Colistin resistance of Acinetobacter baumannii: clinical reports, mechanisms and antimicrobial strategies

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Colistin is the last resort for treatment of multidrug-resistant Acinetobacter baumannii. Unfortunately, resistance to colistin has been reported all over the world. The highest resistance rate was reported in Asia, followed by Europe. The heteroresistance rate of A. baumannii to colistin is generally higher than the resistance rate. The mechanism of resistance might be loss of lipopolysaccharide or/and the PmrAB two-component system. Pharmacokinetic/pharmacodynamic studies revealed that colistin monotherapy is unable to prevent resistance, and combination therapy might be the best antimicrobial strategy against colistin-resistant A. baumannii. Colistin/rifampicin and colistin/carbapenem are the most studied combinations that showed promising results in vitro, in vivo and in the clinic. New peptides showing good activity against colistin-resistant A. baumannii are also being investigated.

Keywords: multidrug resistance, heteroresistance, combination therapy

Introduction

Acinetobacter baumannii is a Gram-negative pathogen often associated with nosocomial infections, including bacteraemia, pneumonia, meningitis and urinary tract infections. A. baumannii also has been recognized as a worldwide emerging cause of nosocomial outbreaks and is listed as one of the top-priority dangerous microorganisms by the Infectious Diseases Society of America (IDSA).1 Of particular concern is the multidrug resistance of A. baumannii, defined as resistance to almost all available antibiotics, including β-lactams, fluoroquinolones, tetracyclines and aminoglycosides. Colistin and tigecycline remain the only active antibiotics and have become the last resort of treatment for multidrug-resistant (MDR) A. baumannii.2 Although tigecycline was approved for complicated intra-abdominal infections, complicated skin and skin structure infections and community-acquired pneumonia, a recent meta-analysis showed that tigecycline was not better than the usually used antimicrobial agents.3 More disappointingly, resistance was not rare against MDR A. baumannii isolates when tigecycline had not been commercially available in many countries.4 – 7 As a result, clinicians have been forced to turn to colistin, an ‘old’ drug that was used clinically in the late 1950s.8

Colistin is rapidly bactericidal against Gram-negative bacteria, interacting with the lipid A moiety of lipopolysaccharide (LPS) to cause disorganization of the outer membrane.9 Because of nephrotoxicity and neurotoxicity reports and the emergence of less toxic antibiotics such as aminoglycosides, colistin was almost abandoned in clinical use. Researchers re-evaluated the toxicity of colistin and found the incidence of toxicity resulting from the use of colistin is less frequent and severe compared with what has been previously reported. Possible reasons were the improved formulation of colistimethate sodium, avoidance of concurrent administration of nephrotoxic and/or neurotoxic drugs, careful dosing and critical care services.10 In recent years colistin has been used to treat widespread MDR bacteria. Unfortunately colistin heteroresistance and colistin resistance have been described in A. baumannii. Here we review the worldwide reports of colistin resistance of A. baumannii, possible mechanisms of resistance and the strategies against resistance.

Worldwide reports of colistin heteroresistance and resistance of A. baumannii

Since colistin-resistant Acinetobacter spp. was first reported in the Czech Republic in 1999,11 the number of reports all over the world have increased year by year (Table 1). In 2006 Li et al.12 first described colistin heteroresistance of A. baumannii, which was defined as the emergence of resistance to colistin by a subpopulation from an otherwise susceptible (MIC ≤2 mg/L) population. Because heteroresistant detection requires a special method (population analysis profiles) and equipment (automatic spiral plater), most laboratories cannot routinely perform this test. We determined the rate of colistin heteroresistance in A. baumannii, which is usually higher than the rate of resistance, from the only six related reports in the last 6 years.13 – 16 However, the rate of heteroresistance among the six reports varied (from 18.7% to 100%). This may be due to different sampling and different standards to determine the heteroresistance. The detection of colistin heteroresistant
A. baumannii in the clinical isolates provides a strong warning that if colistin is used inappropriately, there may be substantial potential for the rapid development of resistance and therapeu-
tic failure. Moreover, previous use of colistin might be a risk factor for a higher rate of heteroresistance. 17 SENTRY Antimicrobial Surveillance from 2001 to 2011, 16,19,20 which included different centres from the USA, Europe, Latin America and the Asia-Pacific region, revealed the colistin resistance of A. baumannii remained at a low level (0.9%–3.3%). Because only one study in 2009 16 showed the heteroresistance rate, we could not speculate on its variation.

Other reports of colistin-resistant A. baumannii have come from Asia, Europe, North America and South America. Ten reports from Europe, including two case reports, 21,22 provided information on colistin resistance rates. Most report rates of <7%; 11,23–26 however, two reports from Bulgaria 27 and Spain 28 showed high rates of 16.7% and 19.1%, respectively. Surprisingly, another report from Spain showed a quite high resistance rate of 40.7%, whose strains were collected from a tertiary care hospital between May 2000 and November 2006. 29 Seven of eight reports from Asia showed rates of <12%. 26,30–35 Ko et al. 36 reported the highest colistin resistance rate of 30.6% from Korea. Three reports came from the USA 37,38,39 in South America, only three resistant strains were detected from three reports with a resistance rate of no more than 7.1%. 13,14,40 Overall, the colistin heteroresistance rate in A. baumannii was much higher than the resistance rate. However, because of a lack of uniform standards to determine heteroresistance, the rates from different regions varied greatly. Asia and Europe showed the most serious situation of colistin resistance, with more reports and higher rates of resistance, while lower rates and fewer reports of colistin resistance were presented from North and South America.

### Mechanism of colistin resistance of A. baumannii

Modification of lipid A, a component of LPS, with the addition of 4-amino-4-deoxy-l-arabinose (Ara4N) or phosphoethanolamine is considered to be the mechanism of colistin resistance in Gram-negative pathogens, such as Salmonella enteric and A. baumannii.
Pseudomonas aeruginosa. This addition removes the negative charge of lipid A, thus lowering the affinity of positively charged colistin. However, Ara4N biosynthesis and attachment genes are not present in A. baumannii, which suggests that Ara4N modification of lipid A is not suitable to explain colistin resistance in A. baumannii. There is relatively little research on colistin resistance in A. baumannii. Around the key target of colistin, lipid A, there are currently two main hypotheses of the resistance mechanism.

The first is the loss of LPS hypothesis proposed by Moffatt et al. and Henry et al. Initially they found inactivation of a lipid A biosynthesis gene—lpxA, lpxC or lpxD—resulting in complete loss of LPS production in A. baumannii. The strains loss of LPS was tested to be colistin resistance. They further found insertion sequence ISAba11 (GenBank accession number JF309050) in either lpxA or lpxC, resulting in the complete loss of LPS production and a high level of colistin resistance. In response to total LPS loss, A. baumannii alters the expression of critical transport and biosynthesis systems associated with modulating the composition and structure of the bacterial surface. An LPS-deficient colistin-resistant strain with a less negative charge might be the reason for a loss of affinity to colistin.

The second is the PmrAB two-component system-mediated hypothesis. It was first proposed by Adams et al. in 2009. By comparing the DNA sequence of genes encoding PmrA and PmrB between colistin-susceptible and -resistant strains, they showed that mutations in the genes pmrA and pmrB are linked to colistin resistance in A. baumannii. Park et al. also investigated pmrA and pmrB with colistin resistance. However, their results indicated that increased expression of the PmrAB system is essential for colistin resistance in A. baumannii, but amino acid alterations might not be essential for resistance. The most recent research showed a more in-depth result. By analysing PmrCAB in a diverse collection of clinical isolates and laboratory mutants of A. baumannii, they suggest that resistance to colistin requires at least two distinct genetic events: (i) at least one amino acid change in PmrB; and (ii) up-regulated expression of pmrA and pmrB. More importantly, after analysing the composition of lipid A from resistant and susceptible isolates, they found phosphoethanolamine was added to hepta-acylated lipid A. This kind of LPS modification might lead directly to colistin resistance, because this change was previously reported to associate with polymyxins resistance in Salmonella.

There has been no research to clarify the link between these two hypotheses until now, except for differences in selection methods for the development of different mechanisms: fixed concentration of 10 mg/L in agar versus stepwise increased colistin concentrations from 1 to 8 mg/L in Lysogeny broth. However, this still cannot explain the colistin-resistant strains isolated from the clinic.

**Antimicrobial strategies against colistin resistance of A. baumannii**

One view is that colistin resistance is linked to inadequate dosing. This point of view was confirmed by mutant prevention concentration (MPC) tests of colistin against A. baumannii. The MPC at which 90% of the isolates tested were prevented was >128 mg/L, which was much higher than the plasma concentration of colistin at the current recommended dosage and expected to enrich resistant mutant subpopulations. This highlights the importance of optimizing the colistin regimen based on pharmacokinetics (PK)/pharmacodynamics (PD). One study used an in vitro PK/PD model to evaluate three clinically relevant intermittent regimens—8 h, 12 h and 24 h—and a continuous infusion of colistin against two colistin-heteroresistant A. baumannii. After extensive initial killing, regrowth was observed 6 h later in all the regimens. No bactericidal effect was evident after the second and subsequent doses (intermittent regimens). Moreover, resistant subpopulations emerged regardless of the colistin regimen. Another in vitro PD study found regrowth was observed as early as 3 h, and even at concentrations up to 32 or 64 × MIC, substantial regrowth still exists at 24 h. More recently, Duddhie et al. used neutropenic murine thigm and lung infection models to identify the most predictive PK/PD index of the antibacterial activity of colistin against A. baumannii. The results suggested adequate time-averaged exposure to colistin is important and AUC/MIC is the most predictive value of colistin against A. baumannii. However, amplification of colistin-resistant subpopulations was also revealed for all strains in both models after 24 h of colistin treatment. A population PK (PPK) analysis of colistin in 18 critically ill patients showed that colistin displayed a significantly longer half-life than dosing interval. The implications of the findings are that the plasma colistin concentrations are insufficient before steady state and raise the question of whether the administration of a loading dose would benefit critically ill patients. A larger-scale PPK study included 105 patients. It implied that because of the inability to achieve adequate plasma concentrations of formed colistin monotherapy, colistin might best be used as part of a highly active combination, especially when treating an infection caused by an organism with an MIC >0.5 mg/L in a patient with a creatinine clearance >70 mL/min/1.73 m². From all these reports we can conclude that monotherapy of colistin is unable to prevent the emergence of colistin-resistant strains because of the PK/PD characteristics of colistin. Thus, a rational combination therapy of colistin with other antibiotics might be a feasible alternative.

Many in vitro and in vivo studies and case reports have proposed promising colistin combination regimens, although most of these studies have been based on MDR or extensively drug-resistant (XDR) A. baumannii that were still colistin susceptible (Table 2). The most frequently studied combination was colistin with rifampicin. In addition to in vitro, in vivo and clinical reports confirming the validity of the colistin/rifampicin combination, a recent in vitro study confirmed that this combination was synergistic against heteroresistant isolates and prevented the development of colistin-resistant mutants. Following rifampicin, carbapenems (imipenem or meropenem) received the most attention. Moreover, colistin/imipenem was found to be synergistic against heteroresistant A. baumannii, while colistin/meropenem showed synergy against 49 of 52 A. baumannii (including both colistin-susceptible and -resistant isolates). Although tigecycline is a new antibiotic with a wide antimicrobial spectrum, the emergence of tigecycline-resistant A. baumannii has been reported from time to time. The colistin/tigecycline combination showed favourable results against tigecycline-non-susceptible isolates and colistin-resistant or
### Table 2. Reports of colistin combination therapy to A. baumannii

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Antibiotic combination</th>
<th>Type of research</th>
<th>Type of AB</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peck et al. (2012)</td>
<td>imipenem or rifampicin or tigecycline</td>
<td><em>in vitro</em></td>
<td>imipenem-resistant AB, colistin susceptible and colistin resistant</td>
<td>among the combinations of 0.5× MIC antimicrobial agents, colistin plus tigecycline showed synergistic or bactericidal effects against four <em>A. baumannii</em> isolates</td>
</tr>
<tr>
<td>Sheng et al. (2011)</td>
<td>tigecycline</td>
<td><em>in vitro</em></td>
<td>carbapenem-resistant AB, colistin susceptible</td>
<td>the combination of tigecycline and colistin showed good <em>in vitro</em> synergy against carbapenem-resistant AB with high imipenem resistance</td>
</tr>
<tr>
<td>Santimaleeworagun et al. (2011)</td>
<td>fosfomycin or imipenem or sulbactam</td>
<td><em>in vitro</em></td>
<td>carbapenem-resistant AB, colistin susceptible</td>
<td>a chequerboard assay showed the synergistic effects of colistin plus fosfomycin against 12.5% of eight isolates; no synergy between colistin and sulbactam, colistin and imipenem against the tested isolates</td>
</tr>
<tr>
<td>Hornsey et al. (2011)</td>
<td>glycopeptide (vancomycin and teicoplanin)</td>
<td><em>in vivo</em></td>
<td>MDR AB, colistin susceptible</td>
<td>glycopeptide/colistin combinations are highly active both <em>in vitro</em> and in a simple animal (<em>G. mellonella</em> model of infection)</td>
</tr>
<tr>
<td>Shields et al. (2010)</td>
<td>carbenem</td>
<td><em>in vitro</em></td>
<td>XDR AB, colistin susceptible</td>
<td>carbapenem/colistin combination proved to be effective against strains isolated from transplant patients; when this combination was given to these patients, 80% (4/5) of them were treated successfully</td>
</tr>
<tr>
<td>Ozbek et al. (2010)</td>
<td>tigecycline</td>
<td><em>in vitro</em></td>
<td>meropenem-resistant AB</td>
<td>a synergistic interaction was observed for tigecycline/colistin; tigecycline slightly changed the post-antibiotic effect of colistin</td>
</tr>
<tr>
<td>Candel et al. (2010)</td>
<td>tigecycline + meropenem</td>
<td><em>case report</em></td>
<td>MDR AB, colistin susceptible</td>
<td>a renal transplant recipient who developed bacteraemia had a favourable clinical outcome with a tigecycline/colistin/ meropenem combination</td>
</tr>
<tr>
<td>Rodriguez et al. (2010)</td>
<td>rifampicin or imipenem</td>
<td><em>in vitro</em></td>
<td>carbapenem-resistant AB, colistin heteroresistant</td>
<td>colistin/rifampicin and colistin/imipenem were synergistic against heteroresistant isolates and prevented the development of colistin-resistant mutants</td>
</tr>
<tr>
<td>Pongpech et al. (2010)</td>
<td>imipenem or imipenem + sulbactam</td>
<td><em>in vitro</em></td>
<td>MDR AB, colistin susceptible</td>
<td>imipenem/colistin showed best synergy effects, while addition of sulbactam to meropenem and colistin may further improve their antibacterial activity</td>
</tr>
<tr>
<td>Pachon-Ibanez et al. (2010)</td>
<td>rifampicin</td>
<td><em>in vitro/in vivo</em></td>
<td>MDR AB, colistin susceptible</td>
<td>rifampicin/colistin showed efficacy <em>in vitro</em> and in experimental models of pneumonia and meningitis</td>
</tr>
<tr>
<td>Dizbay et al. (2010)</td>
<td>tigecycline</td>
<td><em>in vitro</em></td>
<td>XDR AB, colistin susceptible</td>
<td>the tigecycline/colistin combination was more synergistic than tigecycline/rifampicin and colistin/rifampicin according to the FIC index</td>
</tr>
<tr>
<td>Principe et al. (2009)</td>
<td>tigecycline</td>
<td><em>in vitro</em></td>
<td>tigecycline-non-susceptible AB</td>
<td>tigecycline/colistin was synergistic against five of seven strains</td>
</tr>
<tr>
<td>Arroyo et al. (2009)</td>
<td>tigecycline</td>
<td><em>in vitro</em></td>
<td>colistin-susceptible and colistin-resistant AB</td>
<td>FIC of tigecycline/colistin for 35 <em>A. baumannii</em> isolates (selected by colistin MICs of 0.12 to 4 mg/L) ranged from 0.75 to 2</td>
</tr>
<tr>
<td>Lee et al. (2008)</td>
<td>meropenem or sulbactam or meropenem + sulbactam</td>
<td><em>in vitro</em></td>
<td>MDR AB, colistin susceptible</td>
<td>combined colistin with meropenem and/or sulbactam can inhibit bacterial regrowth at 24 h</td>
</tr>
<tr>
<td>Song et al. (2008)</td>
<td>rifampicin</td>
<td><em>case report</em></td>
<td>carbapenem-resistant AB, colistin susceptible</td>
<td>7 (70%) of 10 patients with ventilator-associated pneumonia benefitted from colistin/rifampicin therapy; six patients were cured microbiologically</td>
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<tr>
<td>Reference</td>
<td>Antibiotic(s)</td>
<td>Study Type</td>
<td>Setting</td>
<td>Outcome</td>
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<tr>
<td>Bassetti et al. (2008)⁷⁸</td>
<td>rifampicin</td>
<td>case report</td>
<td>MDR AB, colistin susceptible</td>
<td>Clinical and microbiological responses were observed in 22 of 29 (76%) critically ill patients with pneumonia and bacteraemia treated with colistin/rifampicin.</td>
</tr>
<tr>
<td>Pankuch et al. (2008)⁵⁷</td>
<td>meropenem</td>
<td><em>in vitro</em></td>
<td>colistin-susceptible and colistin-resistant AB</td>
<td>Subinhibitory meropenem/colistin showed synergy against 49 of 52 strains at 24 h.</td>
</tr>
<tr>
<td>Pantopoulou et al. (2007)⁷⁸</td>
<td>rifampicin</td>
<td><em>in vivo</em></td>
<td>MDR AB, colistin susceptible</td>
<td>Colistin's activity in prolonging survival in an experimental thigh infection in neutropenic rats was enhanced after co-administration with rifampicin.</td>
</tr>
<tr>
<td>Biancofiore et al. (2007)⁸⁰</td>
<td>meropenem + rifampicin</td>
<td>case report</td>
<td>MDR AB, colistin susceptible</td>
<td>Colistin/rifampicin/meropenem successfully treated a case of multifocal (lungs, skin, soft tissues) infection.</td>
</tr>
<tr>
<td>Timurkaynak et al. (2006)⁶¹</td>
<td>rifampicin or meropenem or azithromycin or doxycycline</td>
<td><em>in vitro</em></td>
<td>MDR AB, colistin susceptible</td>
<td>Colistin/rifampicin was fully synergistic against four of five isolates; colistin/meropenem and colistin/azithromycin each showed synergistic activity against three of five isolates; colistin/doxycycline was partially synergistic or additive against five isolates.</td>
</tr>
<tr>
<td>Motaouakkil et al. (2006)⁸¹</td>
<td>rifampicin</td>
<td>clinical trial</td>
<td>MDR AB, colistin susceptible</td>
<td>Colistin/rifampicin was favourable for all 26 nosocomial infection patients.</td>
</tr>
<tr>
<td>Petrosillo et al. (2005)⁸²</td>
<td>rifampicin or rifampicin + ampicillin/sulbactam</td>
<td>case report</td>
<td>carbapenem-resistant AB, colistin susceptible</td>
<td>Therapy with colistin/rifampicin, and with ampicillin/sulbactam in case of susceptibility to this combination, resulted in microbiological clearance of carbapenem-resistant AB in 9 (64%) of 14 critically ill patients, with limited side effects.</td>
</tr>
<tr>
<td>Fulnecky et al. (2005)⁶²</td>
<td>amikacin</td>
<td>case report</td>
<td>MDR AB, colistin susceptible</td>
<td>A 52-year-old man with post-surgical meningitis experienced successful clinical and microbiological outcomes following colistin/amikacin therapy.</td>
</tr>
<tr>
<td>Montero et al. (2004)⁸³</td>
<td>rifampicin</td>
<td><em>in vitro</em></td>
<td>carbapenem-resistant AB, colistin susceptible</td>
<td>For strains highly resistant to imipenem and moderately resistant to rifampicin, colistin/rifampicin may be useful.</td>
</tr>
<tr>
<td>Hogg et al. (1998)⁸⁴</td>
<td>rifampicin</td>
<td><em>in vitro</em></td>
<td>MDR AB, colistin susceptible</td>
<td>Colistin/rifampicin was synergistic against 11 of 13 isolates.</td>
</tr>
</tbody>
</table>

AB, A. baumannii.
colistin-susceptible isolates. Some studies have reported effective combinations of amikacin, fosfomycin and azithromycin. The most unexpected combination was with the glycopeptides vancomycin and teicoplanin. Although A. baumannii strains were highly resistant to vancomycin and teicoplanin, synergy between colistin and both glycopeptides was repeatedly observed in checkerboard assays, with fractional inhibitory concentrations (FICs) of <0.5, and treatment of Galleria mellonella caterpillars infected with lethal doses of A. baumannii resulted in significantly enhanced survival rates when either vancomycin or teicoplanin was given with colistin compared with colistin treatment alone (P < 0.05). The effect is thought to be mediated via a permeabilizing effect of colistin on the A. baumannii outer membrane, facilitating the entry of glycopeptide molecules, which are usually excluded by Gram-negative strains due to their size. Another study revealed that the MICs of vancomycin and teicoplanin for colistin-resistant A. baumannii were greatly decreased compared with their parent colistin-susceptible strains. Considering the safety issues of glycopeptide/colistin combinations, further PK/PD studies in mammalian models are needed to evaluate their feasibility in clinical use.

Many studies are trying to use existing antibiotics more properly to fight against pan-resistant A. baumannii, while some researchers are trying to find effective new antibiotics. Rodriguez-Hernandez et al. found that cecropin A–melittin hybrid peptide and three of its shortened analogues have a fast microbicidal effect on the colistin-resistant A. baumannii isolates by time–killing studies. Further research found that the cecropin A–melittin hybrids have a higher affinity than colistin towards LPS isolated from colistin-resistant A. baumannii strains, and this might be the reason for their superior activity. By screening 15 different peptides, Vila-Farres et al. found mas-toparan showed good activity against both colistin-susceptible and colistin-resistant A. baumannii. Time–killing curve results also showed the bactericidal activity of mas-toparan at MIC ×8 for both colistin-susceptible and colistin-resistant A. baumannii. Although the mechanism of these peptides is not clear, we believe they may lead to the development of new effective antibiotics against colistin-resistant A. baumannii.

Conclusions

Colistin, as the last resort for treatment of MDR A. baumannii, has received much attention in recent years. Unfortunately, however, resistance to colistin has been reported all over the world. The highest resistance rate was reported in Asia, followed by Europe. The heteroresistance rate of A. baumannii to colistin was generally higher than the resistance rate. The mechanism of resistance is not yet clear, but some studies confirm that it might be related to a loss of LPS or/and the PmrAB two-component system. Because PK/PD studies revealed that colistin monotherapy is unable to prevent resistance, combination therapy might be the best strategy against colistin-resistant A. baumannii. Colistin/rifampicin and colistin/carabapenem are the most studied combinations, showing promising results in vitro, in vivo and in the clinic. New peptides showing good activity against colistin-resistant A. baumannii are also being investigated.

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Transparency declarations

None to declare.

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