

ACINETOBACTER BAUMANNII EXTRACELLULAR MATRIX AS AN ANTIBIOFILM AND ANTI-INFECTION TARGET

El Kheloui Raja, El Megdar Soufiane, Laktib Asma, Mimouni Rachida and
Hamadi Fatima*

Laboratory of Microbial Biotechnology and Plants Protection, Department of Biology,
Faculty of Sciences, Ibn Zohr University, Agadir, Morocco.

Article Received on
28 March 2022,

Revised on 17 April 2022,
Accepted on 07 May 2022

DOI: 10.20959/wjpr20226-24004

*Corresponding Author

Dr. Hamadi Fatima

Laboratory of Microbial
Biotechnology and Plants
Protection, Department of
Biology, Faculty of
Sciences, Ibn Zohr
University, Agadir,
Morocco.

ABSTRACT

Acinetobacter baumannii is one of the most worrisome pathogenic organisms in the healthcare environment, it is responsible for several lethal hospital acquired infections worldwide. The success of this bacterium is due to its resistance mechanisms to antimicrobial agents, its persistence and survival in the harshest environments for a prolonged period. This has been mainly related to the ability of this pathogen to form biofilms on biotic and abiotic surfaces. Biofilm gives the bacterial cells a life form embedded in an extracellular polymeric matrix (exopolysaccharides, proteins and extracellular DNA). This matrix is the defense barrier of the biofilm and crossing this barrier is the crucial step to eradicate the biofilm and the infection as a consequence. In this review, we have mentioned some of the works carried out in the framework of the strategies targeting the main

composition of *Acinetobacter baumannii* matrix (Poly-(1-6)-N-acetylglucosamine, Biofilm-associated protein and eDNA) to activate the dispersion of the biofilm and to make the bacterial cells accessible to the antibacterial treatments.

KEYWORDS: *Acinetobacter baumannii*, Biofilm, extracellular polymeric matrix, poly-(1-6)-N-acetylglucosamine, biofilm-associated protein, extracellular DNA.

INTRODUCTION

Acinetobacter baumannii is a coccobacillus that has gained prominence as a common pathogen recovered from a variety of wound infections of United States military personnel deployed to areas such as Iraq.^[1] It has been classified as one of the five "ESKAPE"

pathogens (*Enterococcus faecalis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter spp.*) because of its ability to escape the antimicrobial treatment by resistance genes acquisition.^[2] Moreover, the European Center for Disease Prevention and Control, Infectious Diseases Society of America, World Health Organization, and the Centers for Disease Control and Prevention of America have declared multidrug resistant *A. baumannii* as a critical health threat. Carbapenem resistant *Acinetobacter* was upgraded from a "severe" to "urgent" threat level in 2019.^[3,4] Recently, World Health Organization designated carbapenem resistant *A. baumannii* as a priority critical bacterium in its list of bacteria.^[5]

A. baumannii is frequently associated with wound infections, meningitis, and urinary tract infections, particularly in intensive care unit patients.^[6] In many cases, infections are acquired after exposure to *A. baumannii* (which persists on contaminated hospital equipment) or through contact with a colonized patient.^[7] Furthermore, mortality due to *A. baumannii* infection can vary from 7.8 to 43%.^[8,9] This bacterium, which is ubiquitous in the environment, is typically found in soil, water, human skin, food products and medical devices.^[10,11] The effectiveness of *A. baumannii* is linked to its response to antibiotics and disinfectants used in hospitals. Several *A. baumannii* isolates exhibit high resistance to all widely used antibiotics, including ampicillin-sulbactam^[12], carbapenems^[13], aminoglycosides^[14], tetracyclines^[15], quinolones^[16], colistin^[17], polymyxin B^[18], and tigecycline.^[19] This microorganism is able to resist to desiccation and disinfection and survives for several days on nutrient-limited surfaces.^[20] *A. baumannii* can also survive on fingertips and on inanimate objects such as plastic, glass and other surfaces, even after being exposed to dry conditions, for prolonged periods.^[21-23] Moreover, it is assumed that its ability to persist in these environments, as well as its virulence, is a consequence of its biofilm formation capacity.^[24] In addition, the antibiotic resistance is highly related to biofilm formation.^[25] These properties make it responsible for hospital-acquired infections.^[23]

Biofilm is the predominant mode of growth of bacteria in the environment in general and the hospital environment in particular. Biofilms are multicellular communities of bacteria attached to a tissue or surface, within which the bacterial cells are sheltered in an extracellular polymeric matrix (EPM) rich in self-produced biopolymers.^[26] These are mainly comprised of extracellular DNA (eDNA), exopolysaccharides (EPS) and proteins. This matrix composition help to adhere and colonize surfaces and form a structurally stable biofilm.^[27]

Bacteria in biofilms are protected from stresses that would effectively target their planktonic counterparts such as antibiotics, desiccation, metal cations, ultraviolet light and host immunity. As a result, conventional antibiotic treatments fail to eradicate biofilm infections and standard cleaning procedures are ineffective.^[28] With the emergence of multi-drug resistant *A. baumannii*, there is a need to discover new drugs or targets to treat and prevent *A. baumannii* infection. Biofilm formation is one of the main mechanisms of survival under various conditions and the elimination of biofilms in a host or clinical setting is an ongoing challenge. Due to its crucial role in biofilm formation, maturation and persistence, the EPM can be an effective target. Despite its critical function in *A. baumannii* biofilms, there are a limited number of studies that are interested to *A. baumannii* EMP. This review is a collection of available informations concerning the EPM of this pathogen and the works already done in this field.

BIOFILM AND FORMATION STEPS

Human pathogenic microorganisms have different mechanisms of pathogenesis, virulence and antimicrobial resistance. However, a common feature of most of them is the ability to form biofilms. Bacterial biofilm consists of a densely packed bacterial population in which the cells unite to form a protective barrier against the external environment. This population is encapsulated in a self-secreting matrix of polysaccharides, proteins and DNA that adheres to any surface or tissue.^[27] Since the invention and description of the term biofilm in 1978.^[29], it has been well documented that cells in biofilm mode differ from their planktonic state in terms of transcribed genes.^[30] In biofilms, the cells are physiologically, morphologically and metabolically distinct from their planktonic homologues. Bacteria within a biofilm reach a significantly higher cell density (10^{11} CFU.mL⁻¹) than planktonic bacteria (10^8 CFU.mL⁻¹).^[31] On the other hand, biofilms are developed on a wide range of interfaces, e.g., solid-liquid interface and air-liquid interface. They have been detected on glass, plastic surfaces and medical devices such as catheters and artificial heart valves.^[32] In the natural environment, this is one of the most common growth patterns, especially in the hospital setting. The main defensive barrier of a biofilm is its EPM, which provides a protective layer against environmental stressors, host immunity and antibiotic treatment.^[33]

The passage from the planktonic to the biofilm state implicates a complicated and irreversible process (Fig. 1). It begins when bacteria sense environmental conditions that initiate the transition from planktonic free-swimming mode to a surface-orientated life.^[34]

- (1). Adhesion: the first stage of biofilm formation, the adhesion of the bacteria to the surface, involves cell-surface interactions and a variety of bacterial surface structures.^[35] Furthermore, each surface structure may be specific to an attaching surface with particular properties, and the expression of these structures may change depending on the environment to which the organism is exposed.^[35]
- (2). Aggregation: after binding to a surface, the bacteria begin to proliferate and form microcolonies.
- (3). Irreversible attachment: bacteria adapt to its new life by the synthesis of the matrix biopolymers.
- (4). Maturation: bacteria increased the synthesis and the development of the EPM to form a mature biofilm.
- (5). Dispersal: the final stage of the biofilm process involves the detachment of the biofilm and the colonization of another area. Possible signals that may trigger cell detachment include starvation, enzymes such as alginate lyase in *Pseudomonas aeruginosa*, or loss of EPM or part of it.^[35]

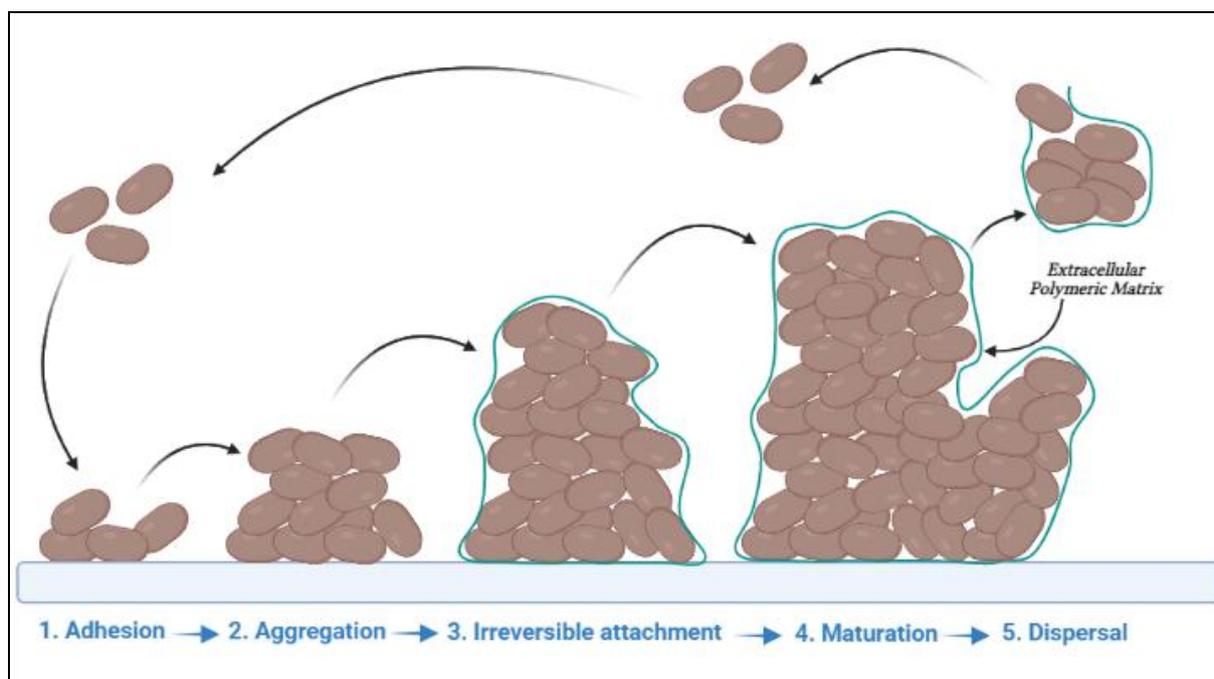


Fig. 1: Bacterial biofilm life cycle.

The antibiotic resistance, hospital survival and high pathological potential of *A. baumannii* can be explained by the fact that it is a prolific producer of biofilms.^[36,37] *A. baumannii* can form biofilms on both abiotic (polystyrene and glass) and biotic surfaces (e.g., epithelial cells and fungal filaments).^[20,38] In *A. baumannii* biofilms, virulence genes, including those

involved in antibiotic resistance, appear to be overexpressed.^[39,40] In addition, a positive correlation has been found between biofilm formation and multiple drug resistance in clinical *A. baumannii* isolates.^[41,42] In fact, the genes required for biofilm formation are widely distributed in clinical strains of *A. baumannii* but are partially or completely absent in environmental isolates.^[43] Indeed, a recent study has proved that 84% of multidrug-resistant *A. baumannii* tested isolates were able to form a biofilm^[44], which contributes to the widespread of this pathogen throughout the globe.^[41] It has also documented that antibiotic-resistant strains were strong biofilm formers.^[41] However, in order to protect themselves from antibiotics, antibiotic-sensitive strains may have the abilities to form biofilm.^[45] Consequently, infectivity and the development of chronic infections are linked to biofilm formation capacity.^[46] More importantly, biofilms may be responsible for the endemic occurrence and subsequent outbreaks of *A. baumannii* in hospitals.^[30,38,47]

EXTRACELLULAR POLYMER MATRIX

The composition of the biofilm matrix varies according to the growth conditions, the bacterial specie and the stage of biofilm development.^[48] EPM usually represents more than 90% of the dry mass of a cell and is mainly composed of biopolymers produced by the bacteria themselves known as extracellular polymeric substances.^[49] More than 99% of the microorganisms on planet live embedded in these biopolymer matrices.^[50] These biopolymers make up only 10% of the matrix and the rest is water.^[51] In general, the biofilm EPM contains EPS, proteins and eDNA. This is also the case for *A. baumannii*, its EPM consists of EPS, eDNA and extracellular proteins including amyloidogenic ones.^[52] This composition will be detailed in the following. One of the factors that has contributed to the success of this bacterium as one of the most troublesome nosocomial pathogens is its ability to produce a matrix of biopolymers that envelops the biofilm.^[53] Previous studies have revealed that the matrix composition (eDNA, EPS and proteins) play a key role in the formation and stability of *A. baumannii* biofilm.^[54–57] It acts as a glue to keep the bacterial cells together^[58] and to facilitate the survival of the cells in their own niches.^[59] EPM molecules use a variety of interactions for cohesion, including electrostatic, ionic and van der Waals. These interactions, together with molecular composition, lead to many important characteristics, including porosity, electrical charge and three-dimensional structure (3D).^[51] The matrix not only fulfils a structural role, but also their components are assembled into supramolecular structures that help to protect the microbes against harsh environments.^[51] EPM has been found to sequester cations, metal ions and toxins and to offer protection against different

environmental stresses such as desiccation, UV radiation, pH change and osmotic shock.^[60] Moreover, the biofilm matrix is implicated in the overall architecture and resistance phenotype of biofilms.^[61,62] It directly protects the biofilm and limits the penetration of antibiotics and other harmful substances^[63], by acting as a protective barrier against attack by blocking, pushing back or capturing the molecules and preventing them from reaching the bacteria.^[51] In particular, it is important to know that two factors are required to inactivate microbial cells with antibiotics; the penetration of the antibiotics and the cells in growth phase (most antibiotics are active against the growth phase of bacteria).^[60] First, EPM acts as a diffusion barrier for these molecules, for example; penetration of ciprofloxacin under normal conditions took 40 seconds while penetration into biofilm containing surfaces took 21 minutes.^[64] Secondly, the bacteria in the biofilm will remain in a dormant phase which will protect the bacterial community from antibiotics.^[60] Furthermore, the EPM contains a set of enzymes that can inactivate or degrade antimicrobials.^[65] Concerning *A. baumannii*, it has been shown that the thick and tenacious matrix of the biofilm makes this pathogen more resistant to antibiotics than its planktonic counterparts.^[66]

In addition, EPM is involved in migration, gene exchange, signaling and ion capture.^[67] On the other hand, the matrix comprises the contents of the lysed bacterial cells, which serve as a source of nutrients under starvation conditions and act as a gene storage for horizontal gene transfer.^[51] Due to the enzymes that can be part of the matrix components, the matrix is considered as a digestive system that can break down biopolymers for recycling or use as a carbon and energy source.^[51] The matrix also contains water channels that provide an efficient means of exchanging nutrients and metabolites by improving the availability of nutrients and the removal of potentially toxic metabolites.^[60] Gene transfer rates in biofilm are reported to be 1000 times higher than those found in the planktonic state.^[68]

Briefly, EPM serves to assemble the biofilm as follows^[65]: (1) Biopolymers formed at the site of adhesion form an initial polymeric matrix that favors microbial aggregation and colonization. (2) Continuation of biopolymers production in situ further enhances the three-dimensional matrix and the core formation of bacterial cells embedded in the EMP. (3) This core offers a supportive scaffold, enabling the development of clusters and microcolonies.

Exopolysaccharides

The term exopolysaccharide (EPS) usually refers to all forms of polysaccharides existing outside the bacterial cell wall.^[69] EPS, as components of the EPM, play a role in primary

biofilm scaffolding, initial attachment and adhesion to surfaces and other cells, and protection of bacteria from host defenses and antimicrobials.^[70-72] EPS, especially high molecular weight EPS, can serve as a basis to which other carbohydrates, proteins and nucleic acids adhere to form the backbone of the biofilm.^[73,74] Additionally, EPS protect bacterial cells from extreme conditions, including temperature, light intensity, pH or osmotic stress.^[75] They also protect bacteria from desiccation, predation, antibodies and bacteriophages.^[76] Due to the hydrated nature of the exopolysaccharides, they can prevent lethal desiccation, protect against moisture variations, contribute to mechanical stability and ensure a longer survival of bacterial cells.^[30,74]

In *A. baumannii* biofilms, the protective matrix consists of extracellular biopolymers, of which the main component is exopolysaccharide (52-86%).^[77] Scanning electron microscopy and Transmission electron microscopy analysis of clinical strains of *A. baumannii*, attached to glass coverslips under dry conditions, showed that the biofilm formed by these strains is surrounded by an exopolysaccharide matrix.^[78] Selective digestion of extracellular proteins (with Proteinase K) or eDNA (with DNase) from EPM did not change the ability of EPM to protect *A. baumannii* biofilms from tobramycin activity.^[53] This finding suggests that EPS are probably responsible for this protective action.^[53] Moreover, it has been shown that a multidrug resistant strain of this bacterium produces more EPS than sensitive strain.^[79]

Poly-(1-6)-N-acetylglucosamine (PNAG) is the well documented exopolysaccharide in *A. baumannii* pathogen.^[53] PNAG is also produced by several pathogens such as *Escherichia coli*^[80], *Staphylococcus aureus*^[81], *Staphylococcus epidermidis*^[82], and it is implicated in their pathogenicity. The synthesis of this polysaccharide was well conserved among clinical isolates and it plays a crucial function in *A. baumannii* biofilm formation.^[57] In other hand, Choi et al (2009) founded that *A. baumannii* contained a *pgaABCD* locus that encodes PNAG synthesis proteins.^[57] PNAG synthesis is initialized by two inner membrane proteins, PgaC and PgaD, which employ a Uridine Diphosphate N-acetylglucosamine to form an N-acetylated precursor.^[83] Subsequently, the PgaA and PgaB proteins interact for the export of polymerized and partially deacetylated PNAG across the cell envelope.^[84] Choi et al. (2009) also showed that deletion of this locus resulted in the loss of the strong biofilm phenotype, which was restored by complementation of this locus.^[57] Furthermore, scanning electronic microscopy studies combined with COMSTAT analysis demonstrated that the biovolume, the thickness of biofilms formed by wild-type *A. baumannii* strains were significantly greater

than those formed by the *pgaABCD* mutant strains.^[57] In addition to its role in biofilm formation, PNAG protects bacteria from host immunity^[85] and is an important virulence factor.^[86] The maintenance of the integrity of *A. baumannii* biofilms in more dynamic environments with higher shear forces is also guaranteed by PNAG.^[57]

Extracellular proteins

Recently, it has been shown that proteins also play an important role in the EPM. In some cases, they can be the dominant components of the EPM.^[87] Biofilm-associated protein (Bap) is a surface protein but it is also a part of the extracellular matrix of *A. baumannii* biofilm and plays an important role in the latter.^[88] Bap was first discovered in *S. aureus* strains.^[89] Thereafter, a larger number of Bap homologs were identified in several other bacteria, including *A. baumannii*.^[90] Over a 10-year period at the Royal Brisbane and Women's Hospital, *bap* was detected in 22 of 24 (92%) clinical *A. baumannii* isolates tested.^[91] Similarly, Loehfelm *et al.* (2008) also revealed that Bap seems to be commonly found in clinical isolates of *A. baumannii*.^[90]

Besides, Bap belongs to a group of surface proteins that have structural and functional characteristics essential for biofilm formation, namely: high molecular weight, core domain of tandem repeats and low isoelectric pH.^[92] Moreover, Bap is encoded by a 25,863 bp gene and is composed of 8620 amino acids, making it one of the largest bacterial proteins ever described.^[90] This protein contributes to bacterial stability under different environmental conditions^[93], and to intercellular adhesion within the mature biofilm and plays an important role in bacterial infection processes.^[94] It is associated with the formation and maturation of the *A. baumannii* biofilm and its maintaining on biotic and abiotic surfaces.^[88] In the same context, another study revealed a correlation between the *bap* gene and biofilm formation by *A. baumannii*.^[41] Brossard and Campagnari (2012) demonstrated that Bap is needed to form a mature *A. baumannii* biofilm on medical devices surfaces, such as polypropylene, polystyrene and titanium.^[56] They also showed, by scanning electron microscopy, that this protein is crucial for the development of the three-dimensional structure and the formation of water channels within *A. baumannii* EPM.^[56] Furthermore, a mutated *bap* gene in *A. baumannii* 307-0294 caused a decrease in adhesion to human bronchial epithelial cells and neonatal keratinocytes and a decrease in biofilm growth.^[56,90] On the other hand, Bap is responsible for increasing the hydrophobicity of the cell surface thus promoting the formation of biofilm.^[56] It has also been suggested that the main interaction targets of this protein are

EPS, which are important for maintaining and strengthening the biofilm structure.^[96] Therefore, it can be deduced that *A. baumannii* Bap plays a key role in the persistence of this pathogen in hospital environments, which explains its induction of nosocomial infections.^[97] For further support, endemic isolates are more susceptible than sporadic ones to carry virulence factors such as Bap, which enhance their survival in health care settings.^[98]

Recently, it has been suggested that Bap is able to form amyloid-like structures.^[99] These amyloids are proteins with a beta-cross structure and polymerize into insoluble fibers. They initially attracted attention because of their application in neurodegenerative diseases, until Larsen et al. (2007) showed that they are abundant in bacterial biofilms.^[100] Amyloid fibers are resistant to degradation by proteases and contribute to the structural integrity of biofilms.^[101,102] Another study demonstrated that overexpression of amyloid in EPM resulted in a 20-fold rise in the rigidity of the *Pseudomonas fluorescens* biofilm.^[103] Bap auto-assembles into functional amyloid fibers, in responding to environmental conditions, to build the biofilm matrix, acting as a scaffold to increase biofilm stability.^[99]

Extracellular DNA

With the succession of EPM investigations, its composition and its structure is becoming increasingly complex and difficult to clarify. Extracellular DNA (eDNA) has been defined as a component of the EPM of several species.^[104–106] This is the case of *A. baumannii* in which eDNA appears as a key component of the EPM.^[107] The origin of this eDNA is not entirely clear; it seems to have been generated from random chromosomal DNA that serves as a cell-to-cell interconnecting element in the biofilm.^[108] In addition, it is believed that autolysis of bacterial cells undergoing in biofilm microcolonies may contribute to eDNA generation.^[109] On the other hand, direct secretion, lysis of a subpopulation by a prophage and liberation of small membrane vesicles are also regarded as potential sources.^[110] It has been suggested that eDNA is mainly situated in high concentrations in the stems of mushroom-shaped microcolonies.^[108]

Like other matrix components, eDNA also serves multiple functions in biofilm formation, such as its contribution to cation gradients, the release of genomic DNA, antibiotic resistance^[111], and it acts as a source of nutrients for bacteria during starvation.^[112] Additionally, the interactions inside EPM with antibiotics have been shown to be through the intervention of eDNA.^[111] Liao et al. (2014) founded that the addition of exogenous eDNA increased glucan (EPS) synthesis by *Streptococcus mutans*.^[113] Moreover, it aided in

strengthen the EPM by interacting with the EPS^[114], and both together are associated with the viscoelasticity of the mature biofilm to make it difficult to detach even under high mechanical pressure or shear stress.^[115] The eDNA acted as a nucleator of amyloids polymerization.^[116]

For *A. baumannii*, eDNA may also be a means of transferring antibiotic resistance genes.^[117] The eDNA constitutes a dynamic gene pool from which bacteria can acquire genetic information by horizontal gene transfer, as transformation requires the availability of DNA in the environment.^[118,119] Consequently, it contributes to the development and spread of antibiotic resistance by horizontal gene transfer.^[120]

Interestingly, eDNA is a carbon source that can influence biofilm dispersion^[121], and a cation chelator that can generate a cation deficient environment promoting antimicrobial resistance.^[122] Using PicoGreen as a dye for a short time, Tang et al. (2013) demonstrated a transient or gradual build-up of eDNA in EPM, suggesting its important dynamic role in the latter.^[123] Surprisingly, the amount of eDNA that accumulates in biofilms does not necessarily reflect its importance because, even at a concentration below the detection limit, it exerted its adhesive effect strongly affecting biofilm initiation.^[123] Adhesion to hydrophobic surfaces and cell aggregation have been promoted by the presence of eDNA using physicochemical interactions.^[116,124,125] In the other hand, eDNA decreased the biofilm susceptibility to a variety of antiseptic and disinfectant agents.^[126,127]

ANTI- EXTRACELLULAR POLYMERIC MATRIX STRATEGIES

Clinically, biofilms are a major problem because of their responsibility for many persistent and chronic infections. This is due to the resistance to antimicrobial agents and the phenotypic variation provided by this phenomenon. Once the biofilm becomes mature, its eradication becomes extremely difficult with traditional antimicrobial agents.^[128] As a result, it is necessary to shift the bacteria from a matrix embedded mode of life to a free, individual and vulnerable mode to eradicate it. After highlighting the roles played by EPM, it will be a beneficial target to design strategies for controlling biofilm formation. In this section we have attempted to summarize some assays performed to counteract *A. baumannii* biofilm formation by targeting different biopolymers of the EPM (Table 1).

Table 1: Anti-*Acinetobacter baumannii* extracellular polymeric matrix strategies.

Anti-EPM strategy	Target	Action mechanism	Reference
5-episinuleptolide (<i>Sinularia leptoclados</i>)	PNAG	Decreasing expression of <i>pgaABCD</i> locus	[129]
<i>Actinidia deliciosa</i> extract	EPM	Reducing the contents of EPM	[52]
Dispersin B	PNAG	Cleaving the -(1-6) linkage of glucosamine and depolymerizing PNAG	[57]
Maltodextrin, Sucrose and Polyethylene glycol	EPS	Dehydration of EPS	[37]
L-Adrenaline	Bap	Binding to monomers and oligomers of Bap	[88]
Phytol and cefotaxime	Bap PNAG	Decreasing the genes expression of <i>bap</i> , <i>pgaA</i> and <i>pgaC</i>	[130]
Zerumbone (<i>Zingiber zerumbet</i> (L.) Smith)	Bap	Reducing <i>bap</i> gene expression	[131]
Antibodies	Bap	Affinity-purified Bap antibodies	[132]
Melittin	Bap	Decreasing <i>bap</i> messenger RNA level	[133]
DNase	EDNA	Cleaving stranded DNA	[55]
Glutathione	EDNA EPS	Increasing acidity	[134]

Targeting Exopolysaccharides

Development of novel solutions that inhibit *A. baumannii* biofilm formation led to the research towards PNAG by being one of its EPM pillars. In this context, Tseng et al. (2016) identified 5-episinuleptolide, isolated from *Sinularia leptoclados*, as an inhibitor of *A. baumannii* biofilm formation by decreasing gene expression at the *pgaABCD* locus encoding PNAG.^[129] Furthermore, the extracellular matrix of the biofilm was significantly reduced by 5-episinuleptolide treatment^[129] On the other hand, this study showed a potentially synergistic activity of the combination therapy between 5-episinuleptolide and Levofloxacin.^[129] *Actinidia deliciosa* extract revealed its anti-biofilm effect by acting against EPM of *A. baumannii*.^[52] It was shown to reduce the contents of EPM in EPS, proteins and eDNA.^[52]

The dispersion enzymatic mechanism has attracted research attention because of its efficacy against biofilm. This mechanism is used by several bacterial species to activate the dispersion of its own biofilm to colonize another location. The bacteria secrete enzymes such as glycosidases, proteases and DNases that degrade various components of the EPM.^[135] Taking as examples alginate lyase, which is produced by *Pseudomonas aeruginosa*, DNase thermonuclease, which is produced by *Staphylococcus aureus*, glycoside hydrolase Dispersin B, produced by *Aggregatibacter actinomycetemcomitans*.^[135] Several of these EPM-degrading enzymes have been studied as potential therapeutic agents. Dispersin B has been

shown to be a hexosaminidase in several bacterial species of medical interest, which consequently inhibits biofilm formation.^[136] Dispersin B confirmed that *A. baumannii* synthesizes PNAG and specifically cleaves the -(1-6) linkage of glucosamine and depolymerizes PNAG.^[57] The combination of Dispersin B with an antimicrobial could be more effective because this enzyme does not have antibacterial activity. Therefore, Gawande et al. (2014) tested the combination of this enzyme with an antimicrobial (Acticoat) to develop a spray to treat chronic wounds.^[137] The results showed that this combination gave significantly better results compared to Acticoat alone.^[137] Alginate lyase was considered to be active against alginate (*Pseudomonas aeruginosa* EPS).^[138] Digestion of this EPS increased the sensitivity of the biofilm formed by this germ to antibiotic.^[138] Moreover, it has been shown that Azithromycin blocks alginate production by *Pseudomonas aeruginosa*.^[139]

Through EPS, EPM is characterized by a hydrated nature that protects the cells inside the biofilm.^[30,74] This hydration of EPS is also explored as an anti-EPM therapy. Falghoush et al. (2017) evaluated the effect of different osmotic compounds namely maltodextrin, sucrose and polyethylene glycol to improve the efficacy of antibiotics against *A. baumannii* biofilms.^[37] They found that the combination of antibiotics with hypertonic concentrations of osmotic compounds for 24 hours reduced the number of *A. baumannii* cells in the biofilm by 5 to 7 log.^[37]

Interestingly, antibodies have been reported as a possible solution against biofilms. Antibodies to the partially de-N-acetylated form of PNAG facilitated the biofilm accessibility and destruction of *Staphylococcus aureus* by human neutrophils.^[140] For *A. baumannii*, Bentancor et al. (2012) demonstrated that PNAG is a target for antibody development in two chronic infections: pneumonia and bacteremia.^[141]

Targeting Biofilm-associated protein

As mentioned before, Bap is the major biofilm matrix protein of *A. baumannii*. Therefore, it is useful to find an anti-Bap solution to inhibit *A. baumannii* biofilm formation. L-Adrenaline has been suggested as a Bap inhibitor of *A. baumannii*, it binds to monomers as well as oligomers with good affinity.^[88] This molecule is a possible discovery of an anti-Bap, anti-Bap amyloid formation and consequently, anti *A. baumannii* biofilm agent.^[88] In another work, transcriptomic analysis revealed a decrease in the expression of different virulence genes in two strains of *A. baumannii* including the *bap* and *pgaA*, *pgaC* genes when treated with a combination of phytol and cefotaxime.^[130] This caused an alteration of EPM of *A.*

baumannii biofilms due to a variation in the amount of PNAG and Bap.^[130] Similarly, Zerumbone, a natural compound derived from the plant *Zingiber zerumbet* (L.) Smith, reduced *bap* gene expression in *A. baumannii*.^[131] *A. baumannii* Biofilm formation was also inhibited by affinity-purified Bap antibodies, indicating the direct participation of Bap in the development of biofilms of this pathogen.^[132] In another study, Bardbari et al. (2018) tested the effect of melittin, a cationic, amphipathic and alpha-helical peptide, against the biofilm of multi-resistant strong biofilm producing strains of *A. baumannii*.^[133] The results indicated a significant decrease in *bap* messenger RNA levels in all isolates.^[133] Furthermore, the synergy between melittin and colistin inhibited biofilm formation entirely, suggesting the possibility of using this combination to treat *A. baumannii*.^[133] The use of proteases to degrade the protein component of the biofilm matrix has been shown to be useful. It is known that endogenous proteases play a role in biofilm dispersion.^[142], it has also been demonstrated that exogenously added proteases can exhibit dispersal activity against established biofilms.^[142]

Surprisingly, anti-amyloid drugs used to target human pathological amyloids, such as in Alzheimer's and Parkinson's diseases, have been transformed into antibiofilm agents due to the recently discovered participation of bacterial amyloids in the EPM.^[143]

Targeting Extracellular DNA

Extracellular DNA, as an important component in the EPM, has been exploited as a possible therapeutic tool to interfere with the formation of biofilm. Nucleases have been examined against a number of bacteria. DNase acts against eDNA by cleaving single and double-stranded DNA preferably at the phosphodiester bonds adjacent to pyrimidine nucleotides.^[144] The presence of DNase reduced the biomass, resulting in a reduction in the number and size of microcolonies as well as a decrease in antibiotic tolerance.^[145] This decrease in antibiotic resistance makes this DNase approach very interesting.^[145] DNase has previously been reported to be effective against eDNA and conceivably *A. baumannii* biofilms.^[55]

Whitchurch et al. (2002) found that the timing of DNase addition influenced its effect against biofilm.^[106] In another way, addition of this enzyme at early stages inhibited biofilm formation which supports the fact that eDNA is crucial for biofilm development. However, its addition to more mature biofilm had no significant effect due to protective interactions within the matrix.^[106] The cause of this temporary sensitivity is probably the presence of other components of the EPM that can replace the eDNA or the creation of interactions

between the other components and eDNA to protect it from degradation.^[146] As a result, a combined approach of several enzymes target the different components of the matrix at the same time may be useful.^[146] In another hypothesis, mature biofilms may produce proteolytic exoenzymes to deactivate DNase, thus making it active only in the early stages.^[120]

Glutathione was tested for its activity in disrupting biofilm formed by multidrug-resistant *A. baumannii* strains by improving the efficacy of antibiotics.^[134] Firstly, biofilm viability showed a decrease of more than 50% with 30 mM of glutathione, and then, confocal imaging showed considerable changes in the biofilm architecture of the isolates.^[134] The action mechanism may be due to an increase in acidity which triggers the cleavage of matrix components; eDNA and polysaccharides. Moreover, glutathione improved the efficacy of amikacin, and its combination with amikacin and DNase showed the greatest reduction in *A. baumannii* biofilm viability.^[134] Furthermore, the combined action of a quorum sensing (bacterial communication) inhibiting enzyme Aii20J and DNase reduced biofilm formation by *A. baumannii*.^[107]

CONCLUSION

After attempts to kill cells in biofilms have failed, attention has turned to disrupting the biofilm matrix to facilitate the penetration of antimicrobial agents or to return cells to their planktonic vulnerable state. Targeting the matrix composition seems to be useful. However, variability in matrix composition and structure depending on strain, metabolic activity, nutrient availability, environmental conditions, and stage of biofilm growth may pose significant obstacles to the development of anti-EPM therapies. In addition, the physicochemical complexity of the biofilm microenvironment demines the efficacy of this approach. On the other hand, the clinical application of these anti-EPM therapeutic strategies must be accompanied by antibiotics to eradicate the bacterial cells, because most of these techniques have no bactericidal action. Consequently, this field requires a thorough evaluation before any application.^[147]

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

REFERENCES

1. Howard A, O'donoghue M, Feeney A, Sleator RD. *Acinetobacter baumannii* An emerging opportunistic pathogen. *Virulence*, 2012; 3(3): 243–50.

2. Boucher HW, Talbot GH, Bradley JS, Edwards JE, Gilbert D, Rice LB, et al. Bad bugs, no drugs: No ESKAPE! An update from the Infectious Diseases Society of America. *Clin Infect Dis*, 2009; 48(1): 1–12.
3. Center for Disease Control. Antibiotic threats in the United States in 2013 [Internet]. *Morbidity and Mortality Weekly Report*. 2013.
4. Rello J, Kalwaje Eshwara V, Lagunes L, Alves J, Wunderink RG, Conway-Morris A, et al. A global priority list of the TOP TEn resistant Microorganisms (TOTEM) study at intensive care: a prioritization exercise based on multi-criteria decision analysis. *Eur J Clin Microbiol Infect Dis*, 2019; 38(2): 319–23.
5. Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelson M, Monnet DL, et al. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *Lancet Infect Dis*, 2018; 18(3): 318–27.
6. Towner KJ. *Acinetobacter*: an old friend, but a new enemy [Internet]. Vol. 73, *Journal of Hospital Infection*. *J Hosp Infect*; 2009. p. 355–63.
7. Dijkshoorn L, Nemec A, Seifert H. Identification and antibiotic resistance of nosocomial bacteria isolated from the hospital environment of two intensive care units. Vol. 5, *Nature Reviews Microbiology*. 2007. p. 939–51.
8. Fagon JY, Chastre J, Hance AJ, Montravers P, Novara A, Gibert C. Nosocomial pneumonia in ventilated patients: A cohort study evaluating attributable mortality and hospital stay. *Am J Med*, 1993; 94(3): 281–8.
9. Falagas ME, Bliziotis IA, Siempos II. Attributable mortality of *Acinetobacter baumannii* infections in critically ill patients: A systematic review of matched cohort and case-control studies. *Crit Care*, 2006; 10(2).
10. Lee HW, Koh YM, Kim J, Lee JC, Lee YC, Seol SY, et al. Capacity of multidrug-resistant clinical isolates of *Acinetobacter baumannii* to form biofilm and adhere to epithelial cell surfaces. *Clin Microbiol Infect*, 2008; 14(1): 49–54.
11. Tomaras AP, Dorsey CW, Edelmann RE, Actis LA. Attachment to and biofilm formation on abiotic surfaces by *Acinetobacter baumannii*: involvement of a novel chaperone-usher pili assembly system. *Microbiology*, 2003; 149(12): 3473–84.
12. Chiu CH, Lee HY, Tseng LY, Chen CL, Chia JH, Su LH, et al. Mechanisms of resistance to ciprofloxacin, ampicillin/sulbactam and imipenem in *Acinetobacter baumannii* clinical isolates in Taiwan. *Int J Antimicrob Agents*, 2010; 35(4): 382–6.
13. Poirel L, Nordmann P. Carbapenem resistance in *Acinetobacter baumannii*: Mechanisms and epidemiology [Internet]. Vol. 12, *Clinical Microbiology and Infection*. Blackwell

- Publishing Ltd; 2006. p. 826–36.
14. Nemeč A, Dolzani L, Brisse S, Van Den Broek P, Dijkshoorn L. Diversity of aminoglycoside-resistance genes and their association with class 1 integrons among strains of pan-European *Acinetobacter baumannii* clones. *J Med Microbiol*, 2004; 53(12): 1233–40.
 15. Huys G, Cnockaert M, Vaneechoutte M, Woodford N, Nemeč A, Dijkshoorn L, et al. Distribution of tetracycline resistance genes in genotypically related and unrelated multidrug-resistant *Acinetobacter baumannii* strains from different European hospitals. *Res Microbiol*, 2005; 156(3): 348–55.
 16. Hujer KM, Hujer AM, Endimiani A, Thomson JM, Adams MD, Goglin K, et al. Rapid determination of quinolone resistance in *Acinetobacter* spp. *J Clin Microbiol*, 2009; 47(5): 1436–42.
 17. Hernan RC, Karina B, Gabriela G, Marcela N, Carlos V, Angela F. Selection of colistin-resistant *Acinetobacter baumannii* isolates in postneurosurgical meningitis in an intensive care unit with high presence of heteroresistance to colistin. *Diagn Microbiol Infect Dis*, 2009; 65(2): 188–91.
 18. Park YK, Peck KR, Cheong HS, Chung DR, Song JH, Ko KS. Extreme drug resistance in *Acinetobacter baumannii* infections in intensive care units, South Korea [Internet]. Vol. 15, *Emerging Infectious Diseases*. Centers for Disease Control and Prevention; 2009. p. 1325–7.
 19. Navon-Venezia S, Leavitt A, Carmeli Y. High tigecycline resistance in multidrug-resistant *Acinetobacter baumannii*. *J Antimicrob Chemother*, 2007; 59(4): 772–4.
 20. Jawad A, Seifert H, Snelling AM, Heritage J, Hawkey PM. Survival of *Acinetobacter baumannii* on dry surfaces: Comparison of outbreak and sporadic isolates. *J Clin Microbiol*, 1998; 36(7): 1938–41.
 21. Wendt C, Dietze B, Dietz E, Rüdén H. Survival of *Acinetobacter baumannii* on dry surfaces. *J Clin Microbiol*, 1997; 35(6): 1394–7.
 22. Rodríguez-Baño J, Martí S, Soto S, Fernández-Cuenca F, Cisneros JM, Pachón J, et al. Biofilm formation in *Acinetobacter baumannii*: Associated features and clinical implications. *Clin Microbiol Infect*, 2008; 14(3): 276–8.
 23. Jawad A, Seifert H, Snelling AM, Heritage J, Hawkey PM. Survival of *Acinetobacter baumannii* on dry surfaces: Comparison of outbreak and sporadic isolates. *J Clin Microbiol*, 1998; 36(7): 1938–41.
 24. Gaddy JA, Actis LA. Regulation of *Acinetobacter baumannii* biofilm formation. *Future*

- Microbiol, 2009; 4(3): 273–8.
25. Roy R, Tiwari M, Donelli G, Tiwari V. Strategies for combating bacterial biofilms: A focus on anti-biofilm agents and their mechanisms of action. Vol. 9, Virulence. Taylor and Francis Inc.; 2018. p. 522–54.
 26. Costerton JW, Lewandowski Z, Caldwell DE, Korber DR, Lappin-Scott HM. Microbial biofilms [Internet]. Vol. 49, Annual Review of Microbiology. Annual Reviews Inc.; 1995. p. 711–45.
 27. Flemming HC, Wingender J. The biofilm matrix. Vol. 8, Nature Reviews Microbiology. 2010. p. 623–33.
 28. Ceri H, Olson ME, Stremick C, Read RR, Morck D, Buret A. The Calgary Biofilm Device: New technology for rapid determination of antibiotic susceptibilities of bacterial biofilms. *J Clin Microbiol*, 1999; 37(6): 1771–6.
 29. Costerton JW, Geesey GG, Cheng KJ. How bacteria stick. *Sci Am*, 1978; 238(1): 86–95.
 30. Donlan RM. Biofilms: Microbial life on surfaces [Internet]. Vol. 8, Emerging Infectious Diseases. Centers for Disease Control and Prevention (CDC); 2002. p. 881–90.
 31. Quave CL, Estévez-Carmona M, Compadre CM, Hobby G, Hendrickson H, Beenken KE, et al. Ellagic acid derivatives from *Rubus ulmifolius* inhibit *Staphylococcus aureus* biofilm formation and improve response to antibiotics. *PLoS One*, 2012; 7(1): e28737.
 32. Tomaras AP, Dorsey CW, Edelmann RE, Actis LA. Attachment to and biofilm formation on abiotic surfaces by *Acinetobacter baumannii*: Involvement of a novel chaperone-usher pili assembly system. *Microbiology*, 2003; 149(12): 3473–84.
 33. Wei Q, Ma LZ. Biofilm matrix and its regulation in *Pseudomonas aeruginosa* [Internet]. Vol. 14, International Journal of Molecular Sciences. *Int J Mol Sci*; 2013. p. 20983–1005.
 34. Wang Y, Lee SM, Dykes G. The physicochemical process of bacterial attachment to abiotic surfaces: Challenges for mechanistic studies, predictability and the development of control strategies [Internet]. Vol. 41, Critical Reviews in Microbiology. Taylor and Francis Ltd; 2015. p. 452–64.
 35. Toole GO, Kaplan HB, Kolter R. Biofilm formation as microbial development. *Annu Rev Microbiol*, 2000; 49–79.
 36. Elkheloui R, Laktib A, Mimouni R, Aitalla A, Hassi M, Elboulani A, et al. *Acinetobacter baumannii* biofilm: intervening factors, persistence, drug resistance, and strategies of treatment. *Mediterr J Infect Microbes Antimicrob*, 2020; 9(July).
 37. Falghoush A, Beyenal H, Besser TE, Omsland A, Call DR. Osmotic compounds enhance antibiotic efficacy against *Acinetobacter baumannii* biofilm communities. *Appl Environ*

- Microbiol, 2017; 83(19): 1–12.
38. Rodríguez-Baño J, Martí S, Soto S, Fernández-Cuenca F, Cisneros JM, Pachón J, et al. Biofilm formation in *Acinetobacter baumannii*: Associated features and clinical implications. *Clin Microbiol Infect*, 2008; 14(3): 276–8.
 39. Marti S, Chabane YN, Alexandre S, Coquet L, Vila J, Jouenne T, et al. Growth of *acinetobacter baumannii* in pellicle enhanced the expression of potential virulence factors. *PLoS One*, 2011; 6(10): e26030.
 40. Rumbo-Feal S, Pérez A, Ramelot TA, Álvarez-Fraga L, Vallejo JA, Beceiro A, et al. Contribution of the *A. baumannii* A1S_0114 Gene to the Interaction with Eukaryotic Cells and Virulence. *Front Cell Infect Microbiol*, 2017; 7(APR): 108.
 41. Yang CH, Su PW, Moi SH, Chuang LY. Biofilm formation in *Acinetobacter baumannii*: Genotype-phenotype correlation. *Molecules*, 2019; 24(10): 1849.
 42. Zeighami H, Valadkhani F, Shapouri R, Samadi E, Haghi F. Virulence characteristics of multidrug resistant biofilm forming *Acinetobacter baumannii* isolated from intensive care unit patients. *BMC Infect Dis*, 2019; 19(1): 1–9.
 43. Yakkala H, Samantarrai D, Gribskov M, Siddavattam D. Comparative genome analysis reveals nichespecific genome expansion in *Acinetobacter baumannii* strains. *PLoS One*, 2019; 14(6): e0218204.
 44. Saadati M, Rahbarnia L, Farajnia S, Naghili B, Mohammadzadeh R. The prevalence of biofilm encoding genes in multidrug-resistant *Acinetobacter baumannii* isolates. *Gene Reports*, 2021; 23(February): 101094.
 45. Shenkutie AM, Yao MZ, Siu GKH, Wong BKC, Leung PHM. Biofilm-induced antibiotic resistance in clinical *acinetobacter baumannii* isolates. *Antibiotics*, 2020; 9(11): 1–15.
 46. Eze EC, Chenia HY, El Zowalaty ME. *Acinetobacter baumannii* biofilms: Effects of physicochemical factors, virulence, antibiotic resistance determinants, gene regulation, and future antimicrobial treatments [Internet]. Vol. 11, *Infection and Drug Resistance*. Dove Medical Press Ltd.; 2018. p. 2277–99.
 47. Hall-Stoodley L, Stoodley P. Evolving concepts in biofilm infections [Internet]. Vol. 11, *Cellular Microbiology*. *Cell Microbiol*; 2009. p. 1034–43.
 48. Flemming HC, Neu TR, Wozniak DJ. The EPS matrix: The “House of Biofilm Cells” [Internet]. Vol. 189, *Journal of Bacteriology*. *J Bacteriol*; 2007. p. 7945–7.
 49. Blackledge MS, Worthington RJ, Melander C. Biologically inspired strategies for combating bacterial biofilms. *Curr Opin Pharmacol*, 2013; 13(5): 699–706.
 50. Vu B, Chen M, Crawford RJ, Ivanova EP. Bacterial extracellular polysaccharides

- involved in biofilm formation. *Molecules*, 2009; 14(7): 2535–54.
51. Flemming HC, Wingender J. The biofilm matrix [Internet]. Vol. 8, *Nature Reviews Microbiology*. Nat Rev Microbiol; 2010. p. 623–33.
 52. Tiwari V, Tiwari D, Patel V, Tiwari M. Effect of secondary metabolite of *Actinidia deliciosa* on the biofilm and extra-cellular matrix components of *Acinetobacter baumannii*. *Microb Pathog*, 2017; 110: 345–51.
 53. Davenport EK, Call DR, Beyenal H. Differential protection from tobramycin by extracellular polymeric substances from *Acinetobacter baumannii* and *Staphylococcus aureus* biofilms. *Antimicrob Agents Chemother*, 2014; 58(8): 4755–61.
 54. Gawande P V., Leung KP, Madhyastha S. Antibiofilm and antimicrobial efficacy of Dispersinb®-KSL-w peptide-based wound gel against chronic wound infection associated bacteria. *Curr Microbiol*, 2014; 68(5): 635–41.
 55. Sahu PK, Iyer PS, Oak AM, Pardesi KR, Chopade BA. Characterization of eDNA from the clinical strain *acinetobacter baumannii* AIIMS 7 and its role in biofilm formation. *Sci World J*, 2012; 2012.
 56. Brossard KA, Campagnari AA. The *Acinetobacter baumannii* biofilm-associated protein plays a role in adherence to human epithelial cells. *Infect Immun*, 2012; 80(1): 228–33.
 57. Choi AHK, Slamti L, Avci FY, Pier GB, Maira-Litrán T. The *pgaABCD* locus of *Acinetobacter baumannii* encodes the production of poly- β -1-6-N-acetylglucosamine, which is critical for biofilm formation. *J Bacteriol*, 2009; 191(19): 5953–63.
 58. Wei Q, Ma LZ. Biofilm matrix and its regulation in *Pseudomonas aeruginosa* [Internet]. Vol. 14, *International Journal of Molecular Sciences*. Int J Mol Sci; 2013. p. 20983–1005.
 59. Cugini C, Shanmugam M, Landge N, Ramasubbu N. The Role of Exopolysaccharides in Oral Biofilms. *J Dent Res*, 2019; 98(7): 739–45.
 60. Kokare CR, Chakraborty S, Khopade AN, Mahadik KR. Biofilm: Importance and applications. Vol. 8, *Indian Journal of Biotechnology*. 2009. p. 159–68.
 61. Branda SS, Vik Å, Friedman L, Kolter R. Biofilms: The matrix revisited [Internet]. Vol. 13, *Trends in Microbiology*. Trends Microbiol; 2005. p. 20–6.
 62. Sutherland IW. The biofilm matrix - An immobilized but dynamic microbial environment [Internet]. Vol. 9, *Trends in Microbiology*. Trends Microbiol; 2001. p. 222–7.
 63. Sutherland IW. Biofilm exopolysaccharides: A strong and sticky framework [Internet]. Vol. 147, *Microbiology*. Microbiology (Reading); 2001. p. 3–9.
 64. ME D, GA O. Microbial biofilms: from ecology to molecular genetics. *Microbiol Mol Biol Rev*, 2000; 64(4): 847–67.

65. Karygianni L, Ren Z, Koo H, Thurnheer T. Biofilm Matrixome: Extracellular Components in Structured Microbial Communities. *Trends Microbiol*, 2020; 28(8): 668–81.
66. Jefferson KK. What drives bacteria to produce a biofilm? *FEMS Microbiol Lett*, 2006; 236(2): 163–73.
67. Dragoš A, Kovács ÁT. The Peculiar Functions of the Bacterial Extracellular Matrix [Internet]. Vol. 25, Trends in Microbiology. *Trends Microbiol*; 2017. p. 257–66.
68. Hausner M, Wuertz S. High rates of conjugation in bacterial biofilms as determined by quantitative in situ analysis. *Appl Environ Microbiol*, 1999; 65(8): 3710–3.
69. Caggianiello G, Kleerebezem M, Spano G. Exopolysaccharides produced by lactic acid bacteria: from health-promoting benefits to stress tolerance mechanisms. *Appl Microbiol Biotechnol*, 2016; 100(9): 3877–86.
70. KM C, VD G, K M, BR B, DJ W, GC W, et al. The pel polysaccharide can serve a structural and protective role in the biofilm matrix of *Pseudomonas aeruginosa*. *PLoS Pathog*, 2011; 7(1).
71. KM C, Y I, CS T, R U, JC W, C R, et al. The Pel and Psl polysaccharides provide *Pseudomonas aeruginosa* structural redundancy within the biofilm matrix. *Environ Microbiol*, 2012; 14(8): 1913–28.
72. Billings N, Millan MR, Caldara M, Rusconi R, Tarasova Y, Stocker R, et al. The Extracellular Matrix Component Psl Provides Fast-Acting Antibiotic Defense in *Pseudomonas aeruginosa* Biofilms. *PLOS Pathog*, 2013; 9(8): e1003526.
73. Matsukawa M, Greenberg EP. Putative exopolysaccharide synthesis genes influence *Pseudomonas aeruginosa* biofilm development. *J Bacteriol*, 2004; 186(14): 4449–56.
74. Sutherland I. Biofilm exopolysaccharides: a strong and sticky framework. *Microbiology*, 2001; 147(Pt 1): 3–9.
75. Donot F, Fontana A, Baccou JC, Schorr-Galindo S. Microbial exopolysaccharides: Main examples of synthesis, excretion, genetics and extraction. Vol. 87, *Carbohydrate Polymers*. Elsevier; 2012. p. 951–62.
76. Vu B, Chen M, Crawford RJ, Ivanova EP. Bacterial extracellular polysaccharides involved in biofilm formation [Internet]. Vol. 14, *Molecules*. *Molecules*; 2009. p. 2535–54.
77. Marti S, Chabane YN, Alexandre S, Coquet L, Vila J, Jouenne T, et al. Growth of *acinetobacter baumannii* in pellicle enhanced the expression of potential virulence factors. *PLoS One*, 2011; 6(10).

78. Espinal P, Martí S, Vila J. Effect of biofilm formation on the survival of *Acinetobacter baumannii* on dry surfaces. *J Hosp Infect*, 2012; 80(1): 56–60.
79. Chopra S, Ramkissoon K, Anderson DC. A systematic quantitative proteomic examination of multidrug resistance in *Acinetobacter baumannii*. *J Proteomics*, 2013; 84: 17–39.
80. Wang X, Preston JF 3rd, Romeo T. The *pgaABCD* locus of *Escherichia coli* promotes the synthesis of a polysaccharide adhesin required for biofilm formation. *J Bacteriol*, 2004; 186(9): 2724–34.
81. O’Gara JP. *ica* and beyond: biofilm mechanisms and regulation in *Staphylococcus epidermidis* and *Staphylococcus aureus*. *FEMS Microbiol Lett*, 2007; 270(2): 179–88.
82. Gerke C, Kraft A, Süßmuth R, Schweitzer O, Götz F. Characterization of the N-acetylglucosaminyltransferase activity involved in the biosynthesis of the *Staphylococcus epidermidis* polysaccharide intercellular adhesin. *J Biol Chem*, 1998; 273(29): 18586–93.
83. Whitney JC, Howell PL. Synthase-dependent exopolysaccharide secretion in Gram-negative bacteria. *Trends Microbiol*, 2013; 21(2): 63–72.
84. Wang Y, Andole Pannuri A, Ni D, Zhou H, Cao X, Lu X, et al. Structural Basis for Translocation of a Biofilm-supporting Exopolysaccharide across the Bacterial Outer Membrane. *J Biol Chem*, 2016; 291(19): 10046–57.
85. Johnson GM, Lee DA, Regelman WE, Gray ED, Peters G, Quie PG. Interference with granulocyte function by *Staphylococcus epidermidis* slime. *Infect Immun*, 1986; 54(1): 13–20.
86. Kropec A, Maira-Litran T, Jefferson KK, Grout M, Cramton SE, Götz F, et al. Poly-N-acetylglucosamine production in *Staphylococcus aureus* is essential for virulence in murine models of systemic infection. *Infect Immun*, 2005; 73(10): 6868–76.
87. Schlafer S, Meyer RL. Confocal microscopy imaging of the biofilm matrix. *J Microbiol Methods*, 2017; 138: 50–9.
88. Tiwari V, Patel V, Tiwari M. In-silico screening and experimental validation reveal L-Adrenaline as anti-biofilm molecule against biofilm-associated protein (Bap) producing *Acinetobacter baumannii*. *Int J Biol Macromol*, 2018; 107(PartA): 1242–52.
89. Cucarella C, Tormo MÁ, Úbeda C, Trotonda MP, Monzón M, Peris C, et al. Role of Biofilm-Associated Protein Bap in the Pathogenesis of Bovine *Staphylococcus aureus*. *Infect Immun*, 2004; 72(4): 2177–85.
90. Loehfelm TW, Luke NR, Campagnari AA. Identification and characterization of an *Acinetobacter baumannii* biofilm-associated protein. *J Bacteriol*, 2008; 190(3): 1036–44.

91. Sharon Goh HM, Beatson SA, Totsika M, Moriel DG, Phan MD, Szubert J, et al. Molecular analysis of the *Acinetobacter baumannii* biofilm-associated protein. *Appl Environ Microbiol*, 2013; 79(21): 6535–43.
92. Latasa C, Solano C, Penadés JR, Lasa I. Biofilm-associated proteins. Vol. 329, *Comptes Rendus - Biologies*. No longer published by Elsevier; 2006. p. 849–57.
93. Rahbar MR, Rasooli I, Gargari SLM, Sandstrom G, Amani J, Fattahian Y, et al. A potential in silico antibody-antigen based diagnostic test for precise identification of *Acinetobacter baumannii*. *J Theor Biol*, 2012; 294: 29–39.
94. Noori E, Rasooli I, Owlia P, Mousavi Gargari SL, Ebrahimizadeh W. A conserved region from biofilm associated protein as a biomarker for detection of *Acinetobacter baumannii*. *Microb Pathog*, 2014; 77: 84–8.
95. Brossard KA, Campagnari AA. The *Acinetobacter baumannii* biofilm-associated protein plays a role in adherence to human epithelial cells. *Infect Immun*, 2012; 80(1): 228–33.
96. Rahbar MR, Rasooli I, Mousavi Gargari SL, Amani J, Fattahian Y. In silico analysis of antibody triggering biofilm associated protein in *Acinetobacter baumannii*. *J Theor Biol*, 2010; 266(2): 275–90.
97. Sánchez-Encinales V, Álvarez-Marín R, Pachón-Ibáñez ME, Fernández-Cuenca F, Pascual A, Garnacho-Montero J, et al. Overproduction of Outer membrane protein a by *Acinetobacter baumannii* as a risk factor for nosocomial pneumonia, bacteremia, and mortality rate increase. *J Infect Dis*, 2017; 215(6): 966–74.
98. Luo TL, Rickard AH, Srinivasan U, Kaye KS, Foxman B. Association of blaOXA-23 and bap with the persistence of *Acinetobacter baumannii* within a major healthcare system. *Front Microbiol*, 2015; 6(MAR): 1–11.
99. Taglialegna A, Navarro S, Ventura S, Garnett JA, Matthews S, Penades JR, et al. Staphylococcal Bap Proteins Build Amyloid Scaffold Biofilm Matrices in Response to Environmental Signals. *PLoS Pathog*, 2016; 12(6): e1005711.
100. Larsen P, Nielsen JL, Dueholm MS, Wetzel R, Otzen D, Nielsen PH. Amyloid adhesins are abundant in natural biofilms. *Environ Microbiol*, 2007; 9(12): 3077–90.
101. Romero D, Aguilar C, Losick R, Kolter R. Amyloid fibers provide structural integrity to *Bacillus subtilis* biofilms. *Proc Natl Acad Sci U S A*, 2010; 107(5): 2230–4.
102. Schwartz K, Syed AK, Stephenson RE, Rickard AH, Boles BR. Functional amyloids composed of phenol soluble modulins stabilize *Staphylococcus aureus* biofilms. *PLoS Pathog*, 2012; 8(6): e1002744.
103. Zeng G, Vad BS, Dueholm MS, Christiansen G, Nilsson M, Tolker-Nielsen T, et al.

- Functional bacterial amyloid increases *Pseudomonas* biofilm hydrophobicity and stiffness. *Front Microbiol*, 2015; 6(OCT): 1099.
104. Harmsen M, Lappann M, Knöchel S, Molin S. Role of extracellular DNA during biofilm formation by *Listeria monocytogenes*. *Appl Environ Microbiol*, 2010; 76(7): 2271–9.
105. Moscoso M, García E, López R. Biofilm formation by *Streptococcus pneumoniae*: Role of choline, extracellular DNA, and capsular polysaccharide in microbial accretion. *J Bacteriol*, 2006; 188(22): 7785–95.
106. Whitchurch CB, Tolker-Nielsen T, Ragas PC, Mattick JS. Extracellular DNA required for bacterial biofilm formation. *Science* (80-), 2002; 295(5559): 1487.
107. Mayer C, Muras A, Parga A, Romero M, Rumbo-Feal S, Poza M, et al. Quorum Sensing as a Target for Controlling Surface Associated Motility and Biofilm Formation in *Acinetobacter baumannii* ATCC® 17978TM. *Front Microbiol*, 2020; 11(September): 1–13.
108. Allesen-Holm M, Barken KB, Yang L, Klausen M, Webb JS, Kjelleberg S, et al. A characterization of DNA release in *Pseudomonas aeruginosa* cultures and biofilms. *Mol Microbiol*, 2006; 59(4): 1114–28.
109. Webb JS, Thompson LS, James S, Charlton T, Tolker-Nielsen T, Koch B, et al. Cell death in *Pseudomonas aeruginosa* biofilm development. *J Bacteriol*, 2003; 185(15): 4585–92.
110. Spoering AL, Gilmore MS. Quorum sensing and DNA release in bacterial biofilms [Internet]. Vol. 9, *Current Opinion in Microbiology*. *Curr Opin Microbiol*; 2006. p. 133–7.
111. Mulcahy H, Charron-Mazenod L, Lewenza S. Extracellular DNA chelates cations and induces antibiotic resistance in *Pseudomonas aeruginosa* biofilms. *PLoS Pathog*, 2008; 4(11): e1000213.
112. Finkel SE, Kolter R. DNA as a nutrient: Novel role for bacterial competence gene homologs. *J Bacteriol*, 2001; 183(21): 6288–93.
113. Liao S, Klein MI, Heim KP, Fan Y, Bitoun JP, Ahn SJ, et al. *Streptococcus mutans* extracellular DNA is upregulated during growth in biofilms, actively released via membrane vesicles, and influenced by components of the protein secretion machinery. *J Bacteriol*, 2014; 196(13): 2355–66.
114. Klein MI, Hwang G, Santos PHS, Campanella OH, Koo H. *Streptococcus mutans*-derived extracellular matrix in cariogenic oral biofilms [Internet]. Vol. 5, *Frontiers in Cellular and Infection Microbiology*. *Front Cell Infect Microbiol*; 2015.

115. Peterson BW, He Y, Ren Y, Zerdoum A, Libera MR, Sharma PK, et al. Viscoelasticity of biofilms and their recalcitrance to mechanical and chemical challenges [Internet]. Vol. 39, FEMS Microbiology Reviews. Oxford Academic; 2015. p. 234–45.
116. Liu HH, Yang YR, Shen XC, Zhang ZL, Shen P, Xie ZX. Amyloid-DNA Composites of Bacterial Biofilms Stimulate Autoimmunity. *Curr Microbiol*, 2008; 57(2): 139–44.
117. Li L, Xia Z di, Hu Z hui, Zhou Z quan, Li H tao. Expression of Class I integrase gene in *Acinetobacter baumannii* and drug-resistance. *J Cent South Univ Med Sci*, 2008; 33(10): 952–7.
118. Itzek A, Zheng L, Chen Z, Merritt J, Kreth J. Hydrogen peroxide-dependent DNA release and transfer of antibiotic resistance genes in *Streptococcus gordonii*. *J Bacteriol*, 2011; 193(24): 6912–22.
119. Lorenz MG, Wackernagel W. Bacterial gene transfer by natural genetic transformation in the environment. *Microbiol Rev*, 1994; 58(3): 563–602.
120. Montanaro L, Poggi A, Visai L, Ravaioli S, Campoccia D, Speziale P, et al. Extracellular DNA in biofilms [Internet]. Vol. 34, International Journal of Artificial Organs. *Int J Artif Organs*; 2011. p. 824–31.
121. DeFrancesco AS, Masloboeva N, Syed AK, DeLoughery A, Bradshaw N, Li GW, et al. Genome-wide screen for genes involved in eDNA release during biofilm formation by *Staphylococcus aureus*. *Proc Natl Acad Sci U S A*, 2017; 114(29): E5969–78.
122. Okshevsky M, Meyer RL. The role of extracellular DNA in the establishment, maintenance and perpetuation of bacterial biofilms [Internet]. Vol. 41, Critical Reviews in Microbiology. *Crit Rev Microbiol*; 2015. p. 341–52.
123. Tang L, Schramm A, Neu TR, Revsbech NP, Meyer RL. Extracellular DNA in adhesion and biofilm formation of four environmental isolates: A quantitative study. *FEMS Microbiol Ecol*, 2013; 86(3): 394–403.
124. Liu HH, Yang YR, Shen XC, Zhang ZL, Shen P, Xie ZX. Role of Extracellular DNA in Initial Bacterial Adhesion and Surface Aggregation. *Curr Microbiol*, 2008; 57(2): 139–44.
125. Liu HH, Yang YR, Shen XC, Zhang ZL, Shen P, Xie ZX. Role of DNA in bacterial aggregation. *Curr Microbiol*, 2008; 57(2): 139–44.
126. Tetz G V., Artemenko NK, Tetz V V. Effect of DNase and antibiotics on biofilm characteristics. *Antimicrob Agents Chemother*, 2009; 53(3): 1204–9.
127. Mulcahy H, Charron-Mazenod L, Lewenza S. Extracellular DNA chelates cations and induces antibiotic resistance in *Pseudomonas aeruginosa* biofilms. *PLoS Pathog*, 2008;

- 4(11): e1000213.
128. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. *Science* (80-), 1999; 284(5418): 1318–22.
 129. Tseng SP, Hung WC, Huang CY, Lin YS, Chan MY, Lu PL, et al. 5-Episinuleptolide decreases the expression Of the extracellular matrix in early biofilm formation of multi-drug resistant *Acinetobacter baumannii*. *Mar Drugs*, 2016; 14(8).
 130. Ramanathan S, Arunachalam K, Chandran S, Selvaraj R, Shunmugiah KP, Arumugam VR. Biofilm inhibitory efficiency of phytol in combination with cefotaxime against nosocomial pathogen *Acinetobacter baumannii*. *J Appl Microbiol*, 2018; 125(1): 56–71.
 131. Kim HR, Shin DS, Jang HI, Eom Y Bin. Anti-biofilm and anti-virulence effects of zerumbone against *acinetobacter baumannii*. *Microbiol (United Kingdom)*, 2020; 166(8): 717–26.
 132. Sharon Goh HM, Beatson SA, Totsika M, Moriel DG, Phan MD, Szubert J, et al. Molecular analysis of the *Acinetobacter baumannii* biofilm-associated protein. *Appl Environ Microbiol*, 2013; 79(21): 6535–43.
 133. Bardbari AM, Arabestani MR, Karami M, Keramat F, Aghazadeh H, Alikhani MY, et al. Highly synergistic activity of melittin with imipenem and colistin in biofilm inhibition against multidrug-resistant strong biofilm producer strains of *acinetobacter baumannii*. *Eur J Clin Microbiol Infect Dis*, 2018; 37(3): 443–54.
 134. Das T, Paino D, Manoharan A, Farrell J, Whiteley G, Kriel FH, et al. Conditions under which glutathione disrupts the biofilms and improves antibiotic efficacy of both ESKAPE and NON-EKAPe species. *Front Microbiol*, 2019; 10(AUG): 1–16.
 135. Kaplan JB. Biofilm Dispersal: Mechanisms, Clinical Implications, and Potential Therapeutic Uses [Internet]. Vol. 89, *Journal of Dental Research*. SAGE Publications; 2010. p. 205–18.
 136. Izano EA, Amarante MA, Kher WB, Kaplan JB. Differential roles of poly-N-acetylglucosamine surface polysaccharide and extracellular DNA in *Staphylococcus aureus* and *Staphylococcus epidermidis* biofilms. *Appl Environ Microbiol*, 2008; 74(2): 470–6.
 137. Gawande P V., Clinton AP, LoVetri K, Yakandawala N, Rumbaugh KP, Madhyastha S. Antibiofilm Efficacy of DispersinB ® Wound Spray Used in Combination with a Silver Wound Dressing . *Microbiol Insights*, 2014; 7: MBI.S13914.
 138. Alkawash MA, Soothill JS, Schiller NL. Alginate lyase enhances antibiotic killing of mucoid *Pseudomonas aeruginosa* in biofilms. *APMIS*, 2006; 114(2): 131–8.

139. Hoffmann N, Lee B, Hentzer M, Rasmussen TB, Song Z, Johansen HK, et al. Azithromycin blocks quorum sensing and alginate polymer formation and increases the sensitivity to serum and stationary-growth-phase killing of *Pseudomonas aeruginosa* and attenuates chronic *P. aeruginosa* lung infection in *Cftr*^{-/-} mice. *Antimicrob Agents Chemother*, 2007; 51(10): 3677–87.
140. Maira-Litrán T, Kropec A, Goldmann DA, Pier GB. Comparative opsonic and protective activities of *Staphylococcus aureus* conjugate vaccines containing native or deacetylated staphylococcal poly-N-acetyl- β -(1-6)-glucosamine. *Infect Immun*, 2005; 73(10): 6752–62.
141. Bentancor L V., O'malley JM, Bozkurt-Guzel C, Pier GB, Maira-Litrán T. Poly-n-acetyl- β -(1-6)-glucosamine is a target for protective immunity against *acinetobacter baumannii* infections. *Infect Immun*, 2012; 80(2): 651–6.
142. Boles BR, Horswill AR. agr-mediated dispersal of *Staphylococcus aureus* biofilms. *PLoS Pathog*, 2008; 4(4): e1000052.
143. Perov S, Lidor O, Salinas N, Golan N, Tayeb- Fligelman E, Deshmukh M, et al. Structural Insights into Curli CsgA Cross- β Fibril Architecture Inspire Repurposing of Anti-amyloid Compounds as Anti-biofilm Agents. *PLOS Pathog*, 2019; 15(8): 1–31.
144. Chen WJ, Liao TH. Structure and Function of Bovine Pancreatic Deoxyribonuclease I. *Protein Pept Lett*, 2006; 13(5): 447–53.
145. Tetz V V., Tetz G V. Effect of extracellular DNA destruction by DNase I on characteristics of forming biofilms. *DNA Cell Biol*, 2010; 29(8): 399–405.
146. Okshevsky M, Regina VR, Meyer RL. Extracellular DNA as a target for biofilm control. *Curr Opin Biotechnol*, 2015; 33: 73–80.
147. Folliero V, Franci G, Dell'annunziata F, Giugliano R, Foglia F, Sperlongano R, et al. Evaluation of Antibiotic Resistance and Biofilm Production among Clinical Strain Isolated from Medical Devices. *Int J Microbiol*, 2021; 2021.