Multidrug resistance and biofilm formation contribute to the nosocomial infections caused by *Acinetobacter baumannii*

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The Gram-negative bacterium *Acinetobacter baumannii* has received much attention in recent years as one of the most troublesome opportunistic pathogens for the global health care institutions by causing a wide range of nosocomial infections associated with high morbidity and mortality rates. As a consequence of a variety of resistance mechanisms against different antibacterial agents, the predominance of clinical *A. baumannii* isolates has been related to the multidrug-resistant (MDR) phenotype. Moreover, many *A. baumannii* strains are capable of attaching their planktonic cells to different surfaces and growing as biofilms, which plays significant roles in the ability of *A. baumannii* to persist and spread in the hospital environment, and results in a higher risk of infections. The better understanding of the molecular mechanisms of MDR and biofilm formation will shed light on the development of novel treatments and prevention strategies against *A. baumannii* infections in hospital.

Keywords: *Acinetobacter baumannii*; nosocomial infections; multidrug resistance; biofilm

1. Introduction

Acinetobacter baumannii is now recognized as one of the most successful opportunistic pathogens for the global health care institutions, while it was previously considered as a low-virulence bacterium [1, 2]. Although many *Acinetobacter* species are ubiquitous and easily recovered from many natual environments, there is no known natural habitat for *A. baumannii* outside the hospital [3]. With the identified multifarious virulence factors, *A. baumannii* has been implicated in a variety of nosocomial infections associated with high morbidity or mortality rates, including ventilator-associated pneumonia, wound infections, bloodstream infections, secondary meningitis, skin and soft tissue infections and urinary tract infections *et al.* [1, 4]. The resistance to multiple antibacterial agents and formation of biofilms on both biotic and abiotic surfaces seem to play important roles in the pathogenicity of *A. baumannii*. The predominance of reported clinical *A. baumannii* isolates has been related to the multidrug-resistant (MDR) phenotype, which is a consequence of a variety of resistance mechanisms against different antibiotics, *i.e.* permeability defects, expression of multidrug efflux pumps, production of antibiotic-degradation/modification enzymes and alteration of drug-targeting sites [5]. According to the recent genomic analyses, the high capacity to acquire new genetic determinants contributes *A. baumannii* to display an open pan genome, which may act a key role in the development of MDR phenotype [6]. Moreover, many *A. baumannii* clinical strains are capable of attaching their planktonic cells to different hydrophilic or hydrophobic substrata, *e.g.* hospital equipment, indwelling medical devices and bronchial epithelial cells, and growing as biofilms at the solid-liquid interface [7]. As the self-produced extracellular matrix (ECM) enclosed microbial accretions that adhere to surfaces, biofilms offer *A. baumannii* a stable and protected lifestyle, and allow it to survive in hostile environments and disperse to colonize new niches, which results in a higher risk of nosocomial infections [8, 9].

2. *A. baumannii* in the habitats associated with hospital

Acinetobacter is a genus of Gram-negative, nonmotile and strictly aerobic coccobacilli, with a DNA G+C content of 39 to 47 mol%, belonging to the γ-proteobacteria class, Pseudomonadales order and Moraxellaceae family [10, 11], and members of which have been classified under a few of different names in the history. Because of the widespread presence of organisms identified as genus *Acinetobacter* in different ecological niches, the common misconception of *A. baumannii* is that its natural habitat is highly prevalent in nature [3]. One of the major reasons to cause that misunderstanding is the inaccuracy in strain identification. The phenotypic methods could not identify the *Acinetobacters* at the species level accurately, and the DNA hybridization was thus used to split up the genus into species [12]. However, *A. baumannii* and its close relatives, *e.g. A. calcoaceticus*, have been grouped into the so-called *A. baumannii* complex for a long time bacause of their high similarities [13]. Due to the different clinical implications by members of the complex, grouping these species together could be misleading in clinical settings [14]. Therefore, several molecular approaches (Table 1) have been employed to establish the identity of different species belonging to the genus *Acinetobacter*. Among these genotypic methods, 16S rRNA gene sequencing is still not sufficiently

polymorphic to distinguish all *Acinetobacter* species [15, 16], although it is most commonly used for bacterial identification. According to the recent results, different methods have shown different reliability in identifying *Acinetobacter* clinical isolates, *i.e.* the accuracy rates of *rpoB* gene sequencing, 16S rRNA gene sequencing, *gyrB* multiplex PCR and VITEK 2 fluorescent system are 98.2%, 93.4%, 77.2%, and 35.9%, respectively [14]. In this case, the results describing isolations of *A. baumannii* should be carefully interpreted, especially for the strains not identified to the species level according to the current taxonomy using validated methods.

Table 1 Methods to identify the species belonging to genus *Acinetobacter*.

Identification System	Target(s)	Reference
DNA-DNA Hybridization	whole genome	[13]
Amplified Fragment Length Polymorphism (AFLP)	whole genome	[17]
Pulsed-field Gel Electrophoresis (PFGE)	whole genome	[18]
Amplified Ribosomal DNA Restriction Analysis (ARDRA)	16S rDNA	[19]
tRNA Spacer Fingerprinting	tRNA spacer	[20]
Ribotyping	rDNA and adjacent regions	[21]
Specific Gene/Region Sequencing	16S rRNA gene	[22]
	16S-23S rRNA intergenic spacer	[23]
	rpoB gene	[24]
	gyrB gene	[25]
	recA gene	[26]
	$bla_{\text{OXA-51-like}}$ gene	[27]
MALDI-TOF[1] Mass Spectrometry	bacterial extracts	[28]
VITEK 2 Fluorescent System[2]	phenotype database	[29]
API 20 NE System[3]	phenotype database	[29]

Note: [1]MALDI-TOF: Matrix-assisted laser desorption/ionization-Time of flight; [2]The VITEK 2 fluorescent system (ID-GNB card, bioMe´rieux, Marcy l'Etoile, France) includes 43 nonenterobacterial gram-negative taxa; [3]The API 20 NE system (bioMe´rieux, Marcy l'Etoile, France) covers 61 nonenterobacterial gram-negative taxa.

Although the other *Acinetobacter* species are ubiquitous and easily recovered from soil, water and animals, *A. baumannii* is a very rare organism in environmental samples and has no known natural habitat outside the hospital [3]. As an opportunistic pathogen, *A. baumannii* can be generally isolated during outbreaks and rarely isolated during non-outbreak periods from patients and hospital environmental sources [3]. With some ecological surveys, it has been demonstrated that *A. baumannii* is widespread on clinical environments, and most notably in intensive care units (ICUs) with critically ill patients and other facilities requiring mechanical ventilation [30, 31]. While the sources of nosocomial infection with *A. baumannii* vary among different cases, the infected patient likely acts as the primary reservoir of infection, who sheds huge numbers of *A. baumannii* cells into the surrounding environment. The ability of *A. baumannii* cells to resist desiccation and disinfectants leads to their long-term persistence on hospital materials and medical devices, and consequently the occurrence of outbreaks of infection involving many patients [3, 4]. In addition to airborne and patient to patient transmissions, several potential sources of infectious *A. baumannii* have been uncovered in hospital, *e.g.* ventilators, hands of staff, gloves, oxygen analyzers, air supply, bronchoscopes, bed frames, sinks and soap *et al.* [3]. Meaning while, multiple factors have been identified to facilitate the spread of *A. baumannii* in hospital, such like the increased length of hospital stay, prior antibiotics, mechanical ventilation, environmental contaminations, understaffing, poor adherence of staff to hand hygiene and the exposure to patients infected with *A. baumannii* [3].

Moreover, the proposal that *A. baumannii* is a bacterial component of the normal human flora has been challenged recently [3]. Despite *A. baumannii* is often associated with skin infections, it is in fact rarely found on skin of healthy human [32]. In an epidemiological study to investigate the colonization with *Acinetobacter spp.* on the skin and mucous membranes of 40 patients and 40 healthy controls, the most frequently isolated species are *A. lwoffii* (87 of total 186 *Acinetobacter* isolates), *A. johnsonii* (39/186) and *A. radioresistens* (22/186), while *A. baumannii* has been found very rarely on human skin (only 1/186) [33]. While *A. baumannii* is considered as an inhabitant of oral biofilms to act as a reservoir for pneumonia and chronic obstructive pulmonary disease, it has been identified with a significantly higher prevalence in patients with periodontal diseases compared with good periodontal health [34]. The presence of *A. baumannii* in conjunction with the traditional periodontal pathogens, *e.g. Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola*, *Aggregatibacter actinomycetemcomitans* and *Pseudomonas aeruginosa*, increases the likelihood of aggressive periodontitis [35].

3. Nosocomial infections caused by *A. baumannii*

Nosocomial infections associated with species belonging to the genus *Acinetobacter* are predominantly caused by *A. baumannii*, *e.g.* ventilator-associated pneumonia, wound infections, bloodstream infections, secondary meningitis, skin and soft tissue infections and urinary tract infections *et al*.

3.1 Pneumonia

The common clinical manifestation infections caused by *A. baumannii* is ventilator associated pneumonia (VAP), which has a mortality rate of about 73% [36]. VAP occurs most in the patients receiving mechanical ventilation in ICU, and it is developed after *A. baumannii* colonization of the airway via environmental exposure [4]. *A. baumannii* can also cause community-acquired pneumonia associated with old age, alcoholism, heavy smoking, diabetes mellitus, chronic obstructive pulmonary disease and renal disease, which is also regarded serious with a high mortality ranging from 40% to 64% [30, 37].

3.2 Wound infections

The other characteristic nosocomial infection caused by *A. baumannii* was wound infection, including severe burns or trauma. According to the records reviewed from January 2003 to December 2008 at US Army Institute of Surgical Research Burn Centre, *A. baumannii* associated with burn injuries was the most prevalent organism recovered (22% of 780 isolates) [38]. Furthermore, a number of severe wound infections, burn infections and osteomyelitis cases caused by *A. baumannii* have been reported to be associated with natural catastrophes or man-made disasters (*e.g.* earthquakes, floods, tsunami, terrorist attacks or military campaigns) because the capacities of hospital for patient care were overloaded and standard hygiene procedures were not followed [4, 39, 40, 41].

3.3 Bloodstream infections

A. baumannii is also a leading cause of bloodstream infections in health care settings. The crude mortality of bloodstream infection caused by *A. baumannii* is about 43% in the ICU and 16% outside the ICU, ranking the third highest mortality in the ICU after *Candida* species and *P. aeruginosa* [42]. Immunosuppression, ventilator use associated with respiratory failure, previous antibiotic therapy, colonization with *A. baumannii*, and invasive procedures are elucidated as risk factors of bloodstream infection caused by *A. baumannii* [4].

3.4 Skin and soft tissue infections

Skin and soft tissue infection (SSTI) caused by *A. baumannii* is becoming common along with continue increased prevalence of this organism in health care system. Comparative analysis revealed that *A. baumannii*-associated SSTI often occurred in patients with underlying comorbidities (*e.g.* trauma wounds) and accompanied by bacteremia. Frequently complicated treatment resulting from MDR phenotype and the presence of co-pathogens lead to substantial mortality as high as 30% [41, 43].

3.5 Other nosocomial infections

In addition, *A. baumannii* is also a cause of urinary tract infection, meningitis, osteomyelitis, or endocarditis [4].

4. Virulence factors of *A. baumannii* correlated with MDR and biofilm formation

While *A. baumannii* was previously considered as a low-virulence bacterium, it has emerged as a successful and global opportunistic pathogen [1, 2]. In recent years, combinatorial approaches associated with the genomic sequencing, phenotypic analysis and animal infection models [4] have made great contributions to identify *A. baumannii* virulence factors (Table 2). Some established factors are responsible for development of the MDR phenotype in *A. baumannii*, *i.e.* lipopolysaccharide (LPS), outer member vesicles (OMVs), and penicillin-binding protein (PBP). As an important virulence factor of *A. baumannii*, LPS cause septic shock and enhance the Toll-like receptor 4 (TLR4) activation and host damage [2], and are also involved in the polymyxins resistance development by its missing in the outer membrane [44] or modifying lipid A part [45]. OMVs are vesicles secreted from the outer membrane of cells and have been suggested to participate in bacterial virulence by several ways. In *A. baumannii*, OMVs have been shown to facilitate the horizontal transfer of carbapenemase gene *OXA*-24, and thus play a role in the spread of antibiotics resistance [46]. PBP is normally associated with binding to and inactivating β-lactam antibiotics, and also contributes to *A. baumannii* cell stability by involvement in biosynthesis of the peptidoglycan layer [47].

Many factors, including biofilm-associated protein (Bap), chaperone-usher pili assembly system, iron uptake systems and outer membrane proteins (OMPs) contribute *A. baumannii* to adhere and form biofilm on solid surface *in vitro*, which facilitate the resistance of cells to desiccation, immune system clearance, antibiotics and other antibacterial

agents [48]. In *A. baumannii*, the initiation and maturation of biofilms are related to pilus assembly and the production of Bap, and the siderophore iron uptake system is also involved in the development of biofilms [5]. Among all OMPs, OmpA is the best-characterized virulence factor of *A. baumannii* with multiple functions [4], which facilitates the survival of cells by promoting biofilm formation and surface motility [49, 50]. Although new valuable insights into the mechanisms of *A. baumannii* pathogenesis have been provided, our understanding is still in the early stages and numerous additional factors remain elusive.

Table 2 Summary of known virulence factors in *A. baumannii*.

Virulence Factor(s)	Proposed Functions in Toxicity and Pathogenicity	Reference(s)
Biofilm-associated protein (Bap)	Adhesion to human epithelial cells and biofilm formation	[51]
Chaperone-usher pili assembly system	Attachment and biofilm formation	[52]
Capsular polysaccharides (CPS)	Resistance to the host innate immune response	[53]
Lipopolysaccharide (LPS)	Stimulators of inflammatory signalling	[54]
	Stimulators of human monocyte activation	[55]
	Resistance to polymyxins (colistin)	[44, 45]
Outer member vesicles (OMVs)	Delivery of virulence factors to host cells	[56]
	Horizontal transfer of antibiotic resistance genes	[46]
Outer membrane proteins (OMPs)	Inducing apoptosis of epithelial cell	[57]
	Epithelial cell invasion	[58]
	Resistance to human serum	[59]
	Targeting the nucleus and inducing cytotoxicity	[60]
	Antibiotics resistance	[61]
	Biofilm formation	[49]
Iron uptake systems	Persistence under iron-limited conditions	[62]
	Biofilm formation	[63]
Phospholipases	Resistance to the human serum	[64]
	Epithelial cell invasion	[64]
	Pathogenesis in pneumonia	[64]
	Toxicity to epithelial cells	[65]
Penicillin-binding protein (PBP)	Resistance to the human serum	[66]
	Binding to and inactivating β-lactam antibiotics	[47]

5. Multi-drug resistance in *A. baumannii*

In decades, an increasing number of *A. baumannii* clinical isolates have been reported as MDR microorganisms, which are defined as those strains that are resistant to three or more types of antibiotics [67]. Many worldwide studies report that a variaty of *A. baumannii* strains are resistant to the two antibiotics most commonly used to treat *A. baumannii* infections, *i.e.* carbapenems and ampicillin-sulbactam. A recent cohort study has shown that 68 patients (25%) with bloodstream infections were caused by carbapenem- and ampicillin-sulbactam-resistant (CASR) *A. baumannii*, and 206 patients (75%) with bloodstream infections were caused by non-CASR *A. baumannii* [68]. Meaning while, MDR *A. baumannii* infections are associated with the additional increases in morbidity, mortality, length of hospital stay and health care costs versus susceptible infections [69]. It is known that *A. baumannii* possesses numerous mechanisms to resist multiple classes of antibiotics (Table 3), *i.e.* production of antibiotic-degradation/modification enzymes, expression of multidrug efflux pumps, permeability defects and alteration of drug targets [4, 5, 70]. Moreover, different mechanisms can also act synergistically to resist a single class of antibiotic, *e.g.* diverse mechanisms have been shown to contribute to the carbapenem-resistance in *A. baumannii* [71]. In some specific cases, certain *A. baumannii* strains are highly resistant to almost all antibiotics available in the clinical practice [72].

5.1 Antibiotic-degradation/modification enzymes

Most resistance to β-lactams in *A. baumannii* is related to the production of β-lactamases, including all four Ambler classes of β-lactamases (class A, B, C and D). In *A. baumannii,* numerous class A β-lactamases are identified, which are considered to play a minor role in its resistant phenotype; broad-range class B β-lactamases confer resistance to the majority of β-lactams; class C β-lactamase AmpC exhibits a typical cephalosporinase substrate profile; and class D β-lactamases are designated OXAs in the literatures due to their preferred substrate oxacillin [5]. Moreover, some class B and D β-lactamases show carbapenem-hydrolyzing activity (a.k.a. carbapenemases) and confer a major carbapenem resistance mechanism to *A. baumannii* [4]. Chemical modification of antibiotics by enzyme is the principal mode of

resistance to aminoglycosides in *A. baumannii*, which is mediated by three classes of aminoglycoside-modifying enzymes (AMEs), *i.e.* acetyltransferases, adenyltransferases and phosphotransferases [90].

5.2 Efflux pumps

As the active transporters localized in the cytoplasmic membrane, efflux pumps contribute greatly to multidrug resistance in bactreia, which are responsible for *A. baumannii* resistance against many different classes of antibiotics, including tigecycline and imipenem [78, 79]. Among five categories of efflux pumps, four superfamilies are related to antimicrobial resistance in *A. baumannii*, including the major facilitator superfamily (MFS), the small multidrug resistance (SMR) family, the resistance-nodulation-division (RND) superfamily and the multidrug and toxic compound extrusion (MATE) family, except the ATP-binding cassette (ABC) superfamily [5].

Table 3 Antibiotics resistance mechanisms in *A. baumannii*.

Antibiotics	General Mechanism	Specific Mechanism	Proteins	Refer.
β-lactams	Degradation enzymes	β-lactamases	ADC, AmpC, CARB, CTX-M, PER, SHV, SCO, SIM, TEM, VEB	[4, 5, 30]
		Carbapenemases	GES, IMP, KPC, NDM, OXA, VIM	[4, 5]
	Efflux pumps	RND[1]	AdeABC, AdeIJK, AdeDE	[73]
		MATE[2]	AbeM	[73]
	Permeability defects	Change of OMPs[3]	OmpA, CarO, OprD-like protein	[4, 5]
	Alteration of targets	Change of PBPs[4]	PBP2	[47]
Aminoglycosides	Modification enzymes	AMEs[5]	AAC, ANT, APH	[4, 5]
	Efflux pumps	RND[1]	AdeABC	[73]
		MATE[2]	AbeM	[73]
	Alteration of targets	16S rRNA methylation	ArmA, RmtB	[5, 74]
Fluoroquinolones	Efflux pumps	RND[1]	AdeABC, AdeFGH	[5, 75]
	Alteration of targets	DNA gyrase	GyrA, ParC	[5]
Tetracyclines	Efflux pumps	RND[1]	AdeABC	[4, 5]
		MFS[6]	Tet A, Tet B	[4, 5]
	Alteration of targets	Ribosomal protection	Tet M	[4, 5]
Polymyxins (colistin)	Alteration of targets	Loss of LPS[7]	LpxA, LpxC, LpxD	[44]
	Alteration of targets	Modification of lipid A	PmrA, PmrB	[45]
Rifampicin	Modification enzymes	ADP-ribosyltransferase	Arr-2	[76]
	Alteration of targets	*rpoB* mutation	RpoB	[77]

Note: [1]RND: Resistance-nodulation-division superfamily; [2]MATE: Multidrug and toxic compound extrusion family; [3]OMPs: outer membrane proteins; [4]PBPs: penicillin-binding proteins; [5]AMEs: aminoglycoside-modifying enzymes; [6]MFS: Major facilitator superfamily; [7]LPS: lipopolysaccharides.

5.3 Permeability defects

As a major group of OMPs in Gram-negative bacteria, β-barrel protein porins act as a pore to allow the diffusion of molecules across lipid bilayer membranes, which influences the virulence of *A. baumannii* and plays an important role in the mechanisms of MDR [5]. It was reported that the loss of a 29 kDa OMP or reduced expression of 22 kDa and 33 kDa porins resulted in imipenem or carbapenem resistance of *A. baumannii*, respectively [80, 81].

5.4 Alteration of drug targets

The mechanisms of alteration of drug targets, such as changes of PBPs [47], DNA gyrase point mutations [82], production of 16S rRNA methylase ArmA [5] and presence of TetM protein [83] have been all identified in *A. baumannii*. For *A. baumannii* MDR infections, colistin (a.k.a. polymyxin E) is often the only remaining treatment, while colistin-resistant clinical isolates have been reported. It was shown that *A. baumannii* could rapidly develop resistance to colistin by complete loss its LPS production or phosphoethanolamine modification of lipid A, which is the initial binding target of colistin suggested by the electrostatic interaction between the positively charged drug and the negatively charged lipid A [44, 45].

5.5 Horizontal gene transfer (HGT) of resistance related genes

In genomic analysis, current *A. baumannii* clinical population exhibits a high capacity to acquire MDR genetic determinants through HGT and results in an open pan genome, which is a crucial factor for this opportunistic pathogen evolving to clinical success [6]. Based on the 12 available *A. baumannii* genomes, comparative analysis shows that among the identified CDSs, 1,455 CDSs are core genes and 7,363 are dispensable genes, indicating that the high capacity to acquire new genes in *A. baumannii* [6]. Indeed, the spread of MDR determinants in *A. baumannii* may be constitutive or acquired via plasmids, integrons and transposons to gain the related resistance phenotypes [84]. For example, *A. baumannii* MDR strain AYE possesses an 86 kb genomic region termed as a resistance island containing a cluster of 45 resistant genes, and most of which have been confirmed to be acquired by HGT from bacterial cells of the genera *Pseudomonas*, *Salmonella* or *Escherichia* [85]. Most genes that encode degradation/modification enzymes and specific efflux pumps are present in some *A. baumannii* strains and are all associated with genetic elements like transposons, integrons or plasmids, which suggests they are transferred by HGT [30]. Furthermore, an open pan genome appears to be more easily for *A. baumannii* to acquire new functions and promote cells to persist in the hospital setting and survive under the strong selection pressure of antimicrobial utilization.

6. Biofilms formed by *A. baumannii* contribute to its pathogenicity

Bacterial biofilms comprise a self-produced and highly structured ECM enclosed microbial cells that adhere to biological or non-biological surfaces, and differs profoundly from their planktonic counterparts at least in terms of physiological responses and developmental dynamics [86]. Biofilm is now accepted as a preferred and protected lifestyle option for many pathogens, which allows the cells to survive in hostile environments and disperse to colonize new niches [9, 87]. It has been proposed that *A. baumannii* persists in medical environments, resists antimicrobial agents and causes nosocomial infections due to its capacity to form biofilms on surfaces [88, 89].

6.1 The attachment of *A. baumannii* cells on solid surface to initialize biofilm formation

For bacteria, the initial attachment to solid surfaces is the first step for their colonization and biofilm formation. A number of studies have revealed a high propensity of *A. baumannii* clinical isolates to adhere to both biotic and abiotic surfaces, *e.g.* bronchial epithelial cells, glass and plastic, and grow as biofilms [7]. The ability to form biofilms varies remarkably among different *A. baumannii* clinical strains, and the formation is also affected by the property of abiotic surface [91], which implies the complexity of the process. Although many details are still missing, some factors have been shown to play roles in the attachment of *A. baumannii* cells and the subsequent biofilm formation. The usher-chaperone pili assembly system (CsuA/BABCDE) is very important for *A. baumannii* initial attachment on abiotic surfaces [7]. The outer membrane protein OmpA and O-linked protein glycosylation system have been demonstrated to be involved in the attachment of *A. baumannii* and promotion of biofilm formation [58, 92]. The adherence on eukaryotic cells is a crucial step for *A. baumannii* to colonize and infect the host [94]. Bap is a protein distributed on the cell surface, which is highly conserved among different *A. baumannii* clinical isolates [93]. During the initial attachment, *A. baumannii* increases its cell surface hydrophobicity with the presence of Bap, and promotes the adherence of cells both to abiotic medically relevant surfaces (*e.g.* polypropylene, polystyrene and titanium) and to biotic surfaces (*e.g.* human bronchial epithelial cells and neonatal keratinocytes). Furthermore, it has been shown that biofilm formation by Bap-positive strain is inhibited by affinity-purified Bap antibodies, which suggests the direct involvement of Bap to *A. baumannii* biofilm formation and maturation [95].

6.2 *A. baumannii* cells in biofilms are resistant to the hostile environments

After forming biofilms on abiotic surfaces, the enclosed *A. baumannii* cells are protected from desiccation and antimicrobial treatment, which allows the cells to survive long stretches of time in hostile environments and contributes to the maintenance of *A. baumannii* populations in hospital settings, therefore, may partly explain its propensity to cause outbreaks. It has been shown that survival of the biofilm-forming *A. baumannii* strains on dry solid surface is much better than that of non-biofilm-forming strains (36 days vs. 15 days, $P < 0.001$) [87], which agrees with that *A. baumannii* biofilms enhance the cell persistence and increase the probability of causing nosocomial infections. It has also been proposed that *A. baumannii* cells are able to survive long periods of desiccation through the presence of cells in a dormant state, and the mechanisms underlining this phenomenon could be affecting control of cell cycling, DNA coiling, transcriptional and translational regulation, protein stabilization, antimicrobial resistance and toxin synthesis. With a few surviving cells embedded in the ECM, *A. baumannii* is able to resume growth and restore the original population in appropriate environmental conditions following a feast-and-famine strategy [96]. Interestingly, in the presence of some antimicrobial agents like ethanol, biofilm formation of *A. baumannii* on abiotic surfaces is stimulated and enhanced [97].

6.3 Correlation between *A. baumannii* biofilm formation and infections

Biofilm formation has been regarded as one of the major virulence factors of *A. baumannii*. Some clinical isolates exhibited a correlation between serum resistance and the ability to form biofilm [98], and in a multicenter cohort study, all catheter-related urinary or blood stream infections due to *A. baumannii* were caused by biofilm-forming strains [7]. However, the medical relevance of these data is still not clear, considering the lack of correlation between the biofilm phenotype of most clinical isolates and their outbreak or epidemic nature [99]. On the other hand, the pathogenicity of *A. baumannii* clinical strains also relies on the ability to survive antibiotic treatment. In order to inactivate the encased cells, antimicrobial molecules have to diffuse through the biofilm matrix. While the exopolysaccharides in ECM of *A. baumannii* biofilm is suggested to be capable of adsorbing antibiotics near the surface and limiting antibiotics diffusion to the bottom [8]. Meaning while, extracellular DNA (eDNA) molecules have been identified in the biofilm formed by an *A. baumannii* clinical strain [100], which are incorporated into ECM and ought to convey the antibiotic resistance to enclosed cells [101]. Despite the increasing importance of *A. baumannii* biofilm associated with infections, our understanding of mechanisms underlining related pathogenesis remains largely unknown, especially comparing with the knowledge from other bacterial pathogens, such as *P. aeruginosa*.

7. Conclusions and perspectives

In consideration of a wide range of nosocomial infections associated with high morbidity and mortality caused by *A. baumannii*, it has emerged as one of the most successful pathogens in the modern health care system. Some new and valuable insights for virulence and pathogenesis of *A. baumannii* have been provided recently, which reveal the significance of MDR and biofilm formation. An increasing number of *A. baumannii* clinical isolates have been reported as MDR microorganisms, and certain strains are even highly resistant to almost all antibiotics available in clinical practice. *A. baumannii* possesses several mechanisms to resist multiple classes of antibiotics respectively, and employs different mechanisms synergistically to be against a single class of antibiotic. Moreover, the ability of forming biofilms in *A. baumannii* plays a pivotal role in the cell survival in hostile environments, maintenance of populations in hospital settings and increasing the probability of causing nosocomial infections. Further investigations should be conducted on the correlation between *A. baumannii* biofilm formation and propensity to cause outbreaks of infections. The better understanding of the molecular mechanisms of MDR and biofilm formation will shed light on the development of novel treatments and prevention strategies against hospital-derived *A. baumannii* infections.

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