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Synergistic combinations of polymyxins

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Abstract

The proliferation of extensively drug-resistant Gram-negative pathogens has necessitated the therapeutic use of colistin and polymyxin B. However, treatment failures with polymyxin monotherapies and the emergence of polymyxin resistance have catalysed the search for polymyxin combinations that synergistically kill polymyxin-susceptible and -resistant organisms. This mini-review examines recent (2011–2016) *in vitro* and *in vivo* studies that have attempted to identify synergistic polymyxin combinations against *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Acinetobacter baumannii*. Clinical evidence for the use of combination regimens is also discussed.

Keywords

Polymyxins; Synergy; Combinations; *Acinetobacter baumannii*; *Klebsiella pneumoniae*; *Pseudomonas aeruginosa*

1. Introduction

After decades of antimicrobial exposure, multidrug-resistant (MDR) pathogens are now emerging with resistance to three or more antibiotic classes [1,2]. Even more troubling are extensively drug-resistant (XDR) Gram-negative pathogens that are non-susceptible to all but one or two antibiotic classes [3]. In the face of such extensive levels of antibiotic resistance, clinicians have been forced to utilise colistin and polymyxin B (PMB) as last-line agents against XDR *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Acinetobacter baumannii* that are capable of resisting carbapenems and most other agents [4,5]. However,

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the emergence of polymyxin heteroresistance and polymyxin-resistant strains has brought the utility of polymyxin monotherapies into question [6,7].

In response to the global decline in polymyxin susceptibilities, clinicians may be tempted to simply increase the dose of a polymyxin to maximise bacterial killing. Unfortunately, polymyxins are highly nephrotoxic agents and the likeliness of renal impairment has been associated with the daily dose of a polymyxin [8,9]. Given the narrow therapeutic indices of polymyxins, a strategy for overcoming attenuated polymyxin susceptibility without increasing polymyxin exposure is the use of polymyxins in combination with other agents. Polymyxins have a unique mechanism of action that involves disruption of the outer membrane integrity of Gram-negative bacteria, which may enhance the activity of other antibiotic classes [1,10]. Despite promising in vitro results, the usefulness of synergistic polymyxin combinations in the clinical setting remains controversial [11,12].

2. Methodology

This review covers recent studies that examined the in vitro and in vivo synergy of polymyxin combinations and evaluates whether any clinical evidence exists to validate the translation of preclinical work into human patients. Studies were retrieved using the search terms 'colistin combination' or 'polymyxin combination' in PubMed, with an emphasis on manuscripts published after 2010. In vitro studies were restricted to more advanced measures of bacterial killing such as time-killing investigations and dynamic models, whereas studies involving minimum inhibitory concentration (MIC) testing, chequerboard synergy and Etest methods were not examined. After consolidating all of the literature, manuscripts were chosen that epitomised recent in vitro, in vivo and clinical studies. To maintain consistency, synergy will be defined as a ≥ 2 log reduction in bacterial counts compared with reductions achieved by individual agents at any time during an experiment unless a time point is specified.

3. *Pseudomonas aeruginosa*

Several in vitro studies that utilised static antibiotic concentrations of polymyxins in combination with an aminoglycoside have recently been published. An investigation of *P. aeruginosa* biofilms found that colistin and tobramycin at $2\times$ their respective MICs ($\text{MIC}_{\text{colistin}} = 2 \text{ mg/L}$; $\text{MIC}_{\text{tobramycin}} = 1 \text{ mg/L}$) separately reduced bacterial counts of a single *P. aeruginosa* strain to $4.59 \log_{10} \text{ CFU/mL}$ and $4.85 \log_{10} \text{ CFU/mL}$ after 24 h from a $7.95 \log_{10} \text{ CFU/mL}$ starting inoculum, whereas the combination of both agents resulted in a 24-h count of $2.60 \log_{10} \text{ CFU/mL}$ [13]. Another study investigated the activity of PMB (2 mg/L), rifampicin (2 mg/L), meropenem (64 mg/L) and amikacin (80 mg/L), alone and in combination, against 22 clinical XDR *P. aeruginosa* isolates collected in Singapore [14]. After 24 h of antibiotic exposure, none of the single agents achieved a 3 log reduction in bacterial counts, whereas the combination of meropenem + PMB resulted in ≥ 3 log reduction in 8/22 strains, and the triple combination of PMB + amikacin + rifampicin (or meropenem) achieved ≥ 3 log reduction in an additional 7 strains (6 strains for meropenem).

Another static time–killing experiment evaluated the potential synergy between fosfomycin (30, 150 or 300 mg/L) and PMB (0.5, 1 or 2 mg/L) against four clinical *P. aeruginosa* isolates and one reference strain at 10^6 CFU/mL [15]. In 24-h experiments, synergistic killing was achieved at fosfomycin concentrations 30 mg/L and at PMB concentrations 1 mg/L against two isolates susceptible to both antibiotics. In contrast, synergistic killing was most evident at fosfomycin concentrations 150 mg/L for the polymyxin-resistant strain, and synergy was only detected in one of two strains that were resistant to both antimicrobials.

Other static time–killing experiments investigated the killing of a polymyxin with a carbapenem. A study examining PMB in combination with doripenem against wild-type *P. aeruginosa* and hypermutator strains ($MIC_{polymyxin} = 1\text{--}2$ mg/L) found that PMB concentrations up to 64 mg/L resulted in re-growth of all six stains at a 10^8 CFU/mL inoculum, whereas 4 mg/L PMB + 8 mg/L doripenem resulted in sustained killing up to 48 h [16]. Similarly, a study investigating colistin + imipenem in clinically relevant concentration arrays observed that several combinations of colistin + imipenem achieved synergy against colistin-resistant and imipenem-resistant *P. aeruginosa* isolates over 48 h both at 10^6 CFU/mL and 10^8 CFU/mL inocula [17].

The combination of a polymyxin and a carbapenem has also been investigated using dynamic in vitro models. In a dynamic biofilm model that utilised three colistin-susceptible *P. aeruginosa* isolates with varying doripenem susceptibilities ($MIC_{doripenem} = 0.125\text{--}128$ mg/L), the combination of doripenem [peak concentration (C_{max}) = 25 mg/L] + colistin (constant at 3.5 mg/L) achieved 1–2 log of additional killing by 4 h compared with either agent alone, and the enhanced killing was sustained up to 72 h in all three strains [18]. A separate investigation used a one-compartment model to investigate doripenem ($C_{max} = 2.5$ mg/L or 25 mg/L) + colistin (constant at 0.5 mg/L or 2 mg/L) against a colistin-heteroresistant strain and a colistin-resistant MDR *P. aeruginosa* strain and observed that combinations of doripenem + colistin were capable of synergistic killing over 96 h against both stains at 10^6 CFU/mL and 10^8 CFU/mL [19]. Lastly, a 10-day hollow-fibre infection model was used to examine the activity of colistin (constant at 2 mg/L or 5 mg/L) + doripenem ($C_{max} = 25$ mg/L) against two colistin-heteroresistant strains and one colistin-resistant isolate with an initial inoculum of 10^9 CFU/mL (Fig. 1) [20]. Both colistin-heteroresistant strains were eradicated by colistin + doripenem by 72 h (monotherapies re-grew), and although combination treatment was unable to eradicate the colistin-resistant strain, colistin + doripenem achieved >4 log reduction compared with either agent alone by 72 h.

In summary, the use of an aminoglycoside, fosfomycin or a carbapenem in conjunction with a polymyxin was able to confer additive or synergistic killing against several *P. aeruginosa* strains in static or dynamic in vitro models. As expected, strains that were susceptible to one or both of the study antimicrobials generally experienced the most rapid killing; however, the presence of aminoglycoside resistance, fosfomycin resistance or carbapenem resistance did not prevent synergistic killing during combination exposure. Encouragingly, the combination of a carbapenem and a polymyxin was able to achieve synergistic killing against polymyxin-resistant *P. aeruginosa* at 10^6 CFU/mL and 10^8 CFU/mL inocula.

4. *Klebsiella pneumoniae*

Several static time–killing studies have recently investigated the activities of polymyxin combinations against metallo- β -lactamase (MBL)-producing *K. pneumoniae*. In a study that examined 12 antibiotics alone and in dual combinations against six NDM-1-producing *K. pneumoniae* strains at 10^5 CFU/mL, the double combinations of meropenem (64 mg/L) + tigecycline (2 mg/L) and of PMB (2 mg/L) + either meropenem or imipenem (32 mg/L) achieved a 3 log reduction in four and three NDM-1-producing strains, respectively, and PMB + meropenem resulted in 2 log reduction in an additional two strains (individual agents resulted in re-growth) [21]. Another investigation analysed the killing of PMB and chloramphenicol against four NDM-producing *K. pneumoniae* isolates at 10^6 CFU/mL with varying degrees of chloramphenicol susceptibility ($\text{MIC}_{\text{chloramphenicol}} = 4\text{--}256$ mg/L) and found that 1 mg/L PMB + 8 mg/L chloramphenicol achieved synergy against all four strains (at the 6 h and 24 h time points) [22]. Lastly, a study that investigated various antibiotic combinations against two VIM-1- and two NDM-1-producing *K. pneumoniae* isolates at 5×10^6 CFU/mL identified the combination of colistin (4.0 mg/L) + meropenem (6.8 mg/L) + rifampicin (1.7 mg/L) as the most effective, with synergistic killing and a 3 log reduction by 24 h in all four isolates [23]. The study also demonstrated synergy between fosfomycin (83 mg/L) and colistin (4.0 mg/L) against one VIM-producing strain (fosfomycin-susceptible) and both NDM-producing strains (fosfomycin-resistant).

In contrast to the synergistic killing observed between fosfomycin and colistin against MBL-producing *K. pneumoniae*, a study evaluating the killing of fosfomycin combinations against KPC-2 producing *K. pneumoniae* observed a relatively low rate of synergy [24]. Using a 10^6 CFU/mL inoculum, fosfomycin (100 mg/L) and colistin (5 mg/L) were evaluated alone and in combination against 17 KPC-2-producing *K. pneumoniae* isolates in static time–killing experiments. Despite fosfomycin susceptibility in 13 strains and colistin susceptibility in 9 strains, synergistic killing was only observed in 2/17 *K. pneumoniae* isolates (11.8%); however, bactericidal activity was observed against 11/17 isolates. The rate of synergy observed in this study (11.8%) may be lower than the MBL investigation owing to rapid bactericidal activity achieved by colistin or fosfomycin in this study, whereas colistin or fosfomycin alone were unable to achieve substantial killing against the VIM- or NDM-producing *K. pneumoniae* potentially due to unknown phenotypic differences between the bacterial strains [23].

Several in vitro studies have explored polymyxin and carbapenem combinations against *K. pneumoniae* carbapenemase (KPC)-producing organisms. A static time–killing study of doripenem (6 mg/L) in combination with colistin or PMB ($2 \times \text{MIC}$; polymyxin MICs ranged from 0.0625 to 0.125 mg/L) against $10^{5.5}$ CFU/mL of polymyxin-susceptible KPC-3-producing *K. pneumoniae* found that polymyxins alone resulted in re-growth by 48 h, whereas a polymyxin + doripenem achieved synergistic reductions compared with a polymyxin alone by 48 h [25]. Another static time–killing investigation utilising doripenem (8 mg/L), colistin (1 mg/L), gentamicin (2 mg/L) and doxy-cycline (2 mg/L) combinations against 12 KPC-producing *K. pneumoniae* strains at 10^6 CFU/mL identified the combination of doripenem + colistin as superior to other combinations [26]. After 24 h of incubation, doripenem + colistin achieved a >3 log reduction in 9/12 strains and synergistic killing in

6/12 strains (4/6 pan-resistant isolates). The utility of colistin + doripenem against *K. pneumoniae* was further validated in a dynamic one-compartment model that examined doripenem ($C_{\max} = 2.5$ mg/L or 25 mg/L) + colistin (constant at 0.5 mg/L or 2 mg/L) against four isolates with varying degrees of colistin and doripenem resistance ($MIC_{\text{doripenem}} < 0.125$ –8 mg/L; $MIC_{\text{colistin}} = 1$ mg/L to >128 mg/L with two heteroresistant isolates) at 10^6 CFU/mL and 10^8 CFU/mL [27]. At the 10^6 CFU/mL inoculum, co-listin + doripenem achieved synergy in all four strains over 72 h (Fig. 2), whereas the combination resulted in synergistic killing of every isolate except the colistin-resistant strain at the 10^8 CFU/mL inoculum.

Experiments have also been conducted against KPC-producing organisms using polymyxin combinations with tigecycline or rifampicin. A static time–killing investigation of tigecycline ($MIC_{\text{tigecycline}} = 0.5$ –2 mg/L) dual combinations with colistin ($MIC_{\text{colistin}} = 0.5$ –1 mg/L) or meropenem ($MIC_{\text{meropenem}} = 4$ –16 mg/L) against four KPC-producing *K. pneumoniae* at 5×10^5 CFU/mL found that tigecycline + colistin (4 $\times$ their respective MICs) resulted in synergistic killing and a 3 log reduction of all four strains by 24 h [28]. In contrast, a study examining dual and triple combinations of co-listin, tigecycline, rifampicin and other agents against eight colistin-resistant KPC-3-producing *K. pneumoniae* ($MIC_{\text{tigecycline}} = 2$ mg/L for 7/8 strains; $MIC_{\text{rifampicin}} > 32$ mg/L) at 5×10^5 CFU/mL found that colistin (2 mg/L) + rifampicin (2 mg/L) achieved synergy against all eight strains (other dual combinations did not achieve synergy), and the addition of tigecycline (0.125 mg/L) did not substantially improve killing for the majority of the strains [29]. Unfortunately, recent in vivo analyses of colistin + tigecycline in murine models have failed to demonstrate synergy [30] and the combination may even be antagonistic against some KPC-producing *K. pneumoniae* strains [31].

In short, in vitro experiments utilising carbapenems, rifampicin and chloramphenicol were able to demonstrate synergy with polymyxins against *K. pneumoniae* that produced KPC or MBL enzymes and displayed a range of polymyxin susceptibilities. The potential synergy between colistin + fosfomycin or tigecycline varied between studies and may suggest that phenotypic differences between *K. pneumoniae* isolates greatly influence the pharmacodynamics of such regimens. In particular, the poor killing achieved by tigecycline in vivo merits additional investigations as multiple studies were unable to demonstrate synergy.

5. *Acinetobacter baumannii*

Despite concerns over an increased risk of nephrotoxicity, the simultaneous use of a polymyxin and a glycopeptide has received considerable attention as an unorthodox strategy to combat MDR and XDR *A. baumannii*. The combination of colistin (1 mg/L) + teicoplanin (20 mg/L) achieved dramatic synergy (>8 log reduction by 24 h in comparison with colistin alone) against five MDR *A. baumannii* strains at $>10^5$ CFU/mL in static time–killing experiments [32]. Using the same methodology, the combination of colistin (1 mg/L) + vancomycin (20 mg/L) was synergistic by 24 h in all 5 strains (4/5 strains at 48 h) [10], and the dual combinations of colistin (2.5 mg/kg) + teicoplanin (10 mg/kg) and colistin + vancomycin (10 mg/kg) significantly improved survival of *Galleria mellonella* inoculated

with 10^4 CFU/larvae of MDR *A. baumannii* ($P < 0.05$ and $P < 0.001$, respectively) [33]. In a static time–killing investigation that utilised four *A. baumannii* strains ($\text{MIC}_{\text{colistin}} = 1\text{--}8$ mg/L; $\text{MIC}_{\text{vancomycin}} = 128$ mg/L to >128 mg/L) at $10^5\text{--}10^6$ CFU/mL, the combination of colistin + vancomycin ($0.25\times$ and $0.5\times$ their respective MICs) resulted in synergistic killing by 24 h in all four strains [34]. Similarly, synergy with a polymyxin + telavancin at clinically relevant concentrations has recently been observed in vitro [35] and in vivo [36], and there is in vitro evidence supporting the use of a polymyxin + daptomycin as well [37].

The combination of a polymyxin and a carbapenem remains an area of active research for the treatment of MDR and XDR *A. baumannii*. Building upon previous studies, investigators at a single institution noted that 10/11 transplant recipients with *A. baumannii* infections died during treatment with various tigecycline and colistin combinations [7]. In response, static time–killing experiments utilising colistin (0.25 mg/L) + doripenem (8 mg/L) against five XDR *A. baumannii* strains at 10^6 CFU/mL resulted in synergistic killing and eradication by 8 h in all five strains, which prompted the successful treatment of *A. baumannii* infections in four of five additional transplant recipients using colistin (5 mg/kg) + doripenem (500 mg every 8 h). A separate static–time killing investigation of imipenem dual combinations against 12 carbapenem-resistant *A. baumannii* isolates ($\text{MIC}_{\text{colistin}} = 0.125\text{--}4$ mg/L; $\text{MIC}_{\text{imipenem}} = 8\text{--}128$ mg/L) at 5×10^5 CFU/mL identified the combination of colistin and imipenem ($0.5 \times$ their respective MICs) as the most synergistic combination (synergy achieved in 9/12 isolates vs. 6/12 for second best combination) [38]. Another study examined dual and triple combinations of colistin, doripenem and sulbactam against six colistin-resistant *A. baumannii* isolates collected from patients previously treated with colistin methanesulfonate and doripenem, and although colistin + doripenem was synergistic against five isolates, the addition of sulbactam to colistin + doripenem resulted in universal synergy and greater bacterial reductions ($P = 0.04$) [39]. In agreement with the previously discussed studies, a recent meta-analysis of polymyxin time–killing experiments against *A. baumannii* calculated that carbapenems had the highest rate of synergy (80.6%), followed by glycopeptides (70.8%), whereas rifampicin (57.2%) and tigecycline (41.6%) had less favourable rates of synergy [40].

In vitro and in vivo studies have also examined the potential synergy of polymyxins in combination with rifampicin. Using a dynamic one-compartment model, colistin (constant at 2 mg/L) + rifampicin ($C_{\text{max}} = 5$ mg/L) achieved synergistic killing over 72 h against two MDR *A. baumannii* strains (colistin-susceptible and colistin-resistant) at 10^6 CFU/mL and 10^8 CFU/mL inocula [41]. Similarly, an in vivo murine thigh infection model was used to evaluate synergistic killing of colistin (20 mg/kg every 8 h) in combination with various antimicrobials against 12 XDR *A. baumannii* strains [42]. Despite rifampicin MICs of 8 mg/L, colistin + rifampicin (25 mg/kg every 6 h) achieved synergy against all 12 strains at 24 h and 28 h time points, whereas colistin in combination with tigecycline, fosfomycin or sulbactam did not demonstrate synergistic killing.

Lastly, multiple in vitro investigations have evaluated the synergistic killing of a polymyxin with either tigecycline or minocycline. A study in a one-compartment model investigated the combination of PMB ($C_{\text{max}} = 1.16$ mg/L) + tigecycline ($C_{\text{max}} = 1.2$ mg/L) against four MDR *A. baumannii* isolates, which resulted in synergistic killing against one isolate and

additive killing against two other isolates [43]. In static time–killing experiments, colistin + tigecycline (0.5× their respective MICs) achieved synergistic activity by 8 h against 10^6 CFU/mL of an XDR *A. baumannii* strain ($\text{MIC}_{\text{colistin}} = 0.25 \text{ mg/L}$; $\text{MIC}_{\text{tigecycline}} = 1 \text{ mg/L}$); however, colistin (1.25 mg/kg every 6 h) + tigecycline (10 mg/kg every 12 h) did not result in significantly improved killing versus monotherapy in rats inoculated with 1.2 mL/kg of 5×10^8 CFU/mL of the XDR strain in a pneumonia model [44]. In a similar investigation, PMB (0.5 mg/L) + minocycline (8 mg/L) was evaluated in static time–killing experiments against four *A. baumannii* strains with various minocycline susceptibilities ($\text{MIC}_{\text{minocycline}} = 0.25\text{--}16 \text{ mg/L}$) at 106 CFU/mL [45]. Of the four *A. baumannii* strains, only two experienced a ca. 2 log reduction with combination exposure compared with either agent alone by 24 h, whereas one of three clinical strains investigated in a murine pneumonia model displayed a similar reduction in bacterial counts.

The synergistic killing of *A. baumannii* has therefore recently been demonstrated in multiple in vitro studies when a polymyxin was paired with a glycopeptide, a carbapenem or rifampicin. Although the majority of the *A. baumannii* strains were susceptible to polymyxins, synergy was achieved against polymyxin-heteroresistant and -resistant strains as well, suggesting that polymyxins may have clinical utility against *A. baumannii* with elevated polymyxin MICs. Similar to the bacterial killing achieved against *K. pneumoniae* when a polymyxin was paired with tigecycline, the combinatorial pharmacodynamics of the combination varied during in vitro studies and failed to achieve synergy in an in vivo pneumonia model, which may potentially offer insight into reports of poor outcomes following use of the combination in the clinic [46].

6. Clinical studies

Although clinical evidence supporting the use of polymyxin combinations against MDR and XDR Gram-negative pathogens has been scarce, recent studies of *K. pneumoniae* treatment have been published that favour combination treatment. A large retrospective study evaluated the 14-day survival of 661 patients treated for KPC-producing *K. pneumoniae* and found that combination therapy was associated with favourable survival compared with monotherapy [odds ratio (OR) = 0.64, 95% confidence interval (CI) 0.45–0.90, $P = 0.01$; colistin = 39% of monotherapies] [47]. Similarly, a small retrospective cohort study evaluated 34 patients with bacteraemia caused by KPC-producing *K. pneumoniae* and found that 28-day mortality was lower in patients receiving combinations (47% of combinations used colistin or PMB) in comparison with patients receiving monotherapies (13.3% vs. 57.8%; $P = 0.01$) [48]. Another retrospective study of 205 patients treated for carbapenemase-producing *K. pneumoniae* bloodstream infections found that all-cause 28-day mortality was higher in patients who received monotherapy (72 patients total, 22 received colistin alone) than in patients who received combination therapy (44.4% vs. 27.2%; $P = 0.018$) [49]. A systematic review of 20 studies that included patients treated for carbapenemase-producing Enterobacteriaceae posited that a reliable meta-analysis was not possible given the heterogeneity of the studies, but a methodical review of each study led the authors to tentatively claim that combination therapy may benefit critically ill patients with severe *K. pneumoniae* infections [50].

There is also emerging evidence for the survival benefit of polymyxin combination therapy for the treatment of MDR and XDR *A. baumannii*. A retrospective cohort study evaluating 101 patients treated for *A. baumannii* and *P. aeruginosa* infections found that 30-day mortality was lower for patients treated with PMB combinations versus patients receiving PMB monotherapy (42.4% vs. 67.7%; $P = 0.03$) [51]. A smaller retrospective study that analysed the clinical treatments of 17 patients with colistin-resistant *A. baumannii* infections found that the combination of colistin + a carbapenem + ampicillin/ sulbactam resulted in a lower 30-day mortality in comparison with other antibiotic regimens (0/7 deaths vs. 6/10; $P = 0.03$) [6]. A multicentre retrospective study of hospitalised patients who received colistin for MDR Gram-negative bacteria (60% *A. baumannii*, 19% *P. aeruginosa* and 15% *K. pneumoniae*) evaluated the rate of mortality for patients receiving colistin alone or those receiving colistin with a concomitant glycopeptide [52]. Using a sample size of 166 patients with confirmed MDR Gram-negative infections, co-administration of a glycopeptide for 5 days was associated with a favourable 30-day mortality for infections due to MDR Gram-negative bacteria [hazard ratio (HR) = 0.42, 95% CI 0.18–0.93] and due to *A. baumannii* specifically (HR = 0.41, 95% CI 0.17–0.98) in multivariate analyses. Another retrospective evaluation of 291 patients treated with colistin for XDR Gram-negative bacillus infections (78% of patients cultured *A. baumannii*, 21% *P. aeruginosa* and 5% *K. pneumoniae*) found that colistin monotherapy and colistin combinations without guidance from rapid time-killing experiments were eight times and six times more likely to result in infection-related mortality (monotherapy OR = 8.49, 95% CI 1.56–46.05; combinations without guidance OR = 5.75, 95% CI 1.25–25.73), respectively, in comparison with patients who received colistin combinations optimised by multiple-combination bactericidal testing [53]. Lastly, a prospective comparison of 55 patients treated for XDR *A. baumannii* found that the combination of colistin + tigecycline resulted in higher mortality than colistin + a carbapenem when the tigecycline MIC of the causative pathogen was >2 mg/L (HR = 6.93, 95% CI 1.61–29.78; $P = 0.009$), suggesting that caution is needed when developing polymyxin combination regimens [46].

In contrast to the encouraging results previously discussed, many clinical studies have failed to demonstrate superiority of polymyxin combinations over monotherapy. A prospective study by Durante-Mangoni et al enrolled 210 patients to randomly receive colistin or colistin + rifampicin for the treatment of life-threatening XDR *A. baumannii* infections and found that the risk of death within 30 days was not significantly lower during combination treatment (OR = 0.88, 95% CI 0.46–1.69; $P = 0.71$) despite significantly better microbiological cure in patients receiving colistin + rifampicin ($P = 0.034$) [54]. A similar outcome was observed in a retrospective study that evaluated 250 patients treated for XDR *A. baumannii* bacteraemia with colistin combinations or colistin alone; 14-day survival was comparable in both groups (68.2% combination vs. 55.5% monotherapy; $P > 0.05$), whereas microbiological cure (79.9% vs. 55.6%; $P = 0.001$) and in-hospital mortality (52.3% vs. 72.2%; $P = 0.03$) were superior during combination treatment [55]. In a small prospective trial of 94 patients with carbapenem-resistant *A. baumannii*, subjects were randomised to receive colistin alone or colistin + fosfomycin and a statistical difference in the infection-related 28-day mortality was not observed (23.1% vs. 16.3%; $P = 0.507$); however, microbiological cure in the first 72 h (65.7% vs. 78.8%; $P = 0.028$) and at the end of

treatment (84.5% vs. 100%; $P = 0.023$) did favour combination therapy [56]. A meta-analysis that evaluated the clinical response of patients with MDR *A. baumannii* treated with colistin alone or in combination found that colistin combinations did not improve clinical response (OR = 1.37, 95% CI 0.86–2.19; $P = 0.18$), but microbiological response did improve with combination therapy (OR = 2.14, 95% CI 1.48–3.07; $P < 0.0001$) [57]. Another meta-analysis evaluated all-cause mortality in patients treated with colistin or colistin combinations for carbapenem-resistant Gram-negative bacteria and found no mortality benefit for colistin paired with rifampicin ($P = 0.81$), carbapenems ($P = 0.91$), tigecycline ($P = 0.77$), sulbactam ($P = 0.81$) or aminoglycosides ($P = 0.07$) [58].

Despite the large amount of clinical literature surrounding the use of a polymyxin alone or in combination, severe limitations in many of the study designs have obscured the interpretation of such studies and may explain the apparent discordance in study results. For example, the investigation by Durante-Mangoni et al [54] has been criticised for not accounting for the addition of a carbapenem or tigecycline to the ‘colistin alone’ arm, and also failing to evaluate whether microbiological cure impacted treatment duration or recurrence [59,60]. Many of the clinical studies that evaluated polymyxin combinations to date utilised small sample sizes, and numerous retrospective analyses are confounded by biases such as sicker or unresponsive patients receiving combination therapy.

7. Conclusions

The proliferation of MDR and XDR Gram-negative pathogens has forced clinicians to revisit the use of the polymyxin drug class. In a desire to improve clinical outcomes with polymyxin therapy, the medical community has investigated the use of polymyxin combinations. An abundance of recent in vitro and preclinical in vivo studies has identified various polymyxin combinations that demonstrated synergistic killing against MDR and XDR *P. aeruginosa*, *K. pneumoniae* and *A. baumannii*. However, the utility of polymyxin combinations in the clinical setting has been obscured by conflicting studies that are typically limited by small sample sizes and retrospective study designs. To address the ambiguity surrounding the use of polymyxin combinations, two large, prospective, randomised clinical trials comparing colistin alone to colistin + meropenem are currently underway in Europe and the USA (ClinicalTrials.gov IDs NCT01732250 and NCT01597973). Both trials will provide valuable insight into the potential benefits of colistin in combination with a carbapenem for MDR and XDR Gram-negative pathogens, but additional prospective trials may be needed to assess synergy between polymyxins and other agents for specific pathogens.

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Fig. 1.

Viable counts from a 240-h hollow-fibre infection model that examined the killing of colistin-heteroresistant *Pseudomonas aeruginosa* strain FADDI PA033 [colistin minimum inhibitory concentration (MIC) = 1 mg/L; doripenem MIC = 0.5 mg/L] at 10^9 CFU/mL [20]. Investigated antibiotic regimens included: (a) growth control; (b) colistin as a continuous infusion that achieved a constant free steady-state concentration (fC_{ss}) of 2 mg/L; (c) colistin given as a continuous infusion with a fC_{ss} of 5 mg/L; (d) doripenem given as a bolus dose with a free peak concentration (fC_{max}) of 25 mg/L every 8 h (q8h) with a simulated half-life of 1.5 h; (e) colistin given as a continuous infusion with a fC_{ss} of 2 mg/L and doripenem given as a bolus dose with fC_{max} of 25 mg/L q8h; and (f) colistin given as a continuous infusion with a fC_{ss} of 5 mg/L and doripenem given as a bolus dose with a fC_{max} of 25 mg/L q8h. In the key, the total population represents viable counts on antibiotic-free agar plates, and colistin concentrations represent the viable counts on colistin-containing agar plates. Symbols represent the observed counts and lines are the fits based on mathematical modelling. Reproduced with permission from Tsuji et al [20].

Fig. 2.

Time-killing experiments utilising a one-compartment model over 72 h that investigated the killing of a colistin-susceptible doripenem-resistant *Klebsiella pneumoniae* strain (left) or a colistin-resistant doripenem-susceptible *K. pneumoniae* strain (right) at 10^6 CFU/mL [27]. Simulated antibiotic regimens included constant colistin (Col) concentrations of 0.5, 2 or 5 mg/L, dynamic doripenem (Dor) regimens with a peak concentration ($C_{\max}$) of 2.5, 25 or 50 mg/L (bolus dosing every 8 h; half-life = 1.5 h) and combinations of doripenem and colistin. Figure adapted with permission from Nation et al [27].