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Combatting *Burkholderia pseudomallei* Biofilms: Enhanced Strategies through Antibiotic Combinations and Anti-Biofilm Agents

(Membanteras Biofilem *Burkholderia pseudomallei*: Strategi Dipertingkatkan melalui Gabungan Antibiotik dan Agen Anti-Biofilem)

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ABSTRACT

Burkholderia pseudomallei causes melioidosis, a disease with high relapse rates, particularly with chronic infections. Treatment for melioidosis relies solely on antibiotics; however, many *B. pseudomallei* strains are resistant to penicillin, ampicillin, gentamicin, first and second-generation cephalosporins, macrolides and colistin, which may lead to bacterial persistence. The efficacy of antibiotics is further compromised because *B. pseudomallei* forms biofilms, which serve as a multi-layered defence barrier that systematically reduces antibiotic penetration and inhibits their effects. Therefore, agents capable of disrupting or eradicating *B. pseudomallei* biofilms are urgently required to ensure effective treatment of melioidosis. This review briefly outlines the known mechanisms of *B. pseudomallei* biofilm formation, its antimicrobial resistance, and further explores potential new *B. pseudomallei* anti-biofilm strategies. These include antimicrobial peptides that hinder biofilm formation and serve as an alternative to reduce the absolute reliance on antibiotics in treating patients with melioidosis. In addition, this review introduces the concept of administering a combination of antimicrobials to improve the reduction of *B. pseudomallei* biofilms. Finally, strategies to combat *B. pseudomallei* biofilms and the outlook for *B. pseudomallei* biofilm reduction are discussed to ensure more effective and efficient clinical treatment of melioidosis.

Keywords: Antibiotic resistance; biofilm; melioidosis; tolerance; treatments

ABSTRAK

Burkholderia pseudomallei ialah agen penyebab melioidosis, penyakit yang sering dikaitkan dengan kadar kambuhan tinggi terutamanya dalam jangkitan kronik. Rawatan melioidosis bergantung sepenuhnya kepada antibiotik; namun banyak strain *B. pseudomallei* menunjukkan rintangan intrinsik terhadap penisilin, ampisilin, gentamisin, sefalosporin generasi pertama dan kedua, makrolida serta kolistin yang boleh menyebabkan bakteria berterusan dalam tubuh pesakit. Keberkesanan antibiotik turut terjejas kerana *B. pseudomallei* menghasilkan biofilem, iaitu penghalang berlapis yang mengurangkan penembusan antibiotik dan menghalang tindakannya. Oleh itu, agen yang dapat memintas atau memusnahkan biofilem *B. pseudomallei* amat diperlukan untuk memastikan rawatan melioidosis lebih berkesan. Ulasan ini menghuraikan secara ringkas mekanisme pembentukan biofilem oleh *B. pseudomallei*, keupayaan rintangannya terhadap antimikrob, serta meneroka strategi baharu anti-biofilem. Antaranya termasuk peptida antimikrob yang menghalang pembentukan biofilem dan berfungsi sebagai alternatif untuk mengurangkan kebergantungan mutlak terhadap antibiotik. Selain itu, ulasan ini memperkenalkan pendekatan gabungan antimikrob bagi meningkatkan keberkesanan penghapusan biofilem *B. pseudomallei*. Akhir sekali, strategi untuk memerangi biofilem *B. pseudomallei* dan prospek masa depan dibincangkan bagi memastikan rawatan klinikal melioidosis lebih berkesan dan cekap.

Kata kunci: Biofilem; melioidosis; rawatan; rintangan terhadap antibiotik; tolerans

INTRODUCTION

Biofilms are now recognised as major contributors to antimicrobial resistance (AMR). Their extracellular polymeric substance (EPS) matrix protects bacteria, impairs host immunity, and reduces antibiotic penetration (Karygianni et al. 2020). Biofilms can increase resistance up to 1000-fold, enabling survival without genetic change (Roy et al. 2018; Westblade, Errington & Dörr 2020). This complicates the reduction of biofilm, allowing regrowth after treatment cessation and driving relapse (Usui et al. 2023). Persistent biofilm infections, which resist antibiotics and immune clearance, often lead to chronic disease, exemplified by melioidosis.

Burkholderia pseudomallei, the causative agent of melioidosis, is endemic in Southeast Asia and can form biofilms *in vivo* (Vorachit et al. 1995; Wiersinga et al. 2018). It invades and survives intracellularly (Allwood et al. 2011), with biofilm formation enhancing AMR (Sawasdidoln et al. 2010). With no licensed vaccine currently available, treatment remains dependent on antibiotics (Galeas-Pena & Morici 2023). However, intrinsic resistance complicates therapy, highlighting the need for non-antibiotic strategies. Antimicrobial peptides (APs), both natural and synthetic, have the potential to reduce biofilm formation and strengthen antibiotic effectiveness (Kanthawong et al. 2012).

This review consolidates current knowledge on *B. pseudomallei* biofilm formation, its impact on antibiotic efficacy, and emerging therapeutic strategies to overcome resistance and tolerance, providing a foundation for improved treatment of melioidosis and other biofilm-associated infections.

GLOBAL IMPACT AND TREATMENT CHALLENGES OF MELIOIDOSIS

Melioidosis, often termed ‘the great mimicker’, presents with nonspecific symptoms resembling tuberculosis and pneumonia, complicating diagnosis (Wiersinga et al. 2018). Despite being severely underreported, global estimates suggest ~165,000 cases and 89,000 deaths annually (Limmathurotsakul et al. 2016). Endemic regions include Southeast Asia and northern Australia, with incidence rates reaching up to 50 cases per 100,000 people. Northeast Thailand and the Northern Territory of Australia account for a substantial proportion of cases (Wiersinga et al. 2018), while South Asia, particularly India and Bangladesh, contributes ~44% of the global burden (Limmathurotsakul et al. 2016). Underreporting remains a challenge due to limited diagnostic tools and surveillance in resource-poor settings.

Clinical manifestations range from fever, cough, and abscesses to severe complications such as joint and brain inflammation, hypotension, and death (Limmathurotsakul et al. 2016). In its latent phase, melioidosis may remain asymptomatic until reactivation. Current therapy involves a two-phase regimen: An intensive 2-8-week course of intravenous ceftazidime (CAZ) or meropenem (MEM), followed by a 3-6-month eradication phase (CDC 2025). The revised Darwin guideline emphasises trimethoprim-sulfamethoxazole (TMP-SMX) as first-line eradication therapy, with doxycycline (DOX) reserved as a second-line alternative in cases of severe allergy or intolerance (Currie et al. 2024). Despite this, relapse occurs in 5.7-19% of cases, depending on the region (Nathan et al. 2018; Sarovich et al. 2014), underscoring the limitations of antibiotic-dependent treatment and the urgent need for improved strategies.

Burkholderia pseudomallei ANTIMICROBIAL RESISTANCE

B. pseudomallei exhibits intrinsic resistance to penicillin (PCN), ampicillin (AMP), first and second-generation cephalosporins, and several other antibiotics (Wiersinga et al. 2018). Resistance to CAZ is rare, reported in 0.1-1.5% of cases in Thailand (Panya et al. 2016; Wuthiekanun et al. 2011) and 0.6-2.4% in Malaysia (Ahmad, Hashim & Mohd Noor 2013; Hassan et al. 2014), though mortality among CAZ-treated patients remains considerable. CAZ-resistant genes, including *PenA* β-lactamase variants, may explain treatment failures (Chattagul et al. 2021). In Northern Australia, resistance to CAZ and TMP/SMX is uncommon (Crowe et al. 2014; Currie 2015), but isolated CAZ-resistant strains have been identified (Sivabalan et al. 2024). Cephiderocol (CFD), a siderophore cephalosporin, has shown potent activity against amoxicillin-clavulanic acid (AMC) resistant isolates, although some strains remain non-susceptible (Burnard et al. 2021).

Resistance mechanisms include mutations in *PenA*, a β-lactamase. Chirakul et al. (2018) reported multiple mutations linked to β-lactam resistance, including gene deletions and amino acid substitutions that broaden substrate specificity, while duplication and overexpression of *PenA* further increased resistance. In contrast, Hii et al. (2023) found resistance uncommon in extensive isolate surveys, reinforcing the continued utility of CAZ and carbapenems. Efflux pump overexpression, particularly the BpeAB-OprB system, also enhances intrinsic resistance and induces multidrug resistance against aminoglycosides and macrolides (Alav, Sutton & Rahman 2018).

Stress-induced small colony variants (SCVs) represent another adaptive mechanism. Al-Maleki et al. (2020) showed that iron limitation, starvation, and temperature shifts trigger SCVs with increased biofilm formation and elevated resistance to DOX, MEM, and TMP/SMX, while generally remaining susceptible to most agents except gentamicin (GEN). Together, these adaptive mechanisms, combined with biofilm development, enhance tolerance and complicate the eradication of *B. pseudomallei* during infection.

Burkholderia pseudomallei BIOFILM FORMATION

B. pseudomallei biofilm formation progresses through four stages: i) attachment, ii) colonisation, iii) maturation, and iv) dispersal (Nyanasegran et al. 2023). In the initial attachment stage, cyclic di-GMP regulates exopolysaccharide synthesis, lipopolysaccharide production, and adhesin activity, while extracellular DNA (eDNA) further supports bacterial adherence (Fazli et al. 2014; Pakkulnan et al. 2019). Colonisation is driven by quorum sensing (QS), which in *B. pseudomallei* relies on N-acyl homoserine lactones (AHLs) and three QS systems (BpsI/BpsR, BpsI2/BpsR2, BpsI3/BpsR3) that coordinate gene expression (Gamage et al. 2011; Horton et al. 2013; Mott, Panchal & Rajamani 2017; Truong et al. 2015). Additional signals such as hydroxy-methyl-alkylquinolines (HMAQs) also contribute to virulence (Butt et al. 2016; Coulon, Zlosnik & Déziel 2021).

During maturation, QS and cyclic di-GMP promote irreversible attachment and EPS matrix development, forming a protective barrier that limits antibiotic penetration (Mangalea, Plumley & Borlee 2017; Nyanasegran et al. 2023). The EPS is composed mainly of glucose, galactose, and mannose (Mongkolrob, Taweechaisupapong & Tungpradabkul 2015), with gene clusters such as *becAR* encoding enzymes for polysaccharide biosynthesis (Borlee et al. 2017; Chin et al. 2015). Other loci, including *wbiA* and adjacent glycosyl transferase genes, contribute to lipopolysaccharide and sugar metabolism (Albesa-Jové & Guerin 2016; Borlee et al. 2017; Lairson et al. 2008). Exopolysaccharide production is heavily dependent on cyclic di-GMP, regulated by cdpA (Lee et al. 2010).

Finally, dispersal occurs when cyclic di-GMP levels decline, often triggered by stress signals such as nutrient depletion, oxygen restriction, or antimicrobial exposure (Nyanasegran et al. 2023; Sauer et al. 2004). This breakdown of the biofilm matrix releases planktonic cells, enabling dissemination and persistence (Kostakioti, Hadjifrangiskou & Hultgren 2013; Uppuluri & Lopez-Ribot 2016). As biofilm development creates a formidable barrier to conventional antibiotics, therapeutic approaches that directly disrupt biofilms or enhance bacterial clearance are urgently needed. Among these, APs have emerged as promising candidates.

THERAPEUTIC STRATEGIES AGAINST *Burkholderia pseudomallei* BIOFILMS

Antimicrobial peptides

APs are small, positively charged, hydrophobic amino acid sequences that occur naturally in the innate immune system or can be synthetically produced. They have attracted considerable interest for their potential in combating AMR (Mahlapuu et al. 2016). APs exhibit strong antimicrobial activity against intracellular bacteria by reducing survival within host cells, inhibiting growth, limiting entry into target cells, and directly decreasing intracellular bacterial numbers (Cappiello et al. 2016; Kang et al. 2015; Longhi et al. 2004).

Although APs are not effective at every stage of *B. pseudomallei* biofilm formation, they can inhibit initiation or reduce mature biofilms. Notable examples include LFchimera (Puknun et al. 2016), LL-37, LL-31 and its D-enantiomer DLL-31 (Kanthawong et al. 2012; Longhi et al. 2004), bactenecin, RTA3, bovine myeloid antimicrobial peptide-18 (BMAP-18), and CA-MA (Madhongsa et al. 2013). LL-37 and LL-31 demonstrate greater bactericidal activity than CAZ, while DLL-31 combined with CAZ further enhances efficacy (Wongkaewkhiaw et al. 2019). LFchimera disrupts biofilm colonisation (Puknun et al. 2016). Tachyplesin-1 (TP1), derived from horseshoe crabs, also significantly inhibited biofilm growth, though cytotoxicity in mammalian cells requires further evaluation (Lee et al. 2016). Other peptides, such as histatin-5, lactoferrin fragments, protegrin-1, and phospholipase A2 inhibitors, show antimicrobial activity but require validation against *B. pseudomallei* biofilms (Kanthawong et al. 2009; Samy et al. 2015; Sim et al. 2011). Promising APs are summarised in Table 1. Beyond peptides alone, combining them with conventional antibiotics or other adjunctive agents has shown potential in biofilm reduction. These synergistic strategies broaden the therapeutic arsenal against *B. pseudomallei*.

Synergistic antibiotic treatment

To effectively eradicate biofilm-producing *B. pseudomallei*, more comprehensive antibiotic protocols are required, often involving synergistic combinations with adjunctive agents. Iron chelators are one such approach: they restrict bacterial growth and, when paired with CAZ or ciprofloxacin (CIP), reduce biofilm formation and tolerance (Chattagul et al. 2021; Guedes et al. 2023a; Luo et al. 2014). Deferiprone (DFP) combined with AMC or MEM has been shown to lower the minimal inhibition concentration (MIC) values and reduce biofilm biomass, though viability was less affected (Guedes et al. 2023a).

Efflux pump inhibitors also enhance antibiotic efficacy. Promethazine and phenylalanine arginine β-naphthylamide (PAβN) inhibited efflux activity, improving the effectiveness of erythromycin (ERY), TMP/SMX, GEN, and CIP (Sidrim et al. 2017; Sirijant, Sermswan & Wongratanacheewin 2016). When CAZ was combined with iron chelators, DLL-31 (Chattagul et al. 2021), PAβN (Sirijant, Sermswan & Wongratanacheewin 2016), farnesol (Castelo-Branco et al. 2016), chitosan, or DNase I (Pakkulnan, Thonglao & Chareonsudjai 2023; Thonglao et al. 2022), its bactericidal and antibiofilm activity increased significantly.

Synergy between APs and antibiotics has also been demonstrated. DLL-31 combined with CAZ reduced IC_{50} values up to 16-fold compared to either agent

TABLE 1. Antimicrobial peptides that attenuate *B. pseudomallei* biofilm formation

Antimicrobial peptide	Discovery	Mechanism of action	Biofilm stage targeted	Evidence level	Limitations/Toxicity	References
LL-37 and LL-31	LL-37 and LL-31 exhibit stronger bactericidal activity than CAZ	Membrane-targeting peptide; reported mechanisms include permeabilization of outer and inner membranes, depolarization, LPS binding, and leakage of intracellular contents	Early and mature biofilm stages	*In vitro*	*LL-37* exhibits high human-cell toxicity	Kanthawong et al. (2012); Ridyard & Overhage (2021); Phophetleb et al. (2023)
Bactenecin, RTA3, BMAP-18 and CA-MA	APs, which reduced biofilm formation comparable to CAZ antimicrobial capacity	Rapid membrane permeabilization is the primary mechanism; bactenecin binds LPS strongly, disrupts the inner membrane, and causes dye leakage consistent with deep membrane insertion	Early-stage biofilm	*In vitro*	No toxicity or *in vivo* data; evidence insufficient for translation	Madhongsa et al. (2013)
Lactoferrin chimera (LFchimera)	LFchimera, a bovine lactoferrin-derived hybrid peptide, demonstrated strong antibiofilm activity and greater bactericidal efficacy than ceftazidime against biofilm-grown *B. pseudomallei*	The mechanism appears predominantly intracellular, with periplasmic swelling, altered DNA density, and intracellular localization without membrane accumulation	Early and mature biofilm stages	*In vitro*	No toxicity or *in vivo* data; evidence insufficient for translation	Puknun et al. (2016)
Tachyplesin 1 (TP1)	Significantly inhibits biofilm growth (4.2 log CFU/biofilm) when compared to untreated bacteria (7.8 log CFU/biofilm)	Likely membrane-active, with membrane blebbing by SEM and additional *in silico* support for LPS-associated binding	Early-stage biofilm	*In vitro*	Mammalian-cell cytotoxicity was reported, representing the key translational limitation	Lee et al. (2016)

[a] APs = antimicrobial peptides; CAZ = ceftazidime; LPS = lipopolysaccharide; SEM = scanning electron microscopy; CFU = colony-forming units; LFchimera = lactoferrin chimaera; BMAP-18 = bovine myeloid antimicrobial peptide-18; CA-MA = cationic antimicrobial peptide; TP1 = tachyplesin-1; MIC = minimum inhibitory concentration; MBEC = minimum biofilm elimination concentration; IC_{50} = half maximal inhibitory concentration; eDNA = extracellular DNA

alone (Wongkaewkhiaw et al. 2019). Similarly, PAβN combined with CAZ and DOX enhanced antibiotic uptake and disrupted biofilms (Sirijant, Sermswan & Wongratanacheewin 2016). Rhamnolipids paired with MEM and AMC reduced mature biofilm biomass (Sidrim et al. 2020).

Additional adjunctive therapies include fluoxetine, which eradicated both growing and mature biofilms while enhancing antibiotic efficacy (Guedes et al. 2023b), and methionine, which induced nuclease expression, depleted eDNA, and promoted dispersal, thereby increasing susceptibility to CAZ (Rattiyaphorn, Auttawit & Sorujsiri 2024). Collectively, these synergistic approaches highlight multiple avenues to disrupt biofilm formation, overcome tolerance, and improve clinical outcomes in melioidosis. Key examples are summarised in Table 2.

Despite these promising therapeutic combinations, biofilm persistence remains a significant challenge. This has prompted exploration of novel, forward-looking strategies that extend beyond conventional antibiotics.

POTENTIAL STRATEGIES AND FUTURE DIRECTIONS TOWARDS DISRUPTING BIOFILMS

In acute melioidosis, *B. pseudomallei* remains susceptible to antibiotics such as CAZ, but in chronic or relapsing infections, biofilm formation impedes penetration and reduces susceptibility. Alternative approaches that disrupt biofilms are therefore critical. Shrestha et al. (2022) categorised these into two main strategies: compounds that inhibit biofilm formation (anti-QS molecules, natural or synthetic metabolites, secondary metabolites, APs), and compounds that disperse established biofilms (photodynamic therapy, nanoparticles).

Quorum quenching (QQ) represents a promising avenue for disrupting QS and attenuating biofilm development in *B. pseudomallei* biofilms. QQ suppresses QS communication in *B. pseudomallei*, lowering biofilm formation by interfering with cell-to-cell signalling (Ramli et al. 2012). Competitive inhibition of the BpsI/BpsR system by AHL analogues such as OHC8HL blocks responses to QS signals (Ramli et al. 2012). Lactonase-mediated QQ reduces biofilm formation, motility, and virulence in *Burkholderia thailandensis*, while soil-derived *Bacillus* isolates with AHL-degrading activity significantly reduced *B. pseudomallei* biofilms (Gonzales et al. 2023). *In vivo* validation of these approaches remains an important future direction.

APs constitute a leading experimental approach, combining direct bactericidal effects with antibiofilm activity. APs can act synergistically with antibiotics to reduce biofilm biomass and eliminate planktonic cells. They have demonstrated intraphagosomal killing, a vital aspect of host defence (Wiesner & Vilcinskas 2010), and they disrupt biofilm structures while targeting planktonic cells (Ageitos et al. 2017). Continued optimisation of peptide design and delivery could enhance their therapeutic potential.

In addition to peptide-based therapies, nanoparticles are being explored as innovative agents capable of penetrating biofilm matrices and enhancing antibiotic efficacy. Silver nanoparticles (AgNPs) eliminate *B. pseudomallei* cells, outperform CAZ against resistant strains, and act synergistically with β-lactams and aminoglycosides (Malawong et al. 2021; Oves et al. 2018; Thammawithan et al. 2022). Their activity is attributed to reactive oxygen species (ROS) generation, membrane disruption, and penetration into the EPS matrix. Gold nanoparticles (AuNPs) have shown biofilm reduction against other gram-negative bacteria (Ali et al. 2020; Qais et al. 2021) and, when conjugated to antigens, enhanced macrophage uptake and immune responses against *B. pseudomallei*. Their immunological potential warrants further investigation.

Monoclonal antibodies targeting extracellular DNA represent a potential adjunctive strategy to weaken biofilms and improve antibiotic efficacy. TRL1068 binds DNABII proteins that stabilise eDNA, compromising biofilm integrity and increasing antibiotic susceptibility (Xiong et al. 2017). Preclinical studies demonstrated efficacy against gram-positive and gram-negative biofilms, highlighting monoclonal antibodies as promising adjunctive therapies. As illustrated in Figure 1, these diverse strategies converge on a multi-target approach that complements conventional antibiotics, reduces biofilm resilience, and lowers relapse risk, underscoring the future potential of integrated anti-biofilm therapies in melioidosis. Together, these strategies highlight a diverse and hopeful pipeline of anti-biofilm approaches that could complement conventional antibiotics and improve outcomes in melioidosis.

CHALLENGES IN TRANSLATING ANTI-BIOFILM THERAPIES TO CLINICAL APPLICATION

Although diverse anti-biofilm strategies for *B. pseudomallei* show promise *in vitro*, their translation into clinical practice faces major obstacles (Sawasdidoln et al. 2010). A critical comparison of current approaches highlights both strengths and weaknesses. APs demonstrate potent bactericidal and antibiofilm activity, but their instability in physiological environments and occasional cytotoxicity limit immediate applicability (Kanthawong et al. 2012; Puknun et al. 2016; Wongkaewkhiaw et al. 2019). Nanoparticles such as AgNPs and AuNPs penetrate biofilm matrices and generate ROS, yet concerns remain regarding long-term toxicity, biodistribution, and immune activation (Ali et al. 2020; Malawong et al. 2021; Oves et al. 2018; Thammawithan et al. 2022; Qais et al. 2021). QS inhibitors effectively reduce biofilm formation by disrupting cell-to-cell signalling, but their specificity and durability *in vivo* are uncertain (Ramli et al. 2012).

TABLE 2. Potential antibiotic therapeutic agents to alleviate *B. pseudomallei* biofilms

Agent	Discovery	Mechanism of action	Biofilm stage targeted	Evidence level	Limitations/ Toxicity	Reference
CAZ + CIP + iron chelator deferoxamine	Iron chelator DFO significantly reduces biofilm formation and associated CAZ tolerance, consistent with disrupted Fe–S/ ROS homeostasis	Iron chelation disrupting iron/Fe–S metabolism and associated stress responses that support CAZ tolerance in biofilm cells	Mature biofilm stages	*In vitro*	No toxicity or *in vivo* data; evidence insufficient for translation	Chattagul et al. (2021)
D-LL-31 + CAZ	D-LL-31 combined with CAZ decreases IC_{50} values for both peptide and antibiotic and disrupts *B. pseudomallei* biofilms	Antimicrobial peptide activity (membrane/peptide-bactericidal effects) that potentiates CAZ killing and promotes biofilm disruption	Early and mature biofilm stages	*In vitro*	No toxicity or *in vivo* data; evidence insufficient for translation	Wongkaewkhiaw et al. (2019)
Iron chelator deferiprone + AMC, MEM	DFP in combination with AMC and MEM reduces biofilm MIC/biomass and enhances antibiotic activity; DFP also reduces mature biofilm biomass without increasing susceptibility of biofilm viability to some tested antibiotics	Iron chelation impairing biofilm-associated iron-dependent physiology, thereby lowering antibiotic MIC/ biomass and potentiating β-lactams	Mature biofilm stages	*In vitro*	No toxicity or *in vivo* data; evidence insufficient for translation	Guedes et al. (2023a)
Phenylalanine arginine β-napthylamide (PAβN) + CAZ and DOX	PAβN decreases biofilm MBECs for CAZ and DOX by up to 2–16 fold versus untreated biofilm, with increased intracellular accumulation of CAZ and DOX in PAβN-treated biofilms	Efflux pump inhibition (universal efflux inhibitor) reducing antibiotic efflux and thereby increasing intracellular antibiotic levels in biofilm cells	Mature biofilm stages	*In vitro*	No toxicity or *in vivo* data; evidence insufficient for translation	Sirijant, Sermswan & Wongratanacheewin (2016)
Promethazine	Promethazine reduces MBEC values (780–3,120 mg/L for promethazine MBEC range) for tested drugs and reduces the MICs of several antibiotics when used in combination; microscopy suggests structural disruption that may enhance antibiotic penetration	Efflux pump inhibition; additionally alters biofilm structure (possible matrix disruption) to facilitate antibiotic access	Mature biofilm stages	*In vitro*	No toxicity or *in vivo* data; evidence insufficient for translation	Sidrim et al. (2017)
Farnesol	Farnesol combined with CAZ, DOX, AMC, and TMP/SMX reduces MBEC values and damages the biofilm matrix	Biofilm matrix disruption/damage (quorum/biofilm regulation effects implied); improves antibiotic effectiveness by weakening matrix barriers	Mature biofilm stages	*In vitro*	No toxicity or *in vivo* data; evidence insufficient for translation	Castelo-Branco et al. (2016)

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DNase I + CAZ, DNase I + Chitosan + CAZ	DNase I given with CAZ at 24 h significantly inhibited 2-day biofilm formation and reduced viable biofilm cell counts embedded in the 48 h biofilm by ~3–4 logs; DNase I plus chitosan linked with CAZ reduced shedding planktonic and biofilm cells	Enzymatic degradation of extracellular DNA (eDNA) to disrupt the biofilm matrix, promoting dispersal and biofilm reduction	Early and mature biofilm stages	*In vitro*	No toxicity or *in vivo* data; evidence insufficient for translation	Pakkulnan, Thonglao & Chareonsudjai (2023)
Chitosan + CAZ	Chitosan linked with CAZ acts synergistically to kill *B. pseudomallei* biofilm cells more effectively than either agent alone, producing ~3–6 log CFU reduction and improved biofilm reduction metrics in biofilm matrix	Biofilm disruption or dispersal, facilitating enhanced CAZ penetration and increased bactericidal activity against embedded biofilm cells	Mature biofilm stages	*In vitro*	No toxicity or *in vivo* data; evidence insufficient for translation	Thonglao et al. (2022)
Rhamnolipid + MEM and AMC	Rhamnolipid significantly reduces mature biofilm biomass and enhances the antimicrobial activity of MEM and AMC against mature biofilms; it also alters protease and siderophore production dynamics	Biosurfactant-mediated biofilm disassembly/disruption and enhanced antibiotic action; modulation of virulence-factor production that correlates with disassembly	Early and mature biofilm stages	*In vitro*	No toxicity or *in vivo* data; evidence insufficient for translation	Sidrim et al. (2020)
Fluoxetine	Fluoxetine reduces growing and mature *B. pseudomallei* biofilms and enhances the anti-biofilm activity of antimicrobial drugs	Fluoxetine exerts antibiofilm activity by reducing metabolic activity and biomass in both growing and mature biofilms, without altering biofilm structure, and enhances antibiotic penetration/effectiveness	Early and mature biofilm stages	*In vitro*	No toxicity or *in vivo* data; evidence insufficient for translation	Guedes et al. (2023b)
Methionine + CAZ	D- and L-methionine disperse 24 h established *B. pseudomallei* biofilms with eDNA depletion; nuclease gene up-regulation is observed, and D-methionine enhances biofilm susceptibility to CAZ	Nuclease induction leading to eDNA degradation/dispersal and improved antibiotic susceptibility	Early and mature biofilm stages	*In vitro*	No toxicity or *in vivo* data; evidence insufficient for translation	Rattiyaphorn, Auttawit & Sorujsiri (2024)

*AMC = amoxicillin/clavulanate; CAZ = ceftazidime; CIP = ciprofloxacin; CFU = colony-forming units; DFO = deferoxamine; DFP = deferiprone; DOX = doxycycline; IC_{50} = half maximal inhibitory concentration; MBEC = minimum biofilm elimination concentration; MEM = meropenem; MIC = minimum inhibitory concentration; PaβN = phenylalanine-arginine β-naphthylamide; ROS = reactive oxygen species; TMP/SMX = trimethoprim/sulfamethoxazole; eDNA = extracellular DNA

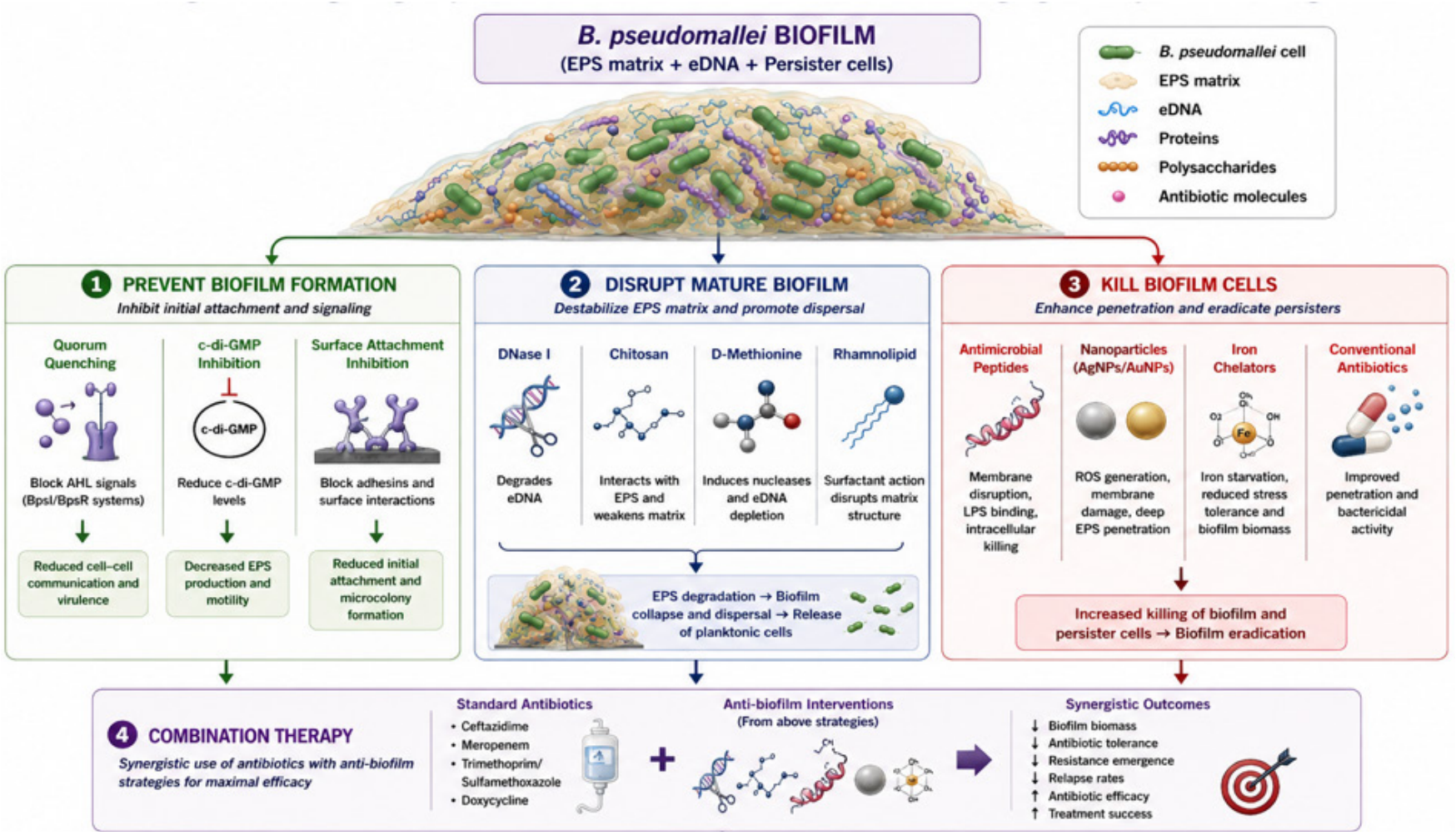

FIGURE 1. Schematic overview of *B. pseudomallei* biofilm architecture and multi-target anti-biofilm strategies. The biofilm consists of cells embedded in an EPS matrix (eDNA, polysaccharides, proteins, and lipids) that promotes structural stability, antimicrobial tolerance, and persistence via persister cells. Four intervention strategies are shown: (1) inhibition of biofilm initiation by targeting attachment, quorum sensing, c-di-GMP signalling, and adhesins; (2) disruption of established biofilms via enzymatic and chemical degradation of the EPS matrix to promote dispersion; (3) eradication of biofilm-embedded cells using enhanced antibiotic delivery, nanoparticles, antimicrobial peptides, iron chelation, and oxidative stress–inducing agents; and (4) combination therapy integrating conventional antibiotics with anti-biofilm agents to achieve synergistic killing, reduced biomass, and lower relapse risk

Monoclonal antibodies targeting extracellular DNA weaken biofilm integrity and enhance antibiotic susceptibility, though delivery systems and regulatory pathways remain underdeveloped (Xiong et al. 2017).

Translational barriers extend beyond compound properties (Nyanasegran et al. 2023). A defining challenge in melioidosis is the dual survival strategy of *B. pseudomallei*. Extracellular biofilm formation combined with intracellular persistence within macrophages and other host cells (Mariappan et al. 2021). This intracellular niche enables immune evasion, long-term latency, and relapse after apparent clearance (Mariappan et al. 2021; Wiersinga et al. 2018). Biofilm and intracellular persistence often coexist in chronic disease, creating overlapping protective barriers that limit drug penetration. Therapies that only target extracellular biofilms may therefore fail to eradicate intracellular reservoirs (Mariappan et al. 2021). Effective strategies must integrate antibiofilm activity with intracellular delivery and host-directed approaches to overcome macrophage survival and immune evasion.

Contradictions between studies further complicate translation. Some reports demonstrate strong synergy between APs and CAZ, whereas others show weaker or more context-dependent effects under different growth conditions (Pibalpakdee et al. 2012; Wongkaewkhiaw et al. 2019). Nanoparticle efficacy varies depending on strain background and experimental design, raising questions about reproducibility (Gomes Bandeira et al. 2013; Pibalpakdee et al. 2012). Even the QQ molecules show inconsistent outcomes depending on the experimental design, reinforcing the need for standardised models and rigorous comparative validation (Gonzales et al. 2023; Ramli et al. 2012). These discrepancies highlight the need for standardised models and rigorous comparative validation.

When evaluating realistic clinical potential, most strategies remain at the preclinical proof-of-concept stage. APs and QQ molecules are promising but require optimisation for stability, delivery, and safety (Al-Emran et al. 2025; Haque et al. 2021). Nanoparticles show potential as adjunctive agents but demand extensive toxicological validation (Al-Emran et al. 2025; Chen et al. 2023). Monoclonal antibodies are conceptually attractive, yet their translation depends on cost, scalability, and regulatory approval (Amara et al. 2011). At present, none of these approaches is close to therapeutic implementation in melioidosis.

While anti-biofilm therapies show potential, their translation is hindered by compound instability, toxicity, delivery challenges, contradictory findings, and the unique host–pathogen dynamics of *B. pseudomallei*. Addressing these barriers will require peptide engineering, nanoparticle optimisation, integrated host-targeted approaches, and robust *in vivo* validation. Only through multidisciplinary research that incorporates both biofilm biology and intracellular persistence can these strategies progress from experimental promise toward clinically viable therapies.

CONCLUSION

B. pseudomallei biofilm formation is a central driver of AMR, antibiotic tolerance, and relapse in melioidosis. Promising strategies such as QQ molecules, APs, nanoparticles, and monoclonal antibodies, together with conventional antibiotics and adjuvants, have shown potential to reduce biofilm biomass and enhance bactericidal activity *in vitro*. However, all anti-biofilm approaches remain experimental. Translation into clinical practice is hindered by compound instability, toxicity, delivery barriers, contradictory findings, and the dual survival strategy of *B. pseudomallei*, which combines extracellular biofilms with intracellular persistence and immune evasion. Future progress will depend on multidisciplinary research that integrates mechanistic insights, host–pathogen dynamics, and rigorous preclinical validation. With sustained effort, these strategies may evolve into adjunctive therapies that complement antibiotics and improve outcomes in melioidosis.

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