

# *Acinetobacter baumannii*: Evolution of Antimicrobial Resistance—Treatment Options

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## Abstract

The first decade of the 20th century witnessed a surge in the incidence of infections due to several highly antimicrobial-resistant bacteria in hospitals worldwide. *Acinetobacter baumannii* is one such organism that turned from an occasional respiratory pathogen into a major nosocomial pathogen. An increasing number of *A. baumannii* genome sequences have broadened our understanding of the genetic makeup of these bacteria and highlighted the extent of horizontal transfer of DNA. Animal models of disease combined with bacterial mutagenesis have provided some valuable insights into mechanisms of *A. baumannii* pathogenesis. Bacterial factors known to be important for disease include outer membrane porins, surface structures including capsule and lipopolysaccharide, enzymes such as phospholipase D, iron acquisition systems, and regulatory proteins. *A. baumannii* has a propensity to accumulate resistance to various groups of antimicrobial agents. In particular, carbapenem resistance has become commonplace, accounting for the majority of *A. baumannii* strains in many hospitals today. Carbapenem-resistant strains are often resistant to all other routinely tested agents. Treatment of carbapenem-resistant *A. baumannii* infection therefore involves the use of combinations of last resort agents such as colistin and tigecycline, but the efficacy and safety of these approaches are yet to be defined. Antimicrobial-resistant *A. baumannii* has high potential to spread among ill patients in intensive care units. Early recognition and timely implementation of appropriate infection control measures is crucial in preventing outbreaks.

## Keywords

- ▶ *Acinetobacter baumannii*
- ▶ virulence
- ▶ MDR
- ▶ polymyxin

*Acinetobacter* species comprise a group of genetically related non-lactose-fermenting, oxidase-negative gram-negative coccobacilli. Among them, *Acinetobacter baumannii* is the most clinically significant *Acinetobacter* species that is implicated in nosocomial infections; however, *Acinetobacter pittii* and *Acinetobacter nosocomialis* are also increasingly recognized in these infections. *A. baumannii* is intrinsically resistant to several classes of antimicrobial agents and also readily acquires resistance to other classes of agents. It is also extremely resistant to desiccation and may survive on inanimate surfaces for months. These traits make it particularly

successful in the hospital environment and have contributed to the spread of multidrug-resistant (MDR) *A. baumannii* clones worldwide. Infections due to MDR *A. baumannii*, in particular carbapenem-resistant strains, have been associated with substantial mortality and hospital costs.<sup>1,2</sup>

## Classification, Epidemiology, and Clinical Relevance

There are now more than 20 *Acinetobacter* species that have been identified, with *A. baumannii* being the most commonly

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encountered in a clinical setting.<sup>3,4</sup> In addition, *A. nosocomialis* (genomospecies 13TU) and *A. pittii* (genomospecies 3) are increasingly implicated in hospital-acquired and health-care-associated infections.<sup>5</sup> In contrast, *Acinetobacter calcoaceticus* is an environmental pathogen of little clinical significance. These four species are biochemically indistinguishable and often lumped together as “*Acinetobacter baumannii* complex,” “*Acinetobacter-baumannii/calcoaceticus*,” or simply “*Acinetobacter baumannii*” in clinical practice. *A. baumannii* has been associated with a higher degree of antimicrobial resistance and higher mortality among patients compared with these related non-*baumannii* species.<sup>5</sup>

*A. baumannii* can cause a wide variety of infections. The majority of the cases involve the respiratory tract, but bacteremia, meningitis, and wound infection may also occur, the last of which was prominently observed in the context of war-related trauma.<sup>3</sup> A survey in U.S. hospitals showed that the majority of the isolates (57.6%) were from the respiratory tract, followed by bloodstream (23.9%) and skin or wound (9.1%) in 2010.<sup>6</sup> *Acinetobacter* species ranked fifth as the causative organism of ventilator-associated pneumonia (6.6%) and thirteenth as the cause of central line-associated bloodstream infection (2.1%).<sup>7</sup>

*Acinetobacter* used to be susceptible to most antimicrobial agents ranging from ampicillin to nalidixic acid up to the early 1970s.<sup>8</sup> However, rates of resistance increased for many classes in the 1980s, and by early 1990s reports on imipenem-resistant isolates appeared. This was a concerning development because it took away the most reliable treatment option for *Acinetobacter* infections. In 2010, 44.7 and 49.0% of isolates were resistant to imipenem and meropenem, respectively, in the earlier-mentioned U.S. survey.<sup>6</sup> *A. baumannii* isolates that are resistant to carbapenems are always resistant to penicillins and cephalosporins and often to aminoglycosides and fluoroquinolones as well. Most isolates remain susceptible to colistin, but again colistin-resistant isolates are increasingly reported, especially following treatment of infection by carbapenem-resistant isolates with this agent.<sup>9</sup>

The worldwide spread of MDR *A. baumannii*, in particular carbapenem-resistant isolates, is understood as a largely clonal phenomenon.<sup>10</sup> In a survey of nearly 500 carbapenem–non-susceptible isolates collected globally in the mid-2000s, about half of them originating from various continents belonged to European Clone II (also called Worldwide Clone 2) by molecular typing.<sup>11</sup> Other Worldwide Clones (WW1 and WW2 through WW8) have also wide distribution and thus contribute to the international spread of carbapenem-resistant *A. baumannii*.

The risk factors for acquiring MDR and carbapenem-resistant isolates include recent exposure to antimicrobial agents (in particular carbapenems), the presence of central venous catheters or urinary catheters, severity of illness, duration of hospital stay, location in an intensive care unit (ICU), larger hospital size, and recent surgery.<sup>12–15</sup> Mortality from invasive *A. baumannii* infection is high, especially when the isolate is resistant to carbapenems. Crude mortality for carbapenem-resistant *A. baumannii* infections ranges from 16 to 76%.<sup>16</sup> Risk factors for mortality among patients with carbapenem-

resistant *A. baumannii* bloodstream infections include the severity of illness, underlying malignancy, history of transplant, higher age, septic shock, concurrent pneumonia, inappropriate antimicrobial therapy, prolonged ICU stay, and renal failure, among others.<sup>17–22</sup> High mortality rates observed in patients with carbapenem-resistant *A. baumannii* infection are attributed to greater severity of illness and higher risk of receiving early inappropriate antimicrobial therapy.<sup>16</sup>

## A. *baumannii* Virulence Mechanisms

Despite the increasing importance of MDR *A. baumannii* disease, our understanding of mechanisms of pathogenesis remains in its infancy. An increasing number of *A. baumannii* genome sequences have broadened our understanding of the genetic makeup of these bacteria and highlighted the extent of horizontal transfer of DNA.<sup>23</sup> Animal models of disease (both mammalian and invertebrate) combined with bacterial mutagenesis have provided some valuable insights into mechanisms of *A. baumannii* pathogenesis. Bacterial factors known to be important for disease are presented in ▶ **Table 1**; these include outer membrane porins,<sup>24–26</sup> surface structures including capsule<sup>27</sup> and lipopolysaccharide,<sup>28</sup> enzymes such as phospholipase D,<sup>29</sup> iron acquisition systems,<sup>30</sup> and regulatory proteins.<sup>31</sup> The following paragraphs will discuss different stages of the infection process and highlight known virulence factors.

Transmission is the initial step in disease. The propensity for biofilm formation is likely to contribute to prolonged survival of *A. baumannii* on abiotic surfaces, leading to transmission. However, a definitive link between outbreak strains, biofilm formation, and adherence to host cells has not been established.<sup>32</sup> *A. baumannii* biofilm formation on indwelling devices, such as urinary catheters, central venous catheters, and endotracheal tubes, may seed infection.<sup>33</sup> Notably, bacteria in biofilms are more resistant to desiccation, immune system clearance, antibiotics, and other antibacterial agents.<sup>34,35</sup> Established factors that contribute to *A. baumannii* biofilm formation include pili, outer membrane proteins, and extracellular polysaccharide (▶ **Table 1**).

Binding to host structures is necessary for colonization. *A. baumannii* has been shown to adhere to a range of host cells in vitro including laryngeal, bronchial, and alveolar respiratory epithelial cells.<sup>24,36,37</sup> In vivo, such binding may be the first stage in the development of pneumonia. The molecular basis for such interactions is being unraveled; adhesins that mediate binding to host cells include OmpA, Bap, and Omp33–36.<sup>26</sup> While specific host ligands for these interactions have not been thoroughly investigated, cellular fibronectin is one adhesin target (▶ **Table 1**).<sup>38</sup> The autotransporter Ata has also been found to adhere to numerous host extracellular matrix proteins, which may also facilitate tissue colonization.<sup>39</sup>

Binding to host cells is followed by cellular damage and *A. baumannii* invasion. While not considered a classical intracellular pathogen, invasion may be a way to avoid immune recognition. Multiple factors including OmpA, Omp33–36, and phospholipase D are necessary for *A. baumannii* cell

**Table 1** *Acinetobacter baumannii* virulence functions and associated bacterial factors

Virulence function	Related protein(s)	Protein function/ description	Mutant attenuated in disease model <sup>a</sup>	Reference	
Biofilm formation	Abal	Autoinducer synthase (quorum sensing)	NT	153	
	Ata	Autotransporter	Mouse septicemia	39	
	Bap	Biofilm maturation and maintenance	NT	154,155	
	BfmS/R	Two-component regulator	NT	47,156	
	CsuA/B ABCDE	Pilus production (usher chaperone)	NT	157	
	GacS	Sensor of two-component regulator	Mouse septicemia	31	
	H-NS	Transcriptional regulator (suppressor)	<i>C. elegans</i>	48	
	OmpA	Outer membrane porin	Mouse pneumonia	24,37	
	PgaABC	Production of poly-β-1,6-N-acetylglucosamine (PNAG)	NT	158	
	PglC	Protein glycosylation, capsule production	Mouse septicemia	159	
	PglL	Protein O-glycosylation	Mouse septicemia	160	
	Adhesion to extra-cellular matrix	Ata	Autotransporter	See above	39
	Adhesion to host cells	Bap	Biofilm maturation and maintenance	NT	154,155
BfmS/R		Two-component regulator	NT	47,156	
H-NS		Transcriptional regulator (repressor)	See above	48	
OmpA		Outer membrane porin	See above	37	
Invasion and intra-cellular survival		BasD, BauA	Siderophore synthesis (iron acquisition)	Mouse septicemia	30
	Omp33–36	Outer membrane porin (perturbation of autophagy)	Mouse septicemia	25,26	
	OmpA	Outer membrane porin	See above	24	
	PlD	Phospholipase D	Mouse septicemia	29	
	Cytotoxicity/induction of apoptosis/cellular necrosis	BasD, BauA	Siderophore synthesis (iron acquisition)	Mouse septicemia	30
	OmpA	Outer membrane porin	See above	37,40,41	
	Omp33–36	Outer membrane porin	See above	26	
	PaaE	Production of toxic epoxide compounds	Mouse septicemia	31	
	Serum resistance	BfmS/R	Two-component regulator	NT	47
		LpsB	LPS synthesis	Rat soft tissue	28
	OmpA	Outer membrane porin	See above	44	
	PglC	Protein O-glycosylation and capsule production	See above	159	
	PlD	Phospholipase D	See above	29	
	Ptk, EpsA	Capsule production	Rat soft tissue	27	

Abbreviation: NT, not tested.

<sup>a</sup>*Caenorhabditis elegans*, nematode model of disease.

invasion.<sup>24,25,29,37</sup> Once inside host cells, BasD and BauA (involved in the synthesis and transport of small, iron chelating molecules called siderophores) are required for survival.<sup>30</sup> Omp33–36 also interferes with autophagy, which may promote survival of *A. baumannii* in host cells.<sup>25</sup> It has been shown that cellular damage can be mediated by *A. baumannii* outer membrane proteins, more specifically OmpA and Omp33–36, which both contribute to apoptosis.<sup>25,37,40,41</sup> These proteins have also been shown to play a role in virulence in mammalian infection models.<sup>24,26</sup>

Bloodstream infection is a common complication of *A. baumannii* infection; accordingly, most clinical isolates are resistant to the bactericidal activity of serum complement.<sup>42</sup> Complement evasion strategies may prevent direct lysis of bacteria by the membrane attack complex, reduce opsonization for phagocytes, and blunt production of complement inflammatory mediators. As with other pathogens, *A. baumannii* serum resistance is multifactorial, conferred in part by lipopolysaccharide and capsule.<sup>27,28,42</sup> LPS is also necessary for defense against antimicrobial peptides such as LL-37.<sup>43</sup> Many bacteria also evade complement activity by binding to soluble mediators of complement regulation such as factor H of the alternative pathway. One study indicated that *A. baumannii* co-opts factor H via several proteins including OmpA.<sup>44</sup> Consistent with this finding, mice infected with an OmpA mutant had 1,000-fold lower burdens of bacteria in the blood consistent with a role in serum resistance.<sup>24</sup> However, a second study found no evidence for factor H binding on the surface of clinical isolates of *A. baumannii*.<sup>45</sup> Proteases can also mediate immune evasion by degrading bound complement molecules and antibodies. Consistent with this, the secreted protease dubbed PKF also contributes to serum resistance.<sup>46</sup>

Regulation of the expression of virulence traits is essential for pathogens. Several regulators of *A. baumannii* virulence have been identified. GacSA is a two-component global virulence regulator essential for disease.<sup>31</sup> Mutation of the sensor kinase gene *gacS* altered the expression of genes involved in pili synthesis, motility, and biofilm formation; there was a consistent defect in the corresponding in vitro phenotypes, in addition to reduced growth in human serum. GacSA was also found to regulate components of a pathway for aromatic amino acid metabolism; mutation of *paaE* from this system confirmed a role in virulence. The BfmRS two-component regulator also controls multiple phenotypes, including biofilm formation, adherence to host cells, and resistance to human serum.<sup>47</sup> The bacterial histone-like nucleoid structuring protein (H-NS) may have a role in regulating hydrophobicity, biofilm formation, adherence to host alveolar macrophages, and motility in *A. baumannii*. An *hns* mutant had significantly increased virulence toward the nematode *Caenorhabditis elegans*, potentially a result of upregulation of the type VI secretion system and a known virulence factor, the *ata* autotransporter.<sup>48</sup> Further study of these and other virulence regulators will facilitate identification of novel virulence factors of *A. baumannii*, thereby broadening our understanding of host–pathogen interactions.

It is likely that many mechanisms of disease pathogenesis remain undiscovered, and are sufficiently novel to evade identification by “genome browsing.” Broad-based methodology for identification of factors by screening of transposon mutant libraries has been successfully applied in various model systems. A screening of 1,324 transposon mutants for virulence against the nematode *C. elegans* and amoeba *Dictyostelium discoideum* identified 14 genes that may have a role in virulence (absent in the environmental organism *Acinetobacter baylyi*).<sup>23</sup> However, the role of these factors in disease has not been verified by complementation with a functional copy of the gene, nor confirmed in a mammalian host. In vivo analysis of a library of 150,000 unique transposon insertion mutants in a high throughput sequencing strategy found 157 genes necessary for persistence in the mouse lung, including previously identified virulence factors OmpA, LPS, BfmRS, and GacA.<sup>49</sup> Several mutants were individually analyzed and found to be attenuated in the mouse. Further validation of these results through mutagenesis and complementation may reveal novel virulence mechanisms. Given the lack of new antimicrobials in the pipeline for problematic MDR organisms, virulence factors constitute novel therapeutic targets for rational drug design.

## Host Immune Responses

Neutrophils play an important role in the immune response to *A. baumannii* infection. In mice, neutrophils are rapidly recruited to lungs during infection, and after clearance of bacteria, neutrophil numbers return to normal.<sup>50</sup> Neutrophil depletion exacerbated disease in systemic and pulmonary models of mouse infection.<sup>50,51</sup> In the study of Breslow et al,<sup>51</sup> increased burdens of bacteria in the lung, decreased production of proinflammatory cytokines, and enhanced bacterial dissemination to extrapulmonary sites were observed. Macrophages also appear to play a significant, but less important, role in the early phase of lung infection through phagocytosis, release of cytokines, and neutrophil recruitment.<sup>52</sup>

Toll-like receptor 4 (TLR4) and CD14 are important for the detection of *A. baumannii* LPS; mice lacking these receptors were more susceptible to pulmonary disease and exhibited delayed lung inflammation and reduced cytokine production.<sup>53</sup> In vitro, TLR4-deficient murine bone-marrow-derived macrophages had reduced ability to kill *A. baumannii*.<sup>54</sup> *A. baumannii* LPS is also a potent stimulator of human THP-1 monocytes in vitro; signaling via TLR4, LPS stimulated the production of IL-8 and TNF- $\alpha$ . TLR2 may also play a role in response to whole *A. baumannii* cells.<sup>55</sup> Recently, it has been shown that during the intracellular phase of *A. baumannii* infection, the intracellular pattern recognition receptors Nod1 and Nod2 are stimulated in airway epithelial cells, resulting in the production of cytokines, chemokines, and  $\beta$ -defensin 2.<sup>56</sup>

It is clear that immunity to *A. baumannii* can be conferred by antibodies. Vaccination with an outer membrane complex of proteins and LPS protected mice against challenge, and animals that received a passive infusion of immune serum

were also protected against disease.<sup>57</sup> Although the specific antigens responsible for protection were not identified in this study, a survey of immunostimulatory antigens of *A. baumannii* in sera from 50 patients identified six dominant outer membrane proteins that were immunogenic.<sup>58</sup> In another study, vaccination with OmpA alone protected a proportion of animals from infection, as did a passive infusion of vaccinate sera, and immune sera increased opsonophagocytosis of *A. baumannii*.<sup>59</sup> In addition to furthering knowledge of mechanisms of immunity toward *A. baumannii*, these studies show the promise of active vaccination and passive immunotherapy (through infusion of antibodies) against *A. baumannii* disease.

## Antimicrobial Resistance Mechanisms

Owing to a propensity for acquisition of foreign DNA, *A. baumannii* can assemble and modulate a host of antimicrobial resistance mechanisms to survive the selective pressure they encounter, providing them with a strong ecological advantage in the hospital environment (► **Table 2**).

## Cephalosporins

A majority of *A. baumannii* clinical isolates are now resistant to cephalosporins, including third-generation (e.g., ceftazidime) and fourth-generation (e.g., cefepime) agents. *A. baumannii* naturally produces AmpC  $\beta$ -lactamase (called ADC [Acinetobacter-derived cephalosporinase]).<sup>60,61</sup> Unlike other gram-negative organisms where production of AmpC can be induced or permanently de-repressed, the ADC  $\beta$ -lactamase in *A. baumannii* is not considered inducible.<sup>61</sup> However, expression of ADC  $\beta$ -lactamase is enhanced when certain insertion sequences are acquired upstream of the gene and provides a strong promoter activity, leading to a clinically relevant level of resistance.<sup>62,63</sup> In addition to ADC  $\beta$ -lactamase, *A. baumannii* may also produce extended-spectrum  $\beta$ -lactamase (ESBL), which also leads to cephalosporin resistance.<sup>64–67</sup>

## Carbapenems

The most significant mechanism of carbapenem resistance in *A. baumannii* is the production of carbapenemases, which can

**Table 2** Mechanisms of antimicrobial resistance in *Acinetobacter baumannii*

Agents	Related protein(s)	Protein function/description	Reference
Cephalosporins	ADC	Intrinsic AmpC $\beta$ -lactamase	60,61
	CTX-M	Acquired extended-spectrum $\beta$ -lactamase	64–67
	PER		
	GES		
	VEB		
Carbapenems	OXA-51-group	Intrinsic serine carbapenemase	68,69
	OXA-23/40/58/143/235-group	Acquired serine carbapenemase	70,71
	NDM	Acquired metallo- $\beta$ -lactamase	72,73
	KPC	Acquired serine carbapenemase	77
Sulbactam	TEM	Acquired serine $\beta$ -lactamase	80
	PBP2	Penicillin-binding protein (reduced expression)	79
	Rifampin	RpoB	RNA polymerase $\beta$ -subunit (amino acid changes)
Aminoglycosides	Unknown	Efflux pump	81
	Arr	ADP-ribosyltransferase	82
	AAC	Aminoglycoside acetyltransferase	83–85
	APH	Aminoglycoside phosphotransferase	
	AAD	Aminoglycoside adenyltransferase	
	ArmA	16S ribosomal RNA methyltransferase	86–88
Fluoroquinolones	RmtB	16S ribosomal RNA methyltransferase	161
	GyrA	DNA gyrase (amino acid changes)	67
	ParC	DNA topoisomerase IV (amino acid changes)	
Colistin	AdeFGH	RND efflux pump	89
	PmrCAB	Two-component regulatory system (amino acid changes)	90–92
	LpxA/C/D	Lipopolysaccharide (complete loss)	93
Tetracyclines	Tet(30)/(39)/(A)/(B)	MFS efflux pump	162
	AdeABC/FGH/IJK	RND efflux pump	95

be either intrinsic or acquired. *A. baumannii* naturally produces chromosomally encoded OXA-51-group carbapenemase at a low level, and acquisition of a stronger promoter by transposition of an insertion sequence, analogous to the case with ADC, upstream of the OXA-51-group gene may lead to elevation of carbapenem MICs.<sup>68,69</sup> *A. baumannii* also becomes resistant to carbapenems when they acquire certain OXA-group  $\beta$ -lactamase genes on plasmids. There are five major groups of acquired OXA-group carbapenemases in *A. baumannii*, including OXA-23, -40, -58, -143, and -235 groups.<sup>70</sup> Among these, OXA-23 group is the most prevalent, often produced by Worldwide Clone 2 isolates.<sup>71</sup>

Recently, non-OXA group carbapenemases that have spread in *Enterobacteriaceae* are also being acquired by *A. baumannii*. The most concerning among these is the metallo- $\beta$ -lactamase NDM-1. Carbapenem-resistant *A. baumannii* producing NDM-1 has been identified worldwide since 2011.<sup>72,73</sup> Other acquired metallo- $\beta$ -lactamases have also been reported on rare occasions.<sup>74–76</sup> Finally, *A. baumannii* producing KPC-group carbapenemase has been reported in Puerto Rico, but there is no evidence that it has spread beyond this island.<sup>77</sup>

### Sulbactam

Sulbactam is a  $\beta$ -lactamase inhibitor which is usually combined with ampicillin or cefoperazone to mitigate their hydrolysis by class A  $\beta$ -lactamases, but it also has intrinsic activity against *Acinetobacter* species including *A. baumannii*, presumably by binding to penicillin-binding protein PBP2.<sup>78</sup> Reduced expression of PBP2 and production of TEM-1  $\beta$ -lactamase have been associated with resistance to sulbactam in *A. baumannii*.<sup>79,80</sup>

### Rifampin

Rifampin exerts its activity by binding to the bacterial RNA polymerase and inhibiting transcription initiation. The major mechanism underlying rifampin resistance is amino acid substitutions in the  $\beta$ -subunit of this target protein.<sup>81</sup> Since this can occur through a single mutation to the *rpoB* gene encoding this subunit, monotherapy with rifampin is contraindicated in any bacteria, and *A. baumannii* is no exception. Besides this mechanism, enzymatic modification of rifampin and active efflux have also been associated with resistance to rifampin.<sup>81,82</sup>

### Aminoglycosides

Aminoglycosides bind to the 16S ribosomal RNA of the 30S ribosomal subunit and inhibits protein synthesis. *A. baumannii* produces various aminoglycoside-modifying enzymes to acquire aminoglycoside resistance.<sup>83–85</sup> Another aminoglycoside resistance mechanism that is emerging is production of 16S ribosomal RNA methyltransferase, especially ArmA. ArmA methylates a guanine residue in the aminoglycoside-binding site (A-site) of 16S rRNA and protects it from binding aminoglycosides.<sup>86</sup> ArmA-producing *A. baumannii* are highly

resistant to gentamicin, tobramycin, and amikacin, and are commonly seen among Worldwide Clone 2 isolates.<sup>87,88</sup>

### Fluoroquinolones

Fluoroquinolones bind to the DNA gyrase and topoisomerase IV and interfere with DNA synthesis leading to cell death. The primary mechanism of resistance to fluoroquinolones is amino acid substitutions in the quinolone resistance determining region of the genes that encode these target proteins.<sup>67</sup> This mechanism results in high-level fluoroquinolone resistance. In addition, *A. baumannii* may overexpress active efflux pumps to gain moderate level of fluoroquinolone resistance.<sup>89</sup>

### Colistin

Colistin is a cyclic cationic antimicrobial peptide that binds to lipid A to initiate its bactericidal activity. Resistance to colistin arises due to modifications of this target in clinical isolates. The addition of phosphoethanolamine to the hepta-acylated lipid A is the commonly reported modification associated with colistin resistance.<sup>90–92</sup> Complete loss of lipopolysaccharide is also proposed as a mechanism leading to colistin resistance, though this phenomenon is mostly observed in laboratory isolates rather than clinical isolates.<sup>93</sup>

### Tetracyclines

Tetracyclines bind to the 30S ribosomal subunit for its activity. Resistance develops by active efflux or target protection by production of Tet proteins that bind to the 70S ribosome.<sup>94</sup> Tigecycline was designed to resist the majority of these mechanisms, but is still prone to efflux by Ade-type efflux pumps in *A. baumannii*, especially when these pumps are overexpressed.<sup>95</sup>

### Prevention of *A. baumannii* Colonization and Infection

*A. baumannii*, in particular carbapenem-resistant or other highly resistant strains, have a propensity to cause outbreaks when they enter the hospital environment. Both antimicrobial and desiccation resistances play an important role in this phenomenon. *A. baumannii* is highly resistant to desiccation and can survive on inanimate surfaces for at least 1 month.<sup>96</sup> Environmental sites may be contaminated with MDR *A. baumannii* in almost half of the hospital rooms accommodating patients with a history of these bacterial infection or colonization.<sup>97</sup> Therefore, transmission occurs both through direct patient contact and contact with the environment of the patient's room. In one study, the gloves and/or gowns of caregivers were contaminated after 39% of all encounters with patients colonized with MDR *A. baumannii*, suggesting that these contact precaution measures are essential in preventing the organism from being transmitted to another patient by healthcare workers.<sup>98</sup>

Once an outbreak is identified, the infection control measures require a bundled approach that typically includes implementation of strict contact precaution, educational activities, enhanced terminal cleaning procedures, and investigation of environmental sources. In addition, molecular typing of the relevant *A. baumannii* isolates should be considered to define the extent of the outbreak, using techniques such as pulsed-field gel electrophoresis or multilocus variable number of tandem repeats analysis. As a case in point, an outbreak of extensively drug-resistant *A. baumannii* at an ICU in a Virginia hospital was brought under control after an intervention was implemented consisting of weekly meetings with the unit personnel, reinforcement of contact precautions and hand hygiene, cohorting of infected patients and their nursing and respiratory care staff, daily chlorhexidine bathing, preemptive contact precaution until negative surveillance culture was obtained, and restriction of carbapenem use.<sup>99</sup>

Unlike other MDR organisms such as methicillin-resistant *Staphylococcus aureus* or vancomycin-resistant enterococci, active surveillance or screening for *A. baumannii* is not conducted routinely at most institutions because of the low sensitivity of screening, which ranges between 55 and 90% depending on the methods.<sup>100,101</sup> Nonetheless, active screening was estimated to reduce *A. baumannii* transmission, infections, and deaths by 48 to 78% depending on the screening methods and to be cost-effective when the carrier prevalence exceeds 2%.<sup>102</sup>

## Treatment Options for *A. baumannii* Infection

Carbapenems have generally been considered the agents of choice for treatment of infections caused by *A. baumannii*, owing to their intrinsic activity against this organism and their favorable safety profile. However, the declining susceptibility to carbapenems has forced clinicians and researchers to explore alternative therapeutic approaches. Adding to the challenge is that by the time *A. baumannii* acquires resistance to carbapenems, there are often resistant to all other commonly used agents as well. Strains that are extensively drug resistant (XDR, resistant to all classes except up to two) usually remain susceptible to polymyxins (colistin or polymyxin B) and tigecycline. Therefore, regimens will include at least one of these two classes of agents with or without a second agent.

## Polymyxins

Polymyxins are amphipathic polypeptides that interact with lipid A of the gram-negative bacterial outer membrane and cause rapid cell death in a concentration-dependent manner. Of the two polymyxins, colistin is administered intravenously as its inactive prodrug colistin methanesulfonate (CMS), whereas polymyxin B is administered as an active drug. CMS is the more commonly used polymyxin formulation worldwide, and thus has more cumulative clinical experience accompanying it. Polymyxin resistance remains relatively rare in *A. baumannii*, though it can develop after treatment

with CMS.<sup>6,9,103</sup> The killing activity of colistin is best correlated with the free area under the curve/MIC (fAUC/MIC).<sup>104</sup> In time-kill studies, colistin exerts a rapid bactericidal effect, but regrowth can occur at colistin concentrations exceeding the MICs.<sup>105</sup> It has been hypothesized that subpopulations with increased tolerance to colistin concentrations higher than the MICs exist in clinical strains and that killing of the susceptible population results in the amplification of these hetero-resistant subpopulations.<sup>106</sup> Also, because CMS has to be converted to colistin in the plasma, patients are exposed to suboptimal concentrations of colistin for 2 to 3 days before the concentrations reach the steady state with an average maximum concentration of approximately 2.3 µg/mL, with large individual variations.<sup>107,108</sup> To mitigate these concerns of inadequate plasma levels of colistin and potential for development of resistance, the use of a loading dose is now advocated for colistin,<sup>107,109</sup> and combination therapy with a second active agent or an agent that is inactive by itself but demonstrates synergy with colistin is widely adopted. Another implication is that, based on these pharmacokinetic properties, the current susceptibility breakpoint of 2 µg/mL, defined by both the Clinical Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST), may not be adequate and a lower breakpoint may be needed. However, nephrotoxicity limits the dosing of intravenous CMS, with approximately 50% of the patients manifesting variable degrees of nephrotoxicity due to this agent.<sup>104</sup> This is a reversible process in most instances.

While clinical data regarding the efficacy of intravenous CMS for *A. baumannii* infection when used alone are scarce, a retrospective study of 35 patients with ventilator-associated pneumonia caused by *A. baumannii* who were treated with either colistin or imipenem according to actual susceptibility has shown comparable clinical cure and in-hospital mortality rates at 57% each and 62 versus 64%, respectively, suggesting that colistin may be as efficacious as imipenem when the latter cannot be used due to resistance.<sup>110</sup> In this study, colistin was dosed at 2.5 to 5 mg/kg/d but without a loading dose.

Given the less than ideal pharmacokinetics of colistin and concerns over nephrotoxicity, direct administration of CMS to the site of infection has been explored. Nebulized CMS is routinely used in the management of chronic airway infection in cystic fibrosis patients, and more recently also for ventilator-associated pneumonia in conjunction with intravenous CMS. Nebulized CMS results in minimal plasma levels of colistin.<sup>111</sup> Observational studies generally suggest that the addition of nebulized CMS to intravenous CMS may expedite clearance of *A. baumannii* from the airway but do not result in survival gain.<sup>112,113</sup> Likewise, a randomized, open-label trial comparing the efficacy of nebulized CMS for 100 patients with gram-negative ventilator-associated pneumonia, 60% of which were caused by *A. baumannii*, also showed favorable microbiological outcome in 60.9% of the nebulized plus intravenous CMS group and 38.2% of the intravenous CMS only group ( $p = 0.03$ ), but the study did not show difference in the rates of favorable clinical outcome (51.0 vs. 53.1%;  $p = 0.84$ ).<sup>114</sup> However, more bronchospasm events were

observed in the nebulized CMS group (7.8 vs. 2.0%;  $p = 0.36$ ). Overall, the clinical benefit of nebulized CMS has not been definitively established.

Colistin has very low penetration into the cerebrospinal fluid, thus intravenous CMS therapy is not expected to be effective for infection of the central nervous system.<sup>115,116</sup> Patients with severe and/or resistant *A. baumannii* CNS infections have been treated with intrathecal or intraventricular colistin.<sup>117</sup> In one recent case series, intraventricular CMS therapy led to cure of ventriculitis and meningitis in all six patients, with sterilization of the cerebrospinal fluid in a median of 2.5 days.<sup>118</sup> Therefore, intrathecal or intraventricular administration of CMS should be considered along with intravenous CMS for central nervous system infection due to *A. baumannii* that is resistant to agents with good cerebrospinal fluid penetration, including carbapenems.

The concerns over the unique pharmacokinetics of CMS and colistin have led to renewed interest in polymyxin B, which is given as the active drug and possesses more predictable pharmacokinetic properties.<sup>119</sup> Furthermore, several observational studies have reported lower nephrotoxicity rates with polymyxin B compared with CMS.<sup>120,121</sup> However, studies comparing the clinical efficacy of these two agents are lacking.

## Tigecycline

Tigecycline is a semisynthetic derivative of minocycline that inhibits protein synthesis by binding to the 30S ribosomal subunit. It is stable against many of the tetracycline resistance mechanisms including efflux pumps, such as Tet(A-E) and Tet(K), and also ribosomal protection, such as Tet(O) and Tet(M), thus has a broader spectrum of activity compared with the earlier tetracyclines.<sup>122</sup> Resistance to tigecycline is relatively rare in *A. baumannii*, but it may develop through overexpression of efflux pumps.<sup>123–125</sup>

The killing activity of tigecycline is predicted by fAUC/MIC as with colistin.<sup>126</sup> Tigecycline has unique pharmacokinetics with a large volume of distribution resulting in a low serum peak concentration of up to 0.8 µg/mL after the standard loading dose of 100 mg.<sup>127</sup> Suboptimal clinical outcome (56% infection-related mortality) has been reported for patients with carbapenem-resistant *A. baumannii* bloodstream infection who were treated with tigecycline despite in vitro susceptibility,<sup>128</sup> and breakthrough bacteremia during therapy has also been observed.<sup>125</sup> The use of tigecycline alone in the treatment of bacteremia is not recommended for these reasons. In addition, a phase 3 trial reported a lower clinical response rate in comparison with imipenem among patients with ventilator-associated pneumonia by *A. baumannii* when the standard approved dose was used.<sup>129</sup> When higher doses of tigecycline (100 mg twice a day or 75 mg twice a day) were used for hospital-acquired pneumonia, they achieved comparable clinical response rate as with imipenem (85, 70, 75%, respectively).<sup>130</sup> These data suggest that the currently approved dose may not be sufficient for the treatment of bacteremia and hospital-acquired pneumonia when used alone.

The largest case series on the use of tigecycline for XDR *A. baumannii* infections described 266 patients who were treated with tigecycline alone or in combination with another agent (a carbapenem, expanded-spectrum cephalosporin, or piperacillin-tazobactam) and 120 patients who were treated with imipenem and sulbactam.<sup>131</sup> In both arms, the isolates were resistant to all antibiotics tested, except tigecycline and colistin. The patients who received tigecycline were significantly less likely to be in an ICU, less likely to be febrile, had lower serum creatinine, less likely to have sepsis, more likely to have pneumonia (64.7 vs. 31.7%), and less likely to have bacteremia (18.0 vs. 43.3%) compared with those who received imipenem and sulbactam. There was no difference in 30-day mortality between the two groups (44.7 vs. 46.7%), whereas favorable clinical outcome was more common in the tigecycline group (69.2 vs. 50.0%,  $p < 0.001$ ). The latter finding needs to be interpreted with caution, however, as the patients in the tigecycline group were generally less ill, the majority of them also received other agents in addition to tigecycline, and the patients in the non-tigecycline group were not on any active agents in vitro.

## Sulbactam

Sulbactam is a β-lactamase inhibitor and also has affinity for penicillin-binding proteins of *A. baumannii* and is active against this species.<sup>132,133</sup> Ampicillin-sulbactam susceptibility of 63.6% has been reported for *Acinetobacter* spp. isolates collected from U.S. hospitals in the early 2000.<sup>134</sup> However, a steady decline in the susceptibility rate of *A. baumannii* from 89% in 2003 to 40% in 2008 was reported from hospitals in Michigan, raising concerns for development of resistance as more sulbactam is used for treatment of *A. baumannii* infections.<sup>135</sup>

The bactericidal activity of *A. baumannii* correlates best with the time that the free drug concentration remains above the MIC (fT > MIC).<sup>136</sup> While the standard dose of ampicillin-sulbactam is 12 g a day, it has been suggested that a dose as high as 27 g of ampicillin-sulbactam (i.e., 9 g of sulbactam) a day using extended infusion may be needed to achieve adequate exposure for treatment of infection due to less susceptible strains (MICs, 32/16 to 64/32 µg/mL).<sup>137,138</sup>

A small controlled study was conducted in Greece to determine the efficacy of ampicillin-sulbactam in 28 patients with ventilator-associated pneumonia due to XDR *A. baumannii*.<sup>139</sup> All isolates were resistant to ampicillin-sulbactam and susceptible to colistin. The patients were randomized to receive either ampicillin-sulbactam (9 g of sulbactam/day) or colistin (270 mg/day). The clinical response rates were comparable with 76.8% for ampicillin-sulbactam versus 73.3% for colistin. Bacteriological success rates, 14- and 28-day mortality rates, and the rates of adverse events were also comparable between the two groups. The study suggested that, consistent with the pharmacokinetic data, a high dose of ampicillin-sulbactam may be efficacious in the treatment of invasive *A. baumannii* infection.

Several observational studies have attempted to explore the efficacy of ampicillin-sulbactam. In a series of *A. baumannii* ventilator-associated pneumonia from the United States,



14 patients were treated with ampicillin-sulbactam and 63 with imipenem.<sup>140</sup> The percentages of successfully treated episodes were similar in the two groups (93 vs. 83%). There were also no differences in the rates of microbiological clearance and mortality. Dosing information was not provided in either of these studies. A study from Brazil examined the efficacy of ampicillin-sulbactam and polymyxins (colistin or polymyxin B) against invasive carbapenem-resistant *A. baumannii* infections, where 85 patients received ampicillin-sulbactam and 82 received polymyxins.<sup>141</sup> Almost 30% also received a carbapenem in both groups, despite all isolates demonstrating carbapenem resistance in vitro. Clinical response was observed in 60% of the ampicillin-sulbactam group and 39% of the polymyxin group, and treatment with polymyxins was an independent risk factor for in-hospital mortality (odds ratio, 2.07;  $p = 0.04$ ). However, the median daily dose of colistin was approximately 150 mg, which is substantially lower than the currently recommended dose of up to 300 mg a day.<sup>107</sup> The median daily dose of ampicillin-sulbactam was 9 g.

These data suggest that the use of sulbactam-containing regimens may have a role in the treatment of infections caused by XDR *A. baumannii*, with efficacy that is at least comparable with polymyxins.

## Rifampin

A potential benefit of adding rifampin to colistin has been demonstrated for *A. baumannii* in multiple in vitro and in vivo studies.<sup>142-145</sup> Two prospective clinical trials have been conducted to test the clinical efficacy of this combination. In Turkey, 43 patients with ventilator-associated pneumonia due to carbapenem-resistant *A. baumannii* were randomized to colistin alone or colistin and rifampin.<sup>146</sup> These two groups were comparable except for the higher mean Sequential Organ Failure Assessment score in the combination group. The crude in-hospital mortality and pneumonia-related mortality were higher for the colistin-alone group (72.7 and 63.6%, respectively) compared with the combination group (61.9 and 38.1%, respectively), but the differences were not statistically significant. Twenty-three percent developed nephrotoxicity, but none had hepatotoxicity from rifampin. The other study was conducted in Italy comparing the same regimens.<sup>147</sup> A total of 210 patients with life-threatening infection due to XDR *A. baumannii* were enrolled. The study was powered to detect 20% absolute difference in 30-day mortality. The baseline characteristics were comparable, with most patients located in ICUs. There was no mortality difference between the two groups (43.4% for the combination group, 42.9% for the colistin group). This was the case even when patients who had rifampin-resistant isolates were excluded. However, the microbiologic eradication rate was significantly higher in the combination group (60.6 vs. 44.8%,  $p = 0.034$ ). On the other hand, there was a trend for a higher rate of hepatic dysfunction in the combination group (20.8 vs. 11.9%). Notably, meropenem was added in the colistin group more frequently than in the combination group (15.9 vs. 3.9%), which may have improved the outcome in the colistin group.

Overall, the beneficial effect of adding rifampin to colistin in the treatment of XDR *A. baumannii* infection has been suggested by in vitro and in vivo studies, but has not been demonstrated in two randomized, controlled trials.

## Fosfomycin

Fosfomycin, a peptidoglycan biosynthesis inhibitor, is not active against *A. baumannii*, but in vitro synergy has been reported between fosfomycin and colistin or sulbactam among carbapenem-resistant *A. baumannii*.<sup>148,149</sup> Based on these observations, a randomized trial of colistin alone and colistin plus fosfomycin was conducted for infections caused by carbapenem-resistant *A. baumannii* in Thailand.<sup>150</sup> In this study, 99 patients were enrolled and 94 were included in the analysis, 47 in each group. Fosfomycin was given at 4 g every 12 hours intravenously to patients in the combination arm, and colistin was given at 5 mg/kg/d to both groups for 7 to 14 days. The combination and colistin-only groups did not differ in favorable clinical outcomes (59.6 vs. 55.3%) or mortality at 28 days (46.8 vs. 57.4%). However, microbiological eradication rates at the end of treatment were significantly higher in the combination group (100 vs. 81.2%,  $p = 0.01$ ). The study was underpowered to detect a relevant difference in mortality, but given the trend for lower mortality with the addition of fosfomycin, this combination may merit further investigation.

## Combination Therapy

Several retrospective studies have documented lower mortality rates after XDR *A. baumannii* infection when more than one agent was given for therapy. In a large retrospective study from Turkey, the clinical outcome of patients with XDR *A. baumannii* bloodstream infections was investigated.<sup>18</sup> Thirty-six of them received colistin monotherapy, whereas 214 received various agents in addition to colistin (102 with a carbapenem, 69 with ampicillin-sulbactam or sulbactam, and 43 with other agents). The baseline characteristics were comparable among the groups, and all isolates were susceptible to colistin. The in-hospital mortality rate was significantly lower in the combination group than in the monotherapy group (52.3 vs. 72.2%,  $p = 0.03$ ), and the rate of microbiological eradication was also significantly higher in the combination group than in the monotherapy group (79.9 vs. 55.6%,  $p = 0.001$ ). In another observational study of 69 patients with solid organ transplantation who developed invasive XDR *A. baumannii* infection, treatment with a combination of colistin and a carbapenem was an independent predictor of survival.<sup>151</sup> On the contrary, in a recent multicenter prospective study of 101 patients with MDR *A. baumannii* sepsis from Spain, there was no difference in the all-cause 30-day mortality between those who received combination therapy (24.2%) and monotherapy (23.5%).<sup>152</sup> The combinations used in this study included, but were not limited to, colistin plus tigecycline and carbapenem plus tigecycline. Therefore, it is not yet clear if any specific

combinations of agents would provide survival benefit for those with XDR *A. baumannii* infection.

## Conclusion

*A. baumannii* has become one of the most problematic hospital-acquired pathogens in the last two decades, helped by its extraordinary ability to accumulate antimicrobial resistance and survive in the modern healthcare environment. An increasing number of *A. baumannii* genome sequences, animal models of disease combined with bacterial mutagenesis, have provided some valuable insights into mechanisms of *A. baumannii* pathogenesis. Early detection and implementation of rigorous infection control measures is a key in preventing major outbreaks due to this organism. Carbapenems have been considered the agents of choice for infections caused by susceptible pathogens, but the rapid increase in carbapenem resistance rates has complicated this issue. The backbone agents when treating carbapenem-resistant cases include polymyxins, tigecycline, and sulbactam. Among these, colistin is the best studied to date, particularly in terms of its pharmacokinetics and pharmacodynamics. Therefore, the most standard approach currently is to treat these infections with pharmacokinetically optimized doses of colistin (including a loading dose), with or without a second agent, particularly a carbapenem, tigecycline, or sulbactam. Nonetheless, there is still a dearth of clinical data to guide clinicians and many questions remain unanswered. It is hoped that ongoing clinical trials and high-quality prospective observational studies will address these questions and improve the care of patients affected by this difficult-to-treat pathogen.

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