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Acinetobacter baumannii: A current review of virulence factors and their clinical significance

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Abstract

The alarming rate of *Acinetobacter baumannii* infections necessitates immediate attention to tackle this issue. The emergence of multidrug-resistant strains has greatly complicated the treatment, making it a challenging endeavour. This problem has been exacerbated by the COVID-19 pandemic, with higher mortality rates observed among COVID-19 patients infected with multidrug-resistant *A. baumannii* during treatment. Infections caused by *A. baumannii* result in a range of health complications, such as urinary tract infections, wound infections, bacteraemia, pneumonia, meningitis, heightened morbidity, and in severe cases, even mortality. The virulence factors and mechanisms of pathogenesis of *A. baumannii* are complex and still encompassing areas of ongoing investigation. Therefore, this review aims to provide a comprehensive overview of the current knowledge regarding key virulence factors, including the secretion of proteases, lipases, catalase, and motility, which contribute to the pathogenesis of *A. baumannii* infection. Additionally, the review explores the organism's resistance and persistence strategy, primarily attributed to its remarkable ability to form biofilms on various surfaces, rendering complete eradication from medical devices an arduous task. Overall, this review emphasizes the importance of *A. baumannii* as a significant pathogen in healthcare settings and underscores the need for continued research to develop more effective strategies for the prevention and treatment of *A. baumannii* infections.

Keywords*:* Acinetobacter baumannii; Biofilm formation; Pathogenesis; Virulence factors

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Acinetobacter baumannii

Acinetobacter baumannii is a Gram-negative aerobic coccobacillus bacterium from the order *Pseudomonadales*, and it is one of the few members of *Acinetobacter* species responsible for human infections (1). This bacterium belongs to the genus *Acinetobacter* whose appearance was first described by Beijerinck back in 1911. Subsequently, it was further classified and differentiated from the genus *Achromobacter* due to its non-motile nature and inability to ferment glucose. The species *A. baumannii* was formally designated in 1986 and is the most researched and well-characterized among five other pathogenic species in the *Acinetobacter calcoaceticus–baumannii* (*Acb*) complex (2). The phenotypic and genotypic similarities between pathogenic species in the *Acb* complex, namely *Acinetobacter calcoaceticus*, *A. baumannii*, *Acinetobacter pittii*, *Acinetobacter nosocomialis*, *Acinetobacter seifertii* and *Acinetobacter dijkshoorniae*, also lead to the sharing of virulence factors, especially among *A. baumannii* and *A. nosocomialis* (3,4).

Identification of *Acinetobacter* species is vital in assessing appropriate treatment in clinical cases of *Acinetobacter* infections. Only a few species of *Acinetobacter* are attributed to clinical manifestations with *A. baumannii* being the most serious concern (5). However, the close relatedness of *Acinetobacter* species makes species identification of these organisms an arduous task. The phenotypic analysis that Bouvet and Grimont first proposed in 1986 failed to identify a more recently found clinically significant *Acinetobacter* strain. Commercially available bacterial identification systems such as API20NE are also incapable of correctly identifying *A. baumannii* (6). Moreover, a case reported by Kim et al. (2018) described the misidentification of *A. baumannii* as *Alcaligenes faecalis* by the VITEK 2 system may lead to unnecessary mistreatment of patients (7). Apart from *A. baumannii*, *Acinetobacter pitti* and *Acinetobacter nosocomialis* are also clinically relevant strains but respond differently to different treatments. This also includes two new strains in the ACB complex isolated from human clinical samples, namely *Acinetobacter seifertii* and *Acinetobacter dijkshoorniae*. Hence, rapid yet accurate identification in a clinical setting is imperative especially when the rate of infections surges during this COVID-19 pandemic (8).

Accurate identification of *A. baumannii* is usually hindered using canonical morphological and fermentation analysis. The increasing usage of matrix-associated laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS), which is based on outer membrane component analysis is capable of identifying *A. baumannii* more accurately (9). Despite the promising usage of MALDI-TOF-MS in expeditiously identifying this species, the research conducted by Álvarez-Buylla et al. (2012) stated that molecular techniques identification is still needed as MALDI-TOF MS is incapable of identifying other important species in the genus (10). Hence, genomic-based identification of *A. baumannii* remains the gold standard in identifying this species. Over the years, several approaches have been developed including tRNA fingerprinting (11), amplified fragment length polymorphism (AFLP) analysis (12), amplified 16S rRNA gene restriction analysis (ARDRA) (13), as well as identification based on the $bla_{\text{OXA-51}}$ like carbapenemase gene (14). However, the laborious work needed for this identification method hindered its use in clinical settings. Moreover, the presence of insertion elements in the $bla_{\text{OXA-51}}$ like gene which was originally thought to be intrinsic to the *A. baumannii* led to false negative results which limited its potential as a marker gene (15). The close relatedness of genetic compositions with the Acb complex also impedes accurate identification of specific strains using canonical 16S RNA sequencing analysis (16).

The *rpoB* gene sequencing showed a higher accuracy rate for identification at the species level using a primer pair specific to the *Acinetobacter* genus. Furthermore, the development of the *gyrB* gene as a gene marker for *A. baumannii* identification was introduced to better discriminate *Acinetobacter* species in the Acb complex (17–19). Recently, specific identification of *A. baumannii* using multiplex *gyrB* PCR assay has been validated against a whole genome sequencing assay, which further confirms its validity in the identification (20). Hence, a combination of *gyrB* multiplex PCR assay and confirmation by *rpoB* gene sequencing could provide a high discriminatory power in identifying *A. baumannii* that can be used in clinical settings to avoid mistreatment due to misidentification of bacterial species.

A. baumannii infections cause a range of catastrophe effects including urinary tract infection, wound infection, bacteremia, pneumonia, meningitis, increased morbidity, and in some serious cases can even lead to mortality (21). The term opportunist pathogen, albeit being commonly asserted with immunocompromised patients, might not be as accurate to describe *A. baumannii* as only a minority of those infected people are neutropenic. However, skin breaching as well as exposure to broad-spectrum antibiotics increase the likelihood of infection by these bacteria (22). Thus, as hospital intervention usually involves defects in anatomical host defenses as well as antibiotic

treatment, the likelihood of being infected with *Acinetobacter* species, primarily *A. baumannii*, will be higher in these cases. Increasing case reports revealing fatal skin infections caused by this multidrug resistance strain of this bacterium are also a concern as this bacterial strain is not normally associated with this complication (23–27).

A coalesce review of *A. baumannii* pathogenesis was conducted by Wong et al. (2017), which discusses the current understanding that *A. baumannii* pathogenicity relies on its capsular composition and abundance, leading to its capability to evade complement and phagocytosis (21). However, the inability to completely remove the bacterial infections by innate immunity resulted in host damage via TLR-4-mediated sepsis due to cytokine storm, which marked the second virulence phase. Thus, effective initial treatment is vital to prevent more severe outcomes from *A. baumannii* infections. Genetic analysis conducted by Smith et al. (2007) (28) identified 16 islands containing virulence genes that accounted for significant genetic compositions attributed to the pathogenicity of *A. baumannii*. The virulence activities of *A. baumannii* were analyzed using several model animals, including wax moth larvae of *Galleria* (29), *Caenorhabditis elegans* (30), zebrafish (31), and mostly on mice (32–34). Despite there were some contradictory observations between *in vivo* and *in vitro* portrayal of *A. baumannii* infections, several virulence factors posed by *A. baumannii* have been accrued, including biofilm formation, cell adhesion, iron acquisition, outer membrane protein phospholipases and, recently, increasing highlights have been given on the role of outer membrane protein A (OmpA).

Virulence

Casadevall and Pirofski (2009) (35) define virulence as the many abilities of bacteria to ensure survival in the host including the ability to invade the host, i) ability to grow in the host environment, ii) ability to evade immune responses, iii) ability to sequester iron and nutrients as well as the ability to sense and adapt to environmental changes. Whole-genome sequencing, as well as transposon-induced mutant analysis, were conducted to uncover the virulence factors in *A. baumannii* (36,37). These include antibiotic resistance genes, motility, iron sequestration, phospholipases, protease, lipase, oxidative stress resistance and biofilm formation. These characteristics are considered virulence factors because they ensure *A. baumannii* survivability during infections. Hence, several antivirulent agents have been studied to successfully target multiple virulence factors of *A. baumannii.* including *LpxC-1*, *Deferiprone* and *Virstatin* (38–40). Recently, more research has been conducted to explore other anti-virulent agents that could be used to inhibit virulence action in *A. baumannii*. Among the potential anti-virulence agents that are also being studied including silver nanoparticles (41), pyrogallol (42), flavonoids and curcumin (43) as well as myrtenol (44).

Antibiotic resistance

The data from the Study for Monitoring Antimicrobial Resistance Trends (SMART) surveillance conducted by MSD (Merck & Co., Inc) revealed an increasing number of multidrug resistance (MDR) *A. baumannii* infections worldwide, especially in the ICU ward settings (45). The emergence of *A. baumannii* MDR strains is attributed to its high evolutionary capabilities driven by many factors, including exposure to non-lethal dosages of antibiotics as well as desiccation-induced mutations (46). In addition, the genome plasticity of this bacterium allows a high mutation rate, which is beneficial for the bacteria to produce resistant strains albeit with random occurrences of mutations.

As one of the ESKAPE organisms, their resistance towards carbapenem, which is usually a highly effective antibiotic has become a concern for many health practitioners. The perilous increase in carbapenem-resistant *A. baumannii* (*CRAB*) is particularly a concern in Southeast Asia and has been widely distributed predominantly in Asia. CDC report on antibiotic resistance threats published in 2019 listed *Carbapenem*-resistant *Acinetobacter* as one of the urgent threats that caused 8500 infections and 700 mortalities in the United States alone (47). Moreover, WHO has listed (CRAB) as a global priority list of bacteria, calling for extensive research on this bacteria (48). Recently, Bartal et al. (2022) highlighted the complex requirement of drug combination therapy in dealing with CRAB infections which further accentuates the severity of this issue(49). The study conducted by Kim et al. (2013) also identified the Global clone 2 (GC2) strain and most of the strains harbored the acquired gene OXA-23 oxacillinase as well as *AbaR* 4-type resistance islands which led to its multi-drug resistance capabilities (50).

The alarming rate of the emergence of multidrug resistance (MDR) strains of *A. baumannii* has made this organism known as Gram-negative Multidrug Resistance *Staphylococcus aureus* (MRSA). The emergence of extensively drug-resistant *A. baumannii* complex (XDRABC) is a serious concern in Taiwan due to the broad spectrum of antibiotics used in normal clinical settings (51). The remarkable ability of *A.*

baumannii to survive antimicrobial drugs is attributed to several means. Three prominent mechanisms cause antimicrobial resistance in *A. baumannii* namely i) enzymatic actions, ii) decrease in antibiotic target access as well as iii) genetic mutations which change the cellular functions that confer resistance to the bacteria.

As discussed in the earlier part of this section, resistance toward antimicrobial agents has been achieved with enzymatic and non-enzymatic action. For enzymatic action, most *A. baumannii* strains harbored genes that encode ß-lactamases, AmpC cephalosporinases, and oxacillinases which made them resistant to a number of antibiotics including carbapenem (52). *A. baumannii* also achieves antimicrobial resistance capability via a non-enzymatic path involving morphological changes such as a change that includes a change in outer membrane protein (OMP), increment in multidrug efflux pump expression, amendment on drug-targeting genes as well as forming a biofilm. These mechanisms lead to the passivation of antibiotics and hinder their application to treat infections. Research conducted by Tang et al. (2020) revealed a significant association between the AbaI/AbaR quorum sensing system and drug resistance, implying a regulatory mechanism for antibiotic resistance actions (53). Effective infections by bacteria were often conducted in density dependent manner to ensure complete evasion from host immune responses. In general, bacteria tend to avoid profligate gene expression without sufficient density corroboration as it will easily be targeted by the host immune system (Czárán et al. 2009) (54). Hence, activation of these genes such as antibiotic resistance genes is thought to be meticulously regulated to ensure successful infection.

AdeFGH efflux systems are generally utilized by *A. baumannii* to flush out fluoroquinolones and meropenem to curb the antimicrobial action (55). A study conducted by He et al. (2015) (55) found that the expression of the AdeFGH efflux pump is significantly increased when the quorum sensing molecule, acyl-homoserine lactones (AHL) is introduced in *A. baumannii* culture (55). These expressions lead to the resistance toward *meropenem* and *levofloxacin* further supporting the role of the quorum sensing and efflux pump toward bacterial resistance. Dou et al. (2017) on the other hand, revealed the regulation of antibiotic resistance genes in a collected clinical strain of *A. baumannii* via a quorum sensing mechanism (56). The study reveals that N-3-hydroxy-dodecanoyl-homoserine lactone (N-3-OH-C12-HSL), the AHL signal produced by *A. baumannii* activates the expression of drug resistance genes particularly responsible for resistance toward meropenem and piperacillin which supported previous reported finding. Among the upregulated genes are *adeA*, *adeB*, *adeC* efflux systems, as well as *AmpC* cephalosporinases and oxacillinases. Hence, this regulatory mechanism that governs the gene expression, which leads to antibiotic resistance, can be a potential target in managing MDR *A. baumannii* infections.

Extended-Spectrum β-Lactamase bla_{PER-1} gene that confers resistance to β-Lactam drugs is widely spread in many *Acinetobacter* species (57). The additional role of the bla_{PER-1} gene in cell adhesion as well as biofilm production was studied by Lee et al. (2008) indicating the vital role of this gene in ensuring successful infections (58). On the other hand, Naeimi Mazraeh et al. (2021) also showed a significant association of bla_{PER-1} with the gelatinase and hemolytic activities in *A. baumannii*, further affirming its importance in *A. baumannii* virulence (59). The team also reported a significant association between quorum sensing and bla_{PER-1} expression, albeit missing details in the statistical analysis. On the other hand, Poirel et al. (2005) reported that expression of the bla_{PER-1} gene in Gram-negative bacteria is influenced by the promoter region in the insertion sequence, suggesting that a modulatory mechanism is employed in the expression which hypothesis can be further tested in this study (57).

Both the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) provide guidelines and standards for determining the antibiotic susceptibility of *A. baumannii* strains. These organizations play a crucial role in guiding clinicians in selecting appropriate antibiotics for the treatment of *A. baumannii* infections. CLSI, widely recognized in North America, has established breakpoints that define the minimum inhibitory concentration (MIC) at which an antibiotic is considered to be effective against the bacterium. These breakpoints help determine whether a strain of *Acinetobacter baumannii* is susceptible, intermediate, or resistant to a specific antibiotic. CLSI breakpoints are determined through a combination of clinical data, pharmacokinetic studies, and laboratory testing (60). On the other hand, EUCAST provides a parallel set of guidelines predominantly followed in Europe and other regions. EUCAST breakpoints also define the MIC values that distinguish susceptibility categories, but they may differ from CLSI breakpoints due to variations in the methodologies and data used in their development. EUCAST employs a more standardized approach, incorporating large data sets and statistical analysis to establish breakpoints (61).

However, the differences in susceptibility

breakpoints between CLSI and EUCAST can result in discrepancies in interpreting antimicrobial susceptibility test results. A strain classified as susceptible according to CLSI breakpoints may be categorized as intermediate or even resistant according to EUCAST guidelines, and vice versa. Such discrepancies can lead to variations in treatment decisions and the choice of antibiotics, potentially impacting patient outcomes (62). To address these discrepancies, efforts are being made to harmonize the breakpoints between CLSI and EUCAST (63,64). Harmonization is essential to ensure consistent and effective treatment practices globally. Healthcare professionals must remain updated with the latest guidelines from both CLSI and EUCAST and consider local resistance patterns when making treatment decisions for *A. baumannii* infections. This comprehensive approach will help optimize therapy and combat the challenges posed by antimicrobial resistance. Some of the suggested antibiotics proposed for the treatment of *A. baumannii* infections are listed in Table 1.

Motility

Motility is imperative in pathogenic bacteria for invasion and dissemination of the bacteria and serves as one of the virulence traits in pathogenic bacteria (65). Complex regulatory networks are often involved when research is conducted on the motility and virulence of bacteria. Although *Acinetobacter* clades are generally regarded as non-motile, *A. baumannii* and *A. nosocomialis* strains exhibited surface-associated motility (66) and twitching motility (67). This trait is linked to virulence as *in vitro* analysis shows that hypermotility strains of *A. baumannii* caused significant impairment and elevated lethality toward the *Caenorhabditis elegans* infection model (68). The twitching motility of *A. baumannii* strains is attributed to repeated cycles of type IV pili extension, retraction and propelling the bacterial cell forward. The presence of *PilA* gene encoding the major pilin subunit of *A. baumannii*, which is mainly responsible for type IV pili, is highly varied among *A. baumannii* isolates and might contribute to the degree of motility capabilities between *A. baumannii* strains (69). Vijayakumar et al. (2016) discovered that *A. baumannii* isolated from blood is more motile as compared to the isolates from respiratory pathways suggesting the regulation of motility depending on environmental cues (70). *A. baumannii* isolated from the respiratory tract exhibited low motility presumably for robust biofilm formation and stronger attachment during invasion on alveolar cells (70). Despite virulence and motility capabilities being linked together, some strains of *A. baumannii* which do not show motility still exhibited virulence in model organisms. *A. baumannii* ACICU strain does not show any motile trait however it shows high virulence potential such as high secretion of protease and iron-chelating compounds (71).

Inhibition of several efflux pumps in *A. baumannii* resulted in reduced inhibition of motility and virulence (72). However, the direct causation of motility in causing damage to the host such as promoting host invasion has not been discussed. This reduction in virulence might also be attributed to disruption of virulence factor secretion extraneous to its motile potential. Blaschke et al. (2021) identified several vital genes for surface-associated motility, which are related to several physiological activities of bacteria, including stress metabolism and oxidative stress (73). Some of these genes, encode a large subunit of carbamoylphosphate synthase (*car*B) which is required for the synthesis of arginine and pyrimidine. Inactivation of this *car*B gene halted pyrimidine synthesis and reduced motility and virulence in a mouse pneumonia model, showing its vital role in these processes (36).

Protease

Pathogenic bacteria generally secrete proteases to ensure survival in the host during infections through myriad proteolytic actions. Some proteases secreted by pathogens cleave antibodies to evade host immunity during infections (74,75). Several proteases secreted via T2SS have been discovered in *A. baumannii*. One of them is zinc-dependent metalloendopeptidase (CpaA), studied by Tilley et al. (2014), which contributes toward the virulence of *A. baumannii* by deregulating blood coagulation in human blood plasma. However, active secretion of this endopeptidase is only present in several clinical strains of *A. baumannii* suggesting that a regulatory mechanism exists in coordinating the secretion (76). The crystal structure of CpaA studied by Urusova et al. (2019) revealed that its activity is inactivated and regulated by the chaperone protein CpaB suggesting that chaperone-substrate complex could be a target in drug development for *A. baumannii* (77). However, the requirement for CpaB in CpaA secretion is not neoteric as the need for CpaB to stabilize CpaA secretion has been reported earlier in Harding et al. (2016) (78). CpaA is a glycoprotease, which virulence involves the cleaving of coagulation factor XII in the host leading to the impairment of blood coagulation. (76,79). Haurat et al. (2020) on the other hand also reported the broad glycoprotease activity of CpaA which involves the cleavage of other glycoproteins in mammalian cells such as mucin and fetuin (80). Moreover, CpaA is

also able to cleave CD55 attached to host cells during infection and delay complement activation by neutrophils (80). Hence, *A. baumannii* encoding gene for this CpaA protease is mostly isolated from clinical settings which can lead to severe ramifications during infection. Resistance toward serum was also attributed to the activity of the plasminogen-binding protein CipA, which is capable of degrading fibrinogen and inhibiting all three complement activation pathways. (81). However, the prevalence of this plasminogen-binding protein in *A. baumannii* strains remains to be elucidated.

Another protease, protein-killing factor serine protease (PKF) secreted by *A. baumannii* was first reported by King et al. (2013) (82). This PKF has been shown to inhibit initial biofilm formation by *A. baumannii* which might confer an advantage for this bacterium to disperse widely before the localization of biofilm structure. This protease also conferred resistance toward complement-mediated killing (82). *A. baumannii* strains harboring the PKF gene were commonly isolated in ICUs as compared to CPA protein (83). However, this protein was also identified in *A. baumannii* isolated from fermented food, suggesting its secretion is also for physiological exigency (84). Carboxy-terminal processing protease, Ctp is another serine protease of *A. baumannii* that also halts biofilm formation in *A. baumannii* (85). The pivotal roles of this protease in membrane cell integration, motility, and virulence in the zebrafish host model were previously described by Roy et al. (2020) (86).

Lipase

Most *Acinetobacter* species secrete esterase and lipase to break the easter bond in triacylglycerols to obtain fatty acids. Microbial pathogens usually secrete lipases for a plethora of infection mechanisms including host cell adhesion and persistence as well as interference with host cell inflammatory responses (87). *A. baumannii* adopts T2SS to export most effector proteins including lipase (78,88). The role of lipase and phospholipases in *A. baumannii* virulence has been shown in various studies showing a virulent reduction in *LipA* and phospholipase knockout mutants in model animals (89–91). Another type of lipase secreted by T2SS encoded the *LipH* gene was also described by Harding et al. (2016) (92). The specific role of this lipase in virulence however was not studied as the virulence analysis on the *Galleria mellonella* model was conducted in the T2SS system mutant as a whole (78). In terms of the regulation of lipase secretion, there is no report on the role of bacterial density, which regulates the secretion; the situation which tactic commonly employed for virulence action. The role of quorum sensing in regulating lipase secretions however has been reported in many microorganisms such as *Burkholderia glumae*, *Pseudomonas psychrophile* as well as *Vibrio harveyi* (93–95). Maria López et al. (2017) on the other hand, reported the regulation of the secretion system via quorum sensing, suggesting its possible involvement with lipase secretion (96).

A. baumannii secreted phospholipase to facilitate the invasion via lysis of the host cell membrane. Phosphatidylcholine, a major phospholipid component in the human cell membrane, is one of the carbon sources utilized by bacterial pathogens including *A. baumannii* (97). Hence, the secretion of phospholipase by *A. baumannii* is vital for carbon acquisition and one of its virulence factors. Three phospholipase D (PLD); PLD1, PLD2, and PLD3 have been identified in *A. baumannii* which work in a concerted manner during the invasion into the host cell. Unlike *Pseudomonas aeruginosa*, the phospholipases however do not confer any advantage during co-infection with other bacterial species as it does not have any effect during interbacterial competition (90,98). *A. baumannii* also secretes two phospholipases C; PLC1 and PLC2 which also play a role in the cytolytic activity of cells during invasion (99). Apart from carbon acquisition, phospholipase secretion also sequesters iron from human erythrocytes whose action is regulated via a ferric uptake regulator. Interestingly, high phosphatidyl content in human erythrocytes shows higher hydrolysis of phospholipases secreted by this bacteria as compared to when sheep blood agar is used, which is generally used in the screening of phospholipases (99). This result further shows the specific action of the substrate for the action of these phospholipases.

Although lipase secreted by *A. baumannii* is generally thought to be responsible for fatty acid utilization in the host, the detailed action of lipase secretion on virulence has not been uncovered. Johnson et al. (2016) however reported the reduction of *in vivo* and *in vitro* fitness of LipA mutant *A. baumannii* showing its possible role in infection pathogenicity (89). Unlike LipH, the secretion of LipA requires membrane-bound chaperone LipB. Another phospholipase, named LipAN has been found in *A. baumannii* 17978 also secreted via Type 2 SS and involved in virulence as shown in a mouse model (100). Therefore, the secretion of lipases by *A. baumannii* is believed to significantly contribute to its pathogenicity, making it a potential target for drug development.

Catalase

Hydrogen peroxide is widely used as a disinfectant due to its strong bactericidal action. Phagocytic activity, which is activated during pathogen invasion in the body, produces hydrogen peroxide which eventually kills invading microorganisms despite causing acute damage to the neighboring tissues (6). Several *Acinetobacter* species encode catalase enzymes, capable of degrading hydrogen peroxide and thus confer resistance toward respiratory bursts from phagocytic activities. Sun et al. (2016) demonstrated that the persistence towards oxidative stress displayed by *A. baumannii* is attributed to *katE* and *katG* that encode catalase, where the knockout of this gene causes high sensitivity of this strain toward oxidative killing (101). Wright et al. (2017) also found an insertion sequence upstream of this *katG* gene, which increased transcription was observed upon exposure to oxidative stress, further validating its role in surviving oxidative stress (102). However, Sun et al. (2016) reported that *A. nosocomialis* with deletion of *katE and katG* stymied killing by neutrophils, leading to more mortality of *G. mellonella*. However, whether this is also true for *A. baumannii* is not known (101). Despite not being classified as a phototrophic microorganism, *A. baumannii* senses and responds to light (103,104). This feature is due to the presence of a blue-light-sensing-using flavin protein (BLUF) *BlsA* which regulates myriad cellular processes (5,103,105). Squire et al. (2022) also reported that the *katE*, encoding catalase-peroxidase enzyme, is also regulated via light which adds to the many processes regulated by *BlsA* (105). *A. baumannii is* also famously known for its ability to resist desiccation. During periods of water limitation, aerobic respiration will be disrupted and many reactive oxygen species (ROS) will be generated. Hence, *A. baumannii* upregulated the secretion of catalase to survive oxidative stress and contributed to its high desiccation tolerance.

Lipooligosaccharides Capsule

As an evasion mechanism against host defenses, many strains of *A. baumannii* possess thick capsular polysaccharides, which have been identified as a major virulence factor for certain strains of *A. baumannii*, as described by Russo et al. (2010) (37). This conclusion is supported by evidence showing that mutants lacking the capsule are highly susceptible to complement-mediated killing by the host immune system. Therefore, the presence of a thick bacterial capsule provides *A. baumannii* with resistance to human serum. Additionally, Geisinger & Isberg (2015) have reported that the two-component system BfmRS plays a role in increasing bacterial capsule synthesis when exposed to subinhibitory concentrations of antibiotics, contributing to its antibacterial resistance properties (106). Furthermore, the glycosylation of Type IV pilin with glycans in some *A. baumannii* strains has also been associated with the shielding from antigenic recognition revealing the strategy to enhance its bacterial capsule for virulence purposes (107).

Glycans, which are bacterial carbohydrates surrounding the bacteria, play a crucial role in biofilm formation. Acting as an interface between bacteria and the surrounding environment, glycans also contribute to the bacteria's virulence mechanisms. Glycoconjugates, which are macromolecules consisting of covalently attached carbohydrates to lipid or protein molecules, are also responsible for regulating the virulence of *A. baumannii* (92). Lipopolysaccharides and peptidoglycan are common examples of glycoconjugates found in bacteria. In the case of *A. baumannii*, the lack of O antigen results in the outer leaflet of the outer membrane being designated as Lipooligosaccharides (LOS), which is a vital structural component ensuring the viability of *A. baumannii*. This LOS acts as a ligand for Toll-like receptor 4 upon infection in the host (108). Consequently, the loss of LOS in *A. baumannii* leads to colistin resistance, which is often used as a last-line drug to treat *A. baumannii* infections.

Mutations in the *pmrAB* gene, encoding another two-component system in *A. baumannii*, have been identified as a key factor in the emergence of colistin-resistant strains (109). These mutations lead to the overexpression of the sensor kinase PmrB, which in turn modifies the lipid A component of lipooligosaccharide (LOS), altering the surface charges of the bacterial cell and impeding the action of colistin. However, LOS is partly involved in surface-associated motility (110), and thus its loss compromises the movement of *A. baumannii* despite its survival against colistin. Therefore, targeting the immobility of *A. baumannii* could be a potential avenue for developing treatments for colistin-resistant strains. Moreover, PmrB is capable of sensing slightly acidic conditions and the absence of Fe3+, triggering changes in the bacterial surface (111). Interestingly, Modarresi et al. (2015) reported a correlation between iron limitation and AHL production in A. *baumannii*, suggesting a possible connection between PmrB and AbaI, the receptor responsible for quorum sensing (112). This finding opens up the possibility of investigating signaling interactions between PmrB and AbaI in *A. baumannii* as discovered recently in BfmRS (113).

The possession of an O-linked protein glycosylation system in addition to its thick capsular polysaccharides leads to an extraordinary

resistance toward the host's innate immune system. *A. baumannii* protein glycosylation system is also responsible for the formation of biofilm as research conducted by Iwashkiw (2012) reveals that the formation of biofilm is deterred in *A. baumannii* strain lacking the glycosylation system.

Environmental persistence

Most research on the *A. baumannii* virulence mechanism was conducted by comparing and identifying homologous virulence mechanisms with other pathogens. Thus, as *A. baumannii* is also one of the ESKAPE organisms, homologous virulence molecular determinants are identified by comparison with other ESKAPE pathogens including *Staphylococcus aureus* and *Pseudomonas aeruginosa* (114). As for *P. aeruginosa*, the infection mode is organized into two discrete phases, acute and chronic phases, where the transition from free-living to biofilm physiology is marked as the beginning of the chronic phase (115). However, there is no such review of distinct infection phases reported in *A. baumannii* despite its homologous virulence mechanism with *P. aeruginosa*. The transition of *A. baumannii* from free-living bacteria to biofilm depends on signals including bacterial density, nutrients, and free cations availability, which has been critically reviewed in (116). The ability of *A. baumannii* to maintain its infectious ability even after a duration of starvation is the main feature attributed to its lucrative chronic phase infection (117). Nevertheless, in contrast with the *P. aeruginosa* chronic phase dormancy strategy, Bravo et al. (2016) research reveals that *A. baumannii* did not transition into a dormant state due to nutrient and water limitation (118). This strategy might have contributed to the high efficiency of *A. baumannii* infection strategy as the bacteria remained viable and persevered even in a desiccated environment.

During the water limitation, there are several mechanisms employed by *A. baumannii* to prevent severe consequences that might arise during dehydration. The similar biosynthetic pathways for capsular polysaccharides between *A. baumannii* and *Acinetobacter baylyi* lead to the prediction that the desiccation resistance mechanism is shared between the two strains. In a desiccation study involving *A. baylyi*, it was found that the bacterial carbohydrates or glycan shield surrounding the bacteria makes them viable despite facing water limitations (119). Its bacterial membrane is composed of highly acylated lipooligosaccharides (LOS) to decrease the membrane compound fluidity, which prevents the leakage of water out of the cell membrane and leads to environmental persistence (120). Meanwhile, DNA damage during dehydration is prevented by the action of the repair protein RecA, which is responsible for genetic combination repair in *Acinetobacter* species (121). However, RecA might have little specificity in its DNA-repairing mechanism due to the high-frequency mutation observed in *A. baumannii* when exposed to dehydration. While this might seem like an inefficient damage repair mechanism, it could also be a kin selection strategy employed by *A. baumannii* to generate bacterial generation with better adaptation to a pressing environment. While persistency is not generally regarded as pathogenicity, high persistence in the environment causes difficult elimination of infection sources as well as onerous clearance by host responses after invasion. This high persistency is attributed to the morphological changes and enzymatic action of bacteria in terms of increment in the thickness of the cell wall as well as the high activity of RecA enzyme in DNA repair during the desiccation period.

Persistency in the environment can also be attributed to the capability of *A. baumannii* to form biofilm. Biofilm is a tertiary structure encasing bacteria in an extracellular matrix consisting of various macromolecules such as proteins, carbohydrates, and nucleic acids formed by the bacterial community (122). Production of biofilm is a vital characteristic employed by *A. baumannii* to survive and the reason that these bacteria thrive in dry conditions (123,124). This biofilm production however aggravated the infection problem as they confer tolerance to antimicrobial agents as well as environmental desiccation. Research conducted by Espinal, Marti, and Vila in 2012 revealed that the biofilm-forming strain of *A. baumannii* has a significantly longer survival time of up to 36 days on dry surfaces compared to the nonbiofilm-forming strain (9). This feature allows *A. baumannii* to survive in the presence of antibiotics even without the presence or acquisition of antibiotic resistance genes. The difference in the mRNA expression of planktonic cells and biofilm of *A. baumannii* indicates distinct metabolic activities employed by this bacterium according to the living conditions (125).

Biofilm formation in *A. baumannii* is intricately regulated by a diverse array of signals, as extensively reviewed by Gaddy and Actis (2009) (116). These signals encompass various factors, including nutrient availability, metal cation presence, quorum sensing, environmental stress (such as temperature), and other cues that coordinate the activation of genes essential for biofilm formation. This capacity for biofilm formation not only contributes to virulence activity but also enables *A. baumannii* to persistently colonize hosts. Another factor aiding biofilm formation in *A.*

baumannii is the production of capsular polysaccharides, specifically poly-B-1,6-N-acetylglucosamine (PNAG) (126). Remarkably, the upregulation of PNAG expression serves as a compensatory mechanism in *A. baumannii* strains lacking lipooligosaccharides (LOS) to confer resistance against colistin, allowing them to maintain viability within the biofilm structure even when exposed to colistin. Several mechanisms involved in the biofilm formation of *A. baumannii* are discussed below.

Biofilm-associated protein (Bap)

Biofilm-associated protein (Bap) is the protein expressed on the bacterial surface secreted by the Type 1 Secretion System. This bap protein plays a role in the development and maturation of biofilm as well as required for cell-cell adhesion. This is supported by Fallah et al. (2016) where the team reported high expression of *bap* gene in a biofilm-forming strain of *A. baumannii* isolated from Iran. Bap protein was first isolated in *Staphylococcus aureus* and found to be involved in cellular adhesion. It also plays a role in the adherence to biotic surfaces like human epithelial cells by increasing the bacterial cell surface hydrophobicity. However, Brossard and Campagnari et al. (2011) reveal that Bap deficient mutant of *A. baumannnii* also sticks on abiotic surface despite being unable to form a tower structure further indicating its role in maturation but not in initial adherence to the abiotic surface. Molecular analysis of *bap* gene also reveals four-fold overexpression of this gene in the iron-limiting environment which shows that iron-limiting condition promotes biofilm formation in this species. The direct contribution of bap in biofilm formation is portrayed in the research conducted by Goh et al. (2013) which shows the inhibition of biofilm formation when *bap*-positive strain of *A. baumannii* ST92 is inhibited by affinity-purified Bap antibodies. However, in contrast with the finding by Brossard and Campagnari (2013), *bap* expressed in this strain does not mediate adhesion to human ECM components suggesting that the role of bap might be strain-specific but the role in its maturation was not discussed. The translocation of bap-like protein to the cell surface in *Pseudomonas fluorescens* is mediated by an ABC transported however in *A. baumannii*, the mechanism of Bap transportation to the surface remains to be studied.

csuA/BABCDE

In the classical laboratory strain of A. *baumannii* 19606, the formation of pili plays a crucial role in biofilm production. These pili are encoded by a polycistronic operon called csuA/BABCDE, which consists of six open reading frames. This operon is also present in other *A. baumannii* strains such as 17978 (28) and AYE (127). The Csu pili, or Type I chaperone-usher pilus system, are protein filaments necessary for adhesion to abiotic surfaces and the initiation of biofilm formation, contributing to the environmental persistence of *A. baumannii* (128). Deletion of the *csuE* gene prevents biofilm formation, highlighting its importance in the initial attachment of bacteria to surfaces before the complete development of the biofilm. The expression of the *csu* operon is regulated by the two-component system BfmRS, which consists of a sensor kinase and a response regulator. BfmRS serves as a regulatory pathway, responding to various environmental stimuli that can either promote or inhibit biofilm formation in *A. baumannii*. While the role of BfmR in controlling the *csu* operon is well-established, the deletion of BfmS does not halt biofilm formation, suggesting that the sensor kinase can be activated independently of BfmR. Additionally, while the *csu* operon-mediated pili formation is necessary for attachment to abiotic surfaces, it is not required for attachment to biotic surfaces such as human alveolar epithelial cells. This observation raises the possibility that another receptor or mechanism is involved in the attachment to biotic surfaces, which does not rely on pili.

Pakharukova et al., (2018) reported the action of csu pili in becoming the structural basis of biofilm formation. It was reported that for this csu pili possess three hydrophobic finger-like loops at the tip of the pilus for bacterial attachment to abiotic surfaces (129). This pili tip plays a vital role in biofilm formation as when an antitip antibody is introduced, biofilm is demolished. This type of flexible fingers in bacterial attachment allows strong adhesion of *A. baumanni* towards surfaces like abiotic surfaces. The important role of csuE which codes for the putative tip adhesion is shown where inactivation of this gene causes total abolishment of pili formation as well as biofilm formation. Thus, the latest research revealing the importance of a finger-like tip for adhesion might explain the action of this pili on highly variable surfaces like abiotic surfaces but not on defined surfaces like biotic surfaces.

BfmRS

The two-component system BfmRS plays a regulatory role in the transcription of the csuA/BABCDE usher-chaperone assembly system, which is responsible for pili formation in *A. baumannii*, as discussed earlier. The activity of BfmRS is controlled by various environmental cues that influence biofilm formation, including nutrient availability and cell density optimization (116). However, while the involvement of csuA/BABCDE in biofilm formation is widely accepted, its role in

surface adherence has been debated, with de Breij et al. (2010) (130) contradicting earlier observations by Tomaras et al. (2003) (128). Additionally, Geisinger et al. (2015) reported the critical role of BfmRS in antibiotic resistance through the modification of exopolysaccharides via a conserved gene cluster known as the K locus (106). More recently, Kim et al. (2022) unveiled a genetic complementarity between BfmRS and the AbaIR system, which regulates various virulence factors in A. *baumannii* (113). Figure 1 illustrates a simplified mechanism proposing the actions of BfmRS in regulating virulence.

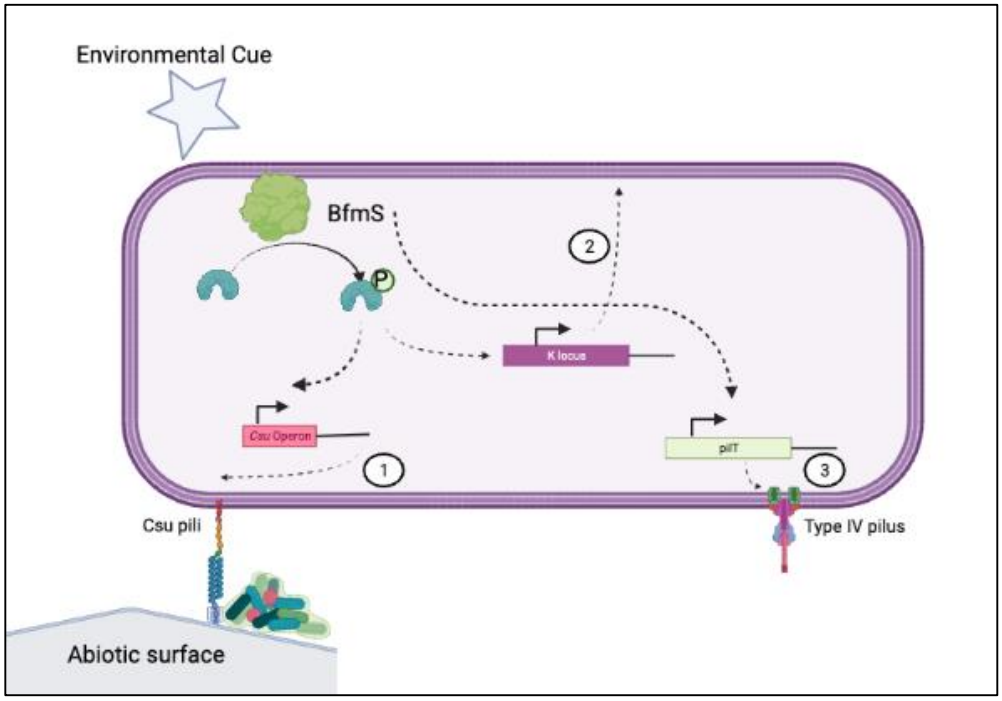

Figure 1. Regulatory network of two-component system BfmRS

1. Two-component system, BfmS phosphorylates its cognate BfmR and activates the transcription of csu operon and subsequently biofilm formation **2**. Phosphorylated BfmR increases the transcription of generic gene K locus and promotes the generation of thick polysaccharides capsule **3.** BfmS activates the transcription of pilT which is responsible for the formation of Type IV pilus for surface motility.

Current and future treatments

The treatment of *A. baumannii* infections, particularly those that exhibit multidrug resistance, poses a significant challenge. One of the primary obstacles is the frequent misidentification of the species due to their high morphological similarities, leading to divergent outcomes (131,132). Existing therapeutic choices for carbapenem-resistant *Acinetobacter baumannii* (CRAB) infections are constrained and encounter challenges related to pharmacokinetics, including substantial toxicity and inadequate plasma concentrations. Treatment approaches utilizing non-canonical drugs, including siderophore cephalosporin (e.g., cefiderocol), improved tetracyclines, beta-lactamase inhibitors, polymyxin B, and apramycin, have demonstrated promising outcomes, albeit with limited widespread effectiveness (133). Additionally, alternative therapeutic approaches like phage therapy (134–136) and monoclonal antibodies (137–139) have been explored. Among these approaches, only *eravacycline* has received FDA approval for the treatment of carbapenem-resistant *Acinetobacter baumannii* (CRAB) infections, while bacteriophage therapy is currently in Phase 3 clinical trials (133). Furthermore, various approaches have been investigated to counteract the virulence activities of *A. baumannii*, aiming to reduce selection pressure and mitigate the emergence of resistance. Several antivirulent agents have been studied to successfully target multiple virulence factors of *A. baumannii* including *LpxC-1*, Deferiprone and Virstatin (38–40). Recently, more research was further conducted in exploring other anti-virulent agents that could be used to inhibit virulence action in *A. baumannii*. Among the potential anti-virulence agents that are being studied including silver nanoparticles (41), pyrogallol (42), flavonoids and curcumin (43) as well as myrtenol

(44). In addition, research has been conducted on the exploitation of *A. baumannii*'s quorum sensing regulation to control its virulence. For instance, the use of quorum sensing inhibitors like acyclase PvdQ (140) and canonical antibiotics such as erythromycin, levamisole, chloroquine, and propranolol(141). Overall, addressing the challenges of *A. baumannii* infections requires a multi-faceted approach, including the development of new antimicrobial agents, combination therapies, and alternative treatment strategies, all aimed at overcoming the resistance mechanisms exhibited by this resilient pathogen.

Table 1: Common antibiotics prescribed for *A. baumannii* infections

Antibiotics	Group	Mechanism of action	Mechanism of resistance	Recommendation
Piperacillin	Penicillin	Inhibit bacterial cell wall synthesis and induce cell lysis	Production of beta-lactamase enzyme Reduced production of Penicillin-binding protein (PBP)	Listed in CLSI but not in EUCAST
Ampicillin-sulbactam	B-lactam Combination Agents	Binding to PBP and inhibit beta-lactamase	β-lactamase synthesis encoded in the gene BlaTEM-1	Listed in CLSI but not in EUCAST
Ceftazidime Cefotaxime Ceftriaxone	Cephems (Cephalosporin)	Binding to PBP and inhibiting bacterial cell wall synthesis	*Acinetobacter*-derived cephalosporinases (ADC) β-lactamase	Listed in CLSI but not in EUCAST
Cefepime	Carbapenems		•ADC does not act on cefepime •Production of extended-spectrum beta-lactamases (ESBLs) and AmpC beta-lactamases •Alteration of penicillin-binding proteins •Efflux pump	Listed in CLSI but not in EUCAST
Cefiderocol	Carbapenems	Uptake into the cell using siderophore and binding to PBP. Inhibit efflux pump synthesis	•Alteration of penicillin-binding proteins •Beta-lactamase that can degrade cefiderocol •Efflux pump overexpression •Altered membrane permeability	Listed in CLSI but not enough evidence for MIC value in EUCAST
Imipenem Meropenem	Carbapenems	Cell wall synthesis inhibition by binding to penicillin-binding proteins (PBPs),	•Overexpression of the carbapenem-hydrolyzing oxacillinase (OXA)-51-like-B-lactamase •Production of RNA 16S ribosomal methyltransferase (ArmA) •Some strains have metallo-β-lactamases •Changing bacterial cell wall permeability •Reduced expression and alteration of penicillin-binding protein •Presence of efflux pumps	EUCAST has different breakpoints based on infection sources
Tobramycin Gentamycin	Aminoglycosides	bind to the RNA 16S of the ribosomal 30S subunit.	•Production of aminoglycoside modifier enzymes •Production of RNA 16S ribosomal methyltransferase (ArmA)	Recommended in both
Tetracyclines	Tetracyclines	Bind to 30S ribosomal subunit and prevent bacterial protein synthesis	•Resistance-nodulation-division (RND)-type efflux pumps •Inhibition of ribosomal binding by Tet proteins	Listed in CLSI but not in EUCAST
Ciprofloxacin Levofloxacin	Fluoroquinolones	Inhibit bacterial DNA replication and transcription by inhibiting enzyme DNA gyrase and topoisomerase IV	•Substitutions of DNA gyrase and DNA topoisomerase IV which hinder the union of fluoroquinolone with protein targeted •Overexpression of efflux active pumps	Both but different MIC were recorded
Colistin	Polymyxin	Disrupting bacterial cell membrane by binding to the lipid component of the outer membrane	•Modification of Lipid A of lipopolysaccharides •Losing its LPS	Listed in CLSI but not in EUCAST

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