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Acinetobacter baumannii Biofilm: Intervening Factors, Persistence, Drug Resistance, and Strategies of Treatment

Acinetobacter baumannii Biyofilmi: Rol Oynayan Faktörler, Persistans, İlaç Direnci ve Tedavi Stratejileri

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Abstract

Acinetobacter baumannii (*A. baumannii*) is a Gram-negative opportunistic and nosocomial pathogen that is associated with most of the hospital epidemics. Its success can be directly attributed to its ability to survive under stressful hospital conditions (desiccation, nutrient starvation, and antimicrobial treatments). This survival ability results from the capacity of *A. baumannii* to form biofilms on the abiotic (polystyrene and glass) and biotic surfaces (epithelial cells and fungal filaments). The purpose of this review is to report different factors implicated in the biofilm formation of *A. baumannii*, notably biofilm-associated protein, CsuA/BABCD chaperone–usher pili system, poly-β-1,6-N-acetylglucosamine, outer membrane protein A, quorum sensing, surface properties, and growing conditions. This review will also discuss the relationship between biofilm formation and multidrug resistance, in addition to several strategies that can be useful in the prevention and treatment of *A. baumannii* biofilm.
Keywords: *Acinetobacter baumannii*, biofilm, genetic determinants, antibiotic resistance, treatment strategies

Öz

Acinetobacter baumannii (A. baumannii), hastane salgınlarının çoğuyla ilişkili Gram-olumsuz fırsatçı ve nozokomiyal bir patojendir. Bu patojenin başarısı, stresli hastane koşullarında (kuruma, besin yokluğu ve antimikrobiyal tedaviler) hayatta kalma kabiliyetine doğrudan bağlanabilir. Bu hayatta kalma yeteneği, *A. baumannii*'nin cansız (polistiren ve cam) ve canlı yüzeylerde (epitel hücreleri ve mantar filamentleri) biyofilmler oluşturma yeteneğinden kaynaklanmaktadır. Bu derlemenin amacı; özellikle biyofilm-ilişkili protein, CsuA/BABCD şaperon-kılavuz pili sistemi, poli-β-1,6-N-asetilglukozamin, dış membran proteini A, çoğunluk algılanması, yüzey özellikleri ve büyüme koşulları gibi *A. baumannii*'nin biyofilm oluşumunda rol oynayan farklı faktörleri bildirmektir. Bu derlemede ayrıca, *A. baumannii* biyofilminin önlenmesi ve tedavisinde yararlı olabilecek çeşitli stratejilere ek olarak, biyofilm oluşumu ve çoklu ilaç direnci arasındaki ilişki tartışılacaktır.
Anahtar Kelimeler: *Acinetobacter baumannii*, biyofilm, genetik belirleyiciler, antibiyotik direnci, tedavi stratejileri

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Introduction

The genus *Acinetobacter* includes a group of bacteria that are nonmotile, Gram-negative coccobacilli, displaying strict aerobic metabolism[1]. This genus is catalase-positive, oxidase-negative, and shows a favorable growth at an incubation temperature of 37 °C[2]. Among its species, *Acinetobacter baumannii* (*A. baumannii*) has emerged as one of the most troublesome pathogens for health care institutions. Over the last years, it has been remarked by its ability to upregulate and acquire the determinants of antibiotic resistance, thereby making it a challenge to the international health care community[2]. *A. baumannii* causes a range of infections, such as respiratory and urinary tract infections, meningitis, endocarditis, wound infections, and bacteremia, especially in the patients admitted in the intensive care units (ICUs)[3-5]. Infections caused by *A. baumannii* account for 1.6% of all healthcare-associated infections in both United States[6] and Europe[7]; however, these rates are twice as high in Asian and the Middle Eastern countries[7]. Biofilm formation is an essential pathogenic mechanism in such infections. The exceptional resistance and survival in the hospital environment of *A. baumannii* may be explained by its potential to form biofilm[2,8]. Consequently, the clinical isolates of *A. baumannii* can survive for longer periods under the highly desiccated conditions on abiotic surfaces[9,10] while forming biofilm on these layers. The purpose of this review is to report the biofilm formation capacity of *A. baumannii* on the surfaces in the hospital environment and its relationship with multidrug resistance, along with the involvement of multidrug resistance in the persistence of this nosocomial pathogen. This review will also present the essential factors involved in the biofilm formation mechanism, namely those related to the bacterium itself or to the surrounding environment, and finally the prevention and treatment strategies initiated by the scientific communities.

Biofilm Formation

Definition

The formation ability of biofilm is one of the major virulence factors of *A. baumannii*. The new definition of a biofilm is a microbial-derived sessile community characterized by the cells that are irreversibly attached to a substratum or interface or to each other. Those attached cells are embedded in a matrix of extracellular polymeric substances produced by them, and exhibit an altered phenotype with respect to the growth rate and gene transcription[11].

Biofilm Formation Steps

Biofilm formation is a step-by-step process that includes three phases: adhesion, maturation, and dispersal[12]. In the adhesion phase, the planktonic cells attach to a surface via their appendages and may also get attached through other physical forces such as van der Waals forces or electrostatic interactions[13]. After primary attachment, the loose cells bind to the site through the molecular interactions between the host molecules (such as fibronectin) and bacterial surface arrangements (such as pili and fimbriae)[14]. After the microorganisms are attached to a biotic or an abiotic surface, this attachment becomes stable, and a process of multiplication and division of microbial cells starts, which is initiated through particular chemical signaling within the exopolysaccharides (EPS)[13]. In the biofilm maturation and dispersal phases, the bacteria produce a high number of EPS, which is the main material in the biofilm's three-dimensional structure. Thereafter, the interstitial voids are produced in the matrix to act as a circulatory system. This system distributes the important nutrients and removes the waste products from the communities of microcolonies in the biofilm[15]. The cells (single or clusters of cells) are then detached and colonized in the adjacent sites, respectively. Biofilm formation on the biotic and abiotic surfaces is an effective strategy to enhance the bacterial survival and persistence under stressed conditions[16,17].

A. baumannii Biofilm

A. baumannii can form biofilms on several abiotic surfaces, such as polystyrene (a polymer that is commonly used in medical devices), polypropylene, polytetrafluoroethylene, and glass[18]. Additionally, several researchers have investigated the ability of *A. baumannii* to adhere and invade the biotic surfaces. The adherence of *A. baumannii* to erythrocytes[19] and human bronchial epithelial cells[20] is considered as the first step in the colonization process of this bacterium. The synergistic effect of an excessive growth on mucosal surfaces and medical devices, such as intravascular catheters and endotracheal tubes, can result in the biofilm formation of *A. baumannii*, which enhances the risk of infection of the bloodstream and airways[18]. Another work has also proved that the *A. baumannii* 19606-type strain adheres to and forms biofilms on the human alveolar epithelial cells and *Candida albicans* filaments, but not on the yeast cells[21].

Researchers have demonstrated that *A. baumannii* is at least three times higher biofilm former at the solid-liquid interface than the other *Acinetobacter* species [number of isolates 48 (80%)-42 (91%) versus 1 (5%)-8 (24%), respectively][22]. It is also hypothesized that the clinical strains can form stronger biofilms than the environmental strains[23], and their ability to survive nutrient availability stress, desiccation, and antimicrobial therapies, is effectuated by the formation of biofilms on the medically relevant surfaces[24]. Ivankovic et al. have examined the susceptibility of biofilms of four hospitals and three environmental *A. baumannii* isolates on ceramics and glass to two disinfectants, namely, benzalkonium chloride and chlorhexidine. According to their research, the hospital

and environmental isolates have the capacity to form biofilm on the two surfaces (glass, ceramics)[25]. Moreover, the biofilms of two isolates (hospital environments) on glass was destroyed by benzalkonium chloride (disinfectant) but on the ceramic surface, the survival rate of two isolates was higher after being exposed to disinfectant[25]. Furthermore, a study, which included 109 isolates of *A. baumannii* sampled in six cities of Southern Croatia in different medical institutions/departments and from different types of clinical samples, showed that the isolates collected from the ICUs and isolated from the respiratory samples were more able to form a biofilm as compared with the isolates from other departments and samples[26].

Intrinsic Factors of Biofilm Formation

Bacterial biofilm, on both abiotic and biotic surfaces, is definitely not a simple adherence of bacterial cells to a surface. Adhesion and biofilm formation are well-orchestrated mechanisms responding to a large variation of factors, and some of them depend on strain (Table 1). In fact, 1,621 genes showed over-expression in biofilms, and 55 genes were exclusively expressed in *A. baumannii* sessile cells[27].

Biofilm-associated Protein (BAP)

Loehfelm et al.[28] were the first ones to identify BAP in *A. baumannii*, it is a protein of cell surface analogous of that of *Staphylococcus*. Biofilm-associated protein is secreted via a type 1 secretion system[29] and involved in the formation and maturation of *A. baumannii* biofilm. It plays a role in the cell-cell adhesion and the development of higher-order structures on the medically relevant materials, such as polystyrene and titanium[28]. The scanning electron microscope analyses of biofilm have shown that the three-dimensional tower structure and water channel formation required BAP on the medically relevant surfaces (e.g., polypropylene, polystyrene, and titanium)[30]. However, BAP-deficient cells remain predominantly in a single layer, thereby constituting a few areas of cellular aggregates[30]. Moreover, the same research showed that BAP increases the adherence of *A. baumannii* by a comparative study of *A. baumannii* strain 307-0294 and *A. baumannii* 307-0294 BAP-deficient mutant[30]. *A. baumannii* strain 307-0294 associated with the normal human bronchial epithelial cells and normal human neonatal keratinocytes at a significantly higher percentage than *A. baumannii* 307-0294 BAP-deficient mutant ($p<0.02$), probably by improving the bacterial cell surface hydrophobicity[30]. Additionally, this protein is conserved among an amount of 98 *Acinetobacter* strains[28]; proving its importance in adhesion and biofilm formation.

CsuA/BABCD Chaperone-usher Pili Assembly System

Pili are homo- or heteropolymeric protein structures present on the surface of bacteria[31], and play a key role in the adhesion of microorganisms[32]. Csu pili are a type 1 chaperone-usher pilus system encoded and produced by a majority of *A. baumannii* strains[33] and regulated by the BfmRS two-component regulatory system[34]. These pili are not required for the adhesion on the biotic surfaces, such as human epithelial cells[35], but are essential for the biofilm formation and maintenance on the abiotic surfaces, including polystyrene. *Csu* operon-positive *A. baumannii* isolates were able to form a biofilm significantly more mature than those of *Csu* operon-negative isolates, thus proving the importance of the *Csu* operon in the biofilm formation[36]. Interestingly, most *A. baumannii* strains appear to carry the CsuA/BABCDE locus (a group of genes coding the different units of the type 1 chaperone–usher pilus system), but a subset of clinical isolates may have lost it[37]. Consequently, these pili may not be critical for biofilm formation and maintenance in all

Table 1. Intrinsic and extrinsic factors implicated in the *A. baumannii* biofilm formation

Intrinsic factors	Functions	Extrinsic factors	Examples
BAP	Cell-cell adhesion[28] Water channel formation and three-dimensional tower structure[30]	**Surface properties**	Surface nature Roughness Physico-chemical properties of the surface Presence of protein films
CsuA/BABCD chaperone–usher pili assembly system	Biofilm formation and maintenance on abiotic surfaces[35]		
PNAG	Capacity and thickness of biofilm formation[39]		
OmpA	Interaction with both human epithelial cells and *Candida albicans* filaments and attachment step of *A. baumannii* on plastics[24,42,44]	**Growing conditions**	pH Osmolarity Iron concentration Oxygen Hydrodynamics of the fluid Temperature Environmental nutritional conditions
Beta-lactamase PER1	Adhesion of *A. baumannii* to both biotic and abiotic surfaces[45]		
Quorum sensing system	Activate/regulate gene expression of virulence factors including biofilm formation[48]		

BAP: Biofilm-associated protein, OmpA: Outer membrane protein A, PNAG: Poly-β-1,6-N-acetylglucosamine

the strains or that other pili systems may functionally replace them[37]. GracSA, a second two-component system, was shown to moderately control the *Csu* gene expression; thus, it indirectly affects biofilm formation[37]. Furthermore, de Breij et al.[35] have stated that *A. baumannii* ATCC 19606 strain produces a short and thin independent CsuA/BABCDE operon pili that may be involved in the biotic surface adhesion such as the human respiratory cells.

Poly-β-1,6-N-acetylglucosamine (PNAG)

Poly-β-1,6-N-acetylglucosamine, encoded by the *pgaABCD* gene cluster, is one of the important structures for biofilm formation in microorganisms (Gram-negative and Gram-positive)[38]. Biofilm development and maturation of the clinical *A. baumannii* isolates also depend on the capacity to produce and secrete this substance as a major component of the biofilm EPS matrix[38]. The expression of *pgaB* was much higher in a clinical *A. baumannii* strain than that of an environmental strain, and was associated with an increase in the capacity and thickness of biofilm formation[39]. Another study has revealed that the deletion of *pgaABCD* induced the absence of PNAG[38]. Moreover, it induces the loss of the strong biofilm phenotype, which was re-established after complementation[38]. Consequently, the antibodies against PNAG[38] can eliminate *A. baumannii* in the opsonophagocytosis assays, suggesting that PNAG might be a potential vaccine target[40].

Outer membrane protein A (OmpA)

The OMP of Gram-negative bacteria have been associated with antibiotic resistance, adaptation, and pathogenicity in the host cells. Some OMPs of the OmpA family have been characterized in the *Acinetobacter* strains and are one of the major OMPs in the genus[41]. The OmpA of *A. baumannii* is a real virulence factor since it is involved in the adhesion and invasion of epithelial cells. These proteins cause their apoptosis by targeting the mitochondria, which leads to the spread of the bacterium through the mucosa. This bacterium is thus disturbed, and it systematically induces infection[41-43]. Outer membrane protein A plays a crucial role in the biofilm formation by promoting the cell surface and cell-to-cell adhesion on both the biotic and abiotic surfaces. This trimeric porin of 38 kDa, acting as a general diffusion pore of size of 1.3 nm, plays a role in the interaction of the pathogen with both the human epithelial cells and *Candida albicans* filaments as well as in the attachment step of *A. baumannii* on plastics[24,42,44].

Beta-lactamase PER1

Lee et al.[45] have shown that the adhesion of *A. baumannii* is enhanced by the presence and expression of the *bla*PER-1 gene to both the biotic surfaces, such as bronchial epithelial cells (initiation of host-pathogen interaction leading to pathogenesis), and to the abiotic surfaces, such as plastic, even if the mechanism by which that occurs remains unstable. On the contrary, only 2 out of 11 human isolates with the *bla*PER-1 gene are robust biofilm formers as compared with the isolates without this genetic determinant[46]. Therefore, these results raise to question the actual intervention of *bla*PER-1 expression in the biofilm formation[46]. This observation will be discussed later in this manuscript.

Quorum sensing (QS) system

In wild life, bacteria share a close association with eukaryotic hosts and other bacteria. Constantly, it is essential to monitor and communicate with neighbors. Hormone-like molecules (autoinducers) are produced by bacteria as the signals to sense the cell density and activate adaptations by QS[47]. Autoinducers act by activating/regulating gene expression by binding to transcriptional regulatory proteins in the organism[48]. Researchers have linked QS system with different processes, notably the production of virulence factors, motility, nodulation, plasmid transfer, antibiotic production, bioemulsan production, bioluminescence, and biofilm formation[49-51].

AbaI and *abaR* are the QS genes acquired horizontally from *Halothiobacillus neapolitanus* and expressed in *A. baumannii* [52]. This QS system involves the AbaR receptor protein that forms a complex with the AbaI (auto-inducer synthase)-generated N-(3-hydroxydodecanoyl)-L-homoserine lactone that regulates the virulence factors (e.g., biofilm formation and surface motility)[53]. Researchers have found that an auto-inducing QS molecule controlled biofilm formation among the clinical isolates of *Acinetobacter* spp.[54]. *AbaI* was present among the isolates that produce the QS signaling molecules and a mutation in the *abaI* influenced biofilm-forming capabilities *Acinetobacter* spp.[54]. Some researchers investigated the association between the effect of *abaR* on the biofilm formation and drug resistance of *A. baumannii*[55]. The upregulation of the expression of *bfmS* and *bfmR* genes is linked to the QS molecules, thereby enhancing the ability of *A. baumannii* to form biofilm on the abiotic surfaces[56]. Furthermore, the QS system can also be a potential target for a new drug by developing many quorum quenching (QQ) strategies targeting the AHL synthase enzyme, the AHL binding receptor, and the AHL itself[57].

Extrinsic Factors of Biofilm Formation

Surface Properties

In the case of adhesion to the abiotic surfaces, *A. baumannii* can form and develop a high biomass biofilms on different surfaces such as stainless steel, polystyrene, and polycarbonate (a thermoplastic material that is often used to construct the medical devices)[58]. Several factors may affect the attachment of bacteria to these surfaces and the formation of a biofilm

(Table 1), such as roughness, the physico-chemical properties of a surface, and the presence of protein films. All the materials used in the manufacturing of implantable medical devices are described as biomaterials. Hundreds of polymers are currently used separately or combination with the manufacturing thereof. As examples, the latex catheters are inexpensive and have a good elasticity, but tend to be more prone to bacterial adhesion[59]. In the case of silicone, it is the standard in terms of biocompatibility and is also mild, non-irritating, and clinically stable, which is ideal for long-term use[59], but silicone is still very sensitive to biofilm formation[60]. The bacterial cells have a negative charge on their cell membrane but this charge is more or less important from one strain to another. The surface charge of the material can be varied by the pH and ionic composition of the surrounding solutions as well as by protein adsorption, which occurs during the early stages of adhesion. This adhesion increases with the hydrophobicity of the support[61,62]. Indeed, the roughness of the surface is important as the colonization by microcolonies[63]. The prior presence of a protein film on a biomaterial such as blood, tears, urine, saliva, interstitial fluid, and respiratory secretions affects the attachment of bacteria to its surface and promotes the formation of biofilm[64].

Growing Conditions

Growing conditions are important characteristics that can strongly modify the adhesion of organisms. pH, osmolarity, iron concentration, and oxygen are important to consider in the biofilm formation process[65]. The hydrodynamics of the fluid also affects the biofilm formation. Indeed, depending on the position of the material in a fluid, it will be more or less exposed to turbulence. The zone of less turbulence, away from the laminar flow, is called the fixing zone. It is precisely in this area where it is easier for the microorganisms to settle on a surface, since they are less subjected to the forces exerted by the fluid[11].

The growth temperature is also an important factor of biofilm formation. *Acinetobacter* spp. biofilm formation was more important at 25 °C than at 37 °C[22]. However, the optimal conditions for biofilm formation by *A. baumannii* were reported to be 30 °C at a pH of 7.0 in a medium containing sodium chloride at a concentration of 5 gL^{-1}[1]. Another study showed that the biofilm formation of *A. baumannii* was high at 28 °C because of the upregulation of certain BAPs such as Csu and iron uptake proteins on the plastic surfaces[66]. Additionally, Eze et al.[67] demonstrated that *A. baumannii* biofilm formation is enhanced when nutrient-poor medium and 26 °C are used with or without agitation.

Interestingly, the *A. baumannii* ATCC 17978 strain produced little or no biofilm on the glass surfaces when it was incubated under blue light, whereas a normal biofilm was observed when the cells were incubated in the darkness[68]. This finding is mediated by the BlsA photoreceptor protein, with a *N*-terminal blue-light-sensing that uses the flavin domain. The mechanisms of light signal transduction and gene expression control of BlsA are not yet known. However, the diverse transcription of *blsA* at 37 °C and 28 °C differentially affects the response of *A. baumannii* biofilm to the light. In addition, this response seems to have a global effect on *A. baumannii* physiology, disturbing biofilm formation and also motility and virulence[68].

Environmental nutritional conditions affect the growth and lifestyle of a bacterial population. Indeed, in a static environment, the concentration of nutrients must be high so that a biofilm can be formed; however, this is not the case for a hydrodynamic environment[69]. Some sources of carbon and cations (Na^+ sodium, Ca^{2+} calcium, and Fe^{3+} ferric ion) also affect the formation of a biofilm[11,70]. The biofilm formation of *A. baumannii* was reported to be affected by environmental stress and growth conditions[71]. *A. baumannii* cultured in a glucose-based medium and exposed to the sub-inhibitory concentrations of antibiotics (e.g., imipenem) can lead to an increased iron uptake and induce biofilm formation in a clinical multidrug-resistant phenotype[71]. Five well-characterized *A. baumannii* strains, cultured in three iron-poor media depending on the strain, have shown different levels of biofilm growth[72]. In the tryptic soy broth dialysate, all the strains produced an increased biofilm as compared with other iron-poor media. Therefore, biofilm formation in *A. baumannii* depends on strain[72]. Also, a significant reduction showed by *A. baumannii* clinical isolates in the adhesiveness and biofilm formation ability on the biotic and abiotic surfaces (i.e., human respiratory epithelial cells and plastics, respectively) when grown with an iron-chelating agent[20,45].

Ethanol had also been an effect on biofilm formation on the abiotic surfaces. In fact, the presence of ethanol increase the production of proteins involved in the lipid and carbohydrate anabolism, thereby raising the carbohydrate biofilm content, decreasing bacterial motility and enhancing biofilm formation[73].

Biofilm and Antibiotic Resistance

A. baumannii is naturally resistant to a large spectrum of antibiotics[74]. In the last two decades, because of the widespread use of antibiotics, multidrug-resistant (MDR) strains, which are defined as an acquired non-susceptibility to at least one agent in three or more antimicrobial categories and extensively drug-resistant (XDR) strains, which are defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e., bacterial isolates remain susceptible to only one or two categories) have emerged. A large number of reports have suggested the worldwide emergence of *A. baumannii* as a critical problem[75,76]. Laktib et al.[77] conducted a study in two ICUs (adult and neonatal ICUs) of The Regional Hospital Center of Agadir,

Morocco and showed that the most frequently isolated strains were of *A. baumannii* (82.8%). Multidrug-resistant *A. baumannii* strains were the most dominant in the adult ICU (42.8%) and held the second position after the extended spectrum beta-lactamase (ESBL)–producing *Enterobacteriaceae* strains (Table 2) in the neonatal ICU[77]. The use of broad-spectrum antibiotics and the transmission of strains among patients are considered as the selective pressures that lead to the emergence of MDR *A. baumannii*[78]. Multidrug-resistant strains are often isolated from the patients treated with broad-spectrum antibiotics and those with compromised immunity[79]. They exhibit various mechanisms that resist multiple classes of antibiotics, especially the production of antibiotic degradation/modification enzymes, active drug efflux pumps, decreased permeability, biofilm formation, and modification in the drug targets[17]. The main common mechanism responsible for carbapenem resistance in *A. baumannii* is the production of carbapenemases, including class B metallo-β-lactamases and class D β-lactamases (oxacillinases) [80,81]. The emergence of MDR and XDR *A. baumannii* leads to the application of limited and potentially toxic alternatives (e.g., colistin, polymyxin B) for treatment, which are correlated with poor outcomes in the patients[82].

The association between biofilm formation and antibiotic resistance phenotypes still disputable. Some studies have demonstrated that biofilm formation appeared to be positively correlated with multidrug resistance[46,83,84]. A study has already reported a positive correlation between the drug resistance and biofilm formation in the form of ESBL *bla*PER-1 gene among the *A. baumannii* isolates[84]. At least 92% of the biofilm-forming isolates of clinical strains isolated from patients with nosocomial infections in three hospitals in Tehran were MDR[84]. Srinivasa Rao et al.[46] mentioned a significant correlation between multidrug resistance and biofilm formation. However, the presence of *bla*PER-1 is more critical for cell adhesion than the formation of bacterial biofilms on the abiotic surfaces[47]. It was observed that the cell adhesiveness and biofilm formation on plastic is higher in strains with the *blaPER-1* gene than in those without this genetic determinant[46]. A significant correlation was determined between multidrug resistance and biofilm formation of environmental and clinical isolates[23], namely, clinical isolates had a higher biofilm formation ability than the environmental isolates[23]. The MDR clinical isolates of *A. baumannii* carrying the *bla*PER-1 gene were reported to adhere to the epithelial cell surface and form biofilm in polystyrene plates higher than those without it[45]. The majority of the clinical *Acinetobacter* spp. isolates from intensive and non-intensive tertiary care hospital units in Bangladesh, especially those isolated from the ICU samples, were reported as MDR and biofilm producers[85]. It was found that 84.7% of the 72 clinical isolates of *A. baumannii* isolated from India were resistant to piperacillin, 80.5% to amikacin, 72.2% to ciprofloxacin, 66.6% to ceftazidime, 36.1% to imipenem, 25% to ampicillin–sulbactam, whereas 62.5% of the isolates produced biofilm[86]. Badave and Kulkarni[86] established that 40 strains of 72 *A. baumannii* tested strains were considered as both MDR and biofilm formers with a significant correlation (p=0.0004).

About 35.5% of the isolates from a tertiary care hospital in Mexico were resistant to meropenem, 50.7% to imipenem and 86% to ciprofloxacin, ceftazidime, and cefotaxime[75]. Of these isolates, 25.7% and 28.3% were positive for the *blaOXA*-72 and *blaOXA*-58 genes, respectively[75]. The work also associated biofilm production with resistance to imipenem (p=0.002) [75]. The ability to produce biofilm in relation to antibiotic resistance in *A. baumannii* was also demonstrated by Kaliterna et al.[26]. Ampicillin/sulbactam-, carbapenems-, and amikacin-resistant strains were found to be biofilm-negative while those were susceptible and intermediately susceptible to ampicillin/sulbactam, carbapenems, and amikacin were biofilm producers[26].

Because of their capacity to extrude the majority of antibiotics from within the cells to the extracellular environment and also in the biofilm formation, efflux pumps have a important implication in antibiotic resistance[87]. It was demonstrated that the synthesis and transport of auto-inducer molecules is linked to the AdeFGH efflux pump during the biofilm formation of *A. baumannii*[88], and in the presence of sub-inhibitory concentrations of tigecycline, the downregulation of the AdeFGH efflux pump resulted in a reduction in *A. baumannii* biofilm[89]. Moreover, AbaF, an efflux transporter of *Escherichia coli*, was cloned and expressed from an efflux-deficient strain *Escherichia coli* KAM32[90]. After its disruption in *A. baumannii* by using a homologous recombination, an increase in fosfomycin susceptibility and a decrease in biofilm formation and virulence were observed[90].

Table 2. Prevalence of multidrug-resistant bacteria isolated from adult and neonatal intensive care units[77]

	MDR bacteria			
Services	**ESBL-producing *Enterobacteriaceae***	**MDR *A. baumannii***	**MDR *S. maltophilia***	**Total**
AICU	5 (35.7%)	6 (42.8%)	3 (24.4%)	14
NICU	12 (60%)	8 (40%)	0	20
Total	-	-	-	34

AICU: Adult intensive care unit, ESBL: Extended spectrum beta-lactamase, MDR: Multidrug-resistant, NICU: Neonatal intensive care unit, *S. maltophilia: Stenotrophomonas maltophilia*

This relationship may be because of the high production of EPS in *A. baumannii*. This EPS production creates a protective barrier that prevents the antibiotic penetration leading to the development of resistance. Furthermore, there are differences in the physiology of the cells inside the biofilm that produces an increased drug resistance[91]. Also, the horizontally genes transfer between bacterial cells is enhanced in biofilm mode, thereby facilitating the spread of antibiotic resistance[11]. *Acinetobacter* is known to exhibit an extraordinary ability to acquire foreign DNA[92]. A study confirmed that *bla*NDM-1 could easily be transfer to strong biofilm-producing *Pseudomonas aeruginosa* and *A. baumannii* by *Enterobacteriaceae* strains in the environment[93]. In general, the biofilm compartment is highly resistant to antibiotics because of the reduced diffusion of antibiotics into the biofilm, the presence of persisted cells, slow growth rates, low metabolism of cells that exist deep within the biofilm, increased horizontal transfer of resistance genes (due to cell vicinity), and a high rate of mutations (in response to stress)[94,95].

Contradictorily, when the MDR *A. baumannii* strain, collected from a Chinese hospital, showed a strong biofilm-forming ability, the resistance to levofloxacin, cefepime, and gentamicin was significantly decreased[96]. Studies have also demonstrated little or no biofilm-producing capacity by resistant *A. baumannii*[1,97]. Colistin reduces biofilm formation on the urinary catheter surfaces at sub-inhibitory concentration[1]. It was shown that the isolates pre-treated with colistin had a significant reduction in their adhesion ability ($p<0.05$)[1]. The cultures treated with 0.5 minimum inhibitory concentration (MIC), as compared to those treated with 0.25 MIC, revealed decreased biofilm formation ($p<0.05$)[1]. In total, 249 isolates of 272 clinical isolates of *A. baumannii* had a biofilm formation ability, of which 63 were stronger biofilm formers than the *A. baumannii* strain type ATCC 19606[97]. Isolates with high levels of resistance were weak biofilm formers, whereas the majority of isolates that formed the biofilms were non-MDR[97]. On the contrary, the over-expression of AdeABC efflux pump largely contributes to multidrug resistance, an altered membrane composition and decreased biofilm production in *A. baumannii*[98].

Through all these facts, it is necessary to more understand the bacterial mechanisms to keep a balance between biofilm formation and antibiotic resistance, and also those implicated in the ability to achieve high levels of biofilm-specific resistance despite producing weak biofilms[97].

Biofilm and Persistence of *A. baumannii*

In addition to antibiotic resistance, biofilms offer protection against the infected host's immunity to the bacteria. The size of biofilms is firstly an important obstacle to the phagocytosis process. Phagocytic cells release enzymes that have a very little effect on the biofilm[99]. The extracellular matrix is also a barrier to the host's immune system as it prevents the antibodies' recognition of bacterial antigens[100]. The proximity of different bacterial strains in the biofilm promotes a genetic exchange[11]. Indeed, the speed of conjugation within the biofilm is very fast, thus suggesting that the evolution by the horizontal transfer of genetic material occurs frequently, which makes the perfect medium for the acquisition of not only determinants of antibiotic resistance but also other virulence factors[101]. *Acinetobacter* is characterized by its important ability to acquire DNA from other species[92].

It is clear that the resistance of desiccation depends on the ability of *A. baumannii* to maintain viability under the conditions of water limitations. Indeed, in *Acinetobacter baylyi,* a non-pathogenic relative of *A. baumannii*, capsular polysaccharides (Ps) promote survival during the periods of desiccation[102]. In *A. baumannii* biofilm, the ability of the capsule to retain water and the presence of a capsular Ps covering the cells play a prominent role in the resistance to desiccation[103]. Globally, *A. baumannii* has increased tolerance to the extracellular stresses within biofilm communities[16,59], thus allowing this bacterium to persist and emerge, especially in hospitals, as one of the phenomenal nosocomial agents.

Strategies of Prevention and Treatment of *A. baumannii* Biofilm

Two strategies can be developed to fight against the biofilm: the prevention or the inhibition of biofilm formation and the dispersion of performed biofilm.

1. Prevention

The inhibition of biofilm formation by pre-adhesion intervention and biofilm formation may be useful.

The elaboration of anti-adhesive or anti-biofouling surfaces by using three different methods i.e., physical, chemical, or biological can be used to fight against the attachment of microorganisms and the formation of biofilm[104].

The physical methods include the modification of the topography or the porosity of a material[104]. The surface chemical composition plays an important role in the adhesion of bacteria affected by the hydrophobicity and the electrostatic charge of the surface. Thus, the presence of specific chemical groups can also have an impact[104]. The addition of a hydrophilic coating (polymeric hydrophilic coatings) such as polyethylene glycol is used for building antifouling surfaces as they minimize the microbial adhesion[105].

Biological mechanisms are also conceivable to combat the development of a biofilm such as the use of non-pathogenic bacteria like *Lactobacillus fermentum*[106]. The presence of this bacterial strain allows the creation of an inhibition zone within the pathogenic biofilm probably because of the partial destruction of the cell membrane[106].

On the contrary, *A. baumannii* contains a *pgaABCD* locus that encodes the proteins that synthesize cell-associated PNAG, which is the major compound of extracellular matrix in the biofilm of *A. baumannii*[38]. The deletion of the *pga* locus led to the loss of the strong biofilm phenotype[38]. Efforts are already under way to further investigate the potential of PNAG as a candidate vaccine against *A. baumannii* [38]. According to antibiotics, Beganovic et al.[107] have tested the anti-biofilm effect of minocycline, polymyxin B, meropenem, and amikacin against *A. baumannii*. Minocycline prevented biofilm formation for 96% of isolates versus 54% for polymyxin B, 29% for meropenem, and 29% for amikacin[107].

2. Dispersion

The dispersion of performed biofilm is the treatment and the elimination of biofilms after their formation by antimicrobial agents, physical forces, and enzymes. In a study conducted by Golberg et al.[108], algal Ps can be useful molecules. Algal-secreted Ps biomaterial patches and metal complex films (MCFs) are examined for their anti-*A. baumannii* and anti-*Pseudomonas aeruginosa* biofilm properties[108]. Polysaccharides moderately reduces biofilm formation; and the Cu-MCF coating has a significant antibiofilm activity ($p<0.001$)[108]. Quantitative analysis showed inhibition rates of 70% and 98% in biofilm formation by *A. baumannii*, and 97% and 99% by *Pseudomonas aeruginosa* on the Ps and Cu-MCF coatings, respectively[108]. Biopanning was conducted with a peptide library on five XDR *A. baumannii* strains to find the antimicrobial peptides against *A. baumannii* growing in the planktonic and in the biofilm mode. These strains were later grown in a medium containing human blood (blood biopanning) and biofilms formed by these strains (biofilm biopanning)[109. Two peptides, namely N10 (from blood biopanning) and NB2 (from biofilm biopanning), were selected[109]. NB2 reduced the biofilm more efficiently (75%) than N10 (50%) but the combination of the two peptides could function better than each peptide alone to prevent the biofilm formation of *A. baumannii*[109]. In another study, the antibiofilm activity of various medicinal plants extracts was evaluated against the carbapenem-resistant strain of *A. baumannii*[110]. The results showed that the polar extract of kiwi (*Actinidia deliciosa*) and clove (*Syzygium aromaticum*) exhibit an effective antibiofilm activity[110]. The antibiofilm effect of *Actinidia deliciosa* extract on the extracellular matrix of *A. baumannii* showed that it reduces EPS, protein, and eDNA contents in the extracellular matrix[110]. The antimicrobial and antibiofilm potential effects of cell-free supernatants obtained from *Clostridium butyricum* were also tested[111]. The results showed not only an inhibition effect against cell growth in the planktonic culture but also the inhibition of the biofilm development, dispersion of mature biofilms, and the suppression of the metabolic activity of biofilm cells[111].

Bacterial extracellular Ps have been shown to mediate many of the cell-to-cell and cell-to-surface interactions that are required for the formation, cohesion, and stabilization of bacterial biofilms[112]. Mutant strains unable to synthesize or export these EPS usually exhibit decreased adherence, decreased biofilm formation, and an increased sensitivity to be killed by biocides and host defenses[112]. These results highlight the importance of EPS in maintaining the integrity of the biofilm and in mediating the pathogenic potential of the biofilm lifestyle[112]. The dispersion or the disorganization of the EPS would be another strategy of combating biofilm[105]. There are specific enzymes that are capable of disrupting the EPS such as Ps lyases and DNases[113], and Dispersin B (a glycoside hydrolase) which works by cleaving the polymers of PNAG that can disperse layers of EPS present on the medical devices[114,115]. This enzyme can offer a better result in combining with silver[116] or with cefamandole nafate[117].

QS is a complex system that regulates different virulence factors including biofilm formation in response to bacterial cell population density. The inhibition of this system also termed as QQ, which may have a significant effect against biofilm. Several strategies have been considered to interrupt and/or disrupt the bacterial QS system. These strategies include the inhibition of signal generation (acyl homoserine lactone synthesis), inhibition of signal diffusion, or inhibition of signal reception[118]. In the biofilm-growing cells, the homoserine lactone synthase (A1S_0109) of *A. baumannii* ATCC 17978 strain was over-expressed with respect to the planktonic cells[27]. Furthermore, many studies have demonstrated the utility of this anti-virulence and antibiofilm strategy. Chow et al.[119] have detected a significant reduction of the biomass of *A. baumannii*-associated biofilms by using QQ lactonase, obtained by directed evolution. Several plant extracts have been considered as anti-QS. For example, *Commiphora leptophloeos* (Burseraceae), *Pityrocarpa moniliformis* (Leguminosae), and *Bauhinia acuruana* (Leguminosae) extracts against *Staphylococcus epidermidis*[120] and *Terminalia catappa* (Combretaceae). The methanolic extract inhibits QS-controlled violacein production and biofilms maturation of *Chromobacterium violaceum* and *Pseudomonas aeruginosa*, respectively[121]. The Australian macroalga *Dilsea pulchra* has the capability to produce a natural furanone that interfered with the bacterial signaling processes by its similarity in the structure to AHL molecules[122]. By binding competitively

to the receptor, it is responsible for affecting the interaction of putative regulatory protein with AHL molecules.

All these possible strategies can be used to develop new drugs or modified medical devices by anchoring the antibiofilm molecules or create more effective disinfectants against the biofilm of *A. baumannii.*

Conclusion

A. baumannii has received much attention in recent years because of its success as a nosocomial pathogen along with its intrinsic and/or acquired resistance to the multiple classes of antibiotics and the ability to form biofilm in both biotic and abiotic surfaces. This ability plays a crucial role in the interactions between host and pathogen and in medical device-associated infections. It involves multiple cell signals, genetic determinants, and environmental factors. Currently, there is no close correlation between the genetic determinants implicated in the initiation of biofilm formation on the abiotic surfaces and those associated with the adherence to biotic surfaces.

With the development of resistance and persistence of *A. baumannii*, the research of effective strategies that procure antibacterial and antibiofilm properties is a real challenge. The abovementioned targets may be feasible and useful techniques for combating the *A. baumannii* biofilm. The targeting of the extracellular matrix polymerics and the QS system could offer very effective antibiofilm routes of action. Also, in basing on the medicinal and aromatic plants, we can develop a technique that is both useful and more secure.

We can also conclude that a combinatory strategy between an antimicrobial and an antibiofilm agent may provide better results by simultaneously offering a bactericidal and antibiofilm effect instead of using a single agent.

Ethics

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Analysis or Interpretation: R.E., A.L., R.M., A.A., M.H., A.E., F.H., Literature Search: R.E., A.L., R.M., A.A., M.H., A.E., F.H., Writing: R.E., A.L., R.M., A.A., M.H., A.E., F.H.

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