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Review

In vitro synergy of polymyxins with other antibiotics for *Acinetobacter baumannii*: A systematic review and meta-analysis

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ABSTRACT

In order to provide preliminary guidance for rational antibiotic combination therapy in the clinic, a systematic review and meta-analysis was performed to evaluate the in vitro synergistic activity of polymyxins combined with other antibiotics against *Acinetobacter baumannii*. An extensive literature search was undertaken without restriction according to region, publication type or language. All available in vitro synergy tests on antibiotic combinations consisting of polymyxins were included. The primary outcome assessed was the in vitro activity of combination therapy on bacterial kill or inhibition. In total, 70 published studies and 31 conference proceedings reporting testing of polymyxins in combination with 11 classes consisting of 28 antibiotic types against 1484 *A. baumannii* strains were included in the analysis. In time–kill studies, high in vitro synergy and bactericidal activity were found for polymyxins combined with several antibiotic classes such as carbapenems and glycopeptides. Carbapenems or rifampicin combination could efficiently suppress the development of colistin resistance and displayed a >50% synergy rate against colistin-resistant strains. Synergy rates of chequerboard microdilution and Etest methods in most antibiotic combinations were generally lower than those of time-kill assays. The benefits of these antibiotic combinations should be further demonstrated by well-designed clinical studies.

1. Introduction

Acinetobacter baumannii is an opportunistic bacterial pathogen that can be easily found in many healthcare environments. Before the 1970s, *A. baumannii* was susceptible to most traditional antibiotics such as broad-spectrum β-lactams, cephalosporins and tetracyclines [1]. Nevertheless, because of its excellent environmental resilience and remarkable ability to develop resistance, *A. baumannii* has become one of the notorious superbugs in recent years [2]. Outbreaks of serious nosocomial infections caused by multidrug-resistant (MDR) *A. baumannii* have been continuously reported from hospitals worldwide, resulting in high mortality rates and bed-day costs [3].

The lack of new potent agents against MDR Gram-negative bacteria has forced clinicians to re-introduce polymyxins, a group of polypeptide antibiotics that was discovered in the 1940s [4]. The polymyxins consist of five chemical compounds (A–E), but only polymyxin E (colistin) and polymyxin B are currently available on the market. Lots of in vitro susceptibility studies show that polymyxins have potent antibacterial activity against MDR *A. baumannii* through disorganising its outer membrane [4]. However, dosing-related nephrotoxicity and neurotoxicity limit its wider clinical application, and the increased usage has led to the emergence of resistant and heteroresistant isolates [5]. Therefore, to improve clinical treatment success and to restrict the emergence of resistance, combination therapies based on polymyxins have been proposed as good options for treating MDR *A. baumannii* infections [6].

In vitro synergy studies can provide preliminary guidance for rational drug combination use in the clinic. A number of in vitro tests have been performed on polymyxins in combination with other antibiotics against *A. baumannii*, yielding various results. The heterogeneity in these tests is likely to arise through the limited number of strains, susceptibility differences, testing methods and clonal diversity of strains among different hospitals and laboratories [7]. To determine which antibiotic combinations might be suitable options to treat MDR *A. baumannii* infections, we

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systematically searched and analysed the literature to evaluate the in vitro synergistic activity of polymyxins with other antibiotics against *A. baumannii*.

2. Materials and methods

2.1. Search strategy

A literature search was performed in July 2014 by two separate reviewers, without restriction according to region, publication type or language. Primary sources were the electronic databases PubMed and Embase. To reduce publication bias, Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), Infectious Diseases Society of America (IDSA) and European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) conference proceedings for the years 2006–2013 were also reviewed. Keywords and Boolean operators used for searches were (colistin OR colistimethate OR polymyxin) AND (*Acinetobacter baumannii* OR *baumannii* OR *A. baumannii*) AND (in vitro OR combination OR chequerboard OR time-kill OR Etest OR microdilution OR agar dilution OR susceptibility). No special search features were used. The related articles function was also used to broaden the search, and the reference lists of the retrieved articles were reviewed for additional studies. When multiple reports describing the same strain population were published, the most recent or complete report was used.

2.2. Inclusion and exclusion criteria

All available in vitro synergy tests of antibiotic combinations consisting of polymyxins were included in this study. Studies using non-traditional testing methods [except for the chequerboard method, Etest and the time-kill assay, which included both the static time-kill and in vitro dynamic pharmacokinetic/pharmacodynamic (PK/PD) model], those testing polymyxins in combination with agents or compounds that are not available on the market worldwide, and those examining combinations with three or more drugs were excluded.

2.3. Outcome measurements

The primary outcome was the in vitro activity of combination therapy on bacterial kill or inhibition. With time-kill assays, synergy for the combination was defined as >2 $\log_{10}$ CFU/mL decrease in comparison with that by the most active constituent of the combined antibiotics, and antagonism was defined as >2 $\log_{10}$ CFU/mL increase. For the chequerboard method and Etest, the fractional inhibitory concentration index (FICI) was calculated with the following formula: FICI = (MIC_{AB}/MIC_A) + (MIC_{BA}/MIC_B), where MIC_{AB} is the minimum inhibitory concentration (MIC) of drug A tested in combination, MIC_A is the MIC of drug A tested alone, MIC_{BA} is the MIC of drug B tested in combination and MIC_B is the MIC of drug B tested alone. Synergy was defined as a FICI ≤0.5, indifference as a FICI between >0.5 and 4 and antagonism as a FICI > 4. The secondary outcomes were bactericidal activity, defined as >3 $\log_{10}$ CFU/mL reduction in the colony count relative to the initial inoculum, and the effect of combination therapy on resistance development.

2.4. Data extraction

For the analysis, the following data were independently extracted by two reviewers: (i) author identification; (ii) year of publication; (iii) synergy testing method; (iv) type of antibiotic(s) used; (v) number of isolates tested; and (vi) MICs of isolates for polymyxins. The breakpoints for polymyxins were those recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST): susceptible, ≤2 mg/L; and resistant, ≥4 mg/L.

2.5. Quantitative data synthesis

All statistical analyses were performed with Comprehensive Meta-Analysis v.2.2 (Biostat Inc., Englewood, NJ). The event rate (synergy rate) with 95% confidence interval (CI) was calculated for each study, and various pooled event rates were calculated both by the fixed-effects model and random-effects model. The I^2 test was used to assess heterogeneity, where I^2 values of 0% indicate no observed heterogeneity whereas larger values indicate increasing heterogeneity. Results of the fixed-effects model are quoted unless substantial heterogeneity is present, in which cases results of the random-effects model are stated [8].

In the statistical analyses, groups were divided by synergy testing method and the classes of antibiotics that polymyxins were combined with. For studies using more than one testing method, the results of different methods were separately collected and analysed in different groups. In each group, the results were subgrouped by antibiotic type and resistance to polymyxins. In time-kill studies performing multiple tests on the same bacterial population and the same antibiotic combination, the one that used a more common bacterial load or clinically achievable drug concentration was chosen.

3. Results

In total, 859 potentially relevant studies were initially identified by the PubMed and Embase searches (Fig. 1). Most of these studies were excluded as they did not report any in vitro tests assessing the synergy of polymyxin combination therapies for *A. baumannii*. Finally, 70 published studies and 31 conference proceedings fulfilled the pre-determined inclusion criteria and were included in the analysis. Table 1 summarises the main characteristics of each included study. In total, 105 time-kill assays, 77 chequerboard microdilution tests and 33 Etests were performed, testing polymyxins in combination with 11 classes consisting of 28 antibiotic types against 1484 *A. baumannii* strains.

3.1. Time-kill data synthesis

For polymyxin–carbapenem combinations (Fig. 2), pooling data from 273 strains in 26 studies showed that the synergy rate was 80.6% (95% CI 64.2–90.6%); 2 isolates showed antagonism, with a rate of 7.1% (95% CI 4.4–11.4%). The rate of bactericidal activity for 170 isolates increased from 26.2% (95% CI 18.6–35.5%) for the most active single agent to 71.8% (95% CI 63.3–79.0%) in combinations. Heterogeneity (I^2) for these studies was 53.4%. The synergy rate of combinations with colistin was 84.9% (95% CI 74.6–91.5%), which was higher than that of polymyxin B combinations (63.4%, 95% CI 37.8–83.2%). Meropenem and doripenem showed synergy rates of 85.2% (95% CI 68.3–93.9%) and 86.6% (95% CI 70.3–94.7%), respectively, whilst imipenem displayed a synergy rate of 66.8% (95% CI 44.2–83.7%). When examining polymyxin-resistant strains (nine studies on 58 isolates), the synergy rate was 79.8% (95% CI 63.2–90.1%), similar to that of polymyxin-susceptible strains.

For polymyxin–rifampicin combinations (Fig. 3), 22 studies tested 280 isolates and yielded a synergy rate of 57.2% (95% CI 50.5–63.6%), whilst 1 isolate was antagonistic, with an antagonism rate of 6.2% (95% CI 3.7–10.4%). Rates of bactericidal activity for 153 isolates increased from 26.4% (95% CI 10.2–53.1%) for the best single agent to 86.7% (95% CI 73.2–94%) in combinations. Heterogeneity (I^2) for these studies was 44.3%. The synergy rate in combinations