Synergy effect of meropenem-based combinations against *Acinetobacter baumannii*: a systematic review and meta-analysis

Zhihui Jiang,^{1,2,*}
Xianxia He,^{3,*}
Jian Li²

¹Institute of Traditional Chinese Medicine and Natural Products, College of Pharmacy, Jinan University, Guangzhou, China; ²Department of Pharmacy, Guangzhou General Hospital of Guangzhou Military Command, Guangzhou, China; ³Department of Drug Certification, Center for Certification and Evaluation, Guangzhou Food and Drug Administration, Guangzhou, China

*These authors contributed equally to this work

Purpose: The main objective of our meta-analysis was to examine the in vitro synergistic effect of meropenem-based combination therapies against *Acinetobacter baumannii* through a systematic review of the existing literature.

Methods: An extensive search was performed with no restrictions on date of publication, language, and publication type. Our study evaluated the main conclusions drawn from various studies describing the synergistic activity of combination therapies in vitro.

Results: In this review, 56 published studies were included. Our report included data on 20 types of antibiotics combined with meropenem in 1,228 *Acinetobacter baumannii* isolates. In time-kill studies, meropenem combined with polymyxin B and rifampicin showed synergy rates of 98.3% (95% CI, 83.7%–100.0%) and 89.4% (95% CI, 57.2%–100.0%), respectively, for *Acinetobacter baumannii*, modest synergy rates were found for meropenem combined with several antibiotics such as colistin and sulbactam, and no synergy effect was displayed in the combination of meropenem and ciprofloxacin, whereas in checkerboard method, the synergy rates of polymyxin B and rifampicin were 37.0% (95% CI, 0.00%–100.0%) and 56.3% (95% CI, 8.7%–97.8%), respectively.

Conclusion: We found that time-kill studies generally identified the greatest synergy, while checkerboard and Etest methods yielded relatively poor synergy rates. Further well-designed in vivo studies should be carried out to confirm these findings.

Keywords: *Acinetobacter baumannii*, meropenem, synergy, combination, in vitro

Introduction

The spread of *Acinetobacter baumannii* (*A. baumannii*), a major pathogen responsible for hospital infections, is difficult to control and its infections are difficult to treat owing to its ability to adapt to different environments and its intrinsic resistance to many antibiotics.¹ Furthermore, an increasing number of multi-drug-resistant (MDR), even pan-drug-resistant *A. baumannii* strains have been isolated.² Infections caused by MDR *A. baumannii* primarily occur in immunosuppressed patients, in patients with serious underlying diseases, and in those who underwent invasive procedures and treatment with broad-spectrum antibiotics.³ Meropenem, as a carbapenem antibiotic, has a low-toxicity profile and is highly resistant to serine β -lactamases produced by many MDR gram-negative bacteria, thus playing a key role in the treatment of various infections that are not readily treated by other antibiotics.⁴ However, reports regarding the yearly increase in meropenem-resistant *A. baumannii* strains have captured the attention of clinicians and microbiologists.⁵ A great focus has been placed on combination therapies to reduce *A. baumannii* resistance, and numerous experiments have been performed with

Correspondence: Jian Li
Department of Pharmacy, Guangzhou General Hospital of Guangzhou Military Command, No. 111 Liuhua Road, Yuexiu District, Guangzhou 510010, China
Tel/Fax +86 20 366 52216
Email jzhxbox1@163.com

meropenem-based therapies.⁶ However, systematic analysis of meropenem-based combination therapies is still lacking. To provide a basis and reference for clinical rationales behind combination therapies using meropenem, we systematically searched and analyzed three mainstream databases to evaluate the in vitro synergistic activity of meropenem with other antibiotics against *A. baumannii*.

Materials and methods

Search strategy and selection criteria

A broad literature search on the PubMed, Embase, and Web of Science databases was performed throughout March 2018 by two separate reviewers. Journal articles were selected without limitations on publication date, language, or publication type. Keywords and Boolean operators used for the searches were (meropenem) AND (*baumannii*) AND (synerg* OR combin*). We also used the related articles function to broaden our search. In addition, reference lists of the selected articles were also manually examined to find relevant studies that were not included in our initial searches.

All meropenem-based in vitro combinations tests (application of traditional testing methods including the checkerboard, Etest, and the time-kill method, which included both the static time-kill and the in vitro dynamic pharmacokinetic/pharmacodynamic model) were included in this study. Those testing meropenem in combination with compounds that are not available on the market worldwide, and the combination therapies with more than two drugs were excluded.

Data collection and statistical analysis

In this meta-analysis, data were independently extracted by two researchers using a premade data extraction form. From each study, the following information was extracted: 1) first author and the publication year; 2) susceptibility to meropenem; 3) types of antibiotics used; 4) total number of isolates tested; 5) in vitro combination testing methods. Antibiotic breakpoints were set based on the Clinical and Laboratory Standards Institute. Meropenem susceptibility and resistance were defined: susceptible, ≤ 2 mg/L; intermediate, 4 mg/L; resistant, ≥ 8 mg/L.

The outcome analyzed in the study was the in vitro synergy activity of combination therapy. For time-kill tests, synergy was defined as $\geq 2 \log_{10}$ (at any time points in 24 hours) colony-forming units/mL decrease between the combination therapy and the most efficient agent, when used alone. With the checkerboard and Etest method, interactions were defined by the fractional inhibitory concentration index (FICI), which was calculated using the following formula: $FICI = (MIC_{AB}/$

$MIC_A) + (MIC_{BA}/MIC_B)$, where MIC_{AB} and MIC_{BA} were the minimum inhibitory concentrations (MIC) of drugs A and B in combination therapy, while MIC_A and MIC_B were the MIC of drugs A and B tested alone. The FICI value was interpreted as follows: ≤ 0.5 , synergy; $>0.5-4$, indifference; >4 , antagonism.⁷

Synergy rates with 95% CI and each isolate reported in a paper were calculated separately for each synergy method, where the number of isolates tested was defined as the sample size and the event was defined as synergy. As the event rates in most studies are 0 or 1, which are not normally distributed, we performed a logit transformation for the event rates. Results from each testing method were subgrouped by antibiotic type. Some time-kill studies utilized multiple drug concentrations on the same bacterial strains, and we chose a more common clinically achievable drug concentration. For studies applying multiple test methods, data of different methods were separately collected and analyzed. All statistical analyses were performed with the Stata 14.0 software (Stata Corporation 2015, College Station, TX, USA). We calculated various pooled synergy rates using both random- and fixed-effects models. Heterogeneity was assessed by P . An P statistic of 0% indicated no observed heterogeneity. We used a fixed-effects model when $P < 50\%$; otherwise, the random-effects model was used.

Results

Studies included

Our search strategy initially yielded 1,161 potentially relevant citations from PubMed, Embase, and Web of Science (Figure 1). Excluding the studies that did not report any in vitro experiments evaluating the synergistic effect of meropenem-based combinations against *A. baumannii*, 56 published studies fulfilled the inclusion criteria and were included in this review.⁷⁻⁶² The characteristics of each included study are described in Table 1. In the analysis, 1,228 *A. baumannii* strains were subjected to 14 Etests, 42 checkerboard microdilution tests, and 40 time-kill assays to evaluate the synergistic activity of meropenem combination therapies using 20 types of antibiotics.

Time-kill data synthesis

Meropenem-polymyxin B combination therapies (Figure 2) were assessed in ten studies using 30 isolates, and yielded a synergy rate of 98.3% (95% CI, 83.7%–100.0%) with no heterogeneity (P); the fixed-effect model was applied in the meta-analysis, and no isolates showed antagonism. As only one study was conducted on the meropenem-susceptible strains, subgroup analysis was not performed for this combination therapy.^{16,17,20,27,34,48,50,57,59,60}

Figure 1 Flow diagram for selection of studies.

For meropenem–rifampicin combinations (Figure 3), pooling data from 20 isolates in four studies showed that the synergy rate was 89.4% (95% CI, 57.2%–100.0%), and no antagonism was observed.^{16,20,24,32} Heterogeneity (I^2) for these studies was 51.9%; thus, the random-effect model was utilized in the analysis.

For meropenem–colistin combinations (Figure 4), tests conducted on 132 isolates in 12 studies yielded a synergy rate of 60.4% (95% CI, 24.7%–91.8%), and no isolate was antagonistic.^{7,14,15,21,23,44,47,53,54,56,61,62} Heterogeneity (I^2) was calculated to be 89.8%, and thus, the random-effect model was applied in the meta-analysis. There are five studies on meropenem–susceptible strains; however, no detailed information on the number of isolates was found in some of these studies, which resulted in the absence of subgroup analysis.

For meropenem–sulbactam combination therapies (Figure 5), tests were performed on 69 isolates in five studies, and yielded a synergy rate of 54.8% (95% CI, 39.7%–69.6%) with no heterogeneity (I^2), and the fixed-effect model was chosen.^{8,14,21,46,53} None of the isolates was antagonistic. Two

studies evaluated the combination of meropenem and ampicillin/sulbactam, and synergy was observed in 0/1 and 1/2 strains, respectively.

For meropenem–tigecycline combinations (Figure S1), six studies were performed on 36 isolates, which yielded a synergy rate of 24.5% (95% CI, 1.0%–58.4%), and no antagonism was found and heterogeneity (I^2) for these studies was 46.5%.^{12,16,20,47,53,56}

Two studies on nine strains tested meropenem–amikacin combinations, and the synergistic effect was found in 0/1 and 8/8 strains, respectively. One study evaluated meropenem–ciprofloxacin combination, and the synergistic activity was found in 18/40 strains.

Checkerboard microdilution data synthesis

For meropenem–polymyxin B combinations (Figure 6), tests were carried out on 99 strains in four studies, and showed a pooled synergy rate of 37.0% (95% CI, 0.0%–100.0%) and heterogeneity (I^2) was 98.1%.^{13,26,39,51} Owing to its high

Table I Characteristics of included studies

Study, year	Meropenem resistance	Combination antibiotics	No of isolates	Test method
Ko et al, ⁸ 2004	R (MIC: 8 mg/L)	Sulbactam (MIC: 8 mg/L)	1	Tks
Kiffe et al, ⁹ 2005	R (n=6); I (n=4); S (n=38) (MIC: 0.125 to >32 mg/L)	Sulbactam (MIC: 2 to >32 mg/L)	48	Checkerboard
Sader et al, ¹⁰ 2005	R (n=4); S (n=1) (MIC: 1 to >8 mg/L)	Aztreonam (MIC: >8 mg/L)	5	Tks
Timurkaynak et al, ¹¹ 2006	R (n=1); I (n=1); S (n=3) (MIC: 1–64 mg/L)	Colistin (R=2, S=3) (MIC: 1–4 mg/L)	5	Checkerboard
Scheetz et al, ¹² 2007	NM	Tigecycline (MIC: 1 mg/L)	1	Tks
Guelfi et al, ¹³ 2008	R (n=5); I (n=1); S (n=4) (MIC: 0.5–256 mg/L)	Gatifloxacin (R=4, I=1, S=5, MIC: 0.03–8 mg/L) Polymyxin B (MIC: 2 mg/L)	10	Checkerboard
Lee et al, ¹⁴ 2008	R (MIC: 256 or 64 mg/L)	Sulbactam (MIC: 128 or 16 mg/L) Colistin (MIC: 1 mg/L)	2	Tks
Pankuch et al, ¹⁵ 2008	R (n=1 or 11); I (n=2); S (n=37 or 38) (MIC: 0.12 or 256 mg/L)	Ciprofloxacin (R=6, I=1, S=33, MIC: 0.06 or 256 mg/L) Colistin (R=21, S=30, MIC: 0.12 or 128 mg/L)	40 or 51	Tks
Lim et al, ¹⁶ 2009	R (MIC: 32 or 64 mg/L)	Polymyxin B (MIC: 1 or 2 mg/L) Tigecycline (MIC: 0.5–4 mg/L) Rifampicin (MIC: 2 or 4 mg/L)	3	Tks
Pankey et al, ¹⁷ 2009	R (MIC: 24 or >32 mg/L)	Polymyxin B (MIC: 0.5 mg/L)	8	Tks, Etest
Kiratisin et al, ¹⁸ 2010	R (n=21); I (n=1); S (n=18) (MIC: 0.19 to >32 mg/L)	Rifampicin (MIC: 1–8 mg/L) Cefoperazone/sulbactam netilmicin, doxycycline, moxifloxacin	40	Etest
Koerber-Irrgang et al, ¹⁹ 2010	NM	Daptomycin	10	Checkerboard
Lim et al, ²⁰ 2010	R	Polymyxin B (MIC: 16–128 mg/L) Tigecycline (R), rifampin (R), Cefepime	5	Tks
Pongpech et al, ²¹ 2010	R (MIC: 64–256 mg/L)	Sulbactam (MIC: 4–64 mg/L) Colistin (MIC: 0.5–2 mg/L)	10	Tks
Sarigüzel et al, ²² 2010	R (n=76); S (n=24) (MIC: 0.125 to >32 mg/L)	Cefoperazone/sulbactam (MIC: 0.25–256 mg/L)	100	Etest
Srisuphaolarn et al, ²³ 2010	R (MIC: 32–128 mg/L)	Colistin (MIC: 0.5–1 mg/L)	3	PK/PD
Chopra et al, ²⁴ 2012	R	Rifampicin	6	Checkerboard; Tks
Deveci et al, ²⁵ 2012	R (n=9); S (n=1) (MIC: 2–64 mg/L)	Sulbactam (MIC: 32–1024 mg/L)	10	Checkerboard
Ozseven et al, ²⁶ 2012	R (MIC: 16–128 mg/L)	Ampicillin/sulbactam (MIC: 32–128 mg/L) Cefoperazone/sulbactam (MIC: 32–512 mg/L) Rifampin (MIC: 2–64 mg/L) Polymyxin B (MIC: 0.0078–0.125 mg/L)	34	Checkerboard
Netto et al, ²⁷ 2013	R (MIC: 32 mg/L)	Polymyxin B (MIC: 0.25 or 2 mg/L)	2	Tks
Turk Dagi et al, ²⁸ 2014	R (n=31); I (n=9) (MIC: 4–64 mg/L)	Sulbactam (MIC: 4 to >128 mg/L)	40	Checkerboard
Frantzeskaki et al, ²⁹ 2014	R	Colistin	5	Checkerboard
Lu et al, ³⁰ 2014	R (MIC: 16–128 mg/L)	Sulbactam (MIC: 16–128 mg/L) Cefoperazone/sulbactam (MIC: 32–256 mg/L) ciprofloxacin, doxycycline	50	Checkerboard
Shah et al, ³¹ 2014	R (MIC: >32 mg/L)	Colistin (MIC: 0.125 mg/L)	1	Etest
Sun et al, ³² 2014	R (MIC: 64 or 128 mg/L)	Cefoperazone/sulbactam (MIC: 16 or 128 mg/L) Amikacin (MIC: 32–128 mg/L) Rifampicin (MIC: 64–128 mg/L) Ciprofloxacin, azithromycin	12	Checkerboard, Tks
Xia et al, ³³ 2014	R	Cefoperazone/sulbactam	60	Checkerboard
Gall et al, ³⁴ 2015	R	Polymyxin B (MIC: 0.5 mg/L)	1	PD
Ke et al, ³⁵ 2015	R (MIC: 16–64 mg/L)	Sulbactam (MIC: 16 or 256 mg/L) Cefoperazone/sulbactam (MIC: 16 or 64 mg/L)	37	Checkerboard

(Continued)

Table 1 (Continued)

Study, year	Meropenem resistance	Combination antibiotics	No of isolates	Test method
Le Minh et al, ³⁶ 2015	R (MIC: 0.19–128 mg/L)	Colistin (MIC: 0.047–0.75 mg/L)	56	Checkerboard
Marie et al, ³⁷ 2015	R (MIC: 16–1024 mg/L)	Sulbactam (MIC: 16–256 mg/L), tazobactam (MIC: 32–512 mg/L)	54	Checkerboard; Etest
Temocin et al, ³⁸ 2015	R (MIC: 16–32 mg/L)	Sulbactam (MIC: 2–256 mg/L)	30	Etest
Teo et al, ³⁹ 2015	R (MIC: 32 to >64 mg/L)	Polymyxin B (MIC: 0.5–2 mg/L)	49	Checkerboard
van Belkum et al, ⁴⁰ 2015	R (n=23); I (n=1); S (n=1) (MIC: 2–64 mg/L)	Colistin (R=17, S=8, MIC: 1–8 mg/L)	25	Checkerboard
Vourli et al, ⁴¹ 2015	R (MIC: 64–256 mg/L)	Ampicillin/sulbactam (MIC: 128–256 mg/L) Colistin (R=2, S=3, MIC: <0.25–16 mg/L)	5	Checkerboard
Yadav et al, ⁴² 2015	S (MIC: 2 mg/L)	Tobramycin, amikacin	1	Tks
Bae et al, ⁴³ 2016	R (MIC: 32–256 mg/L)	Colistin (MIC: 8–1024 mg/L)	9	Checkerboard
Bedenic et al, ⁴⁴ 2016	RI	Colistin (S)	8	Checkerboard; Tks
Hong et al, ⁴⁵ 2016	R (MIC: 8 to >32 mg/L)	Colistin (R=41, S=41, MIC: 0.1 to >256 mg/L) Tigecycline (MIC: 0.3–8 mg/L)	82	Etest
Laishram et al, ⁴⁶ 2016	R (MIC: 16–512 mg/L)	Sulbactam (MIC: 16–128 mg/L)	50	Checkerboard; Tks
Leite et al, ⁴⁷ 2016	R (MIC: 16–128 mg/L)	Colistin (R=7, S=13, MIC: 0.5–64 mg/L) Tigecycline (MIC: 0.25–16 mg/L) Fosfomycin (MIC: 32 to >128 mg/L)	20	Checkerboard; Tks
Lenhard et al, ⁴⁸ 2016	R (MIC: 8–64 mg/L)	Polymyxin B (S)	2	Tks
Liu et al, ⁴⁹ 2016	R (MIC: 16–128 mg/L)	Colistin (MIC: 0.5–2 mg/L)	12	Checkerboard
Lenhard et al, ⁵⁰ 2016	R (n=2); I (n=1); (MIC: 4–64 mg/L)	Polymyxin B (MIC: 0.5 mg/L)	3	Tks
Menegucci et al, ⁵¹ 2016	R (MIC: 64–128 mg/L)	Polymyxin B (R=3, S=3, MIC: 0.5–16 mg/L) Fosfomycin (MIC: 64–512 mg/L)	6	Checkerboard
Wang et al, ⁵² 2016	R	Cefoperazone/sulbactam, sulbactam (R), amikacin (R), ciprofloxacin (R)	116	Checkerboard
Wang et al, ⁵³ 2016	R (n=3); S (n=3); (MIC: 0.5–64 mg/L)	Colistin (MIC: 0.5–2 mg/L) Tigecycline (MIC: 0.5–8 mg/L) Sulbactam (MIC: 2–16 mg/L)	6	Tks
Yang et al, ⁵⁴ 2016	R (MIC: 8–128 mg/L)	Colistin (MIC: 0.5 mg/L) Minocycline (MIC: 16 or 32 mg/L)	4	Checkerboard; Tks
Yavaş et al, ⁵⁵ 2016	R (MIC: ≥32 mg/L)	Colistin (MIC: 0.38 or 1 mg/L) Tigecycline (MIC: 0.75 or 6 mg/L) Sulbactam (MIC: 16 or 96 mg/L)	18	Etest
Büyük et al, ⁵⁶ 2017	RIS	Colistin, tigecycline	15 or 1	Checkerboard; Tks
Gallo et al, ⁵⁷ 2017	S (MIC: 0.25 or 1 mg/L)	Polymyxin B (MIC: 0.5 or 1 mg/L)	3	Tks
Ghazi et al, ⁵⁸ 2017	R (MIC: ≥64 mg/L)	Amikacin (MIC: 64–512 mg/L)	8	Tks
Lenhard et al, ⁵⁹ 2017	R (MIC: 64 mg/L)	Ampicillin/sulbactam (MIC: 32/16 mg/L) Polymyxin B (MIC: 32 mg/L)	1	Tks
Lenhard et al, ⁶⁰ 2017	R (MIC: 64 mg/L)	Ampicillin/sulbactam (MIC: 32/16 mg/L) Polymyxin B (MIC: 32 or 64 mg/L)	2	Tks
Manohar et al, ⁶¹ 2017	R (MIC: >128 mg/L)	Colistin (MIC: 32 mg/L)	2	Tks
Soudeihia et al, ⁶² 2017	RIS	Colistin (RS)	21	Checkerboard; Etest; Tks
Tangden et al, ⁷ 2017	R (n=2); S (n=2); (MIC: 0.5–32 mg/L)	Colistin (MIC: 0.125–1.5 mg/L)	4	Checkerboard; Tks

Abbreviations: I, intermediate; MIC, minimum inhibitory concentration; NM, not mentioned; PK/PD, pharmacokinetic/pharmacodynamic; R, resistant; S, susceptible; Tks, time-kill synergy.

heterogeneity, we used the random-effect model for statistical analysis. No isolates were found to be antagonistic.

Thirteen studies with 189 isolates tested meropenem–colistin combination therapies and showed a pooled synergy rate of 58.8% (95% CI, 29.4%–85.6%) and a heterogeneity (I^2) of 92.3% (Figure 7); thus, the random-effect model was

applied in the meta-analysis.^{7,11,29,36,40,41,43,44,47,49,54,56,62} Two isolates were found to be antagonistic. There are six studies that include meropenem-susceptible strains; however, no detailed number of isolates was found in some of these studies, which resulted in the unavailability of subgroup analysis.

Figure 2 Forest plot and pooled synergy rates for meropenem–polymyxin B combinations in time-kill method.
Abbreviation: ES, effect size.

As shown in Figure 8, tests conducted on 405 isolates in eight studies showed that the synergy rate of meropenem–sulbactam combinations was 25.2% (95% CI, 16.1%–36.2%).^{9,25,28,30,35,37,46,52} A static heterogeneity (I^2) of 79.4% was observed, and as a result, we chose the random-effects model. Three isolates were antagonistic. Considering that there was no detailed number of meropenem-susceptible isolates in three studies, the subgroup analysis was not applied. Six studies were performed on 309 strains in the meropenem–cefoprazone/sulbactam combinations (Figure S2), yielding a pooled synergy rate of 7.4% (95% CI, 1.4%–16.6%). Heterogeneity (I^2) for these studies was 80.6%.^{26,30,32,33,35,52}

Three studies testing meropenem–rifampicin combination therapies (Figure S3) yielded a synergy rate of 56.3% (95% CI, 8.7%–97.8%).^{24,26,32} Heterogeneity (I^2) for these studies was 90.2%. Meropenem–ciprofloxacin combination therapies were tested in three studies (Figure S4), and yielded a pooled synergy rate of 0.3% (95% CI, 0.0%–3.5%).^{30,32,52}

Heterogeneity (I^2) was 33.0%. The combination of meropenem and tigecycline was tested in two studies, and seven of 35 strains showed synergy. Two studies tested the effect of meropenem in combination with amikacin, and synergistic activity was found in 67/128 strains.

Etest data synthesis

Four studies that evaluated the effect of meropenem–colistin combinations (Figure S5) on 122 isolates yielded a pooled synergy rate of 39.2% (95% CI, 0.0%–97.7%), and heterogeneity (I^2) was found to be 95.9%.^{31,45,55,62} Three studies consisting of 102 strains investigated the effect of meropenem–sulbactam combinations (Figure S6), reporting a pooled synergy rate of 35.1% (95% CI, 21.4%–50.1%).^{37,38,55} Heterogeneity (I^2) was 52.3%. One study with 40 strains tested the meropenem–rifampicin and meropenem–moxifloxacin combinations, and synergy was found in 1 and 0 strain, respectively. Two studies reported synergistic effect

Figure 3 Forest plot and pooled synergy rates for meropenem–rifampicin combinations in time-kill method.
Abbreviation: ES, effect size.

Figure 4 Forest plot and pooled synergy rates for meropenem–colistin combinations in time-kill method.
Abbreviation: ES, effect size.

Figure 5 Forest plot and pooled synergy rates for meropenem–sulbactam combinations in time-kill method.

Abbreviation: ES, effect size.

Figure 6 Forest plot and pooled synergy rates for meropenem–polymyxin B combinations in checkerboard method.

Abbreviation: ES, effect size.

Figure 7 Forest plot and pooled synergy rates for meropenem–colistin combinations in checkerboard method.
Abbreviation: ES, effect size.

of 23/100 strains in meropenem–tigecycline combination therapies. Meropenem in combination with cefoprazone/sulbactam resulted in synergistic activity in 61/140 strains.

Discussion

The global emergence of MDR *A. baumannii* has spurred an interest in finding a more effective treatment strategy. The World Health Organization has identified antimicrobial resistance as one of the three most important problems affecting human health and carbapenem-resistant *A. baumannii* as a critical priority pathogen to help in prioritizing the research and development of new and effective antibiotic treatments.⁶³ Combining antimicrobials is an effective treatment approach for MDR *A. baumannii* infections. Reports of meropenem-based combination against *A. baumannii* have been rising steadily during the past few years. Our results indicated that several antibiotics combined with meropenem could act synergistically in vitro against *A. baumannii*, especially for

polymyxin B and rifampicin. Notably, colistin, also known as polymyxin E, showed a lower synergy rate than polymyxin B in the time-kill assays, while an opposite result was found in the checkerboard microdilution method. Several reasons could result in the inconsistent result. First, polymyxin B and colistin are mixture of cyclic polypeptides, which contain up to 39 and 36 distinct lipopeptides, respectively, and differences in the structures of these individual polypeptides and variations in the physicochemical property among different polymyxin products or even batches from the same company can contribute to variability in the antibacterial activity of polymyxin B and colistin.⁶⁴ Second, the small sample size may skew the result though we performed a logit transformation for the event rate. Third, the time-kill method uses colony number as the judgment standard, while checkerboard microdilution and Etest methods use MIC in the formula. With no standardization of synergy test method, comparing results generated from different methods becomes a difficult

Figure 8 Forest plot and pooled synergy rates for meropenem–sulbactam combinations in checkerboard method.
Abbreviation: ES, effect size.

task. As demonstrated in Table 2, it seems that higher rates of synergy are seen with time-kill assays than with checkerboard or Etest assays, which is consistent with the conclusion drawn by Ni et al.⁶⁵ The synergy rate obtained from checkerboard microdilution and Etest methods is a static value and hard to reproduce.⁶⁶ In terms of time-kill assay, it is a useful method to provide us kinetic information of antibiotic interaction with bacteria although time- and labor-intensive for routine use in a clinical diagnostic laboratory. Therefore, developing new kinetic test method such as luciferase-based reporter system or standardizing the interpretation of these existing test methods for synergy evaluation should be a feasible strategy to address these limitations.

In time-kill assays, meropenem–polymyxin B combination showed the highest pooled synergy rate of 98.3%, which can be recognized as a very high degree of synergy to almost all isolates with different resistant profiles. Rifampicin in combination with meropenem displayed up to approximately 90% high synergy rate, and it is worth noting that amikacin may be a good partner of meropenem though only two studies can be found. Polymyxin B and amikacin are both molecules

containing cations, which are capable of binding negatively charged lipopolysaccharides in the outer membrane of gram-negative bacteria, which thus leads to disruption of bacterial membrane permeability.⁶⁷ As to rifampicin, more studies should be conducted to confirm our preliminary finding, and the underlying mechanism of the combination also needs to be addressed. Polymyxins are considered as the last-resort antibiotic for the treatment of gram-negative bacteria; however, they were also reported to be associated with nephrotoxicity and neurotoxicity,⁶⁸ which often hindered their use in clinical settings. To reduce their toxicity, purification of polymyxins product and structure modification have been reported by several groups.⁶⁴

Sulbactam, as a member of serine β -lactamase inhibitor, is unable to inhibit any carbapenemases but displays moderate activity against *A. baumannii*, so a pooled synergy rate of 54.8% generated by meropenem–sulbactam combination was expected. Carbapenemases, especially metallo β -lactamases (MBLs), have caused extensive concern on their rapid dissemination and ability to hydrolyze almost all β -lactam antibiotics except monobactams. Unfortunately, up till now, no

Table 2 Summary of the pooled synergy rates

Meropenem-based combinations	Tks			Checkerboard			Etest		
	Number of studies	Number of isolates	Synergy rate	Number of studies	Number of isolates	Synergy rate	Number of studies	Number of isolates	Synergy rate
Polymyxin B	10	30	98.3% (95% CI, 83.7%–100.0%)	4	99	37.0% (95% CI, 0.0%–100.0%)	1	8	5/8
Colistin	12	132	60.4% (95% CI, 24.7%–91.8%)	13	189	58.8% (95% CI, 29.4%–85.6%)	4	122	39.2% (95% CI, 0.0%–97.7%)
Rifampicin	4	20	89.4% (95% CI, 57.2%–100.0%)	3	52	56.3% (95% CI, 8.7%–97.8%)	1	40	1/40
Tigecycline	6	36	24.5% (95% CI, 1.0%–58.4%)	2	35	7/35	2	100	23/100
Sulbactam	5	69	54.8% (95% CI, 39.7%–69.6%)	8	405	25.2% (95% CI, 16.1%–36.2%)	3	102	35.1% (95% CI, 21.4%–50.1%)
Cefoprazone/sulbactam	0	0	0	6	309	7.4% (95% CI, 1.4%–16.6%)	2	140	61/140
Ciprofloxacin	1	40	18/40	3	178	0.3% (95% CI, 0.0%–3.5%)	0	0	0
Amikacin	2	9	8/9	2	128	67/128	0	0	0

Abbreviation: Tks, time-kill synergy.

MBL inhibitor has been clinically approved and all clinically used β -lactamase inhibitors are not active toward MBLs. It remains a substantial challenge to design MBL inhibitors, especially for broad-spectrum MBL inhibitors.

Conclusion

The pooled synergy data in this review suggested that combination therapies of meropenem with polymyxin B, rifampicin, and possibly amikacin as well could achieve high synergy rates against MDR *A. baumannii* isolates, and colistin and sulbactam could be secondary choice for the combination with meropenem. Tigecycline and ciprofloxacin combination had the least pooled synergy rates. Compared to static checkerboard and Etest method, time-kill assay is more like in vivo dynamic antibacterial process and generally exhibited the greatest of synergisms because of its kinetic synergy calculation method. Combination therapies are future alternatives to traditional monotherapies for infections caused by MDR bacteria. However, the studies included in this review were performed in vitro, which neglects the complicated interactions between antibiotics and hosts. There is still a long way from our results to their applications in clinic, and findings of this review should be verified by more well-designed in vitro and in vivo studies.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (81403084); the Science and

Technology Program of Guangzhou, China (201710010013 and 201509010012).

Disclosure

The authors report no conflicts of interest in this work.

References

- Harding CM, Hennon SW, Feldman MF. Uncovering the mechanisms of *Acinetobacter baumannii* virulence. *Nat Rev Microbiol*. 2018;16(2):91–102.
- Doi Y, Bonomo RA, Hooper DC, et al. Gram-negative bacterial infections: research priorities, accomplishments, and future directions of the Antibacterial Resistance Leadership Group. *Clin Infect Dis*. 2017;64(Suppl 1):S30–S35.
- Dijkshoorn L, Nemec A, Seifert H. An increasing threat in hospitals: multidrug-resistant *Acinetobacter baumannii*. *Nat Rev Microbiol*. 2007;5(12):939–951.
- Papp-Wallace KM, Endimiani A, Taracila MA, Bonomo RA. Carbapenems: past, present, and future. *Antimicrob Agents Chemother*. 2011;55(11):4943–4960.
- El-Gamal MI, Ibrahim I, Hisham N, Aladdin R, Mohammed H, Bahaaeldin A. Recent updates of carbapenem antibiotics. *Eur J Med Chem*. 2017;131:185–195.
- Falagas ME, Mavroudis AD, Vardakas KZ. The antibiotic pipeline for multi-drug resistant gram negative bacteria: what can we expect? *Expert Rev Anti Infect Ther*. 2016;14(8):747–763.
- Tängdén T, Karvanen M, Friberg LE, Odenholt I, Cars O. Assessment of early combination effects of colistin and meropenem against *Pseudomonas aeruginosa* and *Acinetobacter baumannii* in dynamic time-kill experiments. *Infect Dis*. 2017;49(7):521–527.
- Ko WC, Lee HC, Chiang SR, et al. In vitro and in vivo activity of meropenem and sulbactam against a multidrug-resistant *Acinetobacter baumannii* strain. *J Antimicrob Chemother*. 2004;53(2):393–395.
- Kiffer CR, Sampaio JL, Sinto S, et al. In vitro synergy test of meropenem and sulbactam against clinical isolates of *Acinetobacter baumannii*. *Diagn Microbiol Infect Dis*. 2005;52(4):317–322.

10. Sader HS, Rhomberg PR, Jones RN. In vitro activity of beta-lactam antimicrobial agents in combination with aztreonam tested against metallo-beta-lactamase-producing *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. *J Chemother*. 2005;17(6):622–627.
11. Timurkaynak F, Can F, Azap OK, Demirbilek M, Arslan H, Karaman SO. In vitro activities of non-traditional antimicrobials alone or in combination against multidrug-resistant strains of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolated from intensive care units. *Int J Antimicrob Agents*. 2006;27(3):224–228.
12. Scheetz MH, Qi C, Warren JR, et al. In vitro activities of various antimicrobials alone and in combination with tigecycline against carbapenem-intermediate or -resistant *Acinetobacter baumannii*. *Antimicrob Agents Chemother*. 2007;51(5):1621–1626.
13. Guelfi KC, Tognim MC, Cardoso CL, Gales AC, Carrara-Marrone FE, Garcia LB. In vitro evaluation of the antimicrobial activity of meropenem in combination with polymyxin B and gatifloxacin against *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. *J Chemother*. 2008;20(2):180–185.
14. Lee CH, Tang YF, Su LH, Chien CC, Liu JW. Antimicrobial effects of varied combinations of meropenem, sulbactam, and colistin on a multidrug-resistant *Acinetobacter baumannii* isolate that caused meningitis and bacteremia. *Microb Drug Resist*. 2008;14(3):233–237.
15. Pankuch GA, Lin G, Seifert H, Appelbaum PC. Activity of meropenem with and without ciprofloxacin and colistin against *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. *Antimicrob Agents Chemother*. 2008;52(1):333–336.
16. Lim TP, Tan TY, Lee W, et al. In vitro activity of various combinations of antimicrobials against carbapenem-resistant *Acinetobacter* species in Singapore. *J Antibiot*. 2009;62(12):675–679.
17. Pankey GA, Ashcraft DS. The detection of synergy between meropenem and polymyxin B against meropenem-resistant *Acinetobacter baumannii* using Etest and time-kill assay. *Diagn Microbiol Infect Dis*. 2009;63(2):228–232.
18. Kiratisin P, Apisarnthanarak A, Kaewdaeng S. Synergistic activities between carbapenems and other antimicrobial agents against *Acinetobacter baumannii* including multidrug-resistant and extensively drug-resistant isolates. *Int J Antimicrob Agents*. 2010;36(3):243–246.
19. Körber-Irrgang B, Kresken M. In vitro activity of daptomycin combined with other antimicrobial agents against Gram-negative bacteria. *Clin Microbiol Infect*. 2010;16:S156–S157.
20. Lim TP, Lee W, Sasikala S, et al. In vitro activity of antimicrobials in combination against clinical strains of extreme drug-resistant *Acinetobacter baumannii* to all antibiotics including polymyxin B in Singapore. *Clin Microbiol Infect*. 2010;16:S80.
21. Pongpech P, Amornnopparatnakul S, Panapakdee S, et al. Antibacterial activity of carbapenem-based combinations against multidrug-resistant *Acinetobacter baumannii*. *J Med Assoc Thai*. 2010;93(2):161–171.
22. Sarigüzel FM, Sümerkan B, Metan G. In-vitro activity of carbapenem/sulbactam combination against *Acinetobacter baumannii* strains. *Erciyes Tip Dergisi*. 2010;32(1):015–018.
23. Srisupha-Olarn W, Burgess DS. Activity of meropenem and colistin alone and in combination against multidrug resistant *Acinetobacter baumannii* in a pharmacokinetic-pharmacodynamic model. *Pharmacotherapy*. 2010;30(10):401e.
24. Chopra S, Matsuyama K, Tran T, Madrid P. Systematic discovery of synergistic novel antibiotic combinations targeting multidrug-resistant *Acinetobacter baumannii*. *Int J Antimicrob Agents*. 2012;40(4):377–379.
25. Deveci A, Coban AY, Acicbe O, Tanyel E, Yaman G, Durupinar B. In vitro effects of sulbactam combinations with different antibiotic groups against clinical *Acinetobacter baumannii* isolates. *J Chemother*. 2012;24(5):247–252.
26. Ozseven AG, Cetin ES, Aridogan BC, Ozseven L. In vitro synergistic activity of carbapenems in combination with other antimicrobial agents against multidrug-resistant *Acinetobacter baumannii*. *African J Microbiol Res*. 2012;6(12):2985–2992.
27. Netto B, Vieira BJ, Hermes DM, Ribeiro VB, Zavascki AP. In vitro activity of non-bactericidal concentrations of polymyxin B in combination with other antimicrobials against OXA-23-producing carbapenem-resistant *Acinetobacter baumannii*. *Braz J Infect Dis*. 2013;17(4):502–504.
28. Turk Dagi H, Kus H, Arslan U, Tuncer I. In vitro synergistic activity of sulbactam in combination with imipenem, meropenem and cefoperazone against carbapenem-resistant *Acinetobacter baumannii* isolates. *Mikrobiyol Bul*. 2014;48(2):311–315.
29. Frantzeskaki F, Vourli S, Paramythiotou E, et al. In vitro and in vivo synergistic activity of high doses of ampicillin/sulbactam with colistin versus meropenem with colistin in ventilator associated pneumonia (VAP) caused by *Acinetobacter baumannii* (AB). *Intens Care Med*. 2014;40(1):S195.
30. Lu Y, Zhang Y, Zhou H, Yu F, Sun S, Rui Y. Combined drug sensitivity test of 50 strains of extensively drug-resistant *Acinetobacter baumannii*. *Nan Fang Yi Ke Da Xue Xue Bao*. 2014;34(11):1697–1701.
31. Shah PG, Shah SR, Kamat SD, Kamat DV. Colistin-carbapenem combination therapy against carbapenem resistant gram negative bacilli infections: Clinical and an in vitro synergy study. *Int J Pharm Pharmacol Sci*. 2014;6(10):497–500.
32. Sun Y, Wang L, Li J, et al. Synergistic efficacy of meropenem and rifampicin in a murine model of sepsis caused by multidrug-resistant *Acinetobacter baumannii*. *Eur J Pharmacol*. 2014;729:116–122.
33. Xia J, Zhang D, Xu Y, Gong M, Zhou Y, Fang X. A retrospective analysis of carbapenem-resistant *Acinetobacter baumannii*-mediated nosocomial pneumonia and the in vitro therapeutic benefit of cefoperazone/sulbactam. *Int J Infect Dis*. 2014;23:90–93.
34. Gall J, Lenhard J, Holden PN, Tsuji BT. Comparative pharmacodynamics of imipenem, doripenem, meropenem, and ertapenem in combination with polymyxin B against carbapenem-resistant *Acinetobacter baumannii*. *Pharmacotherapy*. 2015;35(11):e304–e305.
35. Ke Q, Lü Y, Wang F. In vitro activity of meropenem in combination with sulbactam or cefoperazone-sulbactam against multidrug resistant *Acinetobacter baumannii*. *Chin J Infect Chemother*. 2015;15(6):548–551.
36. Le Minh V, Thi Khanh Nhu N, Vinh Phat V, et al. In vitro activity of colistin in antimicrobial combination against carbapenem-resistant *Acinetobacter baumannii* isolated from patients with ventilator-associated pneumonia in Vietnam. *J Med Microbiol*. 2015;64(10):1162–1169.
37. Marie MA, Krishnappa LG, Alzahrani AJ, Mubarak MA, Alyousef AA. A prospective evaluation of synergistic effect of sulbactam and tazobactam combination with meropenem or colistin against multidrug resistant *Acinetobacter baumannii*. *Bosn J Basic Med Sci*. 2015;15(4):24–29.
38. Temocin F, Erdinc FS, Tulek N, et al. Synergistic effects of sulbactam in multi-drug-resistant *Acinetobacter baumannii*. *Braz J Microbiol*. 2015;46(4):1119–1124.
39. Teo J, Lim TP, Hsu LY, et al. Extensively drug-resistant *Acinetobacter baumannii* in a Thai hospital: a molecular epidemiologic analysis and identification of bactericidal Polymyxin B-based combinations. *Antimicrob Resist Infect Control*. 2015;4(1):2.
40. van Belkum A, Halimi D, Bonetti EJ, et al. Meropenem/colistin synergy testing for multidrug-resistant *Acinetobacter baumannii* strains by a two-dimensional gradient technique applicable in routine microbiology. *J Antimicrob Chemother*. 2015;70(1):167–172.
41. Vourli S, Frantzeskaki F, Meletiadias J, et al. Synergistic interactions between colistin and meropenem against extensively drug-resistant and pandrug-resistant *Acinetobacter baumannii* isolated from ICU patients. *Int J Antimicrob Agents*. 2015;45(6):670–671.
42. Yadav R, Landersdorfer CB, Nation RL, Boyce JD, Bulitta JB. Novel approach to optimize synergistic carbapenem-aminoglycoside combinations against carbapenem-resistant *Acinetobacter baumannii*. *Antimicrob Agents Chemother*. 2015;59(4):2286–2298.
43. Bae S, Kim MC, Park SJ, et al. In vitro synergistic activity of antimicrobial agents in combination against clinical isolates of colistin-resistant *Acinetobacter baumannii*. *Antimicrob Agents Chemother*. 2016;60(11):6774–6779.

44. Bedenić B, Beader N, Godić-Torkar K, et al. Postantibiotic effect of colistin alone and combined with vancomycin or meropenem against *Acinetobacter* spp. with well defined resistance mechanisms. *J Chemother*. 2016;28(5):375–382.
45. Hong DJ, Kim JO, Lee H, et al. In vitro antimicrobial synergy of colistin with rifampicin and carbapenems against colistin-resistant *Acinetobacter baumannii* clinical isolates. *Diagn Microbiol Infect Dis*. 2016;86(2):184–189.
46. Laishram S, Anandan S, Devi BY, et al. Determination of synergy between sulbactam, meropenem and colistin in carbapenem-resistant *Klebsiella pneumoniae* and *Acinetobacter baumannii* isolates and correlation with the molecular mechanism of resistance. *J Chemother*. 2016;28(4):297–303.
47. Leite GC, Oliveira MS, Perdigão-Neto LV, et al. Antimicrobial combinations against pan-resistant *Acinetobacter baumannii* isolates with different resistance mechanisms. *PLoS One*. 2016;11(3):e0151270.
48. Lenhard JR, Bulitta JB, Connell TD, et al. High-intensity meropenem combinations with polymyxin B: new strategies to overcome carbapenem resistance in *Acinetobacter baumannii*. *J Antimicrob Chemother*. 2017;72(1):153–165.
49. Liu X, Zhao M, Chen Y, et al. Synergistic killing by meropenem and colistin combination of carbapenem-resistant *Acinetobacter baumannii* isolates from Chinese patients in an in vitro pharmacokinetic/pharmacodynamic model. *Int J Antimicrob Agents*. 2016;48(5):559–563.
50. Lenhard JR, Gall JS, Bulitta JB, et al. Comparative pharmacodynamics of four different carbapenems in combination with polymyxin B against carbapenem-resistant *Acinetobacter baumannii*. *Int J Antimicrob Agents*. 2016;48(6):719–724.
51. Menegucci TC, Albiero J, Migliorini LB, et al. Strategies for the treatment of polymyxin B-resistant *Acinetobacter baumannii* infections. *Int J Antimicrob Agents*. 2016;47(5):380–385.
52. Wang FJ, Lyu Y, Liu ZH, Li Y, Cui LQ. In vitro activity of different antibacterial agents in combination with each other against multidrug-resistant *Acinetobacter baumannii*. *Chin Med J*. 2016;129(19):2388–2389.
53. Wang YC, Kuo SC, Yang YS, et al. Individual or combined effects of meropenem, imipenem, sulbactam, colistin, and tigecycline on biofilm-embedded *Acinetobacter baumannii* and biofilm architecture. *Antimicrob Agents Chemother*. 2016;60(8):4670–4676.
54. Yang YS, Lee Y, Tseng KC, et al. In vivo and in vitro efficacy of minocycline-based combination therapy for minocycline-resistant *Acinetobacter baumannii*. *Antimicrob Agents Chemother*. 2016;60(7):4047–4054.
55. Yavaş S, Yetkin MA, Kayaaslan B, et al. Investigating the in vitro synergistic activities of several antibiotic combinations against carbapenem-resistant *Acinetobacter baumannii* isolates. *Turk J Med Sci*. 2016;46(3):892–896.
56. Büyük A, Yılmaz FF, Gül Yurtsever S, Hoşgör Limoncu M. Antibiotic resistance profiles and genotypes of *Acinetobacter baumannii* isolates and in vitro interactions of various antibiotics in combination with tigecycline and colistin. *Tur J Pharm Sci*. 2017;14(1):13–18.
57. Gallo SW, Ferreira CAS, de Oliveira SD. Combination of polymyxin B and meropenem eradicates persister cells from *Acinetobacter baumannii* strains in exponential growth. *J Med Microbiol*. 2017;66(8):1257–1260.
58. Ghazi IM, Grupper M, Nicolau DP. Antibacterial activity of human simulated epithelial lining fluid concentrations of amikacin inhale alone and in combination with meropenem against *Acinetobacter baumannii*. *Infect Dis*. 2017;49(11–12):831–839.
59. Lenhard JR, Smith NM, Bulman ZP, et al. High-dose ampicillin-sulbactam combinations combat polymyxin-resistant *Acinetobacter baumannii* in a hollow-fiber infection model. *Antimicrob Agents Chemother*. 2017;61(3):e01268-16.
60. Lenhard JR, Thamlikitkul V, Silveira FP, et al. Polymyxin-resistant, carbapenem-resistant *Acinetobacter baumannii* is eradicated by a triple combination of agents that lack individual activity. *J Antimicrob Chemother*. 2017;72(5):1415–1420.
61. Manohar P, Shanthini T, Ayyanar R, et al. The distribution of carbapenem- and colistin-resistance in Gram-negative bacteria from the Tamil Nadu region in India. *J Med Microbiol*. 2017;66(7):874–883.
62. Soudeihah MAH, Dahdouh EA, Azar E, Sarkis DK, Daoud Z. In vitro evaluation of the colistin-carbapenem combination in clinical isolates of *A. baumannii* using the checkerboard, Etest, and time-kill curve techniques. *Front Cell Infect Microbiol*. 2017;7:209.
63. *Global Priority List of Antibiotic-Resistant Bacteria to Guide Research, Discovery, and Development of New Antibiotics*. Geneva: World Health Organization; 2017.
64. Roberts KD, Azad MA, Wang J, et al. Antimicrobial activity and toxicity of the major lipopeptide components of polymyxin B and colistin: last-line antibiotics against multidrug-resistant Gram-negative bacteria. *ACS Infect Dis*. 2015;1(11):568–575.
65. Ni W, Shao X, di X, Cui J, Wang R, Liu Y. In vitro synergy of polymyxins with other antibiotics for *Acinetobacter baumannii*: a systematic review and meta-analysis. *Int J Antimicrob Agents*. 2015;45(1):8–18.
66. Rand KH, Houck HJ, Brown P, Bennett D. Reproducibility of the microdilution checkerboard method for antibiotic synergy. *Antimicrob Agents Chemother*. 1993;37(3):613–615.
67. Yadav R, Bulitta JB, Schneider EK, Shin BS, Velkov T, Nation RL, Landersdorfer CB. Aminoglycoside concentrations required for synergy with carbapenems against *Pseudomonas aeruginosa* determined via mechanistic studies and modeling. *Antimicrob Agents Chemother*. 2017;61(12):e00722-17.
68. Yahav D, Farbman L, Leibovici L, Paul M. Colistin: new lessons on an old antibiotic. *Eur Soc Clin Microbiol Infect Dis*. 2012;18(1):18–29.

Infection and Drug Resistance

Publish your work in this journal

Infection and Drug Resistance is an international, peer-reviewed open-access journal that focuses on the optimal treatment of infection (bacterial, fungal and viral) and the development and institution of preventive strategies to minimize the development and spread of resistance. The journal is specifically concerned with the epidemiology of antibiotic

Submit your manuscript here: <https://www.dovepress.com/infection-and-drug-resistance-journal>

resistance and the mechanisms of resistance development and diffusion in both hospitals and the community. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Dovepress