

## ***Acinetobacter baumannii*: A Superbug**

**Michelle Lowings\*<sup>1</sup>, Marthie M. Ehlers<sup>1,2</sup> and Marleen M. Kock<sup>1,2</sup>**

<sup>1</sup> Department of Medical Microbiology, Faculty of Health Sciences, University of Pretoria, Private Bag x323, Arcadia, Pretoria, 0001, Gauteng, South Africa

<sup>2</sup> Department of Medical Microbiology, Tshwane Academic Division, National Health Laboratory Service, Gauteng, South Africa

\* Corresponding author: Miss M Lowings [lowingsmichelle1@gmail.com](mailto:lowingsmichelle1@gmail.com)

*Acinetobacter baumannii* has emerged as a successful opportunistic pathogen, especially in intensive care units (ICU) where *A. baumannii* infects severely ill patients. This micro-organism is known to be a major cause of hospital outbreaks and interinstitutional spread. *Acinetobacter baumannii* is able to survive for extended periods of time in health care settings and can acquire resistance rapidly against antimicrobials. Multidrug resistant (MDR) *A. baumannii* strains are of global concern because these isolates are resistant to at least three classes of antibiotics (carbapenems, cephalosporins, aminoglycosides, fluoroquinolones) and thus limit treatment options.

**Keywords:** virulence factors; antimicrobial resistance genes; resistance mechanisms

### **1. History of the genus *Acinetobacter***

In the beginning of the 20<sup>th</sup> century *Acinetobacter* was known as *Micrococcus calcoaceticus*, discovered by Martinus Willem Beijerinck, a Dutch microbiologist, in 1911 [1]. *Micrococcus calcoaceticus* was isolated from soil and cultivated in a calcium acetate enrichment medium [2–5]. The genus *Acinetobacter* was proposed for the first time in 1954 by Brisou and Prévot to separate the non-motile from the motile bacteria within the genus *Achromobacter* [1,5–8]. *Acinetobacter* originates from the Greek word “akinetos” meaning unable to move; however, *Acinetobacter* does have a characteristic twitching kind of motility on semi-solid media surfaces [7,9,10]. The genus was first listed in *Bergey’s Manual of Systematic Bacteriology* in 1974 with the description of a single species, *Acinetobacter calcoaceticus* [5,7,8]. The *Approved Lists of Bacterial Names* listed two species, *A. calcoaceticus* and *A. lwoffii*, which was included based on the observation that some *Acinetobacter* strains were able to acidify glucose [1,5]. In 1986, twelve DNA hybridisation groups were listed by Bouvet and Grimont after completing DNA-DNA hybridisation studies: *Acinetobacter baumannii*, *A. calcoaceticus*, *A. haemolyticus*, *A. johnsonii*, *A. junii* and *A. lwoffii* were described as species of the genus [1,5]. In 1987 Bouvet and Grimont refined their phenotypic identification scheme, which is based on 28 phenotypic tests and this refined identification scheme included the production of acid from glucose, assimilation of 14 different carbon sources, gelatin hydrolysis and growth at 37°C, 41°C and 44°C [5].

### **2. Classification of the *A. baumannii* complex**

In 1986 *Acinetobacter* species was taxonomically classified by a phenotypic system [11,12]. Currently thirty-nine genomic species of *Acinetobacter* are listed in nomenclature [12]. *Acinetobacter baumannii* forms a complex with three other closely-related species: *A. calcoaceticus*, *A. nosocomialis* and *A. pittii* [8,13,14]. The *A. calcoaceticus*-*baumannii* complex cannot accurately be differentiated with routine phenotypic tests because of this group’s close relatedness [5,8,14–18]. After a long history of taxonomic changes, *Acinetobacter* was moved from the family *Neisseriaceae* to the family *Moraxellaceae* [2,7,17].

### **3. Characteristics of *A. baumannii***

*Acinetobacter baumannii* is a Gram-negative, non-motile, non-fermentative, oxidase-negative, catalase-positive, indole-negative, non-sporeforming, non-pigmented, encapsulated, saprophytic, aerobic coccobacillus [2,8,16,19]. The *A. calcoaceticus*-*baumannii* complex has a DNA G + C content of 38% to 47% [1,8].

### **4. Pathogenesis of *A. baumannii***

The pathogenic mechanisms of *A. baumannii* are not well understood [7]. The infective dosage of *Acinetobacter* species has not been determined for human infections but based on intraperitoneal injections in mice with 40 clinical *Acinetobacter* isolates, results have shown that the lethal dose at 50% (LD<sub>50</sub>) can range from 10<sup>3</sup> to 10<sup>6</sup> viable cells per mouse [7]. *Acinetobacter baumannii* is not known to produce diffusible toxins or cytolysins [7,20]. This bacterium can colonise the skin and respiratory tract without causing an infection [7]. An infection will occur if the host’s first line of

defence is compromised [7]. There is a need to further investigate pathogenic mechanisms for more effective control measures in the clinical setting [7,21].

## 5. Virulence associated characteristics of *A. baumannii*

Only a few virulence factors, such as biofilm formation and the ability to compete for and sequester iron in nutrient deprived environments have been identified in *A. baumannii* [7,10]. *Acinetobacter baumannii* has virulence factors associated with invasiveness, transmissibility or the enhanced ability to colonise immunosuppressed patients [11]. *Acinetobacter baumannii* is capable of forming biofilms, which facilitates colonisation on surfaces and contributes to drug resistance [20]. Jacobs *et al.* [22] conducted studies with a virulent *A. baumannii* strain (AB5075) in animal models. The virulent strain (AB5075) caused more severe infections in the animal models and the survival rates were consistently below 25% [22]. Jones *et al.* [23] isolated a highly virulent extensively drug-resistant (XDR) *A. baumannii* strain, even more virulent than the reported AB5075 strain, from an immunocompetent patient. This strain (Clade B isolate) possesses a unique combination of putative virulence genes involved in iron metabolism, protein secretion and glycosylation [23]. The question to be asked here is whether *A. baumannii* is evolving to become more virulent and is immunocompetent patients now in danger or was this a sporadic case?

### 5.1 Cell surface hydrophobicity

Cell surface hydrophobicity (meaning to repel and not absorb water) is an important determinant in bacterial adhesion [7]. The hydrophobicity of *A. baumannii* protects the bacterium from being phagocytosed and it helps the bacterium to attach to various polymers, such as catheters and prostheses [7]. The hydrophobic properties of bacterial strains depend on the bacterium's cell surface meaning the rougher the cell surface, the greater the hydrophobicity and *vice versa* [7]. Cell surface hydrophobicity is mediated by colonisation factors, complimentary cell surface receptors, cell wall components and cell surface enzymes [7].

### 5.2 Toxic slime polysaccharides

Toxic slime polysaccharides are usually produced during the exponential growth phase and consist of glucose building blocks, such as D-glucose, D-glucuronic acid, D-mannose and L-rhamnose [7]. The function of toxic slime polysaccharides is to inhibit the migration of neutrophils and to inhibit phagocytosis without disrupting the host's immune system [7].

### 5.3 Siderophore mediated iron acquisition

*Acinetobacter*'s siderophores are known as acinetobactins [7,20]. This bacterium's siderophores also produce a hemin utilisation system [7,20]. Siderophores are defined as bacterial iron-binding protein structures that utilise resources, such as iron that is required by *A. baumannii* [7,10]. Iron-acquisition mechanisms and serum resistance are attributes that enable this microorganism to survive in the blood stream [11]. The iron concentration required for bacteria to survive in the human body is  $10^{-6}$  Molar (M) and the normal concentration of free iron in humans is  $10^{-8}$  M [7,24]. *Acinetobacter baumannii* meets its iron requirements by binding exogenous iron by using siderophores or haemophores [7]. Normally the host has low iron availability and to overcome this *A. baumannii* employs the iron-dependent repressor ferric uptake regulator (FUR), which regulates gene expression through the binding of a conserved FUR box DNA sequence upstream of target genes [7,25]. *Acinetobacter baumannii* responds to iron starvation by modifying gene expression for many predicted iron-related genes, as well as for genes involved in various processes, such as biofilm formation, respiration and motility, highlighting the importance of iron levels in *A. baumannii*'s virulence [25].

The first siderophore cluster that was identified in *A. baumannii* strain 8399 consisted of 10 open reading frames (ORFs) [25]. This specific cluster is responsible for the production of a siderophore that chelates oxidised ferric iron from transferrin and restores *A. baumannii*'s growth when iron availability is low [25].

### 5.4 Outer membrane proteins

Outer membrane proteins (OMPs) in some Gram-negative bacteria are known to have essential roles in pathogenesis and adaption in host cells as well as in antibiotic resistance [7,26]. The 38 kDa OMP A was identified in *A. baumannii* isolates and the function of OmpA is to induce apoptosis of epithelial cells through the release of pro-apoptotic molecules [5,10,25,27]. Outer membrane protein A aids additionally in biofilm formation, cell death, serum resistance and surface motility [7,27–30].

### 5.5 Biofilm formation

Biofilms are highly structured communities of bacteria on biological and abiotic surfaces, such as plastic or glass surfaces [11,20,21]. Bacterial biofilms are an arrangement of different cells that differ morphologically, metabolically

and physiologically from their planktonic counterparts [27]. A biofilm matrix is composed of polysaccharide polymers, nucleic acids and biofilm-associated proteins [26,31]. The biofilm serves as a protective mechanism to survive in harsh environments and in hosts, causing infections [26]. Biofilms decrease the bacteria's susceptibility to antibiotics, confer resistance to desiccation or nutritional stress and increase the possibility of the spread of both the bacteria and the antimicrobial resistance genes [20,26,31-34]. Biofilm formation is important in health care associated infections because of the use of ventilators and catheters, which are ideal surfaces for adherence [29,33]. Biofilms provide a niche from which *A. baumannii* colonises intubated or catheterised patients, which gives rise to respiratory tract and blood stream infections or shunt-related meningitis [11,26,31]. Quorum sensing is a system that is used to monitor and communicate with neighbouring cells in the biofilm [27,35]. Signals, such as auto-inducers and hormone-like molecules are produced to sense cell density and activate the required responses [27,35]. Quorum sensing is found in diverse microorganisms and the scale and extent of signalling systems has led to the revelation that the microbial world is in a state of constant and complex communication [35].

## 6. Risk factors for the acquisition of *A. baumannii* infections

Risk factors for hospital-acquired *A. baumannii* infections include burn injuries, enteral feeding, exposure to contaminated medical equipment, ICU admission, immunosuppressed patients, the performance of invasive procedures (central venous or urinary catheters, drainage tubes, mechanical ventilation and surgery), a prolonged length of hospital stay, previous antibiotic usage as well as cytostatics, immunosuppressors and steroids, renal failure and severity of underlying illness [20,36–39].

## 7. Clinical manifestations of *A. baumannii* infections

*Acinetobacter baumannii* is an opportunistic pathogen that has been implicated in various hospital- and community-acquired infections [7,11,40]. Community-acquired infections due to *A. baumannii* are less common than hospital-acquired infections [11,16]. A seasonal variation has been reported in hospital-acquired infections, with an increase during summer months [5,36].

In health care settings, *A. baumannii* mainly affects critically ill patients in ICU's [41–45]. Hospital-acquired infections include: blood stream infections, central nervous system infections, meningitis, skin and soft-tissue infections, urinary tract infections, ventilator-associated pneumonia and wound infections [11,18,25,38,40,44]. Ventilator-associated pneumonia and blood stream infections are the most frequent clinical manifestations of hospital-acquired *A. baumannii* and are associated with high morbidity and mortality rates [2,5,11,39,43,46,47]. Common sources of blood stream infections and disseminated intravascular coagulation are due to intravascular or respiratory tract catheters [5,36,48]. Another characteristic clinical manifestation caused by *A. baumannii* in patients who have had neurosurgery is cerebrospinal-shunt-related meningitis [7,11]. Urinary tract infections are related to indwelling urinary tract catheters and are usually non-threatening [5,11]. *Acinetobacter baumannii* can be the cause of secondary meningitis, specifically in patients with ventricular draining tubes [5,17].

## 8. Different mechanisms of antibiotic resistance in *A. baumannii*

The role of antibiotic resistance mechanisms is to resist the action of a toxic compound or to protect the cell against toxic compounds like antimicrobials [49]. There are multiple mechanisms that *A. baumannii* use to create resistance [19]. *Acinetobacter baumannii* shows resistance to all antibiotic classes and have rapid developing mechanisms [41,50].

### 8.1 $\beta$ -lactamase resistance mechanisms

Resistant bacteria produce distinct enzymes, such as  $\beta$ -lactamases that can inactivate antimicrobials that are present in the cell, periplasmic space or on the outside of the cell [37,51]. The  $\beta$ -lactamases of Gram-negative bacteria belong to Ambler classes A to D [13,31,52]. *Acinetobacter baumannii* possesses different  $\beta$ -lactamases, which hydrolyse and grant resistance to drugs, such as penicillins, cephalosporins and carbapenems [26,37].

#### 8.1.1 Ambler class A

Extended-spectrum  $\beta$ -lactamases (ESBLs) are a heterogeneous group of plasmid-mediated bacterial enzymes that confer resistance to oxyimino-cephalosporin and monobactam antimicrobials [54]. Many class A carbapenemases, including *Klebsiella pneumoniae* carbapenemase (KPC), are less sensitive to the mechanism-based inhibitors, such as clavulanic acid, that are effective against the majority of class A  $\beta$ -lactamases [55]. Widespread and clinically important enzymes, such as the Temoniera (TEM), Sulfhydryl variant (SHV) and Cefotaximase-Munich (CTX-M) families are inhibited by carbapenems through formation of a long lasting acyl-enzyme complex [55].

The most common ESBLs described in *A. baumannii* are the Guyana extended-spectrum  $\beta$ -lactamase (GES), *Pseudomonas* extended resistance (PER) and Vietnam extended-spectrum  $\beta$ -lactamase (VEB) type  $\beta$ -lactamases [56]. The first ESBL identified in *A. baumannii* was PER-1 that was later commonly found in Turkey [56]. The PER-1 gene is also widespread in Belgium, Hungary, Romania, Russia, South Korea and the USA [56]. The first report of VEB-1 and GES-11 producing *A. baumannii* strains was in France [56,57]. On the other hand, the Cefotaximase-Munich (CTX-M), Sulfhydryl variant (SHV) and Temoniera (TEM) type ESBLs, being widespread among *Enterobacteriaceae*, have been rarely identified in *A. baumannii* isolates [56].

### 8.1.2 Ambler class B

Ambler Class B is also known as metallo- $\beta$ -lactamases (MBLs) and confers resistance to all  $\beta$ -lactams with the exception of monobactams (aztreonam) [11,19,53,58–60]. Metallo- $\beta$ -lactamase enzymes are only active in the presence of metal ions, like zinc but are inhibited by metal chelators like ethylenediaminetetra-acetic acid (EDTA) [56,59,61].

Carbapenem resistance in this species is most often linked to the production of carbapenemases [56]. Metallo- $\beta$ -lactamase enzymes are not the most commonly identified carbapenemases in *A. baumannii* [56]. However, if these MBL enzymes are detected it is either Imipenem metallo- $\beta$ -lactamase (IMP), New Delhi metallo- $\beta$ -lactamase (NDM), Seoul imipenem metallo- $\beta$ -lactamase (SIM) or Verona integrin-encoded metallo- $\beta$ -lactamase (VIM) [56].

The *bla*<sub>NDM</sub> gene is usually located on a plasmid in *A. baumannii* [62]. Such strains have been reported in China, India and Germany [19]. The NDM-2 variant was identified in *A. baumannii* strains recovered from Egypt, Israel and UAE [56]. The SIM-1 enzyme, was originally and only once found in *A. baumannii* from South Korea [19,56]. Only a few publications reports the VIM-type enzymes in *A. baumannii* isolates [19].

### 8.1.3 Ambler class C

*Acinetobacter baumannii* naturally produces a gene encoding an Ampicillin class C (AmpC) type cephalosporinase [56]. This gene is usually expressed at a basal and low level, therefore the amount of AmpC produced does not have a significant impact on the activity of expanded-spectrum cephalosporins [56]. The true clinical significance of these enzymes remains unknown [56]. No acquired Amp-C type encoding genes has been identified so far in *A. baumannii* [56].

### 8.1.4 Ambler class D

The class D  $\beta$ -lactamases are the most widespread [11,63]. This class is also known as oxacillinases and are grouped in a heterogeneous class of enzymes either with respect to their structural or biochemical properties [56]. These specific enzymes hydrolyse amoxicillin and cefalotin [56]. Most of class D  $\beta$ -lactamases are not inhibited by  $\beta$ -lactamase inhibitors, such as clavulanic acid, tazobactam, sulbactam, cloxacillin or zinc chelators but are inhibited *in vitro* by NaCl [64].

*Acinetobacter baumannii* possesses naturally occurring class D  $\beta$ -lactamases, known as OXA-51-like enzymes [20,56]. These enzymes exhibit weak carbapenemase activity and are classified as the carbapenem-hydrolysing class D  $\beta$ -lactamases (CHDLs) [56,59]. The OXA-51-like enzymes are able to hydrolyse penicillins and carbapenems [20,29]. High level carbapenem resistance is due to the overexpression of CHDL genes driven by insertion sequences, such as *ISAbal* upstream of the promoters [20,60,65–67]. The role of *ISAbal* and other insertion sequences is to modulate the expression of *A. baumannii*'s resistance genes [20].

Several other acquired class D  $\beta$ -lactamases have been identified as a source of carbapenem resistance [56]. These CHDLs confer only reduced susceptibility to carbapenems [56]. The high resistance to carbapenems observed in *A. baumannii* isolates are due to the association between a CHDL and other resistance mechanisms, such as porin loss and overexpression of efflux pumps [56]. The CHDL carbapenemases in *A. baumannii* can be divided into five subfamilies: OXA-23- (OXA-27 and OXA-49); OXA-40- (OXA-25, OXA-26 and OXA-72); OXA-58- (OXA-96 and OXA-97); OXA-143- and OXA-235- (OXA-236 and OXA-237) like enzymes [13,19,20,56,65,67].

## 8.2 Aminoglycoside resistance mechanisms

Mechanisms of resistance to aminoglycosides include modification of the target by modification of ribosomal proteins or 16S rRNA and enzymatic modification of the drug [68,69]. Resistance are due to acetyltransferases, nucleotidyltransferases and phosphotransferases and have all been found in *A. baumannii* [20,46,69–71]. The enzymes may be located on plasmids, transposons or in association with class I integrons [20].

Another set of enzymes, the 16S rRNA methyltransferases (*armA* and *rmtA*, B, C, D and E), which confer high-level resistance to all formulated aminoglycosides, have been described [20,46,70,72,73]. These genes are mediated by transposons and are translocated to other DNA target sites [73]. The *armA* enzyme has been reported in many *A. baumannii* isolates worldwide, conferring high-level resistance to all aminoglycosides [46,56,71,74]. *Acinetobacter baumannii* isolates containing the *armA* gene have been identified in Algeria, Bulgaria, China, Iran, Italy, Japan, North America, Norway, South Korea and Vietnam [56]. Noteworthy, the *armA*-encoding gene is often

identified among OXA-23 producing *A. baumannii* strains; however, both resistance genes are not physically linked on a single plasmid [56]. Interestingly the 16S rRNA methylase *rmtB* has recently been identified in nine *A. baumannii* isolates from Vietnam [56].

### 8.3 Quinolone resistance mechanisms

Resistance to quinolones in *A. baumannii* is associated with: (i) mutations in the *gyrA* and chromosomal *parC* genes; (ii) changes in drug entry and efflux and (iii) the quinolone protein, which prevents DNA to bind to quinolones [18,75,76]. Plasmid-mediated quinolone resistance genes encode for DNA protection proteins, such as *qnrA*, B, C, D and S [75,77,78]. Isolates that harbour these *qnr* genes are still rare but *qnrA*-producing *A. baumannii* has been reported in Algerian hospitals [76]. The plasmid-mediated quinolone resistance determinants provide resistance [77].

### 8.4 Penicillin-binding proteins

The targets of  $\beta$ -lactamases are known as penicillin-binding proteins (PBPs) [51]. Penicillin-binding proteins are a family of enzymes that catalyse the synthesis of peptidoglycan, the primary component of the bacterial cell wall [26]. If changes occur in the PBPs it will prevent the action of  $\beta$ -lactams [48,51]. The inhibition of PBPs will cause instability in the cell wall of the bacteria, which will lead to growth inhibition or cell lysis [26,79].

### 8.5 Altered structures and porin proteins

In 1976 porins were first characterised in *Escherichia coli* [49]. Porins are found in the outer membrane or waxy layer where the outer membrane is permeable to hydrophilic substances [49]. Porins can act as a potential target for adhesion to other cells and the binding of bactericidal compounds to surfaces [80]. Porins can be divided into several classes including general porins, which are involved in determining the permeability barrier, specific porins (LamB) that enhance the uptake of certain substances like maltose and maltodextrins and iron-regulated porins, which enable the uptake of rare iron complexes [49]. The general porins play a more pertinent role in antibacterial susceptibility and resistance [49]. The concentration of porins in a bacterial cell can be as high as  $10^6$  copies per cell [49]. Porins are diverse and can act as receptors for bacteriocins, bacteriophages and elements of the immune system, including antibodies, epithelial cells and interferons [49].

### 8.6 Efflux pumps

Efflux pumps in bacteria are involved in antibiotic resistance and are usually chromosomally encoded [81]. Efflux pumps are an energy-dependent system that prevents intracellular accumulation of toxic compounds [49]. The most well-known antibiotics that are pumped out of the cell are macrolides, tetracyclines and quinolones [31,49,80]. The efflux pumps can be divided into five families: (i) the adenosine triphosphate (ATP)-binding cassette (ABC) family; (ii) the multi-drug and toxic compound extrusion family (MATE); (iii) the small multi-drug resistance superfamily (SMR); (iv) the major facilitator superfamily (MFS) and (v) the resistance-nodulation cell division (RND) family [26,49,80–82]. Each family uses different energy sources: (i) the ABC transporters are fuelled by ATP hydrolysis; (ii) MFS, RND and SMR use the proton-motive force and (iii) MATE transporters consist of  $\text{Na}^+/\text{H}^+$  drug antiport systems [49,81].

The ABC family is involved in the uptake and efflux of amino acids, antibiotics, ions, polysaccharides, proteins and sugars [49]. This family has a unique energy source as mentioned above [49,81].

The MATE family has 12 transmembrane regions and uses sodium gradients to export toxic compounds [49]. This family comprises of the Abe M-efflux system and pumps out norfloxacin, ofloxacin, ciprofloxacin, gentamicin, 4,6-diamino-2-phenylindole triclosan, acriflavin, doxorubicin, rhodamin 6G and ethidium bromide (EtBr) [7].

The SMR family is small proteins (107 to 110 residues) and each of these proteins contains four transmembrane segments that generally form tetramers in the cytoplasmic membrane [49]. The number of SMR transporters related to antibiotic resistance is fairly small [49].

The MFS family is constituted of a large number of proteins and is the most diverse family [49]. This family comprises of the Tet(A)-efflux system and gives resistance to tetracycline, the Tet(B)-efflux system gives resistance to tetracycline and minocycline and the CamL A-efflux system gives resistance to chloramphenicol [7,20].

The RND family is associated with antibiotic resistance in Gram-negative microorganisms [49,81,83]. These efflux pumps consist of three elements: (i) an inner membrane pump protein with 12 transmembrane regions; (ii) two large periplasmic loops (membrane fusion protein) and (iii) an outer membrane protein [20,49]. Three RND systems, namely AdeABC, AdeFGH and AdeIJK have been associated with MDR in *A. baumannii* [83]. The AdeABC (ATP binding cassettes)-efflux system confers resistance to aminoglycoside,  $\beta$ -lactam, chloramphenicol, erythromycin and tetracycline as well as reduced susceptibility to fluoroquinolones [7,83]. The AdeFGH system pumps out chloramphenicol, clindamycin, fluoroquinolones, sulphonamide, tetracyclines, tigecycline and trimethoprim [83]. The AdeIJK system pumps out  $\beta$ -lactams, chloramphenicol, tetracyclines, erythromycin, fluoroquinolones, novobiocin and trimethoprim [83]. The AdeABC and AdeFGH play a major role in acquired resistance, whereas the AdeIJK is responsible for intrinsic resistance [83].

## 9. Treatment of *A. baumannii* infections

Antibiotics are not a discrete class of molecules, but rather a broad range of structural and chemical families, united by their ability to inhibit microbial growth at high concentrations [35]. The original use of the word “antibiotic” was a generic term that simply reflected the outcome of a laboratory test [35]. In modern terms, an antibiotic has broadly come to mean any synthetic or naturally occurring low molecular weight molecule that inhibits bacterial growth [35].

The prolonged use of antimicrobials over the past 60 years has led to the gradual resistance of *A. baumannii* isolates and left clinicians with little or no effective treatment options [7,35,50,84]. *Acinetobacter baumannii* is generally intrinsically resistant to a number of commonly used antibiotics, including aminopenicillins, first- and second generation cephalosporins (cefalotin and cefoxitin) and chloramphenicol [11]. This situation is worrisome, since the development of new antimicrobial drugs is very slow [85].

Clinicians often start with a broad spectrum antimicrobial agent and later narrow the spectrum of coverage when culture results become available [86]. Unfortunately, it is unclear if and how clinicians can successfully execute this strategy, particularly in light of the fact that *A. baumannii* has become resistant to last resort treatment options, such as colistin and tigecycline [86]. The treatment of serious *A. baumannii* infections should be combination therapy (the use of more than one antimicrobial), based on laboratory antimicrobial susceptibility results [7]. Local antimicrobial prescribing habits should be critically guided by the susceptibility results as inadequate use of antibiotics contributes to the emergence and spread of drug resistance and increases toxic effects and health care costs [7,87]. Rapid and reliable antimicrobial resistance testing is of great relevance for aiding the selection of the most appropriate antibiotic therapy and to ensure the optimised use of antimicrobials [87].

### 9.1 $\beta$ -lactam antibiotics

In 1928, Alexander Fleming observed the bactericidal effect of *Penicillium notatum*, leading to the identification of the first  $\beta$ -lactam antibiotic namely penicillin [88]. Beta-lactams are bactericidal against a variety of bacteria and it inhibits peptidoglycan transpeptidases [51]. The antibacterial agent interferes with the transpeptidation reactions that seal the peptide cross-link between glycan chains [51,88]. Beta-lactams have a  $\beta$ -lactam ring in its structure and is essential for antibacterial activity [51]. Beta-lactams can consist of one  $\beta$ -lactam ring (monobactams), or a  $\beta$ -lactam ring combined to a five-member penem ring (penicillins, carbapenems), or a six-member cephem ring (cephalosporins) [51]. This antimicrobial is clinically used for infections caused by susceptible organisms [89].

#### 9.1.1 Carbapenems

The first-line treatment for *A. baumannii* infections are carbapenem antibiotics, such as imipenem and meropenem [57,87,90,91]. This group of antibiotics have a good bactericidal activity, stability towards a range of  $\beta$ -lactamases, broad-spectrum activity and a good safety profile [92,93]. The first carbapenems discovered were olivanic acids produced by *Streptomyces olivaceus* [94]. This was followed by the discovery of thienamycin in 1976 [94]. Years later, a more stable thienamycin derivative known as imipenem was synthesized and approved for use in 1984 [94]. Other carbapenems for parenteral administration were discovered later and include biapenem, panipenem, lenapenem and ertapenem [94]. Carbapenems are recommended for the empirical treatment of a variety of severe infections, such as nosocomial pneumonia, complicated intra-abdominal infections, septicaemia, complicated skin and urinary tract infections, meningitis and acute exacerbations of cystic fibrosis [94]. Carbapenems are generally well tolerated in the human body but allergic reactions, such as rash and immediate hypersensitivity to  $\beta$ -lactam compounds are the most common side effects [94].

Unfortunately, increasing carbapenem resistance is creating therapeutic challenges, especially considering that most *A. baumannii* strains that are resistant to carbapenems are also resistant to a majority of other antibiotics (except the polymyxins or tigecycline) [45,92]. Imipenem resistance was first described in 1985 and since then carbapenem resistance in *A. baumannii* became increasingly common [87]. Carbapenem resistance are mainly due to the production of chromosome- or plasmid-encoded carbapenemases [87].

### 9.2 $\beta$ -lactamase inhibitors

The discovery of  $\beta$ -lactamase inhibitors is a promising strategy to combat  $\beta$ -lactam resistance [88]. The inhibitors targeting the  $\beta$ -lactamase induction pathway may prevent the emergence of  $\beta$ -lactam resistance and enhance the efficacy of clinical  $\beta$ -lactam antibiotics [88]. Combinations of penicillins with  $\beta$ -lactamase inhibitors also play prominent therapeutic roles, with amoxicillin-clavulanic acid being a major factor in the treatment of community infections and piperacillin-tazobactam being important for serious hospital-acquired infections [95]. Sulbactam (a  $\beta$ -lactamase inhibitor) in combination with ampicillin are used for the treatment of MDR *A. baumannii* infections, such as meningitis, ventilator-associated pneumonia, catheter-related bacteraemia and respiratory tract and urinary tract infections [11,20,92]. The optimal dose to treat serious *A. baumannii* infections is unknown but the recommended dose is 6 g per day for patients with normal renal function [92]. The use of these drugs is also compromised due to resistance [11].

### 9.3 Aminoglycoside antibiotics

Aminoglycosides are used in the treatment of a broad range of life-threatening infections [56]. This antibiotic is polycationic molecules and their positive charge interacts with the negatively charged nucleic acids [68]. This antimicrobial acts by causing translational errors and by inhibiting translocation [68,69]. This antimicrobial class bind to a highly conserved motif of 16S rRNA and this leads to alterations in the ribosome functions [68,96]. Amikacin (not used in our clinical setting) and tobramycin are the two agents that appear to retain activity against many *A. baumannii* isolates [46,92]. However, aminoglycosides are not often used as single agents for treatment and the toxicity profiles often hinder their use, especially for longer treatment courses [92]. Aminoglycosides are used in combination with broad spectrum  $\beta$ -lactams to treat Gram-negative bacterial infections [71].

### 9.4 Tetracycline antibiotics

Minocycline and doxycycline are used for *Acinetobacter* infections (again these antimicrobials are not used in our clinical setting) [92]. Clinical data is limited for minocycline and doxycycline [17,92]. However, minocycline has been found active against *A. baumannii* resistant to tetracycline or doxycycline [17].

### 9.5 Quinolone antibiotics

Other treatment options include quinolones, such as ciprofloxacin and levofloxacin, which induce DNA double-strand breaks by trapping the DNA gyrase and/or topoisomerase IV on the DNA and this leads to fragmentation [76,87]. Quinolones have been widely used in clinical medicine because of their broad efficacy and immuno-modulatory activities [97].

### 9.6 Polymyxin antibiotics

The polymyxins include colistin (polymyxin E) and polymyxin B and are used for the treatment of highly drug-resistant Gram-negative bacteria [92]. Intravenous polymyxins can also be used to treat ventilator-associated pneumonia [11,17]. Colistin has been used with success to treat severe infections, such as bacteraemia, intra-abdominal infection, meningitis, pneumonia, sepsis, urinary tract infections and wound infections [16,20,45]. There are reports that colistin have relatively poor lung and cerebrospinal fluid (CSF) distribution and the clinical outcomes vary for different types of infections [45].

Major side effects for this antibiotic class include nephrotoxicity and neurotoxicity [16,92]. Nephrotoxicity is a particular issue for patients with pre-existing renal impairment, elderly patients and patients who receive concomitant nephrotoxins [92]. Additional work is still required to determine the ideal dosing of intravenous colistin to maintain efficacy and minimise toxicity [92].

### 9.7 Glycylcycline antibiotics

Tigecycline is the first of a new class of antibiotics known as the glycylcyclines [92,98]. It is a semi-synthetic derivative of minocycline and inhibits the 30S ribosomal subunit [17,56,92,98]. Tigecycline has a broader spectrum of activity (compared to tetracyclines) and also have the ability to evade the traditional tetracycline specific resistance mechanisms [17,92]. Tigecycline is used for the treatment of complicated skin and soft-tissue infections and intra-abdominal infections [20,99]. Despite tigecycline's *in vitro* activity against *A. baumannii*, clinical data remains limited [92,99]. For the treatment of MDR *A. baumannii* infections, tigecycline can be used in combination with levofloxacin, amikacin, imipenem and colistin [45,99].

Tigecycline and colistin (polymyxin E) are regarded as the last resort antibiotics in the control of clinical infections caused by MDR *A. baumannii* [45,74,98,100]. Resistance to colistin and tigecycline have been described but is fortunately still rare [11,45].

### 9.8 Combination therapy for *A. baumannii* isolates

Given the lack of new antimicrobials, there has been considerable interest in the use of dual or even triple antimicrobial combinations [20]. Synergy is observed *in vitro* when colistin is combined with ceftazidime, imipenem or minocycline and when sulbactam is combined with meropenem [20]. Studies with tigecycline-containing combinations found synergy with amikacin, colistin, imipenem and levofloxacin but antagonism when tigecycline was combined with piperacillin/tazobactam [20].

Future work should include the use and efficacy of combination therapy as well as the development of novel therapeutic agents [7,29]. Targeting bacterial virulence is an alternative approach to antimicrobial therapy that offers promising opportunities in the treatment of *A. baumannii* infections [22,27].

## 10. Conclusion

Health care associated infections occur worldwide, with significant economic costs and mortality [93]. Treatment and management of *A. baumannii* has become a challenge due to the emergence of resistance to antibiotic agents [65,66,101,102]. There is a need to further investigate virulence factors and pathogenic mechanisms for more effective control measures in the clinical setting [7]. Future work should include the use and efficacy of combination therapy as well as the development of novel therapeutic agents [7,29]. Targeting bacterial virulence is an alternative approach to antimicrobial therapy that offers promising opportunities in the treatment of *A. baumannii* infections [22,27].

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