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Polymyxins combined with other antibiotics for the treatment of multi-resistant Gram negative bacteria: review of the literature

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Abstract:

Background and aims: The emergence of infections due to multi resistant Gram negative bacteria is a major public-health concern worldwide. Polymyxins (colistin and polymyxin B) are among the last available options for the treatment of these infections. However, when used in monotherapy, the development of polymyxin resistant strains and high mortality rates of patients has been observed. Combination of polymyxins with other antibiotic drug classes may lead to synergism enhancing its bactericidal effect and reducing the emergence of resistant strains. This review aims to analyze the main in vitro and clinical studies assessing the potential benefit of combination therapy with this class of drugs.

Methods: We searched on PUBMED database for the mesh terms: "polymyxins", "polymyxin B", "colistin", "combination therapy" and their combinations. Articles published in the last 10 years (2005-2015), in English language, evaluating combination therapy of other antimicrobial classes with polymyxins were included for analyses. Results: Thirty-one articles were selected for review. In vitro studies evaluated combinations with beta-lactams, tigecycline, rifampicin, quinolones, chloramfenicol, vancomycin and daptomycin. Clinical studies evaluated the benefit of different combinations in the treatment of *Klebsiella pneumoniae*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* infections.

Conclusion: Polymyxin combinations regimens seem potentially advantageous in the treatment of multi-resistant Gram-negative bacteria. Combination with beta-lactams, especially carbapenems, were the most studied and yielded positive results. However, data evaluating its benefit are mostly from in vitro and observational studies. Results are not yet conclusive and randomized clinical trials accessing this strategy are urgently needed.

Key-Words: polymyxins, polymyxin B, colistin, combination therapy.

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INTRODUCTION

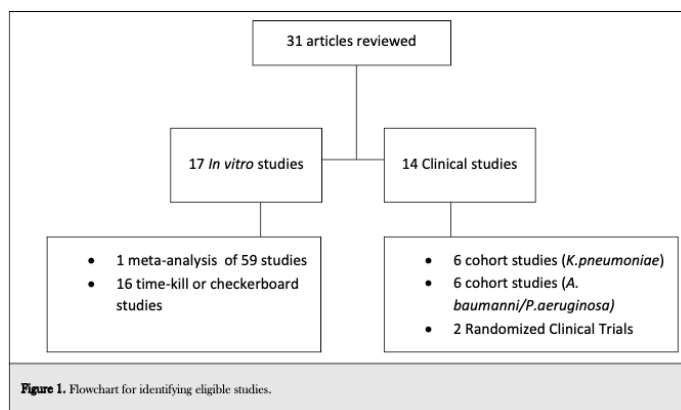
Polymyxins (colistin and polymyxin B) are one of the last available options for the treatment of multi-resistant Gram negative bacterial infections. However, mortality rates ranging from 47 to 67.6% have been reported in patients treated with polymyxins in monotherapy. (Elias,2010; Kvitko,2011; Tuon,2014; Rigatto,2015) Besides these high mortality rates, growing incidence of resistance to polymyxins is a current public health concern worldwide. In infections due to *Klebsiella pneumoniae*, resistance rates to polymyxins in up to 43% of the isolates have been reported. (Monaco, 2014) Pan-resistant bacteria have been isolated with growing frequency, imposing an important therapeutic challenge.

(Bialvaei, 2015) Combination treatment may lead to synergism between antibiotics enhancing its bactericidal effect and reducing the emergence of resistant strains. (Zusman, 2013) Another possible benefit of this strategy is to allow the use lower dose of each antibiotic, reducing adverse effects. In vitro and clinical studies have evaluated the benefit of combining polymyxins with many other antibiotic classes with conflicting results. This review aims to summarize the main studies assessing the potential benefit of combination therapy.

METHODS

Literature search

This manuscript is an updated version adapted from a PhD thesis published at <http://hdl.handle.net/10183/119424>. We searched on PUBMED database- for the mesh terms: “polymyxins”, “polymyxin B”, “colistin”, “combination therapy” and their combinations. Articles published in the last 10 years (2005-2015)- in English language- were selected for analysis if they evaluated combination therapy of other antimicrobial classes with polymyxins. In clinical studies, only the ones that evaluated intravenous use of polymyxins were reviewed. The searched publications were not limited by number of citations or impact factor. The revised manuscripts were divided according to Figure 1.



Definitions

Bactericidal effect and classification of combination as synergic, antagonistic and indifferent, in the in vitro studies, was defined through two main methods: time-kill and Checkerboard.

In time-kill studies, synergism is defined as 2-log reduction in bacterial colony forming units (CFU) when exposed to combination therapy compared to the bacterial activity of the most active antimicrobial alone. Bactericidal activity is defined as 3-log reduction in CFU when exposed to combined therapy compared to the most active agent alone. Antagonism is defined as 2-log increase in CFU after exposure to combination therapy. Bactericidal effect is evaluated after 24 hours of antibiotic exposure. (Zusman, 2013)

In Checkerboard method the minimal inhibitory concentrations (MIC) of each drug combined is added and divided by the MIC of each drug alone. An index $\leq 0,5$ is considered synergism, $>0,5$ and ≤ 1 additive, >1 indifferent e > 4 antagonistic. (Zusman, 2013) achieved combining polymyxins with other antibiotic classes. Time-kill and checkerboard are the most used methods to evaluate synergy in in vitro models, however they do not account for different drug infusion regimens and total body distribution, which might greatly affect bactericidal

capacity. Hollo-fiber models can better simulate these different scenarios and have also shown positive results with combination therapy. However, replicating these findings in a clinical setting is a major challenge considering the complexity of biological systems and the role of immunity, bacteria inoculum and antibiotic distribution and concentrations in different infection sites. Clinical studies are therefore fundamental to evaluate the benefit of these strategies.

RESULTS AND DISCUSSION

In vitro studies

The most studied combination is of polymyxins with beta-lactams, mostly carbapenems. A meta-analysis of 59 studies showed benefit of combination in every bacteria species studied (*Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*). The greater effect was shown in *A. baumannii* species in which synergy occurred in 77% (95%Confidence Interval [CI] 64-87%) of the samples tested, with an increase in bactericidal rate of 26% (95%CI 12-47%) to 74% (95%CI 58-85%) with combination therapy. (Zusman, 2013)

Most studies evaluated colistin, but the few ones that evaluated polymyxin B retrieved fairly similar results. Synergistic effect remained in polymyxin susceptible and carbapenem resistant strains, with rates ranging from 55-71%. Among carbapenems, doripenem had the higher synergistic effect overall, followed by meropenem and imipenem. As an exception, in *P. aeruginosa* strains, imipenem showed greater activity than meropenem. When isolates were resistant to both polymyxins and carbapenems, combination therapy resulted in an increase of bactericidal activity from 14% (95%CI 7-27%) to 43% (95%CI 21-68%). Synergy was evaluated through chekerboard method in 23 studies. A synergy index < 1 was found in 71% and 29% of *A. baumannii* and *P.aeruginosa* strains, respectively. (Zusman, 2013) Hollow-fiber infection models over 10 days simulated colistin and doripenem treatments against two heteroresistant and one resistant *P. aeruginosa* strains. Combinations in various regimens showed greater bactericidal effect in up to 9.38 log₁₀ compared to monotherapy. In the resistant strain, combination led to 6.11 log₁₀ CFU/mL greater bactericidal effect, but regrowth was observed after 72 hours. (Ly, 2015)

Besides increasing bactericidal rates, reducing resistance development during polymyxin therapy was another advantage of combining antibiotics. (Zusman, 2013; Ly, 2015) In biofilms caused by *P.aeruginosa*, the combination of colistin and doripenem resulted in

suppression of resistant strains and synergistic effect that remained for more than 72 hours. (Lora-Tamayo, 2014)

Combining polymyxins with beta-lactams other than carbapenems resulted in much lower synergistic rates: cefoperazone/sulbactam (2 of 50 samples), piperacilin/tazobactan (1 of 50 samples). (Karaoglan, 2013) Decrease in the initial MIC was seen for polymyxins and/or beta-lactam when they were combined in up to 35.29% of associations with ceftazidime, 41% with cefepime and 35.29% with piperacilin. Although MIC decrease was seen with the 3 beta-lactams evaluated, in none of the combinations an index ≤ 0.5 was achieved. (Mitsugui, 2011)

Besides beta-lactams, other antibiotic classes have been studied in combination with polymyxins. The main findings are summarized in Table 1.

In vitro studies show, in general, very promising results regarding combination therapy with polymyxins

in many aspects: higher bactericidal rates, MIC reductions- eventually allowing antibiotic dose reductions to less toxic levels, suppression of resistant strains and regrowth inhibition. More consistent evidence is found with carbapenem combinations, but favorable results were also achieved combining polymyxins with other antibiotic classes. Time-kill and checkerboard are the most used methods to evaluate synergy in in vitro models, however they do not account for different drug infusion regimens and total body distribution, which might greatly affect bactericidal capacity. Hollo-fiber models can better simulate these different scenarios and have also shown positive results with combination therapy. However, replicating these findings in a clinical setting is a major challenge considering the complexity of biological systems and the role of immunity, bacteria inoculum and antibiotic distribution and concentrations in different infection

Table 1. Main results from *in vitro* studies of antibiotics (other than beta-lactams) combined to polymyxins

Antibiotic	Bacteria tested	Main results
Tigecycline	<i>A.baumannii</i>	- Reduction of bacterial inoculum when compared to either drugs alone (3.31±0.71, P<0.001). Suppression of bacterial regrowth curve with tigecycline in doses equivalent to 200mg twice daily. No benefit was seen with lower doses of tigecycline. (Hagihara, 2014) - Synergism rates of 12-29% with this association (Karaoglan, 2013; Lim, 2011; Ni, 2013)
Rifampicin	<i>A.baumannii</i> <i>K.pneumoniae</i>	- Synergic in 13 of 31 isolates (41.9%). (Lim,2011) - Dose dependent increase in bactericidal rates than either drug in monotherapy in high and low bacterial inoculum and also with colistin resistant strains. Emergence of colistin resistance was suppressed. (Lee, 2013) - Antagonism in <i>A.baumannii</i> strains with the combination of polymyxin and rifampicin.(Nageeb, 2015) - Colistin resistant <i>K. pneumoniae</i> KPC- producing strains had their MIC to colistin reduced to susceptibility ranges (≤ 2 mg/L) in 7 of 13 strains tested after the combination with rifampicin. (Tascini, 2013)
Levofloxacin	<i>A. baumannii</i> <i>P. aeruginosa</i>	- Synergistic effect in 90% of <i>P.aeruginosa</i> and 84.8% <i>A. baumannii</i> isolates after 4 and 6 hours of growth in vitro, respectively, regardless of the initial MIC to colistin or levofloxacin. (Safarika, 2015)
Chloramphenicol	<i>K.pneumoniae</i>	- There was a delay in regrowth, synergy in 25 of 28 cases and no emergence of resistant strains.(Abdul Rahim, 2015)
Vancomycin	<i>A.baumannii</i>	- Synergistic effect in 4 of 6 strains. All 34 isolates tested had MIC>256 mg/L when in monotherapy with vancomycin, and reduced to 0.016-48 mg/L when combined to colistin. Regrowth occurred in all samples after 4hours when exposed to colistin alone- the combination inhibited regrowth in 48hours in all but one sample. (Gordon, 2010) - Synergy with an index of ≤ 0.5 was seen in 3 colistin resistant strains and complete bactericidal activity in two of them.(O'Hara, 2013)
Daptomycin	<i>A. baumannii</i>	- Benefit was shown in colistin-susceptible strains only. Daptomycin MIC decreased in 4-128 times and synergistic effect was demonstrated in 16(53.3%) of the isolates in 24hours. Bactericidal activity with colistin alone occurred in 2 samples, whereas it was seen in 9 samples when added colistin. (Galani, 2014)

sites. Clinical studies are therefore fundamental to evaluate the benefit of these strategies.

Clinical studies

In a case series of 18 patients with infections caused by *Klebsiella pneumoniae* carbapenemase (KPC)-producing *K. pneumoniae*, the ones who received combined therapy with polymyxin B (mostly tygecycline) had better survival than the ones treated with monotherapy, 73% versus 14%, respectively (Hisch, 2010). In a study that evaluated 35 patients with *K. pneumoniae* bacteremia treated with in vitro susceptible antibiotics, there were no deaths in the group of 9 patients treated with colistin and tygecycline, while 7 of 15 (46.7%) died in the monotherapy group ($P=0.001$). In the later, mortality rates were similar to the group of patients who received in vitro non-susceptible therapy and emergence of resistant strains were identified.(Zarkotou, 2011) Another study with 41 patients with bacteremia by KPC-producer *K. pneumoniae* showed that in the 28-day survival group 13 of 25 (52%) patients had received combined therapy compared to 2 of 10 (12.5%) in the non-survival group ($P=0.001$). Most of the combinations were polymyxins and carbapenems.(Qureshi, 2012) In a retrospective cohort of 125 patients with KPC- *K. pneumoniae* the mortality rate was 41.6%: 25/46 (54.3%) versus 27/79 (34.1%), in monotherapy and combined therapy groups, respectively ($P=0.002$). The most common combinations were colistin and tygecycline (23 patients) and colistin, tygecycline and meropenem (16 patients). In a multivariate analysis triple therapy was independently protective (Odds Ratio [OR]0.11; 95%CI 0.02–0.69, $P=0.01$). When analyzing only combinations with meropenem, best results were seen with $MIC \leq 4$ mg/L, although the benefit of combination remained even with $MIC \geq 16$ mg/L to this drug.(Tumbarello, 2012) Finally, in a cohort of 205 patients with bloodstream infections due to *K. pneumoniae* (79.5% KPC-producing strains) mortality was lower in combination therapy than in monotherapy group: 27.2% versus 44.4%, respectively ($P=0.018$). Monotherapy remained as an independent risk factor for mortality (adjusted Hazard ratio (aHR)2.08, CI95%1.23-3.51, $P=0.006$). The lowest mortality rates were with carbapenem combination regimens, especially with lower MIC to this drug: 19.3% vs 35.5% with $MIC \leq 8$ mg/L and >8 mg/L, respectively.(Daikos, 2014) Contrasting with these data, in a recent study that evaluated 118 patients with infections due to KPC- *K. pneumoniae* (78 bacteremias)- where monotherapy was used in 57 patients and combined therapy in 61 patients- there was no statistically significant difference between

groups regarding mortality, after adjustment in a multivariate model.(de Oliveira, 2015)

The effect of combined therapy in the treatment of non-fermentative Gram negative bacteria (*A.baumannii* and *P.aeruginosa*) was also evaluated in clinical studies. A randomized clinical trial included 43 patients with ventilator associated pneumonia due to XDR-*A.baumannii* that were treated either with colistin in monotherapy or combined with rifampicin 600mg/day. In the monotherapy group 14 of 22 (63.6%) patients died compared to 8 of 21 (38.1%) patients in the combined therapy group ($P=0.171$). Microbiological eradication was higher in patients that received combined therapy ($P=0.029$). (Aydemir, 2013) Another randomized clinical trial evaluated the same combination including a larger sample in order to increase the study power. Two hundred and ten critically ill patients were analyzed and no statistically significant difference was found in mortality between colistin in monotherapy group versus rifampicin combined therapy group ($P=0.97$). As shown in the previous study, microbiological eradication was higher in the combined therapy group ($P=0.034$). (Durante-Mangoni, 2013)

Other combinations were also evaluated in the treatment of *A.baumannii* infections. A retrospective multicenter study included 36 patients that received colistin in monotherapy and 214 that were treated with combination therapy- most frequently with carbapenems (47.7%). Fourteen-day mortality was comparable between groups in univariate and multivariate analyzes and also after adjustment for a propensity score. The combined therapy group had higher microbiological eradication and lower in-hospital mortality in univariate analysis (52.3% versus 72.2%; $P=0.03$). There was no significant difference in mortality between the combinations: colistin and carbapenem, colistin and sulbactam, colistin and other. (Batirel, 2014) Another study including 101 patients with *A.baumannii* sepsis, in which 68 (67.3%) received monotherapy (with colistin or carbapenem) and 33 (32.7%) combined therapy (most frequently colistin and tygecyclin) also showed no difference in 30-day mortality between groups. In this study patients were classified as receiving combined therapy only when there was in vitro susceptibility to both agents or $MIC < 32$ mg/L for carbapenems - all other patients were considered as receiving monotherapy. (Lopez-Cortes, 2014)

A recent cohort was the first to show benefit of combination therapy in non-fermentative Gram negative bacteria. One-hundred and one critically ill patients with infections due to XDR- *A.baumannii* or *P.aeruginosa* treated either with polymyxin B alone or combined to an

in vitro resistant antibiotic. Thirty-day mortality was 59.4% (60 patients): 42.4% (14 of 33) in combination and 67.6% (46 of 68) in monotherapy groups ($P=0.03$). The mortality protection of combination therapy remained significant in a multivariate model (aHR0.33; 95% CI 0.17- 0.64, $P = 0.001$) along with creatinine clearance of ≥ 60 ml/min, while a higher acute physiology and chronic health evaluation (APACHE II) score and polymicrobial infection were associated with increased mortality. The adjustment with a propensity score did not change the results. Subgroup analysis of combinations with β -lactam or only with carbapenem (mostly meropenem), *A. baumannii* infections and lower respiratory tract infections also showed benefit of combination therapy. (Rigatto, 2015) Another observational study compared the most effective combination regimen in 55 patients with *A. baumannii* bacteremia: colistin-tigecycline or colistin-carbapenem. Fourteen-day mortality was higher in patients in colistin- tigecycline group 35% versus 15% ($P=0.105$). In the subgroup of patients with tigecycline MIC >2 mg/L, the combination with this drug was independently associated with higher 14-day mortality (HR 6.93; 95%CI 1.61-29.78, $P=0.009$). Breakthrough of XDR- *A. baumannii* bacteremia occurred in 18% of patients that received combination with tigecycline and in none of the carbapenem group ($P=0.059$). (Cheng, 2015) The combination of glycopeptides and colistin was evaluated in 2 retrospective studies.(Garnacho-Montero, 2013; Petrosillo,2014) In only one of these studies an independent protective effect of combination was found in patients that survived for ≥ 5 days after the beginning of treatment.(Petrosillo,2014)

Clinical studies assessing combination therapy were observational in all but 2 studies, with prescription bias, imbalance of confounding variables between groups and variability of primary outcomes being important limitations for definitive conclusions. Heterogeneity in the definition of combination therapy is also a major issue when comparing these results. Different drug combinations, lack to report time to begin combination and/or duration of combined regimens affects data interpretation. The combination with in vitro susceptible agents also should be cautiously analyzed, once the benefit may arise of adding a more active drug in the therapeutic scheme rather than of synergy between drugs. Nevertheless, clinical data reviewed in this article systematically points toward benefit of combination especially in KPC-K .pneumoniae infections, with recent studies also suggesting this benefit in non- fermentative Gram- negative bacteria.

CONCLUSION

In times of increasing pan-drug resistance and few therapeutic options to treat severe infections by XDR-Gram negative bacteria, polymyxin combination regimens seems as potentially advantageous in the treatment of these patients. However, data evaluating its benefit are not yet conclusive. Randomized clinical trials assessing different antibiotic combinations in infections by Enterobacteriaceae and non-fermentative Gram negative bacteria are urgently needed.

Conflict of interest and financial disclosure

The authors followed the International Committee or Journal of Medical Journals Editors (ICMJE) form for disclosure of potential conflicts of interest. All listed authors concur with the submission of the manuscript, the final version has been approved by all authors. The authors have no financial or personal conflicts of interest.

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