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Systematic Review and Meta-Analysis of *In Vitro* Synergy of Polymyxins and Carbapenems

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Our objective was to examine the evidence of *in vitro* synergy of polymyxin-carbapenem combination therapy against Gram-negative bacteria (GNB). A systematic review and meta-analysis were performed. All studies examining *in vitro* interactions of antibiotic combinations consisting of any carbapenem with colistin or polymyxin B against any GNB were used. A broad search was conducted with no language, date, or publication status restrictions. Synergy rates, defined as a fractional inhibitory concentration index of ≤0.5 or a >2-log reduction in CFU, were pooled separately for time-kill, checkerboard, and Etest methods in a mixed-effect meta-analysis of rates. We examined whether the synergy rate depended on the testing method, type of antibiotic, bacteria, and resistance to carbapenems. Pooled rates with 95% confidence intervals (CI) are shown. Thirty-nine published studies and 15 conference proceeding were included, reporting on 246 different tests on 1,054 bacterial isolates. In time-kill studies, combination therapy showed synergy rates of 77% (95% CI, 64 to 87%) for *Acinetobacter baumannii*, 44% (95% CI, 30 to 59%) for *Klebsiella pneumoniae*, and 50% (95% CI, 30 to 69%) for *Pseudomonas aeruginosa*, with low antagonism rates for all. Doripenem showed high synergy rates for all three bacteria. For *A. baumannii*, meropenem was more synergistic than imipenem, whereas for *P. aeruginosa* the opposite was true. Checkerboard and Etest studies generally reported lower synergy rates than time-kill studies. The use of combination therapy led to less resistance development *in vitro*. The combination of a carbapenem with a polymyxin against GNB, especially *A. baumannii*, is supported *in vitro* by high synergy rates, with low antagonism and less resistance development. These findings should be examined in clinical studies.

Colistin is an antibiotic of the polymyxin family that demonstrated a resurgence in the past decade for the treatment of multidrug-resistant (MDR) Gram-negative bacteria (GNB) (1, 2). Its efficacy as monotherapy is probably inferior to that of beta-lactams (3). The clinical use of colistin is hindered by side effects, mainly nephrotoxicity (3, 4), in addition to unclear optimal dosing (5). In order to improve clinical success, various combination therapies have been used with colistin (6). One of the antibiotic classes most commonly used in combination with colistin is the carbapenems. The main rationale for this combination, as for other antimicrobial combinations, lies in the existence of *in vitro* synergy.

Several studies examined the *in vitro* interactions between carbapenems and colistin or polymyxin B, with various results. Heterogeneity in their results might be due to testing of different bacteria, different MICs of these bacteria for the various carbapenems and polymyxins, or different methods (e.g., checkerboard microdilution, time-kill, and Etest) used for combination studies. Moreover, since synergy studies are usually done in a specific center and on a limited number of bacterial isolates, the generalization of the data to other geographical areas or bacteria might not be possible.

This review aims to examine available data on the *in vitro* interactions of polymyxins and carbapenems. We aimed to examine whether *in vitro* combination interactions are affected by type of carbapenem or polymyxin, tested bacteria, and study methods.

MATERIALS AND METHODS

Data sources and study selection. We included studies examining the *in vitro* interactions of antibiotic combinations consisting of any carbapenem with colistin or polymyxin B against any GNB. All methods for *in vitro* combination assessment were eligible for inclusion. No language or year restrictions were applied.

PubMed was searched with the following search string: (colistin OR colisti* OR colistimethate OR polymyxin) AND (imipenem OR meropenem OR doripenem OR ertapenem OR carbapenem) AND (pharmacokinetic OR pharmacodynamic OR synergy OR synerg* OR antagonis* OR additive) AND (*in vitro* OR checkerboard OR time-kill OR Etest OR Etest OR microdilution OR agar dilution OR susceptibility). In order to reduce publication bias, Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), Infectious Diseases Society of America (IDSA), and European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) conference proceedings for the years 2007 to 2012 were also reviewed. References of all included studies were reviewed for more eligible studies. The last search was run on 30 March 2013. Each study was screened and reviewed for eligibility independently by two authors.

Outcomes. The primary outcome was the *in vitro* effects of combination therapy on bacterial kill or inhibition. For time-kill analysis, synergy was defined as a >2-log reduction in CFU for a combination compared to the most active single agent, while antagonism was defined as a >2-log

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increase. With checkerboard testing, interactions were expressed as the fractional inhibitory concentration index (FICI; sum of the MIC for each drug in combination divided by the MIC of that drug alone), with synergy defined as ≥0.5, an FICI between >0.5 and ≤1 defined as additive, an FICI of >1 as indifferent, and an FICI of >4 as antagonistic. Results of synergy tests were assessed at 24 h.

Secondary outcomes included bactericidality rates, defined as a >3-log reduction in CFU compared to pretreatment counts in time-kill studies and *in vitro* resistance development, if assessed.

Data extraction. Data were extracted independently and verified by two authors using a predefined data extraction form.

For each study, we sought to extract the method of *in vitro* combination testing, bacterial species, the type of carbapenem and polymyxin used, and number of isolates tested. Reported MICs of study isolates for the carbapenem and polymyxin tested were also extracted, and resistance was determined according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) published breakpoints (http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/Breakpoint_table_v_2.0_120221.pdf).

Synthesis of results and statistical methods. We calculated synergy rates separately for each synergy method, where synergy was counted as an event and the sample size was the number of isolates tested. For each testing method, we subgrouped the results by bacteria, carbapenem type, and resistance to carbapenems and polymyxins. In order to account for influence of resistance on synergy, isolates from each study were subgrouped by resistance to polymyxin or carbapenem and were analyzed separately when possible.

Some studies performed multiple tests on the same bacterial populations with different antimicrobial combinations, different concentrations, or different bacterial loads. In order to avoid bias derived from multiple testing, we chose a representative test for the isolates, such as the one using a more common antibiotic concentration or bacterial load. Some studies used colistin *in vitro* concentrations that are not achievable in clinical practice. In a sensitivity analysis, we excluded these studies. Based on pharmacokinetic/pharmacodynamic (PK/PD) data showing clinically achievable colistin levels (5), we used a cutoff of 4 mg/liter for exclusion.

We used mixed-effect analysis to provide a pooled rate. The I^2 statistic was used to test heterogeneity. Comprehensive Meta-Analysis V2.2 (Biostat, Englewood NJ) was used for analysis.

RESULTS

Identified studies. Our PubMed search yielded 95 citations. After full-text review and evaluation of appropriate references and conference proceedings, 39 published studies and 15 conference proceedings were included (Fig. 1). The main characteristics of included studies are presented in Table 1. Some studies reported more than one synergy testing method, bacterial species with different resistance profiles, various inocula, and different antibiotic concentrations. In total, results reflect 193 time-kill, 18 Etest, and 35 checkerboard tests performed on 1,054 bacterial isolates. Time-kill tests included both static time-kill and dynamic PK/PD models (see Table S4 in the supplemental material for specific inclusion of tests for analysis when multiple tests were performed on the same isolates).

Time-kill data. Forty studies performed on 545 isolates were included (Fig. 2). Polymyxin concentrations ranged from 0.125× MIC (0.5 mg/liter) to 4× MIC (8 mg/liter), while carbapenem concentrations ranged from 0.125× MIC (10 mg/liter) to 2× MIC (64 mg/liter).

For *A. baumannii*, the analysis was performed on 186 isolates, yielding a synergy rate of 77% (95% confidence interval [CI], 64 to 87%). Only 2 isolates showed antagonism, with a pooled antagonism rate of 8% (95% CI, 5 to 13%). The rate of bactericidality for 102 isolates increased from 26% (95% CI, 12 to 47%) for the best single agent to 74% (95% CI, 58 to 85%) in combination. Heterogeneity (I^2) for these studies was 50%. Polymyxin B and colistin produced similar synergy rates. Synergy rates were higher with meropenem and doripenem than with imipenem ($P = 0.008$ for subgroup comparison) (see Table S2 and Fig. S3 in supplemental material). Of note, 3 studies (7–9) examining imipenem and colistin showed very high (100%) bactericidal activity with colistin monotherapy, precluding demonstration of synergy. Exclusion of these studies improved the imipenem-colistin synergy rate to 67%. When examining carbapenem-resistant, colistin-susceptible strains only (111 isolates), the synergy rate was 71% (95% CI, 54 to 84%) (see Table S3).

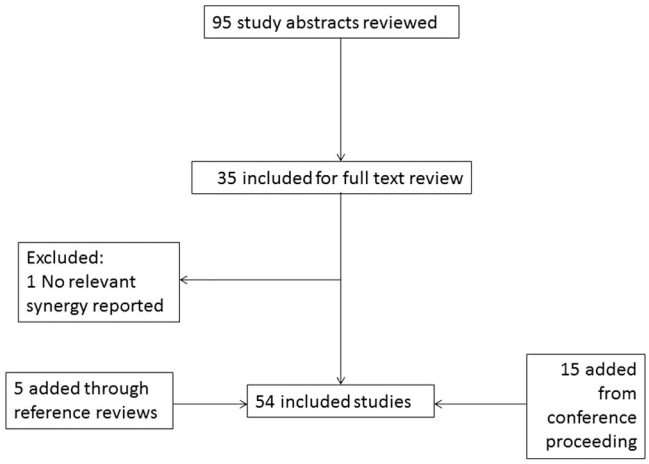

FIG 1 Study flow.

For *K. pneumoniae*, testing on 146 isolates yielded a synergy rate of 44% (95% CI, 30 to 59%); 17 isolates were antagonistic, with a rate of 15% (95% CI, 9 to 25%). Rates of bactericidality for 82 isolates increased from 18% (95% CI, 10 to 29%) for the most active single agent to 63% (95% CI, 50 to 74%) in combination. Heterogeneity (I^2) for *K. pneumoniae* was 51%. Polymyxin B was tested in 3 studies, which showed a synergy rate of 64% (95% CI, 47 to 79%), whereas colistin studies showed a synergy rate of 40% (95% CI, 29 to 52%; $P = 0.04$ for subgroup comparisons). Doripenem showed higher synergy rates than imipenem and meropenem but no significant difference between subgroups (Table 2). Data for ertapenem were available for only 2 tests, with a low synergy rate of 11% (10). When specifically examining carbapenem-resistant, colistin-susceptible *K. pneumoniae* (7 tests on 62 isolates), the overall synergy rate was 55% (95% CI, 36 to 73%) (see Table S3 in supplemental material).

For *P. aeruginosa*, 136 isolates were tested and yielded a synergy rate of 50% (95% CI, 30 to 69%), while 6 isolates were antagonistic, with an antagonism rate of 11% (95% CI, 5 to 21%). Bactericidality for 62 isolates increased from 10% (95% CI, 5 to 21%) for the single most active agent to 49% (95% CI, 31 to 68%) in combination. Heterogeneity (I^2) was 66%. Doripenem and imipenem showed synergy rates of 62% (95% CI, 38 to 81%) and 60% (95% CI, 18 to 91%), respectively, while meropenem displayed only 24% synergy (95% CI, 15 to 38%) (Table 2). When examining

TABLE 1 Study characteristics[a]

Reference	Yr published (conference name)	Polymyxin	Carbapenem	Bacterium(a)	No. of isolates	Resistance status		Synergy method(s)	Outcome reported
						Carbapenem	Polymyxin		
Chan and Zabransky (28)	1987	Col	I	*P. aeruginosa*, *S. maltophilia*	33	R, S	R	Checkerboard, time-kill	FICI
Rynn et al. (15)	1999	Col	M	*P. aeruginosa*	2	S	S	Time-kill	AUKBC
Yoon et al. (29)	2003	PB	I	*A. baumannii*	8	R	R	Checkerboard, time-kill	FICI, tks, b
Landman et al. (30)	2005	PB	I	*P. aeruginosa*	10	R	S	Time-kill	b
Bratu et al. (31)	2005	PB	I	*K. pneumoniae*	16	R	R, S	Time-kill	b, tks
Timurkaynak et al. (32)	2006	Col	M	*A. baumannii*, *P. aeruginosa*	10	R, S	S	Checkerboard	FICI
Wareham et al. (33)	2006	PB	I	*A. baumannii*	5	R	S	Etest	FICI
Tateda et al. (12)	2006	PB	I	*P. aeruginosa*	12	R	R	Checkerboard breakpoint	FICI
Biancofiore et al. (34)	2007	Col	M	*A. baumannii*	1	R	S	Checkerboard	FICI
Cirioni et al. (35)	2007	Col	I	*P. aeruginosa*	2	R, S	R	Checkerboard, time-kill	FICI, tks
Tripodi et al. (9)	2007	Col	I	*A. baumannii*	9	R	S	Time-kill	b, tks
Pankuch et al. (36)	2008	Col	M	*P. aeruginosa*, *A. baumannii*	102	R, S	R, S	Time-kill	tks
Tascini et al. (37)	2008	Col	I	*E. cloaca*	1	S	S	Checkerboard	FICI
Guzel and Gerceker (38)	2008	Col	M	*P. aeruginosa*	50	S	S	Checkerboard	FICI
Guelfi et al. (39)	2008	PB	M	*P. aeruginosa*, *A. baumannii*	20	R, S	S	Checkerboard	FICI
Burgess et al. (40)	2008 (ICAAC)	Col	M	*A. baumannii*	5	R	S	Time-kill	b, tks
Ullman et al. (41)	2008 (ICAAC)	Col	M	*A. baumannii*	3	R, S	S	PK/PD time-kill	b
Pankey and Ashcraft (42)	2009	PB	M	*A. baumannii*	8	R	S	Etest, time-kill	FICI, b, tks
Souli et al. (10)	2009	Col	I	*K. pneumoniae*	42	R, S	R, S	Time-kill	tks
Burgess et al. (43)	2009 (ICAAC)	Col	I	*A. baumannii*	5	R	S	Time-kill	b, tks
Hilliard et al. (44)	2009 (ICAAC)	Col	D	*P. aeruginosa*	2	S	S	Checkerboard	FICI
Milne and Gould (16)	2010	Col	M, I	*P. aeruginosa*	144	R, S	R, S	Etest, sbpi	FICI, SBPI
Pongpech et al. (13)	2010	Col	M, I	*A. baumannii*	30	R	S	Checkerboard, time-kill	FICI
Rodriguez et al. (8)	2010	Col	I	*A. baumannii*	14	R, S	R, S	Time-kill	b, tks
Elemam et al. (45)	2010	PB	I	*K. pneumoniae*	12	R	R	Checkerboard	FICI
Lin et al. (46)	2010	Col	I	*E. cloaca*	1	S	S	Time-kill	b, tks
Shields et al. (47)	2010	Col	I, D	*A. baumannii*	17	R	S	Etest, time-kill	FICI, b, synergy
Sopirala et al. (48)	2010	Col	I	*A. baumannii*	8	R	S	Checkerboard, etest, time-kill	FICI, tks
Urban et al. (49)	2010	PB	D	*K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, *E. coli*	20	R, S	R, S	Time-kill	b
Pankuch et al. (50)	2010	Col	D	*A. baumannii*, *P. aeruginosa*	50	R, S	R, S	Time-kill	tks
Steed et al. (51)	2010 (ECCMID)	Col	I	*A. baumannii*	8	R	S	Time-kill	b, tks
Souli et al. (52)	2010 (ECCMID)	Col	M, E	*K. pneumoniae*	55	R, S	R, S	Time-kill	tks
Khuntayaporn et al. (11)	2010 (ICAAC)	Col	I, M, D	*P. aeruginosa*	57	R		Checkerboard	FICI
Dorobisz et al. (53)	2010 (ICAAC)	Col	D	*A. baumannii*	6	R	R	Checkerboard, time-kill	FICI, b
Srisupha-Olarn and Burgess (54)	2010 (ICAAC)	Col	M	*A. baumannii*	3	R	S	PK/PD time-kill	b, tks
Ly et al. (21)	2011 (ICAAC)	Col	D	*P. aeruginosa*	3	S	R, S	PK/PD time-kill	b
Liang et al. (55)	2011	Col	M	*A. baumannii*	4	R	S	Time-kill	b, tks
Pankey and Ashcraft (56)	2011	PB	M	*K. pneumoniae*	14	R, S	R, S	Etest, time-kill	FICI, b, tks
Sheng et al. (57)	2011	Col	I	*A. baumannii*	18	R	S	Checkerboard, time-kill	FICI, b, tks
Bergen et al. (19)	2011	Col	I	*P. aeruginosa*	6	R, S	R, S	Time-kill	b, tks
Bergen et al. (18)	2011	Col	D	*P. aeruginosa*	2	R, S	R, S	PK/PD time-kill	b, tks
Santimaleeworagun et al. (58)	2011	Col	I	*A. baumannii*	8	R	S	Checkerboard	FICI
Lim et al. (59)	2011	PB	M	*P. aeruginosa*	22	R	S, R	Time-kill	b
Morosini et al. (60)	2011 (ECCMID)	Col	M	*K. pneumoniae*	1	S	S	Time-kill	b, FICI
Poudyal et al. (17)	2011 (ECCMID)	Col	D	*A. baumannii*	3	R, S	S	PK/PD time-kill	b, tks
Teo et al. (61)	2011 (ICAAC)	PB	D	*P. aeruginosa*	16	R		Time-kill	b, tks
Principe et al. (62)	2011 (ICAAC)	Col	D	*A. baumannii*	24	R, S		Checkerboard	Synergy
Mohamed et al. (63)	2011 (ICAAC)	Col	M	*P. aeruginosa*	2	R, S	S	PK/PD time-kill	b, tks
Peck et al. (7)	2012	Col	I	*A. baumannii*	6	R	R, S	Time-kill	b, synergy
Jernigan et al. (14)	2012	Col	D	*K. pneumoniae*	12	R	S, R	Time-kill	b, tks, AUBKC
Deris et al. (20)	2012	Col	D	*K. pneumoniae*	4	R, S	R, S	PK/PD time-kill	b, tks
Ozseven et al. (64)	2012	PB	I, M	*A. baumannii*	34	R	S	Checkerboard	FICI
He et al. (65)	2012	Col	D	*P. aeruginosa*	100	R	S	Etest, time-kill	FICI
Lee and Burgess (66)	2013	Col, PB	D	*K. pneumoniae*	4	R	S	Time-kill	tks, b

[a] Col, colistin; PB, polymyxin B; D, doripenem; E, ertapenem; M, meropenem; I, imipenem; R, resistant; S, susceptible; tks, time-kill synergy; FICI, fractional inhibitory concentration index; b, bactericidality; AUKBC, area under bacterial killing curve.

carbapenem-resistant, colistin-susceptible strains only (43 isolates), synergy was 59% (95% CI, 30 to 83%) (see Table S3). All but one of the studies examined colistin.

When examining 67 carbapenem-resistant and polymyxin-resistant isolates of all species, the rate of bactericidality increased from 14% (95% CI, 7 to 27%) with monotherapy to 43% (95% CI, 21 to 68%) with combination therapy. In a sensitivity analysis excluding studies using concentrations of more than 4 mg/liter of

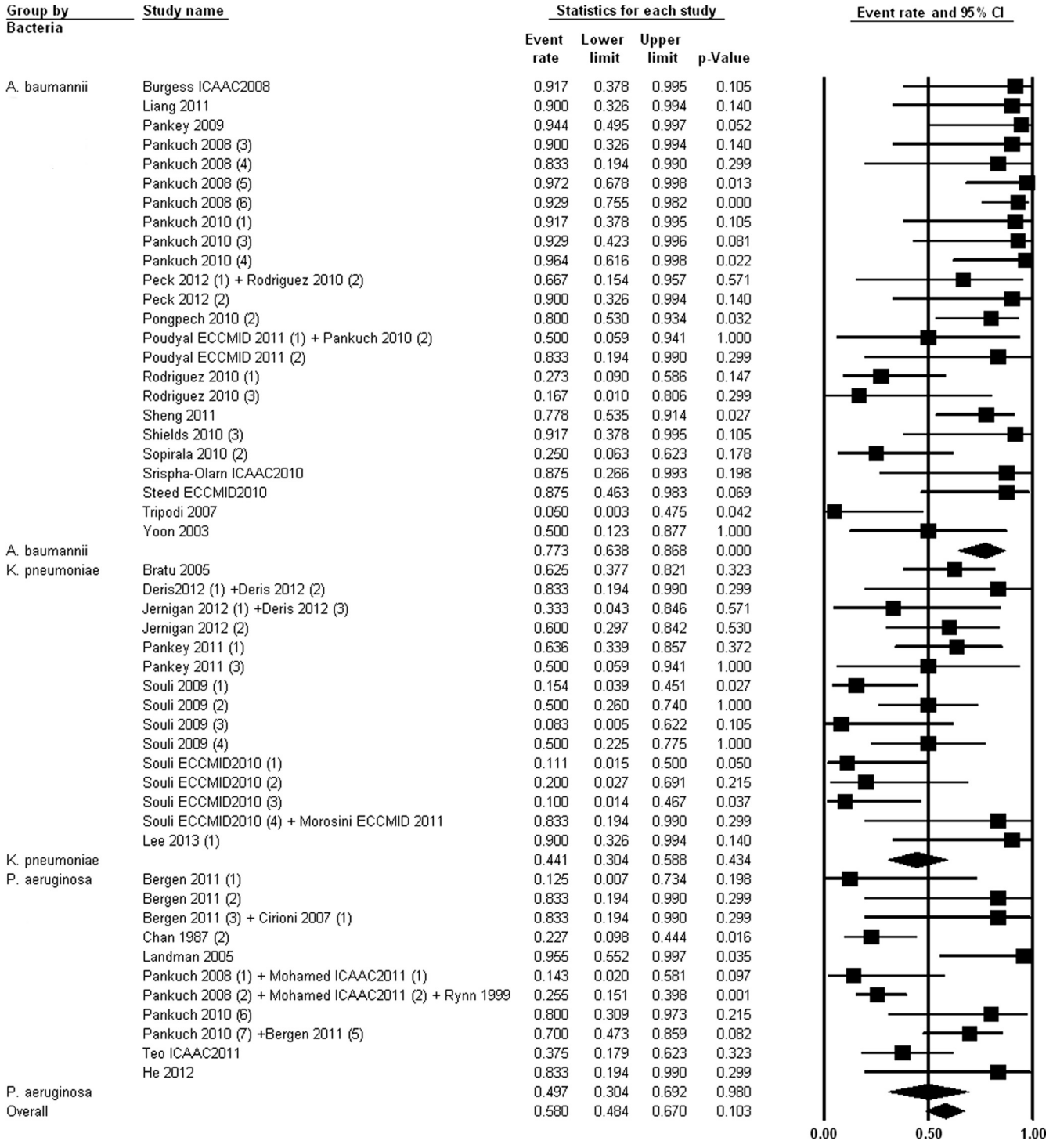

FIG 2 Synergy rates for polymyxin and carbapenem combination by type of bacteria. Study names are comprised of first author and either publication year or convention name and year. Subgroups within studies (according to resistance profile, antibiotic used, etc.; see Materials and Methods) are listed separately and denoted by continuous numbering in parentheses.

colistin, the *A. baumannii* synergy rate was 79%, with bactericidality rising from 22% for the most active single agent to 76%, while the *K. pneumoniae* synergy rate was 62%, with the bactericidality rate rising from 9 to 70% with monotherapy and combination therapy. *P. aeruginosa* rates remained unchanged. Only one study each examined *Enterobacter cloacae*, *Stenotrophomonas maltophilia*, and *Escherichia coli*. These were not included in the meta-analysis.

Checkerboard microdilution data. Twenty-three studies reported checkerboard microdilution testing, of which 11 reported

TABLE 2 Pooled synergy and antagonism rates according to bacterium and carbapenem tested

Bacterium and carbapenem	Synergy		Antagonism		No. of tests	No. of bacteria	Heterogeneity	
	Rate	95% CI	Rate	95% CI			*P* value[a]	I^2 (%)
A. baumannii								
Imipenem	56	35–74	8	4–17	11	82	0.008	48
Meropenem	86	75–93	7	2–17	9	71		
Doripenem	88	70–96	9	3–24	6	33		
K. pneumoniae								
Imipenem	41	23–62	24	7–58	5	58	0.02[b]	51
Meropenem	34	13–64	9	3–23	6	39		
Doripenem	63	39–82	10	2–32	6	19		
Ertapenem	11	3–29	12	3–42	2	30		
P. aeruginosa								
Imipenem	60	18–91	21	11–38	5	39	0.013	66
Meropenem	24	15–38	2	0–16	2	54		
Doripenem	62	38–81	5	1–20	5	43		

[a] Heterogeneity *P* for subgroup comparisons.
[b] The *P* value was 0.44 when ertapenem was excluded.

on *A. baumannii*, 9 on *P. aeruginosa*, and 1 each on *K. pneumoniae*, *S. maltophilia*, and *E. cloacae*.

For *A. baumannii*, the synergy rate for 144 isolates was 32%, while for 100 *P. aeruginosa* isolates the synergy rate was 11%. A minority of studies reported individual strain FICIs, from which mean FICIs could be calculated for *A. baumannii* of 0.8 ± 0.43 (16 isolates) and 1.8 ± 0.5 for *P. aeruginosa* (74 isolates, including and mostly influenced by results from Khuntayaporn et al. [11]). When considering overall synergy or additivity (FICI of <1), rates were 71% for *A. baumannii* and 29% for *P. aeruginosa*. No study reported antagonism with an FICI of >4. Heterogeneity and the limited number of studies and isolates did not permit adequate subgroup analysis.

Etest data. Four studies reporting on *A. baumannii* provided a pooled synergy rate of 17.5% (95% CI, 3 to 60%) and a combined synergy and additivity rate of 42% (95% CI, 14 to 75%). Two studies testing 240 *P. aeruginosa* isolates yielded a synergy rate of 2.5% (95% CI, 1 to 6%) and a combined synergy and additivity rate of 8.5% (95% CI, 0.7 to 55%). One study examining *K. pneumoniae* reported synergy of 6 out of 14 isolates (46%) (56).

Other synergy data. Tateda et al. reported using a breakpoint checkerboard plate method on 12 *P. aeruginosa* isolates tested with imipenem and polymyxin B, with all 12 isolates showing synergy (12). Three studies (13–15) reported using the area under the bacterial killing curve (AUBKC) against *P. aeruginosa*, *K. pneumoniae*, or *A. baumannii*. One study (16) also reported susceptibility breakpoint index (SBPI) data.

Effect of combination on antimicrobial resistance. Comparisons of resistance development between monotherapy and combination therapy were found in one study on 3 *A. baumannii* isolates and four studies on 14 *P. aeruginosa* isolates, all recent time-kill studies. Poudyal et al. (17) reported that colistin monotherapy led to resistance development in almost 100% of strains after as little as 24 h, while with combination therapy, doripenem successfully suppressed colistin-resistant populations, as evidenced by comparing 72-h population analysis profiles (PAPs). Bergen et al. (18) reported a 4-log CFU reduction in the development of colistin-resistant *P. aeruginosa* colonies when combined with doripenem compared to colistin monotherapy. In addition, resistance under monotherapy appeared earlier (24 h) than that with combination therapy (72 h), if at all. When tested on 4 colistin heteroresistant strains (19), similar PAPs where produced with imipenem-colistin combination therapy versus colistin alone. Deris et al. (20) reported suppression of resistance with combination therapy versus 100% resistance development with monotherapy in 2/4 strains. Resistance suppression was also noted in another study on 3 *P. aeruginosa* strains (21).

DISCUSSION

The combination of polymyxins and carbapenems was synergistic against *A. baumannii* strains in 77% of isolates and antagonistic in only 1%. For *K. pneumoniae* and *P. aeruginosa*, synergy rates were also substantial in total and even more so when examining carbapenem-resistant, colistin-susceptible isolates representing a more clinically relevant situation and when excluding studies using unrealistic colistin concentrations. Combination therapy increased bactericidality for all bacteria (from 24 to 75% for *A. baumannii*). For *A. baumannii*, there was an advantage for meropenem or doripenem in combination over imipenem, while meropenem was the least synergistic carbapenem against *P. aeruginosa*. A difference between polymyxin B and colistin was demonstrated only for *K. pneumoniae* in a small number of studies, showing an advantage to polymyxin B. Resistance development for monotherapy versus combination therapy has been assessed mainly in recent studies that showed either suppression or delay of colistin resistance development with combination therapy. With colistin monotherapy, resistance developed sometimes as early as 24 h (22).

Antibacterial combination methods are not fully standardized, and there was no single clear definition for synergy or antagonism in the studies included in this review. Synergy rates were generally higher in studies using the time-kill method than the checkerboard microdilution or Etest method, as was also shown with other antibiotic combinations (23). Discordance is not surprising, since these tests use different outcomes, i.e., inhibition versus killing. It has yet to be determined which combination testing method better predicts *in vivo* efficacy. While Etest and checker-

board reflect MIC values that are used clinically, time-kill by design examines the extent of bacterial killing over time; thus, it might give more information about the nature of interaction. Interaction may depend on the bacterial concentration or inoculum used and the time frame of assessment, which were heterogeneous in the included studies. By definition, in time-kill studies, synergy rates depend on the activity of the most active antibiotic in the combination. In order to improve the ability to integrate results and compare results of different studies, standardization of the methodology of the synergy testing, better definitions for the selection of the various tests, and standardized reporting are urgently required.

While this *in vitro* analysis supports the use of polymyxin and carbapenem combinations, and especially with meropenem or doripenem against *A. baumannii*, it should be recognized that results might not be directly relevant to clinical practice. Due to different pharmacodynamic and pharmacokinetic effects of the drug in the host and different bacterial and drug concentrations in the specific site of infection, one cannot assume straightforward *in vivo* efficacy from *in vitro* studies. Resistance following antibiotic exposure develops readily *in vitro* but probably on a different time scale in clinical practice. In addition, *in vitro* studies cannot examine toxicity, which is highly clinically relevant with colistin combination therapy. Indeed, with beta-lactam-aminoglycoside combination therapy, despite strong *in vitro* data showing synergy and prevention of resistance development with combination therapy against Gram-negative bacteria, clinical studies could not show a benefit for combination therapy. Systematic reviews of randomized, controlled trials of neutropenic and nonneutropenic patients with sepsis showed no advantage of combination therapy with regard to survival or resistance development as assessed by superinfections (24–27). Of note, since EUCAST and CLSI criteria differ, synergy rates described for the carbapenem-resistant, colistin-susceptible group may not be applicable in instances where CLSI breakpoints are used.

In summary, the combination of a carbapenem and a polymyxin against GNB, especially *A. baumannii*, is supported *in vitro* by high synergy and bactericidality rates, with low antagonism and less resistance development. Higher synergy rates might be attained by using specific carbapenems for different bacteria. Strain-to-strain variation suggests that individualized or center-based synergy testing is of value. *In vitro* studies should focus on the clinically relevant questions of carbapenem-resistant GNB. PK/PD studies should be promoted to better characterize the interaction, including the time course of the interaction, especially when the onset of effect and development of resistance may vary. Based on the current analysis, clinical studies examining the addition of a carbapenem to colistin in the treatment of carbapenem-resistant Gram-negative bacteria are warranted. Our systematic review can be used to better guide these clinical trials.

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