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Review

A plethora of carbapenem resistance in *Acinetobacter baumannii*: no end to a long insidious genetic journey

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Acinetobacter baumannii, notorious for causing nosocomial infections especially in patients admitted to intensive care unit (ICU) and burn units, is best at displaying resistance to all existing antibiotic classes. Consequences of high potential for antibiotic resistance has resulted in extensive drug or even pan drug resistant *A. baumannii*. Carbapenems, mainly imipenem and meropenem, the last resort for the treatment of *A. baumannii* infections have fallen short due to the emergence of carbapenem resistant *A. baumannii* (CRAB). Though enzymatic degradation by production of class D β-lactamases (Oxacillinases) and class B β-lactamases (Metallo β-lactamases) is the core mechanism of carbapenem resistance in *A. baumannii*; however over-expression of efflux pumps such as resistance-nodulation cell division (RND) family and variant form of porin proteins such as CarO have been implicated for CRAB inception. Transduction and outer membrane vesicles-mediated transfer play a role in carbapenemase determinants spread. Colistin, considered as the most promising antibacterial agent, nevertheless faces adverse effects flaws. Cefiderocol, eravacycline, new β-lactam antibiotics, non-β-lactam-β-lactamase inhibitors, polymyxin B-derived molecules and bacteriophages are some other new treatment options streamlined.

Keywords: *A. baumannii*, carbapenem, metallo β-lactamase, oxacillinase, efflux pump, porin, treatment options

Introduction

Acinetobacter baumannii is an eminently known pathogen for instigating hospital-associated infections (HAI) including ventilator-associated pneumonia (VAP), surgical-site infections, urinary tract infection (UTI) and secondary meningitis. Though all kind of in-patients are prone to get such infections nevertheless, immunocompromised patients, especially those admitted in intensive care unit (ICU) and burn units are more vulnerable.[1–3]

Aptitude of *A. baumannii* to survive on dry surfaces under nutrient limiting conditions bring about colonization on medical devices and equipments through biofilm formation that could serve as reservoirs in hospital outbreaks. In fact, *A. baumannii* demonstrates increased tolerance to extracellular stressors when part of biofilm communities. Various infections including skin and soft-tissue wounds and even occlusive dressings have been shown to support biofilm-forming *A. baumannii*.[4] The research study conducted on proteins involved in biofilm formation by *A. baumannii* showed that several cell surface proteins (like CarO, OmpA, OprD-like, DcaP-like, PstS, LysM, Omp33), as well as those involved in histidine metabolism (like Urocanase) have been implicated in biofilm formation and among these, urocanase plays a crucial role leading to biofilm formation. OmpA and CarO can act as channels for L-His uptake.[5] Many virulence factors have been involved in bacterial cell adherence, however the

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plasticity observed in *A. baumannii* genomes leads to significant strain specific variations in biofilm formation. Biofilm associated genes observed in *A. baumannii* clinical isolates include the most highly conserved gene *CsuE*, the proposed tip subunit of the chaperone-usher pili (*Csu*), and *OmpA* followed by biofilm-associated protein (Bap) and class A extended β-lactamase $bla_{\text{PER-1}}$ enzyme.[6]

Prolonged period of stay in ICUs along with extensive mechanical modalities and previous antimicrobial therapy are also considered key factors to predispose patients to *A. baumannii* infections.[7–9] Community-acquired (CA) infections caused by *A. baumannii* are not far off. CA pneumonia due to *A. baumannii* has been identified in tropical regions of Australia and Asia during the rainy season in people who have a history of alcohol abuse or have chronic obstructive pulmonary disease.[1–3] Amazingly, the organism which was once the frequently ignored bacterium in most clinical specimens and considered to be a commensal of low-grade pathogenicity has gained fame in the last 20 years, a fact attributed to the worldwide expansion of ICUs and undoubtedly the emergence of resistant strains.[8,10] Year 2004 observed spike in *A. baumannii* bloodstream infections in patients at military medical facilities in which service members injured in the Iraq/Kuwait region during operation Iraqi freedom (OIF) and in Afghanistan during operation enduring freedom (OEF) were treated. The number of these infections and their resistance to multiple antimicrobial agents underscored the importance of infection control during treatment in health-care settings and raised the need to develop new antimicrobial drugs.[11] It eventually dawned on World Health Organization (WHO) to place the organism in the "ESKAPE pathogens" list in the year 2018 against which new antibiotics are urgently needed.[12,13]

Antimicrobial resistance and carbapenems status

Besides survival in the environment for prolonged period of time, resistance of *A. baumannii* to multiple antimicrobial drug classes has made it a suitable candidate to persist in hospitals and retain endemicity as health-care setting pathogen. Carbapenems have been widely used to treat these infections, but a trend of increasing resistance is drastically limiting the range of therapeutic alternatives.[14] WHO warns carbapenem resistance as national and international concern as they are an emerging cause of HAI that pose a significant threat to public health.[15] To date, the spread of multidrug-resistant *A. baumannii* has been mostly through the acquisition of plasmids, transposons or integrons that carry cluster of genes leading to the emergence of resistance to several antibiotic families. Different terminologies like multidrug-resistant (MDR), extensive-drug resistant (XDR), and pandrug resistant (PDR) have been used to describe the degree of antimicrobial resistance for *A. baumannii*.[16] MDR *A. baumannii* refers to bacterium being resistant to a minimum of three classes of antimicrobial drugs e.g. all penicillins and cephalosporins, fluoroquinolones and aminoglycosides. When MDR *A. baumannii* show additional resistance towards carbapenems but retain susceptibility to polymyxins and tigecycline, the organism is defined as XDR. Finally, PDR *A. baumannii* is a term given to the XDR *A. baumannii* that is resistant all antibiotics including polymyxins and tigecycline.[16,17]

The emergence of MDR *A. baumannii* strains has been attributed to its rapid ability to accumulate resistance mechanisms as well as being well suited for genetic exchange. Therefore, this bacterium belongs to a unique class of Gram-negative bacteria that are characterized as naturally transformable.[17,18]

Recently, in a carbapenem-susceptible and extended spectrum β-lactamase (ESBL) producing *A. baumannii* clinical isolate, the largest antibiotic resistance island with more than 40 resistance genes have been identified, demonstrating the genetic plasticity of *A. baumannii* which renders it capable of benefitting from a variety of resistance mechanisms when antibiotic pressure is constant. The high genetic plasticity of *A. baumannii* allows an accumulation of resistance determinants that give rise to multidrug-resistance at an alarming rate.[18,19]

Carbapenem resistance is an on-going concern as carbapenems, including imipenem and meropenem, had a potent activity against *A. baumannii* and were often used as the last resort for the treatment of infections caused by MDR *A. baumannii*. Carbapenems have a good bactericidal activity, are stable towards a range of β-lactamases, possess broad-spectrum activity and a good safety profile.[20,21] The first carbapenem discovered was olivanic acid produced by *Streptomyces olivaceus*. This was followed by the discovery of thienamycin in 1976. Years later, a more stable thienamycin derivative known as imipenem was synthesized and approved for use in 1984. Other carbapenems for parenteral administration were discovered later and included biapenem, panipenem, lenapenem and ertapenem. Carbapenems are recommended for the empirical treatment of a variety of severe infections and they are generally well tolerated in the human body except certain treatable allergic reactions.[21,22] In parallel with the increase in carbapenem use and increase in *A. baumannii* infections there has been an increase in the rise of not only carbapenem resistance, but also resistance towards majority of

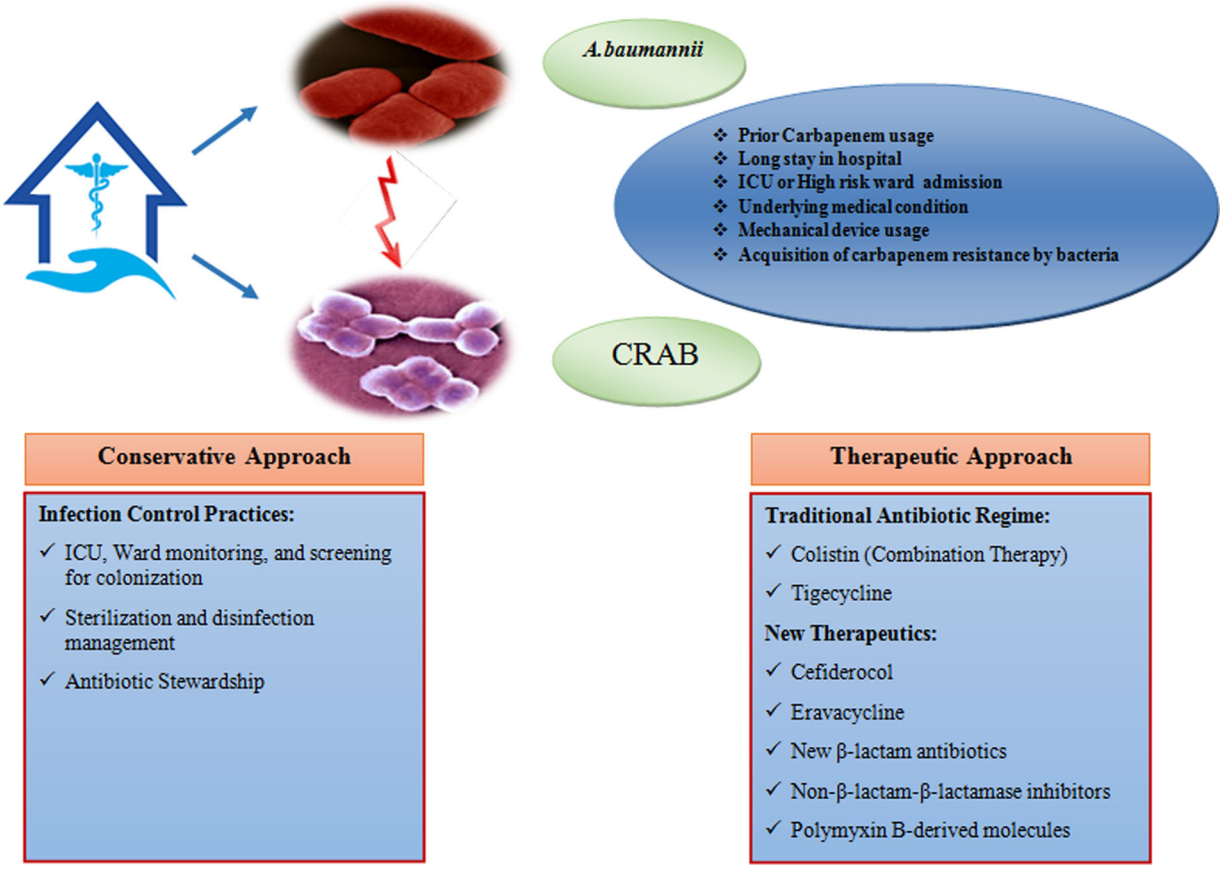

Figure 1. Approaches to confront *A. baumannii* infections in hospital setting.

other antibiotics (except the polymyxins or tigecycline). Imipenem resistance was first described in 1985 and since then carbapenem resistance in *A. baumannii* became increasingly common.[20–22]

Numerous other medical and environmental factors have been responsible to alter an opportunistic bacteria to carbapenem resistant *A. baumannii* (CRAB). Several conservative and therapeutic modalities have also been postulated to overcome the emerging CRAB situation (Figure 1).

Antibiotic resistance mechanisms

A. baumannii has rapidly developed as a multidrug-resistant pathogen after attaining and upregulating substantial resistance mechanisms. The most prevalent *A. baumannii* MDR determinants includes acquisition of genes for efflux pumps, production of class B β-lactamase (metallo-β-lactamase), class C chromosomal β-lactamase (AmpC), class D β-lactamase (Oxacillinase), integrons and associated insertion sequence (IS) elements.[1,2] Penicillin and cephalosporin resistance is usually due to class C chromosomal β-lactamase AmpC. Carbapenem resistance in *A. baumannii* is mediated by the acquisition of a class B or a class D β-lactamase.[3,23] The increased expression of above mentioned three enzymes has been linked to IS*Aba1*, an insertion sequence that is widely distributed in *A. baumannii*.[9,24] Many published reports have focused on the role of efflux pumps in MDR Gram-negative bacteria.[25,26] Three *A. baumannii* efflux pumps (AdeABC, AdeIJK and AbeM) reportedly confer resistance against a wide range of antibacterial agents including β-lactams, aminoglycosides, tetracyclines, fluoroquinolones, chloramphenicol and trimethoprim. Moreover, all major resistance mechanisms reported in other Gram-negative bacteria have also been identified in *A. baumannii* such as modifications of target site, active efflux pumps, enzymatic degradation of drugs and decreased influx.[17–19] This has rendered all current major antibacterial agents such as penicillins, cephalosporins, aminoglycosides and quinolones as an inefficient treatment options for *A. baumannii* infections.[27,28]

The mostly reported recent studies on *A. baumannii* point to natural transformation, transduction and outer membrane vesicles (OMVs) -mediated transfer as mechanisms that may play a role in carbapenemase determinants spread.[29] A published research study demonstrated that the spread of antimicrobial resistance genes (ARGs) among *A. baumannii*, is primarily mediated by transferable plasmids; however, ARGs are frequently integrated into its chromosome. Mechanisms that make the DNA transfer across bacterial cells without cell-cell interaction is

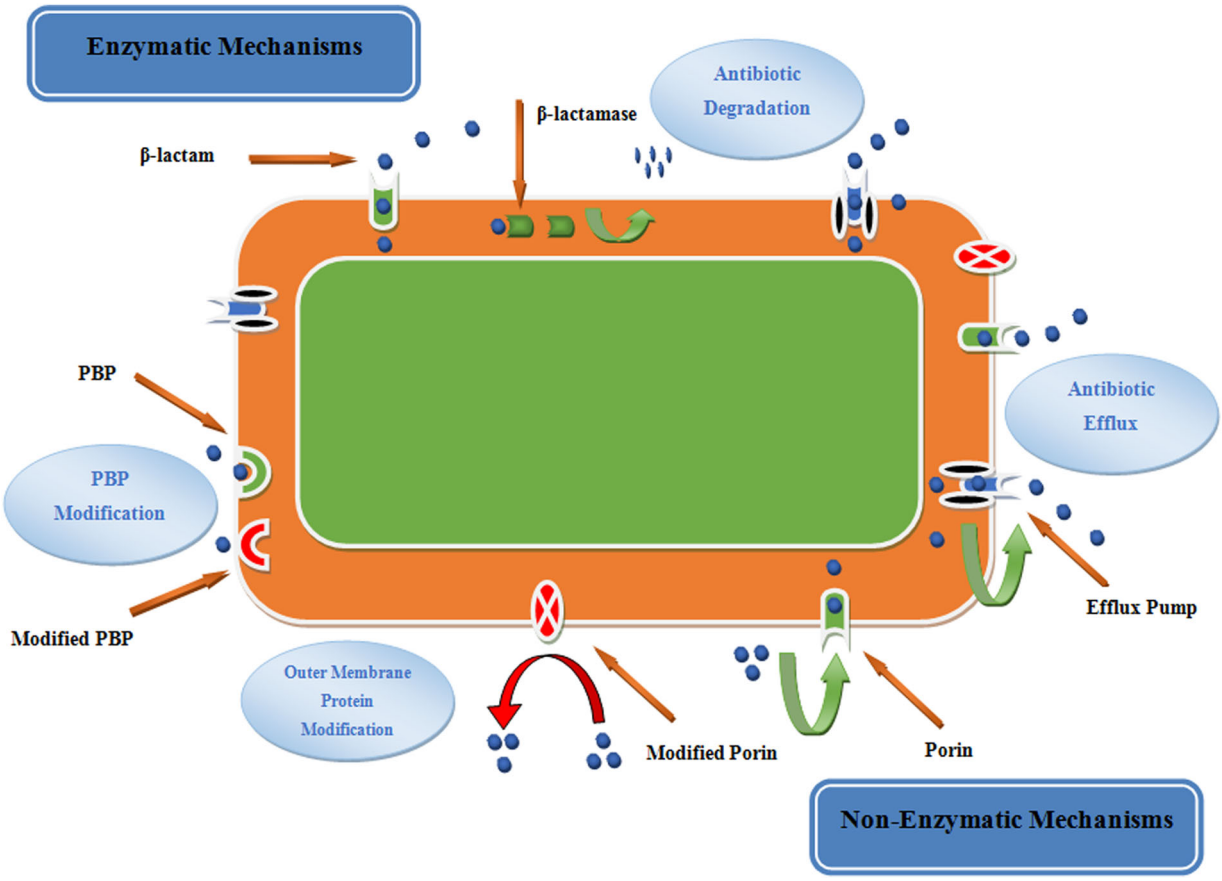

Figure 2. Mechanisms of Carbapenem Resistance in *A. baumannii*.

associated with OMVs.[30] In fact, transfer of bla_{NDM} and bla_{OXA-23} has been described by OMVs.[31,32] Wachino et al (2019) in their study emphasized that the drug resistance in *A. baumannii* occurs by specific prophages that are hidden in the chromosome of MDR and mediate the transfer of a variety of chromosomal ARGs.[30] The ARG transfer is mediated by a generalized transduction mechanism in which shared DNA (including the ARGs) packed in phage particles is discharged by phage-lysis and is transferred to the recipient *A. baumannii* strain. Surprisingly, the advantage offered by the protection of ARGs in phage particles is a subject of concern in a clinical setting as MDR *A. baumannii* strains can disseminate eDNA across the microbial environment without direct cell-to-cell interaction. Another published literature shows the phage-mediated transduction of bla_{NDM} from *A. baumannii* strain R2090 to the recipient.[33] From the four known mechanisms for horizontal gene transfer (conjugation, outer membrane vesicle-mediated transfer, transformation, and transduction), the above–mentioned study ruled out conjugation mechanism on the basis that strain R2090 lacked any plasmid, and a type IV secretion system was not encoded in its chromosome. However, strain R2090 possessed three putative prophages, two of which were predicted to be complete and therefore functional. Accordingly, it was supposed that the transfer of the resistance gene region from the clinical isolate R2090 to the recipient occurred by general transduction facilitated by one of the prophages Bϕ-B1251 present in the R2090 genome. Later, the study found that this strain possessed three putative prophages and one chromosomal segment with phage integrase gene and insertion sequence (IS) elements.

Another hypothesis which emerged for dissemination of A. baumannii was presence of bla_{NDM-1} gene in mobile transposon Tn125.[34] This study suggested that bla_{NDM-1} from Acinetobacter spp. has transferred by horizontal transfer to Enterobacteriaceae and *P. aeruginosa*.

A recent study conducted on MDR *A. baumannii* suggested prophages are commonly found in different lineages *A. baumannii*.[35] Badawy et al. (2020) studied morphological characteristics of *A. baumannii* prophages by transmission electron microscopy, obtained the genomic information, and revealed that the phages belong to the family Siphoviridae.[36]

The summation is that interplay of several enzymatic and non-enzymatic resistance mechanisms in *A. baumannii* has illuminated an escalating antibiotic resistance. Carbapenem resistance in this organism can be attributed mainly due to

acquisition of metallo-β-lactamases and oxacillinases enzymes, changes in outer membrane proteins, modifications of penicillin-binding proteins and efflux pumps along with presence of prophages.[28,37] (Figure 2)

Enzymatic mechanisms (carbapenem-hydrolyzing β-lactamase enzymes)

The most prevalent mechanism of β-lactam resistance in *A. baumannii* is enzymatic degradation by β-lactamase enzymes, resulting in the alteration of β-lactam agent structure. However, in keeping with the complex nature of this organism, multiple mechanisms often work in concert to produce the same phenotype β-lactamases. β-lactamase enzymes of Ambler class A, C, and D have at their active site a serine residue but class B enzymes utilize zinc ions to attack the β-lactam ring.[19,21,37]

The class A β-lactamase enzymes (ESBLs) have the ability to hydrolyze a broad spectrum of antibiotics including third generation cephalosporins, penicillins, carbapenems and monobactams. They are inhibited by β-lactamase inhibitors such as clavulanic acid and tazobactam.[38] The expression of ESBLs in *A. baumannii* may contribute significantly to its resistance to extended-spectrum β-lactams and to the increasingly observed multidrug-resistance profile in this species. The prevalence of the class A β-lactamases in *A. baumannii* may not be a very major problem in comparison to other carbapenemases, however the potential of this species to act as a reservoir for mobile resistance genes in the hospital may be alarming.[21,38,39]

The class C β-lactamase enzymes (AmpC) are commonly found encoded on the chromosome in Gram-negative organisms.[40] In *Acinetobacter* spp. these genes are known as *Acinetobacter* derived cephalosporinases (ADC). These enzymes are able to hydrolyze penicillins, the narrow-spectrum cephalosporins and when over-expressed can confer resistance to the extended-spectrum cephalosporins. The widespread nature of the bla_{ADC} genes is the major reason for high levels of resistance in *A. baumannii* to the penicillins and cephalosporins and as such these drugs are generally not effective for treatment of this organism.[21,38,40]

Metallo-β-lactamases (MBLs)

Ambler Class B β-lactamase are also known as metallo-β-lactamases (MBLs) capable of hydrolyzing carbapenems and other β-lactam antimicrobials with the exception of monobactams (aztreonam). Metallo-β-lactamase enzymes are only active in the presence of metal ions, like zinc ion but are inhibited by metal chelators like ethylene-diamine-tetra-acetic acid (EDTA).[41] Though carbapenem resistance in this bacteria is most often linked to the production of carbapenemases however, metallo-β-lactamase enzymes are not the most commonly identified carbapenemase in *A. baumannii*. MBLs are usually encoded on the gene cassettes harboring class I integron and disseminated easily in bacterial populations.[42] Four examples of MBLs are known in *A. baumannii*, including New Dehli Metallo-β-lactamase (NDM), Imipenemase (IMP), Seoul Imipenemase (SIM) and Verona Integron-encoded Metallo-β-lactamase (VIM).[43–45] Class I integron has been responsible for transferring the gene cassettes harboring MBLs, especially the bla_{VIM} and bla_{IMP} allelic variants suggesting the class I integron has the important role in the horizontal transfer of gene cassettes encoding MBLs.[42,46]

Carbapenem-hydrolyzing oxacillinase (OXAs)

Class D β-lactamases, also known as OXA-type enzymes or oxacillinase or carbapenem hydrolyzing class D β-lactamases (CHDLs), are represented by more than 350 genetically diverse enzymes.[47] Class D β-lactamases (OXAs) uses a catalytically active serine residue for the inactivation of the β-lactam antimicrobials, particularly carbapenems.[48] Among the four β-lactamase molecular classes, class D β-lactamases are the most diverse enzymes, the diversity being observed at both the genetic and biochemical levels. These enzymes are broadly classified into narrow and extended-spectrum enzymes based upon the conferred resistance profile against β-lactam antibiotics. Even though class D includes mostly enzymes with higher hydrolysis rates for cloxacillin and oxacillin than for benzylpenicillin (hence the name oxacillinases), not all class D β-lactamases have this characteristic.[49] The OXA-2 and OXA-10 β-lactamases exemplify the narrow-spectrum enzymes capable of producing resistance to penicillins and some early cephalosporins, nevertheless evidence has been presented that these two β-lactamases, currently regarded as non-carbapenemases, have catalytic efficiencies against carbapenems similar to those well-recognized CHDLs and are capable of conferring resistance to these last-resort antibiotics when expressed in *A. baumannii*. Both narrow and extended spectrum enzymes, however, can extend their substrate profile to produce resistance to extended-spectrum cephalosporins such as ceftazidime, by accumulating one to several amino acid substitutions.[47,49]

Class D carbapenemases represent a further expansion of the substrate profile of class D enzymes to include carbapenem antibiotics. Based on their amino acid sequence identity, CHDLs have been subdivided into several subgroups.

Enzymes belonging to the OXA-23, OXA-24/40, OXA-48, OXA-51, OXA-58 and OXA-143 subgroups are of major clinical importance due to their wide dissemination in bacterial pathogens. The majority of these carbapenemases, except for OXA-48, have been identified in various *Acinetobacter* spp., predominantly in *A. baumannii*.[47]

Most of class D β-lactamases are not inhibited by β-lactamase inhibitors such as clavulanic acid, tazobactam, sulbactam, cloxacillin or zinc chelators (with some exceptions; e.g., OXA-2 and OXA-32 are inhibited by tazobactam but not sulbactam and clavulanate and OXA-53 is inhibited by clavulanate), but interestingly sodium chloride (NaCl) at concentrations of (> 50 to 75 mM) do inhibit some carbapenem hydrolyzing oxacillinases (e.g., OXA-25 and OXA-26).[48,50,51] Site-directed mutagenesis studies suggest that susceptibility to inhibition by NaCl is related to the presence of a (Tyrosine-144) which presumably, may facilitate sodium chloride binding better than the phenylalanine residue found in resistant oxacillinases, although the molecular mechanism remains unexplained.[48]

In fact, the CHDL carbapenemases in *A. baumannii* can be divided into four subfamilies. The first described OXA-type enzyme in *A. baumannii* was ARI-1 (*Acinetobacter* Resistant to Imipenem), obtained from a clinical strain isolated in 1985 from Edinburgh, Scotland. The ARI-1 was encoded on a transferable plasmid and designated as OXA-23. Together with OXA-27 and OXA-49, the first gene cluster of OXA genes ($bla_{\text{OXA-23-like}}$) was defined in *A. baumannii*.[52–54]

The second cluster of OXA enzymes consists of OXA-24 family enzymes (OXA-24, OXA-25, OXA-26, OXA-40 and OXA-72) sharing less than 60% amino acid identity with OXA-23. OXA-24 type enzymes can be either chromosomal or plasmid-encoded.[52,55]

The third cluster consists of OXA-51 family enzymes (OXA-51, OXA-64 to −66, OXA-68 to −71, OXA-75 to −78, OXA-83, OXA-84, OXA-86 to −89, OXA-91, OXA-92, OXA-94 and OXA-95) which are encoded by $bla_{\text{OXA-51-like}}$ genes and are naturally occurring in *A. baumannii*. This cluster of OXA β-lactamases shares less than 63% amino acid identity with OXA-23 and OXA-24 enzymes and are chromosomally encoded.[52,56]

The fourth cluster of OXAs consist of OXA-58, shares less than 50% amino acid identity with other OXA enzyme.[57,58] A novel carbapenem resistance determinant has been identified that was transferable at least between *A. baumannii* strains. This novel CHDL, OXA-143, is the first representative of a novel subclass of CHDLs, even though it is related to OXA-40 enzymes, whose prevalence remains to be determined. OXA-143 hydrolyzes penicillins and carbapenems but not significantly hydrolyze extended-spectrum cephalosporins, as observed with other CHDLs. Despite this weak hydrolysis, it is very likely that OXA-143 significantly contributes to resistance to imipenem and meropenem, as demonstrated previously with OXA-23, OXA-40 and OXA-58.[59] Three other novel CHDLs identified in *A. baumannii* are OXA-235, OXA-236 and OXA-237, which were identified in isolates originated primarily from United States. The $bla_{\text{OXA-235-like}}$ variants encoded on plasmids were isolated from multiple geographical regions and they were able to transform into laboratory strains and reduce carbapenem susceptibility. The expression of OXA-235 in *A. baumannii* led to reduced carbapenem susceptibility, while cephalosporin MICs were unaffected.[60]

Although many class D β-lactamase genes are embedded into class I integrons, indicated that other specific genetic structures, including IS elements such as IS*Aba1* and transposons, may be associated with class D β-lactamase genes.[49] In the absence of IS*Aba1* element, cloning studies suggest a minimal effect on carbapenem susceptibility, even in the presence of an overexpressed multidrug efflux pumps.[61] Most commonly, these elements have been described in association with OXA-23 and OXA-58, but they may also promote carbapenem resistance in association with OXA-51.[61,62] Numerous class D β-lactamase genes have been identified as a source of acquired resistance in Gram-negative bacteria, but recent studies have shown that class D β-lactamases are also naturally produced in clinically significant pathogens and environmental species.[63] Though MBLs, which being less commonly identified in *A. baumannii* than the OXA-type carbapenemases however, their hydrolytic activities toward carbapenems are significantly more potent (100 to 1,000-fold). These enzymes have the capability of hydrolyzing all β-lactams (including carbapenems) except the monobactam aztreonam.[38,49] Prevalence and mechanism of imipenem resistance has not been same in various studies conducted all over the world however, higher prevalence rate of carbapenem hydrolyzing enzymes is a concern.[45,64–77] (Table 1)

Non-enzymatic mechanisms

Resistance to carbapenems in *A. baumannii* may be enhanced by interactions between β-lactamases and other resistance mechanisms, including porin(s) loss, active drug efflux, and (rarely) modification of penicillin-binding proteins (PBPs).[38] Decreased

Table 1. Prevalence of carbapenem hydrolyzing enzymes in *Acinetobacter baumannii*.

Author	Region	Year	Imipenem resistance	Mechanism	Reference
Aksoy et al.	Turkey	2013	100%	OXA-23: 100% OXA-24: Negative OXA-58: Negative MBL: Negative	45
Al-Arfaj et al.	Saudi Arabia	2011	65%	OXA-23: 72.5% OXA-24: 45% OXA-58: 37.5%	52
Elabd et al.	Saudi Arabia	2015	51.9%	OXA-23: 85.7% OXA-24: 5.4% OXA-58: 3.6%	65
Al-Agamy et al.	Egypt	2014	85%	OXA-23: 50% OXA-24: 7.5% OXA-58: 7.5% MBL: Negative	66
Amr et al.	Egypt	2016	75%	OXA-23: 85.7% OXA-24: Negative MBL: Negative	67
Carvalho et al.	Brazil	2009	99.1%	OXA-23: 87.3% OXA-24: Negative OXA-58: Negative MBL: Negative	68
Vilallon et al.	Spain	2012	100%	OXA-23: Negative OXA-24: 57.6% OXA-58: 20.3% MBL: Negative	69
Lean et al.	Malaysia	2014	74.1%	OXA-23 :75.9% OXA-24: Negative OXA-58: Negative MBL: Negative	70
Sohrabi et al.	Iran	2012	62%	OXA-23: 88.7% OXA-24: 1.6% OXA-58: 3.2%	71
Savari et al.	Iran	2017	75.8%	OXA-23: 83.7% OXA-24: 12.2% OXA-58: Negative MBL: Negative	72
Al-Sweih et al.	Kuwait	2012	42.6%	OXA-23: 72.5% OXA-24: Negative OXA-58: Negative MBL: 27.5%	73
Chang et al.	China	2015	60.9%	OXA-23: 80.6% OXA-24: Negative OXA-58: Negative MBL: Negative	74
Khorsi et al.	Algeria	2015	72.5%	OXA-23: 67% OXA-24: 20.2% OXA-58: Negative MBL: Negative	75
Cherkaoui et al.	Italy	2015	100%	OXA-23: 51.8% OXA-24: 7.4% OXA-58: 14.8% MBL: 11.1%	76
Nowak et al.	Poland	2012	100%	OXA-23: 44.2% OXA-24: 46.15% OXA-58: Negative	77

expression of certain porins associated with antimicrobial resistance in *A. baumannii*, including several outer membrane proteins (OMPs) that have some homology with the monomeric OmpA porin found in Enterobactericeae. Porins of this family have been characterized in several species of *Acinetobacter*.[78] Also, efflux-mediated resistance is a common factor affecting antibiotic susceptibility in Gram-negative bacteria and several efflux pumps have been described in *A. baumannii*.[79,80]

Porins

Porins are proteins possessing the ability to form channels to allow the transport of molecules across lipid bilayer membranes while, displaying little permeability for hydrophilic solutes. They are diverse

and divided into general, specific and iron porins depending upon their functional ability.[38,79,81] Amongst them general porins play a more pertinent role in antibacterial susceptibility and resistance. Variations in their structure as a mechanism to escape from the antibacterial pressure or regulation of porin expression in response to the presence of antibiotics are survival strategies that have been developed by many bacteria. The small number and size of porins could explain the decrease in *A. baumannii* outer membrane permeability (less than 5%) when compared with other Gram-negative organisms. The outer membrane in *A. baumannii* is less permeable to antimicrobial agents than that in *Escherichia coli*. Thus, it has been suggested that the intrinsic cause of the resistance to antimicrobial agents could be attributed to the small number of porins as well as their small size.[79] Porin loss has been found to significantly contribute to resistance to carbapenems. Several reports have associated decreased expression of certain porins with antimicrobial resistance in *A. baumannii*, including several OMPs that have some homology with the monomeric OmpA porin found in Enterobactericeae. A tough outer membrane, low numbers of porin proteins and several other constitutive characteristics such as efflux pumps play a role in behavior of *A. baumannii* with regards to the capacity of resistance and survival in the environment. The small number and sizes of the different porins when coupled with efflux provide a significant barrier to the uptake of antibiotics in *A. baumannii*.[38,82,83]

The major outer membrane protein in *Acinetobacter* spp. is heat modified protein-AB (HMP-AB), a member of the OmpA-like family and is a heat-modifiable protein. Sequence comparison of HMP-AB with other OMPs revealed a clear homology with the monomeric OmpA of Enterobactericeae and the OprF of *Pseudomonas aeruginosa*.[78,84] This porin belongs to the OmpA family known as slow porins that allow the penetration of β-lactams and saccharides up to approximately 800 Da. Slow porins with significantly lower efficiency allow a much slower diffusion of small solutes but allow the diffusion of much larger solutes that cannot penetrate through the OmpF channel of *E. coli*. Therefore, in organisms that lack the classical trimeric porin, the protein of this family functions as the major porin and contributes to the high levels of intrinsic resistance.[85,86]

Three other OMPs that have been reported to be missing in the imipenem-resistant strains of *A. baumannii* are 33-36 kDa protein, 29 kDa protein also known as designated CarO and a 43 kDa protein, which shows significant peptide homology with OprD from *P. aeruginosa*.[79] Another OMP in *A. baumannii* is OmpW, which shows high homology with OmpW found in *E. coli* and *P. aeruginosa*. Its function in *A. baumannii* remains unclear however, researchers have found decreased expression of this OMP in an *in vitro* colistin-resistant *A. baumannii* mutant.[38,79]

33-36 Kda protein

The 33-36 kDa outer membrane protein harbors an amino acid sequence and composition typical of Gram-negative porins and has been implicated in carbapenem resistance in clinical strains.[87] Decreased levels of the 33-36 kDa outer membrane protein have been related to carbapenem resistance in *A. baumannii* while, over-expression of the gene encoding this OMP restored a β-lactam sensitive phenotype to the previously resistant isolate.[88,89]

CarO protein

Resistance to imipenem and meropenem in multidrug-resistant *A. baumannii* clinical isolates has also been associated with loss of a heat-modifiable 29-kDa OMP, designated CarO. This is a heat-modifiable protein which co-migrates on SDS-PAGE gels with the HMP-AB protein and when heated, its size alters from 25 to 29 kDa.[79] The lack of CarO in different carbapenem-resistant clinical isolates of *A. baumannii* resulted from the disruption of CarO by distinct insertion elements, supporting the hypothesis that CarO participates in the influx of carbapenem antibiotics in *A. baumannii*.[38,83] The loss or disruption of this protein results in carbapenem resistance has been indicated by other studies but no binding site for imipenem has been identified suggesting that it might function as a nonspecific monomeric channel.[88,90] Studying CarO by mass spectrometry indicated that there is another 25 kDa protein together with CarO that they called Omp25 and both 25/29 kDa proteins adopted a typical β-barrel conformation. However, only one of these proteins (the CarO protein) displayed pore forming properties, but no binding site for imipenem was detected in CarO, suggesting a non-specific monomeric channel function rather than a specific function, as suggested previously.[79,89]

It is important to mention that *A. baumannii* possesses an OprD homologue (a porin, known to be involved in carbapenem resistance in *P. aeruginosa*). It was demonstrated that the imipenem-susceptible *A. baumannii* isolates express naturally an OprD-like protein of 43 kDa which could be modulated by the addition of basic amino acids. Thus, as observed in *P. aeruginosa*, in which the OprD

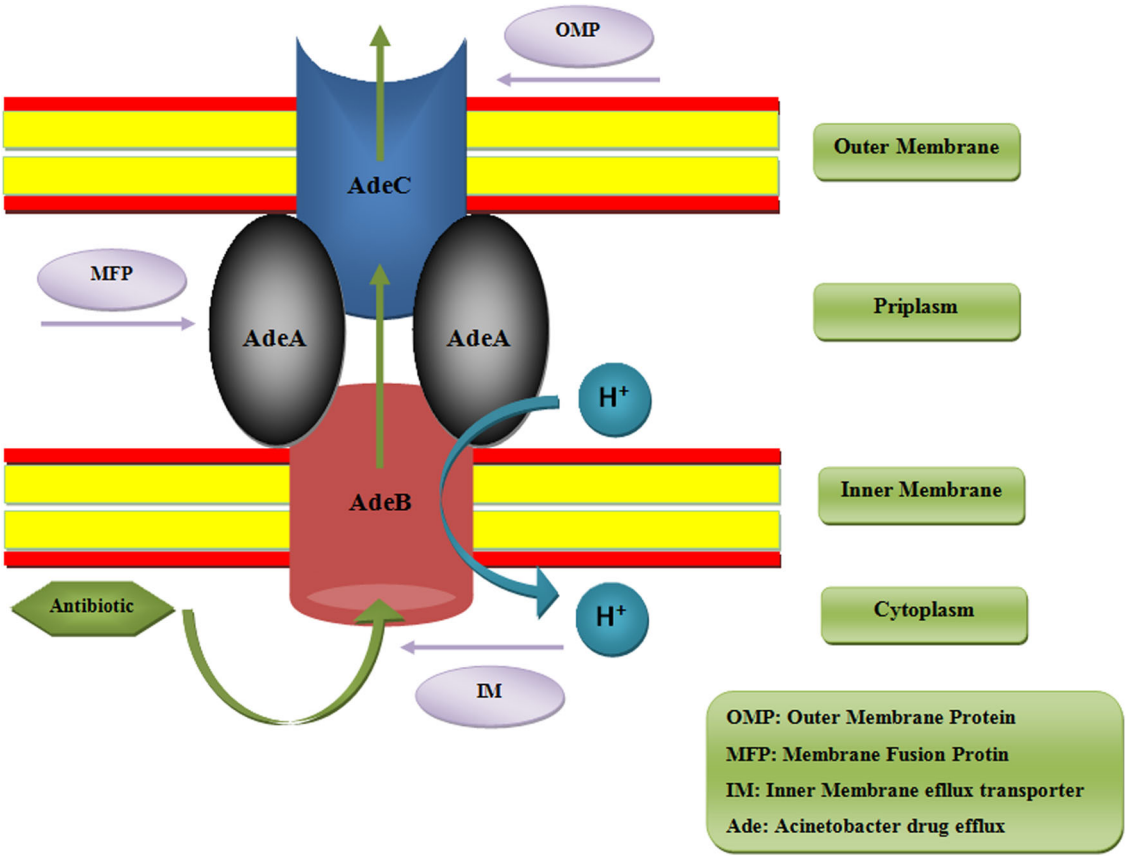

Figure 3. Schematic representation of tripartite RND efflux pump.

porin has a major role in imipenem resistance and is also involved in basic amino acid uptake, carbapenem-susceptible *A. baumannii* isolates express naturally an OprD-like protein of 43 kDa.[79,90] Therefore, CarO may function as a carbapenem-unspecific channel and the OprD-like protein may function as a carbapenem-specific channel.[38,79] It has also been reported that resistance to carbapenems might be associated with reduced expression of two proteins (22 and 33 kDa) in a multi resistant *A. baumannii* isolate that also produced the CHDL OXA-24, so that both mechanisms (carbapenemase production and decreased permeability), could be responsible jointly for the high-level carbapenem resistance observed.[38,79,90]

Efflux pump

Possession or acquisition of efflux pumps stands out against other antibiotic resistance mechanisms in *A. baumannii* making the antimicrobials to be excreted out leading to a reduction in drug accumulation and an increase in minimum inhibitory concentrations (MICs).[91–93] Efflux pumps have multifactorial roles and are important for detoxification of intracellular metabolites, bacterial virulence (in both animal and plant hosts), intercellular signaling and trafficking and cell homeostasis.[94] Multidrug efflux pumps are generally chromosome-encoded and their expressions often result from mutations in regulatory genes. However, drug-specific efflux pumps are encoded by mobile genetic elements whose acquisition is sufficient for resistance.[91] According to the homology of amino acid sequence, five super families of efflux systems are closely related to drug resistance: (i) the adenosine triphosphate (ATP)-binding cassette family (ABC); (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 family (RND). Among the efflux systems, RND pumps are the most prevalent systems in Gram-negative bacteria. It is likely that the RND efflux pumps (AdeABC, AdeIJK, AdeFGH, AdeXYZ and AdeDE) contribute to the virulence and fitness of *Acinetobacter* spp.[92,95]

RND efflux pumps

Efflux pumps of the RND superfamily play a significant role in producing multidrug resistance in Gram-negative bacteria, such as AcrB in *E. coli*, MexB in *P. aeruginosa* and AdeABC, AdeIJK, AdeFGH, AdeDE in *A. baumannii*.[96] These efflux pumps consist of three elements: (i) an inner membrane pump protein (ii) two large periplasmic loops and (iii) an outer membrane protein. Three RND systems, namely AdeABC, AdeFGH and AdeIJK have been associated with MDR in *A. baumannii*.[94]

The AdeABC and AdeFGH play a major role in acquired resistance, whereas the AdeIJK is responsible for intrinsic resistance.[38,97]

AdeABC efflux pump

AdeABC, the major and first identified RND efflux pump, is a kind of ATP-dependent multidrug transporter. This efflux pump expel antimicrobials utilizing the proton motive force.[97,98] The *adeABC* operon encodes *adeA* (Membrane Fusion Proteins, MFP), *adeB* (Inner Membrane Efflux Transporters, IM) and *adeC* (Outer Membrane Proteins, OMP) as contiguous genes. This operon is not expressed in *A. baumannii* natural isolates while, its overexpression results in multidrug resistant isolates. The *adeB* has been proposed to be an epidemiological tool for detecting clinical MDR strains.[99,100] It has been shown that AdeC is not essential for the MDR strains conferred by the pump, because an mutant displays similar resistance to the substrates of AdeABC to that of the parental strain when AdeC is inactivated but AdeC can expel antibiotics out of cell, because it is located in the epicyte and has porin as well.[101] AdeA, which stabilize OMP, allows antibiotics to pass the inner and the outer membranes of the bacteria without accumulating within the periplasm.[97,102] (Figure 3)

AdeABC is very common in both resistant and susceptible *A. baumannii* strains. Expression of *adeABC* is tightly regulated by the two-component regulatory system, *adeR-adeS*. These AdeS and AdeR are sensor kinase and response regulator, respectively.[101,103] They are located upstream of *adeABC* and are transcribed in the opposite direction. Mutations in *adeRS* have been shown to be responsible for constitutive expression of *adeABC*, whereas inactivation of *adeR* or *adeS* is responsible for suscepbility in *A. baumannii*, suggesting that AdeR functions as a transcriptional activator. Mutation in *adeR* (Pro-116→Leu) and *adeS* (Tyr-153→Met or Gly-30→Asp) cause overexpression of *adeABC*, and an insertion of an IS*Aba1* upstream from the operon can also lead to overexpression of *adeABC*, thereby enhancing efflux effect. Transcriptional activation could be due to disruption of *adeS* or to the bringing by IS*Aba1* of a strong promoter for *adeABC* expression.[102,103]

It has been reported that the *adeABC* overexpressing strains confer resistance to aminoglycosides and decrease susceptibility to fluoroquinolones, cefotaxime, tetracycline, chloramphenicol, erythromycin, trimethoprim, minocycline and tigecycline. Recent data suggest that this system decreased netilmicin susceptibility of *A. baumannii*. However, the role of *adeABC* in carbapenem resistance strains raises many controversies.[102,104]

Susceptibility testing with and without efflux pump inhibitors (EPI), such as [Carbonyl cyanide-m-chlorophenylhydrazone (CCCP)], [1-1-naphthylmethyl-piperazine (NMP)], [phenylalanine-arginine β-naphthylamide (PAN)] and reserpine showed no differences in carbapenem activity, whereas a 2 to 8-fold reduction in resistance was found in other work when an EPI was added, suggesting involvement of efflux.[97,104,105] Overexpression of *adeABC* contributes to significantly higher-level carbapenem resistance, notably to imipenem and meropenem, when associated with various class D carbapenemases. Thus, *adeABC* overexpression contributes to carbapenem resistance, but other efflux mechanisms are probably also involved. Efflux pump does not itself confer high-level resistance but weakly increases the MICs, allowing bacteria to reach high-level resistance when associated with other mechanisms. The *adeABC* operon is present in 80% of *A. baumannii* strains. It has not been found in enviromental strains and appears to be associated mainly with clinical isolates.[96,97,106]

AdeIJK efflux pump

AdeIJK, encoded by the *adeIJK* operon, is the second RND efflux system described for *A. baumannii*. *adeIJK* encode a three-component RND efflux system consisting of AdeI, AdeJ and AdeK, which is similar to MFPs, RND and OMFs, as in the AdeABC efflux. As opposed to *adeABC*, *adeIJK* is intrinsic present in all strains of *A. baumannii*. This pump is specific for the species, where it contributes to intrinsic resistance to β-lactams such as ticarcillin, cephalosporins, aztreonam and fluoroquinolones, tetracyclines, tigecycline, lincosamides, rifampin, chloramphenicol, cotrimoxazole, novobiocin and fusidic acid but aminoglycosides are not substrates for this pump.[38,91] AdeIJK was found to act in a synergistic fashion with AdeABC to extrude compounds such as tigecycline. Inactivation of *adeIJK* or overexpressed *adeABC* confers a 3 and 8-fold decrease in the tigecycline MIC, respectively, whereas inactivation of both pumps leads to an 85-fold decrease. It was initially thought that overexpression of *adeIJK* was toxic for the host cell.[97,107] However, spontaneous low-level resistant mutants overexpressing *adeIJK* have been obtained on drug gradients of tetracycline or cefotaxime. No regulatory genes are adjacent to the *adeIJK* operon and no mutations have been detected in the putative promoter region of *adeIJK*-overexpressing mutants. Thus, regulation of

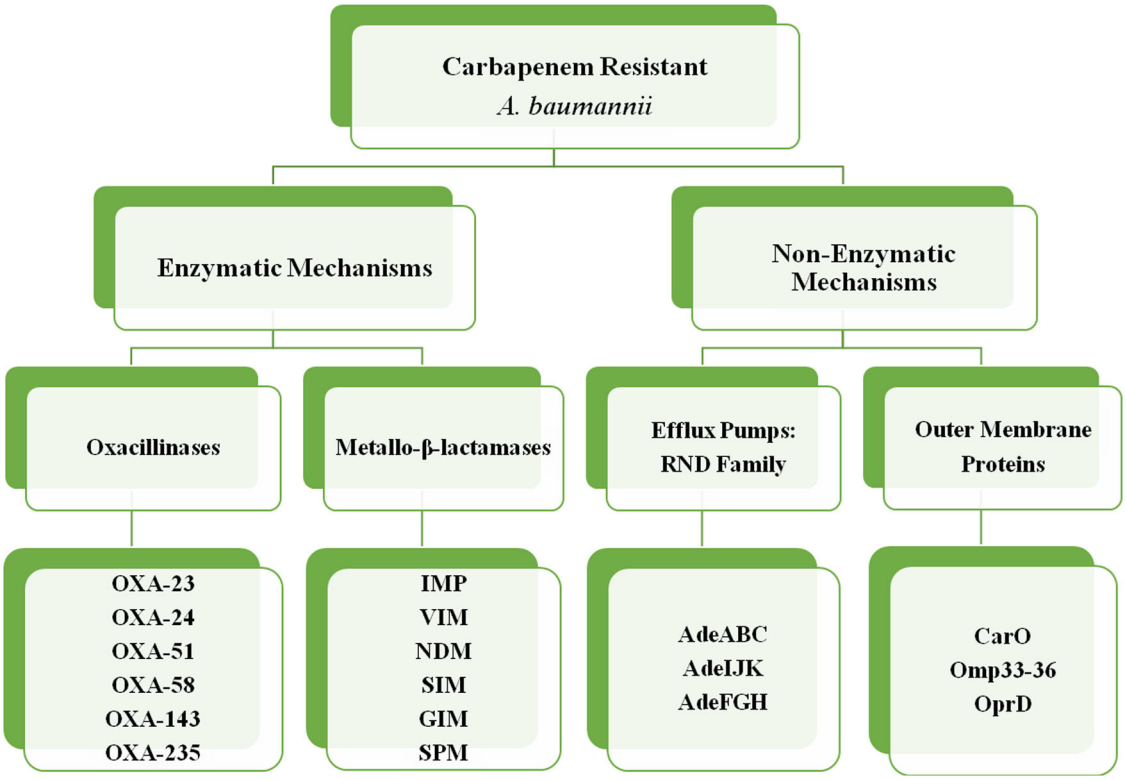

Figure 4. Mechanisms of carbapenem resistance in A. *baumannii*.

the pump and the genetic events leading to overexpression remain unknown.[97,108,109]

AdeFGH efflux pump

A third RND efflux pump, AdeFGH, encoded by the *adeFGH* operon, confers multidrug resistance when overexpressed. Expression of *adeFGH* is regulated by LysR-type transcriptional regulator (LTTR), named *adeL*, which locate upstream from the operon and transcribed in the opposite direction. Mutation in *adeL* may lead to the overexpression of *adeFGH*.[97,110] It is responsible for high-level resistance to fluoroquinolones, tetracyclines, tigecycline, chloramphenicol, trimethoprim, sulfamethoxazole and various dyes such as ethidium bromide, but has little effect when compared to β-lactams, erythromycin, rifampin and aminoglycosides. AdeFGH is also very popular in *A. baumannii*. AdeFGH does not contribute to intrinsic resistance since it is not constitutively expressed in wild-type strains.[97,111]

AdeXYZ efflux pump

AdeXYZ, chromosomally encoded efflux system, shares more than 97% identity with AdeIJK. AdeXYZ is found in *Acinetobacter genomic DNA group 3* (GDG3), *Acinetobacter* (GDG13TU) and *Acinetobacter* (GDG 17). This pump is present in 90% of strains of the species. AdeX, AdeY and AdeZ are similar to MFP, RND and OMF in RND efflux system.[98] Until now, the function of AdeXYZ in antimicrobial resistance is not well understood. The homology with AdeIJK suggests that it could have a similar function, i.e., intrinsic resistance and probable acquisition of higher levels of resistance by overexpression. AdeX, AdeY and AdeZ share 80, 89, and 87% amino acid identity, respectively, with the MFP, RND and OMF proteins of an efflux system from *A. baylyi* ADP1, which has been shown to contribute to intrinsic resistance of the species to β-lactams, ciprofloxacin, tetracycline, rifampin, and chloramphenicol, a substrate range consistent with that of an AdeIJK-like system.[79,97,98]

AdeDE efflux pump

The AdeDE efflux pump has been reported to confer resistance to ceftazidime, amikacin, ciprofloxacin, chloramphenicol, erythromycin, rifampin, meropenem, tetracycline in *Acinetobacter genomic DNA group 3* (GDG3) and imipenem in *A. baumannii.* AdeDE increases the host resistance to ceftazidime and rifampicin while AdeABC protects the host from cefotaxime and thus, differs from AdeABC. Unlike AdeABC efflux systems, the structural gene for the outer membrane proteins is not found in downstream of the *adeDE* gene cluster, suggesting that another OMP could be recruited to form a tripartite efflux pump.[91,112] The AdeDE system, which displays less than 45% identity with AdeABC, extrudes aminoglycosides,

carbapenems, ceftazidime, fluoroquinolones, erythromycin, tetracycline, rifampin and chloramphenicol. Inactivation of *adeE* in a clinical isolate leads to a greater than 4-fold increased susceptibility to these antibiotics.[97,112]

PBPs

The targets of β-lactamases are known as penicillin-binding proteins (PBPs). PBPs are a family of enzymes that catalyse the synthesis of peptidoglycan, the primary component of the bacterial cell wall. If changes occur in the PBPs it will prevent the action of β-lactams. The inhibition of PBPs will cause instability in the cell wall of the bacteria, which will lead to growth inhibition or cell lysis.[38,79] The role of PBPs in conferring antibiotic resistance in *A. baumannii* has been poorly investigated, but the reduced expression or modification of PBPs has been reported to contribute to carbapenem resistance in isolates of *A. baumannii*.[38,79,83,113] Another study found that the modification of PBPs was a source of imipenem resistance.[38,79] Figure 4 displays the comprehensive view of various mechanisms associated with carbapenem resistance.

Treatment options for CRAB infections

CRAB isolates has gained global notoriety as a critically important nosocomial pathogen. Difficulties in treating CRAB infections emanate an alarming resistance profile that leaves available only a few antibiotics of uncertain efficacy such as colistin and tigecycline. CRAB isolates tend to be XDR, that is resistant to all antibiotic classes except polymyxins and tigecycline.[114] CRAB is declared as the top priority pathogen by the WHO for the investment in new drugs.[115] The increasing importance of the species recognized by the WHO classifies CRAB amongst the critical priority pathogens in the 'Global priority list of antibiotic resistant bacteria to guide research, discovery and development of new antibiotics, 2017'.[114] This urgent need for new therapies, in combination with faster FDA approval process have placed several drug candidates in the pipeline.[115]

The therapeutic options for CRAB infections when were first replaced by polymyxins [colistin (polymyxin E) or polymyxin B] as the first choice in the therapeutic arsenals against *A. baumannii*, despite possible severe side effects such as acute renal failure and neurological disorders found polymyxins to have a poor pharmacokinetic profile regardless of the infection site.[116] Tigecycline emerged as an alternative drug for CRAB infections. However, this broad-spectrum antibiotic has a pharmacokinetic profile that is not favorable for severe bloodstream infections. Serum levels of tigecycline are low because of the large distribution volume. This property allows the prescription of tigecycline for surgical site infections but not for bloodstream infection.[115,116] Combined therapy has been suggested as an alternative amongst many controversies. In some studies combined therapy has shown superiority for some strains of *A. baumannii* in animal models and *in vitro* studies. Studies with humans are scarce and too poor quality to suggest the best approach for the treatment of infections caused by multidrug resistant *A. baumannii*.[116] However, the combination of agents and dosing regimens that delivers the best clinical efficacy while minimizing toxicity is yet to be defined. Carbapenems, sulbactam, rifampin and tigecycline have been the most studied in the context of combination therapy.

Bacteriophages

Highly lytic bacteriophages have been considered as alternative option for controlling Acinetobacter associated infections. The acinetobacter phage BS46, which was very active *in vitro*, was protective *in vivo* however, in the process of killing replication of phage was also observed.[117] Since this study other researches have been carried out to confirm the therapeutic usage of bacteriophages.[118–124] Recently, phage Bϕ-R2096 has been used against CRAB infection using *Galleria mellonella* and mouse as an animal model. The survival rate in a mouse model of acute pneumonia showed excellent elimination of the target bacteria. Similarly, statistically significant improvement in survival rates of larvae was a characteristic feature in the larvae treated with phage.[125] Bacteriophage therapy is more tolerable and has been reported to be low resistance. In addition, phages are highly specific to their target microbes, in contrast to broad-spectrum antibiotics. Yet, bacteriophage therapy is not always successful.[126] In an Iranian research, and bacteriophages isolated from waste water were shown to decrease the turbidity significantly and thus, indicated that these isolated phages may be considered as candidates for phage therapy.[127]

In addition, recently several other new therapeutic agents have been introduced, although these compounds are mainly in experimental phase and have not entered clinical trials.[116,128]

Siderophore cephalosporins (cefiderocol)

Cefiderocol is a recently developed novel cephalosporin conjugated with a catechol siderophore on its side chain. Cefiderocol has a distinctive active uptake mechanism and stability against many

β-lactamase classes, which provide enhanced penetration of bacterial cell and activity against highly resistant Gram-negative bacteria including CRAB.[129] In *in vitro* studies, cefiderocol was shown to be potent against OXA-23, OXA-40 and OXA-58 as well as NDM and IMP-producing *A. baumannii* isolates. For *in vivo* studies, the efficacy of humanized exposures of cefiderocol was evaluated in animal infection models. Cefiderocol is likely to be the first of the new agents active against CRAB to be approved for clinical use.[115,129]

Tetracyclines

Eravacycline

In *in vitro* studies, eravacycline, a novel fluorocycline of the tetracycline family, shows activity against a broad range of pathogens, including MDR and XDR Gram-negative, Gram-positive and anaerobic pathogens. Eravacycline MICs were found to be 2 to 8-fold lower than tigecycline MICs against CRAB.[130] The drug is also active against colistin-resistant and ceftazidime-avibactam-resistant strains. Eravacycline, recently placed on the market, has better *in vitro* activity against CRAB than tigecycline.[115,130]

TP-6076

TP-6076 is another fluorocycline antibiotic being developed for the treatment of MDR pathogens. TP-6076 MICs were very low (MIC range, 0.008 to 0.5) against clinical CRAB isolates producing OXA carbapenemases.[115]

New non-β-lactam-β-lactamase inhibitors

ETX2514

ETX2514 is a broad-spectrum diazabicyclooctanone (DBO) β-lactamase inhibitor similar to avibactam and relebactam. ETX2514 inhibits penicillin binding protein 2 (PBP2) and enhances β-lactam activity.[131] It has been developed by modifying the DBO scaffold to cover a broad range of OXA-type β-lactamases. EXT2514 is being developed in combination with sulbactam. Sulbactam-ETX2514 is a potent combination against CRAB, whereas combinations with imipenem and meropenem did not decrease the MICs to susceptible levels.[115,131]

WCK 4234

WCK 4234 is another DBO β-lactamase inhibitor being developed in combination with meropenem. WCK 4234 is active against several carbapenemases from classes A, C, and D, including OXA-23 and OXA-51. The WCK 4234 MIC50/90 values were 2/8 mg/L when combined with meropenem against a large collection of *A. baumannii* isolates.[115,132]

LN-1-255

LN-1-255 is a non-β-lactam-β-lactamase inhibitor from the penicillanic acid sulfone family. It is active against class D β-lactamases *in vitro*. The inhibition efficiency of LN-1-255 was shown to be superior to those of tazobactam and avibactam in kinetic assays. LN-1-255-carbapenem combinations were tested against isogenic CRAB strains and clinical isolates producing various OXA-type carbapenemases, i.e., OXA-23, OXA-40, OXA-58 and OXA-143.[115,133]

WCK 5153 and Zidebactam

Zidebactam (WCK 5107) and WCK 5153 are DBOs that also inhibit PBP2 and show a potent β-lactam enhancer effect against Gram negative pathogens, including *A. baumannii*. They are also active against MBL-producing *K. pneumoniae* strains *in vitro*. WCK 5153 and zidebactam decreased the sulbactam MIC from 16 to 2 mg/L for MDR *A. baumannii*.[115,134]

New β-lactam antibiotics

AIC-499

AIC-499 is a new β-lactam antibiotic being developed in combination with a β-lactamase inhibitor. It is claimed to have activity against MDR *A. baumannii* and MDR *P. aeruginosa* strains.[115]

FSI-1671

FSI-1671 is a new class of carbapenems which possesses activity against *A. baumannii*. The FSI-1671-sulbactam combination was active against clinical *A. baumannii* isolates, including OXA producers, though the number of CRAB isolates is not specified.[115,135]

Polymyxin B-derived molecules

SPR741

SPR741 is a polymyxin B (PMB)-derived antibiotic adjuvant that permeabilizes the Gram-negative membrane. It does not exhibit Gram-negative activity itself and is specifically designed to minimize nephrotoxicity. Potentiation of rifampin activity with SPR741 against *A. baumannii*, including clinical isolates, has been shown in several *in vitro* studies using checkerboard or time-kill analyses.[115,136]

FADDI-287

FADDI-287 is a novel polymyxin analogue with an improved safetyprofile. It has greater potency than PMB against CRAB.[115,137]

Aminoglycoside (Apramycin)

Apramycin is an aminoglycoside antibiotic used in veterinary medicine. Its resistance to inactivation by most aminoglycoside-modifying enzymes makes it an attractive therapeutic option against MDR Gram-negative microorganisms. The MIC50/90 values of apramycin were found to be 16/64 mg/L against carbapenem and aminoglycoside-resistant *A. baumannii* isolates.[115,138]

Conclusion

Acinetobacter baumannii has acquired several carbapenem resistance mechanisms to reach to a danger of becoming too dogmatic. Carbapenem resistance is a multifactorial approach acquired by the bacterium and has attenuated therapeutic regimens. Though several new therapeutic drugs are being developed which may help clinicians to treat the *A. baumannii* infections however, scientists and researchers have to hit stretch goals to lead to medical countermeasure research.

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Conflicts of interest

The authors declare no conflict of interest.

Ethics approval

Ethical Committee of Tabriz University of Medical Sciences approved the project under the code: IR.TBZMED.VCR.REC.1397.042 dated 1396/11/04.

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