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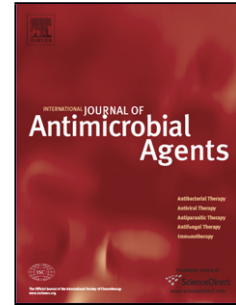
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Confronting multidrug-resistant *Acinetobacter baumannii*: a review

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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.

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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 ≤ 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 $\mu\text{g}/\text{mL}$ and 16 $\mu\text{g}/\text{mL}$ [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 $>400 \mu\text{M}$] 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 (vLD_{99}) of $<10 \mu\text{g}/\text{mL}$, whilst the 7 non-MDR *Acinetobacter* isolates demonstrated an average vLD_{99} of $>10 \mu\text{g}/\text{mL}$. Furthermore, the vLD_{90} ranged between 3.25 $\mu\text{g}/\text{mL}$ and 4.5 $\mu\text{g}/\text{mL}$ for all MDR strains, whilst the vLD_{90} 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

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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.

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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.

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