REVIEW

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Acinetobacter baumannii biofilm and its potential therapeutic targets

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Abstract

Background *Acinetobacter baumannii* (*A. baumannii*) is an opportunistic pathogen that poses dangerous health threat. It is a main cause of biofilm-associated infections that are mostly resistant to antibiotic therapy. Because of its capacity to form biofilm on biotic and abiotic surfaces, it has been linked to most nosocomial infections such as ventilator-associated pneumonia, urinary tract infections, bacteremia, meningitis, wound infections, soft tissue infections, and peritonitis.

Main body of the abstract The biofilm refers to an organized group of microbial cells that are embedded in an exopolymeric substance made of protein, extracellular DNA, and polysaccharide. Bacterial cells in biofilms are resistant to chemicals, phagocytosis, and other elements of the body's innate and acquired immune systems posing treatment challenges. Biofilm formation in *A. baumannii* is a complicated process that is influenced by a variety of factors such as outer membrane protein A, poly-β-(1,6)-*N* acetyl glucosamine (PAGE), biofilm-associated protein, two-component system (Bfm/S BfmR), chaperone–usher (Csu) pilus assembly system of pili, BlaPER-1 belonging to β-lactamase family, extracellular polymeric substance, and the quorum sensing system. Several biofilm-associated genes influence antimicrobial susceptibility, implying a link between biofilm formation and antimicrobial resistance.

Short conclusion This review describes the complex biofilm system of *A. baumannii*, which gives it a survival advantage and increases its colonization ability. Also, it demonstrates various extrinsic and intrinsic factors that function and regulate the biofilm machinery of *A. baumannii*. Furthermore, this study considers prospective ways for preventing biofilm development on relevant medical equipment, as well as potential therapeutic strategies for eradicating mature biofilms, which can aid in the treatment of biofilm-associated *A. baumannii* infection.

Keywords *Acinetobacter baumannii*, Biofilm, Antibiotic resistance, Extracellular polymeric substance, Anti-biofilm, Quorum sensing, Multidrug resistance

Background

Acinetobacter baumannii is a gram-negative, non-fastidious, non-fermentative, non-motile, catalase-positive, and oxidative-negative coccobacillus [1]. It is a member of Eubacteria class Proteobacteria family Moraxellaceae and genus Acinetobacter. In addition, it is also considered one of the ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus (S. aureus), Klebsiella pneumoniae (K. pneumoniae), A. baumannii, Pseudomonas aeruginosa (P. aeruginosa), and Enterobacter* species) that are linked to alarming multidrug-resistant hospital-acquired infections [2, 3]. An estimate of 1 million cases of *A.*

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baumannii infections is reported per year worldwide with death rates ranging from 20 to 80% [4, 5].

A. baumannii has an ability to form biofilm on biotic (host mucosal tissue) and abiotic surfaces (e.g., catheters) which plays a critical role in causing nosocomial infections [1, 6]. The most prevalent nosocomial illnesses caused by Acinetobacter are urinary tract infections (UTI), meningitis, pneumonia, wound, bacteremia, burn, endocarditis, as well as skin and soft tissue infections [7, 8]. *A. baumannii* bloodstream infections and ventilator-associated pneumonia have been connected to substantial death rates of up to 35% [9]. However, community-acquired pneumonia is more dangerous than nosocomial pneumonia and has a 60% mortality rate [10].

A. baumannii infections are frequently associated with multidrug resistance; and recently, it shows resistance to all antibiotics including carbapenem and colistin [9]. There are different resistance mechanisms owned by *A. baumannii* such as modifications of target sites, permeability deficiencies, multidrug efflux pumps, and enzymatic drug degradation, for example, β-lactamases and aminoglycoside-modifying enzymes [10–12]. Currently, a serious threat to public health is posed by the rapid expansion of antibiotic resistance in *A. baumannii*. *A. baumannii*'s capacity to colonize and create biofilm on biotic and abiotic surfaces contributes to long-lasting infections, antibiotic resistance, as well as survival and transmission in hospital settings [13]. As a result, the biofilm matrix, that surrounds the bacteria, enables them to tolerate harsh conditions and resist antibiotic treatments. Consequently, current treatments for infections caused by biofilm-forming *A. baumannii* are most probably unsuccessful [14]. Therefore, there is an urgent need for alternative medications and/or therapies to prevent the establishment of biofilms and inactivate Acinetobacter adherence to biotic or abiotic surfaces. Numerous efficient innovative anti-biofilm treatments have been developed, including antibiotic therapy, quorum sensing inhibitors, natural products/essential oils, antimicrobial peptides, efflux pump inhibitors, nanoparticles, and phage therapy. Additionally, photodynamic treatment (PDT) offers a potential strategy to tackle antibiotic resistance [15].

Main text
A. baumannii pathogenesis and biofilm formation
Biofilm formation and development is a complex process in which microorganism cells change their growth mode from planktonic to sessile. It is fueled by a variety of physical, chemical, and biological processes [16, 17]. The common steps in the formation of a biofilm are summarized in Fig. 1 [18, 19].
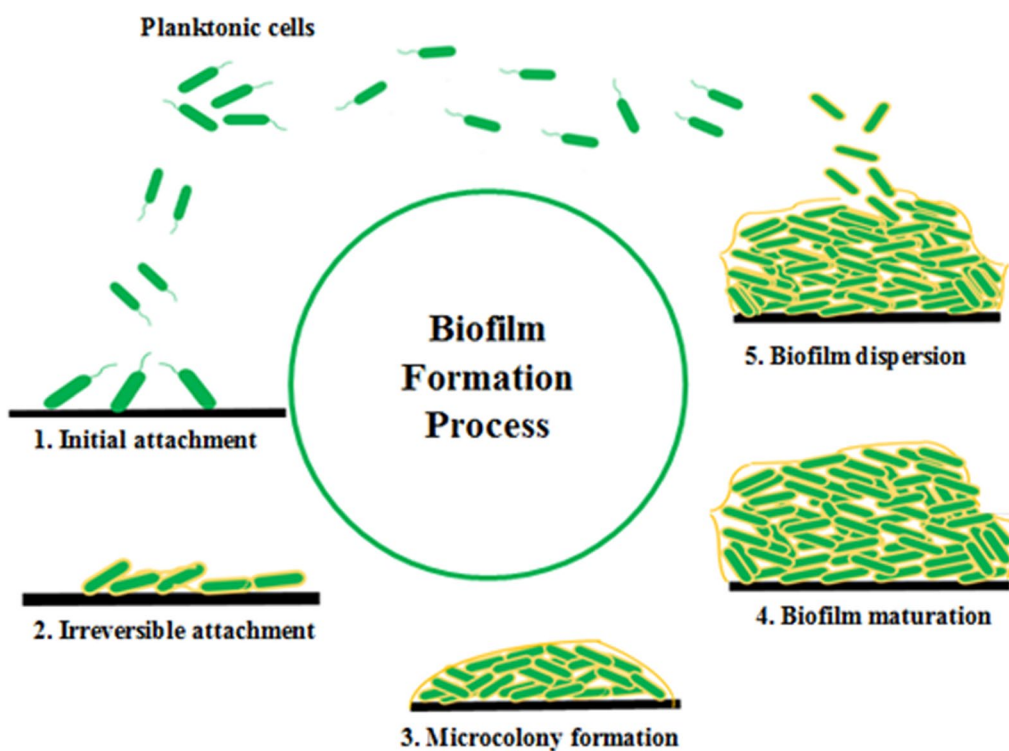
The pathophysiology of *A. baumannii* infections is still inadequately understood, despite the fact that they are a serious clinical practice concern as well as a worldwide health threat. Evidence showed that phospholipases, extracellular polysaccharides, a siderophore-mediated iron acquisition system, outer membrane protein A (OmpA), phospholipids, and the K1 capsule are virulence factors that play a significant part in bacterial pathogenicity [20–24]. Biofilms are responsible for a number of subacute or chronic infections that are difficult to cure [22, 25]. Furthermore, the ability of *A. baumannii* to form biofilms (due to fimbriae and pili) is a significant factor that exacerbates the disease process. In comparison with other species, *A. baumannii* has a biofilm formation rate of nearly 80–91%, whereas the other species has a rate of approximately 5–24% [21, 22, 24]. The assembly of pili and the production of the biofilm-associated protein (*bap*), a surface adhesion protein, are crucial for the development and maturation of biofilms once *A. baumannii* adheres to abiotic surfaces [26]. The ability of cells to adhere to abiotic surfaces such as medical devices and environmental surfaces, as well as the level of expression of outer membrane proteins, mRNAs, is virulence factors for *A. baumannii* pathogenesis [8, 22–24]. Additionally, OmpA interacts with fibronectin on the host cell surface to facilitate bacterial attachment to lung epithelial cells and helps in the formation of biofilms on biotic surfaces like epithelial cells. It may also induce apoptosis in human epithelial cells [24, 27].

A. baumannii biofilm formation and antimicrobial resistance
A. baumannii has been classified as a "red-alert" human pathogen because it can develop resistance to all antimicrobial agents that are currently available in the market [23].

The phrase "biofilm resistance" refers to the ability of cells to persist for extended periods of time while embedded in biofilm when antimicrobials are present. Biofilms are better suited to evading antimicrobials than planktonic cells because they are not easily killed by antimicrobials.

There are different factors that cause biofilm resistance and explain the reasons why biofilm cells survive longer than planktonic cells in the presence of antimicrobials and they are demonstrated in Fig. 2 [28]. However, the relationship between the development of biofilms and the phenotypes of antibiotic resistance is still up for debate [29]. According to some studies, the development of biofilm and multidrug resistance is positively correlated [29, 30]. One study revealed that biofilm formers showed greater resistance to ampicillin–sulbactam, amikacin, ciprofloxacin, and ceftazidime as compared to imipenem

Planktonic cells

Biofilm Formation Process

1. Initial attachment

2. Irreversible attachment

3. Microcolony formation

4. Biofilm maturation

5. Biofilm dispersion

Fig. 1 The common steps of biofilm development. Step 1: The planktonic cells adhere to the biotic or abiotic surface. Step 2: Bacterial cells aggregate and form microcolonies, secrete EPS, and the attachment becomes irreversible. Step 3: A biofilm is formed and matures, and cells form multi-layered clusters. Step 4: The growth of three-dimensional and further maturation of the biofilm, providing protection against the external environment. Step 5: The biofilm reaches a critical mass, and planktonic bacteria disperse to colonize other surfaces

and piperacillin [31]. Another study found that *A. baumannii* clinical isolates exhibit a significant propensity for the development of biofilms, and biofilms have a correlation to multiple drug resistance. It was also revealed that biofilm producers had a greater rate of antibiotic resistance than isolates who did not build biofilms [32]. A similar investigation on the relationship between biofilm generation and drug susceptibility was done on *A. baumannii* strains obtained from hospital-acquired illnesses, where ceftazidime-sensitive bacteria were observed to create less biofilm than ceftazidime-resistant strains, but tobramycin- and amikacin-sensitive strains produced more biofilm than antibiotic-resistant ones. On the other hand, powerful biofilm producers from intensive care units (ICUs) are frequently more vulnerable to antibiotics because bacteria protected in biofilm do not need the resistance mechanisms required by planktonic cells [33]. According to other research, non-biofilm-forming isolates were frequently more resistant to imipenem and ciprofloxacin than biofilm-forming isolates [34]. Similarly, *A. baumannii* isolates that were resistant to meropenem were less able to generate biofilms than isolates that were sensitive to meropenem [35]. Acinetobacter strains with multidrug-resistant (MDR) and biofilm

production often continue to pose a substantial concern in the hospital setting because they quickly gain drug resistance to routinely used drugs, and their potential to produce biofilm is statistically significant with imipenem resistance [36].

Therefore, bacteria are able to tolerate harsh conditions and resist antibiotic treatments owing to the biofilm matrix that surrounds them. Consequently, current treatments for infections caused by *A. baumannii* biofilms are unsuccessful [14]. Many successful innovative anti-biofilm treatments have been developed [28].

Factors influencing *A. baumannii* biofilm formation

Biofilm formation is triggered by three different methods of interaction. Firstly, the interaction between the microbial cells. Secondly, bacterial adherence to the surface of human tissues or other objects, and thirdly, information transfer that takes place in the environment through the acylation of serine lactones. These mechanisms have been impacted by several intrinsic and extrinsic factors, including physicochemical and microbiological determinants, such as the aggregation of substances, adherence of collagen, expression of pili, capsular polysaccharides, and resistance determinants. There are additional elements
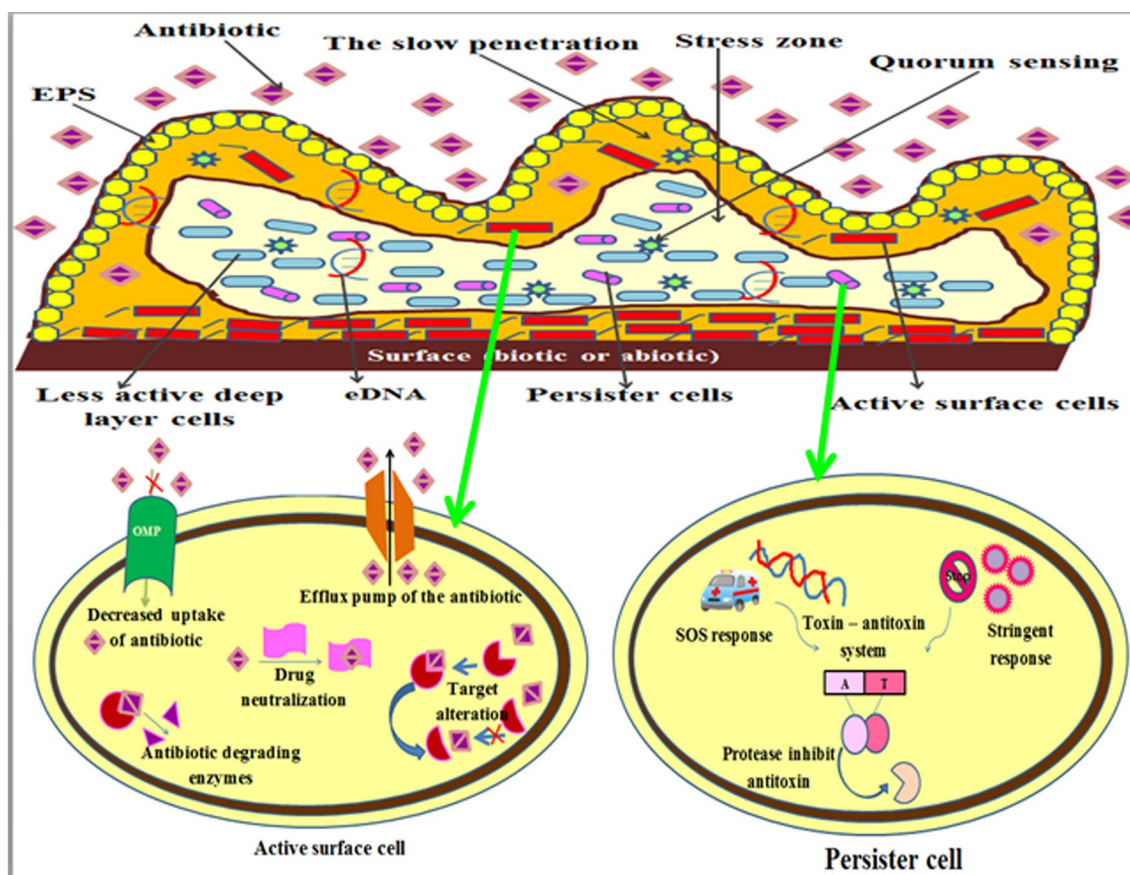
Fig. 2 Diagram depicting the various processes of antibiotic resistance in biofilm ecosystems. Biofilm cells are adhered to the biotic or abiotic surface. In the stress zone where the nutrient and oxygen availabilities are low, the persister cells and less active deep layer cells develop. The figure depicts the following resistance: Antibiotic penetration is slowed by matrix of exopolysaccharides (EPS); extracellular DNA (eDNA); multidrug efflux pumps; outer membrane protein; antibiotic degrading enzymes; target alterations; drug neutralization; quorum sensing; and stress responses (SOS response and stringent response (SR))

that contribute to the biofilm's formation, including macromolecule secretion, cell interaction, and surface-regulated attachment [37].

Intrinsic factors associated with *A. baumannii* biofilm formation

A. baumannii biofilm development is influenced by a number of virulence factors. Genes or proteins that provide pathogenicity, cellular architectures, and phenotypic or genotypic traits are all included. Table 1 summarizes these factors that are related to the development and control of biofilms in *A. baumannii.*

Outer membrane proteins (Omps)

Omps are considered as one of the most important porins that regulate cellular permeability and play a significant role in adaptability, environmental communication, and microbial pathogenicity through the use of drug exclusion mechanisms across outer membrane channels [27].

Numerous OMPs, including CarO, Omp33 OprD-like, and PstS, are present in Acinetobacter species and are associated with pathogenicity and the emergence of antibiotic resistance. OmpA is the primary outer membrane protein in *A. baumannii* which is considered as a beta barrel-shaped monomeric integral outer membrane protein with 8–26 antiparallel strands joined by four loops on the outer membrane surface and three short twists on the periplasmic side [38]. It is also a well-characterized virulence factor because of the numerous essential roles it plays in *A. baumannii's* survival and pathogenesis, including maintaining cell membrane integrity, mediating drug resistance, altering host immune response, starting the formation of biofilms, invading host epithelial cells, and inducing host cell apoptosis [39]. A study demonstrated that *A. baumannii* cells quickly attached to a 96-well plate coated with fibronectin as a result of OmpA's interaction with fibronectin, indicating the beginning of the interaction between *A. baumannii*

Table 1 Summary for intrinsic factors implicated in *A. baumannii* biofilm formation and regulation

Biofilm factors	Function	References
Outer membrane proteins (OmpA)	Well-characterized virulence factor in maintaining cell membrane integrity, mediating drug resistance, altering host immune response, starting the formation of biofilms, invading host epithelial cells, and inducing host cell apoptosis	[38, 39]
Biofilm-associated protein (BAP)	Important for intercellular cell adhesion, bacterial cell aggregation, maintenance, biofilm development, and maturation on different surfaces such as polypropylene, polystyrene, and titanium	[40]
Chaperon–usher (*Csu*) pilus assembly system of pili	Regarded as the most important virulence factor in facilitating the irreversible adhesion of cells to an abiotic surface and biofilm formation	[41]
Bla PER1	It's presence and expression help in the adhesion to both biotic and abiotic surfaces, which promotes the formation of biofilm	[32]
Extracellular polymeric substance (EPS)	Form the biofilm matrix after irreversible cell attachment to the surface and considered the most important component of biofilm matrix	[42]
Quorum sensing (QS) system	It produces the signaling molecules, autoinducers to maintain bacterial cell-to-cell communication, population density, synchronized behavior, and interaction. QS is also responsible for activation and regulation of gene expression of virulence factors, motility, plasmid transfer, drug resistance, and biofilm formation	[43, 44]
Efflux pumps	Essential for the growth and maturation of biofilms through a variety of mechanisms, including the indirect regulation of biofilm-forming genes, the efflux of antibiotics or metabolic intermediates, the efflux of quorum quenching (QQ) molecules, and the efflux of EPSs and quorum-suppressing molecules	[37, 45]
Poly-β-(1, 6)-*N*-acetylglucosamine (PNAG)	Necessary for cell–cell adherence, biofilm development, and thickness of biofilm	[46, 47]
Extracellular DNA (eDNA)	It plays a significant role in cell adhesion, biofilm development, and maintenance	[48]
Alginate	Essential for the protection and stability of the biofilm structure. It also contributes to the preservation of contents of the matrix such as nutrients and water	[49]
Amyloids	Facilitate bacterial cell–host adhesion and biofilm formation and contribute to host cell invasion and adhesion as well as to the host's inflammatory response	[50]
Cyclic di-guanosine monophosphate (c-di-GMP)	Act as a universal positive regulator in the production of biofilms	[51, 52]

biofilm formation on biotic surfaces [53]. OmpA-deficient *A. baumannii* mutants were less virulent than wild-type cells, with reduced adhesion to human airway epithelial cells and decreased biofilm formation [39].

Bap

Bap is a higher molecular cell surface protein that is found on the surface of bacteria with a molecular weight of about 854-kDa and 8620 amino acids. The protein encoded by the *bap* gene is important for intercellular cell adhesion, bacterial cell aggregation, maintenance, biofilm development, and maturation on different surfaces such as polypropylene, polystyrene, and titanium [40]. Bap generates a multidimensional pattern of mature biofilm and creates water pathways between them. A study found that *bap* plays an important role in biofilm maturation rather than the initial stage of adherence since wild-type *A. baumannii* strains produced typical biofilm phenotypes, whereas *bap* mutants failed to produce mature biofilm and remained in a single layer [54, 55]. Furthermore, Bap protein increased *A. baumannii* adhesion to both normal human neonatal keratinocytes and bronchial epithelial cells [55]. Several investigations have shown that *bap* is present in *A. baumannii* strains and that it is associated with significant biofilm formation.

Also, *A. baumannii bap* is a type I secretion system that primarily targets carbohydrates in host cells [56, 57].

Csu pilus assembly system of pili

A. baumannii has been considered as non-motile due to the lack of flagella [58, 59]. However, research reveals that this organism, by using its twitching motility, is able to survive after infection and to spread on hospital surfaces [60]. Type IV pili are used to push cells in media through extension and retraction movements [61]. These pili play a critical role in gene transfer, biofilm formation, and organism adherence to host cells [62]. A. *baumannii* has a clustered gene known as the *Csu* operon that produces confined structures resembling pilus. It is regarded as the most important virulence factor in facilitating the irreversible adhesion of cells to an abiotic surface. The Csu pilus is a polycistronic adhesive surface organelle that is produced by the ancient chaperone–usher (CU) pathway from four protein subunits, CsuA/B, CsuA, CsuB, and CsuE [41]. There are another two subunits such as CsuC and CsuD which act as transporter proteins. Biofilm formation was affected by gene deletion because CsuA/B and CsuE mutants completely lost their pilus structure, whereas *CsuA* and *CsuB* mutants produced small number of abnormal fibers. The deletion mutants failed to

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establish a biofilm on the plastic surface, demonstrating that all four subunits are required for a functioning pilus [63].

PER-1 β-lactamase

The *bla PER1* gene is classified as a class A extended spectrum beta-lactamase. Additionally, this gene's presence and expression help *A. baumanni* adhere to both biotic and abiotic surfaces, which promotes the formation of biofilm [32]. Several studies have found that strains with the *bla PER1* gene have higher cell adhesiveness and biofilm formation than those without this genetic trait [32, 64, 65]. However, Bardbari et al. discovered no relationship between the production of *PER1* beta-lactamase and the development of biofilms [66]. Therefore, the presence of *bla PER1* probably improves the ability of cells that express this gene to adhere, but it does not always aid in the formation of biofilms.

EPS

EPS are organic polymers of microbial origin involved in bacterial cells' interactions with their environment [42]. EPS is made up of polysaccharides, proteins, eDNA, and lipids. Bacterial cells are stimulated to produce EPS, which form the biofilm matrix after irreversible cell attachment to the surface and considered the most important component of biofilm matrix. Bacterial cells use EPS such as glycolipids, glycoproteins, alginate, poly-β-(1,6)-*N* acetyl glucosamine (PNAG), DNA, and bap to form a stable three-dimensional structure of mature biofilm. The development and pathogenicity of biofilms are significantly influenced by EPS. The main components of EPS are alginate and antibiotics hydrolytic enzymes immobilized on biofilm, and they work by preventing antibacterial chemicals from reaching the target and reducing antibacterial action [67]. The negatively charged amino acid side chains of a polypeptide chain, which constitute the majority of EPS, have a proclivity to attract positively charged amino acid side chains. This attraction prevents hydrophilic antibiotics from entering cell bodies and significantly reduces bactericidal activity, making it more difficult to eradicate cells after biofilm formation. EPS has a significant role in the O-glycosylation system and capsule formation of the MDR *A. baumannii* strain [68].

Quorum sensing (QS) system

QS is a cell-to-cell communication process that is influenced by the density of the bacterial population. Several small diffusible signaling molecules known as autoinducers (AI) are involved. These are hormone-like substances, such as acyl homoserine lactones (AHLs), which stimulate the expression of genes that regulate a variety of biological processes, including biofilm formation, motility, pathogenicity, bioluminescence, and sporulation [43, 44]. *A. baumannii* often produces 3-hydroxy-C12-homoserine lactone as an AHL [69]. Additionally, it contains a two-component system called AbaI/AbaR that manages the QS system. This system is similar to the typical LuxI/LuxR system found in gram-negative bacteria. AHL is synthesized by autoinducer synthases, which are catalyzed by the gene *AbaI* and are receptor proteins for AHL, which are encoded by the protein AbaR [70]. According to a study, the interaction of AHL with the AbaR receptor causes enhanced expression of the csu pili and the development of biofilms [71]. Another gene termed *abaM*, an uncharacterized member of the RsaM protein family located between AbaR and AbaI, plays a crucial role in the regulation of *A. baumannii* QS, virulence, surface motility, and biofilm formation. *abaM* has recently been found to regulate both QS-dependent and QS-independent genes in *A. baumannii*. Increased levels of N-(3-hydroxydodecanoyl)-l-HSL positively trigger the expression of *abaM*, a negative auto-regulator that inhibits the expression of AbaI and AbaR and hence adversely controls the generation of AHL. QS deficiency results in thinner biofilm formation and lower EPS production, making bacteria more susceptible to antibiotics. The QS gene, which controls the production of virulence genes and the creation of biofilms, is more highly expressed when there is an iron deficiency [72]. Therefore, developing QS cascade inhibitors or quenchers to treat persistent *A. baumannii* biofilm infections would be a useful strategy [73].

Efflux pumps

Efflux pumps are membrane proteins that can expel a wide variety of compounds including antibiotics, detergents, dyes, poisons, and waste metabolites. The efflux system families identified in *A. baumannii* are major facilitator superfamily (MFS), multidrug and toxin compound extrusion (MATE), AbeM, resistance nodulation division (RND), small multidrug resistance (SMR), ATP-binding cassette (ABC), and MacB [74]. Numerous studies show that efflux pumps are essential for the growth and maturation of biofilms through a variety of mechanisms, including the indirect regulation of biofilm-forming genes, the efflux of antibiotics or metabolic intermediates, the efflux of quorum quenching (QQ) molecules, and the efflux of EPSs and quorum-suppressing molecules [37, 45]. There are three types of RND efflux pumps associated with *A. baumannii*: AdeABC, AdeFGH, and AdeIJK. Yoon et al. discovered that mutant AdeABC, AdeFGH, and AdeIJK efflux pumps generate considerably less biofilm than wild-type AdeABC,

AdeFGH, and AdeIJK efflux pumps. Thus, in order to initiate and maintain biofilm formation in *A. baumannii*, efflux pump genes must be expressed [75]. Another study discovered that mutations in the AdeABC and AdeIJK efflux genes were associated with reduced expression of many pilus system-encoding proteins, including CsuA/B, CsuC, and FimA. These proteins are required for *A. baumannii* adhesion, surface colonization, and biofilm formation [76]. According to Richmond et al., when AdeABC efflux pumps were inhibited in *A. baumannii* mutant strain, biofilm production on mucosal tissue was dramatically reduced compared to the wild-type strain [77]. *A. baumannii's* pilus gene expression, biofilm formation, and altered membrane composition are regulated by the overexpression of the AdeABC and AdeIJK efflux pumps. Additionally, the production and transfer of autoinducer molecules during the development of biofilm in *A. baumannii* are connected to the overexpression of the efflux pump AdeFGH, which confersMDR [76, 78].

PNAG

PNAG is a polysaccharide that is necessary for biofilm development in both gram-positive and gram-negative bacteria [79]. It is encoded by a set of four genes called *pgaA*, *pgaB*, *pgaC*, and *pgaD*, which are related to those in *Yersinia pestis* and *Escherichia coli (E. coli)* [46, 80]. *A. baumannii's* pgaA gene encodes a predicted transmembrane protein with a porin-like domain, suggesting that it may be involved in the movement of PNAG across the outer membrane. The production of biofilms was significantly reduced after the pgaABCD locus was deleted. Numerous studies have, therefore, demonstrated that PNAG is essential for preserving the integrity of *A. baumannii* biofilms in a more dynamic and demanding environment [47, 80].

eDNA

eDNA plays a significant role in cell adhesion, biofilm development, and maintenance. It was first reported by Whitchurch *et al*. in *P. aeruginosa* [48]. Later, it was shown that the presence of eDNA in bacteria serves a crucial role in providing structural stability to the biofilm, increasing the creation of extracellular polymeric compounds, and transforming additional neighboring competent bacterial cells [81]. The role of eDNA in the production of *A. baumannii* biofilms is not well understood. A study using an *A. baumannii* clinical isolate showed that the release of eDNA was promoted either in free form or trapped in outer membrane vesicles, and it did not necessitate cell lysis at an early stage. This suggests that eDNA may assist other adhesion proteins that may later play a role in the earliest stages of adhesion in the formation of biofilm, promoting the adhesion that

occurs in the first stages of the biofilm [82]. Furthermore, DNaseI treatment of a 24-h-old prepared biofilm resulted in its depletion by about 60%, showing the importance of eDNA in biofilm preservation [82, 83].

Alginate

Alginate C (*algC*) is a biofilm-associated gene that controls the synthesis of phosphomannomutase/phosphoglucomutase, a bi-functional enzyme that belongs to the alpha-d-hexomutase superfamily and is necessary for the production of exopolysaccharide alginate and lipopolysaccharide core when Mg^{2+} and the activator glucose 1,6-bisphosphate are present during biofilm formation [84–86].

Alginate lyase is a glycoside hydrolase that degrades alginate, allowing bacteria to escape biofilms and improving the effectiveness of drugs against the *A. baumannii* biofilm. It was discovered that the MDR strain of *A. baumannii* expressed more *algC* in a biofilm mode of development than in a planktonic phase. Biofilm cells showed an 81.59-fold expression compared to planktonic cells, which reach 3.24-fold, according to real-time PCR, which was utilized to characterize the quantitative gene expression pattern [87].

Amyloidogenic proteins

Curli fibers are an amyloid protein involved in matrix formation that is encoded by the curli-specific gene (*csg*) operon. Amyloids have been found in bacteria as well as fungi. Amyloids are known to facilitate bacterial cell–host adhesion and biofilm formation. The amyloid protein is made up of a major subunit encoded by the gene *csgA*. Curli fibers contribute to host cell invasion and adhesion as well as to the host's inflammatory response [50]. The management of bacterial infections caused by biofilms may be aided by focusing on amyloid formations [88].

Second messenger signaling pathway

In the bacterial population, several nucleotide messenger signaling pathways have been found, including cyclic di-guanosine monophosphate (c-di-GMP), c-AMP, (p) pp-G-pp, c-di-AMP, and c-AMP-GMP, which regulate a wide range of physiological features [89]. All bacterial populations share a high degree of conservation for cyclic di-guanosine monophosphate (c-di-GMP) second messenger signaling, which controls physiology including cell motility, cell division, and differentiation as well as harmful features such as virulence and biofilm formation [51, 52]. They are known to act as a universal positive regulator in the production of biofilms in gram-negative bacteria [90]. *A. baumannii* controls the transition of planktonic to biofilm bacteria by using the secondary

messenger cdiGMP. The development of the biofilm matrix components is induced by an elevated amount of cdiGMP, which also controls the formation of the biofilm. The production of biofilm matrix was downregulated, and the distribution of biofilm development was spread due to the decreased level of cdiGMP [90, 91]. Because of its known function in regulating biofilm development and because it is largely conserved in bacteria, targeting c-di-GMP signaling may be effective in inhibiting biofilms [90].

Extrinsic factors associated with *A. baumannii* biofilm formation

Environmental factors that influence biofilm structure and function include physical parameters that control these ecosystems, chemical factors, and biological factors. Light penetration, temperature, and water are examples of the physical features, while pH, nutrient availability, and toxicant effects are chemical factors that influence biofilm formation. Biological factors are community composition (bacteria, algae, and fungi), relative contribution of autotrophs and heterotrophs, biomass thickness, and grazing [92, 93].

Surface property

A. baumannii develops biofilms three times faster than the other Acinetobacter species at the solid–liquid interface. Clinical strains are more capable of developing biofilms than environmental strains, and the formation of biofilm on surfaces with potential medical importance affects the strains' ability to tolerate the availability of nutrients, desiccation, stress, and antibiotic therapy [29]. *A. baumannii* adhesion to abiotic surfaces and biofilm formation are influenced by a variety of factors, including surface roughness, physicochemical properties, and the presence of biological components [94]. Also, pH, ionic content, and biomaterial of protein adsorption have all been connected to *A. baumannii's* capacity to develop mature biofilms on polypropylene, polystyrene, titanium, and other medical-related devices [95]. Blood, sweat, tears, urine, saliva, interstitial fluid, wound cultures, and respiratory secretions are examples of biomaterials that have an impact on how well bacteria adhere to their surfaces and promote the growth of biofilms [96]. Surfaces made of polycarbonate are known to statistically create more biofilm mass than surfaces made of glass, rubber, porcelain, and polypropylene [94]. Latex catheters, despite being less expensive and more elastic, are more susceptible to bacterial adhesion and biofilm formation. Therefore, latex catheters are so favored than silicone catheters [97].

Growth temperature

Temperature has an impact on the development of biofilms. The optimal temperature for bacterial growth is dependent on the rise in the nutrient intake [96]. Also, it is well understood that the nutrient metabolism depends on the presence of enzymes and reaction rates that regulate the development of many physiological and biochemical systems in bacteria. As a result, ideal temperature encourages bacterial growth, resulting in biofilm development [98, 99]. In contrast, when the temperature falls below the optimum range, bacterial growth can be slowed down as a result of a decline in response rates, which may have an effect on the development of biofilms. In addition to enzymes, environmental temperature can influence the physical properties of substances inside and around cells [16]. Temperature can also have an impact on the characteristics of bacterial EPS, such as the viscosity of the polysaccharides. It was discovered that as the temperature of EPS rises, a gel-like substance is produced. After reaching a critical degree, the gel turns into a solution [100]. Thus, lower temperatures may result in the polysaccharides' characteristics being more consistent, which frequently raises the likelihood of bacterial biofilm adherence [16]. On the other hand, it was discovered that some bacteria grew more adhered to the surface when exposed to high temperatures [101]. *A. baumannii* survived effectively at temperatures between 20 and 44 °C [102]. However, other researches have shown that different optimal temperatures for biofilm growth in *A. baumannii*, including 30 °C, at pH 7, in a medium containing sodium chloride (NaCl), or 25 °C [7, 103]. Another study found that the overexpression of specific *baps*, *Csu* pili, and iron-uptake proteins led to a high level of biofilm production in *A. baumannii* on plastic surfaces at 28 °C [95].

Growth media

The growth medium is another element that influences the development of biofilms. The availability of oxygen may play a role in bacterial adherence to submerged surfaces and subsequent biofilm formation [104]. Additionally, the availability of oxygen can affect how much bacteria produce energy, which may have an impact on the development of biofilms. For instance, a lack of oxygen might reduce the metabolic activity of bacteria in biofilms [100]. Therefore, it is thought that the availability of oxygen is a crucial environmental component that might affect the structure and growth of biofilm [105, 106]. Lower oxygen availability typically causes active dispersal, which is essential for the biofilm life cycle [107]. For instance, it was discovered that bacterial cells near the base of a biofilm received less oxygen

than those at the surface, which facilitated their separation from the biofilm's deeper layers [108]. Nutrients can have an impact on the transition between planktonic and biofilm bacterial lifestyles because the bacterial response to develop biofilm or to remain suspended depends on the nutritional condition [98]. A higher concentration of nutrients speeds up the rate of microbial attachment since it has been discovered that the presence of nutrients abundantly in the surrounding media aids bacterial adhesion to surfaces [108]. Furthermore, nutrient levels can influence biofilm development and cell dispersal from biofilms [109, 110]. Additionally, it has been demonstrated that variations in the essential nutrient availability have an effect on the physiology of bacteria in developing biofilms [111]. Numerous methods have been used to investigate how nutrient concentrations affect the formation of bacterial biofilms. For instance, the number of cells in biofilms increase when nutrients were present in high concentrations in drinking water distribution networks [112]. Additionally, the incidence and the amount of *Pseudomonas putida (P. putida)* biofilm forming in a paper mill water stream increased with rising nutrient levels [113]. Also, it has been discovered that adding glucose as a carbon source to the medium promotes the development of biofilm in several bacteria, such as *E. coli* and *P. putida* [111, 113]. On the other hand, adding glucose to different media prevented the development of biofilms in a number of Enterobacteriaceae family species, including *K. pneumoniae, Citrobacter freundii*, and *Salmonella enterica* [114]. According to a research by Rochex and Lebeault, the rate and amount of *P. putida* biofilm formation increased when nitrogen concentration decreased from a carbon/nitrogen ratio of 90–20. On the other hand, the absence of nitrogen caused the *Pseudomonas fluorescens* biofilm to actively detach, as was the case when the amount of glucose was limited [115]. However, variations in yeast extract and peptone concentrations, which are both effective sources of nitrogen, had no discernible effects on the development of *E. coli* biofilms [116]. Increased NaCl concentrations prevented the development of biofilms in other bacteria, including Salmonella species, *Sinorhizobium meliloti, S. aureus, Enterococcus faecalis*, and *P. aeruginosa* [117–119]. According to a research, *A. baumannii* biofilm formation is influenced more by a static environment with high nutrient-containing medium (tryptic soy broth (TSB) or brain–heart infusion broth (BHI)) and components of carbon, glucose, and cation sources (sodium, calcium, and ferric ion) than by hydrodynamic environment. Also, these amendment characteristics of the medium and supplementary sources have an impact on the structural and mechanical characteristics of *A. baumannii* biofilms by reducing stiffness and increasing adhesiveness [120].

Iron concentration

The production of biofilm is hampered by the sources and concentration of iron. Bap is typically increased by the scarcity of iron. A higher iron concentration can make some selective antibiotics more difficult to employ by producing signal or reacting directly with the antibiotics. To scavenge the available iron in the environment, the majority of bacteria produce potent iron-chelating molecules known as siderophores. When siderophores come into contact with iron, an iron–siderophores complex eventually is formed. It binds to the particular outer membrane receptors, which makes it easier for molecules to flow through the outer membrane. To the contrary, when the iron is present in larger concentrations, siderophores are unable to develop complexes and cannot create passageways in the outer membrane, which prevents the antibiotics from dispersing to the outside membrane [121]. Clinical isolates of *A. baumannii* showed a significant decrease in adhesiveness and biofilm development when present on abiotic surfaces with ethanol and an iron-chelating agent [122, 123].

pH

The pH of the environment has a considerable impact on the development of biofilms [124]. It has been shown that this element has an impact on the process of microbial adhesion to surfaces, which is the first step in the formation of biofilms [125, 126]. Additionally, it has been demonstrated that the pH of the medium influences the development of bacterial biofilm, which may affect enzyme function because each enzyme has a preferred pH [127]. Bacterial biofilms are more resistant to pH fluctuations than planktonic cells [128]. For instance, the gel-like structure of the bacterial biofilm can reduce the rapid transport of ions and enable the creation of a pH gradient within the extracellular matrix in severely acidic conditions [129]. However, under alkaline conditions, poorly structured and very thin biofilms have been observed as a result of impaired biofilm maturation, as well as adhesion inhibition for some bacteria such as *S. aureus and Staphylococcus epidermidis* [130]. The ideal pH for *A. baumannii* to develop biofilms in a solution containing NaCl is pH 7 [131].

Strategies for prevention and control *A. baumannii* biofilm

It is difficult to control *A. baumannii* biofilm infections due to increasing rate of biofilms' resistance to antibiotics and limited antibiotics penetration in the matrix. Furthermore, it is crucial to stop these organisms from creating biofilms. So, numerous potent new anti-biofilm treatments have been created to prevent Acinetobacter adherence to biotic as well as abiotic surfaces and to prevent biofilm generation. Three fundamental strategies

have been investigated: inhibition of bacterial attachment to both abiotic and/or biotic surfaces, disrupting targets of biofilms during the maturation steps, and interference with signals or QQ [132]. Properties of biomaterials, chemical and physical, are modified to inhibit initial attachment of biofilm, while different chemicals such as matrix-degrading enzymes, amino acids, surfactants, nitric acid donors, and free fatty acids, in addition to physical forces, can be utilized to remove biofilm. Additionally, inhibition of biofilm via QQ method works by degrading signals of QS, opposing signaling molecules, and inhibiting generation, transduction, and transport of signal [29, 132].

Quorum sensing quenchers
Inhibition of QS signaling pathways reduces the creation of biofilms since QS contributes to the production of biofilms, making it a potential new treatment approach [133]. Enoyl-acyl carrier protein reductase, a crucial enzyme for AHL acyl chains production, may be inhibited by a small amount of the chemical agent having antibacterial qualities triclosan [134]. Vanillin, allin, and patulin/clavacin were discovered to interact with receptors of AHL and prevent QS signals transmission [135–137]. Additionally, AHL analogs, AbaR antagonists (like streptomycin), anoR antagonists (like virstain), as well as antagonists for the enzyme di-guanylate cyclase, which produces cyclic di-GMP, were also discovered to block QS, and hence, development of biofilm is prevented in *A. baumannii* and *Acinetobacter noscomialis* [138–142]. It was discovered that *A. baumannii's* EPS generation, swarming motility, and biofilm formation were inhibited by siphonocholin, a marine steroid with anti-QS activity [143]. AHL lactonase and MomL are two examples of genetically modified QQ enzymes that can effectively suppress QS signal and cause biofilm structure disintegration [144–146].

Natural products/essential oils (EOs)
A. baumannii infections can be successfully reduced by natural materials as animal, microbial, and plant derivatives. Bacterial metabolites were demonstrated to be efficient against the biofilm of *A. baumannii* [147]. A study revealed that crude cell-free supernatants of several bacteria such as *Mycobacterium mucogenicum, Burkholderia cepacia, Sphingomonas capsulate,* Staphylococcus spp., and Methylobacterium spp. were found to inhibit *Acinetobacter calcoaceticus* [148]. *A. baumannii* biofilm development is prevented on medical supplies such as silicone catheters and endotracheal tubes (polyvinyl chloride) by maipomycin A, an iron chelator obtained from actinomycete metabolites [149]. Several bacterial biofilms, including *A. baumannii*, were inhibited by many sets of marine

sponge-derived chemicals using non-microbicidal methods [150]. The 5-episinuleptolide, a soft coral compound obtained from *Sinularia leptoclados*, demonstrated activity against biofilms of *A. baumannii* ATCC R 19606TM and MDR *A. baumannii* strains through the reduction of the pgaABCD locus expression that encodes the PNAG of biofilm structure [151]. Myrtenol, a bicyclic monoterpene derived from diverse plants, has potent anti-biofilm activities against *A. baumannii* clinical strains. Myrtenol particularly decreased biofilm thickness, interfered with the mature biofilm, inhibited the virulence factors associated with the biofilm, and made strains more susceptible to conventional antibiotics. Myrtenol therapy resulted in the reduction of biofilm-related genes such as ompA, bfmR, bap, csuA/B, pgaA, katE, and pgaC [152]. Natural products such as EOs and secondary metabolites of plants may have broad-spectrum antibacterial activity through the disruption of integrity of bacterial membrane and inhibition of ATP synthesis, leading to metabolite/ion leakage [153]. *A. baumannii's* biofilm structure may be severely harmed by various EOs generated from flowering plants such as *Ziziphora tenuior* L. *and Mentha pulegium* L. The main constituents of plant extract were found to be pulegone, menthol, isopulegone, D-isomenthone, and piperitenone [154, 155]. The biofilm inhibition characters of four EO constituents (eugenol, vanillin, carvacrol, and thymol) were tested against food surfaces adhering organisms in meat industry. Thymol and carvacrol demonstrated the highest antimicrobial activity against strains of *A. baumannii* [156]. Additionally, several studies demonstrated that plant-driven EOs that are rich in these constituents (such as cinnamon oil, which contains eugenol, oregano oil, which contains carvacrol and thymol, and eucalyptus camaldulens oil, which contains thymol) were effective against wound infections caused by *A. baumannii* [157, 158]. Strong anti-biofilm action against *A. baumannii* was demonstrated by EO-based nano-emulsions made from the plant *Thymus daenensis* [159]. Shivaprasad et al. demonstrated that the activity of various antibiotics such as cefipime, amikacin, piperacillin/tazobactum, gentamicin, ciprofloxacin, cefoperazone, imipenem, and cotrimoxazole against MDR/extensively drug-resistant (XDR) complexes of *A. baumannii* was enhanced when combined with EO of lemongrass, which demonstrated 65–79% activity against biofilm [160]. The activity of most EOs against bacteria was tested in vitro; however, few examples were examined in animal or cellular models [161]. According to Ismail et al., the EOs extracted from the leaf of *Pimenta dioica* showed a higher activity (85% inhibition) against *A. baumannii* biofilm than EOs extracted from the leaf of *Pimenta racemosa* (34% inhibition). Both extracts demonstrated bactericidal activity against *A. baumannii*.

Furthermore, *A. baumannii* microbial density was significantly reduced in the mouse wound infection model [162].

Antimicrobials peptides (AMPs)

AMPs, cationic peptides with a length of 15–30 amino acids, are alternatives to antibiotics. They are released by the innate immune response and attack the bacterial cell membrane which harbors negative charge [163]. *A. baumannii* biofilm was found to be inhibited by a variety of AMPs with biological sources. Human AMP LL37 is one of the AMPs with activity against *A. baumannii* biofilm [164]. Lactoferrin extracted from human milk and considered an AMP that chelates iron, was less effective for preventing the formation of the *A. baumannii* biofilm than lactoferrin extracted from bovine milk [165]. A derivative (D-RR4) of the short chain synthetic peptide RR, with a length of 12 amino acids, demonstrated high anti-biofilm as well as antibacterial activities against *A. baumannii* and *P. aeruginosa* in both *Caenorhabditis elegans* model and macrophages cells [166]. *A. baumannii* biofilm was inhibited by the antimicrobial peptide magainin 2, which is made up of 23 amino acids and was obtained from the skin of *Xenopus laevis*, an African clawed frog [167]. Many AMPs obtained from flies, including larvae immune peptides derived from *Calliphora vicina*, and cecropin AMP from *Musca domestica,* have also been shown to have potent efficacy against *A. baumannii* biofilm [168, 169]. Jakiewicz et al. examined eight peptides (CAMEL, LL-37, r-omiganan, temporin A, aurein 1.2, citropin 1.1, omiganan, and pexiganan) from various biological origins for their antibacterial activity against the biofilm of *A. baumannii* on tracheal tube segments. Two of these peptides; CAMEL and pexiganan; showed potent anti-biofilm action [170]. Recently, four artificially created chimeric AMPs were demonstrated to exhibit inhibitory actions against the biofilm of MDR *A. baumannii.* Those AMPs inhibited *A. baumannii* biofilm in combination with ciprofloxacin, cefotaxime, or erythromycin [171]. Combining the antibiotics imipenem and tobramycin with WLBU2, a cationic 24-residue synthetic peptide with antimicrobial activity, demonstrated encouraging results against the biofilm as well as the planktonic cells of MDR *A. baumannii* [172]. *P. aeruginosa* and *A. baumannii* persisted cells in the biofilm were destroyed by the synthetic cyclic peptide ZY4, which is 17 amino acids long and exhibits biofilm eradication activity [173]. Some AMPs were used as ointments on the medical devices' surfaces and to prevent superficial tissue infections [167]. Temporin-L demonstrated an anti-biofilm impact in an in vitro trial without causing cell damage, indicating its tremendous potential for clinical application. AMPs have excellent therapeutic treatment potential because they remove biofilms efficiently [174].

Efflux pump inhibitors (EPI)

Many studies have demonstrated that the efflux pumps play different roles in the production of biofilms in ESKAPE infections; as a result, preventing their activity with EPI could also prevent the development of biofilms. When a substance has a wide range of substrate specificities and low off-target toxicity, it can be referred to as a powerful EPI [175]. Phenylalanine–arginine–beta-naphthylamide, one of the most widely applied EPIs against *A. baumannii*, has the ability to prevent the growth of biofilms on the pathogen [176]. It was discovered that the novel serum-associated EPIs, such as ABEPI1 and ABEPI2, enhanced the antibacterial effects of *A. baumannii* cultured in human serum. These substances showed comparable antibiotic synergistic patterns for ciprofloxacin and minocycline as well as reduced pump activity [177]. On the other hand, another study produced a group of substances, named pharmacophores, made of 2-substituted benzothiazoles that significantly inhibited AdeABC efflux pumps when applied in conjunction with ciprofloxacin [178]. The effectiveness of two microbicides, including chlorhexidine and cetrimide, which negatively affected operation and expression of the AdeABC efflux pump in the *A. baumannii* biofilm, was evaluated by Krishnamoorthy et al. Additionally, they demonstrated that the *A. baumannii* negative charge of the cell membrane was reduced through these antimicrobials, resulting in efflux pump inhibition and eventually cell death [179].

Nanoparticles (NPs)

NPs have a very large surface area, a very small size (100 nm), and are exceedingly reactive. They have broad-spectrum antimicrobial activity against gram-positive as well as gram-negative bacteria, and they have occasionally been chosen in place of antibacterial drugs. They have the ability to disrupt the integrity of biofilm through reactive oxygen species (ROS) generation, penetration of bacterial cell membrane, interaction with eDNA, lipids, proteins, and polysaccharides, and ATP depletion [180]. Numerous investigations were conducted to comprehend the function of NPs in inhibiting biofilm of *A. baumannii.* Nitric oxide releasing NPs were used in an in vivo study for the treatment of wound infections associated with *A. baumannii* biofilm in mouse models [181]. According to a different investigation, exposure to a nano-emulsion of the quaternary ammonium salt cetylpyridinium chloride disrupted the *A. baumannii* biofilm [182]. An inhibitory action of NPs against biofilm and planktonic cells was demonstrated when these particles are combined with

metals or extracts of natural product. A specific investigation demonstrated the effectiveness of gold NPs, silver NPs (AgNPs), and silver–gold bimetallic NPs against biofilms of *A. baumannii* with 88% inhibition [183]. Many studies showed the inhibitory action of gNPs against biofilms of Actinobacter, which could be due to the ease of penetration of these NPs through the dense biofilms' EPS. The interaction between negatively charged eDNA and positively charged AgNPs is an important factor in the inhibition of biofilms [184, 185]. Additionally, selenium, curcumin, and aluminum oxide NPs were all found to inhibit the growth of *A. baumannii* biofilms [186–188]. NPs have also been noted that NPs have strong anti-biofilm activity when combined with antibiotics. Since imipenem lyses the bacterial cell wall, it was discovered that AgNPs work in concert with it to promote AgNPs' penetration of bacterial cells [189].

Phage therapy

Bacteriophages are considered another strategy for removing and controlling the biofilms. Different lytic bacteriophages, including vB AbaMIME-AB2 and AB7-IBB2 (family of Podoviridae), were reported to inhibit biofilms of *A. baumannii* (10–8 CFU/well) on both biotic and abiotic surfaces with 60–80% inhibition [190–192]. According to Lood et al., 13 different *A. baumannii* strains caused induction of 21 distinct lysins (prophages). PlyF307 was the most active of these lysins, which resulted in an in vivo and in vitro significant reduction of *A. baumannii* biofilm [193]. Thandar et al. demonstrated that the C-terminal amino acids of P307, a phage lysin, have a high ability (>3-logs) to kill *A. baumannii*, while an enhanced killing activity (>5-logs) was observed for its engineered product (P307SQ-8C) when combined with Polymyxin B [194]. *A. baumannii* biofilms were discovered to be inhibited by B AbaM ISTD and vB AbaM NOVI, two distinct phages obtained from Belgrade wastewaters [195]. When antibiotics commonly used to treat UTIs, such as trimethoprim/sulfamethoxazole, ciprofloxacin, tobramycin, gentamicin, meropenem, and imipenem, and were combined with an environmental phage cocktail, *A. baumannii* biofilm biomass was decreased. While the phage cocktail in combination with levofloxacin and amikacin did not have a synergistic effect [196]. Ran et al. used photodynamic bacteriophages with Nile blue photosensitizers to create an effective way of preventing *A. baumannii* biofilm. ROS production was demonstrated by NB photosensitizers containing sulfur atoms. It was demonstrated, through in vitro and in vivo studies, that NB-phage bioconjugate has the ability to bind to biofilms' main constituents and causes a reduction in drug resistance produced by these biofilms [197].

Photodynamic treatment (PDT) surface modification and physical therapy

Antibiotics are often used for biofilm infections associated with medical devices; however, it is crucial to use new strategies when antibiotics fail to eradicate these biofilms. Physical therapy as well as surface modifications are examples of these strategies that could eradicate and prevent microbial adherence to medical devices. PDT is a powerful physical technique used to prevent biofilm formation and microbial adherence. PDT produces ROS, which prevent the synthesis of several toxic substances that impair bacterial adhesion and biofilm matrix formation, and combat biofilm infections [198, 199]. Also, this method is also used to control infections produced by other implant-related biofilms, such as infections of prosthetic joints and infections brought on by biofilms linked with ventilator-associated pneumonia [200]. By increasing the antibacterial agents' activity, low-intensity ultrasound used at an acceptable level was proven to be effective in biofilm removal [201]. Another efficient physical technique to remove biofilms attached to implants' surfaces was the application of water jets. They can remove biofilms through the mechanical action of pressure and pulse [202]. Surface engineering advances have resulted in the production of antibacterial or anti-adhesion agents that have the ability to coat the surfaces of medical devices and subsequently inhibit microorganisms' growth.

Other biofilm inhibitors

A. baumannii biofilms have also been found to be inhibited by certain additional chemicals or substances that do not precisely belong to the aforementioned classes. Hydrogen peroxide and its formulations were examined to see how well biocides worked to get rid of MDR *A. baumanni* biofilms. In comparison with single-species Acinetobacter biofilm cells, mixed culture biofilms were resistant to various biocides, such as sulfathiazole and hydrogen peroxide. Compared to non-oxidizing biocides such as glutaraldehyde and sulfathiazole, it was found that oxidizing biocides (sodium hypochlorite and hydrogen peroxide) have an increased ability to remove and destroy biofilms [203, 204]. An inhibition of *A. baumannii* biofilms by more than 95% was observed for 2-aminoimidazole compounds when applied at a concentration of 100 M. These compounds could enhance the permeability of many conventional antibiotics through bacterial cell membrane, so they can be utilized in a variety of systems as a novel "drug delivery" approach [205]. The disinfectant octenidine dihydrochloride was found to be efficient in preventing *A. baumannii* biofilms, which were found to accumulate on polystyrene and stainless steel catheters [206]. Orthophthalaldehyde, peracetic acid, sodium

hypochlorite, chlorhexidine, and peracetic acid were investigated for their biocidal activities against *A. baumanni*. Also, biofilm-producing *A. baumannii* was sensitive to all studied antiseptics and disinfectants in about 78% of cases [207]. It was found that replacing 6-position on N2,N4-dibenzylquinazoline-2,4-diamines, inhibitors of dihydrofolate reductase enzyme, with an alkyl or a halide substituent, resulted in a 90% activity against *A. baumannii* biofilms [208]. According to a clinical investigation, reduction in bacterial colonization was observed after using silver alloy, which, in turn, decreased UTIs [209]. Additionally, it was discovered in many clinical trials that nitrofurazone-coated silicon catheters had the ability to minimize UTI during brief use (30 days) [210].

Future prospects
Failure to respond to treatment is brought about by a build-up of resistance via numerous secretory chemicals and cell markers. Situations are getting worse all over the world due to the growth of MDR microbes found in biofilms, which makes this problem a new public health threat. We have covered topics such as how virulence factors of biofilms are increased, the roles of different components involved for biofilm formation, chronic illnesses linked to biofilms, and novel, potentially effective treatments for pathogenic biofilms and biofilm-related infections. In addition, other alternative approaches such as vaccination approach might be future options to protect against the MDR biofilms and prevent the spread of *A. baumannii* infections. In order to immunize against *A. baumannii*, a number of biofilm-associated components, including Bap, PNAG, OmpA, outer membrane vesicles, and Ata, were investigated as prospective candidates for vaccine development [211–216].

Conclusion
A. baumannii is the causative agent of a variety of biofilm-associated and MDR infections both in hospitals and community. For both researchers and clinicians, the genus Acinetobacter is difficult bacteria, because it has the ability to acquire genes responsible for antibiotic resistance and can form biofilms. *A. baumannii* forms biofilms through a multifactorial process that includes both intrinsic and extrinsic variables and is controlled by different regulatory mechanisms that control bacterial cell release from the biofilm, bacterial adhesion, and biofilm maturation. The genes implicated in the development of biofilms were found in both strains that produced biofilms and those that did not; however, these genes were highly expressed in biofilm producers compared to non-producers. Antibiotics are ineffective in treatment due to the development of antibiotic resistance as well as the intricate biofilm matrix structure. To

increase the pipeline for antimicrobial drugs, the current situation urgently calls for innovation, discovery, or repurposing medicinal molecules. Chronic infections produced by preformed biofilms could be managed by exposing the bacterial cells to antibiotics and focusing on matrix components to break down the biofilm's structure. Controlling *A. baumannii* infections linked to biofilms may be possible by interfering with regulatory processes as QS, two-component systems, and nucleotide signaling. Due to the global spread of antibiotic resistance, phage, photodynamic, and nanoparticle therapies have lately attracted attention and demonstrated promising outcomes. To completely minimize the risk associated with these novel techniques, additional in vitro and in vivo researches utilizing them are needed. Due to the fact that biofilms may become polymicrobial and the existing non-invasive in vivo diagnostic techniques have difficulty identifying the etiological bacterium in biofilm-related infections, the management of these infections must include the development of broad-spectrum drugs for enhanced outcomes.

Abbreviations

A. baumannii	*Acinetobacter baumannii*
P. aeruginosa	*Pseudomonas aeruginosa*
P. putida	*Pseudomonas putida*
K. pneumoniae	*Klebsiella pneumoniae*
S. aureus	*Staphylococcus aureus*
E. coli	*Escherichia coli*
ABC	ATP-binding cassette
AgNPs	Silver nanoparticles
AHLs	Acyl homoserine lactones
AI	Autoinducers
NaCl	Sodium chloride
AlgC	Alginate C
AMPs	Antimicrobials peptides
Bap	Biofilm-associated protein
Bla PER1	Beta-lactamase PER-1
C-di-GMP	Cyclic di-guanosine monophosphate
Csg	Curli-specific gene
Csu	Chaperon–usher
eDNA	Extracellular DNA
EO	Essential oil
EPI	Efflux pump inhibitors
EPS	Extracellular polymeric substance
ICUs	Intensive care units
MATE	Multidrug and toxin compound extrusion
MDR	Multidrug-resistant
MFS	Major facilitator superfamily
NPs	Nanoparticles
IN	Inner membrane
OM	Outer membrane
OmpA	Outer membrane protein A
PCR	Polymerase chain reaction
PDT	Photodynamic treatment
PNAG	Poly-β-(1,6)-*N*-acetyl glucosamine
QQ	Quorum quenching
QS	Quorum sensing
RND	Resistance nodulation division
ROS	Reactive oxygen species
SMR	Small multidrug resistance
UTI	Urinary tract infections

XDR	Extensively drug-resistant
TSB	Tryptic soy broth
BHI	Brain–heart infusion broth

Availability of data and materials
The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Declarations

Ethics approval and consent to participate
This article does not contain any studies with human participants or animals performed by any of the authors.

Competing interests
The authors declare that they have no competing interests.

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