
Chapter

2

THE IMPRESSIVE ADAPTABILITY OF *Acinetobacter baumannii*: A PARADIGM OF ANTIMICROBIAL RESISTANCE

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2.1. INTRODUCTION

Nosocomial, or hospital acquired, bacterial pathogens frequently obtain and easily develop mechanisms of resistance to multiple antibiotics, presenting significant clinical and economic challenges. Globally, drug-resistant infections are currently responsible for more than half a million deaths each year. By 2050 -unless new solutions are forthcoming- antimicrobial resistance will have caused the deaths of more than ten million people. Related healthcare costs worldwide are projected to be between 300,000 million and more than 1 billion dollars *per year* [1].

Recently, the World Health Organization (WHO) published a list of "priority" bacterial pathogens demonstrating antibiotic resistance [2]. Among these, *Acinetobacter baumannii* falls into the "maximum priority" category, and is considered one of the opportunistic pathogens most threatening to global health. *A. baumannii*'s high genetic plasticity allows it to adapt quickly to unfavourable contexts, and easily develop antibiotic resistance (which has led to multiple successive changes in therapeutic strategy). This pathogen has been cited by some authors as a paradigm of multidrug-resistance [3,4].

The increasing frequency of *A. baumannii* isolates that are resistant to antibiotics, such as cephalosporins, imipenem, sulbactam, rifampin, colistin or tigecycline, represents a major challenge when selecting appropriate treatment regimens. In recent years, the frequency of infections and hospital outbreaks caused by strains resistant to most available antibiotics has increased. Virtually all strains of *A. baumannii* are resistant to at least two antibiotic classes [5]. The carbapenems have classically been considered a last resort for the treatment of multidrug-resistant strains, but *A. baumannii* resistance to even this class of antibiotics has increased enormously during the first decade of the 21st century. The SENTRY antimicrobial surveillance program revealed a worldwide increase in imipenem resistance from 34–60 % in only 3 years (the period from 2006–2009) [6]. In Spain, imipenem resistance rates were even higher, at 83 %. Possibly the only effective antimicrobial against this pathogen (resistance rates <10 %) is colistin [7], which has clinically-significant shortcomings, such as the high potential for nephrotoxicity and poor bioavailability in the lungs and cerebrospinal fluid [8,9]. Antibiotic misuse and insufficient investment in new drug development have resulted in few novel alternatives for the treatment of multidrug-resistant organisms reaching the market over the last two decades. The design and evaluation of new antibiotic therapies is imperative.

While *A. baumannii* lacks the necessary virulence factors to cause disease in healthy individuals, it can act as an opportunistic pathogen in susceptible individuals (*e.g.* those with underlying disease). Opportunistic pathogens can produce infection, which can only be prevented by the use of antimicrobial

therapies. *A. baumannii* can colonize niches where few other species would survive (e.g. environments with high antibiotic pressure) and can even displace commensal microflora. Antimicrobial resistance can increase the virulence or fitness of certain species in some environments, often helping these species to colonize new niches, such as the hospital environment. This explains why these pathogens are isolated much more frequently than few decades ago. Thus the genetic background of resistant pathogens such as *A. baumannii* allows it to persist in the presence of minimal concentrations of antibiotics or even in their absence [10]. The capacity to develop or acquire resistance and the ability to persist in complicated environments are key factors in explaining the increase in the number of infections in hospitalized patients [10]. Furthermore, in adapting to survival in the presence of antibiotics, species such as *A. baumannii* and *Pseudomonas aeruginosa* have evolved to cause greater host damage [11]. Carrying virulence genes also confers some evolutionary advantage during host colonization and infection, favouring resistant strains and providing a plasticity that allows *A. baumannii* to employ novel strategies in exploring new environments (including providing advantages over commensal microflora).

This review explores intrinsic and acquired resistance mechanisms of *A. baumannii*, with a focus on the main resistance transmission methods known thus far, in order to better understand the plasticity of this species in adapting to survival in the presence of antimicrobials and in a nosocomial environment.

2.2. MECHANISMS OF *A. baumannii* ANTIMICROBIAL RESISTANCE

The most relevant resistance mechanism in *Acinetobacter* spp. is enzymatic hydrolysis of β -lactams. However, due to complex resistance development and acquisition systems, multiple resistance mechanisms can coexist in this pathogen [12-14]. The main resistance mechanisms of *A. baumannii* are summarized in Table 1.

Table 1. Major resistance mechanisms of *A. baumannii* [3,15,16]

Antibiotic	Resistance mechanism	Key examples
β-lactams	Enzymatic inactivation	Penicillinases Extended spectrum β-lactamases (AmpC, TEM, VEB, PER, CTX-M, SHV) Carbapenemases (OXA, VIM, IMP, NDM-1)
	Loss, down-regulation, or alteration of porins	CarO, Omp 33-36, OprD-like
	Alteration of PBP expression	PBP2
	Efflux systems	AdeABC
Aminoglycosides	AMEs	AAC, ANT, APH
	Efflux systems	AdeABC, AdeM
	Ribosomal methylation	ArmA
Tetracyclines and tigecycline	Efflux systems	AdeABC, TetA, TetB
	Ribosomal protection	TetM
Polymyxin E (Colistin)	Lipid A modification	PmrCAB
	Loss of lipopolysaccharide	LpxABC

2.2.1. Resistance to β-lactams

Inactivation of the β-lactams is due to the action of the β-lactamases enzymes, which are grouped into four molecular classes (A–D) following the Ambler's Molecular classification system [17].

2.2.1.1. Class A β-lactamases

A. baumannii exhibits a wide range of class A β-lactamases, including Temoneira (TEM), sulfhydryl variable (SHV), cefotaxime-hydrolyzing β-lactamase (CTX-M), guiana extended-spectrum (GES), self-transferable plasmid from *Escherichia coli* (SCO), *Pseudomonas* extended resistant (PER), vietnam extended-spectrum β-lactamase (VEB), carbenicillin hydrolyzing β-lactamase (CARB), and *Klebsiella pneumoniae* carbapenemase (KPC) [18]. However they use to have a minor role in resistance, especially in resistance to carbapenem antimicrobials. Class A β-lactamases, which are inhibited by clavulanic acid, typically hydrolyse penicillins and cephalosporins more effectively than carbapenems, with the exception of some KPC type enzymes [17].

Some members of this class – such as TEM-1, CARB-4, and SCO-1 – are narrow-spectrum β-lactamases, whereas other enzymes (*e.g.*, PER-1, TEM-92, CARB-10, SHV-5, PER-2, CTX-M-2, CTX-M-15, VEB-1, GES-14, and PER-7) are known

as extended spectrum β -lactamases (ESBLs) [8,19]. Clinically-relevant ESBLs include CTX-M enzymes, which (due to their transmission *via* plasmids or integrons) have a high potential for rapid dissemination and could thus produce drug-resistant infection outbreaks [20,21]. Although prevalent among *Enterobacteriaceae*, ESBLs are less prevalent in *Acinetobacter* spp., but CTX-M-2, CTX-M-43, and CTX-M-15 have been described from Japan, Bolivia and India, respectively [8]. Another relevant ESBL is VEB-1, with reported dissemination in France [22], Belgium and Argentina [8]. Finally, the PER ESBLs, initially observed in *P. aeruginosa*, have been detected in *A. baumannii* in many countries: in Korea, PER-1 is one of the most predominant β -lactamases [23,24]. Additional class A carbapenemases, such as GES-14 and KPC-2, have also been detected in *A. baumannii* [25,26].

A. baumannii also exhibits other class A β -lactamases such as the narrow-spectrum β -lactamases TEM-1 and -2, but they are of lesser clinical significance compared to other resistance mechanisms of *Acinetobacter* spp. [27,28].

2.2.1.2. Class B β -lactamases

This class comprises metallo- β -lactamases (MBLs), which require zinc or another heavy metals for antimicrobial catalysis [17], and are capable of hydrolysing all β -lactams except monobactams [29]. They exhibit broad-range, potent carbapenemase activity, and are resistant to inactivation by classical inhibitors [30].

MBLs are not the predominant carbapenemases in *A. baumannii*, but they do contribute to the high carbapenem resistance levels observed in this species. The firsts MBLs isolated in Gram-negative bacilli were imipenemase (IMP)- and Verona imipenemase (VIM)-type, in Japan and Italy, respectively [8]. IMP-1 and VIM-2 were the first of this class detected in *Acinetobacter* spp., in 1998 [31]. Subsequently, VIM-1, VIM-3, VIM-4, VIM-11, IMP-2, IMP-4, IMP-5, IMP-6, IMP-8, IMP-11, IMP-19 and IMP-24 have all been observed in this pathogen [18]. Seoul imipenemase (SIM)- and New Delhi metallo- β -lactamase (NDM) have also been detected in *Acinetobacter* spp. [8], although of the SIM-type MBLs, only SIM-1 has been detected [32]. Most recently, NDM-1 [33,34] and NDM-2 [35] have been detected in *A. baumannii*. The *bla*_{NDM-1} gene use to be integrated into the chromosome as a transposon, bracketed by two copies of *ISAba125*, which has facilitated its rapid dissemination [33,36,37].

The MBLs are especially predominant in non-*baumannii* *Acinetobacter* spp. Other genomic species (*e.g.* genomic sp. 13TU and genomic sp. 3) represent the majority of MBL-carrying *Acinetobacter* spp. isolates [38,39].

2.2.1.3. Class C β -lactamases

Most Gram-negative bacilli harbour a chromosomally-encoded β -lactamase, and this is usually a cephalosporinase belonging to class C [40]. Class C β -lactamases – like classes A and D – are serine-dependent, and display many

additional specific features, for example an insensitivity to clavulanic acid, and a preference for cephalosporin hydrolysis (although they can also confer resistance to cephamycins and penicillins) [29]. This confers significant therapeutic problems [19]. While the first AmpC β -lactamase characterized in *A. baumannii* was from a Spanish clinical isolate in 2000 [41], all strains actually possess intrinsic AmpC cephalosporinases [42] bearing small sequence differences [43,44]. In 2005, all *Acinetobacter* spp. chromosomal cephalosporinases were uniformly designated as *Acinetobacter*-derived cephalosporinase (ADC) β -lactamases [45]. ADC enzymes play a pivotal role in the antimicrobial resistance of *Acinetobacter* spp., especially in isolates carrying an insertion sequence under the control of a strong promoter (ISAb_a1-like sequence), which usually results in pronounced resistance to ceftazidime [47,48].

2.2.1.4. Class D β -lactamases

Also known as the OXA-type β -lactamases (in reference to their preferred hydrolysis of oxacillin) [29], this is probably the fastest-growing class of β -lactamases with more than 500 reported enzymes [49]. The class D enzymes are organised into three categories, based on substrate specificity: narrow-spectrum (e.g. OXA-1 and -10), extended-spectrum β -lactamases (ESBLs, e.g. OXA-13 and -17), and carbapenem-hydrolysing class D β -lactamases (CHDLs, e.g. OXA-23 and -24/40) [49,50]. Approximately 50 % of OXA β -lactamases possess carbapenemase activity [51]. However, due to the multiple mechanisms of antibiotic resistance exhibited by *A. baumannii*, it is difficult to conclusively determine the relative contribution of CHDLs [52].

Until a decade ago, the most clinically relevant β -lactamases were the AmpC and extended-spectrum β -lactamases, but the number of known carbapenemase β -lactamases has increased in recent years, with CHDLs being especially problematic. Since the description of the first OXA enzyme (OXA-23) in *A. baumannii* in 1993, the number of CHDLs discovered worldwide in clinically-problematic Gram-negative pathogens, such as the *Enterobacteriaceae*, *P. aeruginosa*, and (largely) *A. baumannii* has increased dramatically [53,54]. Among the four classes of β -lactamases, MBLs and CHDLs are the main groups of carbapenemases occurring in *A. baumannii*, and degradation is the most common mechanism of carbapenem resistance [55,56].

A. baumannii possesses six CHDL families: OXA-23-like, OXA24/40-like, OXA-58-like, OXA-143-like, OXA-235-like and OXA-51-like [57-60]. Of these, OXA-23-like is the major family of CHDLs, the most disseminated worldwide, and the main source of carbapenem resistance in this pathogen [61]. Other prevalent groups include OXA24/40-like and -58-like. Nearly all strains of *A. baumannii* possess OXA-51-like chromosomal enzymes with weak carbapenemase activity. However, if the gene acquires a strong promoter *via*

upstream insertion sequence *ISAbal*, or is located in a plasmid, it can confer carbapenem resistance [59,62]. The aforementioned insertion sequence has also been described to increase expression of OXA-23 and -58 [63].

The OXA-24/40 CHDL group comprises OXA-24/40, OXA-25, OXA-26, and OXA-72 [58]. OXA24/40 was initially identified in Spain in 2000 [64]. Later, OXA-25, OXA-26 and OXA-27 were identified in *A. baumannii* clinical isolates originating in Spain, Belgium and Singapore, respectively [65]. This group of carbapenemases has now been identified worldwide [18].

OXA-58 was first identified in France, encoded within a plasmid from a multidrug-resistant *A. baumannii* isolate, in 2005 [66]. Since then, it has been found that OXA-58-like CHDL-producing *A. baumannii* isolates are widely distributed [168].

Given that carbapenems have been utilized as last-resort antibiotics for the treatment of multidrug-resistant *Acinetobacter* infections, the prevalence and plasmid-mediated dissemination of CHDLs are an important clinical challenge, motivating evaluation of potential alternate antibiotics (including β -lactamase inhibitors, aminoglycosides, tigecycline and polymyxins) in the treatment of such infections.

2.2.1.6. Outer membrane proteins

Although β -lactamases with carbapenemase activity are the main carbapenem resistance mechanism, porins are also thought to be involved. Their reduced expression plays a role in the resistance to these antibiotics. Few outer membrane proteins (OMPs) have been reported, their functions remain unclear [67], and – compared to other pathogens – little is known about the porins of *A. baumannii*. Indeed, *A. baumannii* exhibits very low outer membrane permeability, with a small number and size of porins relative to other Gram-negative organisms [68]. Several reports describe down-regulated expression of some OMPs, implicating these in antimicrobial resistance [13,14]. The major OMP of *A. baumannii*, reported to date, is the heat-modifiable protein HMP-AB, which exhibits homology with the monomeric OmpA of *Enterobacteriaceae* and with OMP-F (OprF) of *P. aeruginosa* [69]. Three additional porins implicated in carbapenem resistance are the 33–36 kDa protein [70], the 29 kDa protein (CarO: carbapenem resistance-associated outer membrane protein of *A. baumannii*) [71] and the 43 kDa protein, which exhibits homology with OprD of *P. aeruginosa* [72]. It has been suggested that CarO may function as a carbapenem-nonspecific channel, while OprD-like porin may function as a carbapenem-specific channel [73]. Clinical outbreaks of carbapenem-resistant *A. baumannii* isolates have been described in association with porin loss, including loss other OMPs, such as 47-, 44-, and 37-kDa OMPs in *A. baumannii* isolates in New York City [12], and loss of 22- and 33-kDa OMPs in association with OXA-24/40 in Spain [14].

2.2.1.7. Multidrug efflux pumps

While the outer membrane can limit the entry of antimicrobials into the bacterial cell, multidrug efflux pumps actively export multiple classes of antimicrobials from the cell [73]. While transport proteins involved in metabolic function exhibit a high degree of substrate specificity, multidrug efflux systems are more promiscuous [74]. Thus, efflux pumps work synergistically with the low permeability of the outer membrane [75]. Expression of efflux pumps is associated with an increased minimum inhibitory concentration (MIC) of many different antibiotic classes, including resistance to tigecycline, aminoglycosides and carbapenem in *A. baumannii* [76].

The major efflux pumps involved in multidrug resistance in *A. baumannii* belong to the group of proton-motive-force-dependent exporters, especially the major facilitator superfamily (MFS) and resistance-nodulation-cell division superfamily (RND) families [73]. Several MFS-family efflux pumps have been characterized in *A. baumannii*, including TetA, CmlA, MdfA, CraA and AmvA, which mediate resistance to different types of antibiotics (including β -lactams) [18]. An example of an RND family member is (chromosomally-encoded) AdeABC of *A. baumannii*, the best studied member, thus far. AdeABC overexpression is a major mechanism for decreased susceptibility to various antibiotic classes [76], and efflux inhibitors (*e.g.* phenyl-arginine- β -naphthylamide, carbonyl cyanide 3-chlorophenylhydrazone) have been shown to reverse resistance [77].

Since antibiotics act as AdeABC substrates, they can increase the expression of the AdeABC genes, which are chromosomally encoded, leading to multidrug resistance. Treatment failure and death due to *A. baumannii* infection are common when efflux pumps (especially the RND family) are implicated in antibiotic resistance [78]. In addition to the AdeABC efflux pump, other RND-type efflux pumps, including AdeFGH [79] and AdeIJK [80], are implicated in multidrug resistance in *A. baumannii*.

2.2.1.8. Penicillin-binding proteins

Another relevant mechanism of resistance to β -lactams is direct modification of their targets: the penicillin-binding proteins (PBPs), which catalyse peptidoglycan synthesis and are associated with cell morphogenesis and the cell division complex [81]. β -Lactams are suicide inhibitors which bind covalently and irreversibly to PBPs [82]. There is limited information regarding this resistance mechanism in *A. baumannii*, but no differences were found between the sequences of susceptible and resistant *A. baumannii* strains [83]. Although confirmatory studies are required, this suggests that PBP mutations are not as important a resistance mechanism in *A. baumannii* as in other pathogens (*e.g.* *P. Aeruginosa*) [84].

However the impact of PBP alterations on bacterial virulence should be further investigated, as PBP7/8 contributes to both the *in vitro* and *in vivo* survival of *A. baumannii*. A PBP7/8 mutant strain exhibited poorer survival in *in vivo* models, compared to the isogenic wild-type strain [85].

2.2.1.9. Resistance to β -lactamase inhibitors

Most β -lactamase inhibitors are ineffective against *A. baumannii*: no commercial inhibitors exist with activity against its class B and D carbapenemases. An exception is sulbactam, which has affinity for *A. baumannii*'s PBPs [13,86] and possesses bactericidal activity against *A. baumannii*. It is usually used in combination with ampicillin; however, the contribution of sulbactam is more relevant than the contribution of ampicillin as antimicrobial agent against this pathogen. While no breakpoints have been defined for *A. baumannii*, it is considered susceptible when $\text{MIC} \leq 4 \text{ mg L}^{-1}$ [87]. Sulbactam monotherapy is not recommended in patients with serious infections, and resistance to sulbactam has already been described in *Acinetobacter* [87]. Reduced PBP2 expression is associated with resistance to this compound [13], although production of the non-ESBL β -lactamase TEM-1 has also been suggested to contribute to sulbactam resistance [88]. Clavulanic acid, too, shows bactericidal activity against a percentage of *A. baumannii* isolates [89]. New agents, which specifically inhibit *A. baumannii* β -lactamases, are being developed (*e.g.* ETX2514 [90] and LN-1-255 [60]), but additional preclinical assays are required to evaluate their *in vivo* efficacy.

2.2.2. Resistance to aminoglycosides

Aminoglycosides are effective against both Gram-negative and -positive pathogens. During *A. baumannii* infection, specifically, tobramycin and amikacin are used (in combination with other antimicrobials) [9]. Aminoglycoside antibiotics target ribosomal 16S rRNA, modifying its structure to produce a loss of translation fidelity, production of erroneous proteins, and, finally, bacterial cell death [91]. Bacteria exhibit increasing levels of resistance to aminoglycosides, mainly by acquiring genes encoding aminoglycoside-modifying enzymes (AME), *N*-acetyltransferases (AAC), phosphotransferases (APH) or *O*-adenyltransferases (ANT), all of which are typically encoded by transposable elements. Other implicated mechanisms of resistance include mutation of ribosomal proteins or RNA. Ribosomal methylases – which methylate the aminoacyl site of 16S rRNA – also confer high resistance to aminoglycosides, and six acquired 16S rRNA methyltransferases have been described: *armA*, *rmtA*, *rmtB*, *rmtC*, *rmtD* and *rmtE* [92]. An additional mechanism of aminoglycoside resistance is reduction of intracellular accumulation by means of outer membrane alterations, two-component systems and efflux pumps [*e.g.* the AbeM pump, a member of the multidrug and toxic compound extrusion (MATE) family] [93].

Many *A. baumannii* isolates express a combination of aminoglycoside-modifying enzymes [94,95]. A multidrug resistant *A. baumannii* isolate carrying genes for four aminoglycoside-modifying enzymes was described in China [96], and Japanese and Greek studies have shown that the majority of multidrug-resistant isolates carry at least one gene encoding an aminoglycoside-modifying enzyme [95,97]. In a 2007 outbreak of highly aminoglycoside-resistant *A. baumannii* isolates, the strains carried the genes for *armA* together with Per-1 and OXA-23 carbapenemases [23]. Similarly, multidrug-resistant *A. baumannii* isolates have been described that carry both a *bla*_{OXA-23}-like gene (*aac(6')-Ib*) and the 16S rRNA methylase *armA*. Such isolates show that *A. baumannii* can employ multiple simultaneous mechanisms to elude the action of antimicrobials.

2.2.3. Resistance to tigecycline

Tigecycline is a relatively new broad-spectrum glycylycylcycline antibiotic, which has demonstrated *in vitro* and *in vivo* activity against *A. baumannii* [98]. It is a derivative of minocycline, with structural modifications that improve ribosomal binding-site affinity and orientation relative to both minocycline and tetracycline [99]. Tigecycline is one of the few agents developed recently that has enhanced activity against problematic Gram-negative organisms. In the 2005–2011 Tigecycline Evaluation and Surveillance Trial investigating multidrug-resistant *A. baumannii* strains, tigecycline's MIC₅₀ was 0.5 mg L⁻¹, and its MIC₉₀ was 1 mg L⁻¹ [100].

Tigecycline can often overcome active efflux and ribosomal protein resistance mechanisms which inactivate the tetracyclines (*tet(A)* to *tet(E)* efflux pump genes) [101]. However, resistance has been detected in some strains, due to high expression levels of chromosomally-encoded efflux pumps; tigecycline is susceptible to efflux by overexpressed multidrug efflux systems such as AdeABC and AdeIJK [101,102]. Several studies have already described high rates of tigecycline-resistant strains in *A. baumannii* [103,104], including development of resistance during tigecycline treatment [105]. However, little is known about the specific tigecycline resistance mechanisms of *A. baumannii*, necessitating further investigation.

2.2.4. Resistance to polymyxins

The increasingly frequent isolation of strains resistant to carbapenems, sulbactam, rifampin or tigecycline [106,107] has driven the therapeutic use of polymyxins. Colistin and polymyxin B bind to the portion of the cell membrane interacting with the lipid A moiety of lipopolysaccharide (LPS) to cause outer membrane disorganization and hyper-permeability, leading to rapid Gram-negative bacterial death [108]. Colistin – discovered in the 1940s – was used mainly in the 1960s and 1970s, before being largely abandoned due to

nephron- and neurotoxicity; recent studies have demonstrated that revised dosing regimens can minimize this problem [109].

Polymyxins are active against most *A. baumannii* isolates, though colistin-resistant isolates have been described *in vitro* and *in vivo* [110-114]. For example, of the *A. baumannii* isolates recovered at two Korean hospitals, 30.6 % exhibited colistin resistance [115], and outbreaks of polymyxin-resistant *A. baumannii* have been already reported [116-118]. Two primary colistin-resistance mechanisms have been described in *A. baumannii* to date. The most common is modification of the lipid A moiety of LPS with phosphoethanolamine (PEtN) as a result of mutations in the *pmrA/pmrB* two-component system [119]. The modifications of lipid A by the addition of PEtN confer a positive charge to LPS, preventing colistin binding. The mutations in *pmrA* or *pmrB* induce expression of *pmrA*, in turn leading to up-regulated expression of the *pmrCAB* operon, and subsequent synthesis and addition of PEtN to the lipid A portion of LPS [119-121]. The second mechanism is the complete loss of lipopolysaccharide caused by either mutation or insertional inactivation of the lipid A biosynthesis genes. In colistin-resistant *A. baumannii*, mutations in lipid A biosynthesis genes (*lpxA*, *lpxC* and *lpxD*) due to nucleotide substitution, deletion or insertional inactivation by sequence *ISAbA11*, completely abrogate production of LPS with high colistin resistance (MIC > 128 mg L⁻¹) [113,122]. Additionally, polymyxin B-resistant *A. baumannii* isolates have been shown to carry mutations in the *lpxC* and *lpxD* genes, in addition to mutations in the *lpsB* gene which encodes a glycosyltransferase involved in LPS core biosynthesis [123]. However, colistin-resistant *A. baumannii* *lpx* mutants (lacking LPS) also demonstrate reduced virulence and fitness compared to colistin-resistant *A. baumannii* *pmrA/pmrB* mutants (PEtN-modified LPSs) [124], indicating that *lpx* mutation comes with a biological cost.

Other colistin-resistance mechanisms have been recently suggested, including modification of Gram-negative outer membrane asymmetric lipid distribution (essential for outer membrane functions) [125]. Also, two studies have suggested that efflux pumps may be involved in the colistin resistance phenotype in *A. baumannii* [126,127].

2.3. VIRULENT AND MULTIDRUG-RESISTANT DISSEMINATED CLONES OF *A. baumannii*

Multidrug-resistant microorganisms, such as *A. baumannii*, are opportunistic pathogens, able to compete in new niches where previously only commensals or non-pathogenic microorganisms existed. The ability to adapt to survival and persistence in nosocomial environments, encompassing patients with

weakened immune systems and an environment with high antimicrobial pressure, has led to the emergence of *A. baumannii* as a key pathogen, whereas a few decades ago, it caused practically no disease. At least in these specific settings, the incidence of multidrug-resistant and virulent clones of *A. baumannii* is also increasing worldwide.

The population of clinical isolates of *A. baumannii* is dominated by three lineages: European or International clones I, II and III, corresponding to clonal complex 1 (CC1, comprising ST1, ST7, ST8, ST19 and ST20), clonal complex 2 (CC2, comprising ST2, ST45 and ST47) and clonal complex 3 (CC3, comprising ST3 and ST14) [128,129]. The predominance of a few successful multidrug-resistant lineages worldwide underlines the importance of studying *A. baumannii* epidemiology.

Outbreaks are most frequently attributable to European clones I and II [130]. The evolutionary advantage of these predominant clones is due to their capacity for acquiring resistance determinants. Clone II is particularly well-adapted to hospital environments [131], and CC2 is the most frequent genetic lineage observed in European, Asian and North American carbapenem-resistant *Acinetobacter* isolates (with carbapenem resistance mainly mediated by class D β -lactamases such as OXA-23, OXA-24/40 and OXA-58, or relevant metallo- β -lactamase-type carbapenemase genes) [132-134]. Other CCs that exhibit narrower distribution patterns than European clones I-III do exist, such as clone ST15. The predominance and geographic distribution of certain clones is usually associated with their capacity to acquire multiple resistance mechanisms [128], either chromosomal or plasmid-encoded [135]. Regarding geographic distribution of acquired OXA genes, OXA-58-like genes are associated with Greece and Italy, OXA-24/40-like genes are associated with Spain and Portugal, and OXA-23-like genes are associated with Northern European countries, Asia and South America [129]. The ability of these clonal lineages to adapt and acquire these mechanisms is a key role in the successes of their distribution.

Frequently, internationally-distributed resistant clones demonstrate efficient pathogenic factors. For instance, an isolate carrying the OXA-23 carbapenemase gene, and belonging to international clone II, led to the death of a patient within only six days [136]. Some clones can cause outbreaks, rapidly affecting up to hundreds of patients. For example, the high-risk clone ST56, which is susceptible only to tigecycline and colistin, and carries the carbapenemase OXA-24/40, as well as overexpressing two putative virulence factors (septicolysin and the TonB-dependent receptor). This strain spread extremely rapidly, causing the largest nosocomial outbreak ever reported, with 377 patients becoming colonized or infected with *A. baumannii* [137].

While β -lactamase-mediated resistance to β -lactams is of great therapeutic concern, few studies have analysed the impact of these enzymes on *A. baumannii* virulence. There is, however, a relationship between carrying

carbapenemase PER-1 and increased cell adhesion in *A. baumannii* strains, although the exact mechanism of the association remains unknown [138]. Other resistance factors are also implicated in virulence. Porins, for example, provide trans-membrane passage for molecules, such as nutrients, toxins and antibiotics, and have clear roles in both virulence and resistance [139]. Furthermore, the *A. baumannii* OmpA (HMP-AB) protein, which has been associated with cephalosporin resistance [140], has also been reported to induce human epithelial and dendritic cell death *via* mitochondrial targeting [141], and is involved in biofilm formation [142]. The Omp33-36 protein of *A. baumannii*, involved in carbapenem resistance, has also been suggested to be involved in apoptosis and modulation of autophagy [143]. Moreover, both CarO and Omp33-36 porins are implicated in biofilm formation [144], and both OmpA and Omp33-36 proteins (as well as the TonB-dependent copper receptor) are fibronectin-binding proteins (FBPs) [145].

2.4. ANTIMICROBIAL RESISTANCE AND VIRULENCE MECHANISM CO-SELECTION

A. baumannii is naturally competent at incorporating DNA from other bacterial species [19]. It is commonly-known that the horizontal transfer of multiple resistance genes can occur simultaneously. Similarly, horizontal transfer is a relevant mechanism to exchange virulence factors. The co-selection of virulence and resistance factors has been observed during pathogen evolution in the post-antibiotic era, and could contribute to bacterial adaptation to new environments. The distribution of such transfer elements (*e.g.* plasmids, integrons and transposons) in pathogens, such as *Acinetobacter*, may become a major clinical challenge in the future.

Plasmids are extra-chromosomal, self-replicating elements, which are non-essential and are usually implicated in functions, such as virulence, resistance or persistence under extreme conditions [3]. Conjugative plasmids have a relevant role in the evolution of *A. baumannii* due to their horizontal transmission, including resistance genes (mainly during the last decades), highlighting the key role of the plasmids [146,147]. Evidence suggests that *A. baumannii* strains may bear distinct sets of plasmid types and thus a broad heterogeneity of resistance plasmids [148,149]. An example of virulence and resistance co-selection in *A. baumannii* has been described in clone ST56, which caused a large 2006 outbreak in a Spanish hospital. The plasmid pMMA2, isolated from the main outbreak clone, harboured the *bla*_{OXA-24/40} gene, as well as two genes implicated in virulence (a septicolysin-like gene encoding a pore-forming toxin, and a TonB-dependent receptor gene encoding an outer membrane protein involved in iron uptake) [137].

Genetic elements associated with antibiotic resistance gene acquisition by *Acinetobacter* include integrons and transposons. Integrons contain site-specific recombination systems through which they can include resistance genes, and transposons are able to integrate and move genes. These structures are usually found as part of the chromosome or plasmids [150-152]. There is a high prevalence of class 1 and 2 integrons in *A. baumannii* [153,154]. Most acquired MBL genes in *A. baumannii* occur within class 1 integrons, which often contain several resistance genes. Many genes encoding aminoglycoside-modifying enzymes are associated with transposons. Moreover, transposons can concomitantly carry other resistance determinants [24].

IS elements, too, play an important role in *A. baumannii* resistance. *ISAbal*, *ISAbal2*, *ISAbal3*, *ISAbal4* and *IS18* are associated with carbapenemase gene expression in *A. baumannii*. As intimated above, *ISAbal1* is the most highly-prevalent such element of this pathogen [155]. Frequently, IS element-encoded promoter regions increase expression of genes such as *bla*_{OXA-23}-like, *bla*_{OXA-51}-like, *bla*_{OXA-58}-like and *bla*_{AmpC} [151].

Gram-negative bacilli secrete vesicles, which mediate interactions with other bacteria and eukaryotic cells in the local environment. The *A. baumannii* secretome is also implicated in both virulence and resistance; two examples of outer membrane vesicles (OMVs) of the secretion systems transporting virulence and resistance factors have been described. OMV-mediated toxin delivery is a potent virulence mechanism employed by various Gram-negative organisms. The OMVs can thus be employed as a means to deliver virulence factors, and it appears they may also be relevant to transmission of resistance genes in *A. baumannii*. The release of OMVs favours the spread of antibiotic resistance genes to other bacteria *via* horizontal DNA transfer (*e.g.* transmission of OMVs harbouring the *bla*_{OXA-24/40} carbapenemase gene in an *A. baumannii* isolate). Furthermore, the *A. baumannii* ATCC 17978 strain, transformed with OMVs from clinical strains, exhibits an antibiotic-resistant profile due to expression of OXA-24/40 carbapenemase. Thus, in *A. baumannii*, OMVs represent a new mechanism of antibiotic resistance gene dissemination, in addition to the previously-known mechanisms (conjugation, transformation, and transduction) [156].

Lastly, some Gram-negative bacteria utilize the antibacterial type VI secretion system (T6SS) to kill competitors. In *A. baumannii* strains, T6SS exhibits variable expression, including a self-transmissible plasmid that carries T6SS negative regulators, as well as resistance factors. This secretion system is inactivated in resistant cells, but some cells lose this plasmid, thereby de-repressing expression of the secretion system and T6SS-mediated killing of the rest of surrounding microflora. In this way, harbouring of resistance genes can lead to elimination of competing bacteria [157].

2.5. CONCLUDING REMARKS

This review highlights the major resistance mechanisms of *A. baumannii*, and their role in the spread of this organism. Future considerations include the urgent requirement for new antibiotics to treat emerging bacteria that are resistant to almost all existing (including last-resort) antibiotics. Very few truly new antibiotics have been developed against such Gram-negative pathogens that have emerged since the 1990s. Development of new antibacterial therapies need not be limited to antibiotics; innovative anti-virulence therapies (including novel vaccines and the revival of antibacterial phage therapy) are a promising alternative to antibiotic treatment in combating resistant and/or virulent pathogens.

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