REVISITING DRUG RESISTANCE MECHANISMS OF A NOTORIOUS NOSOCOMIAL PATHOGEN: *Acinetobacter baumannii*

ABSTRACT

Acinetobacter baumannii (*A. baumannii*) has a propensity to develop, acquire and transmit antibiotic resistance-associated genes. This ability has enabled the proliferation of the species in harsh living conditions like the hospital environment. It is well known that a quasi-permanent contact between the bacterium and antibiotics has contributed to the improvement of expressed resistance mechanisms, but also, literature highlights the natural living conditions in which survival strategies have led the species to develop mechanisms and systems to establish their niche, sometimes in very competitive environment. All these mechanisms and strategies which are expressed, sometimes in response to antibiotics exposure or to just sustain viability, have enabled the rise of this bacteria species as a successful nosocomial pathogen. Here we review drug resistance mechanisms and strategies for environmental survival employed by this bacterium to consolidate information relevant for the current search for alternative management of infections caused by *A. baumannii*.

1. INTRODUCTION

Originally, the genus Acinetobacter was described as *Micrococcus calco-aceticus*.[1] It embeds Gram-negative, aerobic, non-fermenting, non-fastidious, non-motile, catalase-positive, oxidative positive and negative bacteria with a DNA G + C content of 39% to 47%.[2,3] *Acinetobacter baumannii* (*A. baumannii*) is ubiquitous in nature, found in environmental elements such as soil, and in food such as vegetables, meat, and fish. The bacterium shares the common features of Acinetobacter genus thus, it is oxidase negative and morphologically ranges from bacillus to coco-bacillus.[4] *A. baumannii*

forms a complex with three other clinically significant species that are closely related; *A. calcoaceticus, A. nosocomialis* and *A. pittti*.[5,2] These species are difficult to differentiate phenotypically. Therefore, various molecular techniques are applied to differentiate isolates of the *A. baumannii* complex using hierarchical cluster analysis.[6,7,8] *A. baumannii* causes healthcare-associated infections (HCAI) such as hospital-acquired pneumonia, catheter-associated bloodstream infections, catheter-associated urinary tract infections, surgical site infections, antibiotic-associated diarrhoea and puerperal sepsis in immunocompromised individuals, particularly in Intensive Care Units (ICU) and high care wards.[9,10,11]

In healthier humans, *A. baumannii* has been found to be part of the normal skin flora;[2] particularly in moist regions such as the axillae, groin, and toe webs. The carriage rate of *Acinetobacter spp.* in healthy people apart from on the skin, is normally low, but colonization of skin and mucous membrane has been reported at high rate among hospital personnel.[12] The carriage rate of *Acinetobacter spp.* in patients hospitalized in non-intensive care units has also been reportedly high; but this is due to various sources of colonization or infection (hands of the hospital staff, respiratory therapy equipment, food (including hospital food), tap water, fomites, etc…) with multidrug-resistant Acinetobacter species in hospitalized patients.[13] The reservoirs of *A. baumannii* are poorly understood but its ability to survive for long periods on both dry and moist surfaces enable the organism to survive in hospital environments and grow at a range of different temperatures and pH values.[14,15,16] Amongst the several risk factors for colonization or infection with multidrug-resistant *Acinetobacter sp*, some factors such as a prolonged hospital stay, undergoing antimicrobial therapy (by using antibiotics that have little or no activity against Acinetobacter),[17] exposure to an intensive care unit (ICU), recipient of mechanical ventilation, having had recent

surgery, underlying severity of illness and invasive procedures place people at higher risks of getting colonized by, or infected with the bacteria.[16,18,15] To survive in hostile environments such as hospitals, *A. baumannii* has developed multidrug resistance mechanisms to maintain its viability in the permanent presence of antibiotics. This communication reviews commonly reported mechanisms and/or strategies of survival for MDRAB to offer a comprehensive understanding of this pathogen.

2. MULTIDRUG RESISTANT *A. baumannii*

Bacteria are qualified as resistant to an antimicrobial agent when they can survive and multiply in the presence of an antimicrobial agent which loses its ability to inhibit bacterial growth effectively at therapeutic doses.[19] These bacteria can be categorised as 'multidrug resistant (MDR)', 'extreme drug resistant', 'extensive, extensively or extremely drug resistant (XDR)' and 'pandrug-resistant' (PDR).[20] However, no consensus has been reached on the definition or criteria used to classify an organism as MDR, XDR and PDR.[21,22,23] The European Committee on Antimicrobial Susceptibility Testing (EUCAST) classifies bacteria using clinical Minimum Inhibitory Concentration (MIC) breakpoints as interpretive criteria to categorise bacteria (Table 1).[24] Bacteria are categorised into three categories: S - Susceptible, I – Intermediate, R – Resistant or Non-susceptible. A strain is categorised S-susceptible when isolates with a MIC that is below or at the breakpoint are inhibited by achievable concentrations when the standard dosing regimen is used. It is classified I – Intermediate when there is a high likelihood of therapeutic success because exposure to the agent is increased by adjusting the dosing regimen or by its concentration at the site of infection. R - Resistant when there is a high likelihood of therapeutic failure even when there is increased exposure to the regimen.[24] Exposure relies on the mode of administration,

dose, dosing interval, infusion time as well as distribution and excretion of the antimicrobial agent influencing the infecting organism at the site of infection.[24]

A panel of international experts came together through a joint initiative by the European Centre for Disease Prevention and Control (ECDC) and the Centre for Disease Control and Prevention (CDC), to create a standardized international terminology and classification criteria for MDR, XDR and PDR. Their approach combined technical parameters used in Clinical Laboratory Standards Institute (CLSI), EUCAST and the United States Food and Drug Administration (FDA) guidelines; applicable when an isolate demonstrates resistance to multiple regimen.[20] Based on this approach, MDR *A. baumannii* was defined as an isolate that is non-susceptible to at least one agent in three or more antimicrobial categories. XDR *A. baumannii* was defined as an isolate that is non-susceptible to at least one agent in all but two antimicrobial categories. PDR *A. baumannii* was defined as an isolate of *A. baumannii* that is non-susceptible to all agents in all antimicrobial categories.[20,24]

Table 1: EUCAST worksheet for categorizing *Acinetobacter spp* isolates.

Antimicrobial category	Antimicrobial agent	MIC breakpoints (mg/L)		Zone diameter breakpoints (mm)		Disk content (µg)
		S≤	R>	S≥	R <	
Aminoglycosides [20]	[HE]Gentamicin[20]	4[24]	4[24]	17[24]	17[24]	10[24]
	[HE]Tobramycin[20]	4[24]	4[24]	17[24]	17[24]	10[24]
	[HE]Amikacin[20]	8[24]	16[24]	19[24]	17[24]	30[24]
	[HE]Netilmicin[20]	4[24]	4[24]	16[24]	16 [24]	10 [24]
Antipseudomonal Carbapenems[20]	Imipenem[20]	2[24]	4[24]	24[24]	21[24]	10[24]
	Meropenem[20]	2[24]	8[24]	21[24]	15[24]	10[24]
	Doripenem[20]	# [24]	# [24]	# [24]	# [24]	
Antipseudomonal	Ciprofloxacin[20]	0.06[24]	1[24]	50[24]	21[24]	5[24]
	Levofloxacin[20]	0.5[24]	1[24]	23[24]	20[24]	5[24]

fluoroquinolone[20]						
Antipseudomonal penicillins + betalactamase inhibitors[20]	Ticarcillin-clavulanic acid[20]	IE 24	IE 24	IE 24	IE 24	
	Piperacillin-tazobactam[20]	IE 24	IE 24	IE 24	IE 24	
Extended spectrum cephalosporins[20]	Cefotaxime[20]	- 24	- 24	- 24	- 24	
	Ceftriaxone[20]	- 24	- 24	- 24	- 24	
	Ceftazidime[20]	- 24	- 24	- 24	- 24	
	Cefepime[20]	- 24	- 24	- 24	- 24	
Folate pathway inhibitors[20]	Trimethoprim-sulphamethoxazole[20]	2[24]	4[24]	14[24]	11[24]	1.25-23.75[24]
Penicillins + betalactamase inhibitors[20]	Ampicillin-sulbactam[20]	IE 24	IE 24	IE 24	IE 24	
Polymyxins[20]	Colistin[20]	2[24]	2[24]	A 24	A 24	
	Polymyxin B[20]	NT 24	NT 24	NT 24	NT 24	
Tetracyclines[20]	Tetracycline[20]	- 24	- 24	- 24	- 24	
	Doxycycline[20]	- 24	- 24	- 24	- 24	

Minocycline[20]	IE 24	IE 24	IE 24	IE 24

[HE] High exposure for agent.

[#]breakpoints are based on higher dose therapy.

[IE] Insufficient evidence that the organism or group is a good target for therapy with the agent.

[-]No breakpoints. Susceptibility testing is not recommended.

[A] Use an MIC broth microdilution method only.

[NT] Not tested.

3. COMMON ANTIMICROBIAL RESISTANCE MECHANISMS IN MDRAB

A resistance mechanism is a set of biochemical reactions and/or enzymatic interactions that enable a micro-organism to resist and/or escape any threat to its survival.[25] A resistance mechanism can affect different antibiotic classes, while several different resistance mechanisms can synergistically work to resist a single antibiotic class.[26] This technique has increased the number of antibiotic classes that are unable to kill or inhibit the growth of MDRAB.[27] MDRAB has developed and/or acquired several resistance mechanisms that are associated with specific genes.[28] These antimicrobial resistance genes are mainly spread by mobile genetic elements such as plasmids, transposons or integrons.[28] In MDRAB, there are three main mechanisms of resistance: production of antibiotics inactivating enzymes; reduced entry to the target site and alteration of the target site or cellular functions due to mutations.

3.1 Production of antibiotic inactivating enzymes

Drug resistance through enzyme-mediated degradation is the major mechanism employed by MDRAB.[29] Production of beta-lactamases by MDRAB inhibit the action of beta-lactam antibiotics. Beta-lactamase enzymes in Gram-negative organisms are

produced in the periplasmic space.[30] They deactivate the effect of beta-lactam antibiotics by hydrolysis of beta-lactams ring (Figure 1).

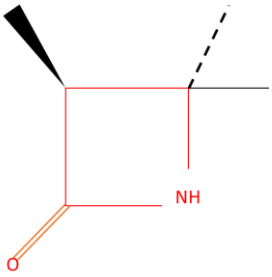

Figure 1: Beta-lactam ring, characteristic of beta-lactam drug

These enzymes have been named according to divers' criteria such as: the strain or plasmid that produced them; peculiarities of sequence; location of the gene on the chromosome; discovery location; patient's names; and the names of the individual(s) who discovered them or named after substrates that are hydrolysed. Consequently, some were designated by more than one name.[31] With the use of advanced molecular techniques such as Whole Genome Sequencing (WGS), more than 2,770 unique beta-lactamases have been identified in wild-type isolates.[32] Therefore, it is important to have reliable and easily understandable nomenclature to refer to these enzymes.

There are two standardized classification schemes for the classification and nomenclature of beta-lactamase enzymes. The first one is based on molecular characteristics and the second one is based on the functional properties.[33,34] The molecular characteristics-based classification relies on amino-acid sequence homology that categorizes beta-lactamase enzymes into four Ambler molecular classes (A, B, C and D).[34] Ambler classes A, C and D hydrolyse beta-lactam substrates through active serine site; while class B beta-lactamases, also known as metallo-beta-lactamases, utilise an active zinc ion to hydrolyse beta-lactams. The classification scheme by functionality also called The Bush–Jacoby system resulted in three major

groups: Group 1 cephalosphorinases (Class C), Group 2 serine beta-lactamases (Class A and Class D), and Group 3 metallo-beta-lactamases (Class B). Each of them is also divided into several different subgroups. The functionality-based classes of the beta-lactamases were determined according to the hydrolysis rates of some pre-defined antibacterial such as EDTA and benzylpenicillin.[35]

3.1.1 Mechanism of action of beta-lactam antibiotics:

To adequately understand the hydrolysis mechanism by which beta-lactamases deactivate beta-lactam drugs, it is important to briefly review the beta-lactam mechanism of action. Beta-lactam antibiotics inhibit a family of related enzymes (four to eight in different bacteria), each involved in different aspects of cell wall synthesis.[36] The cell wall is comprised of alternate N-acetylmuramic acid (NAM) and N-acetylglucosamine (NAG) units, these units are linked by a trans glycosidases, and a pentapeptide is attached to each NAM unit. The penicillin binding proteins (PBPs) act as transpeptidases to catalyse the cross-linking of two D-alanine-D-alanine NAM pentapeptides (Figure 2 and Figure 3). The mosaic is essential to maintaining cell shape, sustains its rigidity and confers osmotic stability in hypertonic environments. Enzymes that mediate autolysis of peptidoglycan are normally present in the bacterial cell wall but are strictly regulated to allow breakdown of the peptidoglycan only at growing points.[37] There is a sterical similarity between the D-alanine-D-alanine of the NAM pentapeptide and beta-lactam structure.[38] As a result, PBPs “mistakenly” use the beta-lactam as a substrate “building block” during cell wall synthesis. This “error” results in acylation of the PBPs that will induce constitutive continuous peptidoglycan autolysis. Consequently, the cell-wall integrity is compromised, and its permeability is increased. This way beta-lactam-mediated inhibition of transpeptidation causes cell lysis.

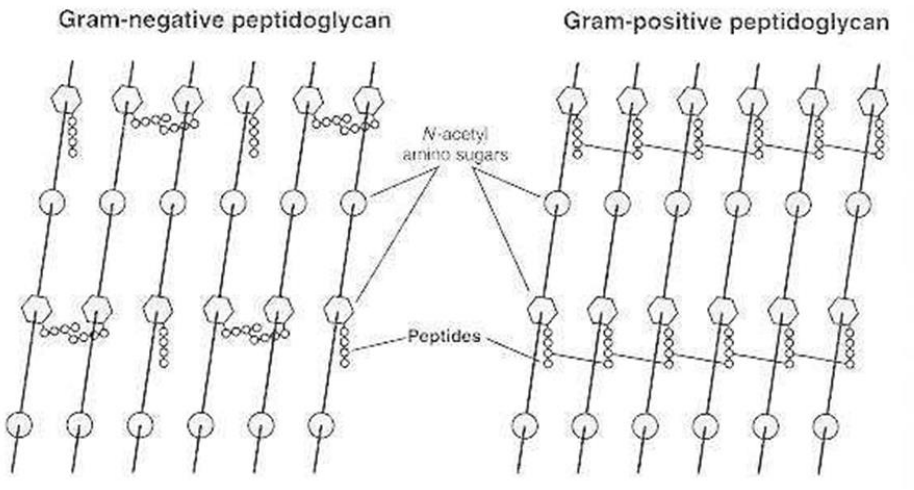

Figure 2: Diagrammatic representation of peptidoglycan structures with adjacent glycan strands cross-linked directly from the carboxyterminal D-alanine to the Ɛ-amino group of an adjacent tetrapeptide or through a peptide cross bridge, N-acetylmuramic acid, N-acetylglucosamine[39]

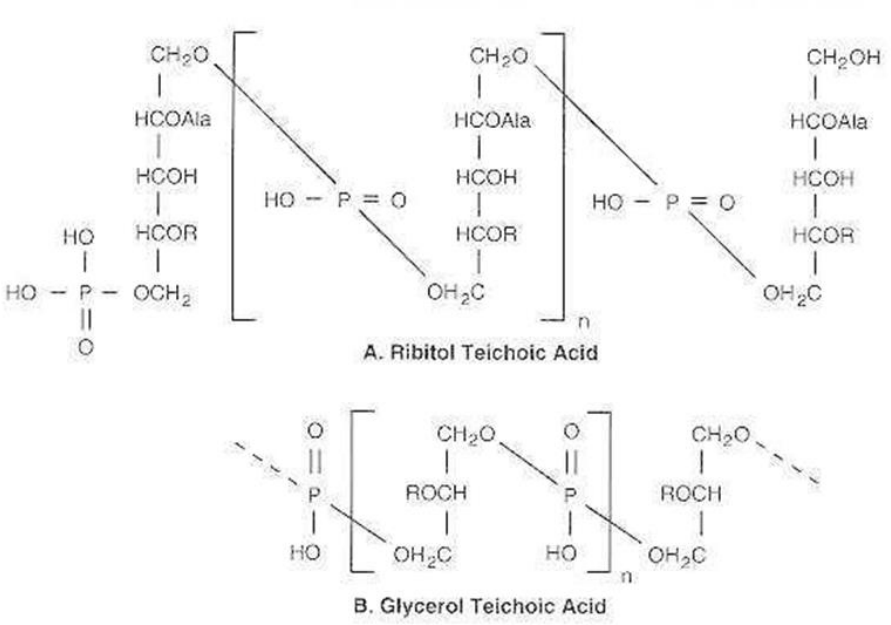

Figure 3: Structures of cell wall teichoic acids. (A) Ribitol teichoic acid with repeating units of 1,5-phosphodiester linkages of D-ribitol and D-alanyl ester on position 2 and glycosyl substituents (R) on position 4. (B) Glycerol teichoic acid with 1,3-phosphodiester linkages of glycerol repeating units (1,2-linkages in some species)[39]

3.1.2 Ambler class A, C and D hydrolysis mechanism:

Classes A, C and D of beta-lactamases are serine-based enzymes. Nucleophilic attack by the active site serine on C – N beta-lactam bond of the beta-lactam antibiotic results in a high-energy acylation intermediate. Next, this intermediate “transitions” into a lower energy covalent acyl enzyme. Following this, a catalytic water molecule attacks the covalent complex and leads to a high-energy deacylation intermediate, with subsequent hydrolysis of the bond between the beta-lactam carbonyl and the serine oxygen. Lastly, deacylation regenerates the active enzyme and renders the beta-lactam inactive (Figure 4).[32] The distinction between these three-serine dependent beta-lactamase classes is in the process by which their active site serine is activated as a nucleophile for acylation and water is activated as a nucleophile for deacylation.

A catalytic residue is required during these two reactions. This residue facilitates the movement of protons during catalysis. Each class uses different catalytic residue(s): class A uses a glutamate-lysine pair; class C uses lysine-tyrosine pair;[40] class D uses a carbamate anion.[41]

Class A beta-lactamases are inhibited by clavulanate; they hydrolyze penicillin and cephalosporins more efficiently than carbapenems, except for some *Klebsiella pneumoniae* carbapenemase (KPC) type enzymes.[42] A wide range of beta-lactamases such as Temoneira (TEM), sulfhydryl variable (SHV), cefotaxime hydrolyzing capabilities (CTX-M), Guiana extended-spectrum (GES), self-transferable plasmid from *E. coli* (SCO), *Pseudomonas* extended resistant (PER), Vietnam extended-spectrum beta-lactamase (VEB), carbenicillin hydrolyzing beta-lactamase (CARB) and KPC have been identified in *A. baumannii* (Table 2).[27] Among the latter enzymes TEM-1, CARB-4 and SCO-1 are narrow-spectrum beta-lactamases; while PER-1, TEM-92, CARB-10, SHV-5, PER-2, CTX-M-2, CTX-M-15, VEB-1, GES-14, and PER-7 are responsible for the hydrolysis of extended-spectrum beta-lactams (ESBL). They were regarded as playing a minor role in its resistance phenotype, especially in carbapenem resistance.[27]

Class C beta-lactamases confer resistance to Cephamycin (Cefoxitin and Cefotetan), Penicillin, Cephalosporins and combinations of beta-lactamase inhibitors. Thus, an insignificant inhibition during clinical application of combination of beta-lactam inhibitor such as clavulanic acid was noted.[42] In the year 2000 in Spain, for the first time the chromosomal cephalosporinase gene which encodes an AmpC beta-lactamase, was described in *A. baumannii*.[43] Since then, several isolates of *A. baumannii* have shown

similar AmpC sequences.[44] Phylogenetic analysis demonstrates that Acinetobacter *ampC* genes are genetically related and are different from *ampC* gene found in other bacterial species.[45] Typically, class C chromosomal beta-lactamase AmpC in *A. baumannii* are the substrate profile of cephalosporinases.[45] A high percentage of drug-resistant *A. baumannii* isolates possess bla_{ampc}.[46] The presence and overexpression of *Ampc* gene results in high level resistance to ceftazidime.[47] A strong promoter containing IS*Aba1*-like sequence has been associated with this mechanism.[44]

Class D beta-lactamases are known as oxacillinase or OXA enzymes. The name originated from the first described OXA enzymes which had a high affinity for isoxazolylpenicillin oxacillin as compared to benzylpenicillin during hydrolysis reactions of the beta-lactam ring.[48] Currently the OXA beta-lactamase class has the highest clonal expansion.[49] Over 400 variants are currently recognized and novel variants are still being described.[50] These genes have easily been disseminated throughout the world[51,52,53]. Despite its heterogeneous composition, this class can be organized based on amino acid identity. The genes associated with this class of enzymes are found in chromosomes and plasmids of various bacterial species[54] and sometimes in integrons.[55] Metallobeta-lactamases (MBL) and Carbapenem-hydrolysing class D beta-lactamases (CHDL) are the two main groups of carbapenemases in *A. baumannii*.[3] Nine subgroups of OXA carbapenemases classified according to their amino acid homologies have been described.[54] In 2004, a subgroup with carbapenemase activity link to upstream presence of ISAba1 in the region of the promoter emerged. Further studies have shown that this subgroup in which belongs beta-lactamases Oxa-51/69 is chromosomal; intrinsic to *A. baumannii*.[56,54] The OXA 51 group contains novel variant oxacillinases cluster that have been reported in several

studies.[57,58] The OXA-40/OXA-24 CHDL group is made up of OXA-25, OXA-26, OXA-40, and OXA-72. These enzymes only differ by a few amino acid substitutions.[54] In several groups of OXA enzymes, expansion of spectrum activity is due to the substitution of only a single amino acid.[59] When for the first time in Spain in 2000 OXA-40/OXA-24 was identified, it was described as chromosomally encoded in a carbapenem-resistant *A. baumannii* isolate.[60] Later, OXA-25, OXA-26 and OXA-27 were characterized with association to carbapenem resistant *A. baumannii*.[61] OXA-58, a plasmid borne enzyme was first described in France; it is associated with multidrug resistance.[62] Thereafter it has been reported globally.[63,64,65] Two variants of OXA-58 due to a point mutation in amino acid have been described; OXA-97 in Tunisia[66] and OXA-96 in Singapore.[67] OXA-23, OXA-40/24, OXA-51 and OXA-58 clusters are the most prevalent Class D beta-lactamases.[54]

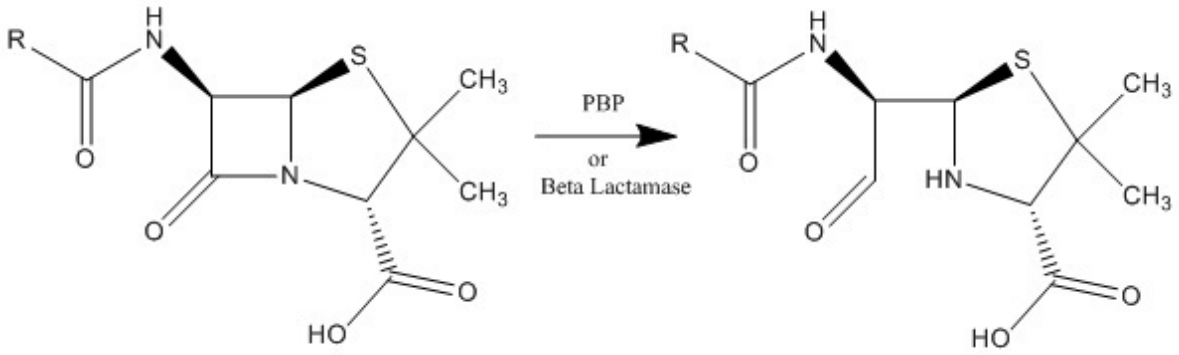

Figure 4: Hydrolysis of beta-lactam ring

3.1.3 Amber class B hydrolysis mechanism:

Class B metallo-beta-lactamase (MBL), like serine beta-lactamases, catalyses the overall reaction by breaking the amide bond. The distinctive trait of this class is the hydrolysis reaction which is based on the interaction of the beta-lactams with zinc ions

in the active site of the enzyme (Figure 5).[68] Due to zinc ion dependence, catalysis is inhibited in the presence of metal-chelating agents like EDTA. The hydrolysis mechanism of beta-lactamases is not effective on monobactams. They are not susceptible to hydrolytic attack.[42] Although MBLs are not the predominant carbapenemases in *A. baumannii*, there is a dramatic increase in the detection and spread of the acquired or transferable families of these metalloenzymes. Globally, MBLs IMP, VIM, and NDM are widely distributed.[3,68] MBL *bla* genes are located on the chromosome, plasmid, and integrons[32] which may explain their rapid spread globally.

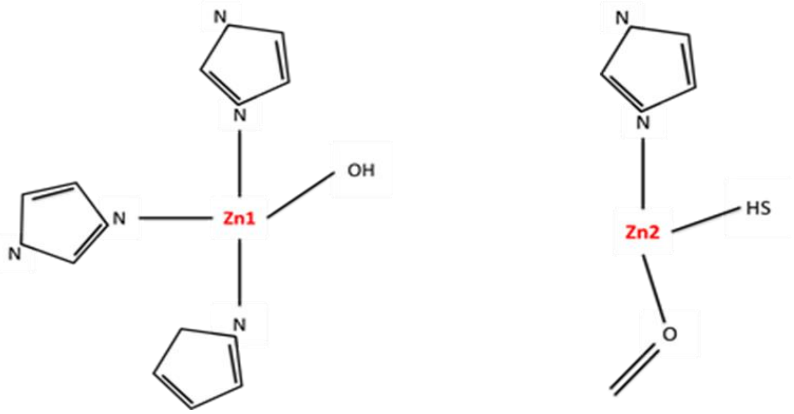

Figure 5: Metallo-beta-lactamases active site

3.2 Reduced entry into the antibiotic target site:

The presence of porin channels and other outer membrane proteins help in the delivery of the drugs to target sites in the cytoplasm. Unfortunately, the porin channels in *A. baumannii* are smaller and lesser, thus preventing the entry of some drug molecules, hence conferring resistance.[69] The use of porins combined with production of beta-lactamases work together to confer resistance. Along with these factors, efflux pumps also contribute to reduced entry into the target site for antibiotics by pumping the drugs

out of the bacteria. Point mutations occurring in the genes coding for the target proteins, namely the enzymes or the porin channels, decrease the affinity or up-regulating cellular functions involved in the production of efflux pumps. Change in affinity for binding was documented in the case of colistin resistance.[70]

3.2.1 Permeability defect:

The permeability of bacterial outer membrane can play an active role in drug resistance. Porins are protein-based channels that are located within the outer membrane. They can form channels to allow transport of molecules across lipid bilayer membranes. By regulating porins, bacteria can limit actions of antibacterial drugs; especially those that have intracellularly located target sites.[71,72] Reduced or loss of expression of porins such as CarO, Omp22-33, Omp33-36, Omp37, Omp43, Omp44, and Omp47 is associated with resistance to carbapenems in *A. baumannii* (Table 2).[73,74] Loss of Omp29 is associated with imipenem resistance in *A. baumannii*.[75] Resistance to aztreonam, chloramphenicol, and nalidixic acid by *A. baumannii* is associated with OmpA loss or decrease expression (Table 2).[76] Other mechanisms through which porins can induce resistance have been reported. Different porins can physically interact to raise antibiotic resistance. A study by Wu et al.,[77] showed that physical interaction of OmpA and CarO with OXA-23 carbapenemase induce antibiotic resistance in *A. baumannii* strain AB5075 (Table 2).[77] In the presence of OXA carbapenemases including OXA-51 or OXA-23, the loss of the 29-kDa outer-membrane protein will result in imipenem resistance *A. baumannii*.[75] A study from Australia demonstrated that loss of Lipopolysaccharide (LPS) from the outer membrane resulted in colistin-resistance in a clinical isolate of *A. baumannii* (Table 2).[78]

3.2.2 Overexpression of efflux pump:

Known to be one of the virulence factors of *A. baumannii*, efflux pumps are also associated with multidrug resistance.[79,80] The importance of efflux pumps in multidrug resistant *A. baumannii* was demonstrated by reversing the resistance pattern of the bacteria when using an efflux pump inhibitor.[81] A study demonstrated that overexpression of AdeABC efflux pumps is a prevalent mechanism for decreased susceptibility to tigecycline.[82] The resistance-nodulation-division (RND) superfamily, the major facilitator superfamily (MFS), the multidrug and toxic compound extrusion (MATE) family and the small multidrug resistance (SMR) family transporters are the 4 categories of efflux pumps reported and associated with multidrug resistance in *A. baumannii* (Figure 6).[83] RND and MFS are the most prevalent. RND-type efflux pump is associated with aminoglycoside resistance and decreasing susceptibility to several antimicrobials, including tigecycline (Table 2).[84,85] In *A. baumannii*, up-regulation and overexpression of *adeABC* genes affect bacteria antimicrobial patterns by increasing its resistance to tigecycline and non-fluoroquinolone antibiotics.[86,87] In wild-type *A. baumannii*, the AdeRS two-component system tightly controls AdeABC, but a critical amino acid substitution or insertion of the *ISAba1* sequence in the *adeS* gene leads to an overexpression of the AdeABC pump.[88] The actions of cell density and BaeSR two-component system during envelope stress response to external stimuli interfere in regulation of *adeA* gene transcription, which affects *A. baumannii* tigecycline susceptibility pattern.[89,90] AdeFGH and AdeIJK efflux pumps belonging to resistance-nodulation-division efflux pump superfamily, contribute concomitantly to enhance *A. baumannii* resistance to tigecycline. Regulation of *AdeFGH* gene expression is due to a gene named *adeL* located upstream on the *adeFGH* operon. Mutation in *adeL* gene induces an overexpression of AdeFGH which enhances *A. baumannii* resistance to

tigecycline.[91] The *adeN* gene is located 813 kbp upstream from *adeIJK*, which encodes a TetR transcriptional regulator. The overexpression of *adeN* gene represses AdeIJK expression, resulting in enhancing *A. baumannii* resistance to tigecycline (Table 2).[92] It has been reported that *adeE* and *adeB* coexist in some *A. baumannii* isolates. The RND efflux pump AdeDE was initially identified in *Acinetobacter* genomic group 3.[93,94] A study demonstrated a link between low dose of antimicrobial therapy and the overexpression of AdeFGH efflux pump in *A. baumannii*.[95] This study highlighted the responsibility of low dose anti-biotherapy in the emergence of resistance mechanisms from *A. baumannii*. The major facilitator superfamily (MFS) including CmlA and CraA,[96] Tet(A) and Tet(B) are involved in tetracycline, minocycline and chloramphenicol resistance;[97] and AbeM are involved in resistance to imipenem and fluoroquinolones.[98] Novel efflux pumps such as AmvA and AbeS belonging to MFS category enable resistance of *A. baumannii* to different classes of antibiotics and disinfectants, detergents, and dyes.[99,100] Other efflux pumps such as A1S_1535, A1S_2795, and ABAYE_0913 which confer resistance to gentamicin, kanamycin, chloroxylenol, oxytetracycline, 1,10-phenanthroline, and chloramphenicol[101] have been described. Efflux pumps are topics of interest for several scientists, who focuses on new efflux pump or efflux pump reported in other bacteria but newly described in *A. baumannii.* Case of EmrAB-TolC efflux pump conferring resistance to netilmicin, tobramycin, and imipenem[102] have now been described in *A. baumannii*.

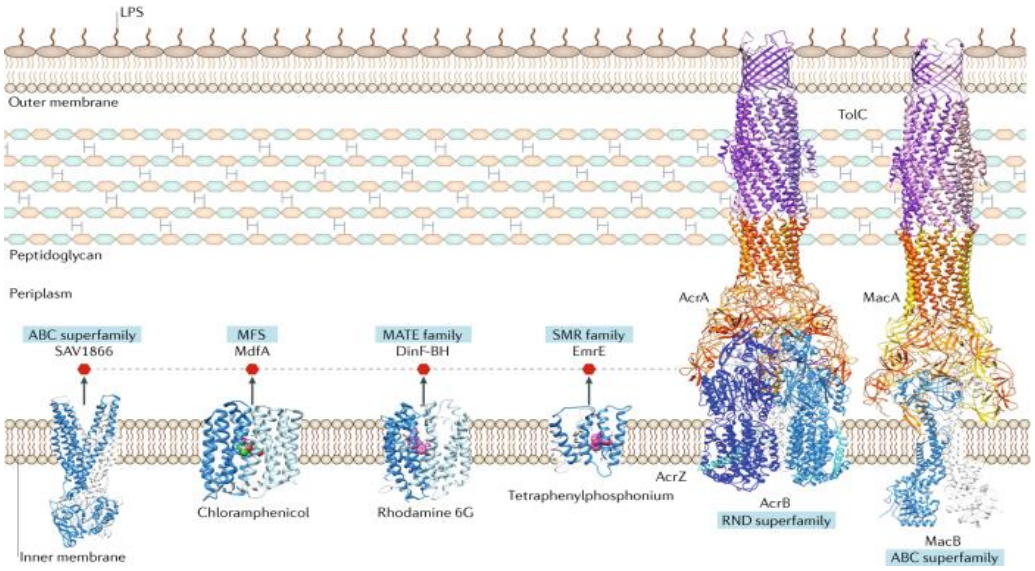

Figure 6: Multidrug efflux pump, structure function and regulation[103]

3.3 Alteration of the target or cellular functions due to mutations:

Alteration and/or modification in antibiotic target sites can induce antibiotic resistance. Mutation in a subunit of DNA gyrase named *gyrA;* and *parC,* a subunit of topoisomerase IV, induces a change in the membrane binding, resulting in a lower affinity for the binding of quinolones to the enzyme-DNA complex. Ultimately this confers resistance against quinolones (Table 2).[69,70,87] In *A. baumannii,* an overexpression of altered penicillin binding proteins called penicillin-binding proteins 2 with a low affinity for imipenem, induces imipenem resistance.[104] *A. baumannii* resistance to tetracycline is associated with mutation in *tetA* and *tetB* expressed through efflux pumps.[105,80] The presence of a *tetM* gene isolated from *A. baumannii* and which showed 100% homology with *S. aureus tetM* had been pointed out as another resistance mechanism of *A. baumannii* to tetracycline.[106] Nosocomial MDRAB isolate have been reported to have plasmids containing *folA* genes and integrons harbouring *dfr* or *dhfr* genes reported to induce resistance to Trimethoprim through dihydrofolate reductase.[105,107] Coexistence of the 16S rRNA methylase *armA* gene and

genes encoding OXA type carbapenemases have been reported in many countries, where studies have highlighted contributions of the *armA* gene to multidrug resistance in *A. baumannii*.[108,109,110] Table 2 indicates the common resistance mechanisms and associated genes.

Table 2: Common resistance mechanism and genes associated with antimicrobial resistance

Resistance mechanisms	Class/Family	Genes associated	Antibiotics affected
Production of antibiotics inactivating enzymes (Beta-lactamases)	Class A	*bla*$_{GES}$; *bla*$_{PER}$; *bla*$_{SHV}$; *bla*$_{KPC}$; *bla*$_{TEM}$	Penicillins and Cephalosporins more efficiently than Carbapenems
	Class B	*bla*$_{VIM}$; *bla*$_{IMP}$; *bla*$_{SIM}$; *bla*$_{NDM}$	Carbapenems but not monobactams
	Class C	*AmpC*	Cephamycins (Cefoxitin; Cefotetan); penicillins, cephalosporins

	Class D (OXA classes)	*bla*$_{OXA-51}$; *bla*$_{OXA-23}$; *bla*$_{OXA-58}$; etc..	Carbapenem; penicillins; cephalosporins
Reduced entry into the target site of bacteria	Permeability defect	*CarO*; *OmpA*; *OMP*; Lipopolysaccharide (LPS)	Imipenem; aztreonam; chloramphenicol; nalidixic acid; colistin
	Efflux pumps (Class RND)	*adeABC*; *adeFGH*; *adeIJK*	Tigecycline; aminoglycosides;
Alteration of the target or cellular functions due to mutations	Change of PBP	*pbp2*	Penicillins
	DNA gyrase	*gyrA/parC*	Quinolones
	16S rRNA methylation	*armA*	Carbapenems

TEM: Temoneira; SHV: Sulfhydryl variable; CTX-M: Cefotaxime hydrolysing capabilities; GES: Guiana extended-spectrum; PER: *Pseudomonas* extended resistant; VEB: Vietnam extended-spectrum beta-lactamase; KPC: *K. pneumoniae* carbapenemase; VIM: Verona integron-encoded metallobeta-lactamase; IMP: Imipenemase; SIM: Seoul imipenemase; NDM: New Deli metallobeta-lactamase; AmpC: Ampicillin class C beta-lactamase; CHDL: Carbapenem-hydrolysing class D beta-lactamase; OXA: Oxacillinase; RND: Resistance-nodulation-division; Ade: *A.*

baumannii multidrug-resistant efflux pump; TetA: Tetracycline resistant Acinetobacter; CarO: Carbapenem-associated outer membrane protein; OMP: Outer membrane protein; PBP: Penicillin binding protein; GyrA/ParC: DNA Gyrase/partitioning of the nucleoid partition; FolA: Folate; ArmA: Armillaria mellea.

3.4 Other resistance mechanisms to antibiotics:

Resistance to antimicrobial agents by MDRAB is known to be associated with specific resistance mechanisms. However, reports of resistance to some antimicrobial agents have been made without observing the associated mechanism of resistance.[85,111,112] These reports suggest the existence of other resistance mechanisms other than the common ones documented for MDRAB. Resistance to tigecycline is known to be associated with overexpression of AdeABC efflux pump;[85] however, a study reported that clinical isolates of *A. baumannii* have decreased their susceptibility to tigecycline without overexpression of AdeABC, AdeFGH, and AdeIJK. In fact, it has been demonstrated that deletion mutation in the *trm* gene encoding for S-adenosyl-L-methionine-dependent methyltransferase, decreases susceptibility to tigecycline as another mechanism of resistance.[111] It has also been documented that a frameshift mutation in *plsC*, encoding 1-acyl-*sn*-glycerol-3-phosphate acyltransferase, is associated with decreased susceptibility to tigecycline.[112] Deletion of the novel *abrp* gene encoding for peptidase C13 family, results in modifications to the cell membrane. *A. baumannii* cell membrane permeability is increased, displaying slower cell growth rate and decreased susceptibility to tetracycline, minocycline, doxycycline, tigecycline, chloramphenicol, and fosfomycin.[101] SOS operon regulation involved in DNA damage response in which RecA plays a role, seems to acquire antibiotic resistance under clinically relevant DNA-damaging conditions in *A.baumannii*.[113,114] The *blhA* is a novel gene singular to *Acinetobacter spp* involved in cell division, as well as *zipA, zapA*, and

ftsK. Any mutation on these genes increases beta-lactam susceptibility in *A. baumannii*. There is, therefore, a relation between cell division and intrinsic beta-lactam resistance in *A. baumannii*.[115]

3.4.1 Integrons:

Horizontal gene transfer is a successful mechanism for transmission and dissemination of multidrug resistance associated genes among bacteria.[25] Genetic structures called integrons are associated with the acquisition of resistance genes by the recipient cell. These DNA fragments carried by either bacterial chromosomes or plasmids, acquire open reading frames (ORFs) embedded in exogenous genetic tapes and convert them to functional genes by ensuring their correct expression.[116] Integrons have the unique ability to collect, integrate and allow recipient bacteria to express the acquired resistance genes.[116] This is the mainstay of these DNA fragments in the acquisition and dissemination of resistance genes. The combination of a system of gene capture and expression, coupled with the vertical and horizontal transmission capacity of resistance genes, is a potent weapon used by bacteria to overcome antibiotics.[117] So far, four classes of integrons have been reported, with class I being the most frequently encountered globally.[118] Studies suggested that epidemic strains of *A. baumannii* contain more integrons than non-epidemic strains.[119] They therefore could be useful markers during investigation of outbreaks due to *A. baumannii* strains.[120] Despite the genetic diversity of integrons among *A. baumannii*, a hypothesis suggests that all these integrons are clonally related.[121] However, the same integrons can be present in unrelated strains,[107] and related strains can have different integrons.[122] The relationship between integrons and class of antibiotics are different from one class to another.[10] However, association was evidenced between aminoglycoside and choramphenicol resistance patterns and a particular type of

integron in *A. baumannii*.[118] Additionally, association between class I integrons and genes responsible for aminoglycoside resistance in *A. baumannii* was established in a study involving three Pan-European clones.[123] The two studies highlight the implication of horizontal gene transfer as a major role in the dissemination of resistance associated genes. Integrons are also documented to be associated with imipenem resistance in *A. baumannii*[124] and carry MBLs encoding genes responsible for carbapenemases such as bla_{VIM}, bla_{IMP}, bla_{SIM}, various bla_{IMP}, bla_{SIM-1}, and several CHDL genes bla_{OXA} encoding for oxacillinases. Integrons may bear *catB2*, *catB3,* and *catB8* genes that are associated with resistance to chloramphenicol in *A. baumannii*[3].

Environmental persistence of *A. baumannii* has generally been accepted as a virulence strategy. However, associated genes and mechanisms enable strains of MDRAB to survive unfavourable living conditions, thus, can be associated to the survival skills of this specie.

4. SURVIVING ENVIRONMENTAL CHALLENGES:

Apart from the above-mentioned mechanisms of resistance that allow MDRAB to survive antimicrobial agents attack, the bacteria have developed mechanisms and strategies to survive and persist in unfavourable environmental conditions.

4.1 Protein secretion:

In Gram-negative bacteria, various protein secretion systems have been described. Their compositions and functions are varied as well as their role in the survival of the bacterium in a challenging condition. A range of protein secretion system has been described in *A. baumannii*.[125] Several strains of *Acinetobacter spp.* can secrete proteins or protein substrate such as type II secretion system, type V system auto-transporter and type VI secretion (T6SS).[125] Activation and secretion of T6SS protein

happens for reasons related to the living environment of the bacteria. Within an environment where several microbial communities meet and co-exist, insufficient nutrients for growth may induce a hard competition for the survival of bacterial species.[126] Bacteria such as *Vibrio cholerae*, *Pseudomonas aeruginosa* and *A. baumannii* secrete proteins like T6SS that have antagonistic effects on the growth of their potential opponents.[127] By killing the opponent bacteria, sometimes strains from the same species; they ensure their niche establishment.[128] T6SS genes are remarkably well conserved across *Acinetobacter spp.*.[129] They have been reported in *A. baumannii* ATCC 19606 and M2 strain.[130] T6SS is composed of approximately 15 conserved structural proteins and variable number of accessory factors.[131] Two of the major components of T6SS are Hcp and VgrGs. Hcp forms a polymerized tubular structure; and VgrGs is present at the ends of T6SS and it facilitates the effector secretion.[132] T6SS expression is often tightly controlled and is activated only under certain conditions. The molecular mechanisms used to achieve this regulation are extremely diverse, complex and differ from one bacterium to another and even between strains of the same species.[132] T6SS are plasmid mediated. Research suggest that in some specific cases *A. baumannii* uses either T6SS activation and secretion or antibiotic resistance mechanism to guarantee its survival when facing unfavourable living condition.[125]

4.2 Tolerance to desiccation and oxidative stress/ Resistance to disinfectant agents:

In healthcare settings, regularly applied disinfection regimes expose micro-organisms to prolonged periods of desiccation and repeated attack from disinfectant agents at doses higher than the ones applied by antibiotics. MDRAB have developed survival strategies to resist and be established in such inhospitable conditions.[133] Desiccation

tolerance in *A. baumannii*, is the ability of the bacteria to maintain viability for several days under dry conditions.[134] This resistance mechanism is multifactorial thus remains to be fully characterized. However, the ability to retain water during desiccation period seems to play a key role in the survival process as well as the role played by *BfmR*.[135] A study demonstrated that capsular polysaccharides composed of repeating carbohydrate units, work as capsule to retain water in *A. baumannii* under dry conditions.[136] Another study highlighted the link between the compositions of the outer membrane and resistance to desiccation. The authors demonstrated that altered lipid composition due to mutation, results in increased membrane fluidity leading to water leakage and loss of hydrophilic nutrients from the intracellular compartments.[137] Water loss leads to decreased turgor pressure and biochemical changes that can damage cell membranes. Sequestering water to avoid dehydration during drying period mitigates the damage of cell integrity.

Originally identified as one of the key role players in controlling biofilm formation, and progressively recognised to be involved in formation of pili, motility, complement resistance, antibiotic susceptibility, and virulence;[138] alteration of *bfmR* leads to increased sensitivity to desiccation.[135] The structural and biochemical mechanisms of BfmR makes it an ideal target for a new approach in the search for pharmaceutical compounds in order to treat *A. baumannii* infections.[139]

Oxidative stress is also induced during desiccation periods; reactive oxygen species generated can damage both proteins and DNA. To survive, *A. baumannii* substantially up-regulates proteins that are associated with detoxifying reactive oxygen species.[140] Some *Acinetobacter spp.* demonstrate highest tolerance to hydrogen peroxide than spore forming Gram positive bacteria.[141] Following hydrogen peroxide exposure,

authors have observed that *ISAba* insertions adjacent to *katG* resulted in more than 20-fold overexpression of the gene and increased hydrogen peroxide tolerance.[142]

The process from desiccation to rehydration is responsible for various DNA alterations such as alkylation, oxidation, crosslinking, base removal and strand breaks.[143] These DNA lesions induce the activation of DNA repair pathway in *A. baumannii* involving *RecA* genes;[144] The DNA repair pathway activation induces an ~50-fold increase in the mutation frequency during a round of desiccation and rehydration in *A. baumannii*. This mechanism leads to multidrug resistance phenotypes of *A. baumannii*.[114] Authors hypothesised that there is a link between desiccation and raise of MDRAB.[142]

4.3 Biofilm formation:

Microbial biofilms are aggregates of microbial communities that are surrounded by self-produced exopolysaccharide matrices. Biofilms demonstrate greater protection against antibiotics, host immune defence, and adverse environmental conditions than the free-living cells.[145] *A. baumannii* can form biofilm on most biotic and abiotic surfaces, including healthcare associated equipment.[146] The cell's surface of biofilm producing organisms is a mix of adhesins and capsular polysaccharide that contribute to the formation, maintenance and increased tolerance to extracellular stresses and resistance to antibiotic attack.[147] Several common factors that contribute to the formation of biofilm in *A. baumannii* have been identified. Csu pili, a type I chaperone-usher pilus system regulated by the BfmRS is crucial in the formation and maintenance of *A. baumannii* microbial biofilm community on abiotic surface[148] but not required on biotic surface.[149] Yet, reports have been made about clinical strains that lost their Csu cluster but can still produce biofilm.[150] This observation indicates that there is an existent substitute mechanism that enables bacteria to produce biofilm even in cases of Csu pili loss, or that other pili systems may functionally replace them.[150] Another

study demonstrated that GacSA, a two-component system, moderately controls *csu* gene expression and thus indirectly, biofilm formation. Moreover, improper use of antibiotics may promote a planktonic lifestyle by completely repressing the expression of Csu pili.[151]

The protein secretion system type I (T1SS secretion system) in *A. baumannii* is the origin of a protein involved in the formation and maturation of biofilm.[152] Biofilm-associated protein (Bap_{Ab}) is involved in cell−cell adhesion and for the development of biofilm on abiotic surfaces such as polystyrene and titanium.[152] In medically relevant *Acinetobacter spp*, T1SS secrete a repetitive RTX domain-containing protein that mediates biofilm development.[153] Other notable factors in *A. baumannii* that might be crucial for biofilm formation include the production of poly-β-1,6-N-acetylglucosamine (PNAG);[154] and other putative chaperone-usher pili systems and Pap pili systems, which are homologous to the P pili of *Escherichia coli*, have been implicated in formation and maintenance of *A. baumannii* biofilm.[156]

5. CONCLUSION

Several mechanisms and strategies have enabled *A. baumannii* to survive various challenges within different environments. Research conducted on *Acinetobacter sp* still reveals new and/or enhanced mechanisms that enabled the specie to thrive within inhospitable environments. *A. baumannii* are becoming increasingly resistant to the best available antibiotics and increasingly threaten human health. Infection prevention and control services as well as epidemiological surveillance should be reinforced as the bacteria seem to be ahead in the battle against antibiotics. More efforts should be made in the search for, and development of new antibacterial compounds. All alternatives with potential antimicrobial activity should be considered to tackle this current threat to human health.

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