

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}

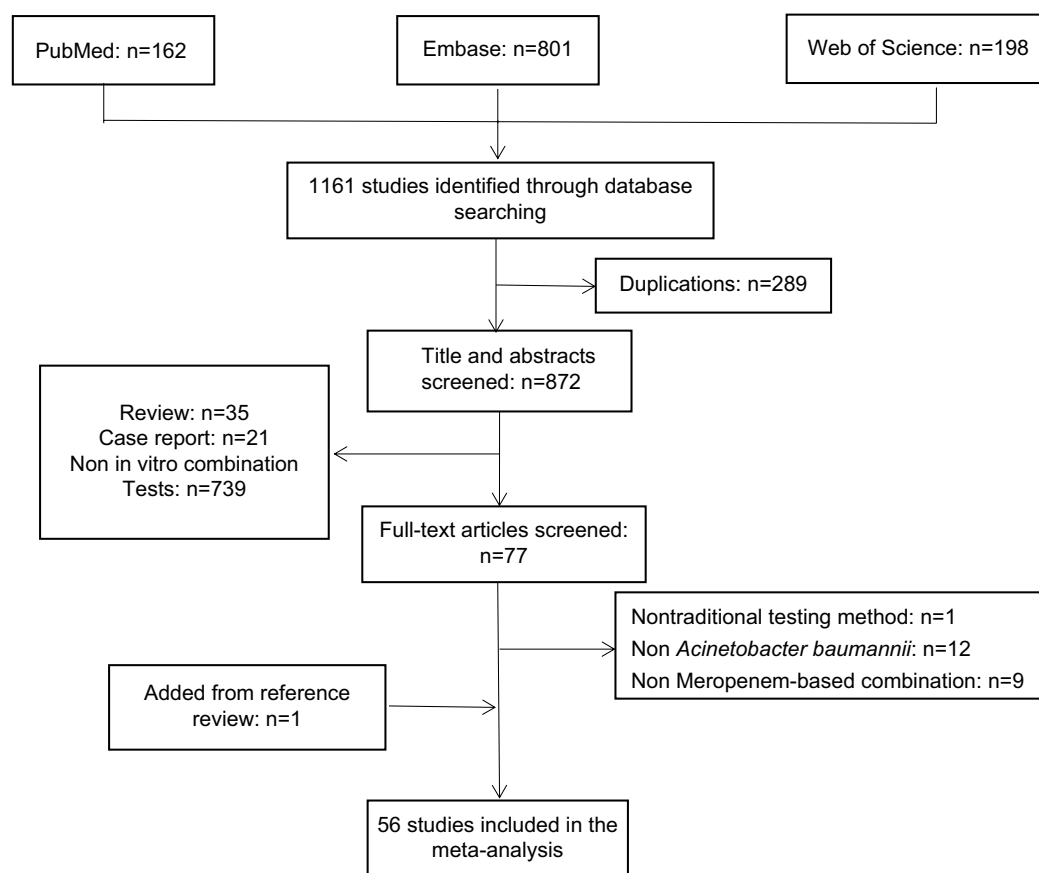


Figure 1 Flow diagram for selection of studies.

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 1 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
Soudeihha 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.

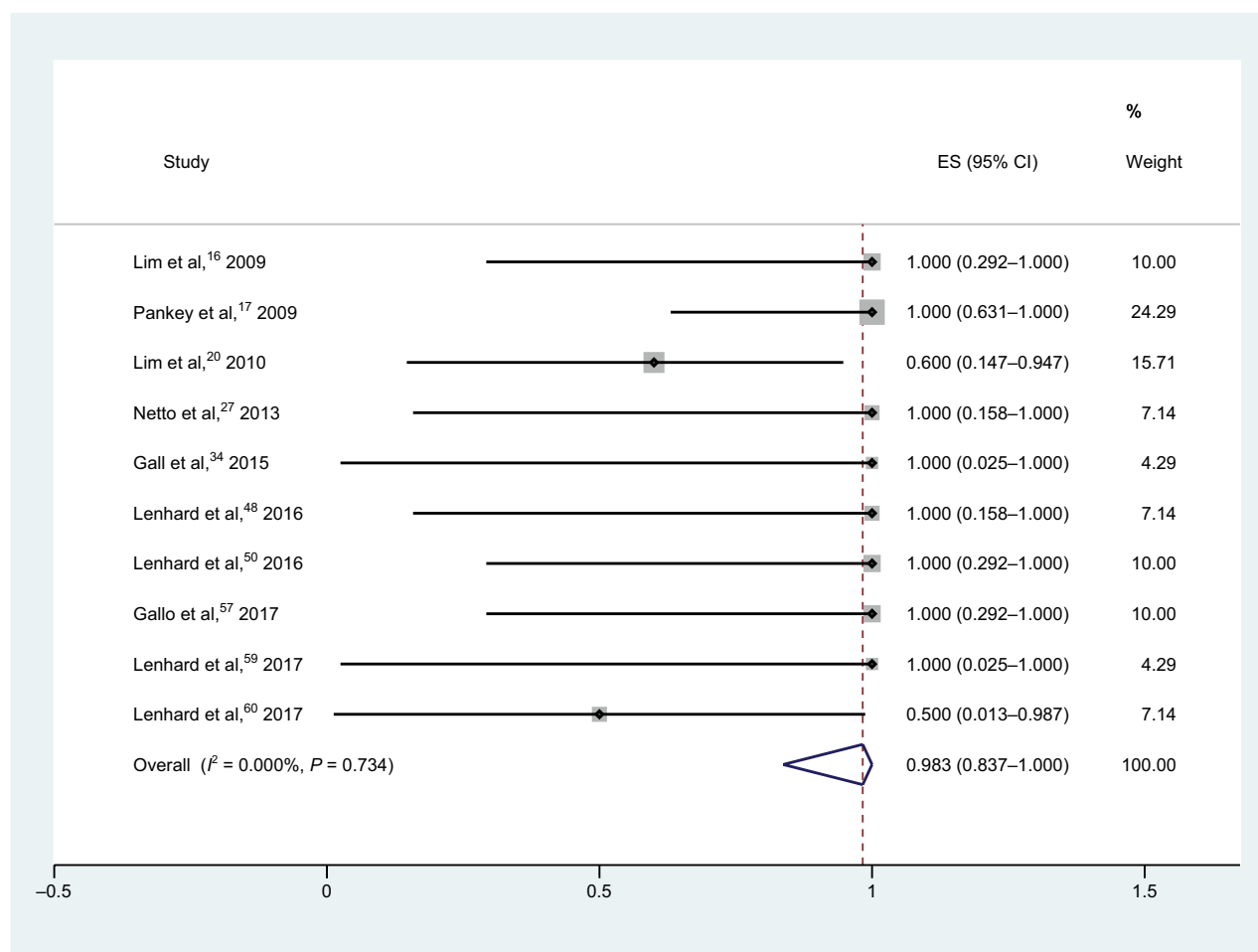


Figure 2 Forest plot and pooled synergy rates for meropenem–polymyxin B combinations in time-kill method.
Abbreviation: ES, effect size.

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

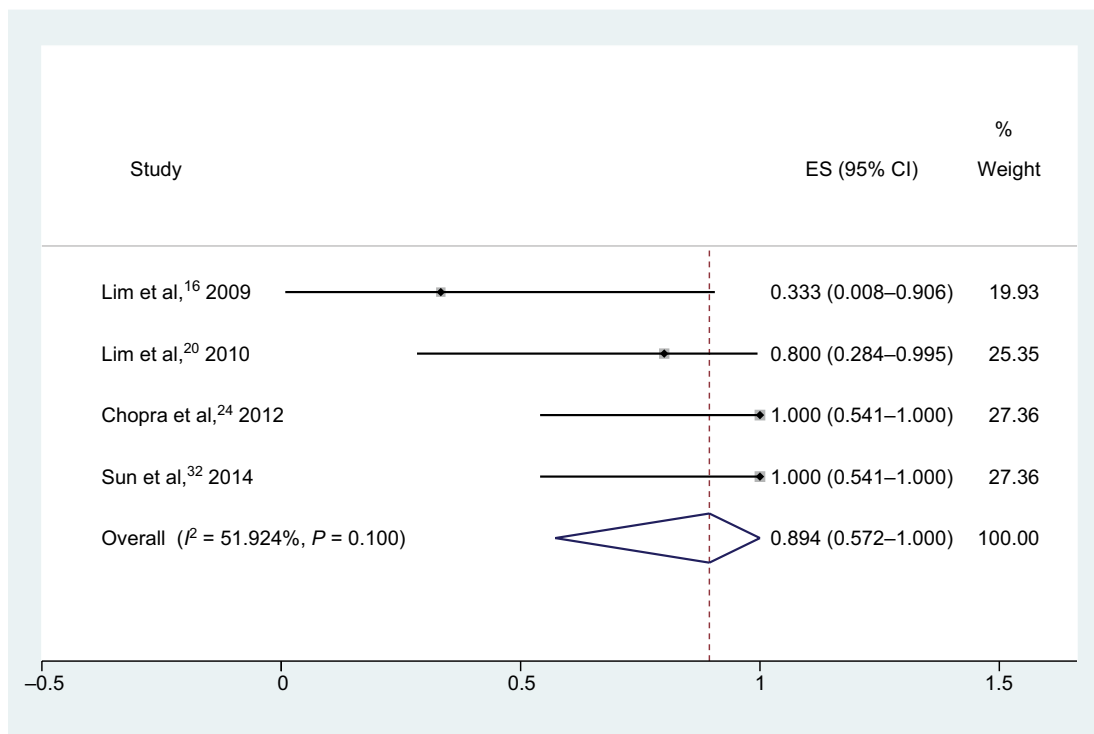


Figure 3 Forest plot and pooled synergy rates for meropenem–rifampicin combinations in time-kill method.
Abbreviation: ES, effect size.

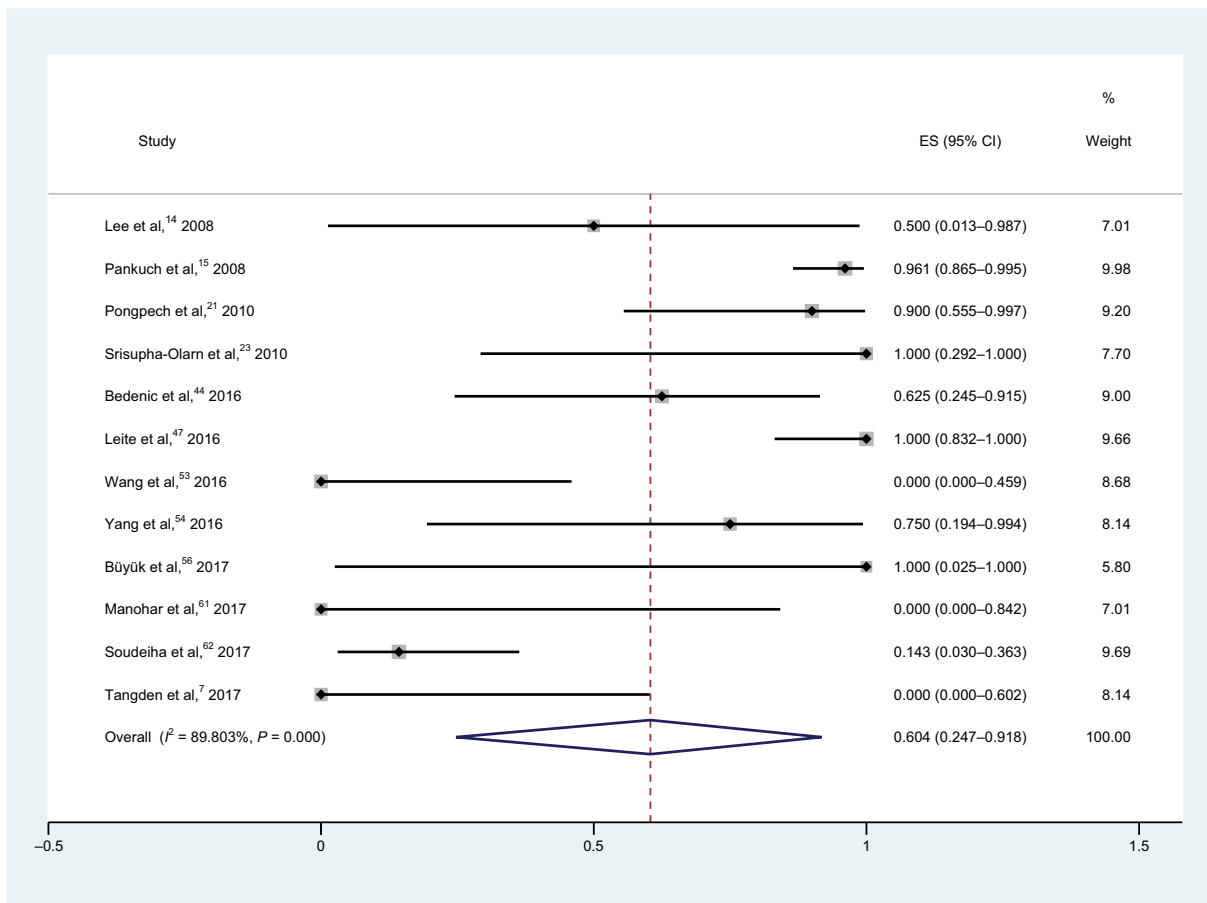


Figure 4 Forest plot and pooled synergy rates for meropenem–colistin combinations in time-kill method.
Abbreviation: ES, effect size.

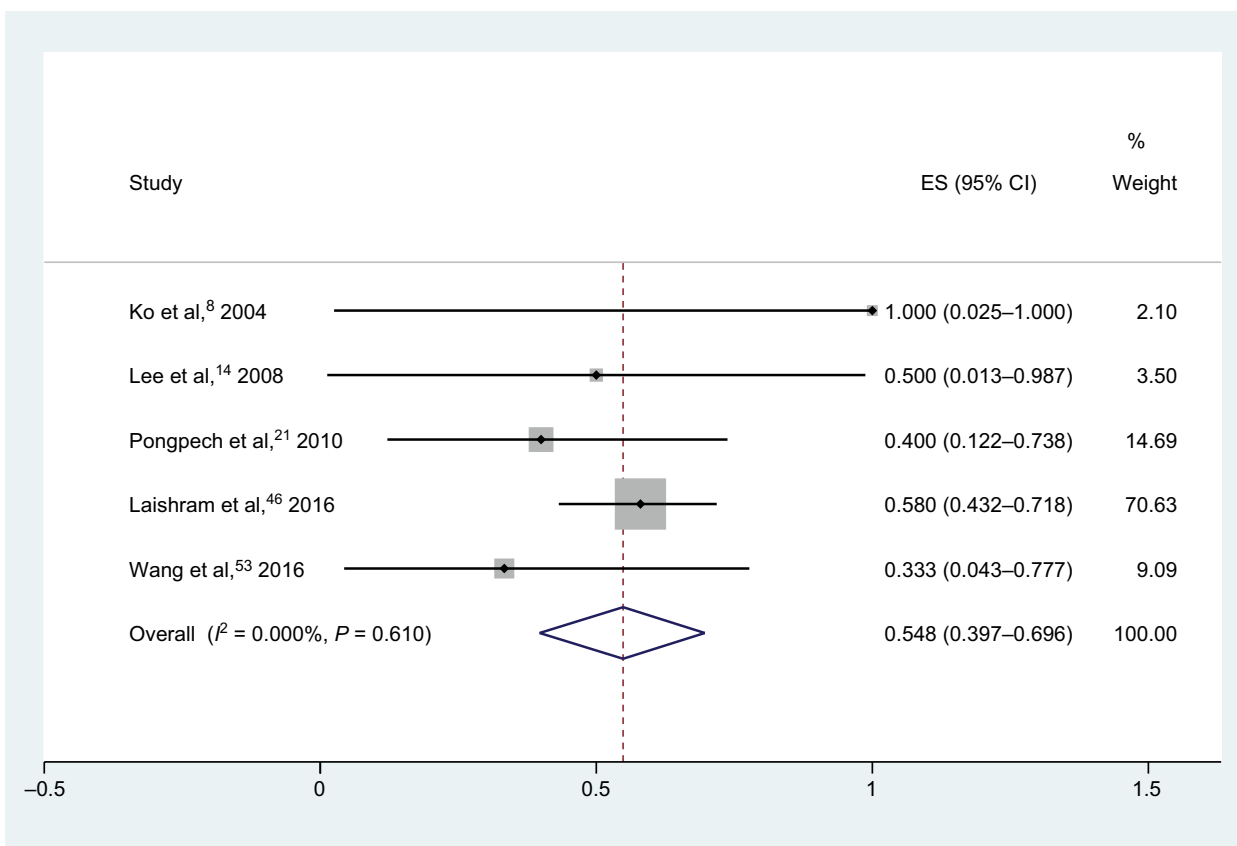


Figure 5 Forest plot and pooled synergy rates for meropenem–sulbactam combinations in time-kill method.

Abbreviation: ES, effect size.

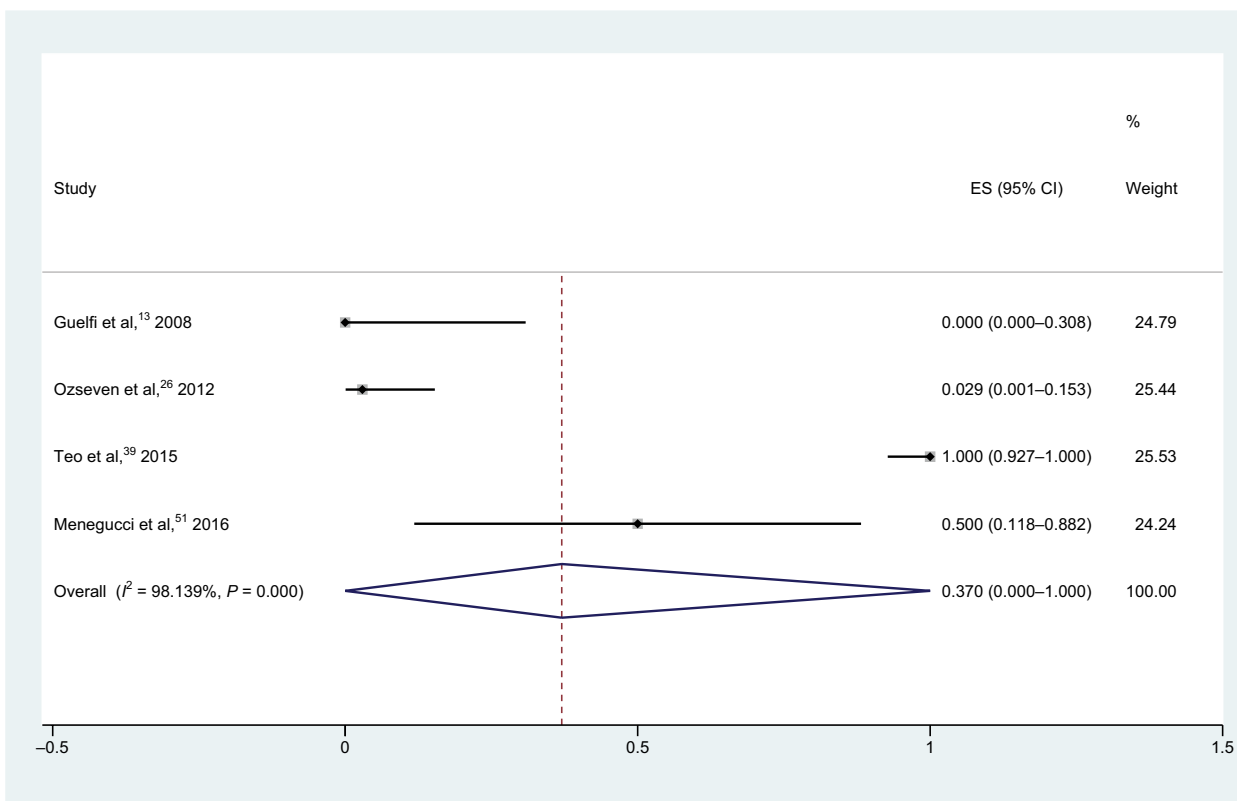


Figure 6 Forest plot and pooled synergy rates for meropenem–polymyxin B combinations in checkerboard method.

Abbreviation: ES, effect size.

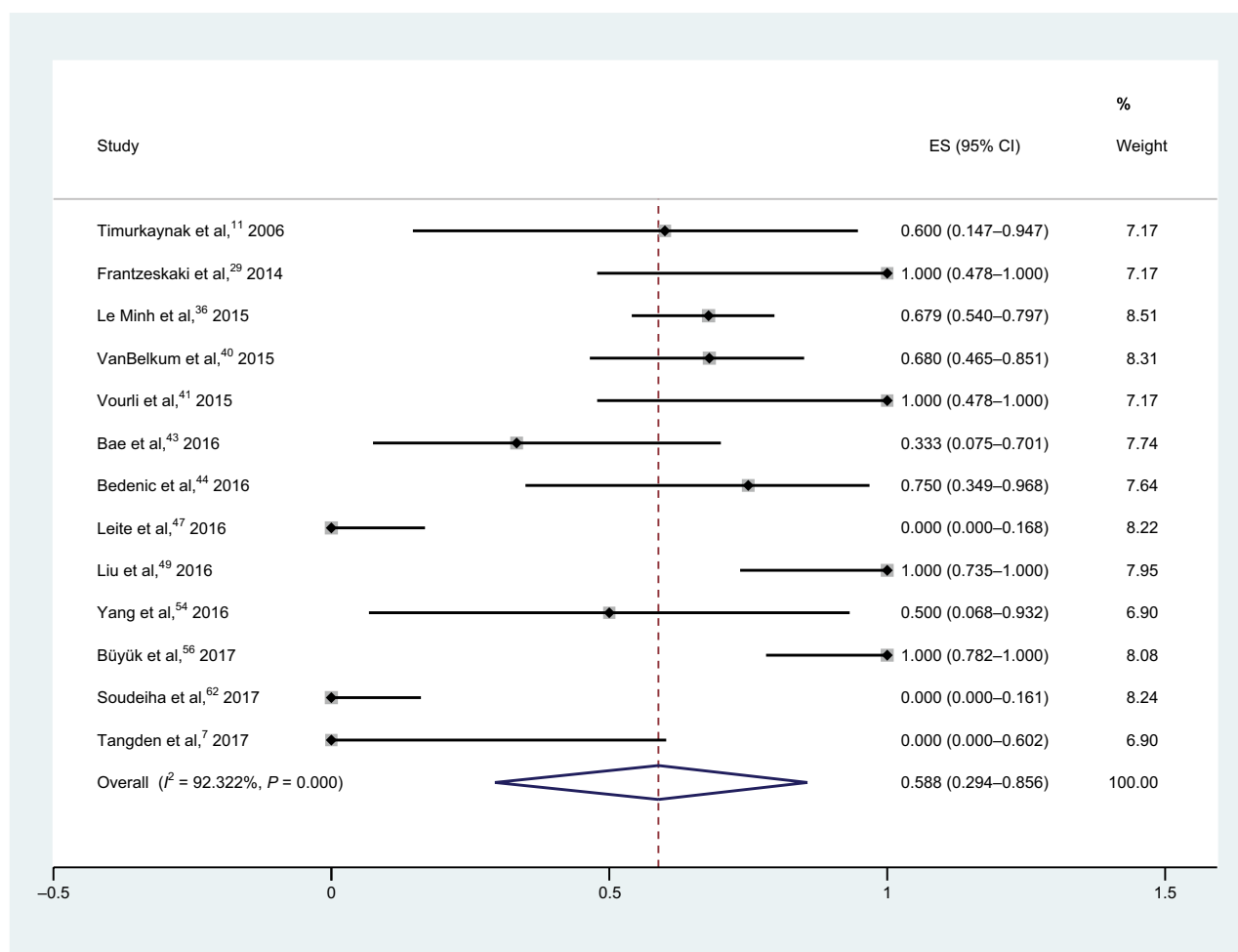


Figure 7 Forest plot and pooled synergy rates for meropenem–colistin combinations in checkerboard method.
Abbreviation: ES, effect size.

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

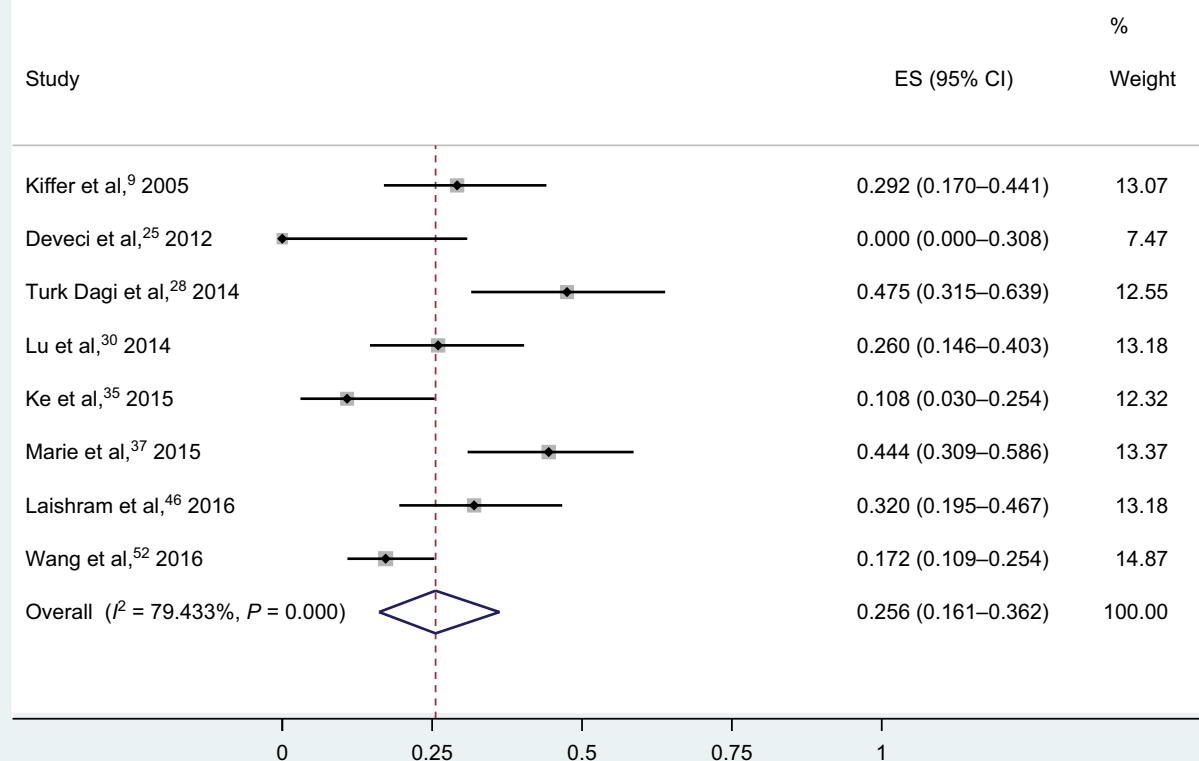


Figure 8 Forest plot and pooled synergy rates for meropenem–sulbactam combinations in checkerboard method.
Abbreviation: ES, effect size.

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.

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Disclosure

The authors report no conflicts of interest in this work.

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