Pharmacological strategies for targeting biofilms in otorhinolaryngologic infections and overcoming antimicrobial resistance (Review)

MAULANA A. EMPITU[1,2], IKA N. KADARISWANTININGSIH[3,4] and NADHIRAH MOHD SHAKRI[5]

[1]Division of Pharmacology, Faculty of Medicine, Airlangga University, Surabaya, East Java 60131, Indonesia; [2]Faculty of Health, Medicine and Natural Sciences (FIKKIA), Airlangga University, Banyuwangi 68425, Indonesia; [3]Department of Medical Microbiology, Faculty of Medicine, Airlangga University, Surabaya 60131, Indonesia; [4]Clinical Microbiology Residency Program, Dr. Soetomo Regional Hospital/Faculty of Medicine, Airlangga University, Surabaya 60131, Indonesia; [5]Department of Otorhinolaryngology-Head and Neck Surgery, Faculty of Medicine, National University of Malaysia, Kuala Lumpur 50300, Malaysia

Received February 20, 2025; Accepted March 31, 2025

DOI: 10.3892/br.2025.1973

Abstract. Biofilm formation is a key factor in the persistence and recurrence of otorhinolaryngology (ORL) infections, driving antimicrobial resistance and treatment failure. Chronic conditions, such as rhinosinusitis, otitis media and tonsillitis, are linked to biofilm-producing pathogens, forming protective extracellular matrices that shield bacteria from immune defenses and antibiotics. The present review explores emerging pharmacological strategies to disrupt biofilm integrity and improve treatment outcomes. Strategies such as quorum sensing inhibitors, antibiofilm peptides, enzymatic dispersal agents, and drug repurposing can potentially disrupt biofilms and counter-resistance mechanisms. Furthermore, novel therapies (including nanotechnology-based drug delivery systems, phage therapy and immunomodulation) offer innovative alternatives for managing biofilm-associated infections. However, clinical implementation remains challenging. Future research should prioritize optimizing drug formulations, refining delivery techniques, and exploring synergistic combinations to enhance biofilm eradication. Implementing these innovative strategies can improve the management of chronic ORL infections, reducing recurrence rates and enhancing patient outcomes.

Contents

Correspondence to: Dr Maulana A. Empitu, Division of Pharmacology, Faculty of Medicine, Airlangga University, Mayjend, Prof. Dr. Moestopo 47, Surabaya, East Java 60131, Indonesia
E-mail: maulana.antiyan@fk.unair.ac.id

Key words: biofilm-associated infections, otorhinolaryngology, antimicrobial resistance, infectious diseases

1. Introduction

Biofilm-associated infections in otorhinolaryngology (ORL) pose a significant clinical challenge due to their inherent resistance to antimicrobial therapy and immune defenses. These infections contribute to chronic and recurrent conditions, such as chronic rhinosinusitis, otitis media and tonsillitis, leading to prolonged patient morbidity and a growing healthcare burden (1-5). Biofilms also frequently develop on tracheostomy and airway stents, often forming within 1 week postoperatively, regardless of material type. This can facilitate biofilm spread to the lower airways, increasing the risk of pneumonia and sepsis (1-5). Despite conventional treatment approaches, biofilm persistence in ORL structures hinders disease resolution, necessitating alternative therapeutic strategies (6-9). Consequently, biofilm-associated infections pose significant challenges, highlighting the urgent need for innovative pharmacological strategies to disrupt biofilm formation.

The mechanisms underlying biofilm formation are complex, involving changes in phenotypic traits, antibiotic resistance and gene expression (1-3). Pathogens, such as *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Staphylococcus aureus* (*S. aureus*), are frequently implicated, exhibiting strong biofilm formation in the sinuses and middle ears (1,4-6). The intricate anatomy of ORL structures, including the narrow sinus ostia, middle ear cavity and deep tonsillar crypts, fosters bacterial adhesion and biofilm formation. These niches shield bacteria from immune defenses and antibiotic penetration, contributing to recurrent infections and treatment failures (3,6).

Effectively managing biofilm-associated infections requires innovative pharmacological approaches beyond

conventional antibiotics. Recent research highlights promising strategies, such as quorum sensing inhibitors (QSIs), that disrupt bacterial communication essential for biofilm formation (7-9). Antibiofilm peptides, such as natural and synthetic cationic peptides, effectively hinder biofilm growth and enhance antimicrobial efficacy by disrupting bacterial membranes or modulating gene expression (10,11). Additionally, repurposing non-antibiotic drugs, such as statins and non-steroidal anti-inflammatory drugs (NSAIDs), has emerged as a potential for targeting biofilm pathogens while complementing existing therapies (12-14).

Biofilm formation plays a crucial role in the persistence of ORL infections, necessitating the development of innovative therapeutic strategies. Advancing our understanding of biofilm development mechanisms and exploring alternative approaches (such as QSIs, antibiofilm peptides and drug repurposing) presents promising opportunities to improve treatment outcomes. The present review examines current drug mechanisms targeting biofilm formation and highlights the need for further research into biofilms in ORL infections.

2. Biofilm formation in ORL infections

Mechanisms of biofilm formation. ORL bacterial infections can arise from either planktonic (free-floating) or biofilm-associated bacteria. Planktonic bacteria are typically more susceptible to antibiotics and immune defenses. Conversely, biofilm-associated bacteria form structured communities encased in an extracellular polymeric matrix, which offers increased resistance to antimicrobial agents and immune system clearance (15,16). This difference is crucial, as biofilm-associated infections are often persistent and recurrent, playing a significant role in chronic disease progression (15,16).

The mechanisms of biofilm formation in ORL involve dynamic processes that manifest either as surface-attached structures or free-floating aggregates, reflecting the broader framework of biofilm formation. These processes are divided into three key phases (attachment, growth and disaggregation) each playing a critical role in biofilm persistence and enhancing bacterial resistance to treatment (Fig. 1) (15,16).

The attachment phase represents the initial step in biofilm formation, during which pathogens adhere to epithelial surfaces or medical devices. Surface structures, such as pili and fimbriae, play a key role in this process by enabling bacteria to anchor firmly to surfaces (15,16). In ORL, biofilms have been identified on the mucosal surfaces of the tonsils and adenoids, as well as in the middle ear, where pathogens, such as *S. aureus* and *Haemophilus influenzae* can colonize (5,6,17,18). The presence of biofilms in these regions is closely linked to chronic conditions, such as chronic tonsillitis and otitis media, highlighting the significance of the attachment phase in the development and persistence of these infections (5,6,17,18).

In the growth phase, bacterial communities proliferate and secrete extracellular polymeric substances (EPS), which assemble into a protective matrix. This matrix not only ensures structural stability but also retains nutrients and establishes gradients of oxygen and metabolites, fostering diverse microenvironments within the biofilm (19,20). Quorum sensing (QS) regulates gene expression, enabling bacteria to adjust their metabolism and enhance antibiotic resistance (19,21). Biofilms in ORL infections mature over time, growing more resistant and persistent. Consequently, they become less susceptible to antimicrobial treatments and immune defenses (22).

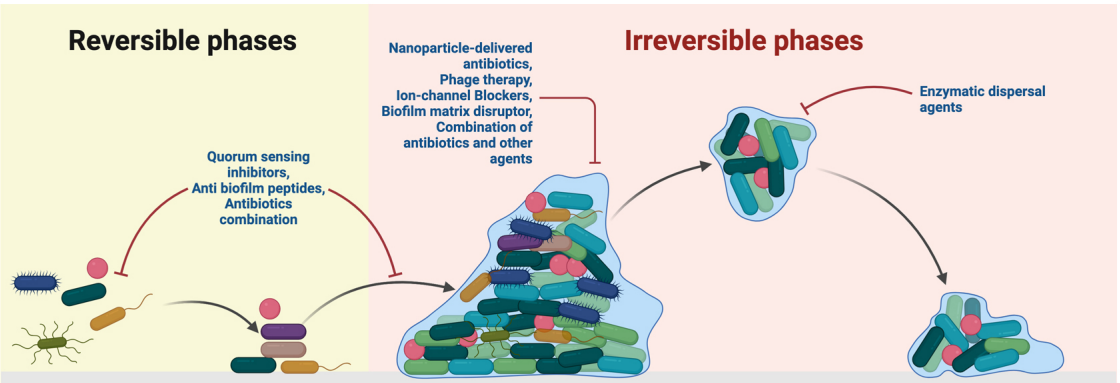
Previous studies highlight that biofilm growth is not limited to surface attachment. Free-floating aggregates formed by bacterial EPS or host-derived polymers can develop within host tissues and secretions. These aggregates exhibit features similar to surface-attached biofilms, including antibiotic tolerance and matrix production, expanding the conceptual understanding of biofilm formation (15).

The final phase-disaggregation-involves the release of planktonic cells or fragments from mature biofilms, facilitating the colonization of new sites and contributing to infection recurrence (23-25). This dispersal process is often triggered by environmental changes, such as nutrient depletion or antibiotic exposure (23-25). Recent findings challenge the traditional model by showing that disaggregation can occur in both surface-attached and free-floating biofilms, further complicating treatment strategies (15). Understanding the formation of biofilms is essential, as their structural complexity directly contributes to antibiotic resistance and complicates treatment. This highlights the need for strategies specifically designed to address the unique challenges posed by biofilms, as discussed in the following section.

In summary, biofilm formation involves dynamic and adaptive processes encompassing surface-associated and free-floating mechanisms. This revised framework highlights the importance of developing therapies that target biofilms in all forms. Understanding these processes is crucial for developing effective strategies to combat chronic ORL infections.

Pathogens involved in ORL biofilms. Numerous bacterial and fungal pathogens are frequently linked to biofilm formation in ORL infections. Key bacterial species, such as *P. aeruginosa*, *Streptococcus pneumoniae* (*S. pneumoniae*) and *S. aureus,* are commonly implicated in chronic conditions such as rhinosinusitis, otitis media and tonsillitis (Table I) (4,5,18,22,26-29). These pathogens adhere to epithelial surfaces and form biofilms, enabling them to evade host immune defenses and antimicrobial treatments. *Haemophilus influenzae* is a significant pathogen in recurrent otitis media, with its biofilm-forming ability associated with persistent middle ear infections (18). In fungal biofilms, *Candida* spp. and *Aspergillus* spp. are often implicated in chronic fungal sinusitis, particularly in immunocompromised patients or those with underlying conditions, such as allergic fungal sinusitis (30-33).

Specific environmental and host-related factors significantly influence the prevalence of biofilm-forming pathogens. Recurrent infections, prior antibiotic use and medical procedures such as tympanostomy tube placement create ideal conditions for biofilm formation. Additionally, biofilms are more prevalent in patients with chronic inflammation or structural abnormalities, as these conditions impair normal mucosal defenses (34-36). This persistence highlights the need for targeted therapies that simultaneously disrupt biofilm structure and inhibit microbial activity to achieve effective treatment outcomes.



Biofilm formation step / Resistance mechanism	Attachment	Growth	Disaggregation
	Pathogens adhere to medical device or epithelial surface, eg. in the surface of tonsil or adenoids. Bacterial structure such as pili and fimbriae facilitate this process.	Bacterial communities produced EPS that provide structural integrity and retains nutrients.	Planctonic cells or free-floating aggregates are released, enabling the colonization of the new sites.
Extracellular Polymeric Substance (EPS)	EPS barrier not yet formed, bacteria begin secreting EPS	EPS barrier is fully developed, blocks antibiotic penetration and immune attack	EPS matrix starts breaking down, reducing protection
Quorum Sensing (QS)	Bacteria begin releasing signaling molecules to coordinate biofilm formation	QS regulates biofilm growth, virulence, and resistance activation	Some QS signals may trigger dispersal of biofilm fragments
Efflux Pumps	Low activity, planktonic bacteria may express basic efflux mechanisms	Highly active, bacteria pump out antibiotics, reducing intracellular drug levels	Some efflux pump activity remains, but bacteria are more susceptible in planktonic form
Altered Metabolism	Bacteria begin adapting to low-nutrient conditions	Some cells enter dormant states, reducing antibiotic effectiveness	Dormant bacteria may reawaken once conditions improve
Horizontal Gene Transfer (HGT)	Low occurrence, but initial bacterial clusters allow gene exchange	Low occurrence, but initial bacterial clusters allow gene exchange	Resistant bacteria can detach and spread resistant genes to new locations
Host Immune Evasion	Planktonic bacteria are susceptible to immune attack	EPS matrix shields bacteria from phagocytosis and immune cells	Dispersed bacteria are more vulnerable to immune defenses

Figure 1. Biofilm formation, resistance mechanisms and therapeutic strategies in ORL infections. There are three key phases of biofilm development in otorhinolaryngologic infections: Attachment, growth and disaggregation. During the growth phase, bacteria produce extracellular polymeric substances, enabling resistance through quorum sensing, efflux pumps, altered metabolism, horizontal gene transfer and immune evasion. Various antibiofilm strategies have been shown, targeting different stages, including quorum sensing inhibitors, nanoparticle-based antibiotics, phage therapy, and enzymatic dispersal agents. This schematic highlights the persistence of biofilm infections and the need for targeted therapies.

3. Challenges in treating biofilm-associated infections

Challenges related to biofilm development and treatment agents. A significant challenge in treating biofilm-associated infections is the increased antibiotic resistance observed within biofilms. Biofilm-associated infections hinder effective treatment by restricting antibiotic penetration, fostering antibiotic resistance, and altering bacterial metabolic activity, which reduces susceptibility to therapeutic agents. Biofilm matrices act as physical barriers, shielding embedded bacteria from antibiotics, while the metabolic dormancy of biofilm cells further diminishes their responsiveness to treatment.

Bacteria within biofilms often exhibit significantly higher resistance levels than their planktonic counterparts, primarily due to the protective biofilm matrix and the presence of resistance genes, such as those encoding multidrug efflux pumps (37-39). The EPS matrix acts as a formidable barrier to drug penetration. The dense structure of the biofilm, composed of polysaccharides, proteins and extracellular DNA (eDNA), hinders the diffusion of antimicrobial agents and reduces their efficacy (40-42). Consequently, even when antibiotics are administered, they often fail to achieve the concentrations required to effectively eliminate bacteria within the biofilm (43,44). This resistance complicates treatment strategies, as conventional antibiotics often cannot penetrate the biofilm adequately, enabling bacterial persistence and leading to chronic infections.

Additionally, biofilms exhibit phenotypic variations that give rise to subpopulations capable of withstanding antimicrobial treatments, making elimination more difficult. Within biofilms, cells often exist in diverse metabolic states, with numerous entering a dormant or slow-growing phase. This heterogeneity significantly reduces the effectiveness of antibiotics, which typically target actively dividing cells (15,42). To address these challenges, innovative therapeutic strategies (such as combining physical disruption, enzymatic degradation, and targeted drug delivery systems) are essential for improving treatment outcomes.

Table I. Common pathogens involved in biofilm-associated ENT infections.

Pathogen	Infection type	Antibiotic resistance features	(Refs.)
Pseudomonas aeruginosa	Chronic rhinosinusitis, otitis media	Multidrug resistance, quorum sensing, efflux pumps	(4,5,17,18, 26,52)
Staphylococcus aureus	Tonsillitis, otitis media, sinus infections	Methicillin-resistance, EPS production, efflux pumps	(5,17,18,35, 38,39)
Haemophilus influenzae	Otitis media, sinus infections	β-lactam resistance, biofilm-forming ability	(5,17,18)
Streptococcus pneumoniae	Otitis media, sinus infections	Penicillin resistance, efflux pumps, biofilm formation	(4,5,18)
Moraxella catarrhalis	Otitis media, sinus infections	β-lactam resistance, biofilm development	(5,18)
Klebsiella pneumoniae	Chronic rhinosinusitis, sinus infections	Carbapenem resistance, extended-spectrum beta-lactamase production	(4,5,38,39)
Escherichia coli	Sinus infections, tonsillitis	β-lactam resistance, efflux pumps, quorum sensing	(3,17,38,39)
Candida spp.	Chronic fungal sinusitis	Azole resistance, biofilm growth on mucosal surfaces	(30-33)
Aspergillus spp.	Fungal sinusitis, chronic invasive infections	Azole resistance, biofilm-like growth in immunocompromised hosts	(30-33)
Proteus mirabilis	Otitis media, sinus infections	Biofilm production, multidrug resistance	(5,18)
Acinetobacter baumannii	Chronic rhinosinusitis, sinus infections	Carbapenem resistance, efflux pumps, biofilm-related resistance	(38,39)
Enterococcus faecalis	Chronic ear infections, sinus infections	Vancomycin resistance, iron acquisition systems facilitating biofilm growth	(38)

Current pharmacological strategies focus on three key objectives-preventing biofilm formation, disrupting established biofilms, and enhancing the susceptibility of biofilm-embedded pathogens to antimicrobial agents. Innovative approaches, such as antibiotic-based strategies, QSIs, antibiofilm peptides, enzymatic dispersal agents and repurposed drugs, offer promising avenues for addressing the challenges posed by biofilm-associated infections.

Challenges related to anatomical and clinical aspects. The persistence of biofilm-associated infections in ORL is closely linked to the distinct anatomical features of the ear, nose and throat, which create protective niches ideal for microbial colonization. For instance, the sinonasal cavities, characterized by narrow ostia and complex mucosal folds, create an environment conducive to biofilm formation and immune evasion (43,44). In chronic rhinosinusitis, biofilms drive persistent inflammation and antibiotic resistance, leading to recurrent infections even after prolonged medical treatment (43,44). Similarly, in chronic tonsillitis, the tonsillar crypts act as reservoirs for bacterial biofilms, harboring deep-seated microbial communities that shield pathogens from host defenses and antimicrobial agents (45).

In the middle ear, biofilms are a key factor in recurrent otitis media, particularly in children. Their shorter and more horizontal Eustachian tubes make it easier for pathogen entry and persistence (46-48). Biofilm-forming bacteria, such as *Haemophilus influenzae* and *S. pneumoniae,* often lead to chronic infections that are challenging to eliminate, necessitating tympanostomy tube placement (46-48). The resistance of these biofilms to antibiotic penetration results in frequent recurrences and extended disease duration, posing a significant challenge in clinical management (46-48).

The complex ear, nose and throat anatomy, characterized by narrow and intricate spaces, poses significant challenges for interventional procedures. These confined regions impede the effective delivery of enzymatic dispersal agents, rendering antibiotic-only approaches frequently inadequate for managing such infections (46-48). These limitations necessitate a multifaceted treatment approach. Surgical interventions, including precise mechanical debridement, are often utilized to physically remove biofilm-affected tissues.

Given the significant challenges posed by biofilm-associated infections (such as antibiotic resistance, poor drug penetration and metabolic adaptations) conventional treatments often prove inadequate. Therefore, novel therapeutic strategies aimed at inhibiting biofilm formation, disrupting established biofilms, and eliminating biofilm-associated bacteria have been explored. The following sections outline promising pharmacological approaches, including antibiotic-based strategies, QSIs, enzymatic dispersal agents, antibiofilm peptides and drug repurposing. These strategies are designed to enhance treatment efficacy and improve patient outcomes.

4. Antibiotic-based strategies

Conventional antibiotics. Conventional antibiotics have long been the foundation for treating bacterial infections, including those associated with biofilms. However, their efficacy is often limited in biofilm environments. Biofilm-associated

bacteria exhibit significantly higher resistance to antibiotics than planktonic bacteria due to factors such as hindered penetration of antibiotics through the biofilm matrix and altered metabolic activity of bacteria embedded within the biofilm structure (37-39). Vancomycin, a standard treatment for methicillin-resistant *S. aureus*, is less effective against biofilm-associated infections, highlighting the need for alternative strategies to enhance its efficacy (49-51). The protective EPS matrix and the presence of persister cells within biofilms hinder antibiotic penetration and reduce metabolic activity, further diminishing antibiotic effectiveness. Moreover, prolonged or inappropriate antibiotic use increases the risk of resistance development, leading to persistent and recurrent infections. This resistance poses significant challenges in clinical management.

Combination therapies of antibiotics with antibiofilm properties. Combination therapy has become a promising approach for treating biofilm-associated infections. This approach involves using two or more antimicrobial agents that target different aspects of bacterial physiology or biofilm structure. For example, the combination of colistin and tobramycin has shown greater efficacy in eliminating biofilm-forming *P. aeruginosa* than treatments with single agents, as it targets the metabolic heterogeneity of bacteria within biofilms (52).

Synergistic effects occur when two antibiotics with distinct mechanisms of action are combined. For example, combining aminoglycosides, which disrupt protein synthesis, with beta-lactams, which inhibit cell wall synthesis, enhances antibacterial activity (53,54). Synergy is further amplified when one antibiotic exhibits strong biofilm penetration. Notably, rifampin, known for its ability to penetrate biofilm and target dormant bacterial populations, has been effectively combined with vancomycin or daptomycin to treat biofilm-associated infections caused by *Staphylococcus* species (55-57). These combinations improve biofilm penetration, target both metabolically active and dormant bacterial cells, and enhance treatment efficacy while reducing the risk of resistance development.

Combining antibiotics with biofilm-targeting agents, such as chelating agents, has emerged as a promising therapeutic strategy. For example, combining edetic acid (PubChem CID: 6049) with broad-spectrum antibiotics, such as minocycline has shown the ability to disrupt biofilm integrity and enhance antibiotic penetration, thereby improving treatment outcomes (58). Furthermore, topical antibiotics and silver-containing wound dressings have shown efficacy in reducing biofilm formation and promoting wound healing in infected tissues, although supporting evidence remains limited (59,60).

Recent advancements have yielded antibiotics with targeted antibiofilm properties, designed to either disrupt the biofilm matrix or inhibit mechanisms critical to biofilm formation. For example, macrolides, such as azithromycin, exert antibiofilm effects by interfering with QS, a key bacterial communication system that regulates biofilm formation and maintenance. By inhibiting QS, macrolides reduce biofilm integrity and increase bacterial susceptibility to other antibiotics, particularly in infections caused by *P. aeruginosa* and *E. coli* (61,62). Tigecycline, a glycyl-cycline antibiotic, is effective against multidrug-resistant pathogens and exhibits strong biofilm penetration, targeting both Gram-positive and Gram-negative bacteria (63). It has proven particularly effective against MDR *Acinetobacter baumannii* and *Enterobacteriaceae* species (63). Additionally, combining ceftolozane/tazobactam inhibits biofilm formation and eradicates established biofilms formed by *P. aeruginosa* under both aerobic and anaerobic conditions (64).

Effectively managing biofilm-associated infections in ORL requires a comprehensive understanding of the limitations of conventional antibiotic therapies, alongside the adoption of combination strategies and innovative agents with antibiofilm properties. By combining these approaches, healthcare providers can enhance treatment efficacy and improve patient outcomes.

5. Biofilm formation disruptor

QSIs. Bacteria communicate using chemical signaling molecules called autoinducers, whose concentration rises with increasing cell density. This process, known as QS, involves synthesizing, releasing, detecting, and responding to autoinducers. QS serves as a bacterial communication system that regulates gene expression based on population density, playing a crucial role in biofilm formation and virulence (65). In Gram-negative bacteria, such as *P. aeruginosa*, QS systems comprise genes encoding transcriptional regulators (R genes) and autoinducer synthesis enzymes (I genes). The I genes, such as *lasI* and *rhlI*, facilitate the production of specific autoinducers, including 3-oxo-C12-HSL and N-butyryl-l-homoserine lactone (C4-HSL) (65). These autoinducers play a pivotal role in regulating the expression of virulence factors and promoting biofilm formation.

In ORL infections, targeting QS presents a promising strategy for combating biofilm-associated infections, particularly in chronic conditions such as rhinosinusitis and otitis media. QSIs disrupt the signaling pathways bacteria use to communicate and coordinate their behaviors, which are essential for biofilm formation. By disrupting these pathways, QSIs can suppress the expression of virulence factors and inhibit biofilm maturation, rendering bacteria more vulnerable to antibiotics and the host immune system (8,66). For instance, QSIs can block the production of key signaling molecules, such as N-acyl homoserine lactones (AHLs), indole, autoinducer-2 (AI-2), autoinducing peptide, and diffusible signal factors, which are crucial for regulating biofilm development in pathogens such as *P. aeruginosa* (8,66). By inhibiting these communication systems, QSIs reduce bacterial virulence and destabilize biofilms, ultimately enhancing treatment outcomes for chronic infections.

Several compounds have shown efficacy as QSIs. Natural substances, such as furanone, baicalin and iberin, have been shown to disrupt QS in various bacterial species, including *P. aeruginosa*, by interfering with the synthesis of AHLs (67-69). The application of QSIs holds particular promise in treating chronic rhinosinusitis and otitis media. Chronic rhinosinusitis, for instance, is often linked to biofilm-forming bacteria, contributing to persistent inflammation and recurrent infections (70,71). By targeting QS mechanisms, QSIs can disrupt biofilm structures, thereby enhancing the efficacy of conventional antibiotic therapies (9).

Similarly, in otitis media, biofilm formation by pathogens such as *S. pneumoniae* and *Haemophilus influenzae* often complicate treatment (72). For instance, the LuxS/AI-2 QS system in *S. pneumoniae* plays a critical role in biofilm formation and pathogenicity in middle ear infections (72). QSIs have been shown to reduce bacterial loads and improve patient outcomes (73), making them a promising therapeutic strategy for biofilm-associated infections in ORL. By targeting and disrupting bacterial communication systems, QSIs can enhance the efficacy of conventional antibiotics and provide a precise approach to treating chronic infections.

QSIs can potentially prevent biofilm formation, but their clinical application is hindered by challenges such as bacterial adaptation and off-target effects. Although certain QSIs, such as azithromycin, exhibit biofilm-disrupting capabilities, their efficacy in treating chronic ORL infections requires further study. Similarly, enzymatic dispersal agents, including DNase I and Dispersin B, have shown promise in degrading biofilms *in vitro*. However, issues related to their stability and methods for localized delivery pose significant barriers to their broader adoption in clinical settings.

Biofilm dispersal agents and enzymatic disruption of EPS. Biofilm dispersal agents and enzymatic approaches focus on disrupting the EPS that forms the structural backbone of biofilms. Enzymatic methods offer a direct approach to EPS disruption. DNase I degrades eDNA, a key structural component that stabilizes biofilms, thereby compromising their integrity (74,75). eDNA is essential for maintaining biofilm stability and integrity, and its degradation by DNase I disrupts biofilm structure, promoting dispersal and enhancing bacterial susceptibility to antimicrobials (74,75). Furthermore, treating DNase I can prevent biofilm reformation by degrading eDNA, which inhibits bacterial adhesion and aggregation (74,75).

Dispersin B is an enzyme that targets and hydrolyzes β-1,6-linked N-acetylglucosamine, a key polysaccharide in biofilm matrices, effectively dispersing biofilms formed by various bacteria, including *S. aureus* and *P. aeruginosa*. When combined with antibiotics, Dispersin B exhibits a synergistic effect, significantly reducing bacterial viability compared with treatment with antibiotics alone (76). Similarly, alginate lyase degrades alginate, a major component of *P. aeruginosa* biofilms. This enzymatic degradation disrupts biofilm structure and improves antibiotic penetration, particularly in cystic fibrosis-related infections (77,78).

Nitric oxide (NO) disrupts biofilms by acting as a signaling molecule that triggers dispersal in *P. aeruginosa*. Research indicates that low sublethal concentrations of NO donors, such as sodium nitroprusside, effectively induce biofilm dispersal while enhancing bacteria susceptibility to antimicrobial agents (79). This process is associated with changes in bacterial metabolism and oxidative stress responses, destabilizing biofilm structures and facilitating sessile cell transition to a planktonic state. Consequently, treatment efficacy against biofilm-associated infections is significantly enhanced (79).

Combining biofilm-disruption strategies (such as enzymatic agents and chemical dispersal compounds) with conventional antimicrobial therapies can significantly improve treatment outcomes in chronic infections. However, further research is needed to optimize delivery systems and ensure their safety and efficacy across various clinical settings. Additionally, future research should investigate whether combining biofilm dispersal agents with conventional saline irrigation in biofilm-associated sinonasal cavity infections can enhance mucociliary clearance, reduce inflammation, and ultimately improve patients' quality of life.

Antibiofilm peptides and bacterial membrane disruptors. Antibiofilm peptides function by compromising the structural integrity of biofilms and the membranes of the bacteria they contain. These peptides can penetrate the biofilm matrix (composed of polysaccharides, proteins and eDNA) leading to biofilm destabilization and dispersal (80). Furthermore, certain peptides directly interact with bacterial membranes, inducing membrane disruption and subsequent cell lysis (80,81). This dual mechanism, targeting both the biofilm matrix and bacterial membranes, enhances the effectiveness of antibiofilm peptides in combating persistent infections.

Numerous antibiofilm peptides have exhibited significant potential for clinical applications. Among these, LL-37, a human cathelicidin peptide, stands out for its broad-spectrum antimicrobial and antibiofilm activities, disrupting bacterial membranes and modulating immune defenses. Notably, LL-37 has shown promise in disrupting biofilms formed by *S. aureus* and *P. aeruginosa*, enhancing the effectiveness of conventional antibiotics (82). In a PREVIOUS study, LL-37 effectively reduced *S. aureus* biofilm colony counts by over 4 logs at concentrations of 10 μM or higher within 24 h, outperforming conventional antibiotics such as gentamicin and vancomycin (82).

Bacteriocins, ribosomally synthesized peptides produced by bacteria, exhibit potent antibacterial effects against closely related species. For example, bacteriocins derived from *Lactobacillus* species have been shown to effectively inhibit biofilm formation by pathogenic bacteria in various settings (83,84). Specifically, *Lactobacillus acidophilus* VB1 exhibits antibacterial and antibiofilm activities against pathogens linked to chronic otitis media (85). This strain exerts this effect through co-aggregation with pathogens and the production of metabolites, such as cell-free supernatants and biosurfactants, which disrupt biofilm formation and synergistically enhance the efficacy of antibiotics such as ciprofloxacin (85).

Synthetic antimicrobial peptides (AMPs) also show significant potential for eliminating biofilms by disrupting bacterial membranes or inhibiting biofilm-related gene expression. For instance, peptide 1018 has proven effective in eliminating mature biofilms formed by both Gram-negative and Gram-positive bacteria at concentrations below the minimum inhibitory concentration. At 0.8 μg/ml, peptide 1018 induced biofilm dispersal, while higher concentrations (10 μg/ml) achieved near-complete elimination of biofilm-associated cells (80). Moreover, synthetic peptides can be engineered to enhance their stability and broaden their efficacy against diverse biofilm-forming pathogens. A notable example is the novel antibiofilm peptide, BiF, which was developed through hybridization with a lipid-binding motif. This dual-function antimicrobial-antibiofilm peptide hybrid has shown effectiveness against biofilm-forming *Staphylococcus epidermidis* (86).

Antibiofilm peptides hold significant potential in preventing and managing recurrent otitis media, a prevalent

ear infection often associated with biofilm formation in the middle ear. Recurrent otitis media is often linked to biofilm formation on the tympanic membrane and within the middle ear, resulting in persistent infections that are challenging to treat using conventional antibiotics (5,6,17,18). Further investigation is warranted to determine whether the targeted delivery of LL-37 and other AMPs, combined with standard treatment protocols, could significantly reduce the recurrence of otitis media and sinus cavity infections.

Ion channel blockers. Ion channel blockers offer a novel therapeutic strategy for treating biofilm-associated infections, particularly chronic ORL infections. These agents target bacterial ion transport systems, which are essential for maintaining biofilm integrity. By disrupting these systems, ion channel blockers can destabilize biofilms, thereby enhancing the effectiveness of conventional treatments.

Bacterial biofilms utilize ion gradients to regulate various cellular processes, including nutrient uptake, waste removal and membrane potential maintenance, while also facilitating antibiotic resistance mechanisms. For instance, NorA, an efflux pump belonging to the major facilitator superfamily of transporters, expels toxic compounds, including antimicrobial agents, from bacterial cells (87-89). Overexpression of NorA in *S. aureus* enhances its resistance to antimicrobial treatments and facilitates biofilm formation (87-89). Similarly, FeoB in *Enterococcus faecalis* mediates iron uptake, enabling enhanced biofilm growth under iron-rich conditions (90,91). Disrupting these ion transport systems can compromise biofilm formation and stability, making bacteria more susceptible to antimicrobial agents.

Gallium compounds have emerged as promising ion channel blockers due to their ability to mimic iron and disrupt iron-dependent bacterial processes. Iron serves as a crucial cofactor for bacterial enzymes involved in DNA synthesis, respiration and virulence factor production. The structural similarity of gallium to ferric iron (Fe^{3+}) allows it to compete for uptake through bacterial iron transport systems, such as the FeoB transporter and siderophore-mediated pathways (92,93). Unlike iron, gallium cannot be reduced from Fe^{3+} to Fe^{2+} under physiological conditions, which inhibit key iron-dependent metabolic enzymes, including ribonucleotide reductase and superoxide dismutase (93). This disruption impairs bacterial DNA synthesis, oxidative stress defense and overall biofilm stability. Consequently, gallium not only prevents biofilm formation but also enhances bacterial susceptibility to antibiotics. Studies have shown that gallium compounds, such as gallium nitrate, effectively inhibit *P. aeruginosa* biofilms and exhibit synergistic effects when combined with conventional antimicrobial agents (92,93).

Calcium channel blockers and proton pump inhibitors (PPIs) destabilize biofilms by disrupting calcium- and cation-dependent signaling pathways. Agents such as the calcium channel blockers nimodipine and verapamil, alongside PPI dexlansoprazole, block calcium channels associated with the cyclic-di-GMP signaling cascade (94,95). This disruption reduces EPS production, weakens biofilm structure, and promotes the dispersal of biofilms formed by *S. aureus*, *P. aeruginosa* and *Candida albicans* (94,95).

Ion channel blockers represent a promising strategy for managing biofilm-associated infections in ORL. Future research should explore their applicability, safety and efficacy when combined with conventional antibiotic therapy for chronic rhinosinusitis and otitis media.

6. Drug repurposing strategies

Drug repurposing, or drug repositioning, involves exploring existing medications for new therapeutic applications beyond their original use. This strategy is particularly beneficial for biofilm-associated infections, as it enables the use of non-antibiotic drugs with antibiofilm properties. Leveraging the established safety profiles of these drugs, as well as their mechanisms of action, clinicians may improve treatment outcomes for conditions such as recurrent tonsillitis and otitis media.

Drug repurposing for biofilm management leverages the ability of certain non-antibiotic drugs to disrupt biofilm formation and stability. These drugs can exert anti-inflammatory effects, modulate immune defenses, or interfere with bacterial signaling pathways, ultimately impairing biofilm formation and persistence.

Several drug classes have been identified as promising candidates for repurposing in biofilm-associated infections. Statins, such as atorvastatin and simvastatin, have been shown to inhibit biofilm formation in various bacterial species, including *S. aureus* and *P. aeruginosa*. Atorvastatin suppresses the toll-like receptor 4 (TLR4)/MyD88/NF-κB signaling pathway, promoting anti-inflammatory responses (96,97). Simvastatin shows bacteriostatic and bactericidal activity against methicillin-sensitive and methicillin-resistant *S. aureus* and reduces biofilm formation (98).

Similarly, NSAIDs not only reduce inflammation but may also disrupt biofilm integrity by modifying the bacterial microenvironment. Combining ibuprofen with fluoroquinolones enhances the inhibition of biofilm-associated regulators, including Alg44 and AlgT/U, as well as the MexB and OprM efflux pump (12,99). Another study showed that diclofenac significantly reduced biofilm formation, with inhibition rates ranging from 22.67-70%, compared with controls (100).

Drug repurposing offers a promising approach for managing chronic ORL infections, such as recurrent tonsillitis and otitis media, where biofilm formation drives antibiotic resistance and treatment failure. Combining repurposed drugs with conventional antibiotics can enhance biofilm disruption, reduce bacterial load, and minimize recurrence. However, current evidence on NSAID and statins primarily stems from *in vitro* studies, highlighting the need for further *in vivo* and clinical investigations.

7. Emerging and experimental therapies

Advancements in biofilm-associated infection management have led to the development of innovative and experimental therapeutic strategies. Notably, nanotechnology-based therapies and phage therapy offer distinct mechanisms to disrupt biofilm formation and enhance treatment efficacy.

Nanotechnology. Nanotechnology-based therapies leverage nanoparticles to enhance drug delivery and enhance the

Table II. Anti-biofilm therapies and their mechanisms of action.

Therapy type	Mechanism of action	Example agents	(Refs.)
Quorum sensing inhibitors	Disrupt bacterial communication, preventing biofilm formation and virulence factor expression.	Furanones, Baicalin, Iberin, Azithromycin	(8,65-67)
Enzymatic dispersal agents	Degrade extracellular polymeric substances and eDNA, breaking biofilm structure.	DNase I, Dispersin B, Alginate lyase	(74-77)
Anti-biofilm peptides	Penetrate biofilm, disrupt bacterial membranes, and modulate gene expression to inhibit biofilm formation.	LL-37, Bacteriocins, Synthetic AMPs (e.g., peptide 1018)	(81,82, 84)
Drug repurposing strategies	Reuse existing drugs with anti-inflammatory or biofilm-disrupting properties, enhancing antibiotic effects.	Statins (Atorvastatin, Simvastatin), NSAIDs (Ibuprofen)	(12,97, 100)
Combination antibiotic therapy	Target different aspects of bacterial physiology to penetrate biofilm and eradicate bacteria.	Colistin + Tobramycin, Vancomycin + Rifampin	(52,54)
Nanotechnology approaches	Deliver drugs using nanoparticles to enhance penetration, stability, and antimicrobial action.	Silver nanoparticles, Magnetite-based carriers	(102,105-107)
Phage therapy	Use bacteriophages to target and lyse bacteria specifically within lyse bacteria specifically within biofilms. biofilms.	Bacteriophages for *P. aeruginosa, S. aureus*	(111,112)
Ion channel blockers	Disrupt ion gradients and efflux pump activity, weakening biofilm integrity.	Gallium compounds, Verapamil, Proton pump inhibitors	(87-89, 92,95)
Biofilm matrix disruptors	Target EPS components to weaken matrix stability, enabling antibiotic penetration.	Chelating agents (e.g., EDTA), Nitric oxide donors	(89)
Immunomodulatory approaches	Enhance immune responses to target biofilm-associated infections and modulate inflammation.	Immunostimulatory peptides, Toll-like receptor modulators	(10,13, 97)

penetration of antibiotics and antibiofilm agents. These nanoparticles can be engineered to improve the solubility, stability and bioavailability of therapeutic agents. By facilitating the targeted delivery of antibiotics to infection sites, nanoparticles help overcome biofilm-related barriers to treatment (101-103). Studies have shown that silver nanoparticles exhibit significant antimicrobial and antibiofilm properties, effectively disrupting biofilm formation by pathogens such as *P. aeruginosa* and *S. aureus* (101,102,104,105). Magnetite nanoparticles combined with antibiotics, such as streptomycin and neomycin, and encapsulated within biopolymeric spheres exhibit promising antimicrobial properties and enhanced biocompatibility. These characteristics make them highly suitable for targeted delivery systems in ENT infection (106). Furthermore, polymeric nanoparticles can optimize the controlled release of antibiofilm agents, promoting sustained therapeutic effects while minimizing the need for frequent administration (107).

Nanotechnology-based drug delivery systems offer a promising approach to addressing the limitations of conventional antibiotics in ORL biofilm infections. Nanoparticles, such as silver nanoparticles and liposomal antibiotic carriers, have been shown to penetrate biofilms and enhance antimicrobial efficacy. For instance, in chronic rhinosinusitis, nanoparticle-based drug formulations have shown improved retention in sinonasal mucosa, enhancing local drug concentrations and reducing systemic toxicity (106,108). However, several challenges, such as rapid clearance by mucociliary mechanisms, potential cytotoxic effects on the respiratory epithelium, and the lack of large-scale clinical trials, limit their immediate clinical application. Additionally, the regulatory approval process for phage therapy remains complex due to strain-specific variations and the need for personalized phage cocktails. Although nanoparticle-based formulations have shown efficacy in preclinical models of chronic rhinosinusitis and otitis media,

Table III. Summary of effectiveness and limitation of anti-biofilm therapies.

Therapy type	Effectiveness	Limitations
Quorum sensing Inhibitors	Effective in early biofilm stages; reduces virulence	Limited clinical trials; potential bacterial resistance
Enzymatic dispersal agents	Directly breaks down biofilms; enhances antibiotic effects	Specificity issues; potential immune response interference
Anti-biofilm peptides	Broad-spectrum activity; effective in resistant strains	Expensive; potential cytotoxicity
Drug repurposing strategies	Cost-effective; enhances conventional therapy	Limited data on long-term effects
Combination antibiotic therapy	Synergistic effect; useful for resistant infections	Risk of toxicity and antibiotic resistance
Nanotechnology approaches	High penetration; effective at lower doses	Requires advanced formulation; regulatory concerns
Phage therapy	Specific targeting; minimal side effects	Narrow spectrum; requires strain-specific selection
Ion channel blockers	Weakens biofilm structure; enhances antibiotic action	Limited *in vivo* evidence; possible side effects
Biofilm matrix disruptors	Directly weakens biofilm structure	Risk of toxicity; variable effectiveness
Immunomodulatory approaches	Potential for long-term infection control	Complexity of immune modulation; needs further research

their clinical validation remains limited (106,108). Future clinical trials evaluating the safety and efficacy of nanocarriers in targeting biofilm-associated ORL infections are essential to advance their translational potential.

Phage therapy. Phage therapy is an innovative approach that employs bacteriophages (viruses that specifically infect and eliminate bacteria) to target biofilm-specific pathogens. This method is particularly promising in addressing antibiotic resistance, as bacteriophages can effectively disrupt biofilms while eliminating resistant bacterial strains, all without harming beneficial microbiota (109-111). Studies have shown the efficacy of phage therapy in treating chronic infections caused by biofilm-forming bacteria, such as *P. aeruginosa*, *S. aureus* and *Klebsiella sp*., in chronic wound infection (109-111). The high specificity of bacteriophages enables targeted treatment, minimizing collateral damage to surrounding tissues and reducing the adverse effects often associated with conventional antibiotics (112). By enhancing the efficacy of existing treatments and offering alternatives in the face of increasing antibiotic resistance, phage therapy has significant potential for improving patient outcomes in chronic ORL conditions.

It has been previously revealed that phages can effectively disrupt biofilms in patients with chronic otitis media and sinus infections by degrading EPS and lysing bacterial cells (113). However, the clinical application of phage therapy in ORL faces significant challenges, including concerns about phage stability in mucosal environments, potential immune responses and regulatory constraints (114). Although compassionate-use cases have yielded promising outcomes, large-scale clinical trials are needed to develop standardized phage formulations, dosing protocols and comprehensive safety profiles (114). Addressing challenges, such as mucosal penetration, host immune interactions and large-scale production, is crucial to integrating these innovative therapies into mainstream ORL clinical practice.

8. Conclusion and future directions

The present review highlights the pivotal role of biofilm formation in the persistence and recurrence of ORL infections, particularly its contribution to antimicrobial resistance and treatment failure. Emerging pharmacological strategies, such as QSIs, antibiofilm peptides, enzymatic dispersal agents and repurposed drugs, offer promising approaches to disrupt biofilm structural integrity and enhance treatment outcomes (Tables II and III).

Despite these advancements, translating these findings into clinical practice remains challenging, necessitating further research to validate safety, refine delivery systems, and assess long-term efficacy. QSIs, for instance, exhibit potential in preventing biofilm formation, but their clinical application is hindered by concerns over bacterial adaptation and off-target effects. Although certain QSIs, such as azithromycin, exhibit biofilm-disrupting capabilities, their efficacy in treating chronic ORL infections is still being studied (115). Similarly, enzymatic dispersal agents, such as DNase I and Dispersin B, have shown promise in degrading biofilms *in vitro,* but their stability and methods for localized delivery remain significant barriers to their broader clinical application (116).

Antibiofilm peptides exhibit broad-spectrum activity, but their clinical use is hindered by high production costs and potential cytotoxicity. While some synthetic AMPs have advanced to preclinical testing, their pharmacokinetics and potential immunogenicity require further validation before clinical adoption (117). Drug repurposing strategies, such as statins and NSAIDs, have garnered interest due to their dual anti-inflammatory and biofilm-disrupting effects (12-14). However, their effectiveness in treating biofilm-associated ORL infections remains unclear, as most supporting evidence derives from *in vitro* studies and animal models rather than large-scale human trials.

While *in vitro* and preclinical studies have shown promising biofilm-targeting strategies, their efficacy and practicality in real-world clinical settings remain largely unverified. Future research must focus on conducting well-designed clinical trials to assess the effectiveness, safety and pharmacokinetics of these therapies in patients with chronic biofilm-associated infections. Additionally, it is essential to explore patient-specific factors (such as comorbidities, immune responses and treatment adherence) to ensure successful clinical implementation. Integrating advanced technologies, such as nanocarriers, phage therapy and immunomodulatory approaches into clinically relevant frameworks, will enhance treatment precision and help translate laboratory findings into practical, patient-centered applications.

Acknowledgements

The authors would like to thank Ms Rahmati Putri Yaniafari (Nanyang Technological University, Singapore) for her assistance in accessing several sources used in the present review.

Funding

No funding was received.

Availability of data and materials

The data generated in the present study are included in the figures and/or tables of this article.

Authors' contributions

MAE, INK and NMS conceptualized the study, drafted and revised the manuscript. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Use of artificial intelligence tools

During the preparation of this work, artificial intelligence tools were used to improve the readability and language of the manuscript or to generate images, and subsequently, the authors revised and edited the content produced by the artificial intelligence tools as necessary, taking full responsibility for the ultimate content of the present manuscript.

References

1. Drenkard E and Ausubel FM: Pseudomonas biofilm formation and antibiotic resistance are linked to phenotypic variation. Nature 416: 740-743, 2002.
2. Behzadi P, Gajdács M, Pallós P, Ónodi B, Stájer A, Matusovits D, Kárpáti K, Burián K, Battah B, Ferrari M, *et al*: Relationship between biofilm-formation, phenotypic virulence factors and antibiotic resistance in environmental *Pseudomonas aeruginosa*. Pathogens 11: 1015, 2022.
3. Chen C, Liao X, Jiang H, Zhu H, Yue L, Li S, Fang B and Liu Y: Characteristics of Escherichia coli biofilm production, genetic typing, drug resistance pattern and gene expression under aminoglycoside pressures. Environ Toxicol Pharmacol 30: 5-10, 2010.
4. Sanderson AR, Leid JG and Hunsaker D: Bacterial biofilms on the sinus mucosa of human subjects with chronic rhinosinusitis. Laryngoscope 116: 1121-1126, 2006.
5. Mohammed RQ and Abdullah PB: Infection with acute otitis media caused by *Pseudomonas aeruginosa* (MDR) and *Staphylococcus aureus* (MRSA). Biochem Cell Arch 20: 905-908, 2020.
6. Bendouah Z, Barbeau J, Hamad WA and Desrosiers M: Biofilm formation by *Staphylococcus aureus* and *Pseudomonas aeruginosa* is associated with an unfavorable evolution after surgery for chronic sinusitis and nasal polyposis. Otolaryngol Head Neck Surg 134: 991-996, 2006.
7. Carradori S, Di Giacomo N, Lobefalo M, Luisi G, Campestre C and Sisto F: Biofilm and quorum sensing inhibitors: The road so far. Expert Opin Ther Pat 30: 917-930, 2020.
8. Wang Y, Bian Z and Wang Y: Biofilm formation and inhibition mediated by bacterial quorum sensing. Appl Microbiol Biotechnol 106: 6365-6381, 2022.
9. Zhou L, Zhang Y, Ge Y, Zhu X and Pan J: Regulatory mechanisms and promising applications of quorum sensing-inhibiting agents in control of bacterial biofilm formation. Front Microbiol 11: 589640, 2020.
10. Ridyard KE and Overhage J: The potential of human peptide LL-37 as an antimicrobial and anti-biofilm agent. Antibiotics (Basel) 10: 650, 2021.
11. Martínez M, Polizzotto A, Flores N, Semorile L and Maffía PC: Antibacterial, anti-biofilm and in vivo activities of the antimicrobial peptides P5 and P6.2. Microb Pathog 139: 103886, 2020.
12. Paes Leme RC and da Silva RB: Antimicrobial activity of non-steroidal anti-inflammatory drugs on biofilm: Current evidence and potential for drug repurposing. Front Microbiol 12: 707629, 2021.
13. Schelz Z, Muddather HF and Zupkó I: Repositioning of HMG-CoA reductase inhibitors as adjuvants in the modulation of efflux pump-mediated bacterial and tumor resistance. Antibiotics (Basel) 12: 1468, 2023.
14. Kumar A, Alam A, Grover S, Pandey S, Tripathi D, Kumari M, Rani M, Singh A, Akhter Y, Ehtesham NZ and Hasnain SE: Peptidyl-prolyl isomerase-B is involved in Mycobacterium tuberculosis biofilm formation and a generic target for drug repurposing-based intervention. NPJ Biofilms Microbiomes 5: 3, 2019.
15. Sauer K, Stoodley P, Goeres DM, Hall-Stoodley L, Burmølle M, Stewart PS and Bjarnsholt T: The biofilm life cycle: Expanding the conceptual model of biofilm formation. Nat Rev Microbiol 20: 608-620, 2022.
16. Irie Y, Borlee BR, O'Connor JR, Hill PJ, Harwood CS, Wozniak DJ and Parsek MR: Self-produced exopolysaccharide is a signal that stimulates biofilm formation in *Pseudomonas aeruginosa*. Proc Natl Acad Sci USA 109: 20632-20636, 2012.
17. Parastan R, Kargar M, Solhjoo K and Kafilzadeh F: *Staphylococcus aureus* biofilms: Structures, antibiotic resistance, inhibition, and vaccines. Gene Rep 20: 100739, 2020.
18. Galli J, Calò L, Ardito F, Imperiali M, Bassotti E, Fadda G and Paludetti G: Biofilm formation by *Haemophilus influenzae* isolated from adeno-tonsil tissue samples, and its role in recurrent adenotonsillitis. Acta Otorhinolaryngol Ital 27: 134-138, 2007.
19. Davenport EK, Call DR and Beyenal H: Differential protection from tobramycin by extracellular polymeric substances from *Acinetobacter baumannii* and *Staphylococcus aureus* biofilms. Antimicrob Agents Chemother 58: 4755-4761, 2014.
20. Serra DO, Klauck G and Hengge R: Vertical stratification of matrix production is essential for physical integrity and architecture of macrocolony biofilms of Escherichia coli. Environ Microbiol 17: 5073-5088, 2015.

21. Parsek MR and Greenberg EP: Acyl-homoserine lactone quorum sensing in gram-negative bacteria: A signaling mechanism involved in associations with higher organisms. Proc Natl Acad Sci USA 97: 8789-8793, 2000.
22. Singh GB, Malhotra S, Yadav SC, Kaur R, Kwatra D and Kumar S: The role of biofilms in chronic otitis media-active squamosal disease: An evaluative study. Otol Neurotol 42: e1279-e1285, 2021.
23. Dar D, Dar N, Cai L and Newman DK: Spatial transcriptomics of planktonic and sessile bacterial populations at single-cell resolution. Science 373: eabi4882, 2021.
24. Cornforth DM, Dees JL, Ibberson CB, Huse HK, Mathiesen IH, Kirketerp-Møller K, Wolcott RD, Rumbaugh KP, Bjarnsholt T and Whiteley M: *Pseudomonas aeruginosa* transcriptome during human infection. Proc Natl Acad Sci USA 115: E5125-E5134, 2018.
25. Dal Co A, Van Vliet S and Ackermann M: Emergent microscale gradients give rise to metabolic cross-feeding and antibiotic tolerance in clonal bacterial populations. Philos Trans R Soc Lond B Biol Sci 374: 20190080, 2019.
26. Boase S, Foreman A, Cleland E, Tan L, Melton-Kreft R, Pant H, Hu FZ, Ehrlich GD and Wormald PJ: The microbiome of chronic rhinosinusitis: Culture, molecular diagnostics and biofilm detection. BMC Infect Dis 13: 210, 2013.
27. Kostić M, Ivanov M, Babić SS, Tepavčević Z, Radanović O, Soković M and Ćirić A: Analysis of tonsil tissues from patients diagnosed with chronic tonsillitis-microbiological profile, biofilm-forming capacity and histology. Antibiotics (Basel) 11: 1747, 2022.
28. Lee MR, Pawlowski KS, Luong A, Furze AD and Roland PS: Biofilm presence in humans with chronic suppurative otitis media. Otolaryngol Head Neck Surg 141: 567-571, 2009.
29. Hoa M, Syamal M, Schaeffer MA, Sachdeva L, Berk R and Coticchia J: Biofilms and chronic otitis media: An initial exploration into the role of biofilms in the pathogenesis of chronic otitis media. Am J Otolaryngol 31: 241-245, 2010.
30. Karthikeyan P and Nirmal Coumare V: Incidence and presentation of fungal sinusitis in patient diagnosed with chronic rhinosinusitis. Indian J Otolaryngol Head Neck Surg 62: 381-385, 2010.
31. Bahethi R, Talmor G, Choudhry H, Lemdani M, Singh P, Patel R and Hsueh W: Chronic invasive fungal rhinosinusitis and granulomatous invasive fungal sinusitis: A systematic review of symptomatology and outcomes. Am J Otolaryngol 45: 104064, 2024.
32. Yang SW, Luo CM and Cheng TC: Fungal abscess of anterior nasal septum complicating maxillary sinus fungal ball rhinosinusitis caused by *Aspergillus flavus*: Case report and review of literature. J Fungi (Basel) 10: 497, 2024.
33. Leszczyńska J, Stryjewska-Makuch G, Lisowska G, Kolebacz B and Michalak-Kolarz M: Fungal sinusitis among patients with chronic rhinosinusitis who underwent endoscopic sinus surgery. Otolaryngol Pol 72: 35-41, 2018.
34. Marom T, Habashi N, Cohen R and Tamir SO: Role of biofilms in post-tympanostomy tube otorrhea. Ear Nose Throat J 99 (1 Suppl): 22S-29S, 2020.
35. Manasherob R, Mooney JA, Lowenberg DW, Bollyky PL and Amanatullah DF: Tolerant small-colony variants form prior to resistance within a *Staphylococcus aureus* biofilm based on antibiotic selective pressure. Clin Orthop Relat Res 479: 1471-1481, 2021.
36. Habashi N, Marom T, Steinberg D, Zacks B and Tamir SO: Biofilm distribution on tympanostomy tubes: An ex vivo descriptive study. Int J Pediatr Otorhinolaryngol 138: 110350, 2020.
37. Mah TF, Pitts B, Pellock B, Walker GC, Stewart PS and O'Toole GA: A genetic basis for *Pseudomonas aeruginosa* biofilm antibiotic resistance. Nature 426: 306-310, 2003.
38. Kvist M, Hancock V and Klemm P: Inactivation of efflux pumps abolishes bacterial biofilm formation. Appl Environ Microbiol 74: 7376-7382, 2008.
39. Tang M, Wei X, Wan X, Ding Z, Ding Y and Liu J: The role and relationship with efflux pump of biofilm formation in *Klebsiella pneumoniae*. Microb Pathog 147: 104244, 2020.
40. Powell LC, Abdulkarim M, Stokniene J, Yang QE, Walsh TR, Hill KE, Gumbleton M and Thomas DW: Quantifying the effects of antibiotic treatment on the extracellular polymer network of antimicrobial resistant and sensitive biofilms using multiple particle tracking. NPJ Biofilms Microbiomes 7: 13, 2021.
41. Kosztołowicz T and Metzler R: Diffusion of antibiotics through a biofilm in the presence of diffusion and absorption barriers. Phys Rev E 102: 032408, 2020.
42. Tuon FF, Dantas LR, Suss PH and Tasca Ribeiro VST: Pathogenesis of the *Pseudomonas aeruginosa* biofilm: A review. Pathogens 11: 300, 2022.
43. Denton O, Wan Y, Beattie L, Jack T, McGoldrick P, McAllister H, Mullan C, Douglas CM and Shu W: Understanding the role of biofilms in acute recurrent tonsillitis through 3D bioprinting of a novel gelatin-PEGDA hydrogel. Bioengineering (Basel) 11: 202, 2024.
44. Huang Y, Qin F, Li S, Yin J, Hu L, Zheng S, He L, Xia H, Liu J and Hu W: The mechanisms of biofilm antibiotic resistance in chronic rhinosinusitis: A review. Medicine (Baltimore) 101: e32168, 2022.
45. Abu Bakar M, McKimm J, Haque SZ, Majumder MAA and Haque M: Chronic tonsillitis and biofilms: A brief overview of treatment modalities. J Inflam Res 11: 329-337, 2018.
46. Schilder AGM, Chonmaitree T, Cripps AW, Rosenfeld RM, Casselbrant ML, Haggard MP and Venekamp RP: Otitis media. Nat Rev Dis Primers 2: 16063, 2016.
47. Duff AF, Jurcisek JA, Kurbatfinski N, Chiang T, Goodman SD, Bakaletz LO and Bailey MT: Oral and middle ear delivery of otitis media standard of care antibiotics, but not biofilm-targeted antibodies, alter chinchilla nasopharyngeal and fecal microbiomes. NPJ Biofilms Microbiomes 10: 10, 2024.
48. Niedzielski A, Chmielik LP and Stankiewicz T: The formation of biofilm and bacteriology in otitis media with effusion in children: A prospective cross-sectional study. Int J Environ Res Public Health 18: 3555, 2021.
49. Abdelhady W, Bayer AS, Seidl K, Moormeier DE, Bayles KW, Cheung AL, Yeaman MR and Xiong YQ: Impact of vancomycin on sarA-mediated biofilm formation: Role in persistent endovascular infections due to methicillin-resistant *Staphylococcus aureus*. J Infect Dis 209: 1231-1240, 2014.
50. Rose WE and Poppens PT: Impact of biofilm on the in vitro activity of vancomycin alone and in combination with tigecycline and rifampicin against *Staphylococcus aureus*. J Antimicrob Chemother 63: 485-488, 2009.
51. Cho OH, Bae IG, Moon SM, Park SY, Kwak YG, Kim BN, Yu SN, Jeon MH, Kim T, Choo EJ, *et al*: Therapeutic outcome of spinal implant infections caused by *Staphylococcus aureus*: A retrospective observational study. Medicine (Baltimore) 97: e12629, 2018.
52. Herrmann G, Yang L, Wu H, Song Z, Wang H, Høiby N, Ulrich M, Molin S, Riethmüller J and Döring G: Colistin-tobramycin combinations are superior to monotherapy concerning the killing of biofilm *Pseudomonas aeruginosa*. J Infect Dis 202: 1585-1592, 2010.
53. Giamarellou H, Zissis NP, Tagari G and Bouzos J: In vitro synergistic activities of aminoglycosides and new beta-lactams against multiresistant *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 25: 534-536, 1984.
54. Giamarellou H: Aminoglycosides plus beta-lactams against gram-negative organisms. Evaluation of in vitro synergy and chemical interactions. Am J Med 80: 126-137, 1986.
55. Olson ME, Slater SR, Rupp ME and Fey PD: Rifampicin enhances activity of daptomycin and vancomycin against both a polysaccharide intercellular adhesin (PIA)-dependent and -independent *Staphylococcus epidermidis* biofilm. J Antimicrob Chemother 65: 2164-2171, 2010.
56. Zimmerli W and Sendi P: Role of rifampin against staphylococcal biofilm infections in vitro, in animal models, and in orthopedic-device-related infections. Antimicrob Agents Chemother 63: e01746-18, 2019.
57. Niska JA, Shahbazian JH, Ramos RI, Francis KP, Bernthal NM and Miller LS: Vancomycin-rifampin combination therapy has enhanced efficacy against an experimental *Staphylococcus aureus* prosthetic joint infection. Antimicrob Agents Chemother 57: 5080-5086, 2013.
58. Ferreira Chacon JM, Hato de Almeida E, de Lourdes Simões R, Lazzarin C Ozório V, Alves BC, Mello de Andréa ML, Santiago Biernat M and Biernat JC: Randomized study of minocycline and edetic acid as a locking solution for central line (port-a-cath) in children with cancer. Chemotherapy 57: 285-291, 2011.
59. Vermeulen H, van Hattem JM, Storm-Versloot MN and Ubbink DT: Topical silver for treating infected wounds. Cochrane Database Syst Rev: CD005486, 2007.
60. Jiang Y, Zhang Q, Wang H, Välimäki M, Zhou Q, Dai W and Guo J: Effectiveness of silver and iodine dressings on wound healing: A systematic review and meta-analysis. BMJ Open 14: e077902, 2024.

61. Tateda K, Comte R, Pechere JC, Köhler T, Yamaguchi K and Van Delden C: Azithromycin inhibits quorum sensing in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 45: 1930-1933, 2001.
62. Hoffmann N, Lee B, Hentzer M, Rasmussen TB, Song Z, Johansen HK, Givskov M and Høiby N: Azithromycin blocks quorum sensing and alginate polymer formation and increases the sensitivity to serum and stationary-growth-phase killing of *Pseudomonas aeruginosa* and attenuates chronic *P. aeruginosa* lung infection in Cftr(-/-) mice. Antimicrob Agents Chemother 51: 3677-3687, 2007.
63. Gupta S, Aruna C, Nagaraj S, Dias M and Muralidharan S: In vitro activity of tigecycline against multidrug-resistant gram-negative blood culture isolates from critically ill patients. J Antimicrob Chemother 67: 1293-1295, 2012.
64. Kostoulias X, Fu Y, Morris FC, Yu C, Qu Y, Chang CC, Blakeway L, Landersdorfer CB, Abbott IJ, Wang L, *et al*: Ceftolozane/tazobactam disrupts *Pseudomonas aeruginosa* biofilms under static and dynamic conditions. J Antimicrob Chemother 80: 372-380, 2025.
65. Miller MB and Bassler BL: Quorum sensing in bacteria. Annu Rev Microbiol 55: 165-199, 2001.
66. Köhler T, Perron GG, Buckling A and van Delden C: Quorum sensing inhibition selects for virulence and cooperation in *Pseudomonas aeruginosa*. PLOS Pathog 6: e1000883, 2010.
67. Tsikopoulos A, Petinaki E, Festas C, Tsikopoulos K, Meroni G, Drago L and Skoulakis C: In vitro inhibition of biofilm formation on silicon rubber voice prosthesis: A systematic review and meta-analysis. ORL J Otorhinolaryngol Relat Spec 84: 10-29, 2022.
68. Jakobsen TH, Bragason SK, Phipps RK, Christensen LD, van Gennip M, Alhede M, Skindersoe M, Larsen TO, Høiby N, Bjarnsholt T and Givskov M: Food as a source for quorum sensing inhibitors: Iberin from horseradish revealed as a quorum sensing inhibitor of *Pseudomonas aeruginosa*. Appl Environ Microbiol 78: 2410-2421, 2012.
69. Luo J, Dong B, Wang K, Cai S, Liu T, Cheng X, Lei D, Chen Y, Li Y, Kong J and Chen Y: Baicalin inhibits biofilm formation, attenuates the quorum sensing-controlled virulence and enhances *Pseudomonas aeruginosa* clearance in a mouse peritoneal implant infection model. PLoS One 12: e0176883, 2017.
70. Prince AA, Steiger JD, Khalid AN, Dogrhamji L, Reger C, Eau Claire SE, Chiu AG, Kennedy DW, Palmer JN and Cohen NA: Prevalence of biofilm-forming bacteria in chronic rhinosinusitis. Am J Rhinol 22: 239-245, 2008.
71. Foreman A, Holtappels G, Psaltis AJ, Jervis-Bardy J, Field J, Wormald PJ and Bachert C: Adaptive immune responses in *Staphylococcus aureus* biofilm-associated chronic rhinosinusitis. Allergy 66: 1449-1456, 2011.
72. Yadav MK, Vidal JE, Go YY, Kim SH, Chae SW and Song JJ: The LuxS/AI-2 quorum-sensing system of *Streptococcus pneumoniae* is required to cause disease, and to regulate virulence- and metabolism-related genes in a rat model of middle ear infection. Front Cell Infect Microbiol 8: 138, 2018.
73. Dawit G, Mequanent S and Makonnen E: Efficacy and safety of azithromycin and amoxicillin/clavulanate for otitis media in children: A systematic review and meta-analysis of randomized controlled trials. Ann Clin Microbiol Antimicrob 20: 28, 2021.
74. Brown HL, Hanman K, Reuter M, Betts RP and Van Vliet AHM: Campylobacter jejuni biofilms contain extracellular DNA and are sensitive to DNase I treatment. Front Microbiol 6: 699, 2015.
75. Tetz GV, Artemenko NK and Tetz VV: Effect of DNase and antibiotics on biofilm characteristics. Antimicrob Agents Chemother 53: 1204-1209, 2009.
76. Gawande PV, Leung KP and Madhyastha S: Antibiofilm and antimicrobial efficacy of DispersinB®-KSL-W peptide-based wound gel against chronic wound infection associated bacteria. Curr Microbiol 68: 635-641, 2014.
77. Lamppa JW and Griswold KE: Alginate lyase exhibits catalysis-independent biofilm dispersion and antibiotic synergy. Antimicrob Agents Chemother 57: 137-145, 2013.
78. Daboor SM, Rohde JR and Cheng Z: Disruption of the extracellular polymeric network of *Pseudomonas aeruginosa* biofilms by alginate lyase enhances pathogen eradication by antibiotics. J Cyst Fibros 20: 264-270, 2021.
79. Barraud N, Hassett DJ, Hwang SH, Rice SA, Kjelleberg S and Webb JS: Involvement of nitric oxide in biofilm dispersal of *Pseudomonas aeruginosa*. J Bacteriol 188: 7344-7353, 2006.
80. Reffuveille F, de la Fuente-Núñez C, Mansour S and Hancock REW: A broad-spectrum antibiofilm peptide enhances Antibiotic Action against bacterial biofilms. Antimicrob Agents Chemother 58: 5363-5371, 2014.
81. Le CF, Fang CM and Sekaran SD: Intracellular targeting mechanisms by antimicrobial peptides. Antimicrob Agents Chemother 61: e02340-16, 2017.
82. Kang J, Dietz MJ and Li B: Antimicrobial peptide LL-37 is bactericidal against *Staphylococcus aureus* biofilms. PLoS One 14: e0216676, 2019.
83. Jalilsood T, Baradaran A, Song AAL, Foo HL, Mustafa S, Saad WZ, Yusoff K and Rahim RA: Inhibition of pathogenic and spoilage bacteria by a novel biofilm-forming Lactobacillus isolate: A potential host for the expression of heterologous proteins. Microb Cell Fact 14: 96, 2015.
84. Li J, Zhang Q, Zhao J, Zhang H and Chen W: Lactobacillus-derived components for inhibiting biofilm formation in the food industry. World J Microbiol Biotechnol 40: 117, 2024.
85. Algburi AR, Jassim SM, Popov IV, Weeks R and Chikindas ML: *Lactobacillus acidophilus* VB1 co-aggregates and inhibits biofilm formation of chronic otitis media-associated pathogens. Braz J Microbiol 55: 2581-2592, 2024.
86. Wongchai M, Wongkaewkhiaw S, Kanthawong S, Roytrakul S and Aunpad R: Dual-function antimicrobial-antibiofilm peptide hybrid to tackle biofilm-forming *Staphylococcus epidermidis*. Ann Clin Microbiol Antimicrob 23: 44, 2024.
87. Tintino SR, Souza VCAD, Silva JMAD, Oliveira-Tintino CDDM, Pereira PS, Leal-Balbino TC, Pereira-Neves A, Siqueira-Junior JP, da Costa JGM, Rodrigues FFG, *et al*: Effect of vitamin K_3 inhibiting the function of NorA efflux pump and its gene expression on *Staphylococcus aureus*. Membranes (Basel) 10: 130, 2020.
88. Monteiro KLC, de Aquino TM and Mendonça Junior FJB: An update on *Staphylococcus aureus* NorA efflux pump inhibitors. Curr Top Med Chem 20: 2168-2185, 2020.
89. Lu X, Wang G, Xie Y, Tang W, Liu B and Zhang J: Efflux pump inhibitor combined with ofloxacin decreases MRSA biofilm formation by regulating the gene expression of NorA and quorum sensing. RSC Adv 13: 2707-2717, 2023.
90. Govindarajan DK, Meghanathan Y, Sivaramakrishnan M, Kothandan R, Muthusamy A, Seviour TW and Kandaswamy K: *Enterococcus faecalis* thrives in dual-species biofilm models under iron-rich conditions. Arch Microbiol 204: 710, 2022.
91. Brunson DN, Colomer-Winter C, Lam LN and Lemos JA: Identification of multiple iron uptake mechanisms in *Enterococcus faecalis* and their relationship to virulence. Infect Immun 91: e0049622, 2023.
92. Kaneko Y, Thoendel M, Olakanmi O, Britigan BE and Singh PK: The transition metal gallium disrupts *Pseudomonas aeruginosa* iron metabolism and has antimicrobial and antibiofilm activity. J Clin Invest 117: 877-888, 2007.
93. Goss CH, Kaneko Y, Khuu L, Anderson GD, Ravishankar S, Aitken ML, Lechtzin N, Zhou G, Czyz DM, McLean K, *et al*: Gallium disrupts bacterial iron metabolism and has therapeutic effects in mice and humans with lung infections. Sci Transl Med 10: eaat7520, 2018.
94. Nobile CJ, Ennis CL, Hartooni N, Johnson AD and Lohse MB: A selective serotonin reuptake inhibitor, a proton pump inhibitor, and two calcium channel blockers inhibit *Candida albicans* biofilms. Microorganisms 8: 756, 2020.
95. Yu Q, Ding X, Xu N, Cheng X, Qian K, Zhang B, Xing L and Li M: In vitro activity of verapamil alone and in combination with fluconazole or tunicamycin against *Candida albicans* biofilms. Int J Antimicrob Agents 41: 179-182, 2013.
96. Koushki K, Shahbaz SK, Mashayekhi K, Sadeghi M, Zayeri ZD, Taba MY, Banach M, Al-Rasadi K, Johnston TP and Sahebkar A: Anti-inflammatory action of statins in cardiovascular disease: The role of inflammasome and toll-like receptor pathways. Clin Rev Allergy Immunol 60: 175-199, 2021.
97. Kong F, Ye B, Lin L, Cai X, Huang W and Huang Z: Atorvastatin suppresses NLRP3 inflammasome activation via TLR4/MyD88/NF-κB signaling in PMA-stimulated THP-1 monocytes. Biomed Pharmacother 82: 167-172, 2016.
98. Graziano TS, Cuzzullin MC, Franco GC, Schwartz-Filho HO, de Andrade ED, Groppo FC and Cogo-Müller K: Statins and antimicrobial effects: Simvastatin as a potential drug against *Staphylococcus aureus* biofilm. PLoS One 10: e0128098, 2015.
99. Khodaparast S, Ghanbari F and Zamani H: Evaluation of the effect of ibuprofen in combination with ciprofloxacin on the virulence-associated traits, and efflux pump genes of *Pseudomonas aeruginosa*. World J Microbiol Biotechnol 38: 125, 2022.

100. Abbas HA, Atallah H, El-Sayed MA and El-Ganiny AM: Diclofenac mitigates virulence of multidrug-resistant *Staphylococcus aureus*. Arch Microbiol 202: 2751-2760, 2020.
101. Severino P, Silveira EF, Loureiro K, Chaud MV, Antonini D, Lancellotti M, Sarmento VH, da Silva CF, Santana MHA and Souto EB: Antimicrobial activity of polymyxin-loaded solid lipid nanoparticles (PLX-SLN): Characterization of physicochemical properties and in vitro efficacy. Eur J Pharm Sci 106: 177-184, 2017.
102. Wang XF, Zhang SL, Zhu LY, Xie SY, Dong Z, Wang Y and Zhou WZ: Enhancement of antibacterial activity of tilmicosin against *Staphylococcus aureus* by solid lipid nanoparticles in vitro and in vivo. Vet J 191: 115-120, 2012.
103. Kotrange H, Najda A, Bains A, Gruszecki R, Chawla P and Tosif MM: Metal and metal oxide nanoparticle as a novel antibiotic carrier for the direct delivery of antibiotics. Int J Mol Sci 22: 9596, 2021.
104. Patro SK, Panda NK and Sharma M: Drug repurposing for, ENT and head and neck, infectious and oncologic diseases: Current practices and future possibilities. In: Sobti RC, Lal SK and Goyal RK (eds). Drug Repurposing for Emerging Infectious Diseases and Cancer. Singapore: Springer Nature, pp253-282, 2023.
105. Kora AJ and Arunachalam J: Assessment of antibacterial activity of silver nanoparticles on *Pseudomonas aeruginosa* and its mechanism of action. World J Microbiol Biotechnol 27: 1209-1216, 2011.
106. Caciandone M, Niculescu AG, Grumezescu V, Bîrcă AC, Ghica IC, Vasile BȘ, Oprea O, Nica IC, Stan MS, Holban AM, *et al*: Magnetite nanoparticles functionalized with therapeutic agents for enhanced ENT antimicrobial properties. Antibiotics (Basel) 11: 623, 2022.
107. García-Alvarez R, Izquierdo-Barba I and Vallet-Regí M: 3D scaffold with effective multidrug sequential release against bacteria biofilm. Acta Biomater 49: 113-126, 2017.
108. Lee M, Park CG, Huh BK, Kim SN, Lee SH, Khalmuratova R, Park JW, Shin HW and Choy YB: Sinonasal delivery of resveratrol via mucoadhesive nanostructured microparticles in a nasal polyp mouse model. Sci Rep 7: 40249, 2017.
109. Cano EJ, Caflisch KM, Bollyky PL, Van Belleghem JD, Patel R, Fackler J, Brownstein MJ, Horne B, Biswas B, Henry M, *et al*: Phage therapy for limb-threatening prosthetic knee *Klebsiella pneumoniae* infection: Case report and in vitro characterization of anti-biofilm activity. Clin Infect Dis 73: e144-e151, 2021.
110. Manoharadas S, Altaf M, Alrefaei AF, Hussain SA, Devasia RM, Badjah Hadj AYM and Abuhasil MSA: Microscopic analysis of the inhibition of staphylococcal biofilm formation by Escherichia coli and the disruption of preformed staphylococcal biofilm by bacteriophage. Microsc Res Tech 84: 1513-1521, 2021.
111. Morris J, Kelly N, Elliott L, Grant A, Wilkinson M, Hazratwala K and McEwen P: Evaluation of bacteriophage anti-biofilm activity for potential control of orthopedic implant-related infections caused by *Staphylococcus aureus*. Surg Infect (Larchmt) 20: 16-24, 2019.
112. Zhao M, Li H, Gan D, Wang M, Deng H and Yang QE: Antibacterial effect of phage cocktails and phage-antibiotic synergy against pathogenic *Klebsiella pneumoniae*. mSystems 9: e0060724, 2024.
113. Fong SA, Drilling A, Morales S, Cornet ME, Woodworth BA, Fokkens WJ, Psaltis AJ, Vreugde S and Wormald PJ: Activity of bacteriophages in removing biofilms of *Pseudomonas aeruginosa* isolates from chronic rhinosinusitis patients. Front Cell Infect Microbiol 7: 418, 2017.
114. Gordon M and Ramirez P: Efficacy and experience of bacteriophages in biofilm-related infections. Antibiotics (Basel) 13: 125, 2024.
115. Lim DJ, Skinner D, Mclemore J, Rivers N, Elder JB, Allen M, Koch C, West J, Zhang S, Thompson HM, *et al*: In-vitro evaluation of a ciprofloxacin and azithromycin sinus stent for *Pseudomonas aeruginosa* biofilms. Int Forum Allergy Rhinol 10: 121-127, 2020.
116. Pakkulnan R, Thonglao N and Chareonsudjai S: DNase I and chitosan enhance efficacy of ceftazidime to eradicate Burkholderia pseudomallei biofilm cells. Sci Rep 13: 1059, 2023.
117. Cresti L, Falciani C, Cappello G, Brunetti J, Vailati S, Melloni E, Bracci L and Pini A: Safety evaluations of a synthetic antimicrobial peptide administered intravenously in rats and dogs. Sci Rep 12: 19294, 2022.