Acinetobacter baumannii biofilms: effects of physicochemical factors, virulence, antibiotic resistance determinants, gene regulation, and future antimicrobial treatments

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Abstract: *Acinetobacter baumannii* is a leading cause of nosocomial infections due to its increased antibiotic resistance and virulence. The ability of *A. baumannii* to form biofilms contributes to its survival in adverse environmental conditions including hospital environments and medical devices. *A. baumannii* has undoubtedly propelled the interest of biomedical researchers due to its broad range of associated infections especially in hospital intensive care units. The interplay among microbial physicochemistry, alterations in the phenotype and genotypic determinants, and the impact of existing ecological niche and the chemistry of antimicrobial agents has led to enhanced biofilm formation resulting in limited access of drugs to their specific targets. Understanding the triggers to biofilm formation is a step towards limiting and containing biofilm-associated infections and development of biofilm-specific countermeasures. The present review therefore focused on explaining the impact of environmental factors, antimicrobial resistance, gene alteration and regulation, and the prevailing microbial ecology in *A. baumannii* biofilm formation and gives insights into prospective anti-infective treatments.

Keywords: *Acinetobacter baumannii*, biofilm, physicochemical, antibiotics, resistance, in vivo model, virulence, treatment

Introduction

Acinetobacter baumannii belongs to the family *Moraxellaceae* in the class *Proteobacteria* of *Eubacteria*.[1] The acceptable phylogeny, classification, and interpretation of the taxonomy of the genus, *Acinetobacter*, has been confused due to the myriads of classification systems established by scientists early on,[2] with over 40 known species within the genus.[3] The most clinically important *Acinetobacter* sp. is *Acinetobacter baumannii*,[4] because of its association with hospital acquired-infections.[5] Others include: *Acinetobacter baylyi*, *Acinetobacter grimontii*, *Acinetobacter junii*, and *Acinetobacter calcoaceticus-A. baumannii* (ACB) complex.[6,7] *A. baumannii* is a Gram-negative, non-motile, strictly aerobic, non-fermentative, non-sporing, coccobacillus, which is notorious for its ability to survive in a variety of environmental conditions.[8] The notorious bacterium *A. baumannii* has been referred to as "Iraqibacter" since its emergence in the US military treatment facilities when it caused serious problematic infections among soldiers during the Iraq and Afghanistan war-zone medical facilities.[8] Hospital-acquired pneumonia, skin and soft tissue infections, and nosocomial meningitis are but a few infections caused by *A. baumannii*.[9] It became a serious issue in medical treatment due

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to its exceedingly increased resistance to almost all known antibiotics and host immune responses.[10] The possession of virulence factors such as the ability to form biofilm and survive in dry and desiccated environmental conditions have created challenges and epidemics in the health treatment, especially in critically ill patients in hospital intensive care units (ICUs).[11] Here, we review the parameters that trigger biofilm formation with a view to understanding *A. baumannii* biofilm formation and also to assess the relationships between biofilm formation, virulence, and antimicrobial resistance.

Search strategy

We searched Academic Search Complete, CINAHL with Full Text, and PubMed (Medline) databases through Ebscohost and Google Scholar, for articles on *A. baumannii* biofilm published in English between the years 2008 and 2018, using the keywords "*Acinetobacter baumannii*", "biofilm", "antibiotic resistance", "physicochemical factors", "in vivo studies" "virulence", and "treatment". Articles on biofilms from bacteria other than *A. baumannii* and the dates stated were excluded except when necessary.

Biofilm formation on biotic and abiotic surfaces

Biofilms are aggregates of microbial cells that are surrounded by self-produced exopolysaccharide matrices on the surfaces of biotic or abiotic surfaces. Biofilms demonstrate greater protection against antibiotics, host immune defense, and adverse environmental conditions than the free-living cells.[12] It is estimated that 65–80% of human infectious are caused by biofilm-forming bacteria.[13] Biofilm-associated infections are chronic infections, hence, they require higher doses of antibiotics for treatment than planktonic cells' acute infection, with the resulting antimicrobial resistance having led to increased death, prolonged hospital stays, considerable economic loss, and loss of protection for patients.[14] The factors that lead to enhanced antibiotic resistance in biofilm phenotype include impaired drug diffusion due to microbial aggregations, over-expression of the exopolymeric substance (EPS) matrix, alterations in microbial phenotypic and genotypic features due to stress responses, and physiological heterogeneity due to physicochemical gradients and persisters.[15] The phenotypic and genotypic features are triggered when bacterial cells produce quorum sensing (QS) signals in a cell density-dependent manner which helps in cell-to-cell communication during changes in various environmental factors such as temperature, oxygen level, acidity, and the quality of growth medium.[16] Stressors, including antibiotics when

administered at concentrations below the minimum inhibitory concentration (MIC), also induce biofilm formation in a variety of bacterial species.[17]

It is highly likely that biofilm formation plays an important role in the interactions of *A. baumannii* with its host, and contributes to medical-device-associated infections.[18] *A. baumannii* demonstrates increased tolerance to extracellular stressors when part of biofilm communities.[18] Based on a review of literatures, a number of factors: physicochemical and microbial (surface appendages, adhesins, capsular polysaccharides, virulence and resistance determinants), contribute to the formation and maintenance of *A. baumannii* biofilms.

Ecology and its influence on the formation of biofilms by *A. baumannii*

Various ecological niches have been reported to support biofilm-forming *A. baumannii*. Bacterial cells of *A. baumannii* demonstrated strong biofilm formation in skin and soft-tissue infections, as well as in wounds and on occlusive dressings.[18] *A. baumannii* biofilm communities were also observed on abiotic surfaces, including health care-associated equipment (endotracheal tubes), as well as polycarbonate and stainless steel.[18]

Bacterial isolates from urinary catheters, hospitalized patients in ICUs, and the environment are among the top biofilm producers.[19,20] *A. baumannii* recovered from blood, urine, sputum, catheters, cerebrospinal fluid, bronchoalveolar lavage, exudates, and burned skin were equally shown to produce biofilms.[21] Environmental isolates collected from patients' rooms at Federal University of Uberlandia Hospital in Brazil were also reported to produce biofilm.[22] These may suggest that the source of microbial cells may have a great impact on the capacity of the cells to produce biofilm.

Studies have reported the influence of environmental factors, strain location, and ecological niche on the distribution and capacity of bacterial strains to produce biofilm.[23] Although biofilm-producing bacteria exist in various environments and habitats, biofilm-forming *A. baumannii* exist almost exclusively in hospital environments. Within the same hospital, bacteria at a specific location may produce more biofilm than in another location. For instance, a statistically significant difference was observed between biofilm producers in surgical and medical wards ($P<0.05$) and the groups ($P<0.05$) despite the bacteria are of similar strain.[23] Skin ulceration has been reported to harbor *A. baumannii* strains with biofilm-producing property which has been related to the major virulence factors, promoting bacterial persistence

and chronicity in a separate and specific manner different from multidrug resistant (MDR) phenotypes.[25]

It was observed that transposition of insertion sequence (IS) elements influenced the ability of bacteria to overcome environmental challenges and adapt to new environmental niches,[26,27] and this can, therefore, be a contributing factor to the existence of biofilm-specific strains in particular environments.

Effect of polymicrobial existence

Polymicrobial communities and associated genetic diversities create synergy within and among bacterial species during biofilm formation, with the polymicrobial existence of bacterial species therfore having significant implications for the clinical treatment of infections.[28] Bacteria in polymicrobial communities produced more biofilms than when grown individually. A polymicrobial community of *Pseudomonas* and *Enterococcus, Acinetobacter* and *Staphylococcus* or *Corynebacterium* and *Staphylococcus* produced more biofilm than that formed by *Enterococcus faecalis* and *Staphylococcus* and *E. faecalis* and *Corynebacterium*.[29] A study investigated the interspecies biofilm-forming abilities of *Leptospira interrogans* with 21 bacterial isolates belonging to ten genera.[29] It was reported that though *Azospirillum brasilense* RMRCPB showed maximum interspecies co-aggregation with *Leptospira* strains (>75%, visual score of +4), isolates belonging to the genera *Acinetobacter, Sphingomonas, Micrococcus, Brevundimonas,* and *Paracoccus* formed significant biofilm and co-aggregation.[29] Microbial species lacking in one or more enzymes necessary for metabolism, such as polysaccharide-degrading enzymes, may thrive in the presence of another bacterium producing such active enzymes during polymicrobial aggregation. For example, it was shown that biofilm formation by *Acinetobacter oleivorans* DR1, when a naphthalene-degrading *Pseudomonas* spp. AS1 was present, increased due to exopolysaccharides generated by the latter.[30] *A. oleivorans* DR1, which could not grow due to its inability to degrade naphthalene, grew under naphthalene-amended conditions in the presence of naphthalene-degrading *Pseudomonas* sp. AS1.[30] Transformation and conjugation experiments using *A. baumannii* harboring plasmid-mediated antibiotic resistant genes were used to demonstrate the possibility of intergeneric gene transfer and there was no difference observed between the wild-type and plasmid-cured strains with respect to their biofilm-forming ability.[31]

Physicochemical factors affecting biofilm formation

The physical and chemical alterations in the environment influence not only microbial phenotypic features but also the expression of important functions.[32] Factors such as surface hydrophobicity, temperature, and oxygen concentration have been reported to influence biofilm formation in *A. baumanni* and other bacteria such as *Clavibacter michiganensis*.[33,34] The general effects of the important physicochemical factors affecting *A. baumannii* biofilm formation are summarized in Table 1.

Temperature

The influence of temperature on the capacity of microbial cells to adhere to and form biofilms on surfaces has been widely reported.[38,80] It was observed that *Acinetobacter* spp. biofilm formation was more prolific at 25°C than at 37°C.[37] However, the optimum temperature for biofilm formation by *A. baumannii* was reported to be 30°C at pH 7.0 in a medium containing sodium chloride at a concentration of 5 gL^{-1}.[31] The factors that influenced biofilm formation of *A. baumannii* on medical devices in the ICU of an Algerian university hospital included temperature, hydrophobicity of the bacterial cell wall, duration of implantation, and nature of the medical device.[40] A report demonstrated that *A. baumannii* strains formed biofilm better at 30°C than at 37°C while another showed that biofilm formation on plastic surfaces was high at 28°C due to the up-regulation of certain biofilm-associated proteins (Baps) such as Csu and iron-uptake proteins in *A. baumannii*.[39]

Growth media

Biofilm formation of *A. baumannii* was reported to be influenced by growth conditions and environmental stress factors.[48] The choice of growth medium for biofilm growth may depend on several factors such as the bacterial species and incubation conditions. For instance, *A. baumannii* cultured in glucose-based medium and exposed to sub-inhibitory concentrations of antibiotics such as imipenem can induce biofilm formation and lead to increased iron uptake in a clinical MDR phenotype.[48] It was reported that there was a substantial inter-strain variability and growth medium-dependence for biofilm formation in *A. baumannii* from both veterinary and clinical sources.[33] Different levels of biofilm growth were observed with five well-characterized *A. baumannii* strains cultured in three iron-poor media depending on the strain.[33] Although all strains produced increased biofilm in Tryptic Soy Broth dialysate when compared with other iron-poor media, the report supports the notion that biofilm formation in *A. baumannii* is strain-dependent.[33]

Media that limit its growth, such as drinking water were reported to support biofilm-producing *Acinetobacter* species in water distribution systems.[81] For instance, a study in Shanghai, China characterized the community diversity and abun-

Table 1 The effects of physicochemical factors on biofilm formation in *Acinetobacter baumannii*

Physicochemical factors	General effects	References
Temperature	High temperature enhances cell hydrophobicity which results in biofilm formation.	35–42
	High temperature increases nutrient uptake which leads to increased biofilm formation.	
	Production of chaperon-usher assembly is high at 28°C in *Acinetobacter baumannii*	
	High temperature induces the production of polysaccharides.	
Type and nutrient availability	Concentration-dependent effect on biofilm accumulation.	41, 43–49
	Higher nutrient concentrations decrease biofilm accumulation due to detachment and reduced competition among bacterial isolates.	
	Poor nutrient media increases biofilm production.	
	Presence of sucrose, calcium, and phosphate enhances biofilm formation.	
	Extreme nutrient limitation results in decreased exopolysaccharide synthesis and thus decreased biofilm formation.	
	Influences metal-ion-mediated cross-linking required for EPS synthesis.	
Growth surface	Surface roughness shelters bacteria against shear forces, allows time for change to irreversible attachment, and hence facilitates biofilm formation.	48, 50–56
	Smooth surface coating could lead to reduced biofilm formation.	
	Chemical and nutritional composition of organic surfaces promotes better bacterial adherence.	
	Surface charge and hydrophobicity influence biofilm formation in electroactive microbes.	
Iron concentration	Has a strain-dependent association with biofilm formation and type of iron source.	57–63
	Iron limitation triggers up-regulation of Baps.	
	Higher iron concentration allows for increased resistance to certain antibiotics through signaling or interacting with the antibiotics themselves.	
	Stimulates EPS production via repression of rhamnolipid production or inhibition of transcription regulator that leads to alginate synthesis.	
	Iron limitation enhances acyl-homoserine lactone production.	
Antimicrobial agents/ Disinfectants	Constitutes stress for enhanced induced gene regulation.	64–66
	Offers fitness advantage for resistant strains, resulting in biofilm formation.	
	Hydrophilic antimicrobial coating repels bacterial adhesion, reduces biofilm formation.	
Agitation	Flow movement influences the composition and cohesiveness of the EPS matrix formed during biofilm production.	67–69
	Influences the composition and physical properties by affecting the elasticity of the biofilm.	
Quorum sensing molecules	It modulates cell functions such as pathogenesis, nutrient uptake, motility, cell communication, and production of secondary metabolites.	70–73
Electrostatic interactions	Bacteria attach rapidly and tightly to positively charged surfaces.	74–76
	Electrostatic repulsion disturbs cell contact with negatively charged surfaces.	
pH and ion content	Divalent cationic ions enhance bacterial attachment to surfaces.	77–79
	Cations stabilize the interaction between negative charge surface and anionic substrates.	

Abbreviation: EPS, exopolymeric substance.

dance of biofilms in a full-scale drinking water distribution system by denaturing gradient gel electrophoresis and analysis of the 16S rRNA sequences with heterotrophic plate count methods. It was observed that *Acidovorax, Ralstonia* and *Acinetobacter* were the most predominant biofilm-forming species.[82] Among other bacterial species, *A. baumannii* was the most common species in biofilms formed in drinking water pipe systems.[83] Though *A. baumannii* persists and prospers in very diverse ecological niches including medical environments, it was demonstrated that biofilm formation is not important for the epidemic spread of *A. baumannii*.[84]

Surfaces

The biofilm-forming abilities of *Acinetobacter* species, both at air-liquid and solid-liquid interphases, and at different temperatures were compared.[37] It was observed that not only did isolates belonging to ACB-complex form biofilm at the air-liquid interface, but also demonstrated greater biofilm formation than other species at solid-liquid interfaces.[37] In addition, within the complex, biofilm formation by *A. baumannii* and *Acinetobacter* G13TU was four times greater than that of *Acinetobacter* G3,[37] confirming the former as a higher biofilm-producing species of the genus *Acinetobacter*.

A. baumannii has the ability to form biofilms on different surfaces and develop high biomass biofilm on stainless steel, polystyrene, and polycarbonate, a thermoplastic material that is often used to construct medical devices.[53] Although *A. baumanni* can form biofilm on most abiotic surfaces, clinical isolates have preference for polypropylene surfaces.[85] A

comparative study on the ability of *A. baumannii* to produce biofilm on two different surfaces, ie, polycarbonate and polypropylene, revealed that *A. baumanni* isolates formed more biofilm on polypropylene under agitation than on the polycarbonate surfaces.[85] It was demonstrated that *A. baumannii* isolated from wound cultures formed strong biofilm on stainless steel surfaces.[54] The ability of *A. baumanni* to form matured biofilms on polypropylene, polystyrene, titanium, and other medical-associated devices has been associated with several factors including the presence of Bap.[55,56] The extent to which these factors promote biofilm production, especially in the human cell environment, is not clearly understood. However, certain studies have shown that these factors may have variable influence on different microbial genera and among microbial species in epithelial cells. For instance, a study reported that the capacity for biofilm formation and the clinical success of *A. baumannii* on epithelial cells varied between the different *A. baumannii* strains.[86] This is probably due to the extent of the contribution of diverse factors including Bap to inflammatory cytokine induction by different strains of *A. baumannii*.[87]

Iron concentration and QS

Iron limitation enhances the expression of the QS genes which control density-dependent virulence factors in microbial species.[88] QS signaling molecules in some bacterial species such as *Pseudomonas aeruginosa* are strong chelators of iron, which causes limitation of iron leading to a stress response.[63] Besides iron chelation, siderophores are reported to have non-classical functions such as antibiotic activity and act as zincophores, chalkophores and metallophores for other metals, metal toxin sequester and oxidative stress.[89]

A. baumannii has one QS system that makes use of the AbaR receptor protein and *N*-(3-hydroxydodecanoyl)-l-homoserine lactone, thereby regulating virulence factors such as biofilm formation and surface motility.[70,71] The influence of iron-III limitation on *N*-acyl homoserine lactone (AHL), siderophore, and catechol on the virulence factors of *A. baumannii* biofilm-forming clinical strains was investigated.[59] A higher amount of AHL (70%) was detected in the presence of low concentrations of iron-III (20 μM). Furthermore, screening for *luxI* and *luxR* showed that the presence of both genes was required in the isolates for high AHL activity.[59] Both AHL production and biofilm formation were found to be regulated by iron concentration in a dose-dependent manner.[59] Hence, biofilm-formers including *A. baumannii* persist in limited iron environments.[59] Analysis of *bap* expression by real-time reverse transcription polymerase chain reaction (RT-qPCR) showed that some tested *A. baumannii* isolates had four-fold

bap over-expression in the presence of low iron concentration (20 μM).[90] However, the study showed that iron starvation was not the only factor required to stimulate biofilm production in *A. baumannii*, but that its formation was affected by growth medium and strain identity.[33]

Virulence determinants in *A. baumannii*

Microbial functions such as cell communication, surface-regulated attachments, and secretion of macromolecules are essential factors for biofilm formation.[91,92] *A. baumannii* expresses several virulence genes which make it a successful pathogen in humans and animals.[93] Some of these factors such as K1 capsular polysaccharides, surface antigen protein 1, iron acquisition systems, outer membrane porins, acinetobactin transporters together with resistance to the majority of antibiotics have made *A. baumannii* an increasingly important pathogen.[93] *A. baumannii* harbors repertoires of biofilm-related virulence genes and proteins which contribute to its ability to adhere and form biofilms on biotic and abiotic surfaces.[96] The presence of these genes makes *A. baumannii* remain viable in diverse environments and a formidable force against antimicrobials and immune cells.[97] These multiple genetic elements are controlled and regulated by complex regulatory networks, including those based on the presence of antibiotic resistance genes, environmental conditions, or cell density. The virulence determinants associated with biofilm and biofilm-specific resistance in *A. baumannii* are summarized in Table 2. Although factors that contribute to biofilm formation seem to be strain-dependent,[93] some common factors have been identified.

Bap

Bap (biofilm-associated protein) is required for the three-dimensional structure tower and water channel formation in biofilm.[95] Bap is a cell-surface protein identical to the *Staphylococcus* Bap and was first identified in *A. baumannii* by Loehfelm et al.[90] Bap, which is secreted via a type I secretion system, mediates *A. baumannii* biofilm formation and maturation.[93] Bap plays a role in cell−cell adhesion and is required for the development of higher-order structures on medically relevant materials, such as polystyrene and titanium.[90]

Although most sequenced strains of *A. baumannii* carry a *bap* gene, many seem to have disrupted or truncated *bap* sequences, which might be attributed to recombination events or sequence alignment errors that are common to the highly repetitive elements of *bap* coding sequences.[56] *bap* has been shown to express a highly variable protein.

Table 2 Gene determinants associated with virulence and antibiotics resistance in *Acinetobacter baumannii*

Virulence determinants	Type/Designation	Functions	References
Bap	A bap	Required for the three-dimensional structure tower and water channel formation in biofilm	60,90,135
AdeABC	AdeA – outer membrane protein associated with carbapenem resistance AdeB – an efflux pump membrane transporter AdeC – outer membrane factor of the AdeABC multidrug efflux complex	Involved in multidrug resistance and biofilm formation	98,99,100,101
AdeFGH	A resistance-nodulation-cell division (RND) antibiotics efflux system	Involved in synthesis and transport of auto-inducer molecules Involved in multidrug resistance	127,142
AdeIJK	An RND antibiotics efflux system	Involved in multidrug resistance	
PgaABCD	Outer membrane secretion involved in biogenesis of poly-beta-1, 6-N-acetyl-D-glucosamine	Substrate-specific transmembrane transporter activity and also involved in biofilm formation	102
CsuA/ BABCDE	A member of usher-chaperone fimbriae CusD – a member of the usher operon involved in outer membrane protein	Pili-biogenesis and biofilm formation	103
AbaIR	A quorum sensing system	Regulates factors such as biofilm formation and surface motility	154,155
OmpA/M	Outer membrane protein which maintains cell membrane integrity and enhances cell adhesion to surfaces	Necessary for antibiotic resistance, biofilm formation; modulates biogenesis of outer membrane vesicles	104,105
OprB/C	Porin proteins which maintain cell membrane integrity and functions	Involved in antibiotic efflux system	106
SmpA	Belongs to the SmpA/OmpA family protein domain in the fgr 9 which enhances adhesion and membrane integrity in bacteria.	Vital for adhesion and complement evasion	107,108
CarO	A membrane fusion protein	Involved in carbapenem resistance. Also essential for L-ornithine uptake	94,109
Pili	A type IV pili system	Required for twitching motility	18
EpsA	A capsular polysaccharide protein	Putative polysaccharide export outer membrane protein Involved in K1-capsular poly saccharide biogenesis	110
Ptk	Putative protein tyrosine kinase	Involved in K1-capsular poly saccharide biogenesis	110
TssB (VipA)	VI polysaccharide biosynthesis protein	Involved in inter-bacterial competition and responses	111
Wzx	Transport protein in K locus	Involved in the synthesis assembly and translocation of oligosaccharides repeat units	112,113
GntK	Thermo-resistant gluconokinase	Regulation of stress responses	114
Lux	Quorum sensing gene	Regulates cell-to-cell communication necessary for signaling during biofilm formation	26,71,105
PilA	Type IV pili gene	Involved in twitching motility and surface-associated motility	92
BarA	GacS-like sensor kinase	Involved in transportation of iron and nutrients	115
BarB	ABC transporter	Involved in nutrient and iron transportation	115
BasC	Probable acinetobactin biosynthesis protein	Involved in cellular transportation of iron and nutrients	115
BasD	Non-ribosomal peptide synthetase	Involved in iron acquisition in iron limiting condition	116
BauA	Ferric acinetobactin receptor	Associated with siderophore-mediated iron uptake	117,118
AmpC	AmpC β-lactamase	Present in a form of ADC type AmpC genes	119, 120
Bla-OXA-69	Carbapenem-hydrolyzing oxacillinase	Antibiotic resistance	121,122
Bla-OXA-75	Carbapenem-hydrolyzing oxacillinase	Antibiotic resistance	122
MVim	A metallo-beta-lactamase protein	Involved in multidrug resistance	121,123

Partial sequencing of *bap* performed on the index case strain MS1968, revealed that *bap* is a large and highly repetitive gene of approximately 16 kb in size.[56] The fully sequenced open reading frame was 25,863 bp and it encoded a protein with a predicted mass of 854 kDa.[56] Bap could be identified through a repetitive structure identical to a bacterial cell

surface adhesion protein. Using phylogenetic analysis, it was shown that *bap* from strain MS1968 clusters with *bap* sequences from clonal complex 2 (CC2) strains ACICU, TCDC-AB0715, and 1656-2, were distinct from *bap* in CC1 strains,[56] revealing that *bap* was clonal. Further analysis of the *bap*-positive strain showed that *Bap* is expressed at the cell surface and that it is associated with biofilm formation and could be inhibited with affinity-purified antibodies.[56] Time course confocal laser scanning microscopy and three-dimensional image analysis were used to observe that *A. baumannii* mutant in the coding sequence of *bap* was unable to sustain biofilm thickness and volume.[90]

The prevalence, expression, and function of Bap in 24 carbapenem-resistant *A. baumannii* ST92 strains isolated from a single institution were previously investigated.[56] Out of the 24 strains, 22 expressed Bap. The biofilm-forming ability of 65 MDR *A. baumannii* isolates and its association with biofilm-related genes was assessed using RT-qPCR. *ompA* and *csuE* genes were detected in all isolates, however, *bap* and *bla*PER-1 were detected in 43 (66%) and 42 (64%) of the phenotypes that showed strong and moderate biofilm activities ($P \leq 0.05$), respectively.[90] It was found that the frequency of genes related to biofilm formation in 27 *A. baumannii* isolates were *ompA* (100%), *bap* (30%), and *bla*PER-1 (44%).[124] Western blotting revealed that all the isolates expressed OmpA and only eight isolates were positive for Bap factor.[124] Double-negative *bap/bla*PER-1 isolates were recovered from bloodstream infections and had low biofilm-forming capability, unlike the clinical isolates from throat infections which were *bla*PER-1-positive and had a high biofilm forming ability.[124]

Efflux systems

Efflux systems in *A. baumannii* are chromosomally-encoded, two component efflux systems and are responsible for resistance to several microbicides when over-expressed.[125] The genes encoding these systems are carried by various genetic elements such as *tetA* and *cmlA* efflux pumps which are also responsible for tetracycline and chloramphenicol resistance.[126] There are five subfamilies of efflux systems which play crucial roles in antibiotic resistance: ABC transporters, SMR, MATE, MFS, and RND families.[127] Among these five "superfamilies", RND efflux systems are the most prevalent in MDR *A. baumannii* and have been reported to play a role in the efflux of a wide range of substrates which include antibiotics, dyes, biocides, detergents, and antiseptics.[127] There are three types of RND pumps that

are associated with *A. baumannii*, these being, AdeABC, AdeFGH, and AdeIJK.

The AdeABC (*Acinetobacter* drug efflux) pump comprises three components: an inner membrane pump (AdeA) which links the major fusion protein to the outer membrane factor (AdeC).[127] The operon that encodes these systems is only expressed by MDR *A. baumannii* phenotype isolates when the efflux pump is over-expressed.[99] AdeABC is tightly regulated by AdeR-AdeS which is encoded by the *adeRS* operon and contributes to the acquisition of antibiotic resistance when over-produced in *A. baumannii*.[99] Over-expression of AdeABC contributes largely to multidrug resistance, decreased biofilm production, and altered membrane composition in *A. baumannii*.[99] Aminoglycosides, beta-lactams, fluoroquinolones, tetracycline, tigecycline, macrolides, chloramphenicol, and trimethoprim resistant phenotypes are associated with mutations in the *adeRS* operon.[128] AdeRS has been linked to the capacity of *A. baumannii* to produce biofilm.[101] It was reported that inactivation of *adeRS* led to the loss of biofilm formation in an *A. baumannii* isolate due to decreased expression of *adeABC*, *pilI*, *com*, and a *pgaC*-like gene, which influenced its antimicrobial resistance and virulence interactions.[101] Deletion of *adeAB* influenced the ability of *A. baumannii* to form biofilms on plastic, in a strain-specific manner.[101] It was observed that increasing amounts of microbicides alone or in combination resulted in the modification of expression, and function of *adeB* gene of the RND-type efflux pump AdeABC and hence, the destruction of the biofilm mass and viability.[129]

AdeFGH is proposed to be regulated by a LysR-type transcriptional regulator and confers multidrug resistance in *A. baumannii* when over-expressed.[127] The AdeFGH efflux pump is linked to the synthesis and transport of auto-inducer molecules during *A. baumannii* biofilm formation.[130] Up-regulation of *adeG* correlated with biofilm induction especially when treated with low-dose antimicrobial therapy such as fluoroquinolone.[130] However, the level of gene transcription varied between bacterial genotypes, antibiotics, and antibiotic concentrations.[130] It was reported that the down-regulation of efflux pumps, especially the AdeFGH efflux pump resulted in a reduction in *A. baumannii* biofilm in the presence of sub-inhibitory concentrations of tigecycline.[125] When the effect of sub-inhibitory tigecycline concentrations was investigated using RT-qPCR, a positive correlation was established between biofilm formation induced by sub-inhibitory concentrations and the expression of biofilm-associated genes, especially AdeFGH efflux pump genes.[125]

AdeIJK is intrinsic to *A. baumannii* and contributes to natural resistance when over-expressed above a certain threshold.[128,131] It is the second RND type efflux pump in *A. baumannii* and comprises *adeI, adeJ,* and *adeK* genes.[132] The AdeIJK pump contributes to resistance to beta-lactams, chloramphenicol, tetracycline, erythromycin, lincosamides, fluoroquinolones, fusidic acid, novobiocin, rifampin, trimethoprim, acridine, safranin, pyronine, and sodium dodecyl sulfate.[128] It was suggested that over-expression of AdeIJK contributes largely to multidrug resistance, decreased biofilm production, and altered membrane composition in *A. baumannii*.[99]

There are other non-RND efflux systems, such as CraA, AmvA, AbeM, and AbeS which have also been characterized for *A. baumannii*, while AdeXYZ and AdeDE efflux pumps have been characterized for other *Acinetobacter* spp.,[127] but have not been implicated in biofilm formation.

PgaABCD gene clusters

The *pgaABCD* gene cluster encodes poly-(1-6)-N-acetylglucosamine (PNAG), one of the most important structures for biofilm formation in both Gram-negative and Gram-positive microorganisms.[102] The association of the expression of *pgaABC* gene clusters and altered biofilm phenotypes was investigated.[131] It was observed that the expression of *pgaB* in a clinical *A. baumannii* strain was much higher than that of a standard strain, and was associated with an increase in the capacity and thickness of biofilm formation.[133] The influence of altered expression of the QS gene *abaI* and *pgaB* in *A. baumannii* strains isolated from burn patients on biofilm formation, extracellular matrix expression, and drug resistance was investigated using RT-qPCR.[133] The relative expression of *abaI* and *pgaB* in the MDR strain was much higher than that of a drug-sensitive strain.[133] The biofilm thickness of the MDR strain was thicker than that of the standard strain.[133] The increased expression of QS *abaI* in the drug-resistant *A. baumannii* strains may have up-regulated the expression of *pgaB*, which possibly leads to increased production of extracellular matrix and hence increased biofilm formation and enhanced drug resistance in *A. baumannii*.[133] Another study aimed to confirm if the *pgaABCD* locus was responsible for cell-associated PNAG and to investigate its involvement in biofilm production.[102] It was revealed that not only did the deletion of *pgaABCD* result in an absence of PNAG, but also led to the loss of the strong biofilm phenotype, which was restored after complementation.[102]

CsuA/BABCD chaperone-usher pilli assembly system

The majority of *A. baumannii* strains encode and produce a type I chaperone-usher pilus system designated Csu pili.[18] Csu pili, which are regulated by the BfmRS two-component regulatory system,[147] are not required for association with biotic surfaces, such as human epithelial cells,[87] but are critical for biofilm formation and maintenance on abiotic surfaces, including polystyrene.

Although many *A. baumannii* strains seem to carry the *csuA/BABCDE* locus, a subset of clinical isolates have lost the *csu* cluster.[134] This might suggest that these pili may not be required for biofilm formation and maintenance in all strains or that other pili systems may functionally replace them.[134] A second two-component system termed GacSA was shown to moderately control *csu* gene expression and thus indirectly affects biofilm formation.[93] The expression of Csu pili in *A. baumannii* is completely repressed by sub-inhibitory concentrations of trimethoprim−sulfamethoxazole, which is indicative that improper use of antibiotics can alter population-level behaviors and may promote a planktonic lifestyle.[93]

QS molecules have been linked with up-regulation in gene expression of the *bfmS* and *bfmR* system, which has been predicted to contribute to the enhanced biofilm-forming capacity of strains on abiotic surfaces.[135] The possible role of QS signaling molecules to regulate pili formation and the ability of *A. baumannii* to form biofilms on abiotic surfaces was investigated.[136] Using transmission electron microscopy analysis, cells cultured in Mueller-Hinton broth in the presence of *N*-hexanoyl-L-homoserine lactone (C6-HSL) showed more abundant pili-like structures than the control strain.[136] In another knock-out and complementation study, the role of the response regulator component of the two-component system and sensor kinase, *bfmS*, in the type strain *A. baumannii* ATCC 17978 was investigated.[137] The *bfmS* knock-out mutant showed a drastic reduction in biofilm formation, lost its adherence to eukaryotic cells, and demonstrated greater sensitivity to serum killing compared with the parental strain.[137] Proteomic analysis also revealed that the release of outer membrane proteins (OMPs) including CarO and OmpA, was associated with the transposon-mediated inactivation of *bfmS* in *A. baumannii* strain.[137]

A biofilm hyper-producing *A. baumannii* strain (MAR002) was investigated to identify the role of the chaperone-usher pili assembly systems in pilus biogenesis pathways and the ability to form biofilm and attachment to

A549 human alveolar epithelial cells.[138] Putative protein-coding genes involved in pili formation were identified based on the newly sequenced genome of MAR002 strain (JRHB01000001/2 or NZ_JRHB01000001/2).[138] When assessed with RT-qPCR, LH92_11085 gene which belongs to the operon, LH92_11070-11085, was over-expressed (~25-fold more) in biofilm-associated cells compared to exponential planktonic cells.[139] Transmission electron microscopy analysis of the LH92_11085 mutant showed the absence of long pili formations normally present in the wild-type.[139]

AbaIR QS system

Co-colonization of same infection sites by *A. baumannii* and *P. aeruginosa* have been observed to employ AHL-based QS systems that co-ordinate biofilm the formation and co-infection of both species resulting in increased severity of the infection.[140]

abaI and *abaR* are QS genes expressed in *A. baumannii* but acquired horizontally from *Halothiobacillus neapolitanus*.[141] This QS system is the only one in *A. baumannii* and involves the AbaR receptor protein which forms a complex with the AbaI (auto-inducer synthase)-generated N-(^{3}-hydroxydodecanoyl)-L-homoserine lactone that regulates virulence factors such as biofilm formation and surface motility.[142]

It was found that biofilm formation among clinical isolates of *Acinetobacter* spp. was under the control of an auto-inducing QS molecule.[143] The auto-inducer synthase gene, *abaI*, was present among the isolates that produce QS signaling molecules and a mutation in *aba1* influenced *Acinetobacter* spp. biofilm-forming capabilities.[143] The association between drug-resistance and the influence of *abaR* on biofilm formation of *A. baumannii* was investigated.[160] It was found that the *abaR* in *A. baumannii* was associated with biofilm formation.[160] The *abaR* gene sequence of *A. baumannii*, ATCC 17978, was compared with *aqsR* (the LuxR-type receptor) of *A. oleivorans* DR1 and it was observed that the similarity to LuxR-type receptor was 80%.[144,145]

QS molecules have been linked to up-regulation of the expression of *bfmS* and *bfmR* genes, thereby enhancing the ability of *A. baumannii* to form biofilm on abiotic surfaces. The possible role of QS signaling molecules in regulating pili formation and its ability in *A. baumannii* to form biofilms on abiotic surfaces was identified using transmission electron microscopy.[136] There were more abundant pili-like structures in the co-cultured cells than the control strain.[136]

Another study linked the AdeFGH efflux pump to the synthesis and transport of auto-inducer molecules during biofilm formation.[130] According to the study, up-regulation of *adeG* was correlated with biofilm induction, especially when *A. baumannii* was treated with low-dose antimicrobial therapy such as fluoroquinolone antibiotics.[130]

Porins

Porins play a very important role in microbial virulence through drug exclusion mechanisms across the outer membrane channels.[106] OmpA is a prominent porin in *A. baumannii* and contributes to drug resistance, adhesion to epithelial cells, and biofilm formation.[146] Two-dimensional gel electrophoresis revealed that OMP receptors were up-regulated during biofilm formation.[147]

Several novel proteins such as CarO, OprD-like, DcaP-like, PstS, LysM, and Omp33, and histidine metabolism (like urocanase) were involved in biofilm formation in *A. baumannii*[148] and its reduced drug permeability.[104] It was reported that OMPs such as CarO and OmpA can interact physically with OXA-23 carbapenemase which can lead to enhanced antibiotic resistance.[185]

Other biofilm-associated genes

algC is a biofilm-associated gene that mediates the synthesis of bi-functional enzyme, phosphomannomutase/phosphoglucomutase, which is required to produce exo-polysaccharide alginate and lipopolysaccharide core during biofilm formation.[144] It was reported that the expression level of *algC* in the MDR strain of *A. baumannii* was up-regulated in a biofilm mode of growth compared to a planktonic mode.[144] Characterizing the quantitative gene expression pattern (using the relative quantification method) in real-time PCR revealed that biofilm cells showed an 81.59-fold expression compared with a 3.24-fold in planktonic cells ($p<0.05$).[144]

It was found that the *A. baumannii* virulence factor, associated with LHp2_11085 gene is required for biofilm formation on abiotic structures and attachment to epithelial cell surfaces.[145] However, validation of this is suggested through an in vivo infection model such as the larvae of the wax moth *Galleria mellonella,* which allows the study of *A. baumannii* pathogenesis and therapeutics.[145] When validated, the protein encoded by the LHp2_11085 gene of MAR002 strain could be a target for therapeutic development.[145]

A recent study linked the *A. baumannii* ATCC 17978 A1S_0112-A1S_0119 gene cluster to biofilm formation and

surface motility.[149] Using microscopy, it was observed that a derivative lacking these genes was unable to form matured biofilm.[149] The A1S_0114 gene is involved in expression of genes coding for surface proteins and pili-assembly systems.[150] Experimental animal models, including vertebrate and invertebrate hosts, were used to demonstrate that A1S_0114 gene has a significant role in *A. baumannii* virulence.[150] It was found that the A1S_0114 gene was necessary for proper attachment to A549 cells.[150]

Antibiotic resistance and the *A. baumannii* biofilm phenotype

A. baumannii is resistant to several classes of currently available antibiotics including β-lactams, aminoglycosides, cefotaxime, tetracycline, erythromycin, chloramphenicol, trimethoprim, and fluoroquinolones.[11] MDR strains are often isolated from immuno-compromised patients and those treated with a broad spectrum of antibiotics.[151]

The type of association that exists between biofilm formation and antibiotic resistance phenotypes remains controversial. According to some studies, the type and the reservoir of resistance determinants harbored by *Acinetobacter* spp. can influence its capacity to form biofilm. Biofilm formation is more strongly associated with MDR *A. baumannii* strains than with the susceptible strains.[21,152] A study performed on clinical isolates from patients with nosocomial infections in three hospitals in Tehran showed that at least 92% of the biofilm-forming isolates were MDR.[21] A significant correlation between multidrug resistance and biofilm formation of clinical and environmental isolates was established,[152] ie, clinical isolates had a higher ability to form strong biofilm than environmental isolates.[152] However, in vitro susceptibility can be influenced by the method used. For instance, when the resistance profiles of 65 clinical *Acinetobacter* spp. strains were assessed by both disk diffusion and minimum inhibitory concentration (MIC) methods, 46% were resistant by the MIC method compared with 32.3% using the disk diffusion assay.[153] When the biofilm-forming ability was tested using the microtiter plate method, seven strains were strong biofilm producers, 18 were moderate biofilm producers, 20 were weak biofilm producers, and 20 were non-biofilm producers.[153] A statistically significant association was observed between biofilm formation and imipenem resistance.[153] It was found that 25% of the 72 clinical isolates of *A. baumannii* isolated from India were resistant to ampicillin-sulbactam, 36.1% to imipenem, 66.6% to ceftazidime, 72.2% to ciprofloxacin,

80.5% to amikacin, and 84.7% to piperacillin while 62.5% (45/72) of isolates produced biofilm.[154] They established that the correlation between antibiotic resistance and biofilm formation was significant (P=0.0004).[154] Biofilm formation in clinical isolates of *Acinetobacter* spp. from intensive and non-intensive tertiary care hospital units in Bangladesh and its correlation with antibiotic resistance were reported. The majority of the clinical *Acinetobacter* spp. isolates were reported as MDR and biofilm producers, especially those isolated from ICU samples.[155] More than 86% of the isolates from a tertiary care hospital in Mexico were resistant to ciprofloxacin, ceftazidime, and cefotaxime, and 50.7% and 35.5% of the isolates were resistant to imipenem and meropenem, respectively.[156] Of these isolates, 28.3% and 25.7% were positive for the *bla*OXA-58 and *bla*OXA-72 genes, respectively.[156] The report also associated biofilm production with resistance to imipenem (P=0.002).[156] It was observed that drug-resistant *A. baumannii* associated with burn wounds readily formed biofilms, preventing antibacterial activity of topical agents mafenide acetate and chlorhexidine acetate commonly used for burn treatment.[157] The combined use of ambroxol (secretolytic agent used in the treatment of respiratory diseases associated with viscid or excessive mucus) with topical agents provided a synergistic effect, with increased killing of wound-associated *A. baumannii* in biofilms.[157] It was observed that *A. baumannii* isolated from sputum of elderly patients were all biofilm-formers and showed different levels of antibiotic resistance, while strong biofilm formation correlated with resistance to gentamicin, minocycline, and ceftazidime (P<0.05).[158] It was demonstrated that 12 out of 17 clinical *A. baumannii* isolates from a Portuguese hospital demonstrated multidrug resistance, while 74.7% of the MDR isolates showed biofilm forming ability.[23] The majority of the isolates from urine samples showed resistance to gentamicin, tobramycin, and trimethoprim/sulfamethoxazole and readily form biofilm compared to susceptible strains and those from other sources.[23] However, no statistical significance was observed between the ability to form biofilms and antibiotic resistance. Of the 100 clinical *A. baumannii* isolates examined in Korea, 77% exhibited enhanced biofilm formation relative to the *A. baumannii* strain type ATCC 19606.[66] *A. baumannii* strains that exhibited increased biofilm formation showed greater differences in their MICs and minimal biofilm-inhibitory concentrations (MBIC) of colistin.[62] While most isolates were susceptible to colistin when in the planktonic state, MBIC was 64 mg/mL when in the biofilm state. Thus, when

growing in a biofilm, a much higher bactericidal concentration of colistin is required.[62]

A strong biofilm-forming MDR *A. baumannii* strain collected from a Chinese hospital showed that resistance to levofloxacin, cefepime, and gentamicin significantly decreased when biofilm-forming ability was strong.[159] Biofilm-related genes such as *bap*, *bfs*, and *intI*1 were observed to contribute to biofilm formation.[150] Biofilm-forming *A. baumannii* strains were associated with reduced resistance to imipenem and ciprofloxacin with such strains possibly being less virulent.[160] Most of the non-biofilm forming isolates were associated with ciprofloxacin resistance, ICU treatment, and isolated from respiratory tract infections while biofilm-formers were all associated with catheter-related infections, blood stream infections, and previous aminoglycoside use.[160] This explains the view that biofilm-forming bacterial strains do not depend on antibiotic resistance and colonization characteristics as do non-biofilm formers for their survival in a hospital setting.[160,161]

Studies have also reported resistant *A. baumannii* strains that demonstrated little or no biofilm-producing capacity. Colistin, at sub-inhibitory concentration reduces biofilm formation on urinary catheter surfaces.[31] It was shown that isolates pretreated with colistin had their adhesion ability reduced significantly and the cultures treated with 0.5 × MIC showed decreased biofilm formation compared to those treated with 0.25 × MIC.[31] Clinical colistin-resistant (Cstr) strains demonstrated biofilm formation that was significantly decreased in static and dynamic assays ($P<0.001$) with lower relative fitness ($P<0.05$) compared with colistin-sensitive counterparts.[162] This was attributed to conversion of a stop codon to lysine in lipopolysacharide synthesis protein, LpsB in one Cstr strain while there exist a frameshift mutation in CarO together with loss of a 47,969 bp element containing multiple genes associated with biofilm production in a second resistant strain.[162] Environmental *A. baumannii* and *Klebsiella pneumoniae* strains recovered from a university hospital in Brazil from 2009 to 2014 were investigated.[22] Ten strains of each species were assessed for their biofilm formation capacity, biofilm biomass, and initial adhesion degree in three independent experiments. Although all *A. baumannii* strains were able to attach to polystyrene plates, some had a lesser degree of adherence than the control.[22] None of the isolates were strong biofilm producers and no relationship was observed between multidrug resistance and biofilm production.[22] It was reported that only one of the three clinical MDR *A. baumannii* strains with diverse resistance mechanisms (OXA-51, IMP-1, and VIM-2 type

β-lactamases) produced strong biofilm compared to the control.[199] The relationship between antibiotic resistance, biofilm formation, and biofilm-specific resistance of 272 clinical isolates of *A. baumannii* was investigated.[163] Of these, 249 isolates exhibited biofilm formation, of which 63 were stronger biofilm-formers than the *A. baumannii* strain type ATCC 19606.[164] The majority of the isolates that formed biofilms were non-MDR, while isolates with high levels of resistance were weak biofilm-formers.[164] These findings raise questions regarding the mechanisms by which bacteria maintain a balance between biofilm-forming capacity and antibiotic resistance, as well as how resistant strains achieve high levels of biofilm-specific resistance despite producing weak biofilms.[164]

Resistance determinants that contribute to biofilm formation

Antibiotic resistance is associated with resistance determinants that could be either intrinsic such as the chromosomally-encoded beta-lactamases, drug efflux system, and porins or acquired through insertion elements and integrons.[164] Some of the resistance determinants identified for *A. baumannii* include AmpC, VIM, and OXA-10 beta-lactamases, efflux pumps and aminoglycoside-modifying enzymes.[165] *A. baumannii* resistance is also linked to the capacity of the bacteria to produce biofilms.[163]

Beta-lactamases

The ability of *A. baumannii* to form strong biofilm has been associated with class-D OXA carbapenemase genes.[166,167] A study showed that clinical isolates were able to form stronger biofilm than environmental isolates.[152] Statistical analysis revealed a significant correlation between the frequency of MDR isolates and biofilm-forming ability ($P=0.008$). The correlation of biofilm formation and the frequency of biofilm-related genes in clinical and environmental samples revealed a significant association between the frequency of biofilm-associated genes such as *bla*OXA-51 and the capacity for biofilm formation ($P=0.008$).[152] PCR analysis of biofilm-related genes revealed that some genes such as *bla*OXA-51, *bla*OXA-23, *bla*OXA-24, *bla*OXA-58, *bla*PER-1, *bap,* and *omp*A could be amplified from isolates with the frequencies of *omp*A, *bla*PER-1, and *bap* in clinical and environmental isolates being 100%, 53.3%, 82.7%, and 100%, 37.5%, and 84.4%, respectively.[152] It was observed that *A. baumannii* containing both *bla*OXA-23 and *bla*-OXA-24/40-like genes showed greater initial attachment to microtiter plates and biofilm-forming ability than the imipenem-sensitive

isolates.[163] Imipenem-resistant strains were found to harbor *bla*OXA-24/40-like gene and efficiently attached to microtiter plates, unlike *bla*OXA-24/40-negative, or imipenem-sensitive strains which had weak or no biofilm-forming ability.[163] The *bla*OXA-51, *bla*OXA-23, and *bla*OXA-24/40-like genes could co-exist in strong biofilm-producing, MDR *A. baumannii,* especially in isolates from hospitalized patients in ICU.[163] The relationship between biofilm-related genes and antimicrobial resistance of *A. baumannii* samples from 122 patients with lower respiratory tract infections at Fujian Longyan First Hospital, China from January 2013 to September 2014 was examined.[96] It was observed that the detection rates of *abaI* and *csuE* were both 59.8%, and those of *ompA* and *bla*PER1were 100% and 0%, respectively.[96] The study further revealed that resistance to amikacin, ampicillin/sulbactam, and 14 other types of antimicrobials was higher in *abaI*- and *csuE*-positive strains than in *abaI*- and *csuE*-negative strains ($P<0.05$).[96] *A. baumannii* carrying extended-spectrum beta-lactamase genes formed significantly more biofilms than those without this gene ($P<0.005$ and $P<0.001$, respectively).[96] The ability of MDR, clinical isolates of *A. baumannii* to adhere to the epithelial cell surface and to form biofilm in polystyrene plates was reported to be higher in isolates carrying the *bla*PER-1 gene than those without it.[168] RT-PCR analysis revealed a positive correlation between the level of expression of the *bla*PER-1 gene and biofilm formation ($r=0.89$; $P<0.0001$) and cell adhesiveness ($r=0.74$, $P<0.006$).[170] *A. baumannii* chromosome also encodes an intrinsic *bla*AmpC and *bla*OXA-51-like genes that are weakly expressed but do not confer resistance to carbapenems.[171] However, it was demonstrated that the insertion of an ISAba1 element upstream of the gene conferred carbapenem resistance.[171] IS elements such as ISAba1, ISAba2, ISAba3, and IS18, have been found to increase the expression of class D beta-lactamase genes (including *bla*OXA-23-like and *bla*OXA-58-like genes) when they were inserted immediately upstream due to the presence of an outward-directing promoter at the ends of these IS elements.[169,172]

The revelation that the presence of resistance determinants is one of the key factors that contribute to the ability of *A. baumannii* to produce biofilm, is a serious concern considering the proximity of the isolates within a biofilm and that can facilitate the distribution of resistance genes and plasmids. The ability of a dual-species biofilm to spread *bla*NDM-1 between both clinical and environmental settings was reported.[173] The study confirmed that *Enterobacte-*

riaceae strains could transfer *bla*NDM-1 readily to strong biofilm-producing *P. aeruginosa* and *A. baumannii* in the environment.[173]

Integrons

A. baumannii has the ability to acquire both narrow- and extended-spectrum beta-lactamases as well as the capacity to acquire integrons, transposons, and insertion elements which can contribute widely to antibiotic resistance.[174] Some of these resistance determinants have also been associated with the capacity of *A. baumannii* to produce biofilm. For instance, it was revealed that *intI1* gene mRNA was ~4 times higher in biofilm cells than that in the planktonic cultured cells and would therefore be much easier for bacteria under biofilm conditions to capture gene cassettes than planktonic cells.[175] Class I integrons are highly distributed among *A. baumannii* strains and antibiotic resistance is strongly associated with *intI1* gene cassette-positive strains compared to that of gene cassette-negative strains.[175] It was reported that MDR *A. baumannii* may harbour integrons that may differ in different strains. For instance, class 1 and 2 integrons dominate in *A. baumannii* clonal complex 113/79 (CC109/1 and CC113/79 respectively) isolated from Latin America.[176] Although these determinants are associated with MDR *A. baumannii*, their relationship with biofilm-forming ability requires further investigation.

Impact of clonality on *A. baumannii* biofilm formation

Associations between biofilm formation and specific clonal lineage were reported.[48,177] In a study to investigate the factors that contribute to the epidemicity of carbapenem-resistant *A. baumannii,* biofilm formation, expression of biofilm-associated genes, and resistance of different epidemic clones: major, minor, and sporadic were compared. The study revealed that the major epidemic clone produced stronger biofilm than the minor and sporadic ones.[178] Biofilm-associated genes such as *abaI, bap, pgaABCD*, and *csu*A/*BABCDE* were highly expressed in the major and minor epidemic clones compared to the sporadic clones in sessile conditions. However, the reverse was true in the planktonic state where the minor clone expressed more biofilm-associated genes than the major epidemic clone. The major epidemic clone expressed more *csuC, csuD*, and *csuE* genes than the minor epidemic clone.[178] This may account for the epidemicity of CRAB ST191 clone in the hospital environment. Biofilm formation by SMAL, a cluster of a specific clone within MDR

A. baumannii strains from an Italian hospital showed that the capacity of *A. baumannii* to form biofilm, represented as the ability to adhere to polypropylene surface, was influenced by the growth medium.[48] Biofilm-production was favored in glucose-based medium and increased the intracellular iron concentration than in rich medium and decreased iron concentration.[48] A study identified ST2 (international clone II), ST25, and ST78 which produced more biofilm than other sequence types (STs).[180] In the report that aimed to compare virulence-related traits that dominate in the international clonal lineages of I-III and the emerging genotypes ST25 and ST78, not only did ST78 invade A549 cells more than others, but it also produced more biofilm.[180] In another study to test if biofilm formation is a major contributor to the success of the international clone 2 (IC2) in infection, it was found that IC2 strains produced less biofilm than other isolates.[84] This study revealed that biofilm formation may not be the major reason for the great success of a particular clone.[84] For instance, *A. baumannii Bap* is polymorphic and can be differentiated in three main clonal types both in the repetitive and the COOH region.[55] A survey of 541 *A. baumannii* sequenced strains that belong to 108 STs revealed that 29% featured type-1 *bap*, 40% type-2 *bap*, 11% type-3 *bap*, while 20% lacked *bap*.[55] It was observed that different *A. baumannii* strains showed high variability in biofilm formation.[181] Although no relationship existed among carbapenem resistance, isolation site, disease severity of the clinical strains and biofilm production, an increase in biofilm formation was detected in *A. baumannii* isolates cultured from the same patient upon prolonged hospitalization.[181] In another study that aimed to investigate the relationship between antibiotic resistance, biofilm formation, and biofilm-specific resistance genes of 272 clinical isolates of *A. baumannii* from a Chinese hospital, pulsed field gel electrophoresis typing yielded 167 different patterns and 103 clusters with a similarity of 80%.[164] The isolates with the strongest biofilm-producing ability belonged to cluster 9 and 34 with four of the isolates demonstrating the strongest biofilm-producing ability.[164]

It was suggested that biofilm formation is a heterogeneous property among *A. baumannii*, and is not associated with certain clonal types.[182] A study to link a particular clonal lineage to carbapenem resistance and biofilm formation with *A. baumannii* isolated from Southern Croatia, revealed that no significant difference exists between them.[177] However, the study showed that higher biofilm producers were isolated from ICUs and respiratory tract infections while lower biofilm producers were isolated from other sources.[177] The genotype relatedness, antibiotic resistance, and biofilm formation of *A. baumannii* isolated from a Portuguese hospital showed that no relationship existed between biofilm formation and pulse type.[23] However, the study showed that urine isolates demonstrated a higher biofilm-forming capacity than those from other sources.[23]

Genomics and proteomics

Understanding the factors responsible for biofilm formation has been improved by genomic and proteomic studies conducted on genes that are differentially regulated during biofilm formation. Complete genome sequence analysis of a high biofilm-producing *A. baumannii* strain 1656-2 provided insight into the molecular basis of biofilm formation.[183] With a genome size of 3,940,614 bp, the strain had a guanine-cytosine content of 39.2%, a coding region of 88.0% with 3,715 open reading frames, 18 predicted rRNA genes, and 71 predicted tRNA genes.[183] The draft genome sequence of a hyper biofilm-producing *A. baumannii* strain MAR002 (GEIH/REIPI-Ab2010 Spain) was reported to have an approximate length of 3,717,929 bp and 3,300 protein-coding sequences, with a CG content of 39.09%.[138] The pan-genome of 30 completed *A. baumannii* genome sequences was studied and it was reported that they share both intra- and inter-genomic similarities and evolutionary relationships.[184] The genes responsible for pilus assembly protein, pili assembly chaperone, AdeK, PonA, OmpA, as well as other genes associated with biofilm formation were found to be conserved.[184] Genes responsible for oxidative stress resistance and alkyl hydroperoxide reductase (AhpC) were up-regulated during biofilm mode compared to the planktonic cells at 48 hours, whereas the AhpC was higher in the planktonic than the biofilm mode at 24 hours.[179] The AhpC mutant showed a severe growth defect in rich media, promoted early biofilm formation through increased production of EPS, and increased the endogenous hydrogen peroxide production unlike that observed in the minimal media.[179]

A. baumannii 1656-2, a high biofilm-producing clinical isolate was used to determine which proteins are differentially regulated in biofilm cells as compared to planktonic conditions using two-dimensional gel electrophoresis.[147] Protein identification using MALDI-ToF mass spectrometry revealed that: 1) proteins involved in the process of environmental responses such as Omp, iron transporters, diguanylate cyclase, and sensor histidine kinase/response regulator (PAS-GGEDF-EAL domain); 2) metabolic proteins such as NAD-linked malate dehydrogenase, putative GalE, ProFAR isomerase, as well as nucleoside-diphosphate sugar epimerase, and *N*-acetylmuramoyl-l-alanine amidase;

3) antibiotic resistance determinant-related proteins; and 4) gene repair proteins such as exodeoxyribonuclease III and GidA were differentially expressed.[147] In another study that investigated the mechanisms associated with the ability of *A. baumannii* to form biofilm, a comparative proteomics analysis of *A. baumannii* strain ATCC 17978 was performed at three different conditions: exponential, late stationary and biofilm growth phase by two-dimensional gel electrophoresis, MALDI-ToF, and iTRAQ/SCX-LC–MS/MS mass spectrometry analysis. The results were compared with the alterations in the proteome resulting from exposure to salicylate, a 'biofilm-inhibitory compound.[159] Several novel proteins as well as Baps such as CarO, OmpA, OprD-like, DcaP-like, PstS, LysM, and Omp33, and histidine metabolism (like urocanase) were implicated in biofilm formation.[148] When MDR and drug-sensitive strains of *A. baumannii* were compared to determine the protein expression levels in *A. baumannii* resistant phenotype, it was revealed that 114 of the 1,484 proteins present were 2-fold to 66-fold more abundant in the drug-resistant strain, and 99 were 2-fold to 50-fold less abundant.[185] The proteins that were over-expressed included porins, drug- and metal-resistant as well as stress- and biofilm-related proteins.[185]

The gene expression pattern of *A. baumannii* ATCC 17978 in the biofilm phenotype was different from that in planktonic cells.[186] The total mRNA from the planktonic (both exponential and stationary phase cultures) and sessile (biofilm) cultures were obtained, six libraries were prepared using mRNA-Seq protocols from Illumina, and reads were obtained in a HiScanSQ platform and mapped against the complete genome.[186] Assessing the mRNA expression profiles in biofilm by RNA-sequencing, 1,621 genes were reported to be over-expressed in the biofilms relative to the stationary phase cells and 55 genes were expressed only in the biofilms.[186] This could explain important changes in amino acid and fatty acid metabolism, motility, active transport, DNA-methylation, iron acquisition, transcriptional regulation, and QS, among other processes.[186] Comparison of the proteome of drug-resistant *A. baumannii* DU202 with *A. baumannii* ATCC 17978 strain revealed 72 genes that encode proteins including β-lactamases, a multidrug resistance efflux pump, and RND multidrug efflux proteins.[187] In an in vivo transcriptomic study conducted by injecting a mouse intra-peritoneally with *A. baumannii* ATCC 17978 and assessing the transcriptome after 8 hours with an RNA-Seq with an Illumina platform, 557 genes were found to be down-regulated while 329 genes were up-regulated.[188] According to the study, up-regulated genes included siderophore iron uptake clusters, genes related

to chaperone and protein turnover, while all genes related to types I and IV pili, QS components, and proteins involved in biofilm formation showed reduced expression.[188]

The global metabolite profiling of both planktonic and biofilm forms of the same strain, *A. baumannii* 1656-2 was compared, using a high-resolution nuclear magnetic resonance spectroscopy and multivariate statistical analysis to investigate the metabolic patterns leading to biofilm formation.[189] Principal components analysis and orthogonal partial least-squares discriminant analysis score plots showed a distinct separation between planktonic and biofilm cells.[189] It was concluded that metabolites such as acetates, pyruvate, succinate, and lysine as well as UDP-glucose, AMP, and glutamate are involved in energy metabolism during biofilm formation.[189]

Treatment options of *A. baumannii* biofilm-associated infections
Current and prospective treatments

The current treatment options available for MDR *A. baumannii* are suggested for prescribers based on best guess or definitive measures.[190] A joint working meeting consisting of the British Society for Antimicrobial Chemotherapy, Healthcare Infection Society, and the British Infection Association recommended ampicillin/sulbactam for carbapenem-resistant but sulbactam-susceptible *A. baumannii* infections.[190] Ceftazidime/avibactam has been reported to have potent activity against Gram-negative bacteria isolated from ICUs including MDR *Acinetobacter* spp.[191] The synergistic activity of cefoperazone-sulbactam combined with tigecycline showed good activity against MDR *A. baumannii* in in vitro studies and could therefore offer a good treatment regime in the future.[192] Relebactam in combination with imipenem/cilastatin appeared to be a prospective treatment having currently being used in a clinical trial against imipenem-resistant strains.[193]

The treatment of biofilm-associated *A. baumannii* infections may present a greater challenge due to the chronic nature of such infections. For instance, in a study that aimed to determine the antimicrobial activity of chlorhexidine against bacteria and fungi biofilms, it was observed that although chlorhexidine demonstrated excellent antimicrobial activity for most microorganisms tested in their free form, it was less effective against biofilms of *A. baumannii, Escherichia coli*, methicillin-resistant *Staphylococcus aureus,* and *P. aeruginosa*.[194] Antibiotics and metal resistance profiles of clinical isolates of *Acinetobacter haemolyticus* in planktonic and biofilm forms revealed a 4-256-fold increase in antibiotic

and metal concentration, respectively, in order to inhibit biofilm formation over a period of 72 hours compared to the susceptible planktonic counterparts.[195]

Although biofilm formation enhances resistance to antibiotics, combination therapy may be effective to treat biofilm-associated infections. The in vitro stability and efficacy of colistin, tigecycline, and levofloxacin alone or in combination with clarithromycin and/or heparin as lock solutions against biofilm-embedded *A. baumannii* strains was assessed.[196] It was reported that catheter lock solution mixed with colistin is effective for treating biofilm-producing *A. baumannii* associated with catheter infections, while their combination with clarithromycin showed bactericidal activity against the biofilm-embedded *A. baumannii* strain.[196] Each antibiotic at 400 times the MIC combined with clarithromycin (200 mg/mL) and/or heparin (1,000 U/mL) was compatible.[196] When compared with other antibiotics alone, the lock solution consisting of colistin was the best agent to eradicate *A. baumannii* embedded in the catheter model.[196] A comparative in vitro efficacy study of colistin-levofloxacin-, colistin-tigecycline-, and tigecycline-levofloxacin-based catheter lock solutions to treat biofilm-associated *A. baumannii* infections was conducted. Although each antibiotic combination tested at 400× MIC clearly demonstrated bactericidal activity, colistin-levofloxacin had the most potent antibacterial activity against biofilm-associated *A. baumannii* catheter infections.[197] The antimicrobial activity of imipenem and rifampicin alone and in combination against clinical isolates of *A. baumannii* grown in planktonic and biofilm culture was investigated.[198] The study revealed that imipenem combined with rifampicin demonstrated great capacity and promise for use as a combinatorial therapy for infectious diseases caused by *A. baumannii*.[198] Hence, there is a need to analyze the relationship of imipenem and rifampicin combination therapy and to also study the effect of this combination therapy on each component of the biofilm, such as PNAG or bacteria in the biofilms using increased clinical isolates.[198] It was observed that none of the antibiotic regimens of imipenem, colistin, and rifampicin used alone reduced biofilm formation at the MIC of each of the antimicrobial agents, and *A. baumannii* biofilms were not susceptible to imipenem, colistin or rifampicin as the sole treatment regimen.[199] However, at 48 hours after incubation, tigecycline (0.76±0.23), imipenem-rifampicin (1.07±0.31), and colistin-rifampicin (1.47±0.54) showed a significant inhibition of biofilm formation compared to the positive controls (*P*<0.01).[199] Meropenem plus sulbactam significantly decreased biofilm biomass and mean thickness, and increased the roughness coefficient of *A. baumannii* bio-

film.[200] However, sulbactam plus tigecycline decreased only the maximum and mean biofilm thickness and not biofilm mass when compared with these antibiotics used alone.[200] According to the report, these combinations suggested good alternative treatment regimens for carbapenem-resistant *A. baumannii* and carbapenem-susceptible *A. baumannii* biofilms.[200]

Alternative treatments
Quorum quenching

QS in *A. baumannii* has been linked to the expression of important proteins such as ppGpp synthase and histidine kinase sensors.[125] Using proteomic analysis of a rifampicin-resistant variant *Acinetobacter* sp. strain DR1, it was observed that an AHL lactonase responsible for degrading QS signals was highly up-regulated in both the DR1 and an *aqsI* mutant, strain causing reduced biofilm formation in the *aqsI* mutant.[201] This report represents the first study on the critical role of QS in hexadecane degradation and biofilm formation.[201] A recombinant lactonase was demonstrated to successfully disrupt biofilm formation of clinical isolates of *A. baumannii*.[202] This was the first demonstration of the use of recombinant quorum quenching enzymes in the disruption of biofilm formation by the human bacterial pathogen, *A. baumannii*.[202] The recombinant quorum quenching enzyme, MomL, an enzyme that degrades several QS molecules, reduced biofilm formation while increasing the susceptibility of *P. aeruginosa* PAO1 and *A. baumannii* LMG 10531 biofilm to different antibiotics in microtiter plates in vitro.[203] However, MomL was ineffective in treating mixed species and wound-associated biofilm infections.[203]

Natural and organic products

Natural products such as microbial, plant, and animal derivatives have been demonstrated to be effective to treat *A. baumannii* infections. Bacterial secondary metabolites have been demonstrated to be effective against *A. baumannii* biofilm production.[30] The effects of metabolites produced by bacteria isolated from drinking water on the biofilms of *A. calcoaceticus, Burkholderia cepacia, Methylobacterium* sp.*, Mycobacterium mucogenicum, Sphingomonas capsulata*, and *Staphylococcus* sp were studied.[82] It was found that *A. calcoaceticus, B. cepacia, Methylobacterium* sp., and *M. mucogenicum* biofilms were strongly inhibited by crude cell-free supernatants from the other bacteria.[81] The isolation and characterization of a new lytic *A. baumannii* bacteriophage that was effective on biofilm were reported.[204] The phage could

inhibit *A. baumannii* biofilm formation and also disrupt pre-formed biofilm.[205]

The effect of the antimicrobial peptide (AMP) LL-37 on the integrity of *A. baumannii* biofilm revealed that LL-37 caused structural damage of the biofilm at a low concentration of 2.5 µg/mL.[206] A natural AMP complex produced by the blowfly maggot *Calliphora vicina* exhibited strong cell killing and matrix-destroying activity against human pathogenic, antibiotic resistant *E. coli*, *S. aureus*, and *A. baumannii* biofilms, and it was non-toxic to human immune cells.[207] The complex is reported to contain AMPs from defensin, cecropin, diptericin, and proline-rich peptide families which were likely expressed as a result of bacterial infection.[207] The anti-biofilm, antimicrobial, and antioxidant activities of the *Salvia glutinosa* essential oil were effective against MDR *A. baumannii* with MIC and minimal bactericidal concentration values between 1.25–2.5 µL/mL and 5–10 µL/mL, respectively.[208] A 2-aminoimidazole-based anti-biofilm agent, one of the constituents of a polymeric formulation, was effective against *A. baumannii* biofilm formation and offered no leaching of the 2-aminoimidazole derivative. It was not hemolytic and proved effective in reducing biofilm on indwelling medical devices.[209]

Inorganic substances

In order to determine the effectiveness of hydrogen peroxide and hydrogen peroxide-based formulations for the eradication of biofilms of three different Gram-negative pathogens associated with MDR phenotypes, it was observed that planktonic cultures and single-species 24-hour biofilms of seven strains of *Acinetobacter* spp., including clinical isolates, were reduced at the working concentrations of hydrogen peroxide and hydrogen peroxide-based formulations for 1 minute to 24 hours.[204] However, it was demonstrated that the mixed-culture biofilm cells were more resistant to removal and killing by some biocides, such as hydrogen peroxide and sulfathiazole, than the single-species *Acinetobacter* biofilm cells.[210] Oxidizing biocides, such as sodium hypochlorite and hydrogen peroxide, demonstrated a higher potential for biofilm removal and killing than non-oxidizing biocides (sulfathiazole and glutaraldehyde).[211]

Cyclic di-GMP is a well-known and highly conserved second-messenger signal transduction that plays a role in biofilm formation. In silico pharmacophore-based screening identified small-molecule inhibitors of DGC enzymes that synthesize c-di-GMP.[212] Four small molecules, LP 3134, LP 3145, LP 4010, and LP 1062 act against c-di-GMP and inhibit biofilm formation in *P. aeruginosa* and *A. bauman-*

nii in a continuous-flow system and with no toxic effect on eukaryotic cells.[212] Although the compounds identified from structure-activity relationship studies of the anti-biofilm compound, LP3134, demonstrated an anti-biofilm effect on a silicon catheter, they had no effect on the planktonic growth of *A. baumannii*.[212] 5-episinuleptolide, isolated from *Sinularia leptoclados*, demonstrated a biofilm-inhibitory effect on *A. baumannii* ATCC 19606 and three MDR *A. baumannii* strains.[213] The mechanism of biofilm inhibition was shown to correlate with decreased expression of the *pgaABCD* locus, which encodes the extracellular polysaccharide, poly-PNAG.[213] It was observed that optimized N^2,N^4-disubstituted quinazoline-2,4-diamines, a dihydrofolate reductase inhibitor, reduced up to 90% of cells within a biofilm at or near MICs.[214] The molecule, which has also demonstrated potency in vitro and in vivo against methicillin-resistant *S. aureus* strains, showed great activity against *A. baumannii* and its biofilm at very low MIC when the 6-position was replaced with a halide or an alkyl substituent.[214] Using a murine model, it was reported that N^2,N^4-disubstituted quinazoline-2,4-diamines proved more effective than tigecycline.[214] Gallium nitrate (16 µM) drastically reduced *A. baumannii* growth and biofilm formation in human serum, whereas 64 µM caused massive disruption of the preformed *A. baumannii* biofilm.[210] This may be associated with the limiting iron concentration in human serum as iron concentration influences *A. baumannii* biofilm formation in a strain-dependent manner. The effect of silver nanoparticles alone and in combination with biocides and imipenem against planktonic and biofilms of *A. baumannii*[85] was studied and it was reported that 29.3% of isolates were susceptible to silver nanoparticles and the agent could inhibit growth and eradicate biofilms produced by the isolates.[85] The result demonstrated the antagonistic impact of silver nanoparticles against biofilms.[85] Virstatin at a concentration of 100 mM led to a 38% decrease of biofilms formed by *A. baumannii* ATCC17978 when grown under static mode.[228] According to the report, the production of biofilms grown under dynamic mode with virstatin was also delayed and reduced.[215]

A. baumannii in the African perspective

Limited to no data are available in scientific literature on biofilm formation of *Acinetobacter baumannii* isolated from South Africa. This review serves as the first comprehensive and updated information on the physicochemical parameters that affect *Acinetobacter baumannii* biofilm formation. However, antimicrobial resistance of *A. baummanii* from Africa has been previously reported. The prevalence of

carbapenem-resistant *A. baumannii* at Mulago Hospital in Kampala Uganda was investigated.[216] The genes responsible for carbapenem resistance such as *bla*OXA-23-like (60%; 9/15), *bla*OXA-24-like (7%; 1/15), *bla*OXA-58-like (13%; 2/15), and *bla*VIM-like (13%; 2/15) as well as the class 1 integron in carbapenem-resistant *A. baumannii* were identified.[217]

A variant of NDM-1 in a clinical isolate of *A. baumannii* that had a C to G substitution at position 82 resulting in an amino acid substitution of proline to alanine at position 28 was also reported.[230] Multilocus sequence typing revealed that the isolate haboured a gene belonging to a new sequence type, ST103, and this variant was designated NDM-2.[218] The prevalence of different Ambler class β-lactamase-encoding genes such as *bla*TEM, *bla*SHV, *bla*CTX-M, *bla*VEB, *bla*PER, *bla*GES, *bla*VIM, *bla*IMP, *bla*SIM, *bla*SPM, *bla*GIM, *bla*NDM, *bla*ADC, *bla*OXA-23, *bla*OXA-24, *bla*OXA-51, and *bla*OXA-58, ISAba1, and *int*1 among 40 carbapenem-resistant *A. baumannii* in Egypt were studied.[219] *bla*ADC- and *bla*OXA-51-like genes were present in all the isolates while SHV, CTX-M, VEB, KPC, and MBL encoding genes were absent. *bla*OXA-23 (50%), *bla*OXA-24 (7.5%), *bla*TEM-(87.5%), *bla*PER- (55%), and *bla*GES-like (27.5%) genes were also detected among the isolates.[219] The isolates were resistant to amoxicillin-clavulanate, aztreonam, cefepime, cefotaxime, and ceftazidime.[219] 16S rRNA methylase *armA* was observed together with *bla*NDM-1 and *bla*OXA-23 in *A. baumannii* isolated from tertiary care hospital in Egypt.[220] The emergence and spread of ST236 and a carbapenemase that resides in clones belonging to ST884, ST945, and ST1096 in Saudi Arabia and Egypt was reported. From Egypt and Saudi Arabia respectively, it was reported that ST236 (CC104), ST208 (CC92), and ST884 were widespread.[231] Resistance genes detected were ISAba1 (19%), *bla*OXA-23 (65%), *bla*OXA-24-like (9%), and *bla*NDM-1 genes.[221]

The activity of tigecycline against carbapenem-resistant *A. baumannii* was studied using Vitek-2 colorimetric compact system with Advanced Expert System and modified Hodge test for susceptibility testing and carbapenemase production testing, respectively.[216] The study revealed that out of the 232 carbapenem-resistant clinical strains, 169 (75.8%) were susceptible to tigecycline, 37 (16.6%) were intermediately resistant, and 17 (7.6%) were resistant.[216] *A. baumannii* isolates were susceptible to colistin and none of the carbapenem-resistant isolates were susceptible to ampicillin, amoxicillin/clavulanic acid, piperacillin/tazobactam, cefuroxime, cefoxitin, cefepime and nitrofurantoin.[216] High prevalence of oxacillinases in clinical MDR *A. baumannii* from Tshwane region, in South Africa was reported. Another report indicated that South African clinical *A. baumannii*

isolates were 100% resistant to ampicillin, amoxicillin, cefuroxime, cefotaxime, and nitrofurantoin.[222] Although the isolates showed 67% or higher resistance rates to ciprofloxacin, imipenem, meropenem, ceftazidime, and cefepime, none were resistant to colistin.[222] Surprisingly, none of the isolates harbored GES, KPC, NDM, VIM, SPM SIM, PER, and IMP genes and only carried OXA-51 and OXA-23 genes.

Conclusion

A. baumannii is the first in the critical priority list of pathogens for new antibiotics according to the World Health organization.[223,224] The extent and levels of MDR *A. baumannii* in hospitals and the influence of antibiotic resistance on biofilm phenotype is worrisome. Biofilms are estimated to be 1000 times more resistant to antibiotics than the free-living cells.[225–227] Biofilm formation has been explained to be triggered by the interplay between bacterial cells and environmental factors. *A. baumannii* is now known to resist almost all currently available antibiotics making it a "superbug" in the hospital setting, specifically in ICUs. There is an urgent need to comprehensively understand the processes and causes of the biofilm phenotype in bacterial cells including *A. baumannii*, in order to develop antibiofilm-specific drugs. Some of the resistance gene determinants are associated with biofilm formation and serve as a fitness cost in a strain-dependent manner, making it difficult to establish a consensus on the relationships that exist between biofilm formation and environmental triggers, antibiotic resistance, virulence mechanisms as well as the contributions of the source of the bacterial isolates. Hence, future studies should evaluate the influence of bacterial source, environmental factors, antibiotic resistance, and virulence determinants on biofilm formation.

Acknowledgments

Disclosure

The authors report no conflicts of interest in this work.

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