J Antimicrob Chemother 2012; **67**: 1607–1615
doi:10.1093/jac/dks084 Advance Access publication 22 March 2012

Journal of Antimicrobial Chemotherapy

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

Yun Cai, Dong Chai, Rui Wang*, Beibei Liang and Nan Bai

Department of Clinical Pharmacology, the PLA General Hospital, Beijing 100853, People's Republic of China

*Corresponding author. Tel: +86-10-6693-7909; Fax: +86-10-8821-4425; E-mail: caicaihh@sohu.com

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 six 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 $\leq$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.[12–16] 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

Table 1. Worldwide reports of colistin heteroresistance and resistance of *A. baumannii*

Author (year)	Country/region	Heteroresistance (no. of strains/%)	Resistance (no. of strains/%)
Chang *et al.* (2012)[34]	Taiwan	—	14/10.4
Lee *et al.* (2011)[35]	Taiwan	—	2/9.5
Gales *et al.* (2011)[20]	SENTRY	—	42/0.9
Herrera *et al.* (2011)[13a]	Argentina	14/18.7	0/0
Rolain *et al.* (2011)[21]	France	—	1/—
Al-Sweih *et al.* (2011)[30a]	Kuwait	—	30/12
Lopez-Rojas *et al.* (2011)[22]	Spain		1/—
Keen *et al.* (2010)[38]	USA	—	1/0.1
Rodriguez *et al.* (2010)[14]	Argentina	6/42.9	1/7.1
Rodriguez *et al.* (2009)[15]	Argentina	13/46.4	—
Arroyo *et al.* (2009)[29]	Spain	—	61/40.6
Doi *et al.* (2009)[37]	USA	—	1/—
Eser *et al.* (2009)[31a]	Turkish	—	14/11.3
Gomez-Garces *et al.* (2009)[23a]	Spain	—	1/1.2
Lee *et al.* (2009)[32]	Taiwan	—	2/1.1
Yau *et al.* (2009)[16]	SENTRY	7/23.3	1/3.3
Hawley *et al.* (2008)[18a]	USA	21/100	—
Mezzatesta *et al.* (2008)[24]	Italy	—	1/0.9
Hawley *et al.* (2007)[39a]	USA	—	3/2.1
Quinteira *et al.* (2007)[25]	Portugal	—	10/6.7
Ko *et al.* (2007)[36]	Korea	—	66/30.6
Sung *et al.* (2007)[33]	Korea	—	5/9.1
Li *et al.* (2006)[12]	Australia	15/93.6	—
Dobrewski *et al.* (2006)[27]	Bulgaria	—	3/16.7
Garcia-Penuela *et al.* (2006)[26]	Hong Kong	—	1/3.34
Garcia-Penuela *et al.* (2006)[26]	Spain	—	1/1.36
Tognim *et al.* (2006)[40a]	Brazil	—	2/3
Arroyo *et al.* (2005)[28]	Spain	—	22/19.1
Gales *et al.* (2001)[19a]	SENTRY	—	2/3.3
Hejnar *et al.* (1999)[11a]	Czech Republic	—	5/5.9

[a]*Acinetobacter* spp., including *A. baumannii*.

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 therapeutic failure.[17] Moreover, previous use of colistin might be a risk factor for a higher rate of heteroresistance.[18]

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 reported 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] two of these reported a relatively low rate of resistance of no more than 2.1%.[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/and phosphoethanolamine is considered to be the mechanism of colistin resistance in Gram-negative pathogens, such as *Salmonella enteric* and

Pseudomonas aeruginosa.[41] 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*.[42] 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*.[43,44] and Henry *et al*.[45] 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.[43] They further found insertion sequence ISA*ba11* (GenBank accession number JF309050) in either *lpxA* or *lpxC*, resulting in the complete loss of LPS production and a high level of colistin resistance.[44] 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.[45] An LPS-deficient colistin-resistant strain with a less negative charge might be the reason for a loss of affinity to colistin.[46]

The second is the PmrAB two-component system-mediated hypothesis. It was first proposed by Adams *et al*.[42] 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*.[47] also investigated *pmrA* and *pmrB* with colistin resistance. However, their results indicate 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.[47] 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.[48] This kind of LPS modification might lead directly to colistin resistance, because this change was previously reported to associate with polymyxins resistance in *Salmonella*.[49]

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[43] versus stepwise increased colistin concentrations from 1 to 8 mg/L in Lysogeny broth.[48] 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.[50] 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.[51] 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.[52] 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.[53] More recently, Dudhani *et al*.[54] used neutropenic murine thigh 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 *f*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.[55] 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 m^2.[56] 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.[14] Following rifampicin, carbapenems (imipenem or meropenem) received the most attention. Moreover, colistin/imipenem was found to be synergistic against heteroresistant *A. baumannii*,[14] while colistin/meropenem showed synergy against 49 of 52 *A. baumannii* (including both colistin-susceptible and -resistant isolates).[57] 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[58] and colistin-resistant or

Table 2. Reports of colistin combination therapy to *A. baumannii*

Author (year)	Antibiotic combination	Type of research	Type of AB	Findings
Peck *et al.* (2012)[59]	imipenem or rifampicin or tigecycline	*in vitro*	imipenem-resistant AB, colistin susceptible and colistin resistant	among the combinations of 0.5× MIC antimicrobial agents, colistin plus tigecycline showed synergistic or bactericidal effects against four *A. baumannii* isolates
Sheng *et al.* (2011)[60]	tigecycline	*in vitro*	carbapenem-resistant AB, colistin susceptible	the combination of tigecycline and colistin showed good *in vitro* synergy against carbapenem-resistant AB with high imipenem resistance
Santimaleeworagun *et al.* (2011)[63]	fosfomycin or imipenem or sulbactam	*in vitro*	carbapenem-resistant AB, colistin susceptible	a chequerboard assay showed the synergistic effects of colistin plus fosfomycin against 12.5% of eight isolates; no synergism between colistin and sulbactam, colistin and imipenem against the tested isolates
Hornsey *et al.* (2011)[69]	glycopeptide (vancomycin and teicoplanin)	*in vivo*	MDR AB, colistin susceptible	glycopeptide/colistin combinations are highly active both *in vitro* and in a simple animal (*G. mellonella*) model of infection
Shields *et al.* (2010)[70]	carbapenem	*in vitro*/ case report	XDR AB, colistin susceptible	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
Ozbek *et al.* (2010)[71]	tigecycline	*in vitro*	meropenem-resistant AB	a synergistic interaction was observed for tigecycline/colistin; tigecycline slightly changed the post-antibiotic effect of colistin
Candel *et al.* (2010)[72]	tigecycline + meropenem	case report	MDR AB, colistin susceptible	a renal transplant recipient who developed bacteraemia had a favourable clinical outcome with a tigecycline/colistin/ meropenem combination
Rodriguez *et al.* (2010)[14]	rifampicin or imipenem	*in vitro*	carbapenem-resistant AB, colistin heteroresistant	colistin/rifampicin and colistin/imipenem were synergistic against heteroresistant isolates and prevented the development of colistin-resistant mutants
Pongpech *et al.* (2010)[73]	imipenem or imipenem + sulbactam	*in vitro*	MDR AB, colistin susceptible	imipenem/colistin showed best synergy effects, while addition of sulbactam to meropenem and colistin may further improve their antibacterial activity
Pachon-Ibanez *et al.* (2010)[74]	rifampicin	*in vitro*/*in vivo*	MDR AB, colistin susceptible	rifampicin/colistin showed efficacy *in vitro* and in experimental models of pneumonia and meningitis
Dizbay *et al.* (2010)[75]	tigecycline	*in vitro*	XDR AB, colistin susceptible	the tigecycline/colistin combination was more synergistic than tigecycline/rifampicin and colistin/rifampicin according to the FIC index
Principe *et al.* (2009)[58]	tigecycline	*in vitro*	tigecycline-non-susceptible AB	tigecycline/colistin was synergistic against five of seven strains
Arroyo *et al.* (2009)[29]	tigecycline	*in vitro*	colistin-susceptible and colistin-resistant AB	FIC of tigecycline/colistin for 35 *A. baumannii* isolates (selected by colistin MICs of 0.12 to 4 mg/L) ranged from 0.75 to 2
Lee *et al.* (2008)[76]	meropenem or sulbactam or meropenem + sulbactam	*in vitro*/ case report	MDR AB, colistin susceptible	combined colistin with meropenem and/or sulbactam can inhibit bacterial regrowth at 24 h
Song *et al.* (2008)[77]	rifampicin	case report	carbapenem-resistant AB, colistin susceptible	7 (70%) of 10 patients with ventilator-associated pneumonia benefitted from colistin/rifampicin therapy; six patients were cured microbiologically

Bassetti *et al*. (2008)[78]	rifampicin	case report	MDR AB, colistin susceptible	clinical and microbiological responses were observed in 22 of 29 (76%) critically ill patients with pneumonia and bacteraemia treated with colistin/rifampicin
Pankuch *et al*. (2008)[57]	meropenem	*in vitro*	colistin-susceptible and colistin-resistant AB	subinhibitory meropenem/colistin showed synergy against 49 of 52 strains at 24 h
Pantopoulou *et al*. (2007)[79]	rifampicin	*in vivo*	MDR AB, colistin susceptible	colistin's activity in prolonging survival in an experimental thigh infection in neutropenic rats was enhanced after co-administration with rifampicin
Biancofiore *et al*. (2007)[80]	meropenem + rifampicin	case report	MDR AB, colistin susceptible	colistin/rifampicin/meropenem successfully treated a case of multifocal (lungs, skin, soft tissues) infection
Timurkaynak *et al*. (2006)[61]	rifampicin or meropenem or azithromycin or doxycycline	*in vitro*	MDR AB, colistin susceptible	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
Motaouakkil *et al*. (2006)[81]	rifampicin	clinical trial	MDR AB, colistin susceptible	colistin/rifampicin was favourable for all 26 nosocomial infection patients
Petrosillo *et al*. (2005)[82]	rifampicin or rifampicin + ampicillin/sulbactam	case report	carbapenem-resistant AB, colistin susceptible	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
Fulnecky *et al*. (2005)[62]	amikacin	case report	MDR AB, colistin susceptible	a 52-year-old man with post-surgical meningitis experienced successful clinical and microbiological outcomes following colistin/amikacin therapy
Montero *et al*. (2004)[83]	rifampicin	*in vitro*	carbapenem-resistant AB, colistin susceptible	for strains highly resistant to imipenem and moderately resistant to rifampicin, colistin/rifampicin may be useful
Hogg *et al*. (1998)[84]	rifampicin	*in vitro*	MDR AB, colistin susceptible	colistin/rifampicin was synergistic against 11 of 13 isolates

AB, *A. baumannii*.

colistin-susceptible isolates.[29,59,60] Some studies have reported effective combinations of amikacin, fosfomycin and azithromycin.[61–63] 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 chequerboard 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.[64] 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.[65] 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. Rodríguez-Hernández *et al.*[66] 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–kill 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.[67] By screening 15 different peptides, Vila-Farres *et al.*[68] found mastoparan showed good activity against both colistin-susceptible and colistin-resistant *A. baumannii*. Time–killing curve results also showed the bactericidal activity of mastoparan 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/carbapenem 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.

Funding

This study was supported by grant 81000755 from the National Natural Science Foundation of China, 7122167 from Beijing Natural Science Foundation of China and 2010B079 from the Beijing Science and Technology New Star Program of China.

Transparency declarations

None to declare.

References

1 Boucher HW, Talbot GH, Bradley JS *et al*. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. *Clin Infect Dis* 2009; **48**: 1–12.

2 Gordon NC, Wareham DW. Multidrug-resistant *Acinetobacter baumannii*: mechanisms of virulence and resistance. *Int J Antimicrob Agents* 2010; **35**: 219–26.

3 Tasina E, Haidich AB, Kokkali S *et al*. Efficacy and safety of tigecycline for the treatment of infectious diseases: a meta-analysis. *Lancet Infect Dis* 2011; **11**: 834–44.

4 Hornsey M, Ellington MJ, Doumith M *et al*. AdeABC-mediated efflux and tigecycline MICs for epidemic clones of *Acinetobacter baumannii*. *J Antimicrob Chemother* 2010; **65**: 1589–93.

5 Dizbay M, Altuncekic A, Sezer BE *et al*. Colistin and tigecycline susceptibility among multidrug-resistant *Acinetobacter baumannii* isolated from ventilator-associated pneumonia. *Int J Antimicrob Agents* 2008; **32**: 29–32.

6 Kulah C, Celebi G, Aktas E *et al*. Unexpected tigecycline resistance among *Acinetobacter baumannii* isolates: high minor error rate by Etest. *J Chemother* 2009; **21**: 390–5.

7 Navon-Venezia S, Leavitt A, Carmeli Y. High tigecycline resistance in multidrug-resistant *Acinetobacter baumannii*. *J Antimicrob Chemother* 2007; **59**: 772–4.

8 Falagas ME, Kasiakou SK. Colistin: the revival of polymyxins for the management of multidrug-resistant Gram-negative bacterial infections. *Clin Infect Dis* 2005; **40**: 1333–41.

9 Hancock RE. Peptide antibiotics. *Lancet* 1997; **349**: 418–22.

10 Falagas ME, Kasiakou SK. Toxicity of polymyxins: a systematic review of the evidence from old and recent studies. *Crit Care* 2006; **10**: R27.

11 Hejnar P, Kolar M, Hajek V. Characteristics of *Acinetobacter* strains (phenotype classification, antibiotic susceptibility and production of β-lactamases) isolated from haemocultures from patients at the Teaching Hospital in Olomouc. *Acta Univ Palacki Olomuc Fac Med* 1999; **142**: 73–7.

12 Li J, Rayner CR, Nation RL *et al*. Heteroresistance to colistin in multidrug-resistant *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2006; **50**: 2946–50.

13 Herrera ME, Mobilia LN, Posse GR. Comparative evaluation of the sensitivity of *Acinetobacter* to colistin, using the prediffusion and minimum inhibitory concentration methods: detection of heteroresistant isolates. *Rev Argent Microbiol* 2011; **43**: 115–9.

14 Rodriguez CH, De Ambrosio A, Bajuk M *et al*. In vitro antimicrobials activity against endemic *Acinetobacter baumannii* multiresistant clones. *J Infect Dev Ctries* 2010; **4**: 164–7.

15 Rodriguez CH, Bombicino K, Granados G *et al*. Selection of colistin-resistant *Acinetobacter baumannii* isolates in postneurosurgical meningitis in an intensive care unit with high presence of heteroresistance to colistin. *Diagn Microbiol Infect Dis* 2009; **65**: 188–91.

16 Yau W, Owen RJ, Poudyal A *et al*. Colistin hetero-resistance in multidrug-resistant *Acinetobacter baumannii* clinical isolates from the Western Pacific region in the SENTRY antimicrobial surveillance programme. *J Infect* 2009; **58**: 138–44.

17 Li J, Rayner CR, Nation RL *et al*. Pharmacokinetics of colistin methanesulfonate and colistin in a critically ill patient receiving continuous venovenous hemodiafiltration. *Antimicrob Agents Chemother* 2005; **49**: 4814–5.

18 Hawley JS, Murray CK, Jorgensen JH. Colistin heteroresistance in *Acinetobacter* and its association with previous colistin therapy. *Antimicrob Agents Chemother* 2008; **52**: 351–2.

19 Gales AC, Reis AO, Jones RN. Contemporary assessment of antimicrobial susceptibility testing methods for polymyxin B and colistin: review of available interpretative criteria and quality control guidelines. *J Clin Microbiol* 2001; **39**: 183–90.

20 Gales AC, Jones RN, Sader HS. Contemporary activity of colistin and polymyxin B against a worldwide collection of Gram-negative pathogens: results from the SENTRY Antimicrobial Surveillance Program (2006–09). *J Antimicrob Chemother* 2011; **66**: 2070–4.

21 Rolain JM, Roch A, Castanier M *et al*. *Acinetobacter baumannii* Resistant to colistin with impaired virulence: a case report from France. *J Infect Dis* 2011; **204**: 1146–7.

22 Lopez-Rojas R, Jimenez-Mejias ME, Lepe JA *et al*. *Acinetobacter baumannii* resistant to colistin alters its antibiotic resistance profile: a case report from Spain. *J Infect Dis* 2011; **204**: 1147–8.

23 Gomez-Garces JL, Aracil B, Gil Y *et al*. Susceptibility of 228 non-fermenting Gram-negative rods to tigecycline and six other antimicrobial drugs. *J Chemother* 2009; **21**: 267–71.

24 Mezzatesta ML, Trovato G, Gona F *et al*. In vitro activity of tigecycline and comparators against carbapenem-susceptible and resistant *Acinetobacter baumannii* clinical isolates in Italy. *Ann Clin Microbiol Antimicrob* 2008; **7**: 4.

25 Quinteira S, Grosso F, Ramos H *et al*. Molecular epidemiology of imipenem-resistant *Acinetobacter haemolyticus* and *Acinetobacter baumannii* isolates carrying plasmid-mediated OXA-40 from a Portuguese hospital. *Antimicrob Agents Chemother* 2007; **51**: 3465–6.

26 Garcia-Penuela E, Aznar E, Alarcon T *et al*. [Susceptibility pattern of *Acinetobacter baumannii* clinical isolates in Madrid vs. Hong Kong]. *Rev Esp Quimioter* 2006; **19**: 45–50.

27 Dobrewski R, Savov E, Bernards AT *et al*. Genotypic diversity and antibiotic susceptibility of *Acinetobacter baumannii* isolates in a Bulgarian hospital. *Clin Microbiol Infect* 2006; **12**: 1135–7.

28 Arroyo LA, Garcia-Curiel A, Pachon-Ibanez ME *et al*. Reliability of the E-test method for detection of colistin resistance in clinical isolates of *Acinetobacter baumannii*. *J Clin Microbiol* 2005; **43**: 903–5.

29 Arroyo LA, Mateos I, Gonzalez V *et al*. In vitro activities of tigecycline, minocycline, and colistin-tigecycline combination against multi- and pandrug-resistant clinical isolates of *Acinetobacter baumannii* group. *Antimicrob Agents Chemother* 2009; **53**: 1295–6.

30 Al-Sweih NA, Al-Hubail MA, Rotimi VO. Emergence of tigecycline and colistin resistance in *Acinetobacter* species isolated from patients in Kuwait hospitals. *J Chemother* 2011; **23**: 13–6.

31 Eser OK, Ergin A, Hascelik G. [Antimicrobial resistance and existence of metallo-β-lactamase in *Acinetobacter* species isolated from adult patients]. *Mikrobiyol Bul* 2009; **43**: 383–90.

32 Lee YT, Huang LY, Chiang DH *et al*. Differences in phenotypic and genotypic characteristics among imipenem-non-susceptible *Acinetobacter* isolates belonging to different genomic species in Taiwan. *Int J Antimicrob Agents* 2009; **34**: 580–4.

33 Sung H, Choi SJ, Yoo S *et al*. [In vitro antimicrobial synergy against imipenem-resistant *Acinetobacter baumannii*]. *Korean J Lab Med* 2007; **27**: 111–7.

34 Chang KC, Lin MF, Lin NT *et al*. Clonal spread of multidrug-resistant *Acinetobacter baumannii* in eastern Taiwan. *J Microbiol Immunol Infect* 2012; **45**: 37–42.

35 Lee SC, Huang SS, See LC *et al*. In vitro activities of nine current antibiotics against culprit bacteria in nosocomial infections in an institution in northern Taiwan. *Chang Gung Med J* 2011; **34**: 580–9.

36 Ko KS, Suh JY, Kwon KT *et al*. High rates of resistance to colistin and polymyxin B in subgroups of *Acinetobacter baumannii* isolates from Korea. *J Antimicrob Chemother* 2007; **60**: 1163–7.

37 Doi Y, Husain S, Potoski BA *et al*. Extensively drug-resistant *Acinetobacter baumannii*. *Emerg Infect Dis* 2009; **15**: 980–2.

38 Keen EF III, Robinson BJ, Hospenthal DR *et al*. Prevalence of multidrug-resistant organisms recovered at a military burn center. *Burns* 2010; **36**: 819–25.

39 Hawley JS, Murray CK, Griffith ME *et al*. Susceptibility of *Acinetobacter* strains isolated from deployed U.S. military personnel. *Antimicrob Agents Chemother* 2007; **51**: 376–8.

40 Tognim MC, Gales AC, Penteado AP *et al*. Dissemination of IMP-1 metallo-β-lactamase-producing *Acinetobacter* species in a Brazilian teaching hospital. *Infect Control Hosp Epidemiol* 2006; **27**: 742–7.

41 Raetz CR, Whitfield C. Lipopolysaccharide endotoxins. *Annu Rev Biochem* 2002; **71**: 635–700.

42 Adams MD, Nickel GC, Bajaksouzian S *et al*. Resistance to colistin in *Acinetobacter baumannii* associated with mutations in the PmrAB two-component system. *Antimicrob Agents Chemother* 2009; **53**: 3628–34.

43 Moffatt JH, Harper M, Harrison P *et al*. Colistin resistance in *Acinetobacter baumannii* is mediated by complete loss of lipopolysaccharide production. *Antimicrob Agents Chemother* 2010; **54**: 4971–7.

44 Moffatt JH, Harper M, Adler B *et al*. Insertion sequence ISAba11 is involved in colistin resistance and loss of lipopolysaccharide in *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2011; **55**: 3022–4.

45 Henry R, Vithanage N, Harrison P *et al*. Colistin-resistant, lipopolysaccharide-deficient *Acinetobacter baumannii* responds to lipopolysaccharide loss through increased expression of genes involved in the synthesis and transport of lipoproteins, phospholipids, and poly-β-1,6-*N*-acetylglucosamine. *Antimicrob Agents Chemother* 2012; **56**: 59–69.

46 Soon RL, Nation RL, Cockram S *et al*. Different surface charge of colistin-susceptible and -resistant *Acinetobacter baumannii* cells measured with zeta potential as a function of growth phase and colistin treatment. *J Antimicrob Chemother* 2011; **66**: 126–33.

47 Park YK, Choi JY, Shin D *et al*. Correlation between overexpression and amino acid substitution of the PmrAB locus and colistin resistance in *Acinetobacter baumannii*. *Int J Antimicrob Agents* 2011; **37**: 525–30.

48 Beceiro A, Llobet E, Aranda J *et al*. Phosphoethanolamine modification of lipid A in colistin-resistant variants of *Acinetobacter baumannii* mediated by the pmrAB two-component regulatory system. *Antimicrob Agents Chemother* 2011; **55**: 3370–9.

49 Gunn JS. The Salmonella PmrAB regulon: lipopolysaccharide modifications, antimicrobial peptide resistance and more. *Trends Microbiol* 2008; **16**: 284–90.

50 Lim LM, Ly N, Anderson D *et al*. Resurgence of colistin: a review of resistance, toxicity, pharmacodynamics, and dosing. *Pharmacotherapy* 2010; **30**: 1279–91.

51 Cai Y, Li R, Liang B *et al*. In vitro antimicrobial activity and mutant prevention concentration of colistin against *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2010; **54**: 3998–9.

52 Tan CH, Li J, Nation RL. Activity of colistin against heteroresistant *Acinetobacter baumannii* and emergence of resistance in an in vitro pharmacokinetic/pharmacodynamic model. *Antimicrob Agents Chemother* 2007; **51**: 3413–5.

53 Owen RJ, Li J, Nation RL *et al*. *In vitro* pharmacodynamics of colistin against *Acinetobacter baumannii* clinical isolates. *J Antimicrob Chemother* 2007; **59**: 473–7.

54 Dudhani RV, Turnidge JD, Nation RL *et al*. fAUC/MIC is the most predictive pharmacokinetic/pharmacodynamic index of colistin against *Acinetobacter baumannii* in murine thigh and lung infection models. *J Antimicrob Chemother* 2010; **65**: 1984–90.

55 Plachouras D, Karvanen M, Friberg LE *et al*. Population pharmacokinetic analysis of colistin methanesulfonate and colistin after intravenous administration in critically ill patients with infections caused by Gram-negative bacteria. *Antimicrob Agents Chemother* 2009; **53**: 3430–6.

56 Garonzik SM, Li J, Thamlikitkul V *et al*. Population pharmacokinetics of colistin methanesulfonate and formed colistin in critically ill patients from a multicenter study provide dosing suggestions for various categories of patients. *Antimicrob Agents Chemother* 2011; **55**: 3284–94.

57 Pankuch GA, Lin G, Seifert H *et al*. Activity of meropenem with and without ciprofloxacin and colistin against *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2008; **52**: 333–6.

58 Principe L, D'Arezzo S, Capone A *et al*. In vitro activity of tigecycline in combination with various antimicrobials against multidrug resistant *Acinetobacter baumannii*. *Ann Clin Microbiol Antimicrob* 2009; **8**: 18.

59 Peck KR, Kim MJ, Choi JY *et al*. In vitro time-kill studies of antimicrobial agents against blood isolates of imipenem-resistant *Acinetobacter baumannii* including colistin- or tigecycline-resistant isolates. *J Med Microbiol* 2012; **61**: 353–60.

60 Sheng WH, Wang JT, Li SY *et al*. Comparative in vitro antimicrobial susceptibilities and synergistic activities of antimicrobial combinations against carbapenem-resistant *Acinetobacter* species: *Acinetobacter baumannii* versus *Acinetobacter* genospecies 3 and 13TU. *Diagn Microbiol Infect Dis* 2011; **70**: 380–6.

61 Timurkaynak F, Can F, Azap OK *et al*. In vitro activities of non-traditional antimicrobials alone or in combination against multidrug-resistant strains of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolated from intensive care units. *Int J Antimicrob Agents* 2006; **27**: 224–8.

62 Fulnecky EJ, Wright D, Scheld WM *et al*. Amikacin and colistin for treatment of *Acinetobacter baumannii* meningitis. *J Infect* 2005; **51**: e249–51.

63 Santimaleeworagun W, Wongpoowarak P, Chayakul P *et al*. In vitro activity of colistin or sulbactam in combination with fosfomycin or imipenem against clinical isolates of carbapenem-resistant *Acinetobacter baumannii* producing OXA-23 carbapenemases. *Southeast Asian J Trop Med Public Health* 2011; **42**: 890–900.

64 Gordon NC, Png K, Wareham DW. Potent synergy and sustained bactericidal activity of a vancomycin-colistin combination versus multidrug-resistant strains of *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2010; **54**: 5316–22.

65 Li J, Nation RL, Owen RJ *et al*. Antibiograms of multidrug-resistant clinical *Acinetobacter baumannii*: promising therapeutic options for treatment of infection with colistin-resistant strains. *Clin Infect Dis* 2007; **45**: 594–8.

66 Rodríguez-Hernández MJ, Saugar J, Docobo-Pérez F *et al*. Studies on the antimicrobial activity of cecropin A-melittin hybrid peptides in colistin-resistant clinical isolates of *Acinetobacter baumannii*. *J Antimicrob Chemother* 2006; **58**: 95–100.

67 Saugar JM, Rodriguez-Hernandez MJ, de la Torre BG *et al*. Activity of cecropin A-melittin hybrid peptides against colistin-resistant clinical strains of *Acinetobacter baumannii*: molecular basis for the differential mechanisms of action. *Antimicrob Agents Chemother* 2006; **50**: 1251–6.

68 Vila-Farres X, Garcia de la Maria C, Lopez-Rojas R *et al*. In vitro activity of several antimicrobial peptides against colistin-susceptible and colistin-resistant *Acinetobacter baumannii*. *Clin Microbiol Infect* 2011.

69 Hornsey M, Wareham DW. In vivo efficacy of glycopeptide-colistin combination therapies in a *Galleria mellonella* model of *Acinetobacter baumannii* infection. *Antimicrob Agents Chemother* 2011; **55**: 3534–7.

70 Shields RK, Kwak EJ, Potoski BA *et al*. High mortality rates among solid organ transplant recipients infected with extensively drug-resistant *Acinetobacter baumannii*: using in vitro antibiotic combination testing to identify the combination of a carbapenem and colistin as an effective treatment regimen. *Diagn Microbiol Infect Dis* 2010; **70**: 246–52.

71 Ozbek B, Senturk A. Postantibiotic effects of tigecycline, colistin sulfate, and levofloxacin alone or tigecycline-colistin sulfate and tigecycline-levofloxacin combinations against *Acinetobacter baumannii*. *Chemotherapy* 2010; **56**: 466–71.

72 Candel FJ, Calvo N, Head J *et al*. A combination of tigecycline, colistin, and meropenem against multidrug-resistant *Acinetobacter baumannii* bacteremia in a renal transplant recipient: pharmacodynamic and microbiological aspects. *Rev Esp Quimioter* 2010; **23**: 103–8.

73 Pongpech P, Amornnopparattanakul S, Panapakdee S *et al*. Antibacterial activity of carbapenem-based combinations against multidrug-resistant *Acinetobacter baumannii*. *J Med Assoc Thai* 2010; **93**: 161–71.

74 Pachon-Ibanez ME, Docobo-Perez F, Lopez-Rojas R *et al*. Efficacy of rifampin and its combinations with imipenem, sulbactam, and colistin in experimental models of infection caused by imipenem-resistant *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2010; **54**: 1165–72.

75 Dizbay M, Tozlu DK, Cirak MY *et al*. In vitro synergistic activity of tigecycline and colistin against XDR-*Acinetobacter baumannii*. *J Antibiot (Tokyo)* 2010; **63**: 51–3.

76 Lee CH, Tang YF, Su LH *et al*. Antimicrobial effects of varied combinations of meropenem, sulbactam, and colistin on a multidrug-resistant *Acinetobacter baumannii* isolate that caused meningitis and bacteremia. *Microb Drug Resist* 2008; **14**: 233–7.

77 Song JY, Lee J, Heo JY *et al*. Colistin and rifampicin combination in the treatment of ventilator-associated pneumonia caused by carbapenem-resistant *Acinetobacter baumannii*. *Int J Antimicrob Agents* 2008; **32**: 281–4.

78 Bassetti M, Repetto E, Righi E *et al*. Colistin and rifampicin in the treatment of multidrug-resistant *Acinetobacter baumannii* infections. *J Antimicrob Chemother* 2008; **61**: 417–20.

79 Pantopoulou A, Giamarellos-Bourboulis EJ, Raftogannis M *et al*. Colistin offers prolonged survival in experimental infection by multidrug-resistant *Acinetobacter baumannii*: the significance of co-administration of rifampicin. *Int J Antimicrob Agents* 2007; **29**: 51–5.

80 Biancofiore G, Tascini C, Bisa M *et al*. Colistin, meropenem and rifampin in a combination therapy for multi-drug-resistant *Acinetobacter baumannii* multifocal infection. A case report. *Minerva Anestesiol* 2007; **73**: 181–5.

81 Motaouakkil S, Charra B, Hachimi A *et al*. Colistin and rifampicin in the treatment of nosocomial infections from multiresistant *Acinetobacter baumannii*. *J Infect* 2006; **53**: 274–8.

82 Petrosillo N, Chinello P, Proietti MF *et al*. Combined colistin and rifampicin therapy for carbapenem-resistant *Acinetobacter baumannii* infections: clinical outcome and adverse events. *Clin Microbiol Infect* 2005; **11**: 682–3.

83 Montero A, Ariza J, Corbella X *et al*. Antibiotic combinations for serious infections caused by carbapenem-resistant *Acinetobacter baumannii* in a mouse pneumonia model. *J Antimicrob Chemother* 2004; **54**: 1085–91.

84 Hogg GM, Barr JG, Webb CH. In-vitro activity of the combination of colistin and rifampicin against multidrug-resistant strains of *Acinetobacter baumannii*. *J Antimicrob Chemother* 1998; **41**: 494–5.