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Global Spread of Drug-resistant *Acinetobacter baumannii*

Molecular Epidemiology and Management of Antimicrobial Resistance

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Abstract and Introduction

Abstract

Acinetobacter baumannii is an opportunistic Gram-negative pathogen with increasing relevance in a variety of hospital-acquired infections especially among intensive care unit patients. Resistance to antimicrobial agents is the main reason for *A. baumannii* spread. *A. baumannii* outbreaks described worldwide are caused by a limited number of genotypic clusters of multidrug-resistant strains that successfully spread among hospitals of different cities and countries. In this article, we will focus on the mechanisms responsible for resistance to antimicrobials and disinfectants in *A. baumannii* and the epidemiology of drug-resistant *A. baumannii* in healthcare facilities. We will also discuss the therapeutic and infection control strategies for management of drug-resistant *A. baumannii* epidemics.

Introduction

Acinetobacter spp. are glucose nonfermentative Gram-negative coccobacilli that have emerged in recent years as a major cause of nosocomial infections associated with high morbidity and mortality.[1,2] The genus *Acinetobacter* currently contains up to 32 described named and unnamed (genomic) species.[1] *Acinetobacter baumannii*, genomic species 3 and 13TU, three of the most clinically relevant species, are genetically and phenotypically very similar to an environmental species, *Acinetobacter calcoaceticus*, and are therefore grouped together into the so-called *A. calcoaceticus–Acinetobacter baumannii* complex.[1] However, because *A. calcoaceticus* is not clinically relevant, the designation *A. baumannii* complex might be more appropriate if used in a clinical context.[2] Because phenotypic identification of *Acinetobacter* isolates to the species level has proven to be insufficient, several genotypic methods have been developed for genomic species identification, which include amplified 16S rRNA gene restriction analysis, high-resolution fingerprint analysis by amplified fragment length polymorphism, sequence analysis of the 16S–23S rRNA gene spacer region, *rpoB* sequencing and *gyrB* multiplex PCR.[1–6] The species that is most frequently recognized as a pathogen is *A. baumannii*, which causes a variety of healthcare-associated infections, comprising hospital-acquired and ventilator-associated pneumonia, bacteremia, urinary tract infection and surgical-site infection, especially in intensive care unit patients.[1,2,7–9] *A. baumannii* has simple growth requirements and can survive in dry conditions. This might contribute to the fitness of *A. baumannii* in the hospital environment, which represents the main reservoir of this bacterium.[1,2,10] Hand carriage by healthcare workers has also been implicated as a mode of *A. baumannii* transmission in the hospital setting.[1,2,10] Although *A. baumannii* has been classically recognized as a hospital-acquired pathogen, an increased prevalence of multidrug-resistant *A. baumannii* isolates has been recently observed among older adults in community hospitals and nursing homes in the USA.[11]

Drug-resistant *Acinetobacter baumannii*

Resistance to antimicrobial agents is the main advantage of *A. baumannii* in the nosocomial environment. Multidrug resistance (MDR) in *A. baumannii* has been defined as resistance to more than two of the following five drug classes: antipseudomonal cephalosporins (ceftazidime or cefepime), antipseudomonal carbapenems (imipenem or meropenem), ampicillin-sulbactam, fluoroquinolones (ciprofloxacin or levofloxacin), and aminoglycosides (gentamicin, tobramycin or amikacin).[2] Pandrug resistance was originally defined as resistance to all antimicrobials included in first-line susceptibility testing that have therapeutic potential against *A. baumannii*. This would include all β-lactams (including carbapenems and sulbactam), fluoroquinolones, and aminoglycosides.[2] However, with the increased use of the polymyxins and tigecycline, this definition has to include these two agents too. Therefore, the terms 'pandrug resistance', 'extensive drug resistance', and 'multidrug resistance' among Gram-negative bacilli have been more recently proposed to designate, respectively, resistance of a pathogen to all, resistance to all but one or two, and resistance to ≥3 classes of potentially effective antimicrobial agents.[12]

Multidrug resistance and extensive drug resistance in *A. baumannii* is now an emerging issue worldwide.[1,2] Multidrug-

resistant isolates of *A. baumannii* have been reported increasingly during the last decade, probably as a consequence of extensive use of antimicrobial agents in western countries.[2,7–16] Until recently, most isolates were susceptible to carbapenems but there have been isolated reports of resistance since the early 1990s. Since then, the incidence of imipenem resistance increased over the last 10 years in Europe, North America and Latin America, representing a sentinel event for emerging antimicrobial resistance.[2,17,18] Carbapenem-resistant isolates of *A. baumannii* are usually resistant to all classes of antimicrobials, show intermediate resistance to rifampin, while usually retaining susceptibility to tigecycline and colistin.[1,2,17–20] Also, as recently demonstrated by a retrospective, matched cohort study, patients with infections due to multidrug-resistant *Acinetobacter* show higher mortality rates and longer length of hospitalization than patients infected by susceptible *Acinetobacter*.[7]

Mechanisms Responsible for Antimicrobial Drug Resistance in *A. baumannii*

Mounting evidence indicates that *A. baumannii* possesses a broad range of mechanisms of resistance to all existing antibiotic classes as well as a striking capacity to acquire new determinants of resistance.[1,2] Genome sequence analysis of a number of multidrug-resistant *A. baumannii* clinical strains has shown the presence of several large genomic islands containing multiple resistance genes interspersed with transposons, integrons, and other mobile genetic elements.[13–16,21] Also, plasmids carrying resistance genes and/or resistance determinants involved in horizontal gene transfer have been described in several *A. baumannii* strains.[14,16,22–26] The most relevant mechanisms of resistance to the different classes of antimicrobials in *A. baumannii* are shown in Table 1.

Table 1. Antimicrobial resistance mechanisms in *Acinetobacter baumannii*.

Antimicrobial class	Mechanism	Involved genes	Ref.
Cephalosporins	β-lactam hydrolysis Class C β-lactamase Class A ESBL Class D β-lactamase	ADC 1–7 VEB-1, -2; PER-1,-2; TEM-92,-116; SHV-12,-5; CTX-M-2,-3 OXA-51-like	[28] [30–40] [27]
Carbapenems	β-lactam hydrolysis Class B MBLs CHDLs OXA-51-like intrinsic chromosomal class D β-lactamase Changes in OMPs CarO 33 to 36-kDa OMPs OprD-like OMP	IMP-1, -2, -4, -5, -6, -11; VIM-2; SIM-1; NDM-1 OXA-23 -24/40 -58 -143 clusters. Chromosomal or plasmid genes flanked by IS elements Confers carbapenem resistances if IS elements are inserted upstream of the gene 26 kDa OMP implicated in drug influx Other OMPs associated with carbapenem resistance	[1,2,41] [2,18,21–26,42] [43] [44] [44–47]
Aminoglycosides	Aminoglycoside-modifying enzymes 16S rDNA methyltransferase	AacC1/2; AadA; AadB; Ant1; AphA1; AphA6 ArmA	[49] [50]
Quinolones	Target alteration Gyrase subunit Topoisomerase IV subunit	GyrA ParC	[61,62] [62,63]
Rifampicin	Drug modification	Rifampicin ADP-ribosylating transferase Arr-2	[64]

Trimethoprim/sulfamethoxazole	Dihydropteroate synthase Dihydrofolate reductase	SulI/II FolA	[65] [65]
Broad (aminoglycoside, quinolones, tetracyclines, glycylcyclines)	Efflux pumps RND MATE MFS	AdeABC; AdeFGH; AdeIJK AbeM TetA; TetB	[48,53–59] [60] [51,52]
Polymyxins	Outer membrane modification	Mutations in genes of lipid A biosynthesis pathway and PmrAB two component	[66—68]

CHDL: Carbapenem-hydrolyzing class D β-lactamase; ESBL: Extended spectrum β-lactamase; IS: Insertion sequence; MATE: Multidrug and toxic compound extrusion; MBL: Class B metallo β-lactamase; MFS: Major facilitator superfamily; OMP: Outer membrane protein; RND: Resistance-nodulation-cell division.

Acinetobacter baumannii possesses an intrinsic class D oxacillinase and a noninducible chromosomal AmpC cephalosporinase.[27,28] *A. baumannii* oxacillinases belong to the OXA-51-like group of enzymes, which contains over 40 sequence variants. 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 inactive against expanded-spectrum cephalosporins.[27] The expression of AmpC cephalosporinase has been demonstrated to be upregulated by the insertion element IS*Aba1* upstream of the gene, which is able to act as a strong transcriptional promoter.[29] In addition to upregulation of AmpC, resistance to cephalosporins in *A. baumannii* is mediated by a wide range of class A extended-spectrum β-lactamases, including those of the TEM, SHV, CTX-M, GES, SCO, PER and VEB families (Table 1).[30–40]

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The resistance of *A. baumannii* to carbapenems is mediated by all of the major resistance mechanisms that are known to occur in bacteria, including enzymatic inactivation, active efflux and decreased influx of drugs, and modification of target sites (Table 1). The production of carbapenem-hydrolizing β-lactamases is the most common mechanism responsible for carbapenem resistance in *A. baumannii*. Several carbapenem-hydrolyzing β-lactamases have been identified so far in *A. baumannii*. These include metallo-β-lactamases (VIM-, IMP- and SIM-types), which have been sporadically reported in some parts of the world and have been associated with class 1 integrons.[1,2] Recently, the occurrence of metallo-β-lactamase NDM-1 has been reported in *A. baumannii* clinical isolates from India.[41] Nevertheless, the most widespread carbapenemases in *A. baumannii* are class D β-lactamases. Four major acquired carbapenem-hydrolyzing class D oxacillinase (CHDL) gene clusters have been identified in *A. baumannii*, represented by the *bla* $_{OXA-23}$-, *bla* $_{OXA-24/40}$-, *bla* $_{OXA-58}$-like genes, and *bla* $_{OXA-143}$ gene.[18,21–26,42] CHDL genes have been found either in the chromosome or in plasmids, thus suggesting that they might have been acquired through horizontal gene transfer. A family of insertion sequence elements at the 5' and/or the 3' of CHDL genes, such as IS*Aba1*, IS*Aba2*, IS*Aba3*, or IS*18*, has been demonstrated to regulate their acquisition and expression.[14,21–26] In addition to these CHDL genes, the chromosomal bla$_{OXA-51}$-like gene, intrinsic to *A. baumannii*, has been demonstrated to confer carbapenem resistance when flanked by IS elements.[43] Reduced susceptibility to carbapenems has also been associated with the modification of penicillin-binding proteins and porins or with upregulation of the AdeABC efflux system, and it has been suggested that the interplay of different mechanisms might result in high-level carbapenem resistance in *A. baumannii* (Table 1).[44–48] However, efflux of carbapenems in *A. baumannii* seems only to contribute to the high level of carbapenem resistance induced by CHDL genes.[48] The production of aminoglycoside-modifying enzymes has been demonstrated as the main mechanism responsible for aminoglycoside resistance in *A. baumannii*.[1,2] Acetyltransferases, nucleotidyltransferases and phosphotransferases have all been found in *A. baumannii*, often occurring in combination. In a study of aminoglycoside resistance in epidemic *A. baumannii* clones isolated in Europe, 95% of isolates were found to contain at least one resistance gene and 84% carried between two and five genes.[49] A total of 12 different combinations were found, with *aacC1*, *aadA1* and *aacA4* located in association with class I integrons.[49] Expression of these genes leads to variable susceptibility to different aminoglycosides. Another type of enzyme, the 16S rRNA methyltransferase ArmA, which confer high-level resistance to all formulated aminoglycosides, has recently been described in *A. baumannii*,[49] often found in combination with *bla* $_{OXA-23}$ alone, or with metallo-β-lactamase NDM-1 (Table 1).[35,41] Drug removal by active efflux mechanisms contributes substantially to MDR in *A. baumannii*. Narrow-spectrum pumps of the major facilitator superfamily include those involved in tetracycline (TetA, TetB) and minocycline (TetB) resistance.[51,52] 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. Three systems have been characterized in *A. baumannii*, AdeABC, AdeFGH and AdeIJK. Resistance to

aminoglycosides, β-lactams, chloramphenicol, erythromycin, tetracycline and tigecycline occurs due to overexpression of *adeABC*, a phenomenon under the control of a two-component regulator system encoded by the *adeRS* genes.[48,53–59] Transposition of an IS*aba1* copy into *adeS* can also lead to overexpression of the system as observed in a clinical isolate. Transcriptional activation could be due to insertional disruption of *adeS* or to the functioning of ISA*ba1* as a strong promoter for *adeABC* expression.[54–56] AdeIJK was the second RND efflux system described in *A. baumannii* and has a substrate specificity favoring amphiphilic compounds and contributes synergistically with AdeABC to tigecycline resistance.[57] A third RND efflux pump, AdeFGH, confers MDR when overexpressed. Inactivation of the pump in an *adeFGH*-overexpressing mutant indicated that it confers high level resistance to fluoroquinolones, chloramphenicol, trimethoprim, clindamycin and decreased susceptibility to tetracyclines, tigecycline, and sulfamethoxazole, without affecting β-lactams and aminoglycosides.[58,59] A pump belonging to the multidrug and toxic compound extrusion family, AbeM, has also been characterized. Overexpression of this pump results in reduced susceptibility to quinolones, gentamicin, kanamycin, erythromycin, chloramphenicol and trimethoprim.[60] Additional resistance mechanisms include mutations in DNA gyrase and topoisomerase IV (fluoroquinolone resistance),[61–63] a rifampicin ADP-ribosylating transferase (Arr-2; rifampicin resistance)[64] and a putative dihydrofolate reductase (folA) involved in trimethoprim resistance[65] *A. baumannii* can develop resistance to polymyxin antibiotics by complete loss of the lipid A component of lipopolysaccharide. In fact, mutations within one of the first three genes of the lipid A biosynthesis pathway: *lpxA*, *lpxC* and *lpxD* have been demonstrated in 13 independent colistin-resistant derivatives of *A. baumannii* type strain ATCC 19606; mutation in the *lpxA* gene of a colistin-resistant clinical isolate of *A. baumannii* has also been shown.[66] Another report has shown that resistance to polymyxins can be associated with mutations in a two-component regulator (PmrA/B) (Table 1).[67] In further support of this, it has recently been demonstrated that PmrA activation in *Escherichia coli* and *Salmonella* promotes resistance to polymixin B through inhibition of LpxT-dependent phosphorylation and subsequent phosphoethanolamine decoration of lipid A.[68] The alterations of the permeability barrier provided by anionic lipopolysaccharide molecules as a consequence of mutations in lipid A biosynthesis and phosphorylation might be responsible for the increased susceptibility of colistin-resistant strains to most tested antibiotics.[69]

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	Topoisomerase IV subunit	ParC	[62,63]
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Mechanisms Responsible for Resistance to Antiseptics in *A. baumannii*

Although the majority of *A. baumannii* clinical strains retain their susceptibility to currently used disinfectant,[70,71] a recent study has demonstrated that approximately 10% of isolates demonstrate reduced susceptibility to the concentrations of disinfectants used for the hand hygiene of healthcare workers, and the disinfection of various noncritical medical devices in clinical and healthcare settings.[71] The same study also demonstrated that the isolates with decreased susceptibility to disinfectants also demonstrated considerably higher levels of resistance to ceftazidime, imipenem, ciprofloxacin and/or aminoglycosides.[71]

Several genes responsible for resistance to disinfectants have been identified in *A. baumannii* genomes (Table 2). Two complete operons, one associated with arsenic resistance and the second with mercury resistance have been found in the AbaR1 of AYE strains and in all strains assigned to the ST1 genotype.[13,15] In addition, genes encoding heavy metal efflux pumps, and multiple copies of *qacED1* genes encoding small MDR-family efflux pumps, known to confer a low level of resistance to ammonium antiseptics, are scattered throughout the AbaR1 resistance island as well in the genomes of all epidemic *A. baumannii* strains analyzed so far.[13–15] The *qacED1* genes are usually found at the 3'-conserved region of an integron and are therefore acquired together with multiple antimicrobial resistance genes (Table 2). The augmented efflux pump functions and changes in permeability of the bacterial outer membrane might be responsible for the combined resistance mechanisms to antimicrobials and disinfectants found in a proportion of *A. baumannii* clinical isolates.[71]

Table 2. Antiseptic resistance mechanisms in *Acinetobacter baumannii*.

Antiseptic class (heavy metals)	Genes	Mechanism	Ref.
Arsenic	*arsBC*, *arsHR* *acr*3	Arsenic resistance operon Arsenite efflux pump	[13,15] [13,15]
Mercury	*merA–C*, *merE–R* ACICU_00235	Mercury resistance operon Heavy metal detoxification protein	[13,15] [14]
Cobalt, zinc, cadmium	*CzcD*, Co/Zn/Cd	Efflux pump	[13–16]
Ammonium	*qacE*Δ1	Quaternary ammonium compound small multidrug resistant family efflux pump found at the 3' of integrons	[2,13–16]

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Ammonium	*qacE*Δ1	Quaternary ammonium compound small multidrug resistant family efflux pump found at the 3' of integrons	[2,13–16]

Molecular Epidemiology of Multidrug-resistant *A. baumannii*

Several hospital outbreaks caused by the selection of multiresistant *A. baumannii* clones were reported worldwide and demonstrated to be caused by a limited number of genotypic clusters of strains. Genotypic characterization of *A. baumannii* isolates through amplified fragment length polymorphism analysis has identified clusters of highly similar strains, which were assumed to represent distinct clonal lineages and were defined as European clones I, II and III.[72,73] Similarly, three distinct groups were recently identified among *A. baumannii* isolates from five different countries by sequence-based typing, group 1 corresponding to European clone II, group 2 to European clone I, and group 3 to European clone III.[74] Major outbreak clones initially named European clones I, II and III are now regarded as international,[1,2,19,75–78] and referred to, according to multilocus sequence typing, as clonal complexes 1, 2 and 3, respectively.[75–77] Epidemiological studies showed the prevalence of international clone II lineage worldwide during recent years[19,21,74–78] and the occurrence of epidemics caused by multidrug-resistant strains belonging to novel genotypes not related to the three main clonal complexes in Europe,[77,79–81] North and Latin America and Asia.[19] In the majority of cases, one or two epidemic strains were detected in a given hospital. Transmission of such strains was observed between hospitals in the same city and also on a national or international scale[1,2,19,74–85] and a direct epidemiological link was established in several cases.[2,84,85]

All the outbreak strains assigned to the different genotypes were multidrug-resistant and the majority of them were carbapenem-resistant strains.[19,77–81] Genotypic characterization of carbapenem-resistance genes in *A. baumannii* strains showed the occurrence of *bla* $_{\text{OXA-23}}$-, *bla* $_{\text{OXA-24/40}}$-, *bla* $_{\text{OXA-58}}$-, or *bla* $_{\text{OXA-143}}$-like CHDL genes in multiple isolates from the same hospital or among different hospitals worldwide.[2,19,21,22–26,42,78–83] A plasmid-borne *bla* $_{\text{OXA-58}}$ gene flanked by IS elements was present in the majority of carbapenem-resistant genotypes isolated from Europe and Mediterranean countries.[22,23,79–81] Of note, each of the IS element flanking the 5' end of the *bla* $_{\text{OXA-58}}$ gene occurred in strains of distinct genotypes isolated in the same geographic area, thus suggesting that they might have been acquired through horizontal gene transfer.[79] The spread of carbapenem-resistant *A. baumannii* carrying the *bla* $_{\text{OXA-58}}$ gene might have also been contributed to by international transfer of colonized patients, as recently demonstrated from Greece to Australia,[84] and Iraq to USA military services.[85]

Resistance-guided Therapeutic Approach

Following isolation of MDR *A. baumannii* from clinical specimens, the existence of colonization or infection must be determined, as colonization does not require antibiotic coverage. However, it may be difficult to discriminate between infection and colonization. In cases of documented infection, aggressive treatment has to be started without delay.[86,87]

The ideal therapeutic approach to *A. baumannii* infections should be based on the evaluation of the individual isolate resistance pattern. While strains susceptible to a range of antimicrobial classes may be effectively managed by appropriate doses of carbapenems or cefepime with or without aminoglycosides, fluoroquinolones or tetracyclines, MDR isolates usually require a colistin-based regimen.[86] However, it should be remembered that the currently used methods to detect carbapenem-resistance, including metallo-β-lactamase E-test strips[88] and automated susceptibility testing methods[89] are still not entirely reliable.

On a practical basis, the overall treatment strategy depends on the strain susceptibility to carbapenems. In cases due to imipenem- and/or meropenem-susceptible strains, the highest tolerable, intravenous doses of one of these molecules should be used. In order to avoid or delay the emergence of carbapenem resistance or in cases of heteroresistance, a fluoroquinolone or an aminoglycoside should be added, whenever active, against the isolated strain. In cases of infection due to carbapenem-resistant strains, the only viable therapeutic option may be colistin, as most of these strains show multiple drug or extreme drug resistance.[90–93] Alternative approaches may be based on the results of antimicrobial susceptibility tests or unorthodox antibiotic combinations, as detailed below.

Colistin

Colistin is increasingly recognized as the only *in vitro* active antimicrobial agent available, and as such has mostly been used in a uncontrolled fashion.

The current MIC of colistin for *A. baumannii* is ≤2 mg/l, although full susceptibility is associated with a MIC ≤0.5 mg/l. The optimal colistin dosing is currently unknown. There are two colistin formulations commercially available: colistin sulfate and colistimethate sodium (also known as colistin methanesulfonate sodium or colistin sulfomethate sodium), which may both be given intravenously. Colistimethate sodium may be found formulated in either international units or milligrams of colistin base, and this makes dose recommendations as well as comparison of study results very difficult.[94] Based on the available pharmacokinetic/pharmacodynamic studies, a dose ranging from 2.5 to 5.0 mg/kg of colistin base activity (equal to 6–12 mg/kg of colistimethate sodium in some formulations) or a dose of 30,000–60,000 units/kg of colistimethate sodium in other formulations is currently recommended, although higher doses up to 3,000,000 units every 8 h have also been proposed.[95,96] However, growing evidence suggests that even these high dosages of colistin base do not allow to reach adequate serum concentrations, yielding a suboptimal C_{max}/MIC ratio and failing to reach detectable levels in bronchoalveolar lavage fluid.[97–99] The documented poor lung diffusion of colistin has prompted several groups to use this drug via inhalation, either alone or in combination with intravenous colistin, with overall satisfactory clinical results.[100–102] By the inhalation route, the recommended dosage ranges from 500,000 IU every 12 h to 2,000,000 IU every 8 h.

The major issues of colistin treatment are its potential nephrotoxicity and the emergence of resistance. Recent studies have shown that the rate of nephrotoxicity due to high dose colistin administration is lower than previously thought.[98,103] Risk factors for kidney injury due to colistin are represented by older age and colistin overdosing in obese patients when drug dose is based on actual and not ideal body weight; worse underlying clinical conditions also play a substantial role.[104]

In reported MDR *A. baumannii* isolates, heteroresistance to colistin has been observed *in vitro* and has also developed during therapy,[105–107] raising further concerns that colistin alone may lack sufficient bactericidal activity when used as monotherapy.[108] Moreover, full resistance to colistin has been described and characterized at the molecular level.[66–68] This condition, however, may be counterbalanced by regain of susceptibility to several other antibiotic classes.[69]

Most clinical studies evaluating the efficacy and safety of colistin are weakened by the absence of a prospective and/or unrandomized controlled design. In a retrospective study on critically ill patients with MDR *A. baumannii* infection, colistin was used in 32 patients as the only *in vitro* effective agent and its clinical efficacy was compared with that of tobramycin in an equal number of subjects infected with aminoglycoside-sensitive *A. baumannii* strains. In this retrospective, unrandomized study, colistin did not show a higher clinical effectiveness compared with tobramycin.[109] Based on this observation, the authors suggest the therapeutic use of an aminoglycoside, whenever active *in vitro*. In contrast with this finding, another unrandomized study comparing colistin with other antibiotics against MDR *A. baumannii* found that the clinical success rates were higher and the overall mortality lower in patients given colistin, with no evidence of neurotoxicity and a low incidence of nephrotoxicity.[110] Whether colistin is equal or not inferior to other regimens still remains controversial due to the lack of randomized clinical trials.

In two small retrospective studies, the efficacy of colistin for carbapenem-resistant infections was found to overlap with that exhibited by imipenem in carbapenem-susceptible cases.[111,112] Another retrospective study comparing colistin monotherapy with its combination with meropenem found a better survival in the monotherapy treatment arm.[113]

Tigecycline

Tigecycline, a glycylcycline designed to overcome the major tetracycline resistance mechanisms, is active *in vitro* against most analyzed strains of *A. baumannii*.[19,114,115] Also, tigecycline, with a MIC_{90} of 2 mg/l, was found to be the most active agent against 483 genetically defined carbapenem-resistant *A. baumannii* isolates collected in 35 countries.[116] Because a tygecicline breakpoint has not been determined for *Acinetobacter*, the clinical MIC breakpoint for *Enterobacteriaceae* of ≤2 mg/l, from EUCAST is used[19,114,115] and the recommended dosage is 50 mg intravenously every 12 h with an initial 100 mg loading dose. The only significant tigecycline adverse effect is nausea, which is usually avoided by slow infusion of the drug, while its renal and hepatic safety profile is excellent.

As tigecycline is characterized by a large volume of distribution, a high biliary excretion rate and a poor serum concentration, its potential effectiveness in bacteremia and bloodstream infections due to *A. baumannii* is low. In contrast, abdominal, pulmonary and soft tissue penetration is good.[117] On-treatment development of intermediate

resistance to tigecycline as well as reduced susceptibility of intensive care unit isolates and a tigecycline MIC 'creep' have recently been reported[118,119] and may be related to upregulation of adeABC efflux pumps during treatment.[53,55,56] Also, tigecycline MICs ≤2 mg/l were reported in 10–20% of *A. baumannii* isolates selected without tigecyclin therapy.[19,114,115]

Although highly active *in vitro*, tigecycline is less efficacious than imipenem in the treatment of experimental *A. baumannii* pneumonia caused by imipenem-susceptible and -intermediate strains.[119]

The availability of tigecycline and its extensive activity against MDR *A. baumannii* in susceptibility studies has spurred marked interest in the clinical use of this antimicrobial agent.[120] However, the results of treatment trials have been controversial: rates of clinical improvement have ranged from 50 to 84%, with variable degrees of microbiological eradication, which in some studies were dependent on the actual isolate susceptibility, but which were overall poorly correlated with clinical outcome.[121–126] Therefore, it is currently reasonable to consider tigecycline as a valid option in intra-abdominal and soft-tissue *A. baumannii* infections while further data are needed before a wider use of this drug in lung or bloodstream infections is recommended. As tigecyline has a very good safety profile overall, higher dosage schedules could possibly provide improved clinical and microbiological responses in *A. baumannii* infections and their investigation is surely warranted. However, at variance with these arguments, a recent US FDA safety announcement has warned of a newly documented increased mortality risk associated with tigecycline use compared with that of other molecules employed in serious nosocomial infections.[201]

Sulbactam

Sulbactam is active *in vitro* against a subset of MDR *A. baumannii* strains ranging from 30 to 45%.[2,127] It is not usually tested by automated *in vitro* susceptibility tests and is mostly available in combination with ampicillin in a formulation with a fixed 1:2 concentration ratio. An *in vivo* pharmacodynamic study of healthy volunteers showed that bactericidal or inhibitory levels may be achieved in the serum when at least 2 g of sulbactam are infused every 6 h.[128]

In an uncontrolled clinical report, the administration of sulbactam, from 9 to 12 g daily, in combination with ampicillin, achieved clinical improvement in 66.7% of patients with MDR *A. baumannii* ventilator-associated pneumonia. When compared with colistin at 3 milli units (MU) tid, such high-doses of sulbactam/ampicillin showed similar safety and effectiveness in critically ill patients with ventilator-associated pneumonia due to MDR *A. baumannii*.[129] In a large, retrospective study over 8 years, outcomes of patients with *A. baumannii* infection given ampicillin/sulbactam were compared with those of polymixin-treated patients. In this study, better results were obtained with ampicillin/sulbactam, in terms of clinical cure (29 vs 18%), death during treatment (33 vs 50%) and death during hospitalization (64 vs 77%).[130]

Antimicrobial Combination-based Strategies

One strategy to overcome the extreme or pandrug resistance of *A. baumannii* is the combination of multiple antimicrobial agents that are poorly or not effective when used alone. Combination of antimicrobial agents with different mechanisms of action may exert an enhanced pharmacodynamic effect or synergism, although sometimes a reduced overall effect may be observed, namely antagonism. Combination treatment is indeed widely employed to prevent emergence of resistance in several other bacterial infections.

A number of *in vitro* and animal studies have assessed the activity of polymyxins combined with other antimicrobials. *In vitro* synergy against MDR *A. baumannii* has been demonstrated when colistin is combined with rifampin, minocycline, ceftazidime, imipenem or azithromycin.[20,118,131,132]

Although largely ineffective *in vitro*, rifampicin has shown additive bactericidal activity when combined with colistin. This has prompted its clinical use in double drug schedules with colistin against MDR *A. baumannii*. Three uncontrolled clinical studies have assessed the safety and clinical effectiveness of this approach, showing overall response rates between 64% and 76% and, in one study, a 100% favourable clinical evolution, without major adverse events.[133–135] By contrast, rifampicin has not shown clinical effectiveness when given in combination with meropenem against carbapenem-resistant *A. baumannii* infections.[136] Again, however, no proof for a superiority of colistin–rifampicin combination over colistin monotherapy exists. A randomized controlled study is currently ongoing in our center and will likely provide a definitive answer to this clinical issue.

Combinations of polymyxin B, doripenem and rifampin at subinhibitory concentrations were shown to achieve bactericidal activity for *A. baumannii* resistant to any of these molecules.[137] Synergy between cefepime and amikacin against a single pandrug resistant strain of *A. baumannii* was recently demonstrated by time-kill studies and in the hollow fiber

infection model.[138] However, there is still little evidence to support the clinical use of three or more antibiotics at the same time against *A. baumannii*. Moreover, as *in vitro* studies do not always correlate with *in vivo* efficacy, adequately powered clinical trials are warranted to test each experimental hypothesis.[139]

In conclusion, the major issue related to *A. baumannii* treatment is currently represented by the relative shortage if not absence of efficacy data from randomized, comparative or controlled clinical trials. This is mainly due to the fact that most clinicians are reluctant to use molecules showing no *in vitro* activity in human treatment trials.

Infection Control Strategies

Further to the administration of appropriate antimicrobials, the therapeutic approach to *A. baumannii* infection as well as colonization must include the implementation of measures aimed at preventing intrahospital spread.[140] The most important and effective measures are represented by personnel hand hygiene, physical patient segregation, nursing care from dedicated personnel and use of strictly disposable equipment. Extreme care should be paid to the sterilization of any nondisposable tools used for the treatment of the affected patient. Further effective control measures may include the use of hand-rub antiseptic solutions, microbiologic surveillance of healthcare worker carriage and disinfection of potentially contaminated medical equipment or frequently manipulated surfaces. In selected instances of uncontrolled outbreaks, the closure of hospital wards for thorough sterilization may be warranted..[141–143]

Emerging Therapeutic Approaches

As the development of novel antimicrobial agents potentially effective against MDR Gram-negative pathogens progresses slowly, efforts are being made to evaluate or reconsider currently available molecules, either after chemical modification or within unorthodox drug combination schemes. Moreover, a number of preclinical studies are being performed with the aim to assess the potential usefulness of novel molecules against MDR *A. baumannii*. We will provide a brief overview of the most promising agents (Table 3).

Table 3. Emerging options for the treatment of *Acinetobacter baumannii* infections.

Antimicrobial strategy	Mechanism(s) of action/rationale	Ref.
Chemical modification of old molecules		
Vancomycin encapsulated in fusogenic liposomes	Liposomes allow vancomycin penetration through the outer membrane	[147]
Unorthodox drug combinations		
Colistin + vancomycin	Colistin-induced membrane disruption allows vancomycin penetration	[148]
Novel agents		
Doripenem	Novel carbapenem with extended-spectrum activity	[144,145]
Ceftobiprole	Fifth generation cephalosporin	[146]
BAL30072 (siderophore sulfactam)	β-lactam with iron-chelating activity, competes with siderophores	[149]
ACHN-490 (neoglycoside)	Aminoglycoside derivative resistant to modifying enzymes	[150]
Finafloxacin	Novel fluoroquinolone	[151]
Bis-indole agents	Five novel MICROBIOTIX diarylamidine derivatives, inhibit DNA synthesis	[152]
1-(1-naphthylmethyl)-piperazine (efflux pump inhibitor)	Block of resistance-nodulation-cell division-type multidrug efflux pumps	[159]
Antimicrobial peptides		[153]
Human β-defensin 2	Cell membrane disruption, cellular content loss via pore creation	[154]
Oncocin	Unknown intracellular target specific for Gram-negative bacteria	[155]

Esculentin-1b	Bacterial membrane damage via interaction with phospholipid components	[156]
Cecropin A-melittin	Inner membrane permeation	[157]
A3-APO	Bacterial membrane disintegration, chaperone-assisted protein folding inhibition via binding to DnaK	[158]
Antisense agents		
Several sequence-specific oligonucleotides	mRNA or DNA binding to prevent translation or gene transcription. Resistance gene expression inhibition and restoration of antibiotic susceptibility. Inhibition of genes essential for bacterial survival	[160,161]

In an *in vitro* study, doripenem, a novel carbapenem, was superior to imipenem and meropenem for strains carrying the *bla* $_{OXA-58}$ gene and inactive against isolates expressing the *bla*$_{OXA-24}$ gene.[144]

Bactericidal activities of doripenem, polymyxin B and rifampin against a limited number of multidrug- and carbapenem-resistant *A. baumanni* isolates were studied in *in vitro* double and triple drug synergy studies: although ineffective alone, doripenem in combination with both polymyxin B and rifampin was bactericidal against three of five tested strains.[137] Similar synergism has been demonstrated for doripenem against *A. baumannii* when this novel carbapenem was combined *in vitro* with amikacin and colistin.[145]

In *bla* $_{ADC}$-like negative strains, ceftobiprole, a novel wide-spectrum cephalosporin, showed improved antimicrobial activity compared with ceftazidime and cefepime.[146]

Recent evidence supports the efficacy of vancomycin encapsulated in fusogenic liposomes against a range of Gram-negative pathogens. This unusual effect appears to be due to the facilitated entry of this vancomycin formulation through the otherwise impermeable outer membrane.[147] This hypothesis seems to be supported by early *in vitro* susceptibility data showing a significant synergistic bactericidal effect of small vancomycin concentrations against MDR *A. baumannii* isolates exposed to inhibitory concentrations of colistin.[148]

Interesting perspectives are opened up by the development of novel monobactam and sulfactam derivatives showing an inherent iron-chelating activity that competes with MRD Gram-negative siderophores.[149]

A promising antimicrobial activity against aminoglycoside-resistant *A. baumannii* isolates has been recently reported for ACHN-490, a 'neoglycoside'.[150] This wide-spectrum, next-generation aminoglycoside, has a MIC significantly lower than amikacin or tobramycin and appeared to retain activity against strains producing aminoglycoside-modifying enzymes.[150]

A new investigational fluoroquinolone, finafloxacin, was recently shown to exert a satisfactory antimicrobial activity against both ciprofloxacin-sensitive and -resistant *A. baumannii* isolates, retaining a low MIC even in conditions of acidic pH mimicking those of infected body sites.[151]

Novel bis-indole agents have recently been shown to exert activity against MDR *A. baumannii in vitro*.[152] These compounds target DNA synthesis and exert a bactericidal effect via an unknown mechanism. Interestingly, five of these agents displayed MICs in the range of 0.12–8 mg/l against 18 carbapenem-resistant strains, with the best activity exerted by MBX 1196.[152]

Antimicrobial peptides (AMPs) represent a promising developing tool for the treatment of MDR pathogens.[153] Human β-defensin 2, a 41-amino acid, naturally occurring cationic peptide, exhibited high bactericidal activity against *A. baumannii*, with MDR strains showing higher susceptibility than wild-type strains.[154] A proline-rich AMP, termed oncocin, has recently been developed and has been shown to have bactericidal activity against multiple Gram-negative isolates, including *A. baumannii*. This novel AMP was neither toxic to human cell lines nor hemolytic to human erythrocytes and could freely penetrate bacterial lipid membranes.[155] Several other AMPs derived from amphibian skin have been tested *in vitro* against MDR pathogens, such as temporins, bombinin H2 and esculentin 1b(1-18), with the latter showing potent bactericidal activity against *A. baumannii* that was partially retained even in the presence of human serum.[156]

Another hybrid peptide that has been shown to exert a fast bactericidal activity against colistin-resistant *A. baumannii*

strains is cecropin A-melittin, whose future derivatives could represent a promising option to overcome the emergent polymyxin resistance in this pathogen.[157]

A proline-rich designer AMP, designated A3-APO, displays increased bacterial membrane disintegration activity, alone or in combination with conventional antibiotics. This dimeric peptide binds the intracellular target DnaK inhibiting chaperone-assisted protein folding. It was shown to be effective in mouse models of systemic MDR *Escherichia coli* and *A. baumannii* infections.[158]

The possibility of blocking MDR mechanisms, such as Gram-negative multiple drug efflux pumps, is an active area of investigation. A putative RND-type efflux pump inhibitor, named 1-(1-naphthylmethyl)-piperazine, able to reverse MDR, has shown significant activity against *A. baumannii* MDR strains.[159]

Another very interesting strategy relies on the use of 'antisense' or 'antigene' agents that are able to inhibit resistance mechanisms directly at the nucleic acid level. These are oligonucleotides designed to bind specific mRNA or DNA sequences to block translation or gene transcription, respectively.[160] As they have no intrinsic antimicrobial activity, co-administration of antibiotics is needed. The mechanism of action of these antisense molecular agents is represented by the restoration of antimicrobial susceptibility as a consequence of resistance mechanism suppression. A similar approach is being investigated using antisense oligonucleotide that target genes essential for the bacteria to survive.[161] Much effort is being made to best chemically modify these oligonucleotides in order to allow efficient penetration within the bacterial cell and reduce degradation by nucleases.

Future Perspective

Outbreaks of MDR and extremely drug resistant *A. baumannii* are increasingly reported worldwide. They are sustained by clusters of highly similar strains that successfully spread among different cities and countries and are selected because of the acquisition of antimicrobial resistance genes. Multifacility *A. baumannii* outbreaks can be also sustained by interhospital transfer of colonized patients. This emphasizes the need to adopt surveillance and infection control programs to prevent colonization and infection by drug-resistant *A. baumannii* in the hospital setting. These programs include the study of global epidemiology of resistant *A. baumannii* using molecular typing of bacterial isolates and characterization of antibiotic resistance to identify the most prevalent resistance genotypes within a certain hospital setting and compare them with those found in other locations.[18,19,72–81] The availability of novel molecular tools such as oligonucleotide-based DNA microarray[58] and high throughput DNA sequencing technology[13–16] will make it possible to study the genomic epidemiology of drug-resistant *A. baumannii* epidemic strains and the composition and expression of antibiotic resistance genes in greater detail. This will facilitate the synthesis and identification of new antimicrobials as well as the validation of novel resistance-guided therapeutic approaches.

The identification of specific genetic traits associated with epidemic and extremely resistant *A. baumannii* strains will also give the opportunity to design a vaccine to be administered to high-risk patients to prevent colonization and/or infection.

Drug-resistant *A. baumannii* infection treatment will also need to include a nonremitting and strict adherence to protocols for containment of its spread within hospital and between hospitals. This will include the surveillance of *A. baumannii* outbreaks and *A. baumannii*-associated infections in the hospital and the community as well as the implementation of disinfection and hygiene control measures in high-risk patients. In addition, the prudent use of existing antibiotics for nosocomial infections will remain an important tool to minimize selection of MDR strains and the development of new forms of resistance.

Sidebar

Executive Summary

Relevance of *Acinetobacter baumannii* infections

- *Acinetobacter baumannii* is a major cause of nosocomial infections associated with high morbidity and mortality.
- *A. baumannii* has simple growth requirements, survives in dry conditions and has high fitness in the hospital environment due to antimicrobial and antiseptic resistance.

Multidrug resistance of *A. baumannii*

- Carbapenem resistance has increased over the last 10 years and is associated with resistance to all antimicrobials other than tigecycline and colistin and high mortality rates.

Mechanisms responsible for antimicrobial drug resistance in *A. baumannii*

- *A. baumannii* displays a broad range of resistance mechanisms to all existing antibiotic classes and a striking ability to acquire new determinants of resistance.
- Carbapenem resistance is mediated by enzymatic inactivation, modification of target sites, active drug efflux and decreased drug influx.
- Active efflux mechanisms mediated by pumps confer resistance to several antimicrobials at the same time.
- *A. baumannii* can develop resistance to polymyxin antibiotics by complete loss of the lipid A component of lipopolysaccharide or mutations in a two-component regulator.
- Colistin-resistant strains show increased susceptibility to other antibiotics.

Molecular epidemiology of multidrug-resistant *A. baumannii*

- Several hospital outbreaks caused by the selection of multiresistant *A. baumannii* clones have been reported worldwide and sustained by a limited number of genotypic clusters of strains.
- Outbreak strains are multidrug resistant and mostly carbapenem resistant.

Resistance-guided therapeutic approach

- The major issue related to *A. baumannii* treatment is currently represented by the relative shortage if not absence of efficacy data from randomized, comparative or controlled clinical trials.
- The overall treatment strategy depends on if the strain is susceptibility to carbapenems.
- Colistin is increasingly recognized as the only *in vitro* active antimicrobial agent available.
- The optimal colistin dosing is currently unknown, especially in respiratory infections. High doses of colistin may not reach adequate serum concentrations and detectable levels in the lung. Colistin-induced neurotoxicity, nephrotoxicity and emergence of resistance are not major issues.
- Tigecycline is active *in vitro* against most analyzed strains of *A. baumannii* but treatment trial results are controversial. It could be a valid option in intra-abdominal *A. baumannii* infections.
- Sulbactam may be active *in vitro* against *A. baumannii* and could be an option in selected cases.
- One strategy to overcome the extreme drug resistance of *A. baumannii* is the combination of multiple antimicrobial agents.
- Adequately powered clinical trials are warranted to support the clinical use of two or more antibiotics at the same time against *A. baumannii*.

Emerging therapeutic approaches

- Novel therapeutic options for multidrug resistant *A. baumanii* include monobactam and sulfactam derivatives with iron-chelating activity, neoglycosides, antimicrobial peptides, multidrug efflux pump blockers, 'antisense' or 'antigene' agents and bis-indol agents.

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Papers of special note have been highlighted as:
• of interest

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