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CLINICAL THERAPEUTICS

Comparable Efficacy and Better Safety of Double β-Lactam Combination Therapy versus β-Lactam plus Aminoglycoside in Gram-Negative Bacteria in Randomized, Controlled Trials

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ABSTRACT There is a great need for efficacious therapies against Gram-negative bacteria. Double β-lactam combination(s) (DBL) are relatively safe, and preclinical data are promising; however, their clinical role has not been well defined. We conducted a meta-analysis of the clinical and microbiological efficacy of DBL compared to β-lactam plus aminoglycoside combinations (BLAG). PubMed, Embase, ISI Web of Knowledge, and Cochrane Controlled Trials Register database were searched through July 2018. We included randomized controlled clinical trials that compared DBL with BLAG combinations. Clinical response was used as the primary outcome and microbiological response in Gram-negative bacteria as the secondary outcome; sensitivity analyses were performed for *Pseudomonas aeruginosa*, *Klebsiella* spp., and *Escherichia coli*. Heterogeneity and risk of bias were assessed. Safety results were classified by systems and organs. Thirteen studies evaluated 2,771 cases for clinical response and 665 cases for microbiological response in various Gram-negative species. DBL achieved slightly, but not significantly, better clinical response (risk ratio, 1.05; 95% confidence interval [CI], 0.99 to 1.11) and microbiological response in Gram-negatives (risk ratio, 1.11; 95% CI, 0.99 to 1.25) compared with BLAG. Sensitivity analyses by pathogen showed the same trend. No significant heterogeneity across studies was found. DBL was significantly safer than BLAG regarding renal toxicity (6.6% versus 8.8%, $P = 0.0338$) and ototoxicity (0.7 versus 3.1%, $P = 0.0137$). Other adverse events were largely comparable. Overall, empirically designed DBL showed comparable clinical and microbiological responses across different Gram-negative species, and were significantly safer than BLAG. Therefore, DBL should be rationally optimized via the latest translational approaches, leveraging mechanistic insights and newer β-lactams for future evaluation in clinical trials.

KEYWORDS double beta-lactam, Gram-negative bacteria, beta-lactamase inhibitor, combination therapy, meta-analysis, randomized controlled clinical trial

Antimicrobial resistance is causing a global public health crisis with increasing mortality, morbidity, and medical cost due to serious bacterial infections with resistant strains (1). This situation is more severe for infections with multidrug-resistant

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Gram-negative pathogens (MDRGN), considering the epidemiology and shortage of efficacious antibiotics (2, 3). Combination therapy is widely used to treat serious infections by MDRGN (4) and usually includes antibiotics from different classes to achieve synergistic bacterial killing. However, to target this global health crisis, innovative therapies and new antibiotics are urgently needed. β-Lactam antibiotics are likely to be an indispensable part of these combinations and are safe in patients of all ages. Indeed, against carbapenemase-producing *Klebsiella pneumoniae* strains, better clinical outcomes were observed for carbapenem-containing combinations compared with those for combinations that lacked a carbapenem (5). Presently, extensive efforts are being made to identify novel and efficacious combinations to combat infections with MDRGN (6).

All β-lactams are known to covalently bind to and thereby inactivate one or multiple penicillin-binding proteins (PBPs); however, β-lactams differ greatly in their binding patterns for various PBPs. Combining two β-lactams enables inactivation of multiple PBPs to achieve synergistic bacterial killing and minimize resistance. Such combinations have been widely investigated in preclinical and clinical studies from the 1970s to 1990s (7–10) to target both Gram-negative and Gram-positive pathogens. However, the advantages in spectrum have diminished with the availability of newer broad-spectrum antibiotics, including carbapenems and fluoroquinolones. Unfortunately, this has led to the global emergence of drastic resistance to fluoroquinolone. Since then, the clinical interest in double β-lactam combination(s) (DBL) has declined, and only a small number of clinical case studies have been published.

A series of novel molecular insights and translational approaches now enable us to design and rationally optimize DBL. Comprehensive PBP receptor binding data were recently published for *K. pneumoniae* and *Acinetobacter baumannii* (11–13), and such binding data are available over a series of papers on *Pseudomonas aeruginosa* and *Escherichia coli* (7, 10, 14–17). Some outer membrane permeability data are available for β-lactams in *P. aeruginosa* (18), and novel and efficient permeability assays for β-lactams and β-lactamase inhibitors in MDRGN have been recently developed (19). Addressing the key gaps in our understanding of β-lactam antibiotic action and resistance (13) enables the rational design of mechanistically optimized DBL with or without a β-lactamase inhibitor (11, 20). β-Lactams present the largest antibiotic class with abundant clinical pharmacokinetic (PK) and safety data. This presents a substantial advantage for translating these DBL to patients.

Inspired by these novel mechanistic advances, we performed a systematic review and meta-analysis of the clinical performance of DBL. We aimed to compare clinical and microbiological responses for key Gram-negative pathogens between DBL and β-lactam plus aminoglycoside combinations (BLAG) based on all published randomized controlled clinical trials. The majority of these trials were in patients with febrile neutropenia. The present analysis includes more clinical trials, as well as a meta-analysis that has not been performed in prior reviews (7, 8). The insights gained from these large, early clinical trials add considerable value and a clinical perspective to the future design, optimization, and implementation of innovative DBL that can successfully combat infections by MDRGN.

(Part of this work was presented as an ePoster presentation at the European Congress of Clinical Microbiology and Infectious Diseases [ECCMID] 2017 in Vienna, Austria.)

RESULTS

Study selection. A total of 202 publications were identified during the database searches and by evaluating the references within the identified papers (Fig. 1). Forty-seven duplicates from different databases were removed, and 109 records (e.g., animal and *in vitro* studies) were excluded based on titles and abstracts. Thirty-three records were further removed for other reasons; these were nonclinical studies ($n = 2$), studies reported in another language ($n = 3$), review-only publications ($n = 17$), and trials with designs that did not meet the inclusion criteria (such as trials lacking a comparator

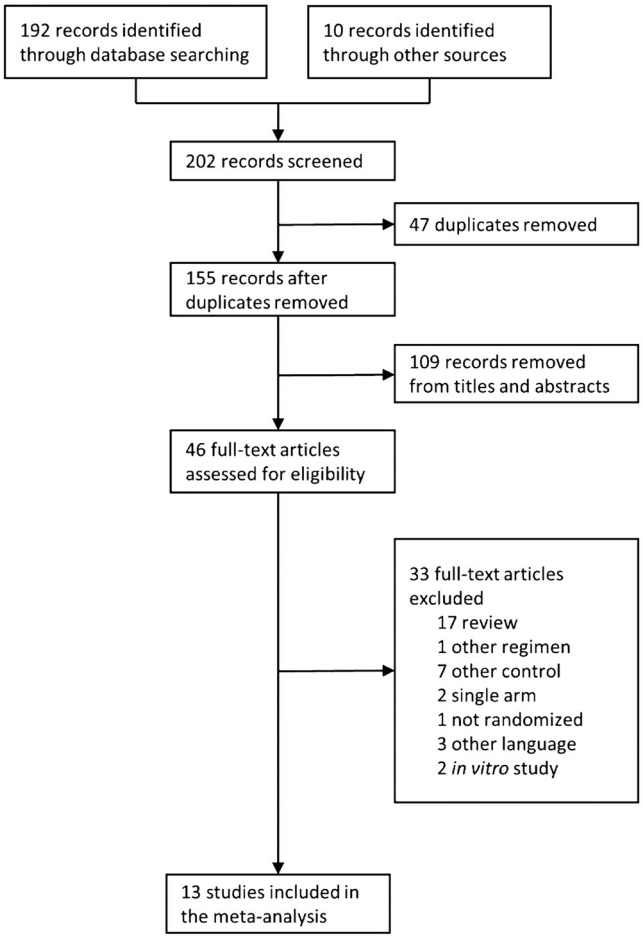

FIG 1 Flow of information in the systematic review.

group; $n = 11$). The final data set included 13 randomized controlled clinical trials reported between 1972 and 1993 (Fig. 1) (21–33).

Four of these thirteen trials were multicenter studies (Table 1), and eleven of these trials assessed febrile neutropenic patients. The latter accounted for 94% of the whole patient population (i.e., 3,257 cases of febrile neutropenia out of 3,476 overall cases). Age, sex, and other demographic variables were distributed evenly between both groups, with the exception of two studies that did not balance sex (22, 31).

A variety of β-lactams and aminoglycosides, as well as their dosage regimens, were investigated by different studies (Table 1). Given the time period in which these trials were performed, the employed β-lactams did not include carbapenems. Definitions for infection and criteria for clinical or microbiological cure were obtained as specified by the respective trial (Tables S1 and S2). The median duration of treatment ranged from 5 to 12 days (Table 1).

Clinical and microbiological response. All pooled risk ratios for the different outcomes were above 1.00 and therefore slightly favored DBL compared to BLAG therapy. The therapeutic advantage of DBL over BLAG was not statistically significant, since all 95% confidence intervals (CIs) included 1.00 (Table 2, Fig. 2). Meta-analysis results from a fixed-effect model (not shown) were nearly identical to those reported in Table 2. Sensitivity analyses of the microbiological responses in the different Gram-negative pathogens (*P. aeruginosa*, *Klebsiella* spp., and *E. coli*) all showed the same trend (Table 2, Fig. S1).

No significant heterogeneity ($P > 0.1$) was detected across studies. For all analyses, the degree of heterogeneity (I^2) was below 25% (Fig. 2, Fig. S1). A sensitivity analysis

TABLE 1 Main characteristics of the trials included in the meta-analysis[k]

Author (yr) (reference)	Trial type[d]	Type of infection	Treatment, dosage frequency[b]		Avg treatment duration (days)	Overall no. of cases[e] (no. of patients)	Demographic characteristic			
							Mean age (yrs)		Men (%)	
			Double β-lactam	β-Lactam plus aminoglycoside (daily doses)			DBL	BLAG	DBL	BLAG
Middleman (1972) (21)	Multicenter	Febrile neutropenia	CAR, 5 g q4h CEF, 3 g q6h	CAR, 5 g q4h KAN, 200 mg/m^2 q8h (14.8 mg/kg/day)	5–10	179 (151)	30.5[f]	36[f]	NA[c]	NA
Klastersky (1975) (22)	Single center	Severe Infection	TIC, 10 g t.i.d. CEF, 3 g t.i.d.	CEF, 3 g t.i.d. TOB, 4.5 mg/kg in 3 doses (4.5 mg/kg/day)	6.3–7.8	186[g] (186)	52.7	67.7	12.5	40
				TIC, 10 g t.i.d. TOB, 4.5 mg/kg in 3 doses (4.5 mg/kg/day)				50.7		25
Schimpff (1976) (23)	Single center	Febrile neutropenia	TIC, 5 g q6h CEF, 3 g q6h	TIC, 5 g q6h GEN, 180 mg/m^2/day in 4 doses (4.45 mg/kg/day)	NA	127 (NA)	40	41	61.3	56.9
EORTC[a] (1978) (24)	Multicenter	Febrile neutropenia	TIC, 5 g q6h CEF, 3 g q6h	TIC, 5 g q6h GEN, 180 mg/m^2/day in 4 doses (4.45 mg/kg/day)	NA	625 (NA)	NA	NA	NA	NA
			CAR, 10 g q6h CEF, 3 g q6h	CAR, 10 g q6h (CEF, 3 g q6h) GEN, 180 mg/m^2/day in 4 doses (4.45 mg/kg/day)			43[h]	43[h]	54[h]	54[h]
				CEF, 3 g q6h GEN, 180 mg/m^2/day in 4 doses (4.45 mg/kg/day)				43[h]		54[h]
Bodey (1979) (25)	Single center	Febrile neutropenia	CAR, 5 g q4h FAM, 3 g loading, followed by 12 g over 24 h	CAR, 5 g q4h TOB, 90 mg/m^2 loading dose followed by 360/m^2/day over 24 h (11.1 mg/kg/day on day 1; then 8.9 mg/kg/day)	6–7[f]	490[i] (NA)	NA	NA	54	60
			CAR, 5 g q4h FAM, 3 g q6h				NA		59	
Wiston (1984) (26)	Single center	Febrile neutropenia	MOX, 57.5 mg/kg q12h (40 mg/kg, q8h) PIP, 75 mg/kg q6h	MOX, 57.5 mg/kg q12h (or 40 mg/kg q8h) AMK, 3.75 mg/kg q6h (15 mg/kg/day)	12	295 (NA)	42[f]	35[f]	58.8	53.4
Fainstein (1984) (27)	Single center	Febrile neutropenia	MOX, 2 g q4h TIC, 4 g q4h	MOX, 2 g q4h TOB, 90 mg/m^2 loading dose followed by 360/m^2/day over 24 h (11.1 mg/kg/day on day 1; then 8.9 mg/kg/day)	8.0	495 (270)	42	40	61.8	55.0
Feld (1985) (28)	Multicenter	Febrile neutropenia	TIC, 3 g q4h MOX, 2 g q4h	TIC, 3 g q4h TOB, 1.25 mg/kg q6h (5 mg/kg/day)	10	277 (244)	50	53	45.3	53.4

(Continued on next page)

TABLE 1 (Continued)

Author (yr) (reference)	Trial type[d]	Type of infection	Treatment, dosage frequency[b]		Avg treatment duration (days)	Overall no. of cases[e] (no. of patients)	Demographic characteristic			
			Double β-lactam	β-Lactam plus aminoglycoside (daily doses)			Mean age (yrs)		Men (%)	
							DBL	BLAG	DBL	BLAG
De Jongh (1986) (29)	Single center	Febrile neutropenia	MOX, 4 g q12h PIP, 5 g q6h	MOX, 4 g q12h AMK, 250 mg q6h (14.3 mg/kg/day)	6	302 (220)	48[f]	48[f]	NA	NA
Rostein (1988) (30)	Multicenter	Febrile neutropenia	CFP, 4 g q12h PIP, 3 g q4h	MEZ, 3 g q4h TOB, 2 mg/kg loading dose, 1.5 mg/kg,[j] (3.5 mg/kg/day on day 1; then 1.5 mg/kg/day)	11	60 (54)	46	47	63.3	43.3
Torres (1989) (31)	Single center	Severe nosocomial pneumonia	CTX, 2 g q6h ATM, 1 g q8h	CTX, 2 g q6h AMK, 500 mg q12h (14.3 mg/kg/day)	NA	33[g] (33)	64	53	84.6	100.0
Kibbler (1989) (32)	Single center	Febrile neutropenia	PIP, 4 g q.i.d. CAZ, 2 g t.i.d.	PIP, 4 g q.i.d. NET, 3.5 mg/kg b.i.d. (7 mg/kg/day)	7–10	202 (130)	28.4	33.2	70.3	65.7
			AZL, 5 g t.i.d. CAZ, 2 g t.i.d.	AZL, 5 g t.i.d. NET, 3.5 mg/kg b.i.d. (7 mg/kg/day)			27.9	32.8	52.7	64.3
Joshi (1993) (33)	Single center	Febrile neutropenia	CAZ, 1.5 g q6h PIP, 5 g q6h	CAZ, 1.5 g q6h TOB, 5 mg/kg/day in 3 doses (5 mg/kg/day)	7	205 (159)	58	50	NA	NA

[a]EORTC, European Organization for Research on Treatment of Cancer.
[b]AZL, azlocillin; ATM, aztreonam; CAR, carbenicillin; CFP, cefoperazone; CTX, cefotaxime; CEF, cephalothin (cefalotin); CAZ, ceftazidime; FAM, cefamandole; MEZ, mezlocillin; MOX, moxalactam; TIC, ticarcillin; PIP, piperacillin; AMK, amikacin; GEN, gentamicin; KAN, kanamycin; NET, netilmicin; TOB, tobramycin; q4h, q6h, q8h, q12h, every 4, 6, 8, or 12 hours, respectively; b.i.d., twice a day; t.i.d., three times a day; q.i.d., four times a day.
[c]NA, not applicable.
[d]All studies were randomized, controlled trials.
[e]Cases included episodes or patient-trials. The number of cases was larger than the number of patients, since some patients had multiple episodes of febrile neutropenia. The number of evaluable cases was used to calculate clinical response.
[f]Median.
[g]The original study only provided the number of subjects but not the number of episodes; the number of subjects was used as the number of cases, since there was usually only one episode of a severe infection per patient in these two studies.
[h]Only overall demographic data were available.
[i]Only 450 episodes were evaluable. Of these, 234 episodes represented fever with unknown origin, and 216 episodes were caused by clinically or microbiologically documented infections.
[j]Dosing interval adjusted by renal function.
[k]Please note: the clinical evaluation time has not been precisely reported in these studies; therefore, it is not presented in this table.

TABLE 2 Summary of clinical and microbiological responses comparing double β-lactam with β-lactam plus aminoglycoside therapy[a]

Outcome	Double β-lactam (% [no. of responses/ total])	β-Lactam plus aminoglycoside (% [no. of responses/ total])	Risk ratio (95% confidence interval)[c]	No. of evaluable cases[b]
Clinical response	67.4 (919/1,364)	64.2 (903/1,407)	1.05 (0.99, 1.11)	2,771
Microbiological response				
Overall	66.5 (374/562)	61.7 (431/699)	1.07 (0.98, 1.16)	1,261
Gram-negative species	65.8 (169/257)	58.6 (239/408)	1.11 (0.99, 1.25)	665
Pseudomonas aeruginosa	58.5 (38/65)	60.6 (60/99)	1.02 (0.81, 1.27)	164
Klebsiella spp.	60.8 (31/51)	50.5 (52/103)	1.16 (0.89, 1.51)	154
Escherichia coli	72.3 (60/83)	65.2 (86/132)	1.08 (0.90, 1.28)	215

[a]From the meta-analysis based on thirteen randomized, controlled clinical trials.
[b]Evaluable cases included evaluable episodes or evaluable patient-trials. The overall number of cases could be equal to or larger than the number of evaluable cases.
[c]Risk ratios were calculated using a random-effects model. There were minor numerical differences between the RevMan and R meta package results, but these did not change any conclusions. This applied especially for comparison of outcomes with relatively small sample size (i.e., microbiological response in the Gram-negative species, *P. aeruginosa*, *Klebsiella* spp., and *E. coli*). Results for the fixed-effect model were identical in both software packages.

was performed for clinical response by excluding the two trials which did not assess febrile neutropenic patients; the risk ratio (95% CI) was 1.04 (0.99 to 1.10) in agreement with the analysis of all thirteen trials (Table 2, Fig. S3). The risk ratio (95% CI) was 1.35 (0.75 to 2.42) in the two trials with severe infections (Fig. S4) (22, 31).

A partial least-squares analysis identified the presence of tobramycin in BLAG to yield favorable results for DBL regarding microbiological response in all bacteria and microbiological response in Gram-negative bacteria. The pooled risk ratio (95% confidence interval) for the six trials with tobramycin was 1.18 (1.08, 1.32) for microbiological response in all bacteria and 1.25 (1.02, 1.52) for microbiological response in Gram-negative bacteria (Fig. S5). Thus, DBL yielded significantly better microbiological responses compared to BLAG regimens that included tobramycin. Likewise, when piperacillin was used in DBL but not in BLAG, the pooled risk ratio (95% confidence interval) for these four studies was 1.17 (0.94, 1.44) for microbiological response in all bacteria and 1.17 (0.93, 1.47) for microbiological response in Gram-negative bacteria (Fig. S6).

Assessment of bias. For selection bias regarding to random sequence generation and allocation concealment, slightly less than half of the studies were at low risk and the other studies at unclear risk of bias (Fig. 3). For performance bias regarding whether participants and personnel were blinded to study conditions, the majority of studies were at high risk of bias, since the dosing schedules differed. For other types of bias, such as blinding of outcome assessment, incomplete outcome data, or selective reporting, the risk of bias was unclear (Fig. 3).

Specifically, for publication bias, symmetric patterns (i.e., visually suggesting no bias) were observed in the funnel plots for clinical response, overall microbiological response, and microbiological response in *P. aeruginosa* and *E. coli* (Fig. 4, Fig. S2). The arcsine-Thompson test showed no significant statistical publication bias for all studied outcomes, including sensitivity analyses in Gram-negative species ($P = 0.246$) and in *Klebsiella* spp. ($P = 0.174$). Due to zero cases for some of the groups, a continuity correction was applied by adding a value of 0.5 to the corresponding observations.

Safety. Significantly lower nephrotoxicity (including renal dysfunction, serum creatinine elevation, and azotemia; $P = 0.0338$, $n = 2,626$) was observed for the DBL (6.6%) than for the BLAG group (8.8%, Table 3). Likewise, ototoxicity was significantly lower ($P = 0.0137$) for the DBL (0.7%) than for the BLAG group (3.1%; Table 3), as expected. Renal toxicity ranged from 2.1% to 7.4% in the four studies with cephalothin in DBL, while this risk was 16.3% to 20.8% in the two studies that combined cephalothin with an aminoglycoside (Table S3). In contrast, when ticarcillin was used in BLAG with gentamicin or tobramycin, nephrotoxicity occurred in 8 of 319 cases (2.5%); these were significantly fewer cases (Fisher's exact test; $P < 0.0001$) than the 117 of 1,110 cases (10.6%) for BLAG therapy without ticarcillin. For BLAG combinations with ticarcillin or

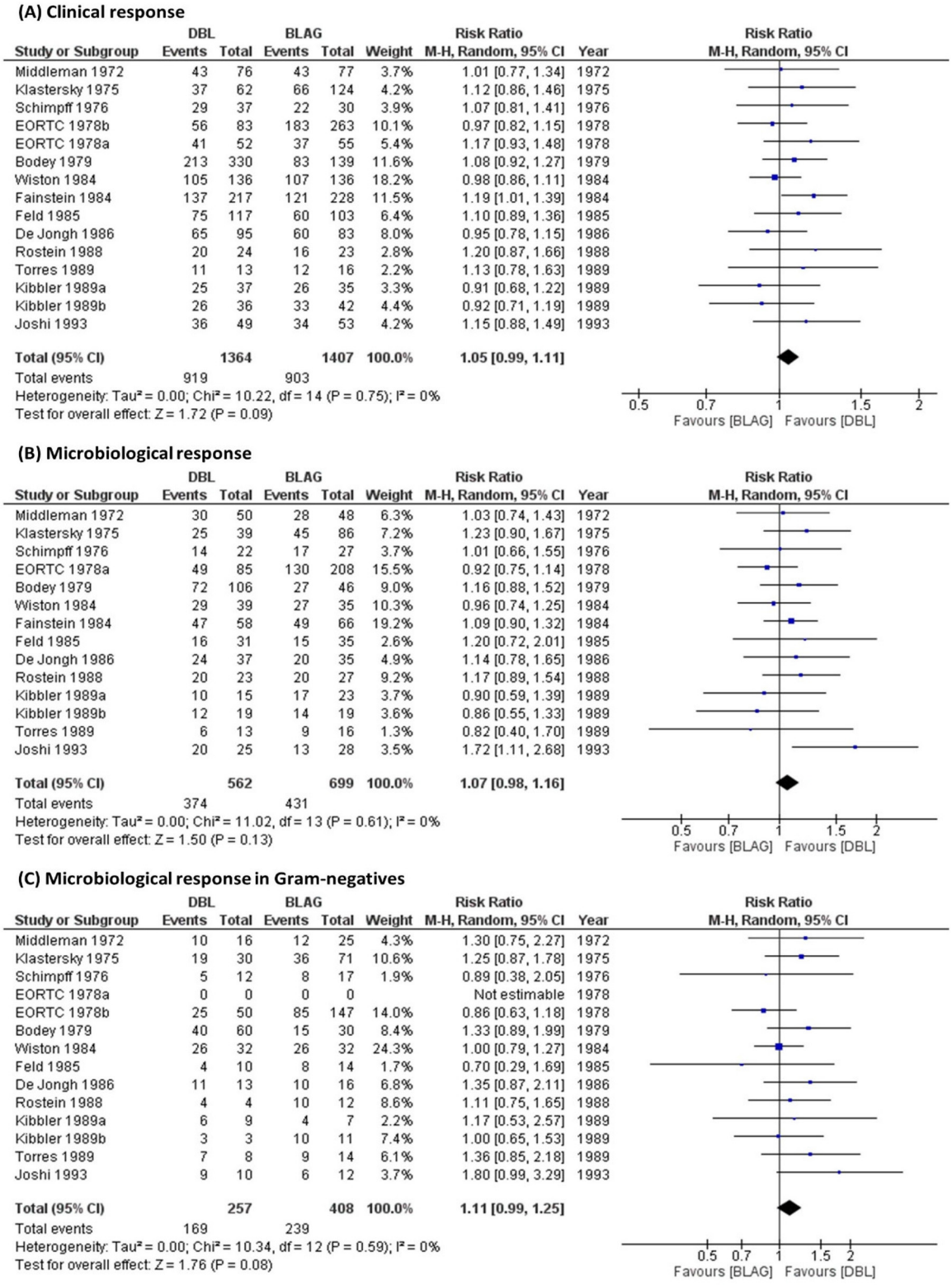

FIG 2 Forest plots for double β-lactam combinations compared with β-lactam plus aminoglycoside combinations for (A) clinical response, (B) microbiological response, and (C) microbiological response in various Gram-negative species. A random-effects model was used. Dots represent the summary measure and 95% confidence interval. The left column shows the numeric values for each study and summary measure.

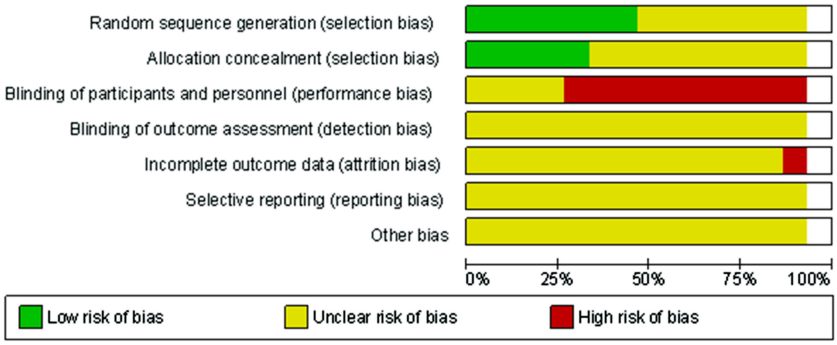

FIG 3 Risk of bias for assessment of the included studies.

carbenicillin, nephrotoxicity occurred in 34 of 540 cases (6.2%) compared to the significantly higher risk ($P = 0.009$) with 91 of 879 cases (10.4%) for BLAG that contained other β-lactams.

Relatively high incidences of coagulation, hypokalemia, and phlebitis were observed for both DBL and BLAG therapy. Coagulopathy was mostly observed in trials with moxalactam (moxalactam versus no moxalactam, 25% [188 of 752 cases] versus 4.9% [25 of 513 cases]; $P < 0.0001$; Table S4) (26–28). Hypokalemia was more common for DBL (32.7%) compared to BLAG (25.5%, $P = 0.0016$). Phlebitis might have been associated with the less advanced quality of early injection formulations. Incidences of superinfection, colonization, and allergy were not significantly higher in the DBL group (Table 3). Other adverse events were relatively minor and comparable between both groups.

DISCUSSION

This meta-analysis showed that empirical, nonoptimized DBL treatments achieved similar clinical and microbiological responses and significantly better safety compared with BLAG therapy (Tables 2 and 3). This is based on thirteen randomized clinical trials reported between 1972 and 1993, before broad-spectrum antibiotics were widely introduced to the clinic. This conclusion was robust in all patients and in patients with febrile neutropenia, and the same trend was observed in two trials studying patients with severe infections (Fig. S3 and S4) (22, 31).

The employed DBL covered a broad range of Gram-negative and Gram-positive species and included at least one β-lactam with antipseudomonal activity; this is still the recommendation in current guidelines for empirical therapy of febrile neutropenia (34). The DBL were chosen empirically. Thus, neither the dose and dosage regimen, nor the PBP receptor binding pattern and resistance mechanisms of the two β-lactams combined were optimized. This highlights the future potential for rationally optimized DBL therapy.

Overall, approximately half of the febrile neutropenia cases had a microbiologically confirmed bacterial infection. Favorable clinical responses may have been partially attributed to less toxicity due to the lack of an aminoglycoside. Only two studies discussed mortality, but neither reported mortality by treatment group (24, 25). The meta-analysis results were robust, considering the large number of cases (Table 2), low heterogeneity, and lack of publication bias. Sensitivity analyses of microbiological responses by pathogen were encouraging despite their relatively small sample size. Typically, antipseudomonal activity was provided by only one of the two β-lactams in DBL therapy but was (at least partially) covered by both the β-lactam and aminoglycoside in BLAG therapy. This may suggest some synergy between the β-lactams used in DBL against *P. aeruginosa*. Mutations in the active site of PBPs are rare (35, 36). Therefore, we expect synergy due to inactivation of optimal sets of PBPs to remain relevant, as long as the same sets of PBPs are inactivated by DBL based on contemporary β-lactams.

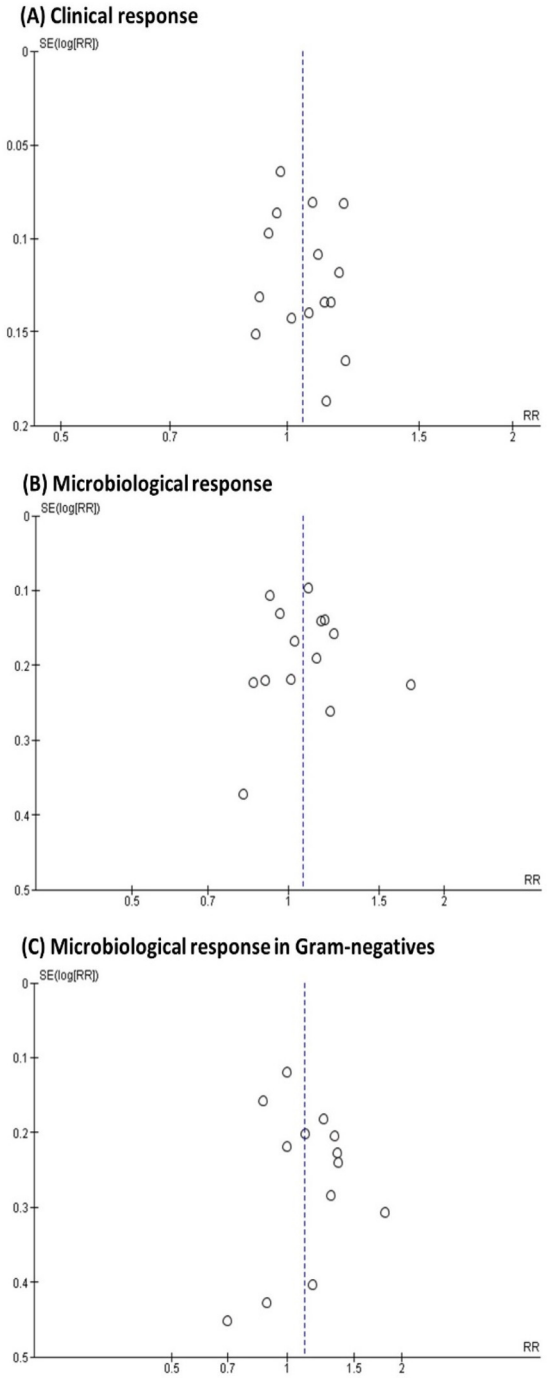

FIG 4 Funnel plots of included trials comparing double β-lactam combinations with β-lactam plus aminoglycoside combinations for (A) clinical response, (B) microbiological response, and (C) microbiological response in specific Gram-negative species. Pooled risk ratios are indicated by the dotted line.

Nowadays, extensive molecular insights and translational pharmacokinetic/pharmacodynamic approaches are available to design and rationally optimize DBL therapy. Binding of PBP4 in *P. aeruginosa* has been shown to extensively and rapidly upregulate the AmpC β-lactamase (37). The PBP4 is the highest-affinity target of carbapenems, cephaloridine, and cefoxitin (14–16), as well as a high-affinity target of cephalothin and moxalactam (also called latamoxef) (17, 38) in *P. aeruginosa*. In eight of 13 clinical trials (Table 1), the latter two β-lactams were used in nonoptimal DBL with carbenicillin, ticarcillin, or piperacillin (i.e., β-lactams that are subject to inactivation by the AmpC

TABLE 3 Safety of double β-lactam compared with β-lactam plus aminoglycoside combinations in 13 published clinical trials[a]

		Treatment							
		Double β-lactam			β-Lactam plus aminoglycoside				
System	Adverse drug events	*n*	Total	%	*n*	Total	%	Overall no. of cases	*P* value[b]
Renal	Renal dysfunction/toxicity	19	292	7.0	43	515	8.3	807	
	Serum creatinine elevation	2	62	3.2	0	65	0.0	127	
	Azotemia	66	739	9.0	63	588	10.7	1,327	
	All renal events	79	1,207	6.6	125	1,419	8.8	2,626	0.0338
Hearing	Ototoxicity	3	411	0.7	14	455	3.1	866	0.0137
Infection	Superinfection	122	821	14.9	118	802	14.7	1,623	
	Colonization	12	113	10.6	28	170	16.5	283	
Systemic	Allergy	7	271	2.6	5	437	1.1	708	
	Fever	7	247	2.8	3	248	1.2	495	
Cutaneous	Rash	65	907	7.2	66	970	6.8	1,877	
Gastrointestinal	Diarrhea	22	320	6.9	20	335	6.0	655	
	Nausea	8	166	4.8	10	166	6.0	332	
Hepatic	Liver dysfunction	11	124	8.9	13	182	7.1	306	
	Liver lab value abnormal	14	111	12.6	12	112	10.7	223	
Coagulation	Bleeding	19	162	11.7	12	226	5.3	388	0.0238
	PT prolongation	31	166	18.7	30	166	18.1	332	
	Coagulation abnormality	79	273	28.9	38	244	15.6	545	0.0003
	All coagulation effects	133	629	21.1	80	636	12.6	1,365	0.0001
Electrolyte	Hypokalemia	240	735	32.7	224	880	25.5	1,615	0.0016
Local	Phlebitis	14	51	27.5	25	103	24.3	154	

[a]The incidence of each adverse drug event was calculated from the number of pooled events divided by the total population evaluated for adverse drug events.
[b]Fisher's exact test (two-tailed); only *P* values below 0.05 were reported.

β-lactamase). To minimize the impact of resistance due to the AmpC β-lactamase, it is likely important to design DBL that include at least one β-lactam that is not inactivated by AmpC. While the chromosomal AmpC β-lactamase, as well as efflux pumps in *P. aeruginosa*, for example, will remain relevant, many new β-lactamases have emerged and spread over the last decades. These and other new resistance mechanisms will need to be considered when designing and rationally optimizing current DBL regimens.

For microbiological response in Gram-negative species, four of five clinical trials that used ticarcillin in DBL therapy favored BLAG (as shown by risk ratios below 1.0; Fig. 2C). Since 1986, ticarcillin (and carbenicillin) were replaced by piperacillin as the antipseudomonal β-lactam (Table 1). The studies that used piperacillin in DBL but not in BLAG favored DBL therapy, with a pooled risk ratio of 1.17 both for microbiological response in all bacteria and that in Gram-negatives (Fig. S6).

The DBL achieved significantly better microbiological responses in all bacteria and in Gram-negative species compared to those for BLAG with tobramycin (Fig. S5). In these six studies, tobramycin was dosed intermittently at 5 mg/kg/day (or less) or was given as a continuous infusion at 8.9 mg/kg/day (with a small loading dose; Table 1). These tobramycin regimens were expected to yield average peak concentrations in plasma of 8.1 mg/liter or less (Table S3). In contrast, amikacin, netilmicin, and kanamycin were given at higher doses than tobramycin and thus achieved higher peaks in plasma (amikacin, 19 or 36 mg/liter; netilmicin, 18 mg/liter; and kanamycin, 14 mg/liter). However, these peak concentrations cannot be used to directly compare the effects of different aminoglycosides on bacterial killing, resistance prevention, and synergy due to enhancing the target site concentration of β-lactams or due to inhibition of protein synthesis (39–43). Future systematic studies are warranted to dissect and elucidate these mechanisms.

Our conclusions (Table 2) were in good agreement with those of two prior reviews (7, 8); however, the latter reviews did not employ a meta-analysis and assessed a smaller collection of clinical trials. Interestingly, our results also agreed with a recent clinical trial on DBL (ampicillin plus ceftriaxone) compared with ampicillin plus gentamicin against an important Gram-positive pathogen, *Enterococcus faecalis* (44). Encouragingly, DBL therapy has been recommended by the latest clinical guideline for *E. faecalis* bloodstream infections and infective endocarditis (45).

The observed safety profile for DBL therapy was generally favorable; the tested combinations had significantly lower renal and ototoxicity compared with BLAG therapy (Table 3). This is attractive for patients with impaired renal function or a risk of toxicity (e.g., due to concomitant use of nephrotoxic agents). Ototoxicity was only reported for a small number of antibiotics (Table 3). Cephalothin is an early β-lactam that is known to cause some nephrotoxicity, especially when combined with aminoglycosides (46, 47). Thus, both cephalothin and the aminoglycoside likely contributed to the overall observed nephrotoxicity. In contrast, double anionic β-lactams, such as ticarcillin and carbenicillin, are dosed as disodium salts. This entails a relatively large sodium concentration that has been shown to provide some protection against aminoglycoside-related nephrotoxicity (48). In agreement with this mechanism, BLAG combinations with ticarcillin or carbenicillin showed a significantly lower risk of nephrotoxicity compared to BLAG with other β-lactams (Table S3). Overall, these nephrotoxicity results need to be interpreted with caution, since the definitions of nephrotoxicity differed between studies.

Most of the reviewed studies from the 1970s to early 1990s (Table 1) used multiple-daily dosing of aminoglycosides, as opposed to contemporary once-daily dosing. The latter is expected to be safer (49–51). Furthermore, therapeutic drug management was likely not widely applied in the reviewed studies and might have improved safety (52–54). Thus, contemporary combination regimens with once-daily aminoglycoside dosing (with or without therapeutic drug management) may show better safety compared to that in the studies reviewed here (Table 3). Moreover, the most popular chemotherapeutic agent at that time (cisplatin) is known to cause renal toxicity and ototoxicity (55, 56). Febrile neutropenia usually occurred 7 to 10 days after anticancer chemotherapy; thus, the toxicity profile of the chemotherapeutic(s) may have overlapped with that of antibiotic therapy.

The relatively high incidence of coagulation abnormalities observed in both groups was associated with moxalactam (Table S4), and possibly also with the hematological toxicity from anticancer chemotherapeutic agents in febrile neutropenic patients. Moxalactam is no longer used clinically. Moxalactam exhibited a significantly higher likelihood of bleeding (odds ratio, 9.9) than other agents in a study with 1,493 patients who received one antibiotic for at least 3 days (57). The underlying mechanisms included inhibition of ADP-induced platelet aggregation and an interference with vitamin K-dependent hepatic metabolism of clotting factors (58, 59).

Hypokalemia might be associated with the high dose of sodium from the β-lactams and may be related to renal potassium loss following the use of β-lactam antibiotics (60). These adverse events are less common for more contemporary β-lactams. Resistance emergence is important both for DBL and BLAG therapy (61); however, only a limited amount of resistance data was published in these early clinical trials. This was in part caused by limited knowledge of the molecular resistance mechanisms for β-lactams at this time. Superinfection and bacterial colonization were reported, and no significant differences were found (Table 3).

Over the last 4 decades, both the clinically prevalent bacterial isolates and the available antibiotics have changed extensively. Thus, caution is required when translating the results from this meta-analysis to the isolates with current resistance mechanisms. While the available aminoglycosides remained unchanged since the 1980s (with exception of the recently approved plazomicin), several newer β-lactams (such as carbapenems and cephalosporins) and newer β-lactamase inhibitors have become available (20). Potential limitations of this meta-analysis further include the time frame of these early, open-label,

randomized clinical trials and that some of the studied β-lactams are no longer (widely) clinically used. The quality of reporting of some of these early clinical trials resulted in an unclear risk of bias when evaluated according to contemporary standards. Furthermore, the diversity of these empirical and nonoptimized DBL and BLAG combinations did not allow us to identify optimal combinations. Future translational research should rationally optimize these combinations, leveraging latest pharmacokinetic/pharmacodynamic principles and molecular insights. However, this meta-analysis clearly showed promising safety profiles of DBL therapy in patients that support an in general favorable safety profile of rationally optimized DBL for contemporary β-lactams.

We employed several approaches to enhance the robustness of this meta-analysis. First, we only used randomized clinical trials with a BLAG control group, since BLAG therapy has a long-established status in treating patients with severe infections by Gram-negative pathogens. Second, we put great care into distinguishing the terms "episode," "patient-trial," and "subject." Third, only the evaluable population was considered when calculating clinical response, whereas the overall population who received the dose was included in the safety analysis. Fourth, multiple approaches were used to evaluate microbiological responses (i.e., overall, in all Gram-negative species, and in specific Gram-negative species). Fifth, an exploratory partial least-squares analysis identified potentially influential drugs in DBL and BLAG regimens which led to sensitivity analyses that were underpinned by pharmacokinetic predictions of the aminoglycoside drug exposures.

In summary, this meta-analysis showed comparable clinical and microbiological efficacy and significantly better safety between empirically designed, nonoptimized DBL and BLAG. These conclusions are based on data from thirteen randomized controlled clinical trials. As expected, DBL showed significantly lower renal and ototoxicity compared with BLAG therapy. While empirical, nonoptimized DBL provided promising safety and efficacy, future research will need to design and rationally optimize DBL using newer β-lactams (including carbapenems) and β-lactamase inhibitors. This translational research should leverage mechanistic insights to combat contemporary MDRGN isolates. The latest findings on PBP receptor binding patterns, molecular insights on resistance mechanisms, and translational approaches now enable the rational optimization of innovative DBL dosing strategies. These optimized DBL hold excellent promise to substantially contribute to combating infections by MDRGN and warrant further systematic nonclinical and clinical evaluations.

MATERIALS AND METHODS

Search strategy. (i) Data sources. An exhaustive literature search was performed in PubMed, Embase, ISI Web of Knowledge, and the Cochrane Central Register of records through 31 July 31 2018, with the search terms "double beta lactam," "two beta lactam," and "dual beta lactam." References cited by these publications identified from this search strategy, relevant reviews, and forward citations were further evaluated.

(ii) Inclusion and exclusion criteria. We searched for published randomized clinical trials that compared DBL with BLAG therapy. No restrictions were imposed on the patient population, clinical diagnosis, infection type, length of follow-up, or the specific β-lactams and aminoglycosides combined. All studies had to clearly define the clinical diagnosis standards and the evaluation criteria for clinical and microbiological responses in both treatment groups. Mortality data were inspected, but a lack of mortality data did not result in exclusion of the respective study. The diagnosis and treatment response criteria are summarized in the supplemental material (Tables S1 and S2) for quality assessment. Nonclinical studies that did not report clinical data were excluded. Furthermore, studies without sufficient assessment of clinical and microbiological efficacy and DBL studies which lacked a comparator group were excluded. Only studies written in English were included. Two reviewers (Y.J. and M.-J.C.) searched for and examined the identified studies independently.

Data extraction. Three spreadsheets were developed to extract data from each study for efficacy, safety, and study characteristics. The study characteristics spreadsheet included the last name of the first author, year of publication, diagnosis for antimicrobial treatment in the population, treatment and control regimens, treatment duration, follow-up time, age, and sex. The efficacy spreadsheet included numbers of subjects with positive response and overall numbers of subject for evaluated outcomes. The safety spreadsheet included the last name of the first author, year of publication, numbers of subjects with adverse events, and overall subject number evaluated for the corresponding adverse events. Two reviewers (Y.J. and M.-J.C.) independently extracted the data from the included studies. Disagreements in interpretation were discussed by the two authors, who consulted with the other coauthors (including J.B.B. and G.L.D.) until a consensus was reached.

Outcome. The primary outcome was clinical response in the clinically assessable population. Clinical response was determined as the improvement of clinical symptoms. Secondary outcomes were microbiological response in all bacteria and in all Gram-negative pathogens. Microbiological response was defined as eradication of pathogens present at baseline in the microbiologically assessable population. The risk ratio was chosen as statistical measure and was calculated as the percentage of clinical or microbiological response for the DBL group divided by the percentage for the BLAG group.

Overall numbers of cases (i.e., episodes, or patient-trials) and overall numbers of patients receiving treatment were provided where available (Table 1). Nevertheless, only evaluable cases were used to calculate the risk ratio for clinical and microbiological responses unless specified otherwise. Definitive nonbacterial pathogen infections (e.g., infections by fungi or virus) were excluded from the clinical assessable cases; however, cases with no evidence of bacterial infection were included (i.e., cases with clinically diagnosed bacterial infection without microbiological evidence).

To assess safety, the overall number of cases (patients) receiving the investigated regimens were taken for evaluation unless specified otherwise. All adverse events reported in the studies were recorded and classified, including superinfection and bacterial colonization. Fisher's exact test was used to compare the incidence between two groups for each adverse event.

Analysis. Meta-analyses were performed in the RevMan software (version 5.1) using a random-effects model. Pooled risk ratios and 95% confidence intervals (CIs) were calculated for all primary and secondary outcomes. The DerSimonian-Laird method was used to calculate the between-study variance estimator, τ^2 (62). Sensitivity analyses for specific Gram-negative species (i.e., *P. aeruginosa*, *Klebsiella* spp., and *E. coli*) were performed.

For statistical heterogeneity, the between-study variance (τ^2) was statistically tested using the *Q* test, and a *P* value below 0.10 was considered significant (63). The degree of heterogeneity was assessed by the I^2 metric, which denotes the proportion of total variability in the point estimate that could be attributed to statistical heterogeneity; we classified a heterogeneity of 25% to 49% as low, 50% to 74% as moderate, and ≥75% as high. Forest plots and the heterogeneity results were presented.

Risk of bias was assessed using the RevMan software. This included selection bias, performance bias, detection bias, attrition bias, and reporting bias. Publication bias was further explored graphically by funnel plots. The arcsine-Thompson test in the R package meta (version 4.9-2) was used (64) because of its improved power in detecting publication bias for dichotomous data compared to that of other tests in studies with small sample sizes.

An exploratory analysis was performed to identify β-lactams and aminoglycosides that affected the microbiological response in all bacteria and that in Gram-negative species. This analysis was performed for β-lactams and aminoglycosides which were part of the DBL or BLAG regimens in at least three trials. The presence or absence of each drug in DBL or BLAG regimens was used as the independent variables in a partial least-squares analysis using the XLSTAT software (version 19.02; Addinsoft, Long Island City, NY); this analysis weighted the studies according to sample size. The risk ratios for microbiological response in all bacteria and microbiological response in Gram-negative species served as dependent variables. Subsequently, sensitivity analyses were performed for trials with antibiotics that were identified as influential by partial least-squares analysis.

To further inform this analysis, we calculated the average drug exposures (i.e., area under the plasma concentration-time curve from 0 to 24 h) and peak concentrations expected for the studied aminoglycoside dosage regimens based on published PK data (65–68).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.00425-19.

SUPPLEMENTAL FILE 1, PDF file, 1.6 MB.

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