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Insights on MDR Mechanism of *Pseudomonas aeruginosa* with Emphasis on Diabetic Foot Ulcer in the Indian Subcontinent

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Abstract

Diabetic foot ulcers (DFU) in patients with uncontrolled diabetes mellitus are considered a global public health menace that is highly associated with morbidity and mortality. Pathogenic microorganisms entrenched deep into diabetic foot wounds are the causative agents for delayed healing and escalation of diabetic foot wound severity. *Pseudomonas aeruginosa* is a common opportunistic pathogen associated with several nosocomial infections, cystic fibrosis, and one of the most critical pathogens often isolated from acute and chronic diabetic foot ulcers. The organism can exhibit resistance to a wide range of antibiotics like ciprofloxacin, cefotaxime, and meropenem, thereby causing severe damage to the host tissues, followed by amputation of the affected foot region. Due to their ability to synthesize biofilms, the wound becomes more chronic and incurable, posing a serious threat to immunocompromised diabetic patients. This review highlights on the insights of pathophysiology and microbiological profile of Diabetic foot ulcers, the resistance mechanisms, and the therapeutics available for dealing with drug-resistant *Pseudomonas*, which could help clinicians in treating DFUs.

Keywords: *Pseudomonas aeruginosa*, Diabetic Foot Ulcers, Biofilm, Antibiotic Resistance, Quorum Sensing

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INTRODUCTION

Diabetes mellitus is one of the oldest metabolic diseases known to mankind. It is characterized by hyperglycaemia resulting from low insulin secretion or increased glucagon production or insulin resistance. Diabetes is mainly of two types based on the absence or minimal secretion of insulin and reduced response to insulin to peripheral receptors. Type I Diabetes Mellitus (T1DM) is caused due to autoimmune destruction of β -cells by ICA8 and anti-GAD65 autoantibodies. Exogenous insulin treatment is necessary because T1DM typically manifests in children and adults before the age of 30, when blood insulin levels are lowered and patients stop responding to the anti-diabetic regimen. Type II Diabetes Mellitus (T2DM) is caused by the development of insulin resistance due to a sedentary lifestyle, comorbidities, and other metabolic disorders.¹ According to an epidemiology survey in 2021, globally, approximately 536 million people are suffering from diabetic mellitus, with a total anticipated cost of 966 billion USD for diabetes-related healthcare.² In India, 77 million are diabetic, which is anticipated to climb to nearly 134 million by 2045.³ Diabetes is associated with many complications and is the primary cause of neurological disease, cardiovascular disease, kidney failure, blindness and lower limb amputation.^{4,5}

Diabetic foot ulcer (DFU), a well-known T2DM-associated complication, is a primary cause of hospitalisation, accounting for 20% of all hospital admissions and morbidity.^{6,7} Approximately 58% of patients with foot ulcers are prone to septicaemia.⁸ Untreated DFU can progress into ulcers and gangrene eventually leading to limb amputation and death.^{8,9} It has been estimated that DFU is the cause of 50 to 70% of limb amputations.¹⁰ In addition to morbidity, DFUs have significant socioeconomic repercussions. The average cost of hospital admission for amputation in the US is around \$100,000.^{11,12} The price of treatment and management varies by nation, from \$188,000 in the US to \$3060 in Tanzania.¹³ The price of DFU therapy in India, which has one of the highest rates of diabetes, is roughly \$1960.¹⁴ In India, it is anticipated that it will take 5.7 years of a patient's income to treat a DFU.¹⁵

A deeper understanding into the systemic progression of the infections, treatment and management of the infection is required. Only few studies are available to understand the correlation of *Pseudomonas* in diabetic foot ulcers. Hence, the present review aims to discuss the pathogenesis of diabetic foot ulcers emphasizing the role of multidrug resistant *Pseudomonas aeruginosa* in the progression of diabetic foot infections and the existing therapeutics available to combat the resistance which could be further studied to deal with DFU complications.

Pathophysiology of diabetic foot ulcer

DFU is often manifested by lesions and abrasions in the skin, but its aetiology is multifactorial. The pathophysiology of DFU is attributed to a complex triad of peripheral neuropathy, vascular foot abnormalities, arterial occlusive damage and decreased immune response to infection.^{16,17} Hyperglycaemia induces aberrant metabolic changes, such as an increase in intracellular glycosylated nerve proteins, protein kinase C activation, increased hexosamine flow, and polyol pathway, all of which lead to nerve injury.¹⁸ Studies have shown that motor neuron damage causes an imbalance in flexor-extensor coordination and the development of anatomic abnormalities such as Charcot's foot, hammerhead toes, and claws.^{18,19} Damage to sensory nerves results in loss of sensation and proprioception, which lowers the pain threshold, making the foot more susceptible to heat and trauma, thereby increasing the risk of foot ulcers. Autonomic nerve damage inhibits sweat glands, and the foot's capacity to moisten skin may deteriorate, resulting in epidermal fissures and skin breakdown, providing viable channel for microbial invasion and infections.^{7,20,21} These neuropathy-related impairments result in "high-pressure" zones at the metatarsal head on the plantar surface of the foot.²² Hyperglycaemia-induced vascular alterations in the peripheral arteries resulted in a decrease in vasodilators and increased plasma thromboxane A2 levels. As a result, peripheral arteries experience vasoconstriction and plasma hypercoagulation, which ultimately increases the risk of ischemia and ulceration.⁵ Additionally, immunological alterations enhance T lymphocyte apoptosis, which lowers the foot ulcers' ability

to heal.²³ Due to these cellular and metabolic changes, diabetic patients experience repetitive trauma from walking in combination with decreased sensation and proprioception. This leads to the dislocation of the protective plantar fat pads, which can result in ulceration and infection from inadequate skin protection or bad footwear.²⁴

Patients' poor attention to their skin, failure to recognise cutaneous injuries (redness, blister formation), or delayed treatment can result in the progression of foot lesions to ulcers and the development of microbial invasive soft tissue infection. Eventually, the infection penetrates into the deep skin layers and spreads to the midfoot muscles, joints, and tendon sheaths. As the infection progresses, the deep tissue fills with pus, leading to tissue necrosis and abscess formation. One-half of major (above- or below-knee) lower extremity amputations in people with diabetes are due to microbial infection.^{7,25} Flowchart depicting the pathophysiology of diabetic foot ulcer is given in Figure 1.

Microbiology profile of diabetic foot ulcers focussing the different regions of India

Diabetes foot wounds have a complicated microbiome. A diabetic foot ulcer is caused by recurring infections from aerobic, anaerobic and fungal microorganisms, either singly or in combination.²⁶ Microorganisms isolated from diabetic food wounds were identified using 16S rRNA, short gun metagenomic and pyrosequencing.^{27,28} According to the American Infectious Disease Society, DFUs are divided into three subcategories: mild infections with superficial symptoms, moderate infections with deeper and pronounced symptoms, and severe infections with systemic symptoms or metabolic abnormalities.²⁹ Mild infections appear to have a simpler microbiota inhabited by common skin commensals such as beta-haemolytic *Streptococcus* (*S. agalactiae*; *S. pyogenes*; *S. mitis*), aerobic Gram-positive cocci (*Staphylococcus aureus*), Coagulase negative *Staphylococcus epidermidis*, which have been identified as

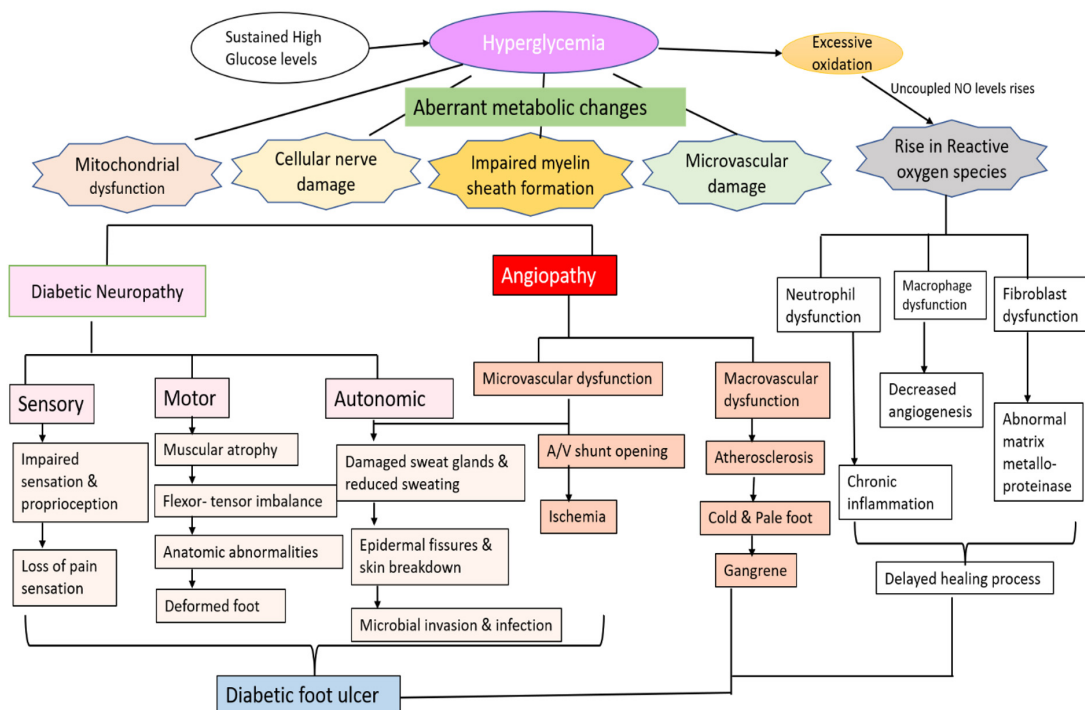


Figure 1. Pathophysiology of Diabetic Foot Ulcer

pathogens in foot ulcers.²⁷ Among these isolates, *S. aureus* was reported to be the most common isolate ranging from 25% to 30% from early diabetic wounds.^{30,31} Group B Streptococcus associated infections are rising in the present days leading to soft tissue damage and severe blistering

cellulitis followed by amputation.³² Moderate infection wounds are primarily colonised by aerobic, non-fermenting Gram-negative bacilli i.e., *Enterobacteriaceae* (*Escherichia coli*, *Morganella morganii*, *Klebsiella pneumonia*, *Proteus mirabilis*), *Pseudomonas aeruginosa*, *Citrobacter* spp.,

Table 1. Microbiology profile of diabetic foot ulcer in different geographical regions of India

Region	North India	South India	North-East India	East India	West India
Total number of patients	102	77	150	148	100
Study duration	Dec, 2008 to Feb, 2010	May, 2003 to March, 2004	Feb, 2015 to Jan, 2016	-	July to Oct 2012
Total Aerobes	152	113	182	240	92
Gram-positive aerobes	55 (36.1%)	48 (62.3%)	73 (40.1%)	88 (36.6%)	20 (21.73%)
<i>Staphylococcus aureus</i>	37 (24.3%)	19 (24.5%)	46 (24.86%)	72 (30%)	6(7%)
<i>Enterococcus faecalis</i>	5 (3.2%)	3 (3.8%)	27 (14.59%)	8 (3.3%)	-
<i>Beta-hemolytic streptococcus</i>	5 (3.2%)	-	-	4 (1.7%)	-
<i>CONS</i>	4 (2.6%)	20 (25.9%)	-	4 (1.7%)	2 (2%)
<i>Coryneform</i> sp.	4 (2.6%)	-	-	-	-
<i>Corynebacterium jeikeium</i>	-	3 (3.8%)	-	-	-
<i>Bacillus subtilis</i>	-	3 (3.8%)	-	-	-
Gram-negative aerobes	97(63.8%)	65 (84.4%)	109 (59.8%)	152 (63.3%)	72 (78.26%)
<i>Escherichia coli</i>	41 (42.2%)	17 (22.0%)	37 (20.0%)	26 (10.8%)	15 (17%)
<i>Pseudomonas aeruginosa</i>	23 (23.7%)	23 (29.8%)	22 (11.89%)	28 (11.7%)	25 (27%)
<i>Klebsiella oxytoca</i>	11 (11.3%)	1 (1.2%)	-	2 (0.8%)	-
<i>Klebsiella pneumoniae</i>	9 (9.2%)	9 (11.6%)	22 (11.89%)	22 (9.2%)	-
<i>Klebsiella</i> sp.	-	-	-	-	20 (22%)
<i>Proteus vulgaris</i>	5 (5.1%)	1 (1.2%)	-	16 (6.7%)	-
<i>Proteus mirabilis</i>	2 (2.0%)	8 (10.3%)	9 (4.86%)	10 (4.2%)	-
<i>Proteus</i> sp.	-	-	-	-	4 (3%)
<i>Acinetobacter</i> spp.	5 (5.1%)	-	7 (3.78%)	12 (5.0%)	2 (2%)
<i>Morganella morganii</i>	1 (1.0%)	-	4 (2.16%)	-	-
<i>Citrobacter koseri</i>	-	2 (1.2%)	-	2 (0.8%)	-
<i>Citrobacter freundii</i>	-	1 (1.2%)	1 (0.54%)	10 (4.2%)	-
<i>Klebsiella ozaenae</i>	-	1 (1.2%)	-	-	-
<i>Enterobacter aerogenes</i>	-	1 (1.2%)	6 (3.24%)	22 (9.2%)	-
<i>Edwardsiella tarda</i>	-	1 (1.2%)	-	-	-
<i>Serratia</i> spp.	-	-	1 (0.54%)	-	-
<i>Stenotrophomonas maltophilia</i>	-	-	-	2 (0.8%)	-
Total Anaerobes	17	5	-	21	-
Gram-positive anaerobes	15 (88.2%)	2 (2.5%)	-	7 (33.3%)	-
<i>Peptostreptococcus</i> sp.	6 (35.2%)	2 (2.5%)	-	7 (33.3%)	-
<i>Peptostreptococcus anaerobius</i>	4 (23.5%)	-	-	-	-
<i>Propionibacterium</i> sp.	3 (17.6%)	-	-	-	-
<i>Clostridium perfringens</i>	1 (5.8%)	-	-	-	-
<i>Eggerthella lenta</i>	1 (5.8%)	-	-	-	-
Gram-negative anerobes	2 (11.7%)	3 (3.8%)	-	14 (66.7%)	-
<i>Bacteroides ureolyticus</i>	2 (11.7%)	-	-	14 (66.7%)	-
<i>Bacteroides fragilis</i>	-	3 (3.8%)	-	-	-
Reference	[38]	[39]	[40]	[41]	[42]

Enterobacter spp. and *Acinetobacter* spp. In several studies, Gram-negative isolates (50%) were found to be higher than Gram-positive isolates (30%).^{33,34} *P. aeruginosa*, which accounts for 14% of the isolates, is a terrible and vital pathogen with antibiotic resistance that can cause significant tissue damage and lead to sepsis and amputation.^{35,36} Chronic infection wounds are inhabited by aerobic Gram-positive cocci, aerobic Gram-negative bacilli and anaerobic pathogens (*Bacteroides fragilis*; *Propionibacterium* spp; *Clostridium* spp; *Peptostreptococcus* spp).³⁷ Table 1 summarises the microbiological profile of diabetic foot ulcers done using microbial culture techniques in different geographical locations of India, where 23.7% of DFU cases in North India,³⁸ 29.8% in South India,³⁹ 11.89% in North-East India,⁴⁰ 11.7% in East India,⁴¹ and 27% in Western India⁴² are caused by *Pseudomonas aeruginosa* at different study periods.

Polymicrobial colonization in diabetic foot Ulcers

Chronic wound infections are typically polymicrobial with varied aerobic Gram-negative bacilli and obligate anaerobic bacteria. According to reported studies, polymicrobial infections were estimated to occur in 75% to 83% of chronic DFU cases.^{34,43} Polymicrobial infections could be induced by anaerobes interacting with aerobes, such as the interaction of *E. coli* with *B. fragilis*. As aerobic bacteria multiply, they use oxygen and enhance the growth conditions for anaerobic bacteria, assisting anaerobes in dealing with the harmful effects of oxygen. Furthermore, microbial isolates from wounds were found to tolerate and thrive at a wider pH range, which helps them circumvent the limitations of the external macroenvironment and promote the growth and survival of microbial communities. As a result, the production of virulence factors such as hemolysins, collagenases, proteinases, and short-chain fatty acids gets increased, which promotes inflammation and hinders the healing of wounds.^{6,44-46} An additional pathogenic property of many organisms is their ability to become enveloped in biofilm. Due to hyperglycaemic condition, *P. aeruginosa* or *S. aureus* synthesizes thick biofilms, decreasing antibiotic susceptibility and hindering wound healing.⁴⁷ These biofilms serve as barriers that inhibit the diffusion of

antibiotics, antimicrobial proteins, lysozymes, and defensins, while simultaneously protecting organisms from phagocytosis and promoting antibiotic resistance.⁴⁸

The growing rate of isolation of antibiotic-resistant pathogens, particularly methicillin-resistant *S. aureus* (MRSA), glycopeptide-intermediate *S. aureus* (GISA), vancomycin-resistant enterococci (VRE) and highly resistant *P. aeruginosa* strains has become a significant problem. Many organisms' capacity to form biofilm encapsulations is another trait that makes them harmful.^{29,49} Also, recent studies showed there was an increased incidence of *P. aeruginosa* in diabetic wounds, especially in geographical locations with hot and humid climates and its management is highly challenging.⁵⁰ Understanding the physiology involved in making the organism highly resistant to antibiotics is critical. The following sections review the role of *Pseudomonas* biofilm production, antibiotic resistance, and treatment options available in managing infections.

Antibiotic resistance mechanism in *Pseudomonas aeruginosa*

Diabetic foot ulcers is a very serious complication of diabetes mellitus which exhibits polymicrobial colonisation and treatment of DFU caused by *Pseudomonas aeruginosa* is extremely challenging due to its propensity for antibiotic resistance. The patients with DFUs are generally treated using empiric antibiotic therapy and antibiotics such as ceftazidime, cefepime, piperacillin-tazobactam, imipenem, or meropenem are commonly used in these scenarios.⁵¹ The organism developed several mechanisms of antibiotic resistance: production of beta-lactamase enzyme for drug inactivation, restrictive outer membrane uptake and efflux mechanism, mutational changes of targeted enzymes or proteins and formation of biofilms.⁵²

Production of beta-lactamase and aminoglycosides modifying enzymes

P. aeruginosa possesses two genes- the inducible *ampC* gene and the regulatory gene *ampR*. Mutations in *ampR* gene trigger the *ampC* gene's overexpression and the production of beta-lactamase protein. Beta-lactamase can hydrolyze the amide bond in the beta-lactam ring,

thereby inactivating beta-lactam antibiotics.⁵³ Downregulation of *ampR* was found to be due to excessive ceftazidime treatment.⁵⁴ Based on Amino acid sequences, beta-lactamases are further classified into A, B, C and D. Class A, C and D have serine Amino acid residue in their active site and were reported to hydrolyze beta-lactam ring. Class B, also known as metallo-lactamases, requires Zn^{2+} ions for hydrolysis of the beta-lactam ring. Some *Pseudomonas* isolates were reported to synthesize another type of beta-lactamases known as extended-spectrum beta-lactamases (ESBLs) that has the ability to hydrolyze beta-lactam ring of majority antibiotics such as cephalosporins, penicillin and aztreonam.⁵⁵⁻⁵⁷

P. aeruginosa is also highly resistant to aminoglycosides antibiotics, which contain an aminocyclitol ring linked to amino sugars by glycosidic bonds. *P. aeruginosa* modifying enzymes-aminoglycoside acetyltransferase, aminoglycoside phospho-transfer and aminoglycoside nucleotide transferase catalyse the structural modifications and inactivation of kanamycin, streptomycin and neomycin.^{58,59}

Outer membrane barrier and efflux mechanism

The outer membrane of *Pseudomonas* is highly selective and made of specific porins (OprB, OprD, OprE, OprO, OprP), non-specific porins (OprF) and efflux porins (OprM, OprN and OprJ).⁵⁹ OprF was found to be a major porin for the transport of ions and carbohydrates but has low permeability to antibiotics.⁶⁰ The OprD is the main porin for the influx of antibiotics, specifically charged lysine molecules. It contains a binding site for carbapenems and its absence in the bacterial cell causes carbapenem resistance. Aminoglycosides, antibiotics and colistin only cross the membrane via binding to lipopolysaccharides outside the cell membrane. Studies on laboratory *Pseudomonas* strains showed that overexpression of OprH (gated porin) blocks lipopolysaccharides, thereby preventing the influx of antibiotics through the membrane.⁶¹

In general, bacterial efflux pumps are mainly used for the extrusion of toxic elements. In *P. aeruginosa*, four main efflux pumps are used for expelling antibiotics.⁶² MexAB-OprM is able to pump out beta-lactam antibiotics and quinolones,⁶³ MexXY-OprM extrusion of

aminoglycosides,⁵⁹ MexEF-OprN expels mainly quinolones,⁶⁴ and MexCD-OprJ expels only β -lactams.⁶⁵ Overexpression of these genes were reported to increase antibiotic resistance contributing to the development of resistance to multiple drugs.⁶⁶

Mutational changes of targeted enzymes or proteins

Pseudomonas aeruginosa poses a significant challenge in clinical settings due to its ability to undergo mutational changes within the genome thereby preventing the binding of specific antibiotics. For example, mutations in the mutations in the *gyrA* and *parC* genes, encoding for bacterial DNA gyrase and topoisomerase IV respectively, prevent the binding of fluoroquinolones.^{67,68} Similarly, mutations in the genes encoding for Penicillin binding proteins (PBPs) confer beta-lactam resistance. Another mechanism involves mutations that reduce the bacterial cell wall permeability towards antibiotics, viz., aminoglycosides and carbapenems, altering the porin channels such as oprD. On the other hand, mutations in *mexR*, *nalC* and *nalD* responsible for the regulation of the mexAB-oprM efflux pump system, results in its overexpression, causing resistance towards antibiotics along with biofilm formation.⁶⁹ These mutational changes in the bacterium plays a crucial role for its survival under stress and hence understanding the underlying mechanisms would be critical in developing strategies towards combatting antibiotic resistance.

Pseudomonas biofilm

Biofilm is a matrix of extracellular polymeric substance (EPS) embedded with aggregate microbial communities and helps in the colonization and attachment of microbial cells to the surface. It safeguards the colonized organisms from fluctuating environmental conditions and prevents antibiotic entry, thereby decreasing antibiotic susceptibility. *Pseudomonas aeruginosa* is well-known for its biofilm synthesis, making it an ideal model for studying biofilm development.

The main components of the *P. aeruginosa* biofilm matrix are polysaccharides, extracellular DNA (eDNA), proteins, and lipids. The three main exopolysaccharides, Psl, Pel, and

alginate, play a major role in the biofilm initiation, attachment of organisms to the surface and maintaining the stability of the biofilm.^{70,71} Pel (pellicle), a cationic glucose-rich polysaccharide and Psl (polysaccharide synthesis locus), a neutral mannose-rich pentapolymer comprising mainly mannose, glucose and rhamnose, are present in non-mucoid strains of *P. aeruginosa* whereas alginate present in only mucoid strains.^{72,73} The primary structural elements of the matrix, Psl and Pel, are important for developing biofilms in early stages, sessile cell adhesion to surfaces, improving cell-to-cell attachment i.e., aggregate formation, and maintaining the structural stability of the biofilm architecture. Additionally, Psl functions

as a signalling molecule to encourage increased cyclic 3'5' GMP production to create thicker and more durable biofilms,⁷⁴ whereas Pel enhances bacteria tolerance to aminoglycoside antibiotics and antibiotic colistin.^{9,75} Both the polysaccharides protect bacteria embedded in biofilm from neutrophil phagocytosis and antimicrobials, creating a powerful defense strategy for the progression of the infection.^{73,76,77} However, *Pseudomonas* strain-specific Psl and Pel switch synthesis depend on the environmental conditions of the wound. Alginate, an acetylated linear, unbranched biopolymer of mannuronic acid and glucuronic acid residues, is reportedly synthesized due to *mucA22* allele mutation in mucoid strains.

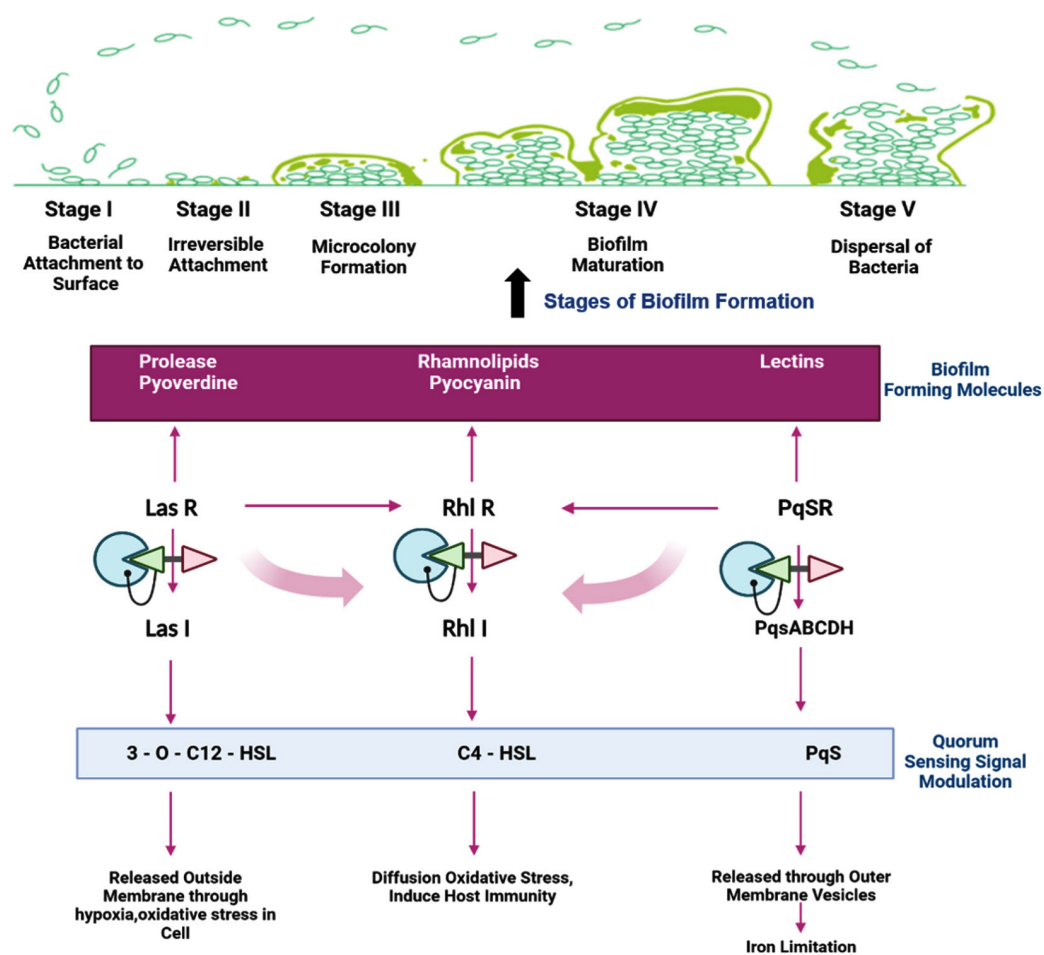


Figure 2. Schematic illustration of biofilm formation in *Pseudomonas aeruginosa* through three main quorum sensing systems-Las, Rhl and PQS systems

Table 2. List of therapeutics, their advantages and limitations targeting *Pseudomonas aeruginosa* in diabetic foot ulcers

Therapeutics	Activity	Advantages	Limitations	Examples	Ref.
Antimicrobial peptides	<ul style="list-style-type: none"> • Antibiofilm • Anti-Quorum sensing 	<ul style="list-style-type: none"> • Interact and penetrate the bacterial cell membrane to cause the death of the bacteria • Broad-spectrum microbicidal activity • C-glycosidic inhibitors of lectin. • Interferes with the quorum sensing and signalling pathways. 	<ul style="list-style-type: none"> • Toxic • Short half-life • Haemolytic activity • Expensive • Complex extraction process • Low availability • Low penetration into biofilm 	LL-37, P5, cationic peptide 1,037, MC1, WLBU2, Fowlicidin-1, BMAP-27, Protegrin PG-5.	[93,94]
Natural products	Antibiofilm agents			Dimethylthiophene 22 (sulphonamide), phenylacetylene bearing thiophene, Ajoene, T6SS, norbougaine, baicalin.	[95-97]
Chemicals	Anti-Quorum sensing agents	<ul style="list-style-type: none"> • Broad-spectrum quorum sensing pathway inhibitors • Inhibit Las A protease and rhamnolipid production. • Gallium disrupts bacterial Fe metabolism and inhibit <i>P. aeruginosa</i> growth • NO creates nitrosative stress or oxidative stress in the biofilm and aids in biofilm dispersal 	<ul style="list-style-type: none"> • Low bactericidal activity • Cytotoxicity • Resistant development 	Gallium, dimethylthiophene 22 (sulphonamide), phenylacetylene bearing thiophene	[93]
Bacteriophages		<ul style="list-style-type: none"> • Narrow spectrum of activity • Safer and better tolerated as they can replicate within target bacterium without infecting mammalian cells • Easy administration • Less expensive • Could be engineered to achieve better efficacy. 	<ul style="list-style-type: none"> • Absence of specificity towards a bacterial strain • Formulation and stabilization of pharmaceutical product is difficult • Gradual emergence of bacterial resistance against bacteriophages. • bacteriophages may also contribute to development of antibiotic resistance 	IME180	[98]
Nanoparticles		<ul style="list-style-type: none"> • Produce reactive oxygen species (ROS) that disrupt replication of the organism. • Inhibit biofilm formation pathways. 	<ul style="list-style-type: none"> • There is no standard testing method for evaluating the antimicrobial activity of the synthesized nanoparticles. • There is no homogeneous culture medium used among different research communities working with the synthesis of nanoparticlebase anti microbials. 	Piper betel (Pb) mediated AgNPs (Pb-AgNPs), Zinc Oxide Nano particles, copper nanoparticles (CuNPs).	[99-102]

Alginate contributes to the maturation of biofilm, protection from phagocytosis and retention of water and nutrients.^{78,79,80}

Extracellular DNA (eDNA) released into the biofilm due to cell lysis is one of the important components of the biofilm matrix with many functions. It is a nutrient source to bacteria in biofilms, acts as an interconnecting compound for the formation of microbial aggregates in biofilm maturation, facilitates twitching motility for biofilm expansion and lastly creates an acidic environment in biofilms, thereby limiting the entry of antimicrobial agents.⁸¹⁻⁸³ The extracellular appendages of *P. aeruginosa*, such as the flagella, type IV pili and fimbriae, in addition to motility, also function as adhesives in the interactions between cells and surfaces as well as in the development of microcolonies in biofilms.^{84,85}

Quorum sensing mechanism and biofilm formation

Quorum sensing is another crucial *P. aeruginosa* regulation mechanism that monitors population size by creating and detecting diffusible signal molecules that control the motility of the organism, synthesis of virulence factors and biofilm formation. Auto signalling synthases activate three main QS systems-Las, Rhl and PQS systems via lactone signaling molecules (3O-C₁₂-HSL, C₄-HSL, 2-heptyl-3-hydroxy-4-quinolone). They trigger the synthesis of functional elements such as rhamnolipid, pyocyanin, pyoverdine, Pel polysaccharides and lectins.^{76,86} Rhamnolipid, a biosurfactant, maintains the pores and channels between microcolonies so that liquid and nutrients can pass through mature biofilms.⁸⁷ Pyoverdine can bind and transport iron, which is essential for the development of biofilms.⁸⁸ The cytotoxic compound pyocyanin lyses cells and releases eDNA, increasing the fluid's viscosity and physicochemical interactions between the biofilm matrix and its environment. It also encourages cellular aggregation.⁸⁹ Pel polysaccharides interact with eDNA through anionic-cationic interactions to strengthen the biofilm.⁸⁸ LecA and lecB, two soluble proteins with adhesive capabilities, enable adhesion to biological surfaces and the retention of both cells and exopolysaccharides in a growing biofilm.^{90,91} In addition, rhl regulation of swarming and twitching motilities in bacterial translocation

has been reported to be an important indirect relationship between biofilm development and QS. In the presence of glutamate or succinate as a carbon source, swarming motility results in flat, uniform biofilms.⁹² Twitching motility, a flagella-independent translocation, resulting in forming *P. aeruginosa* microcolonies in iron-limited media.⁹³ Together with other polymeric elements, these molecular and biological interactions lead to the formation of a developed and robust biofilm (Figure 2).

Current *Pseudomonas* specific therapeutic strategies in controlling diabetic foot ulcer

Biofilms are critical in DFU because the colonizing bacteria interact to generate a synergistic environment conducive for infection progression and as a result, the formation of a chronic wound. Due to ineffective antibiotic therapy, alternatively that has gained the interest of researches is biofilm-based wound therapy. The first step in this therapy is the degradation of the biofilms, followed by the application of antimicrobial drugs to kill or inhibit microorganisms embedded in the wound. As the biofilm bioburden level decreases, so does the inflammatory response (neutrophils and macrophages), proteases and reactive oxygen level decreases. As a result, the wound will transition from a chronic to an active healing condition. This results in active healing of the wound. In 2017, World Health Organization identified *P. aeruginosa* as one of the most harmful bacteria and categorized it as a priority pathogen for the development of new targeted drug deliveries. Current DFU therapies still in clinical trials mainly focus on controlling *Pseudomonas* biofilm formation, inhibiting quorum sensing pathway essential for biofilm formation and other specific enzymes to develop potent antimicrobial therapeutics. Table 2 summarizes therapeutics against *P. aeruginosa* in diabetic foot ulcers.

Potential therapeutics used for dealing antibiotic resistance in *Pseudomonas aeruginosa* Anti-biofilm therapeutics

Enzymatic and synthetic therapeutics are being employed to degrade and disperse biofilms for controlling *Pseudomonas* infections. Microbial enzymes such as alginate lyase,¹⁰³ and glucosyl hydrolases (dextranase and mutanase),^{104,105} were

therapeutically being used singly or in combination with other antibiotics targeting exopolysaccharide polymers and alginate components of the biofilm. Deoxyribonuclease (DNAase) extracted from the human eye were applied for the degradation eDNA.¹⁰⁶ Naturally, Ginger (*Zingiber officinale* Rosc) extracts were reported to inhibit biofilm formation by decreasing the production of cyclic 3'5' GMP.¹⁰⁷ Similarly, ethanol extracts containing casbane diterpene from *Croton nepetaefolius* Baill plant were found to inhibit biofilm formation by interacting with lipopolysaccharides of cell membranes.¹⁰⁸ Marine sponges (Agelas conifer, Agelaceae) synthesize pyrrole-imidazole alkaloids bromoageliferin, which has been shown to prevent the growth of new biofilms and disperse existing ones.¹⁰⁹ Sulphonamides such as dimethylthiophene have potent anti-biofilm activity and are C-glycoside inhibitors with high affinity towards lecB protein of *Pseudomonas*.⁹⁶ Additionally, low dose of nitric oxide gas was found to disperse biofilm and expose microbial species to antibiotics.¹¹⁰ Temporin B from frogs,¹¹¹ indolicidin from the cytoplasmic granules of bovine neutrophils.¹¹² Human beta-defensin 3,¹¹³ and many others have been reported to have anti-biofilm activities. Anti-microbial peptides could be promising alternative therapeutics to deal with antimicrobial resistance in the future.

Anti- Quorum sensing therapeutics

Quorum sensing is a desirable target for biofilm suppression and removal. It was discovered that the naturally available carotenoid zeaxanthin targets the Las and Rhl system and inhibits biofilm.¹¹⁴ The plant flavonoid quercetin is well known for its pharmacological activities, which include lowering pyocyanin synthesis and preventing *P. aeruginosa* from forming biofilms.¹¹⁵ *P. aeruginosa* periplasmic enzyme PvdQ acylase, another quorum-suppressing compound, has been shown to hydrolyze N-acyl homoserine lactone (AHL), reducing virulence and easing infections.¹¹⁶ The fungal metabolite, terrein, derived from *Aspergillus terreus*, has been shown to inhibit both QS system and cyclic 3'5' GMP without impacting bacterial survival.¹¹⁷ Quenching enzymes, QsdA and Aqdc isolated from *Rhodococcus erythropolis* decreased N-acylhomoserine lactone synthesis, inhibiting bioactive compounds required for biofilm formation. Ajoene, a sulfur rich QS

targeting molecule derived from garlic that targets RsmY and RsmZ in *P. aeruginosa*.⁹⁵ Similarly, baicalin flavonoid purified from the roots of *Scutellaria baicalensis*, repressed QS-regulatory genes, including lasI, lasR, rhII, rhIR, pqsR and pqsA in *P. aeruginosa* and minimised the virulence phenotypes such as LasA protease, LasB elastase, pyocyanin, rhamnolipid, motilities and exotoxin A.⁹⁷

Therapeutics against iron metabolism

P. aeruginosa acquires extracellular iron via iron absorption mechanisms (siderophores). Therefore, iron analogues and chelators that target iron metabolism may be effective treatments for *P. aeruginosa* infections. Gallium, which resembles iron structurally, was used as an alternative to iron to obstruct iron uptake, obstruct iron-dependent pathways, affect bacterial survival, and obstruct biofilm formation.¹¹⁸ Deferoxamine and deferasirox, two FDA-approved iron chelation compounds, are used with tobramycin to effectively break up existing biofilms.¹¹⁹

Fifth generation antibiotics

Due to high resistance to conventional antibiotics, fifth-generation antibiotics are used singly or in combination, specifically against *Pseudomonas* species. Cephalosporin or tazobactam was effective against Gram-negative bacteria and being used as an antipseudomonal agent.¹²⁰ A combination of ceftolozane-tazobactam was reported to be effective in the downregulation of *ampC* gene, against the adhesion of colonies to the surface and biofilm formation.^{121,122} Similarly, ureidopenicillin, a beta-lactamase inhibitor found to be effective against *P. aeruginosa*.^{121,123}

Immunotherapeutics

Monoclonal antibodies (mAbs) targeting bacterial DNA binding proteins have been emerging as a promising therapeutic tool against *P. aeruginosa* in mouse models.¹²⁴ KaloBios designed KB001-A, an anti-*P. aeruginosa* mAb against response to the Type III secretion system (T3SS), which is necessary for *P. aeruginosa* pathogenicity and was found to be safe and well-tolerated.^{8,125} Many monoclonal antibodies including MEDI3902 from AstraZeneca targeting *Pseudomonas* biofilms are in clinical trials.¹²⁶

Nanoparticles

Nanoparticles have been employed for the penetration of antibiotics into biofilms. For instance, it has been demonstrated that clinical *P. aeruginosa* strains resistant to specific antibiotics are susceptible to the antibacterial action of silver nanoparticles.¹²⁷ Without altering the development of planktonic cells, zinc ions and ZnO nanoparticles have been shown to prevent the formation of biofilms and also inhibit pyocyanin, pyochelin and hemolytic activity.¹⁰⁰ Methanolic silver nanoparticles showed 85.63% inhibition of *Pseudomonas* biofilm formation.⁹⁹ TTO (Tea of Tree oil) nanoparticles has potential antibiofilm activity against *P. aeruginosa* PAO1,¹²⁸ and D-galactose nanoparticles could inhibit *lecA* of *Pseudomonas* significantly.¹²⁹ Additionally, polyphosphoester nanoparticles, silver acetate, and minocycline significantly improved *P. aeruginosa*'s susceptibility.¹³⁰

CONCLUSION

Diabetes foot ulcers can result in lower limb amputations and significantly negatively impact the socioeconomic and health of diabetic patients. Chronic DFU wounds are polymicrobial with varied organisms. *Pseudomonas aeruginosa* is the dominant pathogen present in medium and chronic diabetic foot ulcers. It is regarded as a highly harmful pathogen due to its ability to form multidrug resistance biofilms. The extraordinary capacity of *P. aeruginosa* to create biofilms is aided by a highly developed quorum-sensing cell communication system and the activation of antibiotic resistance pathways. Nowadays, several therapeutic strategies are developing to prevent resistance such as combination therapy by combining antibiotics of different classes, discovery of novel antibiotics such as ceftolozane-tazobactam, ceftazidime-avibactam, along with designing of novel anti-microbial peptides could be highly promising against multidrug resistant *Pseudomonas aeruginosa*. Future research is needed to create more sophisticated methods that can provide high-throughput and precise treatment at an early stage of *P. aeruginosa* proliferation and biofilm formation.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

DC conceptualized the idea, performed literature review, and wrote the manuscript. SK critically reviewed and revised the manuscript. Both authors read and approved the final manuscript for publication.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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