



HAL
open science

Confronting multidrug-resistant : a review

Ioannis K. Neonakis, Demetrios A. Spandidos, Efthimia Petinaki

► **To cite this version:**

Ioannis K. Neonakis, Demetrios A. Spandidos, Efthimia Petinaki. Confronting multidrug-resistant: a review. *International Journal of Antimicrobial Agents*, 2011, 37 (2), pp.102. 10.1016/j.ijantimicag.2010.10.014 . hal-00659896

HAL Id: hal-00659896

<https://hal.science/hal-00659896>

Submitted on 14 Jan 2012

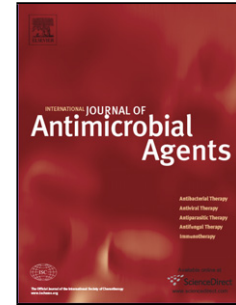
HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Accepted Manuscript

Title: Confronting multidrug-resistant *Acinetobacter baumannii*: a review

Authors: Ioannis K. Neonakis, Demetrios A. Spandidos, Efthimia Petinaki



PII: S0924-8579(10)00461-9
DOI: doi:10.1016/j.ijantimicag.2010.10.014
Reference: ANTAGE 3454

To appear in: *International Journal of Antimicrobial Agents*

Received date: 23-9-2010
Accepted date: 8-10-2010

Please cite this article as: Neonakis IK, Spandidos DA, Petinaki E, Confronting multidrug-resistant *Acinetobacter baumannii*: a review, *International Journal of Antimicrobial Agents* (2010), doi:10.1016/j.ijantimicag.2010.10.014

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Confronting multidrug-resistant *Acinetobacter baumannii*: a review

Ioannis K. Neonakis ^{a,b,*}, Demetrios A. Spandidos ^b, Efthimia Petinaki ^a

^a *Department of Microbiology, Medical School, University of Thessaly, Larissa, Greece*

^b *Department of Microbiology, University Hospital of Heraklion, Heraklion, Crete, Greece*

ARTICLE INFO

Article history:

Received 23 September 2010

Accepted 8 October 2010

Keywords:

Acinetobacter baumannii

Tigecycline

Peptides

Colistin

Rifampicin

Minocycline

* Corresponding author. Present address: Department of Microbiology, University Hospital of Heraklion, 712 01 Heraklion, Crete, Greece. Tel.: +30 281 039 2599; fax: +30 210 725 2922.

[2]

E-mail address: ineonakis@gmail.com (I.K. Neonakis).

Accepted Manuscript

ABSTRACT

Multidrug-resistant *Acinetobacter baumannii* (MDR-AB) infections are difficult to treat owing to the extremely limited armamentarium. The present review reports all available treatment options against MDR-AB, including single molecules, combination schemes, and alternative modes of antimicrobial administration. Additionally, a group of recently reported peptides with anti-MDR-AB activity is described.

Accepted Manuscript

1. Introduction

Over the last 10 years, *Acinetobacter baumannii* has emerged as one of the most problematic pathogens as treatment has been limited to only a few antibiotics. A wide spectrum of antimicrobial resistance mechanisms is exhibited by *A. baumannii*. Apart from its intrinsic resistance mainly due to the low permeability of the outer membrane to certain antibiotics as well as constitutive expression of certain efflux pumps, *A. baumannii* is able to easily acquire and incorporate genetic elements such as plasmids, transposons and integrons [1,2]. Multidrug-resistant (MDR) isolates (i.e. those with non-susceptibility to three or more classes of drugs) are being increasingly reported worldwide [3–8]. Infections due to such resistant microbes are associated with increased morbidity and mortality [4,6,9–11]. Carbapenems, which used to be the antimicrobials of choice, have been increasingly compromised and no longer constitute salvage therapy for *A. baumannii* infections [1,12–14]. The present review aimed to report all available therapeutic options against the MDR *A. baumannii* (MDR-AB) isolates and to present all of the latest developments in the field.

2. Polymyxins

Polymyxins are polycationic lipopeptide antimicrobials that have been available on the market for more than 50 years. They act both on the outer and cytoplasmic membranes leading to loss of membrane integrity. Among them, polymyxin B and polymyxin E [colistin (COL)] are suitable for clinical use. For a long period polymyxins were used infrequently owing to concerns of renal toxicity. However, over the last 5 years the emergence of MDR Gram-negative bacteria and the lack of new

[5]

antimicrobials have led to a revival of polymyxins, especially COL. There are two forms of COL: colistin methanesulfonate (CMS) for intravenous (i.v.) or intramuscular administration; and colistin sulphate for topical skin use (powder) or per os administration. Both COL forms can be inhaled.

Clinical use of polymyxins against *A. baumannii* isolates proved to be extremely successful. An 87% cure rate was reported in a retrospective cohort analysis of patients receiving i.v. COL for microbiologically documented infections, whereas in a large retrospective study a 75% cure rate of ventilator-associated pneumonia (VAP) caused by pandrug-resistant (PDR) *A. baumannii* and *Pseudomonas aeruginosa* isolates susceptible only to COL was reported [15,16]. Similar results have also been reported by other authors [17–19].

Polymyxins have been tested extensively in combination with other agents against MDR-AB. In vitro they have been combined with carbapenems, cefepime, amikacin, azithromycin, minocycline and other antimicrobials, with promising results [20–24]. Clinically, the combination of COL with meropenem (MEM) appears to be superior to the other agents. Falagas et al. [15] reported that patients who were treated with a COL+MEM combination had a better outcome of infection than patients who received COL in combination with other antimicrobials. The authors further noted that although no statistically significant difference in clinical outcome was found between COL monotherapy and COL+MEM combination therapy, use of the combination is preferable since heteroresistance may arise when COL is used alone [15]. Another clinically important combination of COL is that with rifampicin (RIF), which will be described in detail later.

Following is a detailed examination of three issues regarding the treatment of MDR-AB with COL.

2.1. In vitro susceptibility testing

With regard to *in vitro* susceptibility testing of COL, there are three points to be considered. The predictive accuracy of the disk diffusion test is limited owing to the poor agar diffusion characteristics of COL [25]. Hence, the disk diffusion test is reported to be unreliable for detecting COL resistance compared with the gold standard method of agar dilution [25–28]. Another point of concern is that the minimum inhibitory concentrations (MICs) of COL determined by the VITEK[®] 2 system (bioMérieux, Marcy l'Etoile, France), a widely used automated susceptibility testing system, have been reported to be unreliable [29]. However, this matter is controversial since contrary results have been obtained by other researchers [27]. Finally, it should be noted that the currently available breakpoints for COL are based on colistin sulfate and not CMS that is used intravenously [27].

2.2. Resistance: heteroresistance

The mechanisms of resistance to COL have yet to be adequately clarified. However, resistance has been associated with alterations in the outer layer of the bacterial cell, such as a reduction in lipopolysaccharides, reduced levels of specific outer membrane proteins, reduction of lipid content and reduction in cell envelope Mg⁺² and Ca⁺² contents [5,30,31]. Furthermore, efflux of potassium has been correlated with resistance to polymyxin B in *Yersinia* spp. [32].

Resistance to polymyxins is still considered to be rare. However, a number of sporadic cases of infections caused by COL-resistant isolates have been reported [33–36]. Moreover, Gales et al. [37] reported 2.7% and 1.7% polymyxin B-resistant *A. baumannii* isolates collected in Europe and in North and Latin America, respectively. Souli et al. [38] reported a 3% COL resistance rate among 100 *A. baumannii* strains derived from Intensive Care Unit patients in Greece. Notably, Ko et al. [7] reported extremely high resistance rates (27.9%) among 214 isolates of *A. baumannii* from two South Korean hospitals.

For the first time in 2006, Li et al. [39] reported the phenomenon of heteroresistance to COL in MDR-AB clinical isolates. Using population analysis profiles (PAPs) of 16 clinical isolates with MICs of COL in the range 0.25–2 µg/mL, the authors detected the presence of subpopulations (<0.1% of inocula) that were able to grow in the presence of COL at 3–10 µg/mL. The resistant subpopulations were not detected by either the disk diffusion susceptibility test or by commercial automated systems. Owing to resistant subpopulations, significant re-growth was observed at 24 h following the rapid initial killing (<2 h) in time–kill studies. Notably, heteroresistance occurred in almost all the isolates (15/16) [39]. Hawley et al. [40] found a similar high percentage of heteroresistance (100%), whereas in a more recent study by Yau et al. [41] the percentage was determined to be 23%. Li et al. [39] found that in the majority of clinical isolates a subpopulation of 0.00001–0.000001% grew in the presence of 10 µg/mL COL. This growth indicates that the COL plasma concentrations of 0.5–3.5 µg/mL usually achieved with the recommended dosage regimens of CMS may be

[8]

unable to eradicate the more resistant subpopulations [28,42–44]. Hawley et al. [40] found that the degree of heteroresistance among isolates from patients with previous COL treatment was statistically significantly higher than that of isolates not previously exposed to COL. Furthermore, Li et al. [39] reported that following exposure to COL a substantial increase in the proportion of resistant subpopulations was noted. These findings suggest that in order to avoid suboptimal concentrations of COL, further pharmacodynamic investigations on dosage optimisation of CMS are required and that CMS monotherapy should be avoided. In addition, it has been proposed that mini-PAPs with COL at >2 mg/L should be employed for susceptibility reporting in clinical microbiological laboratories [41].

2.3. Side effects

The most common potential toxicities of i.v. CMS administration are nephrotoxicity and neurotoxicity, with the latter being relatively rare [45]. A high incidence of nephrotoxicity was reported in early studies [46,47]. However, a series of recent investigations revealed that COL is not as nephrotoxic as initially believed [48–51]. Falagas et al. [49] analysed data for 19 courses of prolonged i.v. COL administration (mean \pm standard deviation duration of administration 43.4 \pm 14.6 days). The median creatinine value increased by 0.25 mg/dL during treatment compared with the baseline, but returned close to the baseline at the end of treatment [49]. Stein and Raoult [52] reported that a dose of 1 million units of CMS every 8 h was effective and safe, even when administered over a period of 3–6 months for infections associated with orthopaedic devices. In another study, i.v. administration of CMS (5.0 mg/kg/24 h in three doses) was generally well tolerated by critically ill patients with normal

renal function in whom it was used as salvage therapy [51]. Nevertheless, prolonged administration of CMS increases the risk of nephrotoxicity [53]. This risk does not appear to be related to the daily dose of CMS but rather the total cumulative dose [45,53]. Subsequently, careful monitoring of kidney function in patients requiring prolonged therapy with CMS is crucial along with efforts to avoid prolonged treatment courses, whenever possible.

3. Tigecycline (TIG)

TIG is a semisynthetic glycylicycline. It represents a modified minocycline and has activity against a broad spectrum of Gram-positive (including meticillin-resistant staphylococci and vancomycin-resistant enterococci), Gram-negative (including extended-spectrum β -lactamase- and Amp C-producing Enterobacteriaceae) and anaerobic organisms (including *Bacteroides* spp. and *Clostridium perfringens*). TIG inhibits bacterial protein synthesis by binding to the 30S ribosomal subunit [54]. A 100 mg loading dose is recommended, followed by 50 mg i.v. every 12 h. Biliary or faecal excretion accounts for 59% of the administered dose [55,56].

A serious issue regarding TIG is that the disk diffusion technique, when employed either for susceptibility testing or for MIC determination by Etest, may lead to serious discrepancies in the results compared with results by the microdilution method [4,57,58]. The quality of the Mueller–Hinton media plays an important role. TIG activity is affected by the cation concentration of the Mueller–Hinton agar and, moreover, TIG suffers degradation by an oxidation process, leading to loss of

potency [4]. Thus, for correct evaluation of susceptibility to TIG, the Mueller–Hinton agar must be properly and freshly prepared or anaerobically stored [59,60].

Despite its favourable activity against Gram-positive microorganisms, TIG is mainly reserved for use against MDR Gram-negative bacteria owing to the lack of therapeutic alternatives against these bacteria. Its use against *A. baumannii* is off-label. Moreover, no defined susceptibility breakpoints have been established thus far for *A. baumannii*. Even in their latest editions (2010), neither the Clinical and Laboratory Standards Institute (CLSI) [61] nor the British Society for Antimicrobial Chemotherapy (BSAC) [62] propose any specific breakpoints for TIG regarding *A. baumannii* owing to insufficient clinical evidence. However, the limited armamentarium against *A. baumannii* and the relatively low MICs obtained in various studies rendered the use of TIG against *A. baumannii* appealing. In 2007 in a major global study, Reinert et al. [63] reported MIC₉₀ values (MICs for 90% of the isolates) of 1 mg/L for Europe, North America, Latin America and the Asia/Pacific Rim. Recently, using a global collection of 6292 isolates, Garrison et al. [64] reported an MIC₅₀ (MIC for 50% of the isolates) at 0.5 mg/L and an MIC₉₀ at 1 mg/L, with an MIC range of ≤ 0.008 –16 mg/L. Furthermore, when testing only the MDR-AB subset ($n = 582$), the authors reported an MIC₅₀ at 1 mg/L and an MIC₉₀ at 2 mg/L, with an MIC range of ≤ 0.008 –8 mg/L [64]. Similar in vitro results were also reported by Draghi et al. (MIC₉₀ at 2 mg/L) [65], Souli et al. (MIC₉₀ of MDR-AB at 1 mg/L) [38] and Hoban et al. (MIC₉₀ of MDR-AB at 2 mg/L) [66].

Whilst TIG retains good in vitro activity against MDR-AB, its clinical efficacy remains a matter of controversy. The efficacy of TIG in 17 patients with selected serious

infections caused by resistant bacteria was evaluated in a phase III, open-label, non-comparative, multicentre study [67]. The clinical cure rate with TIG therapy observed in microbiologically evaluable patients was 82.4% and the microbiological eradication rate was 64.7% [67]. Gordon and Wareham [68] reported an overall 68% positive clinical outcome in 34 patients with infections involving MDR-AB. Karageorgopoulos et al. [69] reviewed the effectiveness of TIG for MDR-AB infections in eight identified clinical studies, reporting retrospective data regarding 42 severely ill patients, 31 of whom had respiratory tract infection (in 4 cases with secondary bacteraemia) and 4 had bacteraemia. TIG therapy (in combination with other antibiotics in 28 patients) was effective in 32/42 cases. Poulakou et al. [70] retrospectively analysed adult patients who had received TIG for >5 days either as monotherapy (M group) or as presumed active monotherapy (PAM group). In the PAM group, all co-administered antimicrobial(s) were resistant in vitro against the targeted pathogen(s) or failed clinically and microbiologically after ≥ 5 days of therapy despite in vitro susceptibility [70]. Among the 15 cases of MDR-AB isolation from patients in the M group, the clinical response was characterised as improvement or cure in 11 cases. Among the 13 cases of MDR-AB isolation from patients in the PAM group, the clinical response was characterised as improvement/cure in 10 cases, leading to an overall 75% (21/28) successful clinical response rate regarding the 28 *A. baumannii* isolates. In contrast to the aforementioned studies, Gallagher and Rouse [71] reported poor favourable clinical outcome (improvement/cure) with TIG therapy in MDR-AB infections (29%; 8/28 patients). Similarly, a low rate of favourable clinical outcome was reported by Anthony et al. [72]. The authors reported that four of five patients with serious infections by MDR-AB characterised as intermediately resistant to TIG (MIC >2 $\mu\text{g}/\text{mL}$ or <8 $\mu\text{g}/\text{mL}$) succumbed (deaths were related to infection), whereas

[12]

0 of 4 patients with pre-therapy isolates susceptible to TIG (MIC \leq 2 $\mu\text{g}/\text{mL}$) succumbed ($P = 0.048$) [72]. More information on the clinical efficacy of TIG therapy from well designed, prospective studies using a large number of patients is crucial.

In the latter aforementioned study by Anthony et al. [72] it was also reported that one patient with an *A. baumannii* tracheal aspirate isolate that was initially susceptible developed resistance during therapy (MIC increased from 2.00 $\mu\text{g}/\text{mL}$ to 12.00 $\mu\text{g}/\text{mL}$ after 14 days). The difficulty arising with the emergence of resistance among MDR Gram-negative organisms during therapy with TIG has previously been described. Schafer et al. [73] reported that one patient with VAP plus bacteraemia due to MDR-AB developed resistance to TIG during therapy. A similar case was reported by Reid et al. [74] who concluded that clinicians should be aware that TIG MICs for *A. baumannii* isolates may increase during therapy with TIG following only brief exposure to the drug. Patients receiving TIG for *Acinetobacter* should be monitored for the development of clinical resistance and isolates should be continuously monitored [74].

Superinfections with pathogens inherently resistant to TIG (*P. aeruginosa*, *Proteus* spp., *Providencia* spp., etc.) are a matter of great concern. A superinfection is defined as a new isolate that emerges during therapy at the original site of infection, with the emergence or worsening of clinical signs and symptoms of infection [67]. Poulakou et al. [70] reported that among 45 patients treated with TIG for MDR or PDR infections, 10 episodes of superinfections by inherently resistant pathogens (9 bacteraemias and 1 urinary tract infection) occurred. The possibility of

superinfections should be seriously considered, and TIG monotherapy should be avoided when the risk for pseudomonal infection is significant.

Owing to the low mean peak serum concentrations of TIG achievable at recommended doses, therapeutic failure of *A. baumannii* bacteraemia is plausible, even for strains with low MIC values [72,75]. Consequently, TIG treatment should generally be avoided in cases of bacteraemia due to *A. baumannii*, especially for strains with MICs ≥ 1 $\mu\text{g/mL}$. In addition, clinicians should avoid the use of TIG for infections in poorly penetrated anatomical sites such as the urinary tract because therapeutic failure is feasible and because such treatments may promote the development of further resistance [72].

TIG has been investigated in combination with other antibacterials against *A. baumannii* isolates. Enhanced activity or bactericidal synergism occurred with TIG plus carbapenems, levofloxacin, amikacin and RIF in various in vitro studies [76–78]. Results of the combination of TIG with COL are controversial [79–81].

Apart from the in vitro studies, there are reports of single cases of MDR-AB infections successfully treated with combinations of TIG with other agents such as MEM, COL, piperacillin/tazobactam and trimethoprim/sulfamethoxazole [77,82,83]. In a retrospective case series, Schafer et al. [73] reported clinical cure in 9/9 patients (100%) treated with TIG + imipenem (IPM), in 4/7 patients (57%) treated with TIG+COL and in 3/4 patients (75%) treated with a combination of the three drugs. However, the potential clinical role of TIG in combination with other agents against *A. baumannii* infections should be further investigated.

4. Sulbactam

Sulbactam is an Ambler class A β -lactamase inhibitor with a structure similar to β -lactams that carries intrinsic activity against *A. baumannii* by binding to its penicillin-binding protein 2 [4]. A series of studies have reported encouraging results on the efficacy of sulbactam against *A. baumannii* infections and it is generally accepted that in the cases of sulbactam-susceptible *A. baumannii* infections, sulbactam should be considered as the preferred therapeutic solution [11,84–92]. Even in the case of carbapenem-resistant *A. baumannii* infections, sulbactam appears to be more efficacious than polymyxins [93]. Moreover, it has been shown that the pharmaceutical cost is significantly reduced when sulbactam is selected instead of other antibiotics such as IPM [86]. Although more data on optimal dosing and mode of administration are required, it has been proposed that ≥ 8 g of the sulbactam compound in divided doses, assuming normal renal function, should be administered per day [1,4,87,90].

A disadvantage is that in a number of countries sulbactam is commercially available only in combination with ampicillin at a fixed 2:1 ratio. Another difficulty involves the increasing number of sulbactam-resistant *A. baumannii* isolates. In a Spanish multicentre study, only 46.7% of *A. baumannii* isolates were susceptible to this agent [94], whereas in a major recent study from Taiwan ca. 70% of the clinical isolates were resistant to ampicillin/sulbactam (SAM) [95].

Enhanced effectiveness has been found when sulbactam is combined with a series of other molecules such as cefepime, IPM, MEM, amikacin and RIF [87,96–101]. However, these are in vitro studies or studies on animal models without clinical evaluation in humans. More data are therefore imperative to confirm this observation.

5. Rifampicin

RIF is generally considered as an antimicrobial best suited for Gram-positive bacteria. However, in vitro studies and experimental models of infection showed that RIF alone could demonstrate a bactericidal effect on MDR-AB [102–104]. In these experimental models, the MICs of RIF for the *A. baumannii* strains used ranged from 4 mg/L to 8 mg/L, and RIF monotherapy proved to be as efficacious as that of IPM and more efficacious than that of COL monotherapy with regard to bacterial clearance from the lungs [103,104]. However, it is well documented that when used alone RIF shows rapid development of resistance, thus its combination with other antimicrobials is necessary [105,106]. Although an additive effect or synergism have been observed for combinations of RIF with various agents such as carbapenems, TIG or SAM [81,105], the most promising combination is that of RIF with COL.

Early in vitro studies by Giamarellos-Bourboulis et al. [102] revealed the existence of synergy between RIF and COL against MDR-AB. Furthermore, Rodriguez et al. [107] demonstrated that the combination of RIF+COL inhibits resistant subpopulations in heteroresistant isolates irrespective of the RIF MIC values. Apart from the in vitro studies, synergy between COL and RIF has also been shown in experimental models. Using a thigh experimental model of infection by MDR-AB, Pantopoulou et

al. [108] demonstrated that the activity of COL was increased significantly by the presence of RIF and that the combination was effective in prolonging the survival of infected rats. Based on these results, Bassetti et al. [109] conducted a clinical trial including 29 critically ill patients with MDR-AB infections who were treated with a combination of i.v. COL and RIF. Clinical and microbiological favourable responses were observed in 22/29 cases (76%) and the overall infection-related mortality was 21% (6/29 cases). No case developed resistance to either RIF or COL. Of the 29 patients, 3 (10%) developed nephrotoxicity (all of them had previous renal failure), whereas no cases of renal failure were observed among patients with normal baseline renal function [109]. Similar results have been reported in other clinical trials, indicating that the combination of COL+RIF may be considered as a serious therapeutic option [110–112]. However, this conclusion should be confirmed by additional clinical trials with much larger numbers of patients. An issue that should also be clarified is the role of the initial MIC to RIF in the final outcome of combination therapy. This is a critical point, since there are data supporting the fact that in cases with an initial high level of resistance to RIF (MICs ≥ 64 mg/L) a poor microbiological eradication rate is to be expected [112].

6. Minocycline and fosfomycin

Minocycline is a tetracycline derivative introduced in the 1960s. However, its use was limited due to the introduction of more potent agents. Minocycline inhibits bacterial protein synthesis and acts both against Gram-positive and Gram-negative bacteria. Recently, owing to the shortage of therapeutic solutions, its use against minocycline-susceptible MDR-AB isolates has gained new interest. The literature is promising

although limited [113]. In one study, 7/8 patients treated with oral minocycline for traumatic wound infections by MDR-AB yielded positive outcomes [114], whereas in another report i.v. treatment with minocycline was successful in 3/4 patients with MDR-AB VAP [115]. Since minocycline achieves ideal blood and tissue levels and has notable central nervous system penetration, it appears to play a significant role in confronting MDR-AB infections [113]. More in vitro and clinical data regarding minocycline are required to verify this role.

Fosfomycin is an old bactericidal molecule that inhibits synthesis of the bacterial cell wall peptidoglycan. It acts both against Gram-positive and Gram-negative bacteria and its toxicity is very low. Although it has been found to be a reliable solution for MDR Enterobacteriaceae [116], fosfomycin cannot be considered as an option for MDR-AB isolates since the majority of these isolates are not susceptible to this antimicrobial agent [117–120].

7. Continuous administration of intravenous antibiotics

To optimise the effect of available agents, alternative therapeutic approaches have been used. The most promising approach is extended or continuous administration of i.v. antibacterials. It appears that continuous i.v. infusion of antibacterials with time-dependent bacterial killing is superior to normal intermittent i.v. administration [121]. Extended infusion of β -lactams such as carbapenems or cefepime has the ability to achieve drug concentrations above the MIC for a greater time for less susceptible organisms, especially those with an MIC between 4 $\mu\text{g/mL}$ and 16 $\mu\text{g/mL}$ [122–124]. In a meta-analysis of 730 episodes of infection it was shown that when the total daily

[18]

antibiotic dose was the same, clinical failure was significantly lower in the continuous infusion group compared with the intermittent infusion group [125]. Furthermore, there are data suggesting that continuous antibiotic dosing offers more favourable activity against resistant pathogens and may actually reduce the incidence of antibiotic resistance [126]. However, more data regarding *A. baumannii* are necessary before firm conclusions can be drawn.

8. New agents: peptides

A series of new molecules, including natural products and newly synthesised chemical substances, are constantly being screened for their activity against *A. baumannii*, with varying results [127–132]. The most notable and promising group of new substances is that of the peptides reviewed below [133–136].

Peptides normally comprise between 10 and 48 amino acid residues with no conserved domains associated with biological activity [133]. With a few exceptions, peptides are cationic and contain $\geq 50\%$ hydrophobic amino acids [133]. Peptides with antimicrobial activities have been isolated from a wide range of bacterial species, plants and animals [134]. They provide a host defence system to combat infections and play one of the most important roles against pathogenic microorganisms [134]. They bind the negatively charged parts of the bacterial membrane by electrostatic interactions and it is postulated that they exert their inhibitory effects by increasing bacterial membrane permeability, causing leakage of bacterial contents and consequently maximal entry of several other substrates [134]. However, a major obstacle to the development of peptide-based anti-infective drugs,

particularly if they are to be administered systemically, is their short half-lives in the circulation [137]. Efforts are constantly being made to overcome this problem, with varying results [138].

Peptides have presented activity against *A. baumannii* [136]. Here we present three peptides that have recently been reported to exert activity against MDR-AB.

8.1. Alyteserin-1c and its [E4K] analogue

Alyteserin-1c is a 23-amino acid peptide that was first isolated from noradrenaline-stimulated skin secretions from the midwife toad *Alytes obstetricians* [133]. The haemolytic activity of the naturally occurring peptide was relatively low. Colnon et al. [138] reported that it presented bactericidal activity against MDR-AB in the MIC range of 5–10 μM . Following substitution of the Glu4 residue by L-Lys, the created analogue [E4K] was found to have a four-fold increase in potency against all strains of MDR-AB tested by the authors [138]. Moreover, the haemolytic activity of the [E4K] analogue was appreciably lower than the naturally occurring peptide [lethal concentration for 50% of test population >400 μM] and the analogue was extremely soluble in physiological buffers. In a time–kill assay, >99.9% of the bacteria were killed within 30 min by the [E4K] analogue at a concentration of 1 \times the minimum bactericidal concentration [138].

8.2. Buforin II

Buforin II is a peptide isolated from the stomach of the Asian toad *Bufo bufo gargarizans* [139]. Cirioni et al. [134] investigated the in vitro interactions and the in vivo efficacy of buforin II and RIF in a rat model of *A. baumannii* sepsis using both an *A. baumannii* ATCC 19606 strain and a MDR-AB clinical isolate [134]. The two strains showed similar susceptibility to buforin II, with MICs of 8 mg/L, whereas synergy (fractional inhibitory concentration index of 0.312) was observed by testing buforin II combined with RIF. In the rat model of sepsis, following intraperitoneal injection of MDR-AB, the lethality rate in the control group was found to be 100% within 48 h compared with 46.6% for the group treated with buforin II, whereas a rate of 20.0% was observed in the combined group. Buforin II was also significantly superior to the control at reducing blood, spleen, peritoneum, liver and mesenteric lymph node complex bacterial burdens ($P < 0.05$). Furthermore, buforin II alone or combined produced a reduction of 46%, 20% and 28% in tumour necrosis factor (TNF), interleukin-6 and endotoxin plasma levels, respectively ($P < 0.05$). Overall, the combination of buforin II+RIF exhibited the highest antimicrobial activities and the strongest reduction in plasma endotoxin and cytokine levels. Similar results were also obtained when the experiments were performed using *A. baumannii* ATCC 19606 instead of the MDR-AB isolate. In conclusion, buforin II exerted strong antimicrobial activity, good survival rates and, finally, achieved a significant reduction in plasma endotoxin and cytokine concentrations. Moreover, synergism of the buforin II+RIF combination was observed. It appears that buforin II increases the permeability of the membrane thereby facilitating penetration of the otherwise impermeable hydrophobic solutes such as RIF [134].

8.3. Human β -defensin 2 (hBD2)

The defensins are a group of β -sheet-rich, cationic and amphipathic peptides that exert an antimicrobial effect, which appears to be achieved by creating pores or otherwise disrupting the cell membrane of target organisms, leading to the release of their cellular contents and ultimately destruction of the cells by osmolysis [140]. hBD2 is primarily expressed in the epithelial lining of the human urinary and respiratory tracts [140]. Routsias et al. [141] examined the bactericidal activity of hBD2 against epidemiologically unrelated hospital pathogens, of which 21 were *A. baumannii* isolates (14/21 isolates were MDR-AB). Preliminary data showed that incubation with 10 $\mu\text{g}/\text{mL}$ hBD2 for 2 h killed the entire *A. baumannii* population [141]. Notably, the 14 *Acinetobacter* isolates that exhibited a MDR phenotype were all (100%) sensitive to low concentrations of hBD2 with a virtually 99% lethal dose (vLD₉₉) of <10 $\mu\text{g}/\text{mL}$, whilst the 7 non-MDR *Acinetobacter* isolates demonstrated an average vLD₉₉ of >10 $\mu\text{g}/\text{mL}$. Furthermore, the vLD₉₀ ranged between 3.25 $\mu\text{g}/\text{mL}$ and 4.5 $\mu\text{g}/\text{mL}$ for all MDR strains, whilst the vLD₉₀ was significantly higher (ranging from 3.90 $\mu\text{g}/\text{mL}$ to 9.35 $\mu\text{g}/\text{mL}$) for non-MDR strains ($t = -3.74$, $P < 0.01$) [141]. Other than mutations conferring resistance to certain antibiotics, it is possible that MDR strains also possess mutations that affect energy generation required for the maintenance of membrane integrity or affect the membrane structure itself, leading to supersusceptibility to the action of substances such as hBD2 [141].

Apart from its ability to fight MDR *A. baumannii*, hBD2 is an endogenous peptide of the innate immune system and may be less toxic than other molecules such as polymyxins. In addition to their direct antibacterial activity, defensins have the

[22]

potential to neutralise bacterial endotoxin by direct binding and inactivation of lipopolysaccharide, thereby preventing the ability of endotoxin to induce shock. Furthermore, owing to their ability to form channels in lipid membranes, defensins facilitate the penetration of hydrophilic drugs.

A significant disadvantage of hBD2 is that the salt concentration affects its bactericidal effect. It was demonstrated that even 150 mM of sodium chloride was sufficient to decrease the bactericidal effect of hBD2 at least 15-fold, indicating that the maximum activity of hBD2 can only be found in sites of the human body with low salt concentration, such as the airway surface fluid of normal lung [141].

9. Conclusions

Currently, COL constitutes a salvage solution against MDR-AB. It is not as nephrotoxic as initially believed and should be used in combination with other antimicrobials, preferably MEM or RIF. Use of TIG against *A. baumannii* is off-label and its clinical efficacy remains a matter of controversy. Resistance to TIG during therapy may occur and TIG monotherapy should be avoided when the risk of pseudomonal infection is serious. TIG treatment should also be avoided in cases of bacteraemia by isolates with MICs of ≥ 1 $\mu\text{g}/\text{mL}$. In cases of sulbactam-susceptible *A. baumannii* infections, sulbactam should be considered as the preferred therapeutic solution. RIF appears to exert bactericidal activity against MDR-AB. Its combination with other antimicrobials is crucial and the most active combination is considered to be that with COL. Minocycline may play a serious role in confronting minocycline-susceptible MDR-AB infections, however clinical studies on this issue are rare.

[23]

Among the new agents, peptides appear to be the most promising. A series of recently reported peptides have shown strong bactericidal activity against MDR-AB. However, various obstacles should be overcome before they can be used clinically.

Funding

None.

Competing interests

None declared.

Ethical approval

Not required.

Accepted Manuscript

References

- [1] Vila J, Pachon J. Therapeutic options for *Acinetobacter baumannii* infections. *Expert Opin Pharmacother* 2008;9:587–99.
- [2] Giamarellou H, Antoniadou A, Kanellakopoulou K. *Acinetobacter baumannii*: a universal threat to public health? *Int J Antimicrob Agents* 2008;32:106–19.
- [3] Fournier PE, Richet H. The epidemiology and control of *Acinetobacter baumannii* in health care facilities. *Clin Infect Dis* 2006;42:692–9.
- [4] Zavascki AP, Carvalhaes CG, Picao RC, Gales AC. Multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii*: resistance mechanisms and implications for therapy. *Expert Rev Anti Infect Ther* 2010;8:71–93.
- [5] Maviglia R, Nestorini R, Pennisi M. Role of old antibiotics in multidrug resistant bacterial infections. *Curr Drug Targets* 2009;10:895–905.
- [6] Paterson DL. The epidemiological profile of infections with multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter* species. *Clin Infect Dis* 2006;43(Suppl 2):S43–8.
- [7] Ko KS, Suh JY, Kwon KT, Jung SI, Park KH, Kang CI, 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.
- [8] Doi Y, Husain S, Potoski BA, McCurry KR, Paterson DL. Extensively drug-resistant *Acinetobacter baumannii*. *Emerg Infect Dis* 2009;15:980–2.
- [9] Fagon JY, Chastre J, Hance AJ, Montravers P, Novara A, Gibert C. Nosocomial pneumonia in ventilated patients: a cohort study evaluating attributable mortality and hospital stay. *Am J Med* 1993;94:281–8.

- [10] Rello J, Ausina V, Ricart M, Castella J, Prats G. Impact of previous antimicrobial therapy on the etiology and outcome of ventilator-associated pneumonia. *Chest* 1993;104:1230–5.
- [11] Peleg AY, Seifert H, Paterson DL. *Acinetobacter baumannii*: emergence of a successful pathogen. *Clin Microbiol Rev* 2008;21:538–82.
- [12] McDonald LC. Trends in antimicrobial resistance in health care-associated pathogens and effect on treatment. *Clin Infect Dis* 2006;42(Suppl 2):S65–71.
- [13] Bassetti M, Righi E, Esposito S, Petrosillo N, Nicolini L. Drug treatment for multidrug-resistant *Acinetobacter baumannii* infections. *Future Microbiol* 2008;3:649–60.
- [14] Falagas ME, Mourtzoukou EG, Polemis M, Vatopoulos AC. Trends in antimicrobial resistance of *Acinetobacter baumannii* clinical isolates from hospitalised patients in Greece and treatment implications. *Clin Microbiol Infect* 2007;13:816–9.
- [15] Falagas ME, Rafailidis PI, Ioannidou E, Alexiou VG, Matthaïou DK, Karageorgopoulos DE, et al. Colistin therapy for microbiologically documented multidrug-resistant Gram-negative bacterial infections: a retrospective cohort study of 258 patients. *Int J Antimicrob Agents* 2010;35:194–9.
- [16] Kallel H, Hergafi L, Bahloul M, Hakim A, Dammak H, Chelly H, et al. Safety and efficacy of colistin compared with imipenem in the treatment of ventilator-associated pneumonia: a matched case–control study. *Intensive Care Med* 2007;33:1162–7.
- [17] Holloway KP, Roupahel NG, Wells JB, King MD, Blumberg HM. Polymyxin B and doxycycline use in patients with multidrug-resistant *Acinetobacter baumannii* infections in the intensive care unit. *Ann Pharmacother* 2006;40:1939–45.

- [18] Michalopoulos AS, Tsiodras S, Rellos K, Mentzelopoulos S, Falagas ME. Colistin treatment in patients with ICU-acquired infections caused by multiresistant Gram-negative bacteria: the renaissance of an old antibiotic. *Clin Microbiol Infect* 2005;11:115–21.
- [19] Levin AS, Barone AA, Penco J, Santos MV, Marinho IS, Arruda EA, et al. Intravenous colistin as therapy for nosocomial infections caused by multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. *Clin Infect Dis* 1999;28:1008–11.
- [20] Timurkaynak F, Can F, Azap OK, Demirbilek M, Arslan H, Karaman SO. 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.
- [21] Tan TY, Ng LS, Tan E, Huang G. In vitro effect of minocycline and colistin combinations on imipenem-resistant *Acinetobacter baumannii* clinical isolates. *J Antimicrob Chemother* 2007;60:421–3.
- [22] Rahal JJ. Novel antibiotic combinations against infections with almost completely resistant *Pseudomonas aeruginosa* and *Acinetobacter* species. *Clin Infect Dis* 2006;43(Suppl 2):S95–9.
- [23] Yoon J, Urban C, Terzian C, Mariano N, Rahal JJ. In vitro double and triple synergistic activities of polymyxin B, imipenem, and rifampin against multidrug-resistant *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2004;48:753–7.
- [24] Pankuch GA, Lin G, Seifert H, Appelbaum PC. Activity of meropenem with and without ciprofloxacin and colistin against *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2008;52:333–6.

[27]

- [25] Galani I, Kontopidou F, Souli M, Rekatsina PD, Koratzanis E, Deliolanis J, et al. Colistin susceptibility testing by Etest and disk diffusion methods. *Int J Antimicrob Agents* 2008;31:434–9.
- [26] Tan TY, Ng LS. Comparison of three standardized disc susceptibility testing methods for colistin. *J Antimicrob Chemother* 2006;58:864–7.
- [27] Lo-Ten-Foe JR, de Smet AM, Diederens BM, Kluytmans JA, van Keulen PH. Comparative evaluation of the VITEK 2, disk diffusion, Etest, broth microdilution, and agar dilution susceptibility testing methods for colistin in clinical isolates, including heteroresistant *Enterobacter cloacae* and *Acinetobacter baumannii* strains. *Antimicrob Agents Chemother* 2007;51:3726–30.
- [28] Giamarellou H, Poulakou G. Multidrug-resistant Gram-negative infections: what are the treatment options? *Drugs* 2009;69:1879–901.
- [29] Tan TY, Ng SY. Comparison of Etest, Vitek and agar dilution for susceptibility testing of colistin. *Clin Microbiol Infect* 2007;13:541–4.
- [30] Mortensen NP, Fowlkes JD, Sullivan CJ, Allison DP, Larsen NB, Molin S, et al. Effects of colistin on surface ultrastructure and nanomechanics of *Pseudomonas aeruginosa* cells. *Langmuir* 2009;25:3728–33.
- [31] Young ML, Bains M, Bell A, Hancock RE. Role of *Pseudomonas aeruginosa* outer membrane protein OprH in polymyxin and gentamicin resistance: isolation of an OprH-deficient mutant by gene replacement techniques. *Antimicrob Agents Chemother* 1992;36:2566–8.
- [32] Bengoechea JA, Skurnik M. Temperature-regulated efflux pump/potassium antiporter system mediates resistance to cationic antimicrobial peptides in *Yersinia*. *Mol Microbiol* 2000;37:67–80.

[28]

- [33] Falagas ME, Rafailidis PI, Matthaïou DK, Vrtzili S, Nikita D, Michalopoulos A. Pandrug-resistant *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* infections: characteristics and outcome in a series of 28 patients. *Int J Antimicrob Agents* 2008;32:450–4.
- [34] Giamarellou H. Colistin: the loss of the last frontier? *APUA Newslett* 2007;25:5.
- [35] Beno P, Krcmery V, Demitrovicova A. Bacteraemia in cancer patients caused by colistin-resistant Gram-negative bacilli after previous exposure to ciprofloxacin and/or colistin. *Clin Microbiol Infect* 2006;12:497–8.
- [36] Souli M, Galani I, Giamarellou H. Emergence of extensively drug-resistant and pandrug-resistant Gram-negative bacilli in Europe. *Euro Surveill* 2008;13:pii: 19045.
- [37] Gales AC, Jones RN, Sader HS. Global assessment of the antimicrobial activity of polymyxin B against 54 731 clinical isolates of Gram-negative bacilli: report from the SENTRY antimicrobial surveillance programme (2001–2004). *Clin Microbiol Infect* 2006;12:315–21.
- [38] Souli M, Kontopidou FV, Koratzanis E, Antoniadou A, Giannitsioti E, Evangelopoulou P, et al. In vitro activity of tigecycline against multiple-drug-resistant, including pan-resistant, Gram-negative and Gram-positive clinical isolates from Greek hospitals. *Antimicrob Agents Chemother* 2006;50:3166–9.
- [39] Li J, Rayner CR, Nation RL, Owen RJ, Spelman D, Tan KE, et al. Heteroresistance to colistin in multidrug-resistant *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2006;50:2946–50.

- [40] Hawley JS, Murray CK, Jorgensen JH. Colistin heteroresistance in *Acinetobacter* and its association with previous colistin therapy. *Antimicrob Agents Chemother* 2008;52:351–2.
- [41] Yau W, Owen RJ, Poudyal A, Bell JM, Turnidge JD, Yu HH, 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.
- [42] 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.
- [43] Li J, Coulthard K, Milne R, Nation RL, Conway S, Peckham D, et al. Steady-state pharmacokinetics of intravenous colistin methanesulphonate in patients with cystic fibrosis. *J Antimicrob Chemother* 2003;52:987–92.
- [44] Li J, Rayner CR, Nation RL, Deans R, Boots R, Widdecombe N, et al. Pharmacokinetics of colistin methanesulfonate and colistin in a critically ill patient receiving continuous venovenous hemodiafiltration. *Antimicrob Agents Chemother* 2005;49:4814–5.
- [45] Nation RL, Li J. Colistin in the 21st century. *Curr Opin Infect Dis* 2009;22:535–43.
- [46] Wolinsky E, Hines JD. Neurotoxic and nephrotoxic effects of colistin in patients with renal disease. *N Engl J Med* 1962;266:759–62.
- [47] Koch-Weser J, Sidel VW, Federman EB, Kanarek P, Finer DC, Eaton AE. Adverse effects of sodium colistimethate. Manifestations and specific reaction rates during 317 courses of therapy. *Ann Intern Med* 1970;72:857–68.

- [48] Conway SP, Pond MN, Watson A, Etherington C, Robey HL, Goldman MH. Intravenous colistin sulphomethate in acute respiratory exacerbations in adult patients with cystic fibrosis. *Thorax* 1997;52:987–93.
- [49] Falagas ME, Rizos M, Bliziotis IA, Rellos K, Kasiakou SK, Michalopoulos A. Toxicity after prolonged (more than four weeks) administration of intravenous colistin. *BMC Infect Dis* 2005;5:1.
- [50] Kasiakou SK, Michalopoulos A, Soteriades ES, Samonis G, Sermaides GJ, Falagas ME. Combination therapy with intravenous colistin for management of infections due to multidrug-resistant Gram-negative bacteria in patients without cystic fibrosis. *Antimicrob Agents Chemother* 2005;49:3136–46.
- [51] Reina R, Estenssoro E, Saenz G, Canales HS, Gonzalvo R, Vidal G, et al. Safety and efficacy of colistin in *Acinetobacter* and *Pseudomonas* infections: a prospective cohort study. *Intensive Care Med* 2005;31:1058–65.
- [52] Stein A, Raoult D. Colistin: an antimicrobial for the 21st century? *Clin Infect Dis* 2002;35:901–2.
- [53] Hartzell JD, Neff R, Ake J, Howard R, Olson S, Paolino K, et al. Nephrotoxicity associated with intravenous colistin (colistimethate sodium) treatment at a tertiary care medical center. *Clin Infect Dis* 2009;48:1724–8.
- [54] Bergeron J, Ammirati M, Danley D, James L, Norcia M, Retsema J, et al. Glycylcyclines bind to the high-affinity tetracycline ribosomal binding site and evade Tet(M)- and Tet(O)-mediated ribosomal protection. *Antimicrob Agents Chemother* 1996;40:2226–8.
- [55] Kasbekar N. Tigecycline: a new glycylcycline antimicrobial agent. *Am J Health Syst Pharm* 2006;63:1235–43.

- [56] Bhattacharya M, Parakh A, Narang M. Tigecycline. *J Postgrad Med* 2009;55:65–8.
- [57] Casal M, Rodriguez F, Johnson B, Garduno E, Tubau F, de Lejarazu RO, et al. Influence of testing methodology on the tigecycline activity profile against presumably tigecycline-non-susceptible *Acinetobacter* spp. *J Antimicrob Chemother* 2009;64:69–72.
- [58] Canigia LF, Bantar C. Susceptibility testing of tigecycline against *Acinetobacter* spp. by disc diffusion method: withdrawing a therapeutic option by varying the Mueller–Hinton agar? *J Antimicrob Chemother* 2008;62:1463–4.
- [59] Hope R, Warner M, Mushtaq S, Ward ME, Parsons T, Livermore DM. Effect of medium type, age and aeration on the MICs of tigecycline and classical tetracyclines. *J Antimicrob Chemother* 2005;56:1042–6.
- [60] Petersen PJ, Bradford PA. Effect of medium age and supplementation with the biocatalytic oxygen-reducing reagent oxyrase on in vitro activities of tigecycline against recent clinical isolates. *Antimicrob Agents Chemother* 2005;49:3910–8.
- [61] Clinical and Laboratory Standards Institute. *Performance standards for antimicrobial susceptibility testing; twentieth informational supplement*. Document M100-S20. Wayne, PA: CLSI; 2010.
- [62] British Society for Antimicrobial Chemotherapy. *BSAC methods for antimicrobial susceptibility testing. Version 9.1 March 2010*. Birmingham, UK: BSAC; 2010. p. 32.
http://www.bsac.org.uk/Resources/BSAC/Version_9.1_March_2010_final.pdf
[accessed 29 October 2010].
- [63] Reinert RR, Low DE, Rossi F, Zhang X, Wattal C, Dowzicky MJ. Antimicrobial susceptibility among organisms from the Asia/Pacific Rim, Europe and Latin and

[32]

North America collected as part of TEST and the in vitro activity of tigecycline. *J Antimicrob Chemother* 2007;60:1018–29.

- [64] Garrison MW, Mutters R, Dowzicky MJ. In vitro activity of tigecycline and comparator agents against a global collection of Gram-negative and Gram-positive organisms: Tigecycline Evaluation and Surveillance Trial 2004 to 2007. *Diagn Microbiol Infect Dis* 2009;65:288–99.
- [65] Draghi DC, Tench S, Dowzicky MJ, Sahm DF. Baseline in vitro activity of tigecycline among key bacterial pathogens exhibiting multidrug resistance. *Chemotherapy* 2008;54:91–100.
- [66] Hoban DJ, Bouchillon SK, Dowzicky MJ. Antimicrobial susceptibility of extended-spectrum β -lactamase producers and multidrug-resistant *Acinetobacter baumannii* throughout the United States and comparative in vitro activity of tigecycline, a new glycylicycline antimicrobial. *Diagn Microbiol Infect Dis* 2007;57:423–8.
- [67] Vasilev K, Reshedko G, Orasan R, Sanchez M, Teras J, Babinchak T, et al. A Phase 3, open-label, non-comparative study of tigecycline in the treatment of patients with selected serious infections due to resistant Gram-negative organisms including *Enterobacter* species, *Acinetobacter baumannii* and *Klebsiella pneumoniae*. *J Antimicrob Chemother* 2008;62(Suppl 1):i29–40.
- [68] Gordon NC, Wareham DW. A review of clinical and microbiological outcomes following treatment of infections involving multidrug-resistant *Acinetobacter baumannii* with tigecycline. *J Antimicrob Chemother* 2009;63:775–80.
- [69] Karageorgopoulos DE, Kelesidis T, Kelesidis I, Falagas ME. Tigecycline for the treatment of multidrug-resistant (including carbapenem-resistant)

- Acinetobacter* infections: a review of the scientific evidence. J Antimicrob Chemother 2008;62:45–55.
- [70] Poulakou G, Kontopidou FV, Paramythiotou E, Kompoti M, Katsiari M, Mainas E, et al. Tigecycline in the treatment of infections from multi-drug resistant Gram-negative pathogens. J Infect 2009;58:273–84.
- [71] Gallagher JC, Rouse HM. Tigecycline for the treatment of *Acinetobacter* infections: a case series. Ann Pharmacother 2008;42:1188–94.
- [72] Anthony KB, Fishman NO, Linkin DR, Gasink LB, Edelstein PH, Lautenbach E. Clinical and microbiological outcomes of serious infections with multidrug-resistant Gram-negative organisms treated with tigecycline. Clin Infect Dis 2008;46:567–70.
- [73] Schafer JJ, Goff DA, Stevenson KB, Mangino JE. Early experience with tigecycline for ventilator-associated pneumonia and bacteremia caused by multidrug-resistant *Acinetobacter baumannii*. Pharmacotherapy 2007;27:980–7.
- [74] Reid GE, Grim SA, Aldeza CA, Janda WM, Clark NM. Rapid development of *Acinetobacter baumannii* resistance to tigecycline. Pharmacotherapy 2007;27:1198–201.
- [75] Peleg AY, Potoski BA, Rea R, Adams J, Sethi J, Capitano B, et al. *Acinetobacter baumannii* bloodstream infection while receiving tigecycline: a cautionary report. J Antimicrob Chemother 2007;59:128–31.
- [76] Principe L, D'Arezzo S, Capone A, Petrosillo N, Visca P. In vitro activity of tigecycline in combination with various antimicrobials against multidrug resistant *Acinetobacter baumannii*. Ann Clin Microbiol Antimicrob 2009;8:18.

- [77] Entenza JM, Moreillon P. Tigecycline in combination with other antimicrobials: a review of in vitro, animal and case report studies. *Int J Antimicrob Agents* 2009;34:8.e1–9.
- [78] Lim TP, Tan TY, Lee W, Sasikala S, Tan TT, Hsu LY, et al. In vitro activity of various combinations of antimicrobials against carbapenem-resistant *Acinetobacter* species in Singapore. *J Antibiot (Tokyo)* 2009;62:675–9.
- [79] Petersen PJ, Labthavikul P, Jones CH, Bradford PA. In vitro antibacterial activities of tigecycline in combination with other antimicrobial agents determined by checkerboard and time–kill kinetic analysis. *J Antimicrob Chemother* 2006;57:573–6.
- [80] Arroyo LA, Mateos I, Gonzalez V, Aznar J. 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.
- [81] Dizbay M, Tozlu DK, Cirak MY, Isik Y, Ozdemir K, Arman D. In vitro synergistic activity of tigecycline and colistin against XDR-*Acinetobacter baumannii*. *J Antibiot (Tokyo)* 2010;63:51–3.
- [82] Leclerc T, Perez JP, Debien B, Clapson P, Lenoir B. Treatment of a septic shock due to multiresistant *Acinetobacter baumannii* with tigecycline in combination [in French]. *Ann Fr Anesth Reanim* 2007;26:1056–8.
- [83] Taccone FS, Rodriguez-Villalobos H, De Backer D, De Moor V, Deviere J, Vincent JL, et al. Successful treatment of septic shock due to pan-resistant *Acinetobacter baumannii* using combined antimicrobial therapy including tigecycline. *Eur J Clin Microbiol Infect Dis* 2006;25:257–60.

- [84] Choi JY, Kim CO, Park YS, Yoon HJ, Shin SY, Kim YK, et al. Comparison of efficacy of cefoperazone/sulbactam and imipenem/cilastatin for treatment of *Acinetobacter* bacteremia. *Yonsei Med J* 2006;47:63–9.
- [85] Cisneros JM, Reyes MJ, Pachon J, Becerril B, Caballero FJ, Garcia-Garmendia JL, et al. Bacteremia due to *Acinetobacter baumannii*: epidemiology, clinical findings, and prognostic features. *Clin Infect Dis* 1996;22:1026–32.
- [86] Jellison TK, McKinnon PS, Rybak MJ. Epidemiology, resistance, and outcomes of *Acinetobacter baumannii* bacteremia treated with imipenem–cilastatin or ampicillin–sulbactam. *Pharmacotherapy* 2001;21:142–8.
- [87] Peleg AY. Optimizing therapy for *Acinetobacter baumannii*. *Semin Respir Crit Care Med* 2007;28:662–71.
- [88] Smolyakov R, Borer A, Riesenber K, Schlaeffer F, Alkan M, Porath A, et al. Nosocomial multi-drug resistant *Acinetobacter baumannii* bloodstream infection: risk factors and outcome with ampicillin–sulbactam treatment. *J Hosp Infect* 2003;54:32–8.
- [89] Levin AS, Levy CE, Manrique AE, Medeiros EA, Costa SF. Severe nosocomial infections with imipenem-resistant *Acinetobacter baumannii* treated with ampicillin/sulbactam. *Int J Antimicrob Agents* 2003;21:58–62.
- [90] Lode HM. Rational antibiotic therapy and the position of ampicillin/sulbactam. *Int J Antimicrob Agents* 2008;32:10–28.
- [91] Corbella X, Ariza J, Ardanuy C, Vuelta M, Tubau F, Sora M, et al. Efficacy of sulbactam alone and in combination with ampicillin in nosocomial infections caused by multiresistant *Acinetobacter baumannii*. *J Antimicrob Chemother* 1998;42:793–802.

- [92] Wood GC, Hanes SD, Croce MA, Fabian TC, Boucher BA. Comparison of ampicillin–sulbactam and imipenem–cilastatin for the treatment of *Acinetobacter* ventilator-associated pneumonia. *Clin Infect Dis* 2002;34:1425–30.
- [93] Oliveira MS, Prado GV, Costa SF, Grinbaum RS, Levin AS. Ampicillin/sulbactam compared with polymyxins for the treatment of infections caused by carbapenem-resistant *Acinetobacter* spp. *J Antimicrob Chemother* 2008;61:1369–75.
- [94] Fernandez-Cuenca F, Pascual A, Ribera A, Vila J, Bou G, Cisneros JM, et al. Clonal diversity and antimicrobial susceptibility of *Acinetobacter baumannii* isolated in Spain. A nationwide multicenter study: GEIH-Ab project (2000) [in Spanish]. *Enferm Infecc Microbiol Clin* 2004;22:267–71.
- [95] Yang SC, Chang WJ, Chang YH, Tsai YS, Yang TP, Juan CW, et al. Prevalence of antibiotics resistance and OXA carbapenemases genes in multidrug-resistant *Acinetobacter baumannii* isolates in central Taiwan. *Eur J Clin Microbiol Infect Dis* 2010;29:601–4.
- [96] Tong W, Wang R, Chai D, Li Z, Pei F. In vitro activity of cefepime combined with sulbactam against clinical isolates of carbapenem-resistant *Acinetobacter* spp. *Int J Antimicrob Agents* 2006;28:454–6.
- [97] Song JY, Kee SY, Hwang IS, Seo YB, Jeong HW, Kim WJ, et al. In vitro activities of carbapenem/sulbactam combination, colistin, colistin/rifampicin combination and tigecycline against carbapenem-resistant *Acinetobacter baumannii*. *J Antimicrob Chemother* 2007;60:317–22.
- [98] Savov E, Chankova D, Vatcheva R, Dinev N. In vitro investigation of the susceptibility of *Acinetobacter baumannii* strains isolated from clinical specimens

- to ampicillin/sulbactam alone and in combination with amikacin. *Int J Antimicrob Agents* 2002;20:390–2.
- [99] Ko WC, Lee HC, Chiang SR, Yan JJ, Wu JJ, Lu CL, et al. In vitro and in vivo activity of meropenem and sulbactam against a multidrug-resistant *Acinetobacter baumannii* strain. *J Antimicrob Chemother* 2004;53:393–5.
- [100] Appleman MD, Belzberg H, Citron DM, Heseltine PN, Yellin AE, Murray J, et al. In vitro activities of nontraditional antimicrobials against multiresistant *Acinetobacter baumannii* strains isolated in an intensive care unit outbreak. *Antimicrob Agents Chemother* 2000;44:1035–40.
- [101] Kiffer CR, Sampaio JL, Sinto S, Oplustil CP, Koga PC, Arruda AC, et al. In vitro synergy test of meropenem and sulbactam against clinical isolates of *Acinetobacter baumannii*. *Diagn Microbiol Infect Dis* 2005;52:317–22.
- [102] Giamarellos-Bourboulis EJ, Xirouchaki E, Giamarellou H. Interactions of colistin and rifampin on multidrug-resistant *Acinetobacter baumannii*. *Diagn Microbiol Infect Dis* 2001;40:117–20.
- [103] Wolff M, Joly-Guillou ML, Farinotti R, Carbon C. In vivo efficacies of combinations of β -lactams, β -lactamase inhibitors, and rifampin against *Acinetobacter baumannii* in a mouse pneumonia model. *Antimicrob Agents Chemother* 1999;43:1406–11.
- [104] Montero A, Ariza J, Corbella X, Domenech A, Cabellos C, Ayats J, et al. Efficacy of colistin versus β -lactams, aminoglycosides, and rifampin as monotherapy in a mouse model of pneumonia caused by multiresistant *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2002;46:1946–52.

- [105] Tripodi MF, Durante-Mangoni E, Fortunato R, Utili R, Zarrilli R. Comparative activities of colistin, rifampicin, imipenem and sulbactam/ampicillin alone or in combination against epidemic multidrug-resistant *Acinetobacter baumannii* isolates producing OXA-58 carbapenemases. *Int J Antimicrob Agents* 2007;30:537–40.
- [106] Pachon-Ibanez ME, Fernandez-Cuenca F, Docobo-Perez F, Pachon J, Pascual A. Prevention of rifampicin resistance in *Acinetobacter baumannii* in an experimental pneumonia murine model, using rifampicin associated with imipenem or sulbactam. *J Antimicrob Chemother* 2006;58:689–92.
- [107] Rodriguez CH, De Ambrosio A, Bajuk M, Spinozzi M, Nastro M, Bombicino K, et al. In vitro antimicrobials activity against endemic *Acinetobacter baumannii* multiresistant clones. *J Infect Dev Ctries* 2010;4:164–7.
- [108] Pantopoulou A, Giamarellos-Bourboulis EJ, Raftogannis M, Tsaganos T, Dontas I, Koutoukas P, 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.
- [109] Bassetti M, Repetto E, Righi E, Boni S, Diverio M, Molinari MP, et al. Colistin and rifampicin in the treatment of multidrug-resistant *Acinetobacter baumannii* infections. *J Antimicrob Chemother* 2008;61:417–20.
- [110] Petrosillo N, Chinello P, Proietti MF, Cecchini L, Masala M, Franchi C, 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.

- [111] Motaouakkil S, Charra B, Hachimi A, Nejmi H, Benslama A, Elmdaghri N, et al. Colistin and rifampicin in the treatment of nosocomial infections from multiresistant *Acinetobacter baumannii*. *J Infect* 2006;53:274–8.
- [112] Song JY, Lee J, Heo JY, Noh JY, Kim WJ, Cheong HJ, 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.
- [113] Bishburg E, Bishburg K. Minocycline—an old drug for a new century: emphasis on methicillin-resistant *Staphylococcus aureus* (MRSA) and *Acinetobacter baumannii*. *Int J Antimicrob Agents* 2009;34:395–401.
- [114] Griffith ME YH, Horvath LL, Murray CK. Minocycline therapy for traumatic wound infections caused by the multidrug-resistant *Acinetobacter baumannii*–*Acinetobacter calcoaceticus* complex. *Infect Dis Clin Pract* 2008;16:16–9.
- [115] Wood GC, Hanes SD, Boucher BA, Croce MA, Fabian TC. Tetracyclines for treating multidrug-resistant *Acinetobacter baumannii* ventilator-associated pneumonia. *Intensive Care Med* 2003;29:2072–6.
- [116] Falagas ME, Kastoris AC, Kapaskelis AM, Karageorgopoulos DE. Fosfomycin for the treatment of multidrug-resistant, including extended-spectrum β -lactamase producing, Enterobacteriaceae infections: a systematic review. *Lancet Infect Dis* 2010;10:43–50.
- [117] Falagas ME, Kastoris AC, Karageorgopoulos DE, Rafailidis PI. Fosfomycin for the treatment of infections caused by multidrug-resistant non-fermenting Gram-negative bacilli: a systematic review of microbiological, animal and clinical studies. *Int J Antimicrob Agents* 2009;34:111–20.

- [118] Falagas ME, Kanellopoulou MD, Karageorgopoulos DE, Dimopoulos G, Rafailidis PI, Skarmoutsou ND, et al. Antimicrobial susceptibility of multidrug-resistant Gram negative bacteria to fosfomycin. *Eur J Clin Microbiol Infect Dis* 2008;27:439–43.
- [119] Dobrewski R, Savov E, Bernards AT, van den Barselaar M, Nordmann P, van den Broek PJ, et al. Genotypic diversity and antibiotic susceptibility of *Acinetobacter baumannii* isolates in a Bulgarian hospital. *Clin Microbiol Infect* 2006;12:1135–7.
- [120] Martinez-Martinez L, Rodriguez G, Pascual A, Suarez AI, Perea EJ. In-vitro activity of antimicrobial agent combinations against multiresistant *Acinetobacter baumannii*. *J Antimicrob Chemother* 1996;38:1107–8.
- [121] Kasiakou SK, Lawrence KR, Choulis N, Falagas ME. Continuous versus intermittent intravenous administration of antibacterials with time-dependent action: a systematic review of pharmacokinetic and pharmacodynamic parameters. *Drugs* 2005;65:2499–511.
- [122] Fishbain J, Peleg AY. Treatment of *Acinetobacter* infections. *Clin Infect Dis* 2010;51:79–84.
- [123] Li C, Kuti JL, Nightingale CH, Nicolau DP. Population pharmacokinetic analysis and dosing regimen optimization of meropenem in adult patients. *J Clin Pharmacol* 2006;46:1171–8.
- [124] Jaruratanasirikul S, Sriwiriyan S, Punyo J. Comparison of the pharmacodynamics of meropenem in patients with ventilator-associated pneumonia following administration by 3-hour infusion or bolus injection. *Antimicrob Agents Chemother* 2005;49:1337–9.

- [125] Kasiakou SK, Sermaides GJ, Michalopoulos A, Soteriades ES, Falagas ME. Continuous versus intermittent intravenous administration of antibiotics: a meta-analysis of randomised controlled trials. *Lancet Infect Dis* 2005;5:581–9.
- [126] Vergidis PI, Falagas ME. New antibiotic agents for bloodstream infections. *Int J Antimicrob Agents* 2008;32(Suppl 1):S60–5.
- [127] Barbuceanu SF, Almajan GL, Saramet I, Draghici C, Tarcomnicu AI, Bancescu G. Synthesis, characterization and evaluation of antibacterial activity of some thiazolo[3,2-b][1,2,4]triazole incorporating diphenylsulfone moieties. *Eur J Med Chem* 2009;44:4752–7.
- [128] Almajan GL, Barbuceanu SF, Almajan ER, Draghici C, Saramet G. Synthesis, characterization and antibacterial activity of some triazole Mannich bases carrying diphenylsulfone moieties. *Eur J Med Chem* 2009;44:3083–9.
- [129] Duraipandiyan V, Ignacimuthu S. Antibacterial and antifungal activity of flindersine isolated from the traditional medicinal plant, *Toddalia asiatica* (L.) Lam. *J Ethnopharmacol* 2009;123:494–8.
- [130] Tan HT, Rahman RA, Gan SH, Halim AS, Hassan SA, Sulaiman SA, et al. The antibacterial properties of Malaysian tualang honey against wound and enteric microorganisms in comparison to manuka honey. *BMC Complement Altern Med* 2009;9:34.
- [131] Aytemir MD, Ozelik B. A study of cytotoxicity of novel chlorokojoic acid derivatives with their antimicrobial and antiviral activities. *Eur J Med Chem* 2010;45:4089–95.
- [132] Schwan WR, Dunek C, Gebhardt M, Engelbrecht K, Klett T, Monte A, et al. Screening a mushroom extract library for activity against *Acinetobacter baumannii*

- and *Burkholderia cepacia* and the identification of a compound with anti-*Burkholderia* activity. *Ann Clin Microbiol Antimicrob* 2010;9:4.
- [133] Conlon JM, Demandt A, Nielsen PF, Leprince J, Vaudry H, Woodhams DC. The alyteserins: two families of antimicrobial peptides from the skin secretions of the midwife toad *Alytes obstetricans* (Alytidae). *Peptides* 2009;30:1069–73.
- [134] Cirioni O, Silvestri C, Ghiselli R, Orlando F, Riva A, Gabrielli E, et al. Therapeutic efficacy of buforin II and rifampin in a rat model of *Acinetobacter baumannii* sepsis. *Crit Care Med* 2009;37:1403–7.
- [135] Powers JP, Hancock RE. The relationship between peptide structure and antibacterial activity. *Peptides* 2003;24:1681–91.
- [136] Saugar JM, Rodriguez-Hernandez MJ, de la Torre BG, Pachon-Ibanez ME, Fernandez-Reyes M, Andreu D, 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.
- [137] Zasloff M. Antimicrobial peptides in health and disease. *N Engl J Med* 2002;347:1199–200.
- [138] Conlon JM, Ahmed E, Pal T, Sonnevend A. Potent and rapid bactericidal action of alyteserin-1c and its [E4K] analog against multidrug-resistant strains of *Acinetobacter baumannii*. *Peptides* 2010;31:1806–10.
- [139] Park CB, Kim HS, Kim SC. Mechanism of action of the antimicrobial peptide buforin II: buforin II kills microorganisms by penetrating the cell membrane and inhibiting cellular functions. *Biochem Biophys Res Commun* 1998;244:253–7.
- [140] Pazgier M, Hoover DM, Yang D, Lu W, Lubkowski J. Human β -defensins. *Cell Mol Life Sci* 2006;63:1294–313.

[43]

- [141] Routsias JG, Karagounis P, Parvulesku G, Legakis NJ, Tsakris A. In vitro bactericidal activity of human β -defensin 2 against nosocomial strains. *Peptides* 2010;31:1654–60.

Accepted Manuscript