International Journal of Antimicrobial Agents 57 (2021) 106344

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: www.elsevier.com/locate/ijantimicag

Review

Systematic review and meta-analysis of in vitro efficacy of antibiotic combination therapy against carbapenem-resistant Gram-negative bacilli

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ARTICLE INFO

Article history:
Received 20 January 2021
Accepted 3 April 2021

Editor: Dr Jim Gray

Keywords:
In vitro synergy
Antibiotic combination
PK/PD
Time–kill
Carbapenem-resistant bacteria

ABSTRACT

The superiority of combination therapy for carbapenem-resistant Gram-negative bacilli (CR-GNB) infections remains controversial. In vitro models may predict the efficacy of antibiotic regimens against CR-GNB. A systematic review and meta-analysis was performed including pharmacokinetic/pharmacodynamic (PK/PD) and time–kill (TK) studies examining the in vitro efficacy of antibiotic combinations against CR-GNB [PROSPERO registration no. CRD42019128104]. The primary outcome was in vitro synergy based on the effect size (ES): high, ES $\geq$ 0.75, moderate, 0.35 < ES < 0.75; low, ES $\leq$ 0.35; and absent, ES = 0). A network meta-analysis assessed the bactericidal effect and re-growth rate (secondary outcomes). An adapted version of the ToxRTool was used for risk-of-bias assessment. Over 180 combination regimens from 136 studies were included. The most frequently analysed classes were polymyxins and carbapenems. Limited data were available for ceftazidime/avibactam, ceftolozane/tazobactam and imipenem/relebactam. High or moderate synergism was shown for polymyxin/rifampicin against *Acinetobacter baumannii* [ES = 0.91, 95% confidence interval (CI) 0.44–1.00], polymyxin/fosfomycin against *Klebsiella pneumoniae* (ES = 1.00, 95% CI 0.66–1.00) and imipenem/amikacin against *Pseudomonas aeruginosa* (ES = 1.00, 95% CI 0.21–1.00). Compared with monotherapy, increased bactericidal activity and lower re-growth rates were reported for colistin/fosfomycin and polymyxin/rifampicin in *K. pneumoniae* and for imipenem/amikacin or imipenem/tobramycin against *P. aeruginosa*. High quality was documented for 65% and 53% of PK/PD and TK studies, respectively. Well-designed in vitro studies should be encouraged to guide the selection of combination therapies in clinical trials and to improve the armamentarium against carbapenem-resistant bacteria.

1. Introduction

The rapid emergence and dissemination of multidrug-resistant (MDR) Gram-negative bacilli (GNB) is recognised as a major public

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https://doi.org/10.1016/j.ijantimicag.2021.106344

health issue [1,2]. Treatment options against carbapenem-resistant (CR) GNB remain limited [3]. To direct research and development of new antibiotics, in 2017 the World Health Organization (WHO) published a list of pathogens prioritising CR *Acinetobacter baumannii*, CR *Pseudomonas aeruginosa* and CR Enterobacteriaceae [4]. Novel compounds displaying in vitro activity against CR-GNB have been mainly tested in clinical trials, often including carbapenem-susceptible bacteria [3]. Other antibiotic classes (e.g. polymyxins, carbapenems, aminoglycosides) have been used against CR-GNB alone or in combination in observational studies [5,6]. The rationale for combining two or more antibiotics against CR-GNB is based on the possibility to achieve a higher rate of bacterial killing and to reduce the development of resistance. Despite promising results highlighted by some studies, pooled data from meta-analyses have not shown clear evidence to support the use of antibiotic combinations in the treatment of CR-GNB infections [5–8]. Moreover, results from well-designed clinical trials including infections by MDR-GNB are lacking, limiting the evidence on the effectiveness of older compared with novel compounds.

The COHERENCE project (COmbination tHErapy to treat sepsis due to carbapenem-Resistant bacteria in adult and pediatric populations: EvideNCE and common practice) aimed to coherently and comprehensively analyse data on the use of antibiotic combinations for treating severe infections caused by CR-GNB. The project was commissioned by the Global Antibiotic Research and Development Partnership (GARDP) and was intended to have a global perspective from real-world clinical practice assessment of published evidence from in vitro and clinical studies. The present article reports data deriving from the meta-analyses performed on in vitro studies.

In vitro assessments of bacterial killing and antibiotic synergism can be used to support the effectiveness of antibiotic combinations against MDR-GNB [9]. A recent meta-analysis including 26 clinical cases from 11 reports showed that synergy-guided antibiotic combination therapy against MDR-GNB (54% *P. aeruginosa*, 27% Enterobacteriaceae and 19% *A. baumannii*) was significantly associated with survival [odds ratio (OR) = 0.44, 95% confidence interval (CI) 0.20–0.98] [10].

There are currently only two meta-analyses assessing the effectiveness of antibiotic combinations against CR GNB, including in vitro studies up to March 2013 and July 2014, respectively [11,12]. These reviews, however, restricted the search only to selected pathogens and to a limited number of antibiotic dosages and combinations.

The aim of this systematic review and meta-analysis was to evaluate the in vitro activity of all available antibiotic combinations and dosages against CR (or carbapenemase-producing) strains of *A. baumannii, P. aeruginosa* and Enterobacteriaceae from time–kill (TK) and pharmacokinetic/pharmacodynamic (PK/PD) studies.

2. Methods

This review is part of the broader COHERENCE study supported by GARDP, which also includes evidence on combination therapy to treat CR-GNB infections from in vivo and human studies.

2.1. Search strategy

This study was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [13]. This systematic review is registered with the PROSPERO international prospective register of systematic reviews (www.crd.york.ac.uk/PROSPERO/; registration no. CRD42019128104).

We searched PubMed, Scopus and Web of Science databases for publications in any language from inception until 31 December 2018. All search strings were discussed with a qualified librarian. Details of the bibliographic search strategy are listed in the Supplementary methods. Bibliographies of reviews and original publications were hand-searched for further studies. To reduce publication bias, the Infectious Diseases Society of America (IDSA) and European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) conference proceedings for the years 2016–2018 were also reviewed.

2.2. Selection criteria

Reports including data of bacterial killing curves from PK/PD and TK studies analysing combination therapies against CR-GNB were included. Any type of study except reviews, editorials and protocol papers was eligible for inclusion, and all antibiotic dosage schedules and frequencies were considered. Standard inoculum sizes (4×10^5 CFU/mL or the nearest available value) were selected [14]. Gold-standard broth microdilution was considered as the susceptibility testing method.

2.3. Data extraction

Two investigators (MC and DB) independently assessed each potentially relevant study for eligibility. Disagreements were resolved by consultation with a third party (ER). If eligibility could not be determined, the full article was retrieved.

A standardised data extraction method was used to record relevant features of each study in a database, including study characteristics (year of publication, country, type of in vitro combination testing), bacterial characteristics (type of bacterial strain and number of isolated tested, method of antimicrobial susceptibility testing and carbapenemase identification) and antimicrobial therapy (type and dose of antibiotic administration, treatment duration). Bacterial isolates were considered resistant to carbapenems according to the local interpretive criteria. Unless otherwise specified, resistance was determined according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2019 breakpoints [15]. Combination therapy was defined as the association of at least two or more antibiotics used to treat CR-GNB.

Primary outcome assessed was in vitro synergy or antagonism of combination therapy defined as a >2 $\log_{10}$ reduction or increase, respectively, in CFU/mL for a combination compared with the most active single agent on bacterial kill or inhibition. Secondary outcomes included: (i) bactericidal rates, defined as a >3 $\log_{10}$ reduction in CFU/mL compared with pre-treatment counts; and (ii) regrowth rates, defined as ≥ 2 $\log_{10}$ CFU/mL decrease of the initial colony count followed by an increase of ≥ 1 $\log_{10}$ CFU/mL at two subsequent timepoints (12 h and 24 h). In TK analyses reporting multiple carbapenem doses tested within the same study, only relevant carbapenem concentrations (e.g. at least twice the resistance breakpoint for carbapenems) were included to limit heterogeneity. Studies testing single bacterial strains were excluded.

2.4. Quality assessment

An adapted version of the ToxRTool to assess in vitro studies was generated to assess the risk of bias of the included studies [16]. Details of the quality assessment are reported in the Supplementary material. In summary, studies were assigned to four categories according to their relevance and scored 0–18 points (reliable without restrictions, 15–18 points; reliable with restrictions, 11–14 points, not reliable, <11 points; not assignable, if insufficient documentation to assess the study was provided). Studies with a score of ≥ 11 points were classified as high quality.

2.5. Data analysis

We calculated each outcome separately for bacterial species, antibiotic treatment, dose and in vitro testing method. Synergy or antagonism, bactericidal rates and re-growth rates were counted as events (e.g. number of isolates showing the outcome) and the sample size was the number of isolates tested. In each study, pooled analysis of bacterial strains from the same species was performed.

For primary outcomes, pooled proportions and 95% CI of synergistic or antagonist strains were calculated in random-effect models. Due to the large number of studies showing extreme magnitude (0% and 100%) of synergy, bactericidal rates and re-growth rates, the pooled estimate was calculated after Freeman–Tukey double-arcsine transformation to stabilise the variances. CIs were based on score procedures. When applicable, the I^2 statistic was used to test heterogeneity (low, 0–0.25; fair, 0.25–0.5; moderate, 0.5–0.75; and high, >0.75). In case of high heterogeneity, the pooled effect was disregarded and not interpreted. Pooled synergy or antagonism rate was defined based on the effect size (ES) as follows: high, ES $\geq$ 0.75; moderate, 0.35 < ES < 0.75; low, ES $\leq$ 0.35; or absence of synergy, ES = 0. Positive trends were reported for synergistic combination regimens showing no significant 95% CI.

While synergy and antagonism intrinsically compare a combination therapy with its monotherapy, assessment of secondary outcomes may involve the comparison of multiple combinations with different monotherapies. For this reason, a network meta-analysis (NMA) was performed to assess bactericidal and re-growth rates [17]. Connected networks were identified for monotherapies and the corresponding combinations for bactericidal rates (proportion of strains showing bactericidal rates over the total number of strains tested) and re-growth rates (proportion of strains showing re-growth over the total number of strains tested). When applicable, inconsistency was reported. The surface under the cumulative ranking (SUCRA) curve was used for ranking different treatments [18]. Analyses were performed separately for PK/PD and TK studies. The results were clustered by study quality. Publication bias was not assessed. Stata Statistical Software: Release 16 (StataCorp LLC, College Station, TX, USA) was used for analysis.

2.6. Role of the funding source

GARDP supported the entire project, and GARDP employees contributed to study design, data interpretation and drafting the report. All authors had full access to data and had final responsibility to submit for publication.

3. Results

Our search yielded 6849 articles, including 6559 from databases, 100 from conference proceedings and 190 from bibliographies of reviews and original publications. A total of 439 full-texts were assessed and 136 studies (94 TK and 42 PK/PD) were included (Fig. 1) [19–154]. The main characteristics of all included studies and their quality assessment are presented in Supplementary Tables S1a and S1b.

3.1. Type of bacteria and antibiotic treatment

A total of 42 studies reported data on PK/PD (including 24 two-compartment and 18 one-compartment models) and 94 studies on TK; 10 studies reported data on both methods.

A total of 56 studies (43 TK and 13 PK/PD) reported data on *A. baumannii*, 54 (36 TK and 18 PK/PD) on *Klebsiella pneumoniae* and 31 (23 TK and 8 PK/PD) on *P. aeruginosa*. We also retrieved 25 studies (6 PK/PD and 19 TK) on Enterobacteriaceae other than *K. pneumoniae*. However, the data were not sufficient to perform a meta-analysis.

A total of 182 different combination regimens were tested in TK studies: 172 (95%) and 10 (5%) were double and triple combination regimens, respectively. In PK/PD studies, 21 antibiotic agents belonging to 12 classes were combined in 41 different treatments: 37 (90%) and 4 (10%) were combinations of two or three antibiotics, respectively. Table 1 summarises the most common combination regimens tested in TK and PK/PD studies. The complete list of antibiotic combinations and the type of outcome assessed for each pathogen is reported in Supplementary Table S2.

High-quality assessment was shown by 73 studies (54%) and was higher for PK/PD (65%) compared with TK (53%) studies.

3.2. Meta-analysis

Synergy rates for combination treatments were assessed by type of bacteria (e.g. *A. baumannii, K. pneumoniae* and *P. aeruginosa*) and study type (TK or PK/PD). No antagonism was detected for any treatment among the assessed studies.

3.2.1. Acinetobacter baumannii

Table 2 summarises the results for *A. baumannii.* Data from TK studies showed high synergy rates for the following combinations: meropenem and polymyxin B or colistin [ES = 0.82 (95% CI 0.23–1.00) and ES = 0.87 (95% CI 0.48–1.00), respectively], colistin and tigecycline (ES = 0.89, 95% CI 0.61–1.00) and colistin and rifampicin (ES = 0.75, 95% CI 0.41–0.99). High synergy rates were reported from single TK studies for combination of colistin and trimethoprim/sulfamethoxazole (ES = 1.00, 95% CI 0.51–1.00), polymyxin B and amikacin (ES = 1.00, 95% CI 0.34–1.00) and imipenem and tigecycline (ES = 0.80, 95% CI 0.38–0.96). PK/PD studies showed high synergy rates for colistin and rifampicin (ES = 0.91, 95% CI 0.44–1.00) and imipenem and tobramycin (ES = 1.00, 95% CI 0.38–1.00) combinations.

3.2.2. Klebsiella pneumoniae

The results for *K. pneumoniae* are reported in Table 3. TK studies showed high synergy rates for colistin and rifampicin (ES = 1.00, 95% CI 0.95–1.00) and for combination of polymyxin B and imipenem (ES = 1.00, 95% CI 0.67–1.00) or doripenem (ES = 1.00, 95% CI 0.51–1.00). High synergy rates were shown for carbapenem and aminoglycoside combination, specifically imipenem and amikacin (ES = 1.00, 95% CI 0.51–1.00) and meropenem and amikacin (ES = 1.00, 95% CI 0.51–1.00). In PK/PD studies, high synergy rates were displayed by combination of ceftazidime/avibactam and aztreonam (ES = 1.00, 95% CI 0.21–1.00) and polymyxin B and fosfomycin (ES = 1.00, 95% CI 0.66–1.00).

3.2.3. Pseudomonas aeruginosa

Table 4 summarises the results for *P. aeruginosa*. TK studies showed moderate synergy rates for combination of ceftolozane/tazobactam and colistin (ES = 0.50, 95% CI 0.15–0.85), colistin and imipenem (ES = 0.67, 95% CI 0.08–1.00) and meropenem and amikacin (ES = 0.43, 95% CI 0.31–0.55). Similar rates were shown for combination of colistin and meropenem and combination of tobramycin and imipenem. High synergy rates were shown for combination of imipenem and amikacin (ES = 1.00, 95% CI 0.21–1.00), while moderate synergy was reported for combination of colistin and doripenem in PK/PD studies.

3.3. Sensitivity analysis

Sensitivity analysis was performed on high-quality studies (defined as reliable, with or without restriction) showing ToxRTool score $\geq$11.

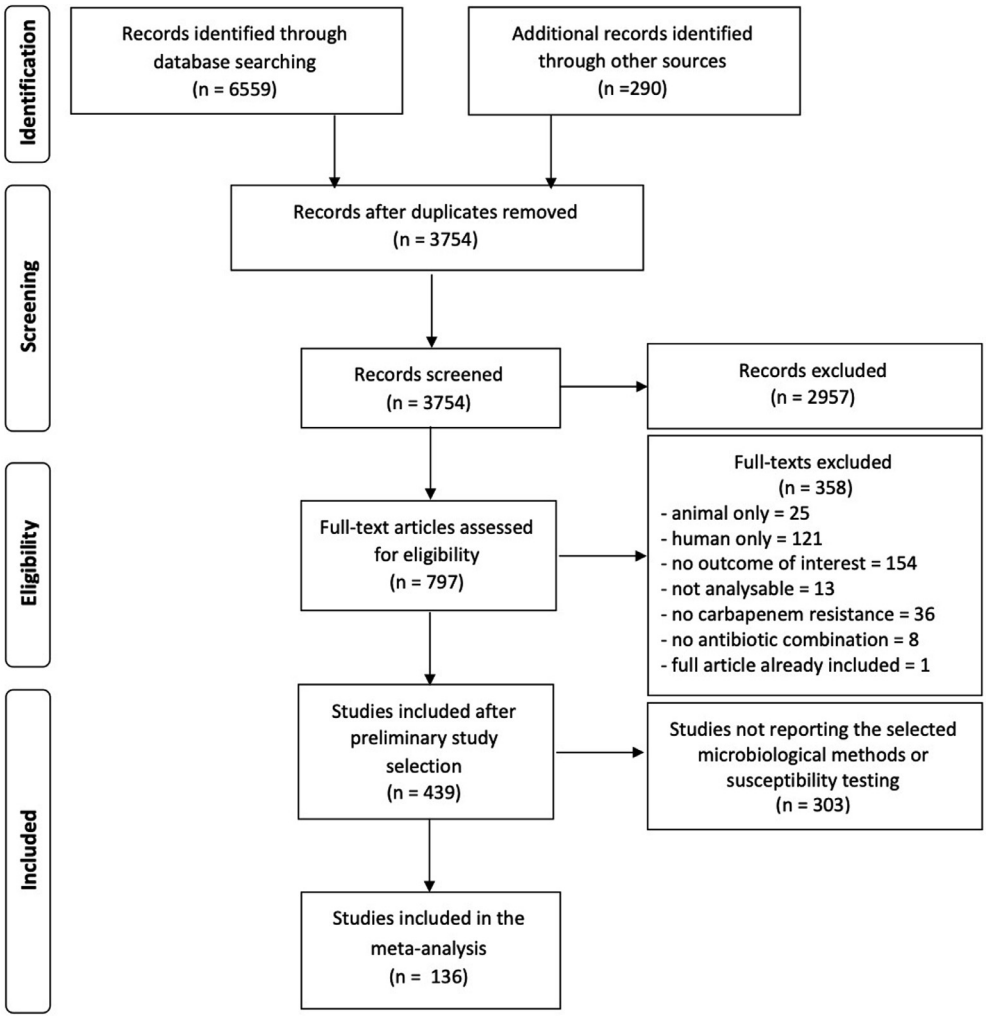

Fig. 1. PRISMA flow diagram of included studies.

Table 1
Most commonly analysed antibiotic combinations for time–kill (TK) and pharmacokinetic/pharmacodynamic (PK/PD) studies

Bacterium/antibiotic combination	TK study	PK/PD study	Total of studies
Acinetobacter baumannii			
Polymyxins + carbapenems	42	7	49
Polymyxins + rifampicin	20	1	21
Carbapenems + rifampicin	14	0	14
Polymyxins + tigecycline	13	2	15
Carbapenems + sulbactam	13	3	16
Total	102	13	115
Klebsiella pneumoniae			
Polymyxins + carbapenems	52	3	55
Double carbapenem	26	1	27
Polymyxins + rifampicin	17	1	18
Polymyxins + fosfomycin	6	5	11
Polymyxins + tigecycline	7	1	8
Total	108	11	119
Pseudomonas aeruginosa			
Carbapenems + aminoglycosides	25	2	27
Carbapenems + fluoroquinolones	22	1	23
Fluoroquinolones + cephalosporins	18	0	18
Polymyxins + carbapenems	8	3	11
Fluoroquinolones + aminoglycosides	10	0	10
Total	83	6	89

Table 2
In vitro synergy of antibiotic combinations against *Acinetobacter baumannii* assessed by pharmacokinetic/pharmacodynamic (PK/PD) and time–kill (TK) studies

Antibiotic regimen	Assay	No. of strains	No. of studies	No. of tests	ES	95% CI	Synergy rate
Colistin + meropenem	PK/PD	3	1	4	0.40	0.00–0.95	Positive trend
Colistin + rifampicin	PK/PD	2	1	4	0.91	0.44–1.00	High
Colistin + tigecycline	PK/PD	4	1	2	0.63	0.24–0.95	Moderate
Imipenem + tobramycin	PK/PD	1	1	3	1.00	0.38–1.00	High
Meropenem + amikacin	PK/PD	1	1	1	0.00	0.00–0.79	No synergy
Polymyxin B + tigecycline	PK/PD	4	1	2	0.08	0.00–0.43	Positive trend
Colistin + ampicillin/sulbactam	TK	2	1	1	0.00	0.00–0.66	No synergy
Colistin + ampicillin/sulbactam + rifampicin	TK	2	1	1	0.50	0.09–0.91	Moderate
Colistin + doripenem	TK	16	2	3	0.39	0.00–0.94	Positive trend
Colistin + imipenem	TK	10	2	2	0.39	0.07–0.76	Moderate
Colistin + meropenem	TK	24	3	4	0.87	0.48–1.00	High
Colistin + rifampicin	TK	24	5	7	0.75	0.41–0.99	High
Colistin + sulbactam	TK	18	1	1	0.56	0.34–0.75	Moderate
Colistin + tigecycline	TK	6	1	2	0.89	0.61–1.00	High
Colistin + trimethoprim/sulfamethoxazole	TK	4	1	1	1.00	0.51–1.00	High
Doripenem + amikacin	TK	8	1	1	0.13	0.02–0.47	Low
Doripenem + sulbactam	TK	18	1	1	0.28	0.12–0.51	Low
Imipenem + rifampicin	TK	4	2	3	0.72	0.00–1.00	Positive trend
Imipenem + tigecycline	TK	5	1	1	0.80	0.38–0.96	High
Meropenem + ampicillin/sulbactam	TK	2	1	1	0.50	0.09–0.91	Moderate
Meropenem + aztreonam	TK	5	1	1	0.00	0.00–0.43	No synergy
Polymyxin B + amikacin	TK	2	1	1	1.00	0.34–1.00	High
Polymyxin B + ampicillin/sulbactam	TK	2	1	1	0.00	0.00–0.66	No synergy
Polymyxin B + imipenem	TK	2	1	1	0.50	0.09–0.91	Moderate
Polymyxin B + meropenem	TK	4	2	2	0.82	0.23–1.00	High
Polymyxin B + meropenem + ampicillin/sulbactam	TK	2	1	1	0.50	0.09–0.91	Moderate
Polymyxin B + meropenem + rifampicin	TK	2	1	1	0.50	0.09–0.91	Moderate
Polymyxin B + rifampicin	TK	33	2	2	0.53	0.32–0.73	Moderate
Polymyxin B + tigecycline	TK	33	2	2	0.34	0.16–0.55	Low

ES, effect size; CI, confidence interval.
NOTE: Pooled synergy or antagonism rate was defined based on the ES as follows: high, ES $\geq$ 0.75; moderate, 0.35 $<$ ES $<$ 0.75; low, ES $\leq$ 0.35; and absence of synergy, ES = 0. Positive trends were reported for synergistic combination regimens showing no significant 95% CI.

Table 3
In vitro synergy of antibiotic combinations against *Klebsiella pneumoniae* assessed by pharmacokinetic/pharmacodynamic (PK/PD) and time–kill (TK) studies

Antibiotic regimen	Assay	No. of strains	No. of studies	No. of tests	ES	95% CI	Synergy rate
Ceftazidime/avibactam + amikacin	PK/PD	3	1	1	0.33	0.06–0.79	Low
Ceftazidime/avibactam + aztreonam	PK/PD	1	1	1	1.00	0.21–1.00	High
Colistin + doripenem	PK/PD	1	1	4	0.50	0.00–1.00	Positive trend
Colistin + fosfomycin	PK/PD	8	3	5	0.58	0.28–0.86	Moderate
Polymyxin B + fosfomycin	PK/PD	4	2	4	1.00	0.66–1.00	High
Meropenem + tigecycline	PK/PD	5	1	1	0.40	0.12–0.77	Moderate
Ceftazidime/avibactam + colistin	TK	16	1	1	0.25	0.10–0.49	Low
Colistin + doripenem	TK	62	5	6	0.50	0.28–0.71	Moderate
Colistin + ertapenem	TK	9	1	2	0.38	0.10–0.70	Moderate
Colistin + fosfomycin	TK	2	1	21	0.60	0.41–0.78	Moderate
Colistin + gentamicin	TK	26	2	2	0.31	0.14–0.50	Low
Colistin + meropenem	TK	9	2	2	0.12	0.00–0.46	No synergy
Colistin + meropenem + tigecycline	TK	6	1	1	0.00	0.00–0.39	No synergy
Colistin + rifampicin	TK	25	2	2	1.00	0.95–1.00	High
Colistin + tigecycline	TK	10	2	3	0.42	0.00–0.98	Positive trend
Colistin + tobramycin	TK	4	1	2	0.37	0.05–0.76	Moderate
Doripenem + ertapenem	TK	12	1	1	0.00	0.00–0.24	No synergy
Doripenem + gentamicin	TK	26	2	2	0.15	0.03–0.32	Low
Imipenem + amikacin	TK	4	1	1	1.00	0.51–1.00	High
Meropenem + amikacin	TK	4	1	1	1.00	0.51–1.00	High
Meropenem + ertapenem	TK	21	1	1	0.43	0.24–0.63	Moderate
Meropenem + gentamicin	TK	13	1	1	0.00	0.00–0.23	No synergy
Meropenem + tigecycline	TK	13	1	1	0.00	0.00–0.23	No synergy
Meropenem + tigecycline + gentamicin	TK	13	1	1	0.00	0.00–0.23	No synergy
Polymyxin B+ doripenem	TK	1	1	4	1.00	0.51–1.00	High
Polymyxin B + imipenem	TK	2	1	3	1.00	0.67–1.00	High
Polymyxin B + meropenem	TK	25	6	50	0.45	0.36–0.53	Moderate

ES, effect size; CI, confidence interval.
NOTE: Pooled synergy or antagonism rate was defined based on the ES as follows: high, ES $\geq$ 0.75; moderate, 0.35 $<$ ES $<$ 0.75; low, ES $\leq$ 0.35; and absence of synergy, ES = 0. Positive trends were reported for synergistic combination regimens showing no significant 95% CI.

Table 4
In vitro synergy of antibiotic combinations against *Pseudomonas aeruginosa* assessed by pharmacokinetic/pharmacodynamic (PK/PD) and time–kill (TK) studies

Antibiotic regimen	Assay	No. of strains	No. of studies	No. of tests	ES	95% CI	Synergy rate
Ceftazidime/avibactam + amikacin	PK/PD	3	1	1	0.33	0.06–0.79	Low
Colistin + doripenem	PK/PD	3	2	6	0.57	0.03–1.00	Moderate
Imipenem + amikacin	PK/PD	1	1	2	1.00	0.21–1.00	High
Ceftolozane/tazobactam + colistin	TK	4	1	1	0.50	0.15–0.85	Moderate
Ceftolozane/tazobactam + aztreonam	TK	4	1	1	0.00	0.00–0.49	No synergy
Ceftolozane/tazobactam + amikacin	TK	4	1	1	0.00	0.00–0.49	No synergy
Colistin + imipenem	TK	2	1	2	0.67	0.08–1.00	Moderate
Colistin + meropenem	TK	7	1	4	0.43	0.16–0.75	Moderate
Imipenem + amikacin	TK	87	4	7	0.35	0.23–0.47	Low
Imipenem + tobramycin	TK	2	1	8	0.39	0.04–0.80	Moderate
Meropenem + amikacin	TK	63	2	1	0.43	0.31–0.55	Moderate

ES, effect size; CI, confidence interval.
NOTE: Pooled synergy or antagonism rate was defined based on the ES as follows: high, ES $\geq$ 0.75; moderate, 0.35 $<$ ES $<$ 0.75; low, ES $\leq$ 0.35; and absence of synergy, ES = 0. Positive trends were reported for synergistic combination regimens showing no significant 95% CI.

3.3.1. Acinetobacter baumannii

Synergy was displayed with the combination of a polymyxin plus a carbapenem in four high-quality TK studies assessing polymyxin B/meropenem combination with or without rifampicin [39], colistin/meropenem [61], colistin/imipenem [71] and colistin/doripenem [96]. These results were not confirmed for combination of colistin and meropenem in one PK/PD study [47]. Synergy rates between 0.68 and 0.91 were confirmed for colistin and rifampicin combination both in PK/PK and TK studies [71,105,113], while colistin and polymyxin B in combination with tigecycline showed conflicting results [36,54]. High synergy rates were confirmed for imipenem plus tobramycin and colistin plus trimethoprim/sulfamethoxazole [38,89].

3.3.2. Klebsiella pneumoniae

Moderate and high synergy rates were displayed with the combination of polymyxins and fosfomycin in one TK and five PK/PD studies [21,27,28,32,40]. Polymyxin/rifampicin and polymyxin/carbapenem combinations showed, respectively, high and moderate-high synergy rates in good-quality TK studies [25,32,33,61,63,84–86,92,95,105,144,143,120].

3.3.3. Pseudomonas aeruginosa

Combination of colistin and doripenem was evaluated in two PK/PD studies [45,124]. Colistin combined with imipenem was assessed in one good-quality TK study [124]. Synergy rates were above 0.50 for both combinations. Other combinations were evaluated in good-quality PK/PD studies confirming the previous results [19,49].

3.4. Network meta-analysis

The list of antibiotic combinations tested for bactericidal activity and re-growth in PK/PD and TK studies are reported in Supplementary Table S2.

3.4.1. Acinetobacter baumannii

Data were available to perform NMAs in 10 and 19 combination regimens from PK/PD and TK studies, respectively, including polymyxin-based and doripenem-based combinations. No overall heterogeneity was identified in pairwise comparison. Significant differences in bactericidal activity between combinations and monotherapies were not shown, although in PK/PD studies a positive trend was displayed by combination of colistin and tigecycline (SUCRA = 0.8). No evidence of significant inconsistency was found. No statistically significant differences in re-growth rates were found for antibiotic combinations tested against CR *A. baumannii*.

3.4.2. Klebsiella pneumoniae

NMAs were performed to evaluate bactericidal rates in 9 and 30 combination regimens from PK/PD and TK studies, respectively. No significant heterogeneity was identified in pairwise comparison for bactericidal rates.

Significantly higher bactericidal rates were shown by polymyxin-based combinations, specifically for polymyxin B/rifampicin ($P < 0.05$) and colistin/gentamicin ($P < 0.05$) compared with a polymyxin used as monotherapy in TK studies. Carbapenem-based combinations did not show significant differences in bactericidal activity compared with monotherapy, however a higher bactericidal trend was displayed by polymyxin B plus imipenem (cumulative probability best 36.8%–SUCRA = 0.8), colistin plus ertapenem (SUCRA = 0.9) and imipenem/relebactam plus amikacin (SUCRA = 0.9). In PK/PD studies, colistin-based combinations did not present significant differences in bactericidal activity compared with colistin alone. No evidence of significant inconsistency was found. In TK studies, significant difference in re-growth rate ($P < 0.05$) was found between colistin plus fosfomycin and monotherapies at 24 h, confirmed by a lower re-growth trend at 12 h in PK/PD studies for both polymyxin B and colistin plus fosfomycin (SUCRA = 0.8). Significant differences in re-growth rates ($P < 0.05$) at 24 h were found for polymyxin B plus meropenem compared with the combination of polymyxin B plus rifampicin and for ceftazidime/avibactam compared with colistin alone in TK studies.

In TK studies, re-growth rates were significantly lower ($P < 0.05$) at 24 h for colistin and fosfomycin compared with colistin and fosfomycin used as monotherapies, for polymyxin B and meropenem compared with polymyxin B and rifampicin combination and for ceftazidime/avibactam compared with colistin monotherapy. Lower re-growth trend at 12 h was shown in PK/PD studies for both polymyxin B and colistin plus fosfomycin (SUCRA = 0.8).

3.4.3. Pseudomonas aeruginosa

Bactericidal rates were analysed for three colistin-based and three carbapenem-based combination regimens in PK/PD and TK studies, respectively.

TK studies showed that combination of meropenem and amikacin or imipenem with either amikacin or tobramycin resulted in significantly higher bactericidal effect compared with monotherapy ($P < 0.05$). In PK/PD studies, colistin-based combination with doripenem, meropenem or ceftolozane/tazobactam resulted as effective as colistin monotherapy in terms of bactericidal effect. An increased bactericidal trend was displayed by combination of colistin and ceftolozane/tazobactam (SUCRA = 0.7). No evidence of significant inconsistency was found.

Statistically significant differences ($P < 0.05$) in re-growth rates were found for combination of imipenem and amikacin or tobramycin compared with monotherapies at 24 h in TK studies. A lower re-growth trend at 24 h in PK/PD studies was shown for meropenem plus tobramycin compared with monotherapies (SUCRA = 0.9).

4. Discussion

Only two meta-analyses reporting in vitro tests assessing antibiotic combinations against CR-GNB are currently available [11,12]. Nevertheless, these studies were limited to single micro-organisms and included only certain antibiotic combinations (e.g. polymyxins plus carbapenems) with no standardisation in the selection of the antibiotic dosage used. To our knowledge, this is the first systematic review and meta-analysis of in vitro studies analysing the effect of over 180 combination therapies against three different CR-GNB.

We selected in vitro methods that are based on killing curves, specifically TK and PK/PD studies. Both methods present advantages in studying the effectiveness of combination therapies. The TK assay is one of the most used, standardised and reproducible static methods for combination testing [119,155]. PK/PD models allow the investigation of bacterial killing and re-growth simulating human drug exposure and can be considered as an accurate and informative in vitro method for studying antibiotic combination regimens [156].

Our meta-analysis showed that colistin-based and carbapenem-based combinations were the most commonly assessed regimens for all bacteria tested. This result is in line with human studies testing various combinations of old antibiotics with in vitro activity against CR-GNB, often used in association with high-dose carbapenems [3].

Few randomised controlled trials (RCTs) have investigated combination treatments against CR *A. baumannii*. One RCT comparing colistin monotherapy with colistin and rifampicin in 210 critically ill patients showed similar 30-day mortality rates (43.3% vs. 42.9%) but increased microbiological eradication among those receiving combination therapy with rifampicin compared with monotherapy (61% vs. 45%; $P = 0.034$) [157]. Similarly, a small RCT including 94 patients with CR *A. baumannii* infections assessed the combination of colistin and fosfomycin compared with colistin monotherapy, showing increased microbiological eradication but similar clinical outcomes [158]. More recently, 406 patients with severe infections caused by CR-GNB (mainly *A. baumannii*) were treated with colistin combined with meropenem, showing similar clinical failure rates compared with colistin alone (73% vs. 79%) [159]. In our study, polymyxin-based combinations with rifampicin or tigecycline resulted in high or moderate synergy against *A. baumannii* both in PK/PD and TK studies, while combination with meropenem showed high or moderate synergy rates in TK studies and was confirmed moderate in two good-quality PK/PD studies including polymyxin B. Increased bactericidal activity was displayed by combination of colistin with tigecycline or imipenem in NMA, although the differences between various combination treatments and colistin alone were not significant. Not enough data were available to investigate treatments involving fosfomycin. Overall, no studies including antibiotics approved in the last 10 years were retrieved for *A. baumannii*.

Various observational studies have analysed double or triple combination regimens against CR *K. pneumoniae*, both including old antibiotics (e.g. tigecycline, polymyxins, aminoglycosides, fosfomycin and high-dose carbapenems) and novel compounds (e.g. ceftazidime/avibactam) administered as monotherapy or as part of combination treatments [3,5,6,160]. Furthermore, few RCTs are currently ongoing comparing polymyxin/carbapenem combination with polymyxin monotherapy in CR-GNB, including CR Enterobacteriaceae [161,162].

In our study, a polymyxin combined with rifampicin or fosfomycin resulted in high or moderate synergy rates against CR *K. pneumoniae*. Association of colistin and fosfomycin, in particular, showed increased bactericidal rates in PK/PD studies and lower re-growth rate at 24 h compared with colistin or fosfomycin monotherapy.

An increased bactericidal rate was also shown for colistin/rifampicin combination compared with monotherapy. Combination of a polymyxin with a carbapenem showed high or moderate synergy rates in good-quality TK studies. Furthermore, pooled data on bactericidal activity from TK studies showed that polymyxin B combined with meropenem was associated with higher killing rates compared with polymyxin B alone and significantly lower re-growth rates at 24 h compared with polymyxin B with rifampicin. Scant data were available for novel compounds that showed in vitro activity against CR *K. pneumoniae*, such as ceftazidime/avibactam and imipenem/relebactam. Although ceftazidime/avibactam was tested in combination with various antibiotics (e.g. polymyxin B, colistin, amikacin and aztreonam), the results were retrieved mainly from single in vitro studies accounting for a limited number of strains.

Colistin, usually in association with meropenem, has been employed to treat MDR *P. aeruginosa* [163]. The use of aminoglycosides also represents a valid alternative in combination therapy, although the high risk of renal toxicity usually limits the combination of an aminoglycoside with colistin in clinical practice [164]. Our review showed that the combination of imipenem with amikacin resulted in a high synergy rate in one good-quality PK/PD study. The combination of a carbapenem with an aminoglycoside demonstrated advantages in terms of bactericidal effect and re-growth rates compared with monotherapies. In terms of bactericidal effect, however, colistin used as monotherapy showed similar results compared with other colistin-based combinations. Data on recently approved antibiotics active against CR *P. aeruginosa* were limited. Synergy for combinations including ceftazidime/avibactam and ceftolozane/tazobactam were evaluated in single studies including PK/PD and both PK/PD and TK analyses, respectively.

While our systematic review highlighted promising results for selected in vitro antibiotic combinations (including polymyxin/tigecycline and polymyxin/rifampicin against *A. baumannii*, polymyxin/fosfomycin and polymyxin/rifampicin against *K. pneumoniae*, and combination of a carbapenem with an aminoglycoside or colistin against *P. aeruginosa*), it also emphasises several drawbacks of the in vitro studies analysed. Specifically, for several combinations, definitive conclusions could not be drawn due to the limited number of reports available assessing in vitro synergism and because of the limited comparability between the available reports. Very few high-quality TK and PK/PD studies have been performed on antibiotic combinations including recently marketed antibiotics that are often used in clinical practice. Even for combinations where a meta-analysis was performed, a generalisable interpretation was hampered by a high study variability.

Our study presents several limitations. First, the high variability in bacterial strains and resistance patterns both in TK and PK/PD studies may limit the generalisability of our results. Second, as previously highlighted by others, the use of different clinical breakpoints to interpret the susceptibility profiles represents an important limitation for the analysis of efficacy of different antibiotic regimens [12]. Third, although re-growth rates were assessed at 12 h and 24 h, the emergence of resistance occurring after 24 h was not analysed since not all studies included reported these data. Finally, the overall quality of TK studies was low. For example, only 39% of TK studies specified the number of repetitions of the experiments.

Due to the propensity of CR-GNB to acquire resistance to multiple antibiotics, support from in vitro studies is important to highlight potential synergies to test in human trials. A major advantage of in vitro studies, and in particular of PK/PD models, includes the possibility to tune the bacterial inoculum and to increase the study duration in order to explore effective antimicrobial regimens. The lack of verification by clinical data, however, remains the main limitation to interpret the significance of in vitro synergies. Furthermore, a correct interpretation of in vitro results and limitations by clinicians is paramount. Results from in vitro data, however, can inform on the potential use of existing methods to explore the efficacy of antibiotic combinations and can help the optimisation of synergy tests that may be of clinical interest also at individual- and strain-specific levels.

In conclusion, we analysed over 180 antibiotic combinations against CR-GNB, most frequently involving the polymyxin and carbapenem classes. Data on combination therapy including recently approved antibiotics with activity against CR-GNB (e.g. ceftazidime/avibactam and ceftolozane/tazobactam) were limited to single studies.

For *A. baumannii*, the most consistently reported synergism was shown by colistin/rifampicin combination both from PK/PK and TK studies, while synergism between a polymyxin with either a carbapenem or tigecycline was not always shown. The association of colistin with rifampicin remains promising and has previously been demonstrated to increase the microbiological clearance of *A. baumannii* in two human RCTs, although no impact on mortality was reported [157,158]. Polymyxin/rifampicin synergism was also shown against *K. pneumoniae*, although the benefit of this combination has not been assessed in a RCT for this pathogen. Regarding *K. pneumoniae*, fosfomycin represents a potential promising option to consider, showing not only synergism but also increased bactericidal activity in association with polymyxins. Finally, the association of an aminoglycoside with imipenem showed increased synergism (e.g. imipenem and amikacin) and bactericidal activity (e.g. imipenem/amikacin or imipenem/tobramycin) against *P. aeruginosa.* Although this combination is often used in clinical practice as empirical therapy in bloodstream infections, limitations are represented by aminoglycoside nephrotoxicity and their limited lung penetration.

Our results encourage the use of high-quality in vitro studies to explore potentially promising combination regimens to be used in clinical practice and to guide the selection of therapies in future RCTs in order to improve the armamentarium against CR-GNB.

Funding: This study was funded by the Global Antibiotic Research and Development Partnership (GARDP).

Competing interests: None declared.

Ethical approval: Not required.

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