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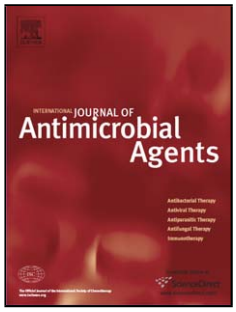

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Multidrug-resistant *Acinetobacter baumannii*: mechanisms of virulence and resistance

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ABSTRACT

Infection due to *Acinetobacter baumannii* has become a significant challenge to modern healthcare systems. The organism shows a formidable capacity to develop antimicrobial resistance, yet the clinical impact of *A. baumannii* infection remains unclear. Much is known about the processes involved in multidrug resistance, but those underlying the pathogenicity and virulence potential of the organism are only beginning to be elucidated. In this article, we provide an overview of current knowledge, focusing on mechanisms of pathogenesis, the molecular basis of resistance and options for treatment in the absence of novel therapeutic agents.

1. Introduction

Acinetobacter baumannii, a Gram-negative non-fermentative coccobacillus belonging to the family Moraxellaceae, has in recent years gained increasing notoriety as a nosocomial pathogen. First described in 1911 by Beijerinck [1], the genus *Acinetobacter* has expanded to contain 32 taxonomically distinct species, most of which are environmental organisms not associated with human disease. However, in the past decade strains of *A. baumannii* often exhibiting multidrug resistance have emerged as a significant clinical problem worldwide [2]. These organisms have been implicated in a diverse range of infections (respiratory tract, bloodstream, skin and soft tissue, prosthetic devices) and are a particular problem in intensive care units where numerous outbreaks have been extremely difficult to control. The rapid emergence and global dissemination of *A. baumannii* as a major nosocomial pathogen is remarkable and demonstrates its successful adaptation to the 21st century hospital environment. In this review, we highlight the mechanisms of pathogenicity and antimicrobial resistance contributing to the astonishing success of this pathogen and discuss the treatment options for infections with multidrug-resistant isolates.

2. Pathogenesis of *Acinetobacter baumannii* infections

The precise mechanisms involved in the establishment and progression of *A. baumannii* infection are unclear. The organism is not known to produce either diffusible toxins or cytolysins, and few virulence factors have been identified. Comparative genomic studies between *A. baumannii* (multidrug-resistant and susceptible) and the environmental *Acinetobacter baylyi* have identified genes

involved in pilus biogenesis, iron uptake and metabolism, quorum sensing and a type IV secretion system as making up part of the organism's 'virulome' [3]. Using a nematode model of microbial virulence and a library of *A. baumannii* transposon mutants to screen for potential virulence genes, Smith et al. [4] were able to identify 16 genes essential for 'ethanol stimulated virulence' (*esv*), which is required for this model. This suggests that there may be a number of novel genes in *A. baumannii* with a significant role in pathogenicity that have yet to be assessed in a mammalian model.

Acinetobacter baumannii readily adheres both to biological and abiotic surfaces, on which it is able to form biofilms [5–7]. This is an important pathogenic feature of many bacteria, facilitating colonisation of prosthetic material and contributing to drug resistance and evasion of the host immune system in vivo. Biofilm formation involves a variety of pathways that are regulated by quorum sensing and a number of two-component regulatory systems [8]. Pili and fimbriae are important for initial adhesion, followed by the production of exopolysaccharide, an important constituent of mature biofilm that suppresses the activity of neutrophils and contributes to serum resistance. Variation in the expression of factors involved in these and other pathways may account for the differing capacity of strains to colonise or infect the host environment. For example, accumulation of certain outer membrane proteins (OMPs) has been associated with the ability to form significant amounts of biofilm [9], and a similar association has been found between biofilm formation and expression of the PER-1 β-lactamase [5]. Conversely, there is a negative correlation between ciprofloxacin resistance and biofilm formation [10].

The interaction of *A. baumannii* with a number of epithelial cell lines has recently been investigated [11]. Although *A. baumannii* readily adheres to bronchial epithelial cells, this appears to occur independently of the mechanisms required for adhesion to abiotic surfaces [12]. Following adhesion, *A. baumannii* is able to invade and promote apoptosis of eukaryotic cells [13], a property attributed to the activity of OmpA (Omp36), which is trafficked to both the mitochondria and the nucleus and induces eukaryotic cell death pathways [14,15]. Purified OmpA elicits a Th_1-mediated immune response [16] and upregulates inducible nitric oxide synthase (iNOS) via a Toll-like receptor (TLR)-2 mediated pathway [17].

The ability of *A. baumannii* to obtain and utilise resources such as iron is an important factor in its ability to survive both in the host and in the environment. *Acinetobacter baumannii* secretes a variety of molecules involved in iron acquisition, including the siderophore acinetobactin, and also produces a hemin utilisation system [18]. There is wide variability in the expression of molecules involved in iron uptake, even between strains isolated during the same outbreak [19]. Specific immunological activity directed towards iron-binding proteins can be found in the convalescent sera of individuals who have recovered from *A. baumannii* bacteraemia [20]; the importance of this is unclear but suggests that these siderophores have at least been expressed in vivo during the infection process.

The inflammatory potential of *A. baumannii* lipopolysaccharide (LPS), a molecule central to the development of Gram-negative sepsis, has also been investigated. LPS derived from *A. baumannii* has been shown to be a potent inducer of pro-inflammatory cytokine expression in human monocytes, via pathways that are

dependent on both TLR-2 and TLR-4 stimulation. Purified LPS stimulates inflammation via the TLR-4 pathway, whereas killed whole cells, or 'crude' LPS extracts, act via both TLR-2 and TLR-4, although the extent to which TLR-2 contamination with surface lipoproteins (such as OmpA) contributes to in vitro stimulation is debatable [21]. Nevertheless, the ability of *A. baumannii* to stimulate an inflammatory response in human monocytes is likely to be a significant contributor to the pathogenesis of infection.

3. Clinical impact

Although *A. baumannii* has been implicated in a wide range of infections at most anatomical sites, ranging in severity from asymptomatic to fulminant sepsis, there is considerable debate regarding its overall clinical impact and attributable mortality. A number of studies have attempted to assess the contribution of *A. baumannii* to patient outcome, but for virtually every study concluding that there is a detrimental effect [22–24] there are similar studies showing little or no effect on equivalent outcomes [25–28]. The lack of consensus on attributable mortality may be due in part to the difficulty in distinguishing between colonisation and infection, which is compounded by the limited data on pathogenesis. There is also wide methodological heterogeneity between studies (prospective versus retrospective), variation in the definitions of cases versus controls (*A. baumannii* infection versus other infection, polymicrobial versus monomicrobial) and often a lack of appropriate matching for underlying co-morbidities. There are also problems with accurate identification of the organism to the species level as well as assessment of the impact of specific clones, which may have different virulence potential. Many of the older studies have only identified isolates to the genus *Acinetobacter*, and in many of the case series

infections are frequently part of a nosocomial outbreak with the majority of organisms belonging to a single clone. The contribution of multidrug resistance is also controversial. Some studies have found that infection with a carbapenem-resistant strain is associated with a worse outcome [29], but empirical treatment with alternative antimicrobial therapy that retains activity in vitro may not provide any additional survival benefit [30], suggesting that *A. baumannii* infection alone may be a surrogate marker for poor clinical outcome rather than a causative factor. Whilst the evidence for an impact on mortality is lacking, most studies do identify an effect on the duration of inpatient stay, particularly in intensive therapy units, a requirement for mechanical ventilation and increased treatment costs [31]. Overall, the outcome for patients both infected with and colonised by *A. baumannii* tends to be poorer [32].

4. Antimicrobial resistance

Interest in *A. baumannii* has intensified owing to its seemingly endless capacity to acquire antimicrobial resistance. Although the precise definition of ‘multidrug’ and ‘pandrug’ resistance is debated [33], there is no question that resistance to all commonly used Gram-negative antimicrobials is now a frequent problem in clinical practice [34–36]. Analysis of the genome sequences of a number of multidrug-resistant (MDR) isolates has revealed the presence of several large genomic islands (AbaR1, R2, R3 and R5) [37–40] containing multiple resistance genes thought to be acquired from other Gram-negative species. Table 1 shows the major mechanisms of resistance that have been identified for the different classes of antibiotics, and these are further discussed below.

4.1. β-Lactamases

Acinetobacter baumannii possesses an intrinsic class D oxacillinase and a non-inducible chromosomal AmpC cephalosporinase. *Acinetobacter baumannii* oxacillinases belong to the OXA-51-like group of enzymes that constitutes over 40 sequence variants [65,66]. The ubiquitous nature of OXA-51-like genes in *A. baumannii* has led to this gene becoming an important genetic marker in identification of the organism to the species level [67]. OXA-51-like enzymes are able to hydrolyse penicillins (benzylpenicillin, ampicillin, ticarcillin and piperacillin) and carbapenems (imipenem and meropenem) but they do this only very weakly. They are not active against expanded-spectrum cephalosporins [68]. A significant contribution to β-lactam resistance by OXA-51-like enzymes therefore requires the presence of an insertion element IS*Aba1* upstream of the gene, able to act as a strong transcriptional promoter [130]. The role of IS*Aba1* and other insertion sequences in modulating expression of *A. baumannii* resistance genes has also been established for genes involved in cephalosporin resistance (*ampC*) [42] and it is also the presumed promoter for *sulII*-mediated sulphonamide resistance [44].

The commonest enzymatic mode of carbapenem resistance is the production of oxacillinases encoded by genes of the *bla*$_{OXA-23}$, *bla*$_{OXA-40}$ and *bla*$_{OXA-58}$-like lineage. These may be plasmid or chromosomally located, are not inhibited by clavulanic acid and have been found in most regions of the world [131]. Potent class B metallo-carbapenemases of the VIM, IMP and SIM type have been found in *A. baumannii*, especially in the Asia Pacific region and Latin America [132]. These confer high-level resistance to carbapenems and all other β-lactams, with the exception of aztreonam, and are usually found in association with complex class 1 integrons.

A wide range of class A extended-spectrum β-lactamases, including those of the TEM, SHV, CTX-M, GES, SCO, PER and VEB families, have all been described (Table 1). Resistance to extended-spectrum cephalosporins owing to production of these enzymes appears to be rarer than upregulation of AmpC, although difficulties in detection in the presence of other modes of resistance mean that their true prevalence may be underestimated [133].

4.2. Aminoglycoside-modifying enzymes

Acetyltransferases, nucleotidyltransferases and phosphotransferases have all been found in *A. baumannii*, often occurring in combination. These may be located on plasmids, transposons or in association with class I integrons [134]. In a study of aminoglycoside resistance in pan-European *A. baumannii* clones (*n* = 105), 95% of isolates were found to contain at least one resistance gene and 84% carried between two and five genes [113]. A total of 12 different combinations were found, with *aacC1*, *aadA1* and *aacA4* located in association with class 1 integrons [113]. Expression of these genes leads to variable susceptibility to different aminoglycosides. Another set of enzymes, the 16s rRNA methyltransferases (ArmA, and RmtA, B, C and D), which confer high-level resistance to all formulated aminoglycosides, have recently been described. Whilst members of the Rmt family have not yet been found in *A. baumannii*, the *armA* gene appears to be relatively widespread [115,116], often found in combination with $bla_{\text{OXA-23}}$ [56,117].

4.3. Permeability defects

Enzymatic resistance may be further enhanced by changes in membrane permeability owing to loss of OMPs that act as porins for the transport of substances across the outer membrane. *Acinetobacter baumannii* has relatively few porins compared with other Gram-negative bacteria, which may account in part for some of its intrinsic antimicrobial resistance [135]. Porin loss has been most frequently implicated in β-lactam resistance, with lack of a 33–36 kDa protein [98], a 29 kDa protein named CarO [9] and a 43 kDa protein homologous to the OprD of *Pseudomonas aeruginosa* [100] implicated in imipenem resistance. The OmpA protein already discussed as a potential virulence determinant is homologous to the OmpA and OmpF porins in Enterobacteriaceae and *P. aeruginosa*, where they serve as slow channels for β-lactams. However, studies in MDR *A. baumannii* have not yet identified a clear role for OmpA in β-lactam resistance [136].

4.4. Multidrug efflux systems

Drug removal by active efflux mechanisms contributes substantially to multidrug resistance. Narrow-spectrum pumps of the major facilitator superfamily (MFS) include those involved in tetracycline (TetA, TetB) and minocycline (TetB) resistance [108] as well as the CmlA system that extrudes chloramphenicol [37]. Neither TetA nor TetB affect tigecycline.

Pumps of the resistance–nodulation–cell division (RND) type are three-component pumps with broad substrate specificity consisting of a common tripartite structure with periplasmic, inner and outer membrane components. Two systems have been

characterised in *A. baumannii*, AdeABC and AdeIJK. Resistance to aminoglycosides, β-lactams, chloramphenicol, erythromycin and tetracyclines occurs due to overexpression of *adeABC*, a phenomenon under the control of a two-component regulator system encoded by the *adeRS* genes [123]. Point mutations in the response regulator (*adeR)* and sensor kinase (*adeS)* genes can lead to overexpression of *adeABC*, but control by other transcriptional regulators is also likely to be important, especially in AdeABC-mediated tigecycline efflux [111]. The second RND pump, AdeIJK, has a substrate specificity favouring amphiphilic compounds and contributes synergistically with AdeABC to tigecycline resistance [124].

A pump belonging to the multidrug and toxic compound extrusion (MATE) family, AbeM, has also been characterised. Overexpression of this results in reduced susceptibility to quinolones, gentamicin, kanamycin, erythromycin, chloramphenicol and trimethoprim [114].

The most recent efflux system to be described, designated AbeS, belongs to the small multidrug resistance family of bacterial integral membrane proteins (BIMP). It has been shown to contribute to resistance to quinolones, macrolides and chloramphenicol [122].

4.5. Miscellaneous resistance mechanisms

Other resistance mechanisms include downregulation of penicillin-binding proteins (PBPs) (β-lactam resistance) [105], mutations in DNA gyrase and topoisomerase IV (fluoroquinolone resistance) [119], a tetracycline ribosomal protection protein (TetM)

[107], a putative dihydrofolate reductase (*folA*) involved in trimethoprim resistance [126] and mutations in a two-component regulator (*pmrA*/*B*) associated with resistance to polymyxins [127].

The relative contribution of each mechanism to overall resistance is likely to vary between strains, with some (IS*Ab1* insertions) having a far greater impact than others (PBP alterations). MDR isolates may possess a number of mechanisms conferring resistance to the same class of antibiotics, adding to the difficulties in finding suitable therapeutic agents.

5. Treatment options

When treatment is required, the choice of antimicrobial agent is severely limited by resistance. Carbapenems are usually considered the treatment of choice where isolates remain susceptible [137], however the rapid dissemination of multidrug resistance means that other options are increasingly used despite a lack of appropriate evidence.

5.1. Monotherapy

5.1.1. Sulbactam

The β-lactamase inhibitor sulbactam has antimicrobial activity against *A. baumannii* via inhibition of PBP2. Preparations containing ampicillin/sulbactam have been shown to be effective in the treatment of bloodstream, respiratory tract and urinary tract infections [138,139], although the contribution of ampicillin is negligible [140]. In a randomised controlled trial of high-dose ampicillin/sulbactam versus colistin for the

treatment of ventilator-associated pneumonia (VAP), comparable safety and efficacy were reported [141]. Combinations of carbapenems and sulbactam have also been shown to be effective even in carbapenem-resistant isolates [142,143]. If isolates are susceptible, sulbactam-containing compounds may be a safe and effective therapeutic option, although as with the other classes the development and spread of resistance is likely to limit future use [144].

5.1.2. Polymyxins

The majority of MDR strains remain susceptible to the polymyxins, resulting in increased reliance on these compounds despite previous concerns regarding toxicity [145]. Colistin (polymyxin E) has proven clinical efficacy in the treatment of bloodstream, wound and urinary tract infections [146] and in modern use has not been associated with significant neurotoxicity, although nephrotoxicity remains a concern [147]. Despite the relatively poor penetration of intravenous colistin into the lung, it has been shown to be comparable with imipenem in the treatment of VAP [148]. Favourable outcomes have also been reported with the use of nebulised colistin [149,150], offering an alternative route of administration where there are concerns over toxicity.

Colistin resistance, particularly heteroresistance, has been reported [151,152] but can be difficult to detect using routine susceptibility testing methodologies [153], meaning current rates of resistance may be underestimated. There is also confusion surrounding the dosing of colistin, a problem that is exacerbated by limited pharmacokinetic information and the fact that current commercial preparations contain different amounts of the active drug. Markou et al. [154] found that dosage

regimens commonly administered to critically ill adult patients were associated with suboptimal peak concentration/minimum inhibitory concentration (C_{max}/MIC) ratios for many strains of Gram-negative bacilli, including *A. baumannii*, which were otherwise considered susceptible. Further work is clearly required to define the optimal dosing regimen and method of administration of this drug.

5.1.3. Tigecycline

The minocycline derivative tigecycline is effective in and licensed for the treatment of complicated skin and soft-tissue infections [155] and intra-abdominal infections [156] and usually demonstrates good in vitro activity against MDR *A. baumannii.* Owing to the lack of alternatives, it has been used for the treatment of *A. baumannii* infections at a variety of other sites outside of the standard indications, including bloodstream and respiratory infections, with reported clinical response rates varying between 68% [157] and 84% [158]. In these retrospective case series, clinical improvement did not necessarily correlate with eradication of the organism from the site of infection, particularly in the treatment of VAP. The use of tigecycline for bloodstream infection is particularly controversial as its enhanced tissue penetration leads to serum concentrations that may be well below the pharmacodynamic breakpoint, resulting in recurrent bacteraemia and in some cases the rapid emergence of resistance [157,159]. However, in the current environment its use in difficult situations seems unavoidable and further prospective evaluation is required to determine the safety and efficacy of higher dosing regimes and its use in drug combinations.

5.2. Combination therapies

Given the lack of new treatments, there has been considerable interest in the use of dual or even triple antimicrobial combinations. Although significant synergy can be observed in vitro when colistin is combined with rifampicin, minocycline, ceftazidime or imipenem, and when sulbactam is combined with meropenem [143], evidence for any clinical benefit is lacking [160]. In some instances less favourable outcomes have been reported with the use of combinations involving imipenem and amikacin or rifampicin [161,162]. Studies with tigecycline-containing combinations found synergy with colistin, levofloxacin, amikacin and imipenem but antagonism when tigecycline was combined with piperacillin/tazobactam [163]. Again, whether these interactions are relevant in vivo is undefined, but given that patients with MDR *A. baumannii* often receive multiple antimicrobial agents these findings should be taken into consideration when formulating treatment regimens to ensure that individuals are not given combinations that are potentially antagonistic.

6. Conclusions

Despite an exponential rise in *A. baumannii* infections over the past decade, many questions remain unanswered. Whilst knowledge of the virulence and particularly the resistance mechanisms is increasing, details of the population at risk and the pathogenesis of severe infection are still poorly understood. Although the available evidence suggests a significant effect on patient outcome in terms of prolonged hospital stay and duration of mechanical ventilation, debate continues regarding the attributable mortality of MDR infections. The dearth of available treatments remains a major concern and although further work on the use and efficacy of combination

therapies is warranted, a more urgent priority must be the development of novel therapeutic agents.

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Table 1

Major mechanisms of resistance identified for the different classes of antibiotics

Antimicrobial class/resistance mechanism	Class/family	Variants	Reference
β-Lactams			
β-Lactamases [a]	Intrinsic cephalosporinase	AmpC (ADC1–7)	[41–45]
	Class A/high-prevalence $ESBL_A$	VEB-1, -2	[46–50]
		PER-1, -2	[49–57]
		TEM-92, -116	[58,59]
		SHV-12, -5	[58,60]
		CTX-M-2, -3	[54,61,62]
	Class A/low-prevalence $ESBL_A$	SCO-1	[63]
	Class D OXA enzymes/$ESBL_{M–D}$	OXA-51-like	[64–68]
Carbapenemases	Class D OXA enzymes/$ESBL_{CARBA-D}$	OXA-23–27, -37, -40, -58-like	[56,69–81]
	$MBLs/ESBL_{CARBA-B}$	VIM	[82–85]
		IMP	[67,83,86–91]
		SIM	[92]
	Class A carbapenemase/$ESBL_{CARBA-A}$	GES-11	[93]
OMP loss	CarO		[94–96]
	HMP-AB		[97]
	33–36 kDa protein		[98,99]
	43 kDa protein		[100]
Efflux pump	AdeABC		[101–103]
Altered PBP expression		PBP2 downregulation	[9,104,105]

Tetracyclines			
Efflux pump	MFS	TetA, TetB	[106–108]
	RND	AdeABC	[101]
Ribosomal protection		TetM	[109]
Glycylcyclines			
Efflux pump	RND	AdeABC	[110,111]
Aminoglycosides			
Enzymatic degradation	Acetyltransferases	AacC1/2, AadA, AadB	[112,113]
	Nucleotidyltransferases	Ant1	
	Phosphotransferases	AphA1, AphA6,	
Efflux pumps	RND	AdeABC	[101]
	MATE	AdeM	[114]
16s rDNA methyltransferases		ArmA	[56,115–118]
Quinolones			
DNA gyrase/topoisomerase mutations		GyrA/ParC	[119–121]
Efflux pumps	RND	AdeABC	[101]
	MATE	AdeM	[114]
	BIMP	AbeS	[122]
Chloramphenicol			
Efflux pumps	RND	AdeABC	[101]
		AdeIJK	[123,124]
	MFS	CmlA	[37]
		CraA	[125]
	BIMP	AbeS	[122]
Trimethoprim/sulfamethoxazole			
Efflux pump	RND	AdeABC, AdeIJK	[124]
Dihydropteroate synthase		SulI/II	[44]
Dihydrofolate reductase		FolA	[126]

Macrolides			
Efflux pumps	MATE	AbeM	[114]
	BIMP	AbeS	[122]
Polymyxins	PmrAB two-component mutation		[127]

MBL, metallo-β-lactamase; OMP, outer membrane protein; HMP, heat modifiable protein; PBP, penicillin-binding protein; MFS, major facilitator superfamily; RND, resistance–nodulation–cell division; MATE, multidrug and toxic compound extrusion; BIMP, bacterial integral membrane proteins.

[a] Extended-spectrum β-lactamase (ESBL) classification as denoted by Bush et al. [128] and Giske et al. [129].