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Factors mediating *Acinetobacter baumannii* biofilm formation: Opportunities for developing therapeutics

Kirti Upmanyu [a,b], Qazi Mohd. Rizwanul Haq [b], Ruchi Singh [a,*]

[a] ICMR-National Institute of Pathology, Safdarjung Hospital Campus, New Delhi, 110029, India
[b] Department of Biosciences, Jamia Millia Islamia, New Delhi, India

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ABSTRACT

Acinetobacter baumannii has notably become a superbug due to its mounting risk of infection and escalating rates of antimicrobial resistance, including colistin, the last-resort antibiotic. Its propensity to form biofilm on biotic and abiotic surfaces has contributed to the majority of nosocomial infections. Bacterial cells in biofilms are resistant to antibiotics and host immune response, and pose challenges in treatment. Therefore current scenario urgently requires the development of novel therapeutic strategies for successful treatment outcomes. This article provides a holistic understanding of sequential events and regulatory mechanisms directing *A. baumannii* biofilm formation. Understanding the key factors functioning and regulating the biofilm machinery of *A. baumannii* will provide us insight to develop novel approaches to combat *A. baumannii* infections. Further, the review article deliberates promising strategies for the prevention of biofilm formation on medically relevant substances and potential therapeutic strategies for the eradication of preformed biofilms which can help tackle biofilm-associated *A. baumannii* infections. Advances in emerging therapeutic opportunities such as phage therapy, nanoparticle therapy and photodynamic therapy are also discussed to comprehend the current scenario and future outlook for the development of successful treatment against biofilm-associated *A. baumannii* infections.

1. Introduction

A rapid surge in the incidence of multidrug-resistant *Acinetobacter baumannii* infections has become a threat for public health worldwide (Dijkshoorn et al., 2007; Peleg et al., 2008). *A. baumannii*, opportunistic gram-negative, aerobic, non-motile, coccobacilli, causes nosocomial and community-acquired infections among immune-compromised patients (Dijkshoorn et al., 2007; Lee et al., 2017; Morris et al., 2019). Its genome plasticity provides an advantage to acclimate various mechanisms of resistance, rendering antibiotics ineffective for treatment. Apart from decking the ESKAPE (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter spp.*) pathogen list, now WHO has declared *A. baumannii* as Group-1 priority pathogen for which new antimicrobials are urgently required (Boucher et al., 2009; World Health Organization. 2017).

Among all the 73 species of *Acinetobacter, A. baumannii* causes a wide range of infections in humans, including urinary tract infection, ventilator-associated pneumonia, skin, wound infection, bloodstream infection and meningitis (Dijkshoorn et al., 2007; Parte et al., 2020). Mortality rates as high as 50% associated with *A. baumannii* infections have been reported depending on the strain and type of infection (Cornejo-Juárez et al., 2020). *A. baumannii* has successfully acquired resistance to all the available antimicrobial drugs, including colistin, the last line of therapy (Cai et al., 2012; Lin and Lan, 2014). Its ability to form biofilm in the hospital environment and on medical equipment is advantageous for colonization and persistent infections that are resistant to antimicrobials and imposes challenges in treatment (Pompilio et al., 2021).

This review discusses the intricate biofilm machinery of *A. baumannii* that provides survival advantage and facilitates its establishment on biotic and abiotic surfaces. It describes the range of infections caused by *A. baumannii* and its strategies to combat the effects of antibiotics. This review presents an account of various extrinsic and intrinsic factors mediating biofilm formation, regulatory mechanisms, including Quorum sensing and two-component systems. Further, the opportunities available through these factors to develop therapeutic strategies to prevent bacterial colonization, inhibit biofilm formation or eradicate preformed biofilm and consequently be beneficial to control and reduce the rate of infection caused by *A. baumannii* are discussed.

* Corresponding author.
E-mail address: qhaque@jmi.ac.in (Q.Mohd.R. Haq).

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2. Clinical importance of *A. baumannii*

A. baumannii is responsible for causing approximately 2% of nosocomial infections in the United States and Europe, twice the rate in Asia and the Middle East (Lake et al., 2018; Lob et al., 2016; Sievert et al., 2013). Due to unrestricted antibiotic overuse, drug resistance against all the available antibiotics has been reported. In no time, the pre-antibiotic era will return if efforts in the direction of novel therapeutic strategies are not made (Falagas et al., 2008). *A. baumannii* employs various strategies to combat the effect of antibiotics, including production of β-lactamases, aminoglycoside modifying enzymes, modification of the target site, efflux pumps and permeability defects. Although the rate of infection by *A. baumannii* is comparatively low than other Gram-negative bacteria, but phenotypes with multidrug resistance are worryingly four times higher than other Gram-negative bacteria such as *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (Harding et al., 2018). Initially, *A. baumannii* isolates were susceptible to carbapenems. However, the rate of carbapenem-resistant *A. baumannii* is reported as high as 90% (Central Asian and Eastern European Surveillance of Antimicrobial Resistance: annual report 2016.World Health Organization). A study conducted in Europe, Eastern Mediterranean and Africa showed that *A. baumannii* and carbapenem-resistant *A. baumannii* accounted for 20.9% and 13.6% of nosocomial infections, respectively (Ayobami et al., 2019). Resistance to antibiotics in *A. baumannii* is contributed by mutability, horizontal gene transfer potential and outer membrane vesicles in the evolution of *A. baumannii* as multidrug resistant (MDR), pan-drug resistant (PDR) and extensively drug resistant (XDR).

3. Biofilm formation and biofilm associated infections

Biofilm is a three-dimensional structure formed by microbial cells that become adhered to biotic or abiotic surfaces under the influence of various (few yet unidentified) physiological and environmental factors. Further, these cells continuously multiply and produce extracellular polymeric substances (EPS), forming a matrix encasing these microbes. Biofilm formation and development involves five main steps (Fig. 1):1) Reversible attachment of bacterial cells to a surface. 2) Irreversible adhesion by cell surface-associated factors. 3) Initial biofilm formation induced by the assembly of extra polymeric substances. 4) Biofilm maturation by continuous cell division and production of extra polymeric substances (EPS) 5) Dispersal of cells from biofilm (Petrova and Sauer, 2012; Armbruster and Parsek, 2018).

Biofilm associated infections can be introduced through contaminated medical devices such as intravascular catheters, cardiac devices, prosthetic joints and shunts, prosthetic vascular grafts or can develop independently through open wounds, dental plaques and native valve endocarditis (Joo and Otto, 2012). *A. baumannii* has the ability to form biofilm on clinically relevant substances, allowing it to persist in the hospital environment and is the root cause for a range of infections, including pneumonia, bacteremia, meningitis, urinary tract infection (UTI) and several other diseases among critically ill patients in intensive care units (ICUs) of hospital settings (Colquhoun and Rather, 2020; Poorzargar et al., 2017; Rodríguez-Baño et al., 2008). These infections are associated with biofilm formation and are a thousand times more resilient to antibiotics than free-living planktonic cells (Eze et al., 2018). Biofilm matrix provides protection to the bacterial cells from the action of antibiotics, bacteriophages and help bacterial cells survive under harsh conditions such as desiccation (Gayoso et al., 2014). Several studies have investigated the relationship between drug resistance patterns and biofilm formation abilities among clinical isolates of *A. baumannii*. A previous study showed that *A. baumannii* isolates with high level of resistance were observed to be weak biofilm formers whereas less resistant *A. baumannii* isolates have tendency to form stronger biofilms (Qi et al., 2016). Later a study by Al-Shamiri et al. showed that resistant strains have the ability to form moderate to strong biofilm but sensitive isolates could produce strong biofilms for 24 h but later their biofilm forming ability abridged and formed weak biofilms (Al-Shamiri et al., 2021). Approximately 65–80% of bacterial infections in humans are biofilm-associated, according to statistics of National Institutes of Health and the Center for Disease and Prevention (Jamal

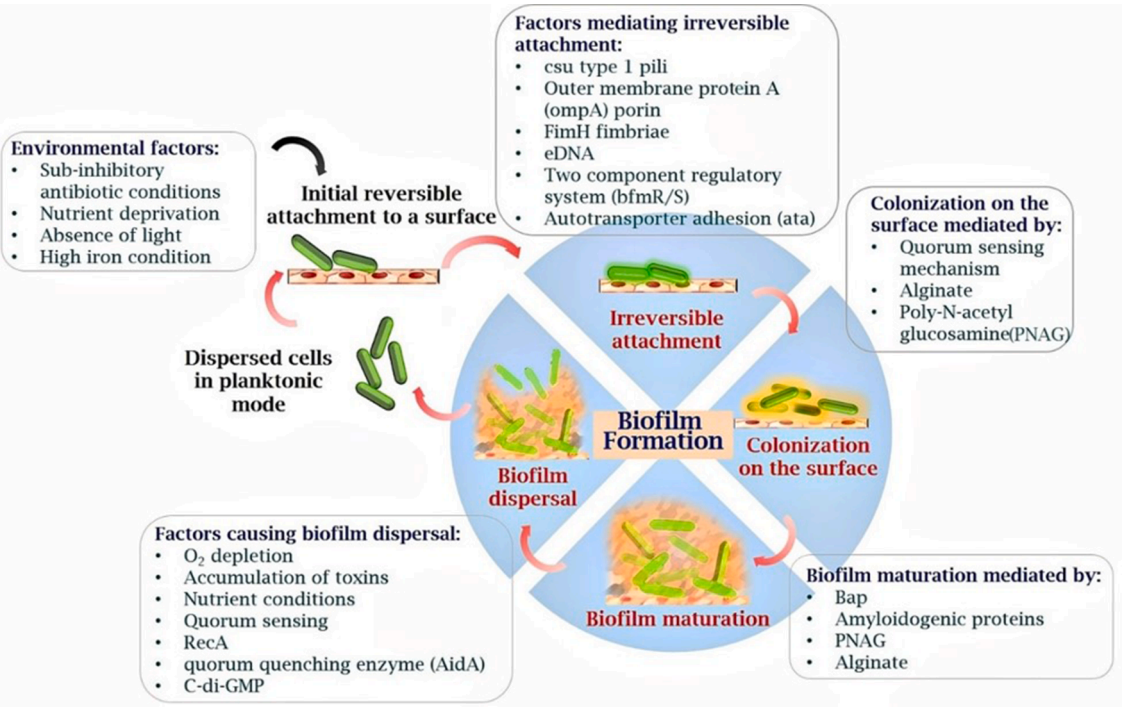

Fig. 1. Intrinsic and extrinsic factors functioning and regulating at each step of *A. baumannii* biofilm formation machinery. Step1: Initial reversible attachment of bacterial cells to a surface. Step 2: Irreversible attachment facilitated by interactions between bacterial cell surface associated factors and biotic or abiotic surface. Step 3: Initial biofilm formation induced by assembly of extra polymeric substances leading to bacterial colonization on the surface. Step 4: Biofilm maturation by continuous cell division and production of extra polymeric substances (EPS) Step 5: Dispersal of cells from biofilm.

et al., 2018). This has also led to substantial economic challenges due to equipment damage, product contamination, energy losses and infections (Wang et al., 2015). Besides challenges in treatment, biofilm-associated infections in tissues also pose diagnostic challenges as the causative microbe cannot be identified by non-invasive imaging techniques. Also, biofilms can become polymicrobial by influencing the surrounding bacteria to associate and colonize (Ryu et al., 2017). Ultimately, treatment involves surgical removal of implants or grafts with biofilm-associated infections.

3.1. Adhesion to biotic and abiotic surface

The switching of bacterial cells from planktonic to sessile mode is driven by multiple biological, chemical and environmental factors. Extrinsic attributes such as nutrient availability, high iron concentration, oxygen depletion, absence of light, temperature, subinhibitory concentrations of antibiotics such as colistin and polymyxin B and intrinsic factors such as drug resistance and carbohydrate metabolism are disclosed to govern this transition of bacterial lifestyle (Eze and El Zowalaty, 2019; Gentile et al., 2014; Sato et al., 2018) . Bacterial cells embedded within biofilm structures can persist there for decades and are able to survive harsh environmental conditions such as desiccation. The initial step is the reversible attachment of bacterial cells to a surface, which is further strengthened by the interactions between the bacterial cell surface proteins described below and the biotic or abiotic surface, resulting in irreversible attachment of the cells.

3.1.1. Csu pili

Surface associated proteins such as pili or porins are the major player in mediating the irreversible attachment of cells to a surface. One such pili system identified in *A. baumannii* is Type 1 chaperon usher pili (csu) encoded by genes clustered together in a polycistronic csu operon assembly system designated csuA/BABCDE (Tomaras et al., 2003). Four subunits, major pilin subunit csuA/B, two adapter subunits, csuA and csuB and the tip csuE, constitute the csu pilus. csuC and csuD function as transport proteins. Impact of each gene deletion on biofilm formation was observed, csuA/B and csuE mutants showed complete abolishment of pilus structure, while csuA and csuB mutants produced few abnormal fibers. The deletion mutants failed to produce biofilm on plastic surface, suggesting that all four subunits are required for a functional pili. csuA/B, csuA, and csuB are predicted to play a role in the assembly of pilus stalk (Tomaras et al., 2003). Besides biofilm formation, csuE also plays a role in twitching motility in *A. baumannii* (Luo et al., 2015; Pakharukova et al., 2018). Structural analysis revealed that csu pili belong to the archaic pili system. Hydrophobic finger-like loops of csuE get inserted into the surface cavities and facilitate irreversible attachment (Pakharukova et al., 2018). The role of the csu pili system in attachment to biotic surfaces is still arguable as earlier studies showed that the csu pili system is not required for the attachment to biotic surfaces such as human epithelial cells (Breij et al., 2009). However, recently, a study by Chen et al. revealed that csu pilus contributes to the adhesion of bacteria to respiratory epithelial cells, and D-mannose significantly inhibited biofilm formation in recombinant *E.coli* JM109/rCsu pilus-producing clone suggesting the sensitivity of csu pilus towards D-mannose (Chen et al., 2021).

The csu pili are known to be regulated by QS signal molecule, acyl-homoserine lactone and a two-component regulatory system, bfmR/S and gacS. On addition of C6-homoserine lactone (HSL), there was 1.5 fold increase in the expression of csuA/B, csuA, csuB, csuC, csuD and csuE genes and 1.33 fold increased expression of chaperon-usher regulators (BfmS and BfmR) (Luo et al., 2015). bfmR mutant cells could not express CsuA/B and were incapable of forming biofilms. Using transcription profiling and functional analysis by genetic mutants, another two-component system called GacSA moderately regulates the expression of csu gene and thus ultimately affects biofilm formation (Cerqueira et al., 2014).

Sub-inhibitory levels of trimethoprim-sulfamethoxazole completely repress the expression of Csu pili in *A. baumannii*, suggesting that inappropriate antibiotic usage can alter population-level behaviours and may trigger the transition to planktonic lifestyle (Moon et al., 2017). Csu pili can be targeted to develop novel compounds for therapy and infection control (Chen et al., 2021).

3.1.2. Outer membrane proteins

Outer membrane proteins (OMPs) of *A. baumannii* such as OmpA, CarO, Omp33 OprD-like, PstS are well-documented to play a role in biofilm formation (Cabral et al., 2011; Eze et al., 2018). Outer membrane receptor proteins were upregulated during biofilm formation when analyzed by two-dimensional gel electrophoresis (Shin et al., 2009). Among several identified OMPs, outer membrane protein A (ompA) is a well-characterized virulence factor owing to its diverse key roles in the survival and pathogenesis of *A. baumannii*, including maintenance of cell membrane integrity, mediating drug resistance, modulation of host immune response, initiation of biofilm formation, invasion of host epithelial cells and triggering host cell apoptosis (Nie et al., 2020). These characteristics make ompA an ideal drug target for controlling *A. baumannii* infections (Nie et al., 2020). OmpA is a beta barrel-shaped monomeric integral outer membrane protein encompassing 8 to 26 antiparallel strands, linked by four loops on the outer membrane surface and three short turns on the periplasmic side (Confer et al., 2013). The role of OmpA in mediating the initial stage of biofilm formation on abiotic surfaces is well defined; besides, it is also required for adhesion to host epithelial cells and facilitates the invasion of *A. baumannii* cells to host epithelial and immune cells (Gaddy et al., 2009). Another study showed that *A. baumannii* cells easily adhered to a 96-well plate coated with fibronectin compared to BSA due to the binding of ompA with fibronectin, suggesting the initial stages of interaction between *A. baumannii* biofilm formation on biotic surfaces (Smani et al., 2012). Choi et al. found that a highly invasive *A. baumannii* 05KA103 exhibited reduced adherence and invasion to epithelial cells when pre-incubated with recombinant AbOmpA. Once *A. baumannii* is internalized within the host cells, it migrates to the nucleus based on the nuclear localization signal (KTKEGRAMNRR) presented by OmpA and mediates host cell apoptosis by causing degradation of chromosomal DNA (Choi et al., 2008). A study showed that immunization of diabetic mice with recombinant OmpA improved survival and reduced bacterial load when later administered with lethal *A. baumannii* infection (Luo et al., 2012). Another mechanism of host cell apoptosis employed by *A. baumannii* Omp38 targets mitochondria and causes the release of proapoptotic molecules such as cytochrome c and other apoptosis-inducing factors (Choi et al., 2005). *A. baumannii* mutants lacking OmpA were comparatively less virulent than wild-type cells showed decreased adherence to human airway epithelium cells, and formed weaker biofilms (Gaddy et al., 2009; Lin et al., 2020).

3.1.3. Extracellular DNA

The presence of extracellular DNA (eDNA) in biofilm and its role in cell adhesion, biofilm formation and maintenance was first studied by Whitchurch et al. in *Pseudomonas aeruginosa* (Whitchurch et al., 2002). Later many studies in other bacterial cells also showed the presence of eDNA in biofilm and its pivotal role in providing structural stability to the biofilm, promoting the production of extracellular polymeric substances and transforming other neighbouring competent bacterial cells (reviewed by Ibáñez de Aldecoa et al., 2017). However, studies on the role of eDNA in *A. baumannii* biofilm are somewhat limited. A study conducted using a clinical isolate of *A. baumannii* AIIMS 7 revealed that eDNA release was facilitated either in free form or encapsulated within membrane vesicles and did not involve cell lysis at an early stage. This implies that eDNA could facilitate the initial steps of adhesion in the formation of biofilm, supporting other adhesion proteins that may subsequently come into play (Sahu et al., 2011). Moreover, treatment with DNaseI on 24 h old preformed biofilm resulted in its depletion by

almost 60%, which signifies the crucial role of eDNA in biofilm maintenance (Sahu et al., 2011; Tetz et al., 2009).

3.2. Quorum sensing regulates biofilm formation in A. baumannii

Quorum sensing (QS) is the eminent property of bacterial cells that facilitates communication within their microenvironment. QS involves the production of small diffusible signaling molecules termed autoinducers (AI), which interact with the receptors of neighbouring cells and induce the expression of targeted genes to respond to a stimuli in a coordinated way (Li and Tian, 2012). Three classes of QS systems have been identified in bacteria: 1) luxI/luxR system in Gram-negative bacteria, which involves acyl-homoserine lactone as an autoinducer type I (AI-1); 2) oligopeptide-two-component-type QS identified in Gram-positive bacteria, uses small peptides as signal molecules; 3) luxS system encoding autoinducer 2 (AI-2) quorum sensing molecule found in both Gram-negative and Gram-positive bacteria (Li and Tian. 2012). In *Acinetobacter spp*. AI-I acyl-homoserine lactone (AHL) QS system has been identified. The first step in QS involves the synthesis of AHL by AbaI synthase. Medium to long chain AHL (C6-C14) are produced by combining the acyl side chain of a specific acyl-acyl side chain protein (acyl-ACP) from a fatty acid biosynthetic machinery to the homocysteine moiety of S-adenosine methionine. The intermediate, N-acyl homoserine, lactonizes to produce acyl-HSL, releasing methylthioadenosine (Li and Tian. 2012). In *A. baumannii* N-(3-hydroxydodecanoyl)-L-HSL (AHL) is produced. In the second and third steps, this signaling molecule diffuses through the membrane in the environment. It interacts with the AHL receptor, abaR, present on the surface of neighbouring bacterial cells. Next, the receptor-signal complex is retrieved from the cell surface, binds to the promoter region, and activates the transcription of pathogenicity and biofilm-related genes (Zhong and He, 2021). A study showed increased expression of csu pili and biofilm formation as a result of AHL interaction with the abaR receptor (Luo et al., 2015). Recently, the role of abaM in regulating QS-dependent and QS- independent genes in *A. baumannii* 5075 has been identified. Increased levels of N-(3-hydroxydodecanoyl)-L-HSL (AHL) positively activate the expression of abaM, which is a negative auto-regulator and negatively regulates the production of AHL by repressing abaI and abaR expression. abaM mutant showed increased surface motility and biofilm formation by reduced virulence in *Galleria mellonela* compared to wild type (López-Martín et al., 2021). Iron depletion leads to increased expression of the QS gene, which regulates virulence gene expression and biofilm formation (Kim et al., 2013). Developing strategies targeting quorum sensing cascade using QS inhibitors or QS quenchers would be a practical approach for treating persistent *A. baumannii* biofilm-associated infections (Zhou et al., 2020).

3.3. Secretion of extracellular polymeric substances for maturation and maintenance of biofilm

Following irreversible attachment to the surface, bacterial cells are triggered to produce extra polymeric substances (EPS) that creates the biofilm matrix. Bacterial cells form the stable three-dimensional structure of mature biofilm with EPS such as glycoproteins, glycolipids, poly-N-acetyl glucosamine (PNAG), alginate, DNA and Biofilm associated proteins (Bap).

3.3.1. Poly- N-acetyl glucosamine (PNAG)

PNAG contributes as a major component of the biofilm matrix encoded by the *pgaABCD* locus (Choi et al., 2009). *A. baumannii pgaA* encodes for a predicted transmembrane protein containing a porin like domain, suggesting its role in the translocation of PNAG across the outer membrane. *pgaB* encodes for 510 amino acids with a putative polysaccharide deacetylase domain. Recently, a study on pgaB revealed that the C-terminal domain of the protein has a glycoside hydrolase activity, which might play a role in the disruption of PNAG (Little et al., 2018). *pgaC* encodes for a 392 amino acid N glycosyltransferase that belongs to the glycosyltransferase 2 family and is involved in the biosynthesis of PNAG. PgaC contains conserved five amino acids (Asp112, Asp205, Gln241, Arg244 and Trp245) critical for the glycosyltransferase activity of the protein. Gene *pgaD* encodes for a 150 amino acid protein that localizes in the cytoplasm and assists pgaC in the synthesis of PNAG (Itoh et al., 2008).

In the same study, deletion of *pgaABCD* locus resulted in a significant decrease in the biofilm formation of cultures grown in glass tubes with vigorous shaking. However, no significant difference was observed between the *A. baumannii* wild type strain and its PNAG negative counterpart when cultured in polystyrene tissue culture wells under static conditions suggesting the role of PNAG in maintaining the integrity of biofilm in a dynamic environment with high shear forces. Another study showed that prior passive immunization in murine pneumonia and murine bacteremia model using a synthetic oligosaccharide conjugate vaccine against PNAG resulted in high levels of the opsonic killing of various PNAG positive A. baumannii strains and reduced CFU levels in the lungs and blood of mice following *A. baumannii* infection (L.V. Bentancor et al., 2012).

Using the glycomics microarray technique, it was found that the presentation of PNAG on *A. baumannii* surface involves PNAG binding lectins, which may also play a role in biofilm formation and pathogenesis (Flannery et al., 2020).

3.3.2. Biofilm associated protein (BAP)

Bap (Biofilm associated protein) is a high molecular weight (854 kDa) protein identified in *A. baumannii* as a homolog of Bap produced by *Staphylococcus aureus* (Loehfelm et al., 2008). Being a hydrophobic cell surface protein, it helps in forming biofilms on human cell surfaces and medically relevant substances such as polystyrene, polypropylene and titanium (Brossard and Campagnari, 2012; Goh et al., 2013; Loehfelm et al., 2008). Bap forms a multidimensional arrangement of mature biofilm and creates water channels in between. This was affirmed by a study where wild type *A. baumannii* strain 307–0294, displayed typical biofilm phenotype, whereas bap mutants failed to produce mature biofilm and remained in a single layer, indicating the role of bap in biofilm maturation rather than the initial stage of adherence (Brossard and Campagnari, 2012; Loehfelm et al., 2008). It is one of the largest proteins yet described in the bacterial population comprising of 8621 amino acids. On sequence analysis, Bap protein was found consisting tandemly arranged repeated domains. The first half comprised A to C modules arranged in an alternate fashion. The second half was composed of 28 direct tandem repeats of module D. Each module belongs to an immunoglobulin-like fold superfamily (Loehfelm et al., 2008). It is highly polymorphic and classified into three main types based on the changes in the repetitive and carboxyl-terminal end. Analysis of different STs, revealed that 29% of *A. baumannii* feature type-1 Bap, 40% type-2 Bap, 11% type-3 Bap and 20% of the strains lack Bap (Gregorio et al., 2015). Recently, bap-like proteins (blp) have been identified in *A. baumannii*, displaying similar characteristics as Bap. A recent study found that blp1 coding sequences vary across *A. baumannii* lineages resulting in functional differences during biofilm maturation with some types exhibiting enhanced adherence and others forming highly complex biofilm structures (Skerniškytė et al., 2019). A study showed recombinant Bap containing 371 amino acid conserved immunodominant region as an effective vaccine candidate for immunization against *A. baumannii* (Fattahian et al., 2011). Another study identified 9 potent vaccine peptides using immuno-informatics approach targeting *A. baumannii* Bap. However, further *in vivo* studies are required to assess the immune response and memory for its application against *A. baumannii* infections (Girija et al., 2021).

3.3.3. Efflux pumps

Efflux systems have gained importance as antimicrobial resistance determinants mediating resistance by pumping out the antibiotic and

other metabolites and toxins out of the cell. Five major subfamilies of the efflux system have been identified in prokaryotes: ATP-binding cassette (ABC), resistance nodulation division (RND), small multidrug resistance (SMR), major facilitator superfamily (MFS), and multidrug and toxin-compound extrusion (MATE). In *A. baumannii*, families of efflux system identified are MFS, MATE, AbeM, RND, AdeABC, SMR, ABC and MacB (Abdi et al., 2020). RND efflux pumps in *A. baumannii* are of three types, AdeABC, AdeFGH, and AdeIJK (Eze et al., 2018). Investigations on biofilm formation mechanisms are directed towards efflux pumps' involvement in the formation of biofilm. Analyzing the whole transcriptome of *A. baumannii* from biofilm and planktonic conditions showed the overexpression of RND (A1S_0009, A1S_0116 and A1S_0538) and MFS (A1S_1316) efflux genes. Some efflux genes such as A1S_1117, A1S_1751 and adeT were expressed only in cells present in biofilm state and not in planktonic cells (Rumbo-Feal et al., 2013). In a clinical *A. baumannii* isolate, AdeFGH efflux pump was overexpressed along with abaI in sessile condition, suggesting its role in the efflux of substrates required for biofilm formation (He et al., 2015). Targeting RND efflux pump with its inhibitor Phenylalanine-arginine beta-naphthylamide (PaβN) significantly reduced the biofilm formation ability of *A. baumannii* and weakly eradicated preformed biofilm (Chen et al., 2020).

3.3.4. Alginate

algC encodes a bi-bifunctional protein phosphomannomutase/phosphoglucomutase (PMM/PGM), belongs to α-D-hexomutase superfamily and synthesize alginate and lipopolysaccharide (LPS) core contributing as components of biofilm matrix (Coyne et al., 1994; Goldberg et al., 1993Lu and Kleckner, 1994). In *P. aeruginosa*, regulation of algC gene expression could depend on attachment to the surface and facilitate biofilm formation on clinically important surfaces, leading to the worsening situation with limited treatment options (Wiens et al., 2014). A study on clinical *A. baumannii* strain showed that PMM/PGM requires Mg2+ and activator glucose 1,6-bisphosphate for its catalyzing alginate and LPS core production. Genetic characterization of algC gene in an MDR strain *A. baumannii* demonstrated a quantifiable differential expression pattern in biofilm cells compared to planktonic counterpart cells over 96 h. Alginate lyase is a glycoside hydrolase that degrades alginate, causes dispersal of bacterial cells from biofilms and increases the antibiotic efficacy effect on *A. baumannii* biofilm (Sahu et al., 2014).

3.3.5. Amyloidogenic proteins

Curli-specific gene operon csg encodes for curli fibres, an amyloid protein contributing to matrix formation. Amyloids have been identified in both bacteria and fungi. Amyloids have been known to assist adhesion between bacterial cells and bacterial-host resulting in the formation of biofilm. The amyloid protein is composed of a major subunit encoded by csgA. Curli fibres play a role in adherence and invasion of host cells and induce host inflammatory response (Barnhart and Chapman, 2006). Targeting amyloid structures may help in controlling biofilm-associated bacterial infections (Jaisankar et al., 2020).

4. Regulatory mechanisms

The transition of microbial cells from planktonic to sessile mode involves various regulatory mechanisms that control attachment of bacterial cells to a surface, production of extracellular matrix and dispersal of bacterial cells from biofilm. These regulatory networks coordinate gene expression profiles among the bacterial population in response to antibiotic exposure, environmental factors and cell density.

4.1. Second messenger signaling pathway

Various nucleotide messenger signaling pathways such as c-di-GMP, c-AMP, (p)pp-G-pp, c-di-AMP, c-AMP-GMP have been identified in the bacterial population and regulate several physiological traits (Hengge et al., 2015). Cyclic di-guanosine monophosphate (c–di-GMP) second messenger signaling is highly conserved across all bacterial population and plays a role in regulation of various bacterial traits associated with pathogenicity such as virulence and biofilm formation and physiology like cell motility, cell division and differentiation (Ryan et al., 2013). Also, in *A. baumannii*, the role of c-di-GMP signaling in biofilm formation and surface motility by regulating csu gene expression has been recently established (Ahmad et al., 2020). Synthesis of c-di-GMP is catalyzed by the diguanylate cyclase (DGC) enzyme containing the active GGDEF (Gly-Gly-Asp-Glu-Phe) domain. Another enzyme, phosphodiesterase (PDE), containing the conserved EAL (Glu- Ala- Leu) domain, catalyzes the degradation of c-di-GMP into two GMP molecules. Increased c-di-GMP levels induce biofilm formation; after reaching a threshold, phosphodiesterase causes degradation of c-di-GMP and downregulation of biofilm-associated genes leading to dispersal of biofilm matrix (Hengge et al, 2009). Further studies on c-di-GMP mediated regulation of biofilm-associated factors are required to exploit c-di-GMP as a drug target.

4.2. Two-component systems

A two-component system, bfmR/S was identified as a central regulator of biofilm formation in *A. baumannii* by Tomaras et al. bfmS is a transmembrane sensor histidine kinase that responds to the yet undefined extracellular or intracellular stimuli. bfmR is a cytosolic response regulator, transduces signals conforming to bfmS and regulates the expression of genes required to initiate and maintain biofilms. bfmR mutant *A. baumannii* cells displayed reduced adherence to the abiotic surface compared to that of wild type strain (Tomaras et al., 2008). The initial stage of biofilm formation requires the expression of pili, helping the attachment of cells to the surface. Deletion of bfmR in *A. baumannii* led to the complete loss of csu expression, and hence the biofilm formation ability was significantly reduced. The structure of bfmR was inferred by combining the X-ray crystallography, solution NMR, chemical crosslinking and mass spectrometry. Phosphorylation (activation) of bfmR at a conserved aspartate residue (Asp58) by bfmS stimulates an increase in bfmR dimerization that binds to the target DNA sequence. A study showed that inactive bfmR (dephosphorylated) binds with a strong affinity to its promoter compared to its active (phosphorylated) state. This signifies that on activation, bfmR prefers binding to its target rather than binding to its promoter in order to control the production of more bfmR (Draughn et al., 2018). Another study by Farrow et al. highlighted the crucial role of bfmR in *A. baumannii* to survive desiccating conditions (Farrow et al., 2018). Compared to the wild type, the ability of bfmR mutant *A. baumannii* to survive drying conditions was greatly reduced and was restored when cells were supplemented with an intact copy of bfmR on a multi-copy plasmid (Farrow et al., 2018). bfmR might also play a role in capsule production since exopolysaccharides play a vital role in bacteria in surviving desiccation. Inactivation or complete loss of bfmS resulted in increased capsular polysaccharide production. However, bfmRS mutant strains did not show this phenotype, suggesting that bfmR may play a role in regulating polysaccharide biosynthesis (Farrow et al., 2018). Recently a study showed that targeting bfmR could be a potential drug target in controlling *A. baumannii* infections (Russo et al., 2016). Another rare phenotype exhibited by *A. baumannii* is the formation of pellicles on liquid-air surface, which is also regulated by bfmR (Krasauskas et al., 2019). Moreover, bfmR down-regulates contact-dependent inhibition and may allow other strains to incorporate. Another two-component system, AdeRS, was shown to regulate *A. baumannii* biofilm formation on plastic and mucosal tissue in an *ex vivo* model by regulating the expression of AdeABC multidrug efflux pump (Richmond et al., 2016).

5. Biofilm dispersal

Biofilm dispersal is a vital phenomenon involving detachment of

cells encased within the matrix, transit to planktonic mode to migrate to another site, consequently spreading infection. This phenomenon may be prompted by environmental cues such as nutrient availability, oxygen deprivation, accumulation of waste products, etc. (Rumbaugh et al., 2020). Intrinsic regulatory mechanisms such as bfmR/S, quorum sensing system, second messenger signaling pathway, catabolite regulatory protein and sRNA regulatory pathway may also govern biofilm dispersal. Studies describing the dispersal mechanism in *A. baumannii* are limited. A study by Runci et al. showed that nutrient limiting conditions favor biofilm dispersal in *A. baumannii*, which contradicts the previous observations by James et al. which showed that high nutrient conditions cause biofilm dispersal. (Runci et al., 2017; James et al., 1995). Employing the "omics" approach to study intrinsic regulatory mechanisms governing this aspect and exploiting them to allow early dispersal and co-administration of antibiotics can be a promising approach for treating biofilm infections (An et al., 2021). A recent study identified mutations in biofilm dispersed cells that were initially exposed to sub-inhibitory concentration of antibiotics for 3 days, and genomic DNA was isolated from 6 days old biofilm cells. The acquired mutations conferred survival advantage in the presence of antibiotic and increased biofilm production. The study showed that many hypothetical proteins with unknown functions were significantly upregulated or downregulated, which may be associated with biofilm formation (Penesyan et al., 2019). Another study identified the role of RecA in regulating biofilm formation, maturation and dispersal through bfmR response regulator. Loss of RecA promoted attachment of cells and production of extracellular matrix during the early stage of biofilm formation resulting in more prominent biofilms. In contrast, increased expression of RecA caused reduced attachment and dispersal of biofilm cells (Ching et al., 2020). Further studies are required to understand the mechanisms of *A. baumannii* biofilm dispersal.

6. Strategies for controlling biofilm-associated *A. baumannii* infections and emerging therapeutic opportunities

Biofilm matrix is believed to restrict the penetration of antibiotics in the deeper layers of biofilm, but some of the studies showed that antibiotic diffusion and the rate of penetration depends on the chemical properties of the antibiotic used (Del Pozo et al., 2007; O'Toole et al., 2001; Shigeta et al., 1997). Moreover, the slowed growth rate and presence of persister cells in the deeper layers within the biofilm escape the effects of antibiotics, particularly targeting the growth phase (Verderosa et al., 2019).

The current treatment strategy for biofilm-associated infections depends upon the involvement of contaminated medical implants or colonization of host tissues. Infections associated with indwelling medical devices require surgical removal of implant for successful treatment results. Other cases when bacteria directly colonize host tissues are chronic infections. In such cases, merely reducing the biofilm by antibiotic treatment is the only possible way presently. Various studies and clinical observations demonstrated that treating solely with antibiotics is insufficient in eradicating preformed biofilm.

New Strategies focusing on inhibiting biofilm formation and eradicating preformed biofilm are urgently required to combat biofilm-associated infections. These strategies may take account of the following approaches (Fig. 2), and the choice may depend on the type of biofilm infection as mentioned above:

1) Developing polymers for medically relevant devices that can inhibit bacterial colonization
2) Novel antibiofilm compounds (sole or in combination) for coating medical devices
3) Targeting EPS of the matrix for biofilm disruption/ dispersal along with antibiotics to target dispersed microbial cells.
4) Targeting regulatory mechanisms such as QS, second messenger signaling and bfmR/S, which regulate biofilm formation.

6.1. *Inhibition of biofilm formation*

6.1.1. *Antibacterial polymers*

An ideal antibacterial polymer should constrain the initial

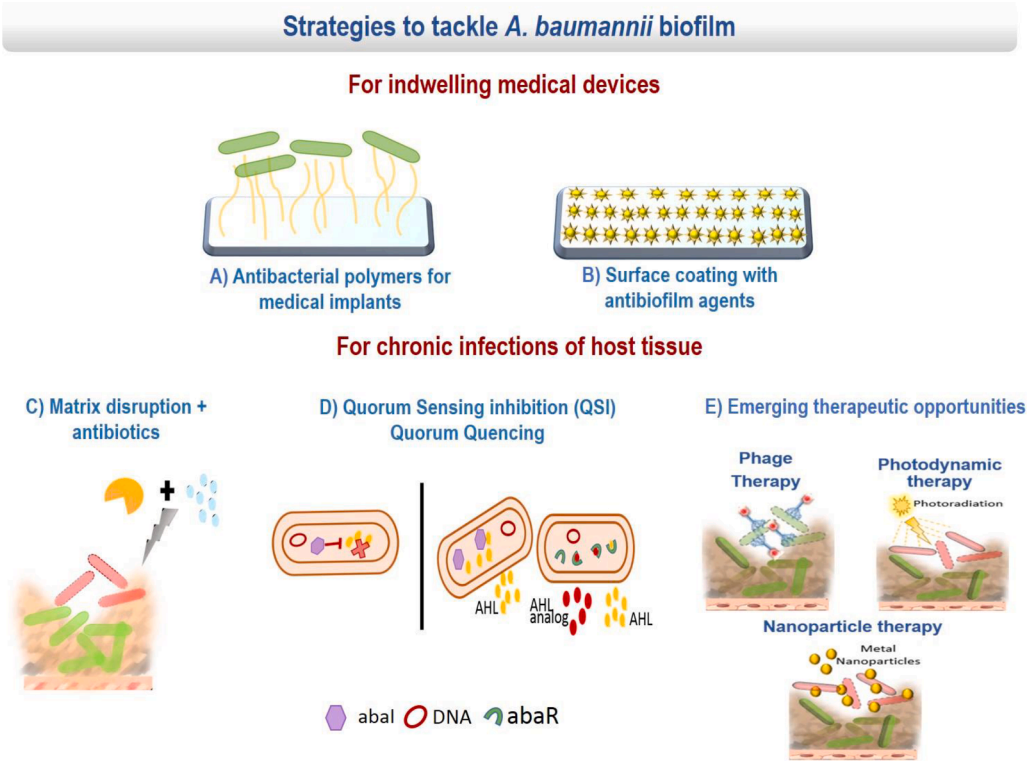

Fig. 2. Strategies to tackle *A. baumannii* biofilms. Preventive strategies using A) antibacterial polymers and B) surface coating with antibiofilm agents to inhibit biofilm formation by *A. baumannii* on medically relevant surfaces and strategies for eradication or dispersal of preformed biofilms of chronic infections by C) degradation of matrix using enzymes or natural or synthetic compounds, D) targeting quorum sensing mechanism of *A. baumannii* by QS inhibition or quorum quenching, and E) emerging therapeutic strategies such as phage therapy, photodynamic therapy and nanoparticle based therapy.

attachment of bacterial cells to prevent colonization or eradicate preformed biofilms on the surface. Efforts toward formulating or modulating existing polymers to develop antibacterial surfaces have been made against several bacteria. Designing polymers with cationic and hydrophobic domains which act on the negatively charged membrane of gram-negative bacteria and the hydrophobicity allows penetration of the polymers into the cell membrane, causing membrane disruption and consequently cell death (Ghosh et al., 2019; Huang et al., 2016; Uppu et al., 2016). In a previous study, methacrylate polymers with 2-aminoimidazole subunit have been shown to prevent colonization by *A. baumannii* (Melander et al, 2011). Another study by Uppu et al. showed that maleic anhydride based amphiphilic polymer with amide side chain could disrupt preformed *A. baumannii* biofilms (Uppu et al., 2016). Hydrogel matrices consisting of polymeric chains with physical or chemical crosslinking can be modified to have antibacterial properties (Li et al., 2018). Silver nanoparticle (Ag NPs) containing hydrogel with N-terminally 2- (naphthalene-6-yl) acetic acid-protected Phe--Phe-Cys-peptide (Nap-FFC), inhibited methicillin-resistant *S. aureus* and *A. baumannii* (Simon et al., 2016). This can be an alternative to current strategies when designed with a combinatorial mechano-chemical approach and prevent the development of resistant phenotypes (Zheng et al., 2021).

6.1.2. Coating medical devices with compounds that inhibit biofilm formation

A preventative approach to control biofilm-associated infections is coating medical devices with antibacterial and antibiofilm blends (Veerachamy et al., 2014). A study by Moon et al. showed that trimethoprim- sulfamethoxazole prevent biofilm formation by inhibiting the expression of csu pilus by inducing folate stress (Moon et al., 2017). Another study by Song et al. described the efficiency of tigecycline, imipenem-rifampicin and colistin-rifampicin to prevent or reduce biofilm formation caused by *A. baumannii* strains (Song et al., 2015). Nevertheless, the option of antibiotics for this purpose seems inappropriate due to the resistance patterns observed against all microbes. Synthetic or natural compounds, including antiseptics, metal oxides, metal nanoparticles, furanones etc. can be utilized for coating medical devices. Additionally, this approach can target adhesion molecules for biofilm inhibition. To achieve this, molecules such as ompA and csuE, can be targeted as they are more prevalent than other adhesion molecules (Table 1). Vila et al. identified AOA-2, a synthetic cyclic hexapeptide that binds to the cavities formed by extracellular loops of ompA and blocks cell adherence in *in vitro* and *in vivo* models (Vila et al. 2017). The same group further showed the efficacy of AOA-2 when co-administered with colistin in murine peritoneal sepsis model against colistin susceptible and colistin-resistant *A. baumannii* (Parra-Millan et al., 2018). In Another study, 15 compounds were identified as *A. baumannii* ompA promoter inhibitors from a library of 7520 compounds with >70% growth inhibition of *A. baumannii* ATCC 17,978. One of these compounds, 62,520 (5-fluoro-1-((1R,3S)−3-(hyroxymethyl)−1.3-dihydroisobenzofuran-1-yl) pyrimidine-2,4-(1H,3H)-dione), was found to downregulate ompA expression, thus suppressing the virulence and biofilm formation ability. Moreover, at higher concentrations, it also showed antimicrobial activity against carbapenem-resistant *A. baumannii* (Na et al., 2021a,b). Another study demonstrated that virstatin, a small chemical compound prevents *A. baumannii* biofilm

Table 1
Prevalence of genetic factors contributing to biofilm formation and maintenance.

Genetic factor	Type	Role	Prevalence in *A. baumannii* isolates	Reference
csuE	Subunit of Type 1 pilus system	Initial adhesion of bacterial cells to the abiotic and biotic surface	85–100%	Silva et al., 2021; Zeighami et al., 2019
ompA	Outer membrane protein A (Porin)	Adhesion to biotic and abiotic surfaces	68.8%−81%	Shenkutie et al., 2020; Zeighami et al., 2019
pgaB	Glycoside hydrolase	Disruption of PNAG	98%	Zeighami et al., 2019
Bap	Extracellular protein	3-dimensional structure of the biofilm	79.2–100%	Goh et al., 2013; Shenkutie et al., 2020; Silva et al., 2021
algC	Gene encoding phosphomannomutase/ phosphoglucomutase	Alginate production	NA	Sahu et al., 2014
bfmS	Sensor kinase	Phosphorylates bfmR, a response regulator involved in biofilm formation.	70–92%	Silva et al., 2021; Zeighami et al., 2019
bla_{PER-1}	B-lactamase	Strains harbouring bla_{PER-1} gene showed increased biofilm formation compared to strains where it was absent.	30.2%	Liu et al., 2016
FimH	Type I Fimbriae protein	Bacterial cell adhesion	6.8 – 50%	Abdullah and Ahmed, 2019; Bentancor et al., 2012; Padmaja et al., 2020
epsA	Polysaccharide export outer membrane protein	Potentially plays a role in the export of polysaccharides during biofilm formation.	95%	Russo et al., 2010; Zeighami et al., 2019
Ptk/wzc	Putative tyrosine kinase	Plays a role in K1 capsular polysaccharide production	95%	Russo et al., 2010; Zeighami et al., 2019
csgA	Curli specific gene A	Amyloidogenic proteins contribute to matrix formation	20.54%−70%	Jaisankar et al., 2020
kpsMII	Group 2 capsule synthesis	Production of capsule	57–75%	Kanaan et al., 2021; Zeighami et al., 2019,
Pap	Pili system	Homologous to *E. coli* pili and associated with *A. baumannii* biofilm formation	80%	Kanaan et al., 2021; Li et al. 2017
Prp	Photoregulated pilus system	biofilm formation in response to light	NA	Wood et al., 2018
Type IV pili	Pili system	adhesion to host cells and stainless steel	NA	Ronish et al., 2019
LHp2–11,085	Gene encoding for protein	Attachment to biotic and abiotic surfaces	NA	Zarrilli et al., 2016
RecA	DNA repair protein	Negatively regulates biofilm formation through bfmR and causes dispersal of cells.		Ching et al., 2020
blsA	Blue light-sensing protein	Inhibits biofilm formation in the presence of blue light	NA	Mussi et al., 2010
Cas3	CRISPR/ cas endonuclease	Genomes with the crispr system were enriched with biofilm associated genes.	NA	Mangas et al., 2019; Tang et al., 2019
Ata	Autotransporter adhesin	Role in adhesion to host cells and infection	NA	Weidensdorfer et al., 2019

formation by inhibiting the synthesis of pilus system (Chabane et al., 2014).

6.2. Therapeutic approach for preformed mature biofilms

6.2.1. Biofilm disruption/ dispersal by targeting EPS of the matrix in combination with antibiotics to target dispersed cells

Degradation of matrix components that encase the target bacteria with EPS degrading molecules along with antibiotics is a promising approach to control preformed biofilms. EPS can be targeted by blocking their production, adhesion inhibition to the surface or degradation of EPS in mature biofilms (Jiang et al., 2020). Antibiotics such as colistin, rifampicin, imipenem and tigecycline have been assessed for their ability to eradicate biofilm embedded *A. baumannii* cells. These studies showed that a combination of imipenem and rifampicin, colistin and a high concentration of tigecycline could eradicate biofilm more efficiently than the individual treatment (Sato et al., 2021; Song et al., 2015). Enzymatic treatments to dissolve the components of biofilm matrix have been widely studied in *A. baumannii* and other Gram-positive and Gram-negative bacteria, as summarized in table 2. Other compounds such as 5-iodoindole could reduce ATCC 17,978 preformed biofilm by 62 ± 8% (Raorane et al., 2020). Chitosan, a natural compound, has been reported to have antibacterial and antibiofilm properties against several bacteria, including *A. baumannii* (Jiang et al., 2020; Costa et al., 2017a; Costa et al., 2017b). Another study showed the anti-biofilm effects of 5-episinuleptolide, isolated from *Sinularia leptoclados* against *A. baumannii* ATCC 19,606 and three other MDR *A. baumannii* strains by decreasing pgaABCD expression, encoding for PNAG (Tseng et al., 2016).

6.2.2. Targeting regulatory mechanisms such as quorum sensing, second messenger signaling and bfmR/S

Biofilm is a highly regulated process governed by multiple regulatory mechanisms. Production of AHL is one of the mechanisms crucially regulating biofilm formation. Quorum quenching by targeting AHL molecules is a practical approach to inhibit or disrupt biofilm. A study showed that AHL lactonase, MomL, is responsible for degrading AHL molecules and causes a reduction in *A. baumannii* biofilm formation (Zhang et al., 2017). Structural analogues such as S-adenosyl methionine, Sinefungin and Butyryl SAM inhibit AHL production by AHL synthase in *P. aeruginosa* (Brackman et al., 2015; Shin et al., 2019). The second messenger signaling molecule, cyclic di-GMP, is highly conserved across gram-negative bacteria and plays a role in the regulation of biofilm formation. Four molecules LP 3134, LP 3145, LP 4010, and LP 1062, were identified in an *in silico* pharmacophore-based screening that inhibited c-di-GMP production by targeting DGC enzymes, causing biofilm inhibition and eradication on silicon catheter. Of these four compounds LP 3134 was the most promising due to broad

Table 2
Antibiofilm agents against ESKAPE pathogens that interfere with different steps of biofilm formation having similarities with biofilm associated factors of *A. baumannii* to inhibit or eradicate preformed biofilms.

Interference with biofilm mechanism	Name of compound/ molecule	Classification	Target molecule	Organism	Reference
Adherence inhibition	AOA-2	Antimicrobial peptide	OmpA	*A. baumannii*	Parra-Millan et al., 2018; Vila et al., 2017;
	Virstatin	Small chemical compound	csuE	*A. baumannii*	Chabane et al., 2014
	zerumbone	Chemical compound	ompA	*A. baumannii*	Kim et al., 2020
	Imidazole	Organic compound	csgA	*A. baumannii*	Jaisankar et al., 2020
Matrix disruption	Aureolysin	metalloprotease	Bap	*Staphylococcus*	Marti et al., 2010
	Serine protease (SspA), Cysteine protease (SspB, Scp)	Enzyme	Bap	*S. aureus*	Marti et al., 2010
	DNase I	Enzyme	DNA	*P. aeruginosa, Acinetobacter baumannii, E. coli, Klebsiella pneumoniae, S. aureus, Enterococcus faecalis,*	Jiang et al., 2020
	Dispersin B	Enzyme	PNAG	*S. aureus, A. baumannii, K. pneumoniae, E. coli, and Pseudomonas fluorescens.*	Jiang et al., 2020
	Alginate lyase	Enzyme	Alginate	*A. buamannii*	Sahu et al., 2014
	L-adrenaline		Bap	*A. buamannii*	Tiwari et al., 2017
	Cec4	Antimicrobial peptide	Bap, csuE, BfmRS, abaI	*A. baumannii*	Liu et al., 2020
Quorum sensing inhibition/quorum quenching	MomL	Enzyme- lactonases	Acyl homoserine lactone (AHL)	*A. baumannii*	Tang et al., 2015
	AiiA lactonase	Enzyme- lactonases	Acyl homoserine lactone (AHL)	*P. aeruginosa*	Rajesh et al., 2016
	Paraoxonases	Enzyme- lactonases	Acyl homoserine lactone (AHL)	*P. aeruginosa*	Camps et al., 2011
	S-adenosyl-homocysteine, Sinefungin, Butyryl SAM	AHL structural analog	AHL Synthase	*P. aeruginosa*	Brackman et al., 2015; Shin et al., 2019
	AidA	Enzyme		*A. baumannii*	López et al., 2017
Nucleotide signaling	LP 3134	Small molecule	Diguanylate cyclase	*A. baumannii* *P. aeruginosa*	Sambanthamoorthy et al., 2014
	DJK-5, DJK-6	Antimicrobial peptide	(p)ppGpp	*P. aeruginosa, A. baumannii, S. enterica and K. pneumoniae.*	Jiang et al., 2020
	1018	Antimicrobial peptide	(p)ppGpp	*P. aeruginosa, E. coli, A. baumannii, K. pneumoniae, S. aureus, Salmonella typhimurium,*	De la Fuente-Núñez et al., 2014
	LL-37	Antimicrobial peptide		*A. baumannii, P. aeruginosa*	
Two component regulatory systems	2-aminoimidazole		bfmR	*A. baumannii*	Rogers et al., 2008; Thompson et al., 2012
	Flavonoids and curcumin		bfmR	*A. baumannii*	Raorane et al., 2019
	cis-2-decenoic acid	Fatty acid		*P. aeruginosa*	Rehmani et al., 2014

range activity and was most efficient in reducing biofilm formation by *P. aeruginosa* and *A. baumannii* (Sambanthamoorthy et al., 2014). A study identified the quorum quenching enzyme, AidA causing biofilm dispersal, in clinical strains of *A. baumannii* by microarray analysis in the presence of the external signal 3-oxo-C12-HSL (López et al., 2017). The dispersal mechanism by AidA may involve hydrolysis of signaling molecules mediating QS between bacterial species (López et al., 2017). Recently, Raorane et al. identified that flavonoids and curcumin have antibiofilm and antivirulence activity against *A. baumannii* by targeting the regulatory component, bfmR, as revealed by molecular docking through *in silico* analysis (Raorane et al., 2019).

7. Emerging therapeutic strategies

7.1. Phage therapy

The occurrence of antimicrobial resistance has shifted the paradigm of research towards phage therapy, as they are natural invaders of bacterial cells. Previously, Yang et al. and Lin et al. described virulent bacteriophages AB1 and AB2, specifically showing lysing activity against *A. baumannii* and their potential as disinfectants in controlling *A. baumannii* infections (Lin et al., 2010; Yang et al., 2010). Later, many *in vitro* and *in vivo* studies have shown the efficacy of phages in treating *A. baumannii* infections. Vukotic et al. described two novel phages, vB_AbaM_ISTD and vB_AbaM_NOVI, isolated from Belgrade wastewaters, with antibiofilm activity against carbapenem-resistant biofilm-producing clinical *A. baumannii* 6077/12 due to their high depolymerizing activity (Vukotic et al., 2020). Application of phage therapy in humans with a cocktail comprising of four phages to treat *A. baumannii* infection showed successful results (Schooley et al., 2017). This prompted and motivated the approach of phage therapy as a therapeutic strategy against several other pathogens (Aslam et al., 2020). Recently, a study showed *in vitro* antibiofilm activity of a capsular polysaccharide (CPS) depolymerase, isolated from the tail spike protein (TSP) of 'AB6 phage, against *A. baumannii* (Shahed-Al-Mahmud et al., 2021). Bacteriophage AB3 and its endolysin LysAB3 have been demonstrated to display antibacterial and antibiofilm activity against *A. baumannii* biofilms, also causing reduction in the number of viable cells within the biofilm (Zhang et al., 2018). Another study showed that the efficacy of φkm18 phage therapy in a murine model resulted in a reduction in bacterial loads, but delayed administration showed reduced therapeutic effects (Shen et al., 2012). A phage cocktail formulated by combining two phages, with lytic activity and another with depolymerase activity showed strong antimicrobial and antibiofilm activity against *A. baumannii* (Blasco et al., 2022). Combination therapy using environmental phage cocktail with antibiotics such as gentamycin, tobramycin, imipenem and meropenem showed significant reduction of *A. baumannii* biofilm biomass (Grygorcewicz et al., 2021). Adapting phage resistance is not a tough job for bacterial cells due to the presence of the CRISPR system that lends an adaptive immunity and is a concerning factor for presenting phages as a treatment alternative.

7.2. Photodynamic therapy

Photodynamic therapy (PDT) is a rapidly emerging, non-invasive and effective therapeutic approach for removing biofilms (Hu et al., 2018). PDT is an old technology that is now revived due to the resistance era (Jia et al., 2019). It involves a nontoxic chemical molecule called photosensitiser (PS) and visible light in the presence of oxygen to generate multiple reactive oxygen species (ROS) within biomolecules. Consequently, excess ROS production causes matrix destruction and membrane damage by causing ROS production in membrane lipids, altering the permeability of outer membrane or intracellular damage such as DNA damage, organelle destruction, and ultimately causing cell death (Hu et al., 2018). Review article by Jia et al. has expertly described the mechanism of various PS for PDT that can be used to treat bacterial infections (Jia et al., 2019). PDT may help treat infections associated with implants, such as prosthetic joint infections and ventilator-associated pneumonia biofilms (Briggs et al., 2018; Vinagreiro et al., 2020). Photodynamic therapy alone has been demonstrated to be ineffective in complete eradication of the pathogen, but synergistic effects along with antimicrobials such as gentamycin, imipenem, and colistin resulted in increased killing of PDR and XDR *A. baumannii* (De Mello et al., 2019; Pourhajibagher et al., 2017; Wozniak et al., 2019). A study showed that *A. baumannii*, when treated with sublethal antimicrobial photodynamic therapy (aPDT) resulted in significantly increased expression of ompA, probably to compensate for its damage and enable bacterial survival (Boluki et al., 2019). Besides antimicrobials, natural compounds such as chitosan have been studied in combination with PDT to effectively eradicate biofilm-associated *A. baumannii* cells (Zhang et al., 2019; Fekrirad et al., 2021). Another study showed the efficacy of methylene blue and protoporphyrin IX as the antibacterial photodynamic therapy photosensitizer against *A. baumannii* biofilms and methylene blue showed relatively significant reduction in colony forming units (Anane et al., 2020). Recently Ran et al. constructed a strategic combination of bacteriophage and PDT using nile blue photosensitizer with excellent antimicrobial properties against *A. baumannii* and was able to eradicate preformed biofilms (Ran et al., 2021). Although the PDT approach is regarded as a safe and reliable strategy, further *in vivo* studies are required to strengthen PDT as a safe method and increase its efficacy in treating biofilm infections (Warrier et al., 2021).

7.3. Nanoparticle therapy

Recently, nanoparticles (NPs), including metal NPs, liposomes, microemulsions, cyclodextrins and polymer NPs have gained attention due to their antibacterial and antibiofilm properties (Ramos et al., 2018). Antibiofilm property of NPs is based on their small size, which allows their penetration into the deeper layers of biofilm and interaction with the microbial cells to cause membrane disruption, inhibition of the metabolic pathway, inactivation of enzymes and alteration in gene expression leading to cell death (Munir et al., 2020; Ramasamy and Lee, 2016; Yin et al., 2020). Besides targeting the microbe, NPs can be exploited to disrupt the matrix by targeting EPS through their intrinsic property or engineered to carry EPS degrading agents such as chitosan. A review by Singh et al. describes the range of nanoparticles exhibiting promising results against *A. baumannii* biofilms and infections (Singh et al., 2016). Silver nanoparticles have been extensively studied as antimicrobial agents against several Gram -positive and Gram-negative bacteria. In a study, silver nanoparticles synthesized using plant extract exhibited antibacterial properties and inhibited *E. coli* growth at MIC 2 μg/disk for *E. coli* and 8 μg/disk for *A. baumannii* and *S. aureus*. Further, biofilm disruption assay showed that these silver nanoparticles could reduce biofilms formed by *A. baumannii, E.coli* and *S. aureus* by 88%, 67% and 78%, respectively (Salunke et al., 2014). Later studies confirmed that besides affecting the growth of *A. baumannii,* subinhibitory levels of silver nanoparticles downregulated the expression of various virulence and biofilm-associated genes such as *kpsMII, afa/-dra*BC, *bap, ompA*, and *csuA/B* genes and reduced their biofilm-forming ability (Hetta et al., 2021). NPs can enhance antimicrobial effects by acting as drug delivery vehicles or catalysts to improve drug penetration into biofilms. Further *in vivo* studies are required to address the cytotoxicity and safety issues associated with NPs before they can be applied to treat infections in humans.

8. Conclusion

A. baumannii is responsible for causing wide range of multidrug resistant and biofilm-associated infections in hospitals and community settings. Biofilm formation by *A. baumannii* is a multifactorial process involving various intrinsic and extrinsic factors governed by regulatory

mechanisms directing bacterial adhesion, biofilm maturation and bacterial cell dispersal from the biofilm. The emergence of antibiotic resistance and the complex structure of the biofilm matrix render antibiotics ineffective. The current scenario urgently necessitates innovation, discovery or repurposing therapeutic compounds to strengthen the antimicrobial pipeline. Vaccination is the most acceptable preventive strategy to control the spread of *A. baumannii* infections. Various biofilm associated factors such as OmpA, Bap, Ata, PNAG and outer membrane vesicles have been studied as promising vaccine candidates for immunization against *A. baumannii* (Ahmad et al., 2016; L.V. Bentancor et al., 2012; Cai et al., 2019; Fattahian et al., 2011; Jun et al., 2013; Luo et al., 2012). Other preventive strategies, such as antibiofilm polymers and coated medical devices are attractive approaches as they can inhibit bacterial colonization and prevent biofilm formation by a broad range of Gram-positive and Gram-negative bacteria. Preformed biofilm-associated chronic infections can be treated by targeting matrix components for dismantling biofilm structure and exposing the bacterial cells to antibiotics. Interference with regulatory mechanisms such as quorum sensing, nucleotide signaling and two-component systems might help in controlling biofilm-associated *A. baumannii* infections. Phage therapy, photodynamic therapy and nanoparticle therapy have recently gained attention due to the emergence of antimicrobial resistance observed globally and has shown promising results. Further *in vitro* and *in vivo* studies employing these strategies are required to effectively eliminate the associated risk with these emerging strategies. Since identifying causative microbe in biofilm-associated infections is difficult with current non-invasive *in vivo* diagnostic methods and biofilms can become polymicrobial, therefore, treatment approach for biofilm-associated infections must emphasize developing broad-spectrum therapeutics for effective outcomes.

CRediT authorship contribution statement

Kirti Upmanyu: Conceptualization, Formal analysis, Writing – original draft. **Qazi Mohd. Rizwanul Haq:** Conceptualization, Writing – review & editing. **Ruchi Singh:** Conceptualization, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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