun advancing targeted biofilm research and interventions (11), Nigeria still lacks a unified synthesis linking biofilm biology, systemic fragility, and antimicrobial resistance. This review addresses that gap by integrating biological, environmental, and policy perspectives within a One Health framework. It identifies context-appropriate, low-cost strategies to mitigate biofilm-associated infections and emphasizes the need for local innovation and interdisciplinary collaboration. By spotlighting Nigeria, the review contributes insights relevant to other resource-limited settings. Figure 1 presents the conceptual framework, while Table 1 outlines specific healthcare system challenges influencing biofilm control in Nigeria.



**Figure 1.** Conceptual framework linking biofilm biology, systemic weaknesses, and antimicrobial resistance leading to chronic infection burden in Nigeria.

Table 1: Healthcare System Challenges in Nigeria and Their Impact on Biofilm Infection Control

Healthcare System Challenge	Specific Impact on Biofilm Infection	Relevant Statistics/Examples
	Control	
Inadequate Healthcare Infrastructure	Limited access to diagnostic equipment for biofilm detection; compromised sterilization and maintenance of medical devices; increased HAIs due to poor facility conditions.	Lack of modern equipment, overcrowding, outdated facilities, poor maintenance, power outages, and lack of clean water (7).
Poor Healthcare Funding	Inability to afford advanced biofilm treatments (e.g., novel antibiofilm agents, specialized equipment); low investment in medical research for local solutions.	Less than 5% of the national budget is allocated to healthcare (7). Public financing covers only 25% of total health spending (12).
Shortage of Medical Professionals & Brain Drain	Reduced capacity for specialized care and research in biofilm-related diseases; increased workload on remaining staff, potentially impacting IPC compliance.	Doctor-to-patient ratio 1:3,500 vs. WHO 1:600 (8). Over 50% of Nigerian doctors work abroad (7).
High Disease Burden	Overwhelmed healthcare facilities; diversion of resources to acute infectious diseases, potentially neglecting chronic biofilm infections.	Malaria, lower respiratory infections, diarrhea diseases, and tuberculosis are leading causes of death (5).
Poor Health Insurance Coverage	Financial burden on patients leading to delayed or incomplete treatment for persistent biofilm infections; reliance on self-medication or unqualified alternatives.	Less than 10% of Nigerians are covered by NHIS (7, 12).
Inadequate Infection Prevention & Control (IPC) Practices	Increased spread of biofilm-associated HAIs; difficulty in implementing effective control measures.	70.5% of doctors and 67.0% of students identified inadequate PPE supply as a barrier (8, 9). IPC materials 78.9% "always not available"(10).
Overcrowding/Lack of Isolation Rooms	Facilitates rapid transmission of biofilm-forming pathogens within healthcare settings.	Overcrowding and lack of isolation rooms are known risk factors for HAIs (10).

### Methodology

### Literature Search Strategy

A comprehensive literature search was conducted between January and June 2025 to gather pertinent peer-reviewed articles, reports, and grey literature on microbial biofilms in resource-limited settings (RLS), with a specific focus on Nigeria. This review focused on biofilms relevant to human health and associated environmental reservoirs that influence infection transmission in Nigeria. The search was primarily conducted across major scientific and public health databases, including PubMed, Scopus, and Google Scholar, and was supplemented by examining grey literature from authoritative sources such as the World Health Organization (WHO), the Centers for Disease Control and Prevention (CDC), the Nigerian Centre for Disease Control and Prevention (NCDC), and relevant environmental health agencies.

The core search strategy utilized combinations of keywords and Boolean operators to ensure comprehensive coverage across the paper's three challenges, opportunities, thematic areas: solutions. Key terms included: (Biofilm OR Biofilms OR "Biofilm-associated infection") AND (Nigeria OR "Sub-Saharan Africa" OR "Low- and Middle-Income Countries" OR LMICs OR "Resource-Limited Settings") AND ("Antimicrobial Resistance" OR AMR OR "Chronic Infection" OR Persistence OR Control OR Management) AND ("Water Sanitation" "Decentralized Solution" OR "Plant-Derived Agents" OR "Natural Products" OR "Community Health") AND ("One Health"). These terms were strategically combined (e.g., (Biofilm AND Nigeria AND AMR); (LMICs AND "Plant-Derived Agents" AND Biofilm); ("One Health" AND Biofilm AND Water)) to retrieve relevant records.

All records retrieved were exported to a reference management software (EndNote 2024) for organization and duplicate removal, and all searches were restricted to articles written in the English language. Two independent researchers initially screened the titles and abstracts of the remaining records for relevance to the review's scope. Full-text articles were then retrieved and assessed for eligibility by the same researchers, with any disagreements resolved through consensus or arbitration from a third reviewer.

### Inclusion and Exclusion Criteria Inclusion Criteria

The selection process was guided by strict inclusion criteria: (1) Language and Publication Date: Articles published in the English language from 2010 to 2025. (2) Subject Focus: Studies, reviews, or reports focusing explicitly on microbial biofilms in human clinical, animal, or environmental contexts. (3) Thematic Relevance: Content that directly addressed the challenges, systemic barriers in Resource-Limited Settings (RLS), or proposed innovative, low-cost solutions adaptable to Nigeria or similar contexts.

### **Exclusion Criteria**

The following criteria led to the exclusion of records: (1) Topical Irrelevance: Studies entirely unrelated to microbial biofilms, antimicrobial resistance, or public health in RLS. (2) Quality and Source: Articles lacking verifiable peer-review (unless originating from designated authoritative public health bodies like WHO or NCDC). (3) Translational Gap: Laboratory studies that did not discuss the practical implications or translational potential for RLS. The authors thoroughly examined the title, abstract, material and methodology, results, and discussion of the selected articles to extract relevant information.

Author (Year)	Setting	Biofilm Source/Organism	Key AMR Findings	Relevance/Remarks
Olalemi et al. (2019)	Groundwater, Ado-Ekiti	E. coli, S. faecalis	High resistance to pefloxacin, septrin, chloramphenicol, augmentin	Waterborne AMR source
Agbabiaka et al. (2021)	Treated water, Ilorin	S. aureus, P. aeruginosa	Complete resistance to ceftriaxone, tetracycline	Environmental health risk
Yaki et al. (2024)	Hospital, Nigeria	Uropathogenic <i>E.</i> coli	Strong biofilm formation, multidrug resistance	Clinical AMR link

Umar et al. (2024)	Burn & wound isolates	S. aureus (MRSA)	mecA gene detected, biofilm producer	Healthcare-associated risk
Nwankwo et al. (2025)	Oral biofilm (children)	A. caviae	Sensitive to plant extract-nanoparticle combo	Innovation potential

### **Search Outcome and Screening Process**

A total of 205 records were retrieved from databases and grey literature sources. After duplicate

removal and screening, 103 full-text articles were reviewed in detail, and 41 studies met the inclusion criteria for narrative synthesis, of which 32 are cited.

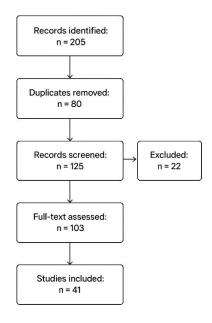


Figure 2. PRISMA-style summary of the literature selection and inclusion process.

### **Data Extraction and Quality Appraisal**

Data were extracted from all included studies standardized template summarizing using authorship, publication year, study setting, type of biofilm or organism investigated, antimicrobial resistance findings, and key contextual relevance to Nigeria. The quality of peer-reviewed empirical studies was appraised using the Joanna Briggs Institute (JBI) critical appraisal checklist, while grey literature (e.g., WHO and NCDC reports) was assessed using the AACODS checklist for authority, accuracy, coverage, objectivity, date, and significance. Discrepancies in assessment were resolved through consensus between two reviewers. Due to the heterogeneity of study types and outcomes, no quantitative synthesis or metaanalysis was undertaken.

### **Data Synthesis**

Given the inherent heterogeneity of the included literature, which spans clinical microbiology, public health policy, and environmental engineering, a

narrative synthesis approach was adopted. This method involved systematically integrating and synthesizing both qualitative and quantitative findings to build a cohesive and comprehensive argument across the review. The synthesis was intentionally structured around three distinct thematic areas that reflect the abstract's scope: (1) Biofilm Pathogenesis Antimicrobial Resistance in Nigerian Healthcare, which addresses the clinical and systemic challenges; (2) Environmental Biofilms and Public Health in Nigeria: A "One Health" Perspective, which links clinical burdens to environmental reservoirs like water; and (3) Low-Cost Strategies and Innovative Opportunities for Biofilm Control in LMICs, which focuses on solutions, leveraging including local biodiversity decentralized engineering. No quantitative metaanalysis was performed due to the diverse nature of the study designs and outcomes.

### Biofilm Pathogenesis and Antimicrobial Resistance in Nigerian Healthcare

Here, we explore the intricate mechanisms by which biofilms contribute to persistent infections and antimicrobial resistance within the Nigerian healthcare landscape, underscoring the diagnostic and treatment challenges encountered.

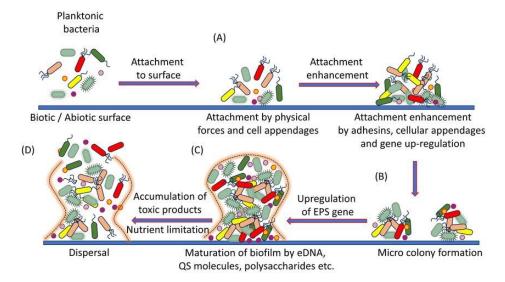
## Mechanisms of Biofilm Formation and Their Role in Persistent Infections

Biofilm formation (Figure 3) is a sophisticated, multi-stage biological process that enables microbial communities to establish and persist (1, 13). It typically commences with a reversible attachment phase, where bacteria non-specifically adhere to a surface. This is followed by an irreversible attachment phase, mediated by bacterial adhesins and structures like fimbriae (1, 2). Subsequently, the maturation phase involves the extensive production of an extracellular polymeric substance (EPS), which forms a protective matrix around the cells. Within this matrix, the biofilm develops a complex social structure, often including permeable water channels that facilitate nutrient and waste transport. Finally, individual cells or clusters can detach from the mature biofilm, dispersing to colonize new sites (4).

The EPS matrix is a critical component, acting as a formidable physical barrier that impedes the penetration of antibiotics and host defense molecules, while also providing protection against various environmental stressors such as UV radiation, pH fluctuations, and desiccation (3). Biofilm formation initiates disease processes through several mechanisms, including the detachment of individual bacterial cells or

aggregates, the production of endotoxins, enhanced evasion of host immune surveillance, and the establishment of a protective environment conducive to the emergence of more virulent phenotypes (4, 13). This inherent protective capacity makes biofilm-associated infections notoriously chronic and difficult to treat, leading to increased morbidity, higher mortality rates, and substantial healthcare costs (1, 4). Clinically, biofilm-associated infections can be broadly categorized into device-associated biofilms (e.g., catheters, ventilators, prosthetic implants) and chronic tissue biofilms (e.g., wounds, chronic otitis, cystic fibrosis infections). This distinction is clinically important because prevention and treatment strategies differ - while the former may require device modification or removal, the latter often necessitate prolonged combination therapy or biofilm-targeted agents (3).

Biofilms are a powerful survival strategy for microorganisms, allowing them to persist against host defenses and antimicrobial treatments. In Nigeria, where healthcare resources are severely limited, the ability of pathogens to form biofilms creates a compounding problem. Persistent infections lead to longer hospital stays and recurrent treatments, draining already scarce resources (11). This means that the biological advantage conferred by biofilm formation directly translates into a significant economic and logistical burden, trapping the healthcare system in a cycle of managing chronic, difficult-to-eradicate infections rather than effectively preventing them.



**Figure 3: Mechanism of Biofilm Formation.** The four stages include: (A) Initial Attachment: Free-living bacteria reversibly attach to a surface, progressing to irreversible binding via adhesins. (B) Microcolony Formation: Bacteria multiply and cluster, upregulating genes for maintained adherence. (C) Biofilm Maturation: Cells produce and are encased by the thick, protective Extracellular Polymeric Substances (EPS) matrix. Quorum Sensing (QS) regulates the structure. (D) Dispersal: Nutrient depletion and waste buildup trigger the release of bacteria, allowing colonization of new sites. Adapted from:(2).

## The Escalating Crisis of Antimicrobial Resistance (AMR) in Nigeria, Exacerbated by Biofilm Presence

Antimicrobial resistance (AMR) constitutes a critical public health challenge in Nigeria, a crisis significantly amplified by the widespread presence of biofilms. The problem is exacerbated by extensive antibiotic use across various sectors, coupled with improper empirical prescriptions and ineffective antimicrobial stewardship programs (14). High antibiotic resistance patterns are consistently detected in bacterial isolates recovered from healthcare settings, food supply chains, and environmental sources across the country (14, 15).

Studies in Nigeria have provided concrete evidence of this link. For instance, bacterial isolates from biofilms in groundwater sources in Ado-Ekiti, including Streptococcus faecalis, Escherichia coli, and Staphylococcus aureus, exhibited high resistance to common antibiotics such as pefloxacin, septrin, chloramphenicol, and augmentin (16). Similarly, biofilm-producing bacteria isolated from treated water supplies in Ilorin displayed complete resistance to ceftriaxone, amoxicillin, tetracycline, cotrimoxazole, further suggesting that biofilm counts could serve as indicators of water quality (17). In clinical settings, strong biofilm-forming uropathogenic E. coli isolates from catheterized patients in a Nigerian hospital were found to be extensively drug-resistant, demonstrating a significant correlation between biofilm formation and resistance to multiple antibiotics, including Augmentin, Ceftazidime, Gentamicin, and Ciprofloxacin (18).

Furthermore, Staphylococcus aureus isolates from burn and wound patients and healthcare workers

in Northern Nigeria were identified as moderate biofilm producers, with half of these isolates carrying the mecA gene, indicative of Methicillin-Resistant Staphylococcus aureus (MRSA) (19). The persistence of "ESKAPE" organisms (e.g., Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Enterobacter spp.), which are frequently associated with multiple antibiotic resistance and biofilm formation, creates an especially challenging scenario in intensive care units (ICUs), where multiple resistant pathogens coexist (19, 20). These findings highlight that biofilm-producing pathogens are not only widespread across hospital environments but also deeply embedded in Nigeria's AMR crisis, where diagnostic infrastructure rarely accounts for biofilm presence.

Nigeria already faces a dire AMR crisis due to systemic issues like poor regulation and misuse of antibiotics. The presence of biofilms, which inherently increase bacterial resistance through physical protection and altered growth rates, acts as an accelerator of this existing AMR problem. This means that even if a bacterial strain is initially susceptible to an antibiotic, its ability to form a biofilm in the body or environment can render standard treatments ineffective, thereby accelerating the selection and spread of multidrug-resistant strains. This dynamic makes the AMR challenge in Nigeria exponentially more difficult to combat, demanding strategies that specifically target the biofilm state rather than solely focusing on planktonic bacteria. Table 3 summarizes the most frequently reported biofilm-forming pathogens in Nigeria, their typical sources, and associated resistance patterns.

Table 3: Key Biofilm-Associated Pathogens and Their Resistance Patterns in Nigerian Clinical and Environmental Settings

Pathogen	Source/Context	Biofilm-Forming	Key Antibiotic Resisted	Relevant
		Capacity		Snippets
Staphylococcus	Wastewater, Surface	High	Varies by strain; MRSA is a major	(21)
aureus (MRSA,	water, Burn/Wound	(environmental);	concern	
MSSA, CoNS)	patients, Healthcare	Moderate (clinical)		
	workers			
Escherichia coli	Groundwater,	High	Pefloxacin, Septrin,	(18)
(Uropathogenic E.	Treated Water	(environmental);	Chloramphenicol, Augmentin,	
coli)	Distribution Systems	Strong (clinical)	Ceftazidime, Ceftriaxone,	
	(DWDS),		Gentamicin, Ciprofloxacin,	
	Catheterized		Ampicillin, Gentamicin	
	patients, Raw meat			
Pseudomonas	Oral cavity, Hospital	Biofilm producer	Gentamicin, Amoxicillin-	(17)
aeruginosa	environment, Treated		clavulanic acid, Cefixime	

	Water Distribution Systems (DWDS)			
Streptococcus faecalis	Groundwater	Biofilm producer	Multiple antibiotic resistance (MAR)	(16)
Enterobacter aerogenes	Groundwater, Hospital environment	Biofilm producer	Pefloxacin, Septrin, Chloramphenicol, Augmentin	(16)
Proteus mirabilis	Groundwater, Oral cavity	Biofilm producer	Pefloxacin, Septrin, Chloramphenicol, Augmentin	(16)
Salmonella typhi	Groundwater	Biofilm producer	Pefloxacin, Septrin, Chloramphenicol, Augmentin	(16)
Shigella dysenteriae	Groundwater, Treated Water Distribution Systems (DWDS)	Biofilm producer	Pefloxacin, Septrin, Chloramphenicol, Augmentin	(16)
Aeromonas caviae	Oral cavity	Biofilm producer	Not specified, but anti-infective nanoparticles/plant extracts are effective	(22)
Serratia marcescens	Oral cavity, Hospital environment, Treated Water Distribution Systems (DWDS)	Biofilm producer	Not specified, but anti-infective nanoparticles/plant extracts are effective	(17)

### Challenges in Diagnosis and Treatment of Biofilm-Associated Infections in Resource-Constrained Healthcare Settings

The effective management of biofilmassociated infections in Nigeria is severely hampered by significant diagnostic and treatment challenges, exacerbated by resource limitations. A primary obstacle is the difficulty in culturing biofilms in laboratory settings, coupled with their inherent complexity and high tolerance to conventional methods (23, 24). This often leads to a critical diagnostic gap: routine tests frequently do not include specific biofilm detection or comprehensive antimicrobial susceptibility assessments, which are crucial for guiding appropriate antibiotic treatment (18). This omission reflects the broader reality that biofilm detection is not part of routine microbiological diagnostics in most Nigerian laboratories, primarily due to limited equipment, lack of training, and absence of standardized protocols (18, 24). Consequently, clinicians may treat infections based on the susceptibility of planktonic (free-floating)

bacteria, unaware that the underlying biofilm structure renders these treatments ineffective (25). This leads to repeated treatment failures, prolonged patient suffering, increased healthcare costs, and ultimately contributes to the proliferation of untreatable infections. Addressing this diagnostic blind spot with accessible, low-cost methods is therefore vital for improving patient outcomes and combating AMR (11).

The high frequency of device-associated infections, such as catheter-associated urinary tract infections (CAUTIs), and the persistence of biofilm-driven infections in intensive care units (ICUs) highlight the pervasive nature of the problem (18, 23). While innovative technologies are warranted to manage the complexities presented by these biofilm infections, such advancements are often unavailable or unaffordable in LMICs (20). Despite ongoing efforts by healthcare providers and policymakers, overcoming biofilm formation in settings like ICUs remains a persistent challenge, contributing significantly to the burden of chronic infections (20).

### Environmental Biofilms and Public Health in Nigeria: A "One Health" Perspective

In this section, we broaden the scope to examine environmental biofilms, emphasizing their profound role in public health within Nigeria through the lens of the "One Health" approach, and considering the exacerbating factors such as climate change.

Prevalence and Impact of Environmental Biofilms on Public Health

Biofilms are ubiquitous in various environmental sectors, including wastewater and surface waters, where their presence can significantly compromise public health (21). Environmental factors such as temperature, pH, nutrient content, salinity, and dissolved oxygen are critical determinants influencing biofilm formation, growth, and persistence (21, 25). These risks are expected to intensify under changing

climatic conditions, where increased temperature, flooding, and drought create favorable conditions for biofilm persistence and antibiotic resistance dissemination (21, 26).

Studies conducted in Nigeria have revealed the widespread presence of antibiotic-resistant bacteria within biofilms in groundwater sources, including boreholes and wells. These findings indicate substantial contamination risks and the potential for widespread waterborne diseases (16). Drinking water distribution systems (DWDS) in Nigeria are particularly concerning, as they can serve as active incubators for the proliferation and dissemination of antibiotic-resistant opportunistic pathogens. Biofilm growth within these systems can visibly alter water quality, affecting turbidity, taste, color, and odor, and can even degrade residual disinfectants, further compromising water safety (17). Among the various environmental reservoirs, hospital effluents, untreated domestic wastewater, and groundwater sources used for drinking and irrigation pose the highest human health risks. These reservoirs act as major convergence points for antibiotic residues and resistant pathogens, facilitating direct human exposure through water consumption or indirect transmission via agriculture and food chains. In contrast, surface water and sediments, while significant, represent secondary reservoirs that sustain environmental persistence (21, 26).

While clinical settings are often the primary focus of discussions on antimicrobial resistance, evidence clearly indicates that environmental biofilms, particularly those in water sources in Nigeria, are not merely passive contaminants but active reservoirs for antibiotic-resistant bacteria and their associated resistance genes (16). This implies that even if clinical infections are successfully treated, continuous exposure to AMR from environmental sources can lead to reinfections or the acquisition of new resistance traits, thereby perpetuating a cycle of resistance. This silent dissemination pathway represents a critical public health challenge, especially in LMICs, where water infrastructure and sanitation are frequently inadequate.

## Role of Environmental Factors in Biofilm Persistence and AMR Dissemination

Environmental factors play a crucial role in the persistence of biofilms and the dissemination of antimicrobial resistance. The presence of low concentrations of antibiotics in environmental reservoirs, often stemming from agricultural runoff and inadequate wastewater treatment, can exert selective pressure on microbial communities. This constant exposure, even at sub-inhibitory levels, contributes significantly to the development and maintenance of

antibiotic resistance within environmental biofilms (15, 27). The complete set of resistance-related genes in a particular system, termed the environmental resistome, is shaped by such exposures (27).

Furthermore, the virome (the collective viral particles within biofilms, particularly bacteriophages) may contribute to the spread of antibiotic resistance genes through transduction, although this area warrants more extensive investigation to fully understand its ecological implications (11, 27). The widespread distribution and persistence of bacteria like Staphylococci in natural environments are largely attributable to their inherent ability to form robust biofilms and tolerate harsh conditions, including dehydration and low water activity (21).

The observation that agricultural practices are major sources of antibiotic resistance transmission to soil and water resistomes, even at sub-inhibitory concentrations. combined with the role environmental biofilms as resistance reservoirs. establishes a critical and often overlooked causal link between agricultural antibiotic use and clinical AMR. In Nigeria, where agriculture is a dominant economic sector and water treatment infrastructure is limited, this means that practices seemingly far removed from hospitals directly contribute to the burden of untreatable infections in patients. These interconnected drivers, ranging from spanning agriculture, water management and environmental microbiology, underscore the necessity of a unified surveillance system that integrates environmental monitoring with clinical and veterinary data.

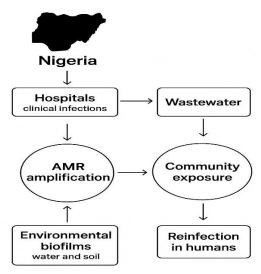
## Interplay Between Human, Animal, and Environmental Health: The "One Health" Approach

The antimicrobial resistance crisis in Nigeria is particularly severe due to the extensive use of antibiotics across human, animal, and agricultural sectors, compounded by ineffective antimicrobial stewardship programs (14). To effectively mitigate this multifaceted crisis, the adoption of a comprehensive "One Health" approach is indispensable. This framework emphasizes collaborative efforts among agencies, healthcare governmental institutions, veterinary experts, farmers, and the broader scientific community, recognizing the intricate convergence of human, animal, and environmental health (14). Such an integrated strategy is crucial for understanding and addressing the complex dynamics of AMR.

Climate variability, manifested through droughts, floods, and erratic rainfall, further undermines Nigeria's water security (27). Such shifts alter temperature, nutrient load, and pH, creating conditions that favor biofilm formation and persistence in environmental reservoirs (21). These effects heighten

waterborne-disease outbreaks and accelerate AMR spread, making climate change a critical amplifying factor in Nigeria's biofilm-related public-health burden. The interplay between clinical, environmental, and animal health domains underscores the necessity of an integrated One Health framework in addressing

biofilm-associated infections. Figure 4 illustrates this interconnected system, showing how biofilms formed in clinical settings, environmental reservoirs, and agricultural systems interact to sustain antimicrobial resistance and perpetuate reinfection cycles in Nigeria.



**Figure 4:** Conceptual link between clinical, environmental, and One Health dimensions of biofilm-associated AMR in Nigeria. Arrows indicate transmission pathways and feedback loops between healthcare facilities, environmental reservoirs, and community interfaces that amplify antimicrobial resistance.

### Low-Cost Strategies and Innovative Opportunities for Biofilm Control in LMICs

Addressing the pervasive challenges posed by biofilms in resource-limited settings like Nigeria necessitates the development and implementation of practical, affordable, and accessible interventions. This section explores several promising strategies that align with the need for low-cost solutions.

### **Experimental and In-Vitro Leads**

Research in developing countries increasingly focusing on the exploration of antiinfective compounds derived from plant sources as a means to control biofilm-induced infections (22). For instance, studies have demonstrated the inhibitory effects of Macrosphyra longistyla extracts and Neem (Azadirachta indica) leaf essential oil against antibioticresistant biofilm-producing bacteria prevalent in Nigeria (28). A particularly promising avenue involves synergistic approaches, where plant extracts are combined with nanoparticles, such as titanium ferrite, to achieve enhanced bioactivity at lower concentrations (22, 29). Furthermore, fungal metabolites enriched with bioactive compounds have shown efficacy in inhibiting multiple factors crucial for biofilm formation, including surface adhesion and cell-to-cell communication through quorum quenching (30).

Nigeria, like many LMICs, possesses rich biodiversity and a long history of traditional medicine. The documented efficacy of local plant extracts and

fungal metabolites against biofilms represents a significant opportunity to develop low-cost, locally sourced antibiofilm agents. This approach reduces reliance on expensive imported pharmaceuticals (31), fostering local research, production, and ultimately contributing to self-sufficiency in combating biofilm infections (29). Such strategies offer sustainable and culturally appropriate healthcare solutions.

Beyond natural products, the repurposing of existing resources and technologies also presents an innovative pathway for LMICs. This includes exploring molecular methods of biofilm dispersal, such as the use of enzymes (proteases, deoxyribonucleases, glycoside hydrolases), antibiofilm peptides, and quorum-sensing inhibitors (30). Although some of these approaches, such as non-thermal plasma therapy, are still technologically advanced, their underlying biological principles can inspire the discovery of affordable local analogues. For example, locally sourced enzymes or plant-derived compounds with comparable antibiofilm activities could be identified and optimized for clinical or environmental applications (32).

While these experimental and laboratory-based innovations hold strong potential, most remain in the preclinical stage, emphasizing the urgent need for translational research and feasibility testing under Nigerian healthcare and environmental conditions.

### **Deployable and Feasible Interventions**

Given the critical role of waterborne biofilms in AMR dissemination and the challenges associated with centralized, high-cost water treatment infrastructure in Nigeria, the development of decentralized and low-cost biofilm management solutions offers a transformative opportunity. These community-scale systems can empower rural and peri-urban populations to locally manage water quality, reducing exposure to pathogenic and antibiotic-resistant biofilms while enhancing public health resilience.

Biofilm technologies such as simple filter systems using readily available materials (e.g., pozzolan and sawdust) have demonstrated high efficiency in removing chemical oxygen demand (COD) and biological oxygen demand (BOD) from wastewater (26). More advanced but still affordable systems, such as moving-bed biological reactors (MBBR) and highly packed biofilm reactors (HPBR), show promise for treating septic tank effluent and rural wastewater with low maintenance requirements (26). Importantly, these systems can harness renewable energy sources like solar and wind power, both abundant in Nigeria, to further minimize operational costs (26). Crucially, these technologies can leverage abundant renewable energy sources, such as solar and wind power, prevalent in many developing countries, to stimulate their treatment processes, further reducing operational expenses (26).

While decentralized solar-powered chlorination and biosand filters have shown promise in other LMICs, evidence of their large-scale implementation in Nigeria remains limited. Adaptation should therefore prioritize pilot testing, local co-design, and integration with Nigeria's water and sanitation agencies to ensure long-term feasibility.

In parallel, effective biofilm control relies on robust community-based public health interventions. Emphasizing preventive measures such as point-of-use water treatment, proper storage practices, and hand hygiene remains essential (16, 24). Addressing barriers to infection prevention and control (IPC), including the availability of personal protective equipment (PPE) and a consistent supply of hygiene materials, is equally important (9, 31). While awareness of hygiene is relatively common, knowledge of biofilm risks and AMR linkage is low among the general population and even among healthcare workers. Hence, culturally sensitive educational campaigns must be coupled with practical training programs to translate scientific understanding into tangible behavioral change.

The feasibility of these interventions depends on local sourcing of materials, training of personnel, and alignment with existing WASH infrastructure. Behavioral change initiatives must also be complemented by material support; awareness alone is insufficient without access to safe water, protective equipment, and essential supplies.

### Conclusion

Biofilms represent a significant and amplified threat in Nigeria, a consequence of their inherent resistance mechanisms converging with systemic healthcare resource limitations and a high burden of infectious diseases. The problem extends beyond clinical settings, permeating environmental reservoirs and necessitating a holistic "One Health" approach to achieve effective control. For sustainable impact, low-cost, context-specific solutions are not merely desirable but essential.

## Future Research Directions and Policy Recommendations

To address the multifaceted challenges posed by biofilms in Nigeria, a concerted effort in both research and policy is required:

• Future Research: Prioritize investigations into Nigeria's indigenous flora to identify novel antibiofilm compounds and elucidate their mechanisms of action. Develop and validate low-cost, rapid diagnostic tools for biofilm infections that are suitable for resource-limited settings. Conduct studies to understand the specific physiological adaptations of biofilms to local environmental

stressors and sub-inhibitory concentrations of antibiotics. Explore the potential of phage therapy and other non-antibiotic strategies as affordable alternatives to conventional antimicrobial treatments. Initiate interdisciplinary studies to assess the impact of climate change on biofilm epidemiology and antimicrobial resistance dissemination in Nigeria. Crucially, focus on translational research to bridge the gap between laboratory discoveries and practical, community-level interventions.

Fostering Interdisciplinary Research and Local Innovation: We recommend the establishment of joint, interdepartmental student research grants and collaborative projects involving departments such as Microbiology, Chemical and Environmental Engineering, and Public Health across Nigerian universities. This cross-sectoral approach should emphasize the discovery and validation of innovative, low-cost antibiofilm technologies that are practical and scalable within local contexts. Key research areas should include: (1) the development of phytochemical-based antibiofilm agents, leveraging Nigeria's rich biodiversity to screen and validate

- plant-derived extracts and purified compounds for their ability to disrupt biofilm formation or degrade mature biofilms; (2) the creation of decentralized diagnostic and monitoring tools, focusing on simple, affordable, and rapid biofilm detection kits suitable for both clinical (e.g., wound care) and environmental (e.g., water source) applications to enable timely and targeted interventions; and (3) engineering-based solutions, exploring novel surface coatings, basic flow dynamics, and locally sourced materials to mitigate biofilm growth in healthcare and water distribution infrastructures. This initiative will not only generate locally relevant data and technologies but also cultivate a skilled, interdisciplinary workforce fluent in the "One Health" approach necessary to address the growing biofilm challenge.
- Integrating Biofilm and AMR Awareness into Grassroots Education: To ensure long-term and sustainable behavioral change in curbing the drivers of antimicrobial resistance (AMR), it is essential to integrate simplified content on biofilms, microbial hygiene, and responsible antibiotic use into foundational education and community programs. This can be achieved through both curriculum adaptation and community literacy initiatives. At the secondary school level, basic concepts of microbial biofilms (such as their connection to persistent infections and water contamination) should be incorporated into existing science subjects like Biology, Chemistry, and Health Science. Introducing Infection Prevention and Control (IPC) principles at this stage helps students develop an early
- understanding of microbial hygiene and the consequences of antibiotic misuse. Complementing this effort, community literacy programs should be designed to deliver simplified and culturally appropriate educational materials that clearly explain the relationship between poor water and sanitation, biofilm reservoirs, and antibiotic misuse. By fostering awareness at both the school and community levels, this integrated approach strengthens the foundation for community-based public health interventions, aligning with the "One Health" framework to promote lasting AMR prevention from the grassroots.
- Policy Recommendations: Advocate for increased governmental and private investment in healthcare and medical research in Nigeria, specifically targeting biofilm-related challenges. Implement robust "One Health" policies to regulate antibiotic use across human, animal, and agricultural sectors, and establish comprehensive surveillance programs for environmental antimicrobial resistance. Strengthen infection prevention and control (IPC) programs in healthcare facilities, ensuring the adequate supply of materials and continuous training for healthcare professionals. Promote the adoption of low-cost, decentralized water treatment technologies in rural and underserved urban areas to improve public health outcomes. Integrate public health education campaigns to raise awareness about biofilms, emphasize the importance of hygiene, and promote responsible antibiotic use among the general populace.

Table 4: Recommended Actions and Implementation Framework

Timeframe	Key Actions	Responsible Actors	
Short-term (0–2 years)	Implement joint AMR–WASH surveillance programs; strengthen infection prevention and control (IPC) training in tertiary hospitals; improve diagnostic capacity for biofilm detection.	NCDC, Federal Ministry of Health, Teaching Hospitals	
Medium-term (3–5 years)	Develop a national biofilm research roadmap; incentivize plant-based compound screening and low-cost intervention trials; enhance intersectoral collaboration among research institutions.	Ministry of Health, NCDC, Universities, Research Institutes	
Long-term (5+ years)	Establish a <i>One Health Biofilm Innovation Centre;</i> integrate  biofilm monitoring into national  AMR surveillance plans;	Federal Government of Nigeria, Ministry of Environment, International Partners (WHO, CDC, UNEP)	

promote sustainable partnerships with international	
organizations for technical and	
financial support.	

The Nigeria Centre for Disease Control and Prevention (NCDC) and the African CDC can play pivotal roles in scaling joint AMR–WASH surveillance and advancing integrated One Health initiatives. Embedding these interventions within national AMR and water safety action plans will translate research

insights into measurable impact. Implementing these actions could significantly strengthen Nigeria's resilience against biofilm-associated infections and support global antimicrobial resistance containment efforts.

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### References

- 1. Montanari E, Bernardo G, Le Noci V, Anselmi M, Pupa SM, Tagliabue E, et al. Biofilm formation by the host microbiota: a protective shield against immunity and its implication in cancer. Mol Cancer. 2025;24(1):148. <a href="https://doi.org/10.1186/s12943-025-02348-0">https://doi.org/10.1186/s12943-025-02348-0</a>
- Almatroudi A. Investigating Biofilms: Advanced Methods for Comprehending Microbial Behavior and Antibiotic Resistance. Front Biosci (Landmark Ed). 2024;29:133. <a href="https://doi.org/10.31083/j.fbl29041">https://doi.org/10.31083/j.fbl29041</a>
   33
- 4. Sharma S, Mohler J, Mahajan SD, Schwartz SA, Bruggemann L, Aalinkeel R. Microbial Biofilm: A Review on Formation, Infection, Antibiotic Resistance, Control Measures, and Innovative Treatment. Microorganisms. 2023;11(6):1614. <a href="https://doi.org/10.3390/microorganisms11061614">https://doi.org/10.3390/microorganisms11061614</a>
- World Health Organization. Nigeria WHO Data [Country overview]. 2025 [updated

- 2025/07/18]. Available from: <a href="https://data.who.int/countries/566">https://data.who.int/countries/566</a>
- 6. Jonah JH, Samuel G, Abdullahi MI, Emmanuel EA, Danladi SS, Ekwuluo CE. Nigeria's public health response to disease outbreaks: A review of strengths and weaknesses. J Public Health Afr. 2024;15(1):773. <a href="https://doi.org/10.4102/jphia.v15i1.773">https://doi.org/10.4102/jphia.v15i1.773</a>
- 7. Nigeria GML. The State of Healthcare in Nigeria: Challenges and Opportunities. 2025 [updated 2025/07/18]. Available from: <a href="https://gml.com.ng/the-state-of-healthcare-in-nigeria-challenges-and-opportunities/">https://gml.com.ng/the-state-of-healthcare-in-nigeria-challenges-and-opportunities/</a>
- Umar AA, Salihu HM, Azuine RE. Crisis of Brain Drain in Nigeria's Health Sector: Challenges, Opportunities, and the Path Forward. Int J MCH AIDS. 14:e011. <a href="https://doi.org/10.25259/IJMA">https://doi.org/10.25259/IJMA</a> 11 202 5
- 9. Ndibuagu EO, Chime OH, Orji CJ, Aneke TJ. Infection Prevention and Control Practices, and Barriers to Compliance among Medical Doctors and Clinical Medical Students, in a State University Teaching Hospital, Southeast Nigeria. J Adv Med Med Res. 2022;34(20):178–

- 90. <a href="https://doi.org/10.9734/jammr/2022/v34i203">https://doi.org/10.9734/jammr/2022/v34i203</a>
  1483
- 10. Caleb G, Okon A, Inah A, Olanrewaju O, Achi F, Bisongedam M. Practices of Infection Prevention and Control among Primary Healthcare Workers and Associated Factors: A Cross-Sectional Study. Trends Med Res. 2023;18:24-
  - 35. https://doi.org/10.3923/tmr.2023.24.35
- 11. Lawal O, Orenolu IO, Ajobiewe MA, Mensah Forson KAA, Agu UF, Fidelix ME, et al. Hospital-Acquired Infections in the Age of Antimicrobial Resistance and Smart Surveillance. Aust J Biomed Res. 2025;1(1):aubm002. <a href="https://doi.org/10.63946/aubiomed/16759">https://doi.org/10.63946/aubiomed/16759</a>
- Eze P, Aniebo CL, Ilechukwu S, Lawani LO. Understanding Unmet Healthcare Needs in Nigeria: Implications for Universal Health Coverage. Health Serv Insights. 2025;18:11786329251330032. <a href="https://doi.org/10.1177/11786329251330032">https://doi.org/10.1177/11786329251330032</a>
- 13. Abbas S, Khan S, Shah M, Aslam J, Nawaz H, Ilyas N, et al. Public Health Threats Posed by Biofilms and Innovative Strategies for their Control. Discoveries.

  2024. https://doi.org/10.15190/d.2024.16
- 14. Alabi ED, Rabiu AG, Adesoji AT. A review of antimicrobial resistance challenges in Nigeria: The need for a one health approach. One Health.
  2025;20:101053. <a href="https://doi.org/10.1016/j.onehlt.2025.101053">https://doi.org/10.1016/j.onehlt.2025.101053</a>
- 15. Lawal O. Antibiogram and Molecular Characterization of Extended-Spectrum Beta-Lactamase-Producing Klebsiella pneumoniae in a Nigerian Teaching Hospital. Microbes Infect Chemother.
- 2025. <a href="https://doi.org/10.54034/mic.e2305">https://doi.org/10.54034/mic.e2305</a>
  16. Olalemi A, Akinruli F, Oluwasusi V.
- Antibiotic Resistance Pattern of Bacteria
  Isolated from Biofilms in Water from
  Groundwater Sources in Ado-Ekiti, Nigeria.
  South Asian J Res Microbiol. 2019:19. https://doi.org/10.9734/sajrm/2019/v3i430093
- 17. Agbabiaka T, Teslim S, Otuyelu F. Biofilmforming bacteria and their antibiotic resistance in treated water supplies in Ilorin Metropolis, Nigeria. Ceylon J Sci.

- 2021;50:199. <a href="https://doi.org/10.4038/cjs.v50i2.78">https://doi.org/10.4038/cjs.v50i2.78</a>
  83
- 18. Yaki LM, Nwabuisi C, Dagang W, Huyop F. Biofilm Associated Uropathogenic Escherichia coli from Catheterised Patients at a Nigerian Hospital. 2024. <a href="https://doi.org/10.21203/rs.3.rs-4007279/v1">https://doi.org/10.21203/rs.3.rs-4007279/v1</a>
- 19. Umar K, Abdullahi IN, Magashi AM, Kawo AH, Usman Y, El-Fulaty Ahmad A, et al. Prevalence and clonal lineages of biofilm-producing Staphylococcus aureus from clinical samples and healthcare workers at Ahmadu Bello University Teaching Hospital, Nigeria. GMS Hyg Infect Control. 2024;19:Doc49. https://doi.org/10.3205/dgkh000504
- 20. Taner F, Baddal B, Theodoridis L, Petrovski S. Biofilm Production in Intensive Care Units: Challenges and Implications. Pathogens. 2024;13(11):954. <a href="https://doi.org/10.3390/pathogens13110954">https://doi.org/10.3390/pathogens13110954</a>
- 21. Silva V, Pereira JE, Maltez L, Poeta P, Igrejas G. Influence of Environmental Factors on Biofilm Formation of Staphylococci Isolated from Wastewater and Surface Water. Pathogens. 2022;11(10). <a href="https://doi.org/10.3390/pathogens11101069">https://doi.org/10.3390/pathogens11101069</a>
- 22. Nwankwo CE, Osho A, Adewuy A, Otuechere C, Olawoye IB, Fayemi SO, et al. Combating oral biofilms in Nigerian schoolchildren: a synergistic approach using Macrosphyra longistyla extracts and titanium-ferrite nanoparticles. GMS Hyg Infect Control. 2025;20:Doc22. https://doi.org/10.3205/dgkh000551
- 23. Mgbodi GO. Inadequate healthcare service administration and management in Nigeria and solutions. 2023. Available from: <a href="https://www.researchgate.net/publication/372769855">https://www.researchgate.net/publication/372769855</a> INADEQUATE HEALTHCARE SERVICE ADMINISTRATION AND MAN AGEMENT IN NIGERIA AND SOLUTION S
- Adedokun AI, Gana SE, Adenrele BO. Bacteria biofilms: Navigating the way forward.
   Microbes Infect Dis.

   2023. <a href="https://doi.org/10.21608/mid.2023.236659">https://doi.org/10.21608/mid.2023.236659</a>

   .1621

- 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;11. <a href="https://doi.org/10.3389/fmicb.2020.00928">https://doi.org/10.3389/fmicb.2020.00928</a>
- Shareef N. Wastewater Treatment by using Biofilm Technologies as a Low-cost Solution for Rural Areas. In: Bui XN, editor. Biomedical Engineering. Cham: Springer International Publishing; 2020. p. 1-13. https://doi.org/10.1007/698\_2022\_870
- Flores-Vargas G, Bergsveinson J, Lawrence JR, Korber DR. Environmental Biofilms as Reservoirs for Antimicrobial Resistance. Front Microbiol.
   2021;12. <a href="https://doi.org/10.3389/fmicb.2021.766">https://doi.org/10.3389/fmicb.2021.766</a>
   242
- 28. Omoya FO, Salami FF, Ajayi KO. Azadirachta indica Leaf Essential Oil as Control of Biofilm-Producing Microorganisms from Hospital Environment Nigeria. SustainE; 2025. Available

from: <a href="https://sustaine.org/azadirachta-indica-leaf-essential-oil-as-control-of-biofilm-">https://sustaine.org/azadirachta-indica-leaf-essential-oil-as-control-of-biofilm-</a>

- producing-microorganisms-from-hospitalenvironment/
- 29. Oluwole OM. BIOFILM: FORMATION AND NATURAL PRODUCTS' APPROACH TO CONTROL A REVIEW. Afr J Infect Dis. 2022;16(2 Suppl):59-71. https://doi.org/10.21010/Ajid.v16i2S.7
- 30. Ghosh S, Nag M, Lahiri D, Sarkar T, Pati S, Joshi S, et al. New holistic approach for the management of biofilm-associated infections by myco-metabolites. J Basic Microbiol. 2022;62(11):1291-306. https://doi.org/10.1002/jobm.202200047
- 31. Srivastava S, Roddam S, Srivastava D, Shehu A, Gogineni KK, Singla R, et al. Exploring Non-antibiotic Interventions for Preventing Urinary Tract Infections During Pregnancy: A Systematic Review. Cureus. 2025;17(10):e93874. <a href="https://doi.org/10.7759/cureus.93874">https://doi.org/10.7759/cureus.93874</a>
- 32. Fleming D, Rumbaugh KP. Approaches to Dispersing Medical Biofilms. Microorganisms. 2017;5(2):15. <a href="https://doi.org/10.3390/microorganisms5020015">https://doi.org/10.3390/microorganisms5020015</a>