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Original Article

Comparison of *in vitro* synergy between polymyxin B or colistin in combination with 16 antimicrobial agents against multidrug-resistant *Acinetobacter baumannii* isolates

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KEYWORDS
Multidrug-resistant *Acinetobacter baumannii*;
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In vitro synergy

Abstract *Purposes:* This study determined the synergy of polymyxin B (POLB) and colistin (COL) with 16 other tested antimicrobial agents in the inhibition of multidrug-resistant *Acinetobacter baumannii* (MDR-AB).
Methods: We used chequerboard assays to determine synergy between the drugs against 50 clinical MDR-AB from a tertiary hospital in the Zhejiang province in 2019, classifying combinations as either antagonistic, independent, additive, or synergistic. The efficacy of hit combinations which showed highest synergistic rate were confirmed using time-kill assays.
Results: Both POLB and COL displayed similar bactericidal effects when used in combination with these 16 tested drugs. Antagonism was only observed for a few strains (2%) exposed to a combination of POLB and cefoperazone/sulbactam (CSL). A higher percentage of synergistic combinations with POLB and COL were observed with rifabutin (RFB; 90%/96%), rifampicin (RIF; 60%/78%) and rifapentine (RFP; 56%/76%). Time-kill assays also confirmed the synergistic effect of POLB and rifamycin class combinations. 1/2 MIC rifamycin exposure can achieve bacterial clearance when combined with 1/2 MIC POLB or COL.
Conclusion: Nearly no antagonism was observed when combining polymyxins with other drugs by both chequerboard and time-kill assays, suggesting that polymyxins may be effective in

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combination therapy. The combinations of POLB/COL with RFB, RIF, and RFP displayed neat synergy, with RFB showing the greatest effect.

Introduction

Acinetobacter baumannii is an opportunistic pathogen, and frequent etiological agent of catheter-associated bacteremia, hospital-acquired or ventilator-associated pneumonia, and urinary tract infections in critically ill patients.[1] *A. baumannii* increasingly present with multidrug-resistant phenotypes. Another key feature of *A. baumannii* is its ability to persist on dry and abiotic surfaces for several months. The long-term existence in the environment, multisite and long-term colonization in the human body, increase the risk of cross and cluster infection. These characteristics lead to the rapid spread of multidrug-resistant *A. baumannii* (MDR-AB).[2,3] In recent years, MDR-AB has emerged as a global threat in the healthcare setting, with one epidemiological study reporting that 45% of *A. baumannii* are resistant to three or more classes of antibiotics, even as high as 70% in Latin America and the Middle East.[4—6] As a result, the number of treatment options are rapidly reducing, leading to the use of nontraditional agents, including polymyxins B (POLB) and E (colistin; COL), for the treatment of patients infected with MDR-AB.[7,8]

The use of polymyxins is limited in the clinic due to high levels of nephrotoxicity and the development of resistance during treatment.[9,10] Moreover, pulmonary infections do not respond well to polymyxin monotherapy.[11] Consequently, to improve the success rate of clinical intervention and avoid the emergence of drug resistance, combinations of polymyxins with low toxicity antibiotics are considered good candidates for the treatment of infections caused by MDR-AB.[11]

Despite the powerful bactericidal activity of polymyxins, supported by both preclinical and clinical studies, it remains important to characterize the synergistic and/or antagonistic effect of polymyxins in combination with other antibiotics to guide empirical use.[12] To date, few studies have concurrently evaluated the activity of all potential polymyxin combination therapies, or whether there are differences between POLB and COL in combination therapy. This study therefore aimed to determine the interaction of polymyxins with a variety of antimicrobial agents against MDR-AB using chequerboard and time-kill assays.

Methods

Strains and antibiotics

50 unduplicated clinical MDR-AB strains were randomly selected from all the 199 unduplicated strains isolated from patient blood from January 2019 to December 2019 in a tertiary hospital in the Zhejiang province. Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry was used to confirm isolates were *A. baumannii*.

The antimicrobial agents used in the study were: POLB, COL, cefepime (FEP), sulbactam (SUL), ampicillin/sulbactam (SAM) (2:1), cefoperazone/sulbactam (CSL) (1:1), ceftazidime/avibactam (CZA), imipenem (IPM), meropenem (MEM), amikacin (AMK), tigecycline (TGC), fosfomycin (FOS), rifampicin (RIF), rifabutin (RFB), rifapentine (RFP), vancomycin (VAN), teicoplanin (TEC), and trimethoprim/sulfamethoxazole (SXT) (1:19). The manufacturer, purity, solvent, and diluent of all antibiotics used in this study are shown in Supplementary Table 1.

Antimicrobial susceptibility testing

The minimum inhibitory concentration (MIC) of all antibiotics in this study to *A. baumannii* were determined using the broth microdilution method. *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) strains were used as quality controls. Broth microdilution was performed with a 2-fold serial dilution of antimicrobial solutions and a final bacterial inoculum of 10^5 colony-forming unit per milliliter (CFU/mL) in each well. Only bacterial suspension in the absence of antibiotic was used as a positive control, whereas wells containing CaMHB only were used as a negative control. After inoculation, the plate was incubated at 37 °C for 16—20 h. TGC MICs were interpreted using the breakpoints for *A. baumannii* defined by the US Food and Drug Administration. And POLB/COL MICs were interpreted using the breakpoints for *A. baumannii* defined by the United States Committee on Antimicrobial Susceptibility Testing (USCAST, 2020). MICs for other drugs were interpreted according to the Clinical and Laboratory Standards Institute (CLSI, 2020). Specific breakpoints are listed in Supplementary Table 1. Of note, SAM, CSL, and SXT consist of drugs in a ratio of 2:1, 1:1, and 1:19, respectively, and the fixed concentration of avibactam in CZA is 4 mg/L,for ease of comparison, MICs are expressed as the sulbactam, ceftazidime, and trimethoprim concentration, respectively.

Chequerboard assay

Antibiotic interactions were determined using the chequerboard MIC assay.[13] Seven 2-fold dilutions (8-1/8 MIC) of POLB/COL and 11 2-fold dilutions (8-1/128 MIC) of the test antimicrobial agents were designed according to the results of single-agent susceptibility testing. After drug dilution, wells were inoculated with 10^5 CFU/mL of *A. baumannii* in a 100 μL final volume and incubated at 37 °C for 16—20 h. Chequerboard assay results were interpreted using the fractional inhibitory concentration index (FICI), which is defined as the sum of the MIC of each drug when used in

combination divided by the MIC of the drug when used alone.[14] Synergy was classified as FICI $\leq$0.5, additive as 0.5 $<$ FICI $\leq$1, indifference as 1 $<$ FICI $\leq$4 and antagonism as FICI $>$ 4.[14]

Time-kill assays

Flasks containing CaMHB and the test compound were inoculated with *A. baumannii* to a density of 10^6 CFU/mL in a final volume of 100 mL before incubating with 190 rpm shaking at 37 °C. Samples for viable counts were taken at 0, 1, 2, 4, 6, 8, 12, and 24 h post-addition of antibiotics. All experiments were performed at least in duplicate and the mean CFU/mL values were analyzed. Data points below the limit of detection (log_{10}10 CFU/mL) were displayed as 0. Time-kill assays were performed with selected combinations of antibiotic displaying highest synergistic rate in the chequerboard assay. One strain showed synergistic effect in selected combinations in the chequerboard assay was randomly selected for the Time-kill assay. The MICs of 18 antibiotics against this selected strain were shown in Supplementary Table 2. Antibiotic combinations were tested at concentrations based on the MIC determined from the chequerboard assay. Drugs alone were used at 1 MIC and 1/2 of the MIC, and drugs were used in combination were at concentrations of 1/2 MIC+1/2 MIC.

A drug combination was classified as synergistic if the bacterial concentration was $\geq$2 log10 CFU/mL lower in combination than the bacterial counts recovered following treatment with the most potent single antibiotic at the 24th h. Combinations were classed as additive if the bacterial reduction was 1–2 log10 CFU/mL.[15] A bactericidal effect was defined as a $\geq$3 log10 CFU/mL reduction in bacterial concentrations compared with the starting inoculum, while a bacteriostatic effect was defined as a $<$3 log10 CFU/mL bacterial reduction.[15]

Population analysis profiles (PAPs)

Antibiotic heteroresistance was analysed by PAPs. In brief, exponential culture of selected strain was grown in 4 mL of LB broth at 37 °C with 180 rpm shaking. Aliquots (50 μL) were taken at 24h post-inoculation. CFUs were enumerated by plating serial dilutions on Mueller-Hinton agar plates with 0, 1/8, 1/4, 1/2, 1, 2, 4, 8 MIC of POLB/COL/RIF/RFB/RFP. Heteroresistance was defined using the criteria published by El-Halfawy et al., which states there should be a more than 8-fold difference between the lowest antibiotic concentration giving maximum growth inhibition and the highest noninhibitory concentration.[16]

Statistical analysis

The FICI values of POLB and COL in combination with test antimicrobial agents were compared using a paired samples t-test. The rank sum test for paired data comparison (Wilcoxon method) was used to compare the synergistic effect of combined schemes. All statistical analyses were performed by using SPSS v. 21.0 software (IBM, Armonk, NY, USA).

Results

MIC distributions and susceptibility profiles

All 50 isolates tested were resistant to FEP, IPM, and MEM, and showed high resistant rates (54%–98%) to SXT, AMK, CSL, and SAM (Table 1). Most isolates exhibited high-level resistance to SUL (MIC_{50} 32 mg/L), CZA (MIC_{50} 32 mg/L), VAN (MIC_{50} 128 mg/L), TEC (MIC_{50} 256 mg/L) and FOS (MIC_{50} 256 mg/L). All isolates exhibited an MDR-phenotype (resistant to three or more classes of antibiotics). By contrast, strains were highly susceptible to polymyxins (POLB and COL), TGC, and rifamycins (RIF, RFB and RFP) (MIC_{50} 1–2 mg/L).

Chequerboard assays

Chequerboard assays of 16 test antibiotics with POLB/COL were performed against the 50 *A. baumannii* isolates. The MIC_{50}/MIC_{90} of 18 antimicrobials in combination with POLB/COL were lower than each antimicrobial alone. When other compounds were combined with polymyxins, the most common effect was additive or independent. Antagonism was only observed in combination of POLB and CSL. A higher percentage of synergistic combinations with POLB or COL were observed with RFB (90%/96%), RIF (60%/78%) and RFP (56%/76%). The greatest synergistic effect was with rifamycins (RFB, RIF, and RFP), with a mean FICI value of 0.28, 0.47, and 0.48 respectively. In combination with RIF and RFP, COL exhibited greater synergy than POLB ($p < 0.001$), whereas no significant difference was observed between COL and POLB in combination with other drugs (Fig. 1).

Cumulative inhibition ratios (CIRs)

CIR curves of POLB/COL shifted markedly to the left when combined with the tested agents compared with POLB/COL alone. The CIRs curves of the tested agents also shifted to the left when combined with the POLB/COL compared with the tested agents alone. Although low doses of POLB/COL reduced the MIC of the tested agents, the combined MIC value was still high for all drugs except RIF, RFB, RFP, and TGC. Importantly, some promising combination schemes (with high synergistic rate) only showed synergy at very high antibiotic concentrations. Compared to combination with POLB, the CIRs curve of RIF and RFP combined with COL had a larger left shift. Instead, the left CIRs shift of other drugs in combination with POLB was no different to that with COL. Together, these data indicate that RFB, RIF, and RFP are potential candidates as combination partners with POLB/COL against MDR-AB (Fig. 2).

Time-kill assays

Synergy in the chequerboard experiments were confirmed by time-kill assays of the most effective combinations, which were POLB/COL (1/2 MIC), combined with RIF, RFB and RFP (1/2 MIC) against a single strain of *A. baumannii*. Of the six combination schemes evaluated in the time-kill assays, all showed a $\geq$2 log_{10} decrease in CFU/mL

Table 1 The MIC distributions of 16 antibiotics against *A. baumannii* alone and in combination with POLB/COL.

	TGC	RIF	RFB	RFP	FOS	FEP	SUL	SAM	CSL	CZA	IPM	MEM	AMK	VAN	TEC	SXT
Single drug MIC																
MIC_{50}	1	2	2	2	256	128	32	64	32	32	32	32	256	128	256	4
MIC_{90}	2	8	4	2	512	256	64	128	64	128	64	64	256	256	256	32
MIC range	0.25–4	0.25–256	0.5–32	1–32	16–512	64–256	8–256	8–256	8–512	8–512	8–256	8–128	0.5–256	128–256	4–512	0.25–32
R (%)	2 (4)	/	/	/	/	50 (100)	/	49 (98)	48 (96)	/	50 (100)	50 (100)	45 (90)	/	/	27 (54)
MIC combined with POLB[a]																
MIC_{50}	0.25	0.5	0.125	0.5	64	64	16	32	16	16	8	16	32	64	64	1
MIC_{90}	0.5	4	0.5	1	256	128	32	64	32	64	64	32	256	64	128	8
MIC range	0.008–2	0.008–16	0.016–1	0.016–8	0.5–512	1–256	0.5–128	1–256	1–256	0.5–256	0.25–128	1–64	0.25–256	2–256	1–256	0.016–16
R (%)	0 (0)	/	/	/	/	44 (88)	/	38 (76)	33 (66)	/	43 (86)	45 (90)	39 (78)	/	/	12 (24)
MIC combined with COL[b]																
MIC_{50}	0.25	0.25	0.125	0.25	32	32	8	32	16	16	8	8	16	32	64	1
MIC_{90}	0.5	2	0.5	1	128	128	32	64	32	64	32	32	256	128	128	16
MIC range	0.016–4	0.008–64	0.03–4	0.016–8	0.5–512	0.5–256	0.5–256	0.5–128	1–128	0.5–512	0.5–128	0.5–64	0.03–512	8–256	0.125–256	0.016–16
R (%)	2 (4)	/	/	/	/	42 (84)	/	35 (70)	34 (68)	/	47 (94)	46 (92)	36 (72)	/	/	15 (30)
POLB MIC combined with test agent[c]																
MIC_{50}	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.25	0.25	0.25	0.25	0.25	0.25	0.125	0.125	0.25
MIC_{90}	0.25	0.5	0.25	0.25	0.5	0.5	1	2	1	1	1	1	0.5	0.5	0.5	1
MIC range	0.016–2	0.06–1	0.03–0.5	0.03–0.5	0.016–1	0.016–4	0.016–2	0.016–4	0.016–4	0.03–4	0.016–2	0.016–2	0.008–1	0.016–1	0.03–0.5	0.008–2
COL MIC combined with test agent[d]																
MIC_{50}	0.25	0.125	0.125	0.125	0.25	0.25	0.25	0.5	0.25	0.25	0.5	0.25	0.25	0.25	0.25	0.12
MIC_{90}	0.5	0.5	0.25	0.5	1	1	1	1	1	1	1	1	0.5	0.5	0.5	1
MIC range	0.03–2	0.03–1	0.03–0.5	0.06–0.5	0.016–2	0.03–2	0.016–4	0.03–4	0.016–4	0.016–4	0.03–4	0.06–2	0.016–2	0.03–1	0.03–2	0.016–4

[a] MIC distributions of 16 antibiotics against *A. baumannii* in combination with POLB.
[b] MIC distributions of 16 antibiotics against *A. baumannii* in combination with COL.
[c] MIC distributions of POLB against *A. baumannii* in combination with 16 antibiotics.
[d] MIC distributions of COL against *A. baumannii* in combination with 16 antibiotics.

Susceptibility breakpoints have not been established for RIF, RFB, RFP, FOS, SUL, CZA, VAN, and TEC against *A. baumannii*; therefore, resistance rates were not calculated.

Abbreviations: POLB, polymyxin B; COL, colistin; FEP, cefepime; SUL, sulbactam; SAM, ampicillin/sulbactam (2:1); CSL, cefoperazone/sulbactam (1:1); CZA, ceftazidime/avibactam; IPM, imipenem; MEM, meropenem; AMK, amikacin; TGC, tigecycline; FOS, fosfomycin; RIF, rifampicin; RFB, rifabutin; RFP, rifapentine; VAN, vancomycin; TEC, teicoplanin; SXT, trimethoprim/sulfamethoxazole.

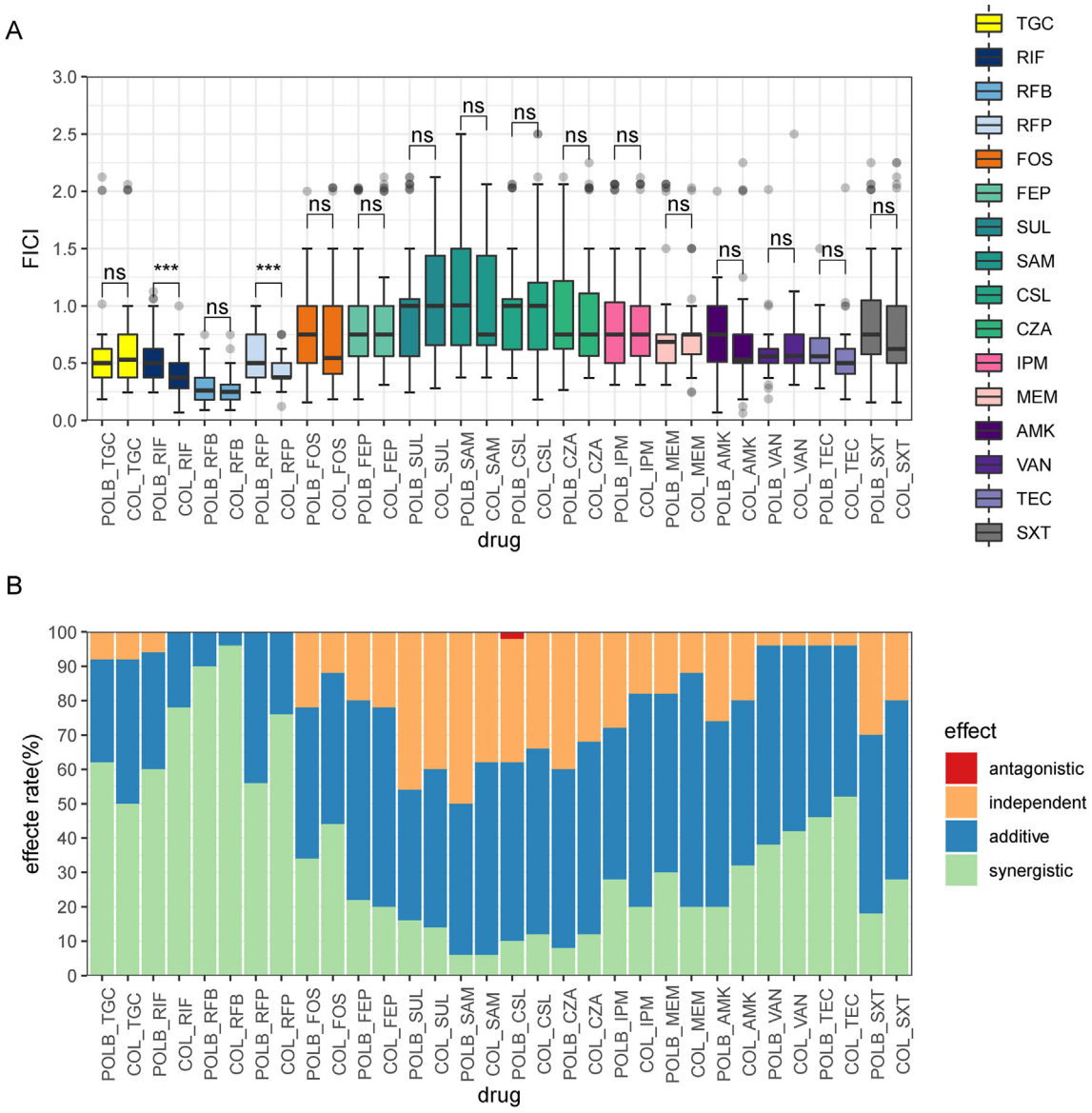

Figure 1. The FICI distributions and synergistic effects of 32 antibiotic combination schemes against 50 *A. baumannii* isolates. (A) Boxplots show the median FICI of 32 antibiotic combination schemes, the first and third quartiles, the interquartile range (IQR), and error bars denoting 1.5 times the IQR. The dots represent data points outside of this range. *, $p < 0.1$; **, $p < 0.01$; ***, $p < 0.001$, ns: nonsignificant. (B) Stacked bar chart summarizing the effects of 32 antibiotic combination schemes against *A. baumannii*. Abbreviations: POLB, polymyxin B; COL, colistin; FEP, cefepime; SUL, sulbactam; SAM, ampicillin/sulbactam (2:1); CSL, cefoperazone/sulbactam (1:1); CZA, ceftazidime/avibactam; IPM, imipenem; MEM, meropenem; AMK, amikacin; TGC, tigecycline; FOS, fosfomycin; RIF, rifampicin; RFB, rifabutin; RFP, rifapentine; VAN, vancomycin; TEC, teicoplanin; SXT, trimethoprim/sulfamethoxazole; FICI, fractional inhibitory concentration index.

compared with the most potent single antibiotic. Therefore, both methods confirmed the synergistic effect of polymyxin and rifamycin combinations. 1/2 MIC POLB, COL, RIF, RFB and RFP alone were bacteriostatic, whereas six combinations (POLB/COL (1/2 MIC), combined with RIF, RFB and RFP (1/2 MIC)) showed $\geq$3 $\log_{10}$ reduction in CFU/mL compared with the starting inoculum, and were considered bactericidal against MDR-AB. When 1/2MIC rifamycin was used in combination with POLB, all combinations eliminated bacteria within 8 h (Fig. 3). The strain had shown a homogeneous response to POLB/COL/RIF/RFB/RFP because the difference between the lowest antibiotic concentration giving maximum growth inhibition and the highest non-inhibitory concentration was less than 8-fold (Fig. S1).

Discussion

Given that MDR-AB frequently harbor multiple resistance mechanisms.[17] leaving few treatment options available, polymyxins are considered a drug of last resort. POLB and COL are polymyxin family antibiotics, which interact with the Gram-negative cell membrane, resulting in rapid changes in permeability leading to cell death.[18] However,

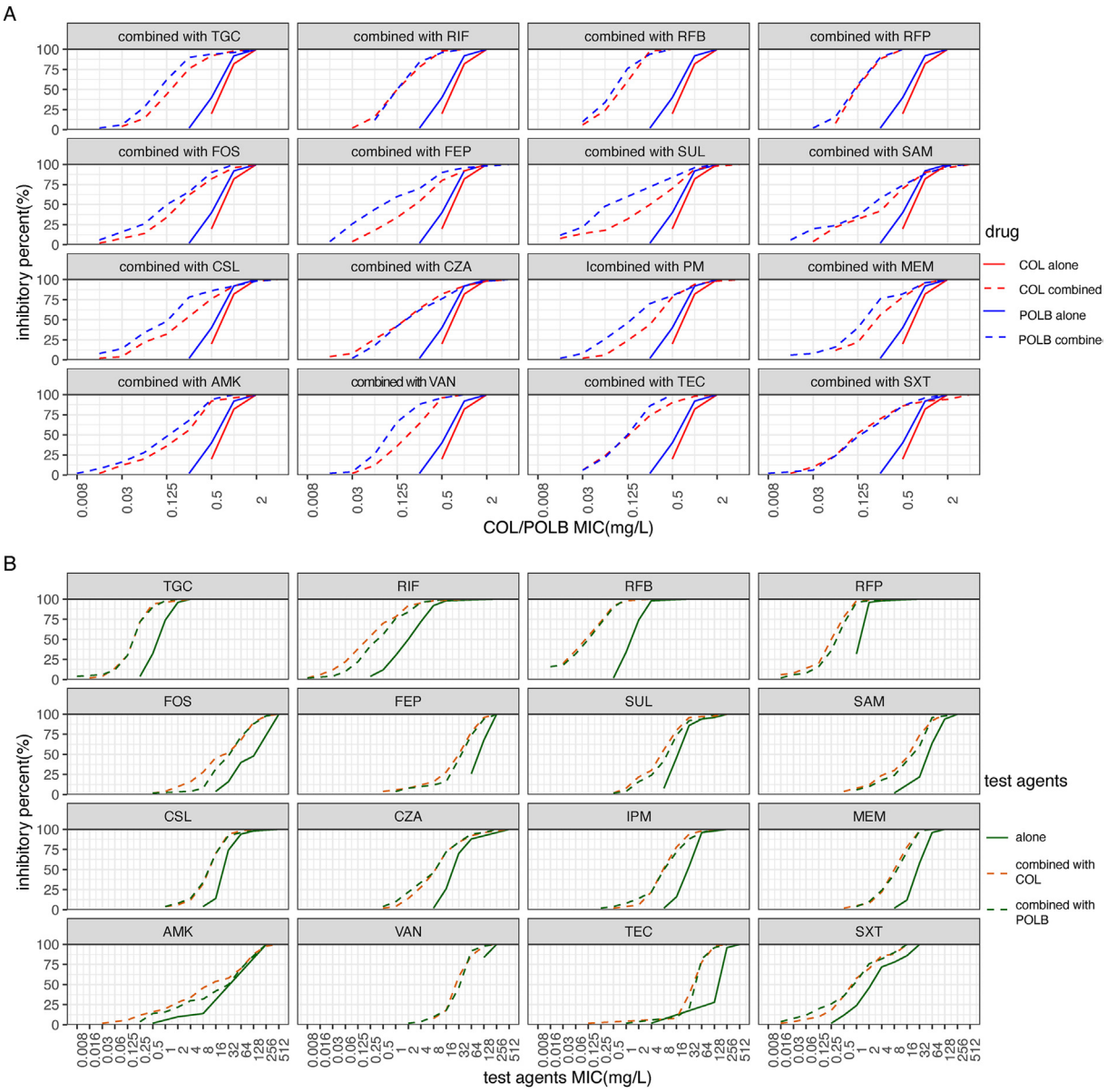

Figure 2. The CIRs of 18 antibiotics against *A. baumannii* alone and in combination with POLB/COL. (A) The CIRs of POLB/COL alone and in combination with test agents against *A. baumannii*. Solid lines represent POLB/COL alone while dotted lines represent the combination between POLB (red), COL (blue) and the test agents. (B) The CIRs of test agents alone and in combination with POLB/COL against *A. baumannii*. Solid lines represent the test agent alone while the dotted lines represent the combination between the test agents and either POLB (green) or COL (orange). Abbreviations: POLB, polymyxin B; COL, colistin; FEP, cefepime; SUL, sulbactam; SAM, ampicillin/sulbactam (2:1); CSL, cefoperazone/sulbactam (1:1); CZA, ceftazidime/avibactam; IPM, imipenem; MEM, meropenem; AMK, amikacin; TGC, tigecycline; FOS, fosfomycin; RIF, rifampicin; RFB, rifabutin; RFP, rifapentine; VAN, vancomycin; TEC, teicoplanin; SXT, trimethoprim/sulfamethoxazole; CIRs, cumulative inhibition ratios.

the efficacy, toxicity, limited penetration of the drug into the lung and the risk of selecting for resistance when used as a monotherapy require careful consideration.[19] Confronted with an increase of MDR-AB infections, selection of effective drugs combined with polymyxins is increasingly important in medical practice, warranting the investigation of novel drug combinations.

This study investigated the combined effects of POLB/COL and 16 other antibiotics against MDR-AB. When used alone, POLB/COL had the greatest *in vitro* activity against the isolates tested, with susceptibility rates of 100%, followed by TGC (96%), RIF (MIC_{50}:1 mg/L), RFB (MIC_{50}:1 mg/L) and RFP (MIC_{50}:1 mg/L). We found enhanced bactericidal activity following combination of POLB/COL with all assayed drugs including some antimicrobials which are indicated for infections caused by Gram-positive bacteria. These drugs, for example, VAN and TEC do not penetrate the outer membrane of Gram-negative bacteria with large size and complex structure. However, when combined with polymyxins, the MIC_{50}, MIC_{90}, and MIC range were clearly

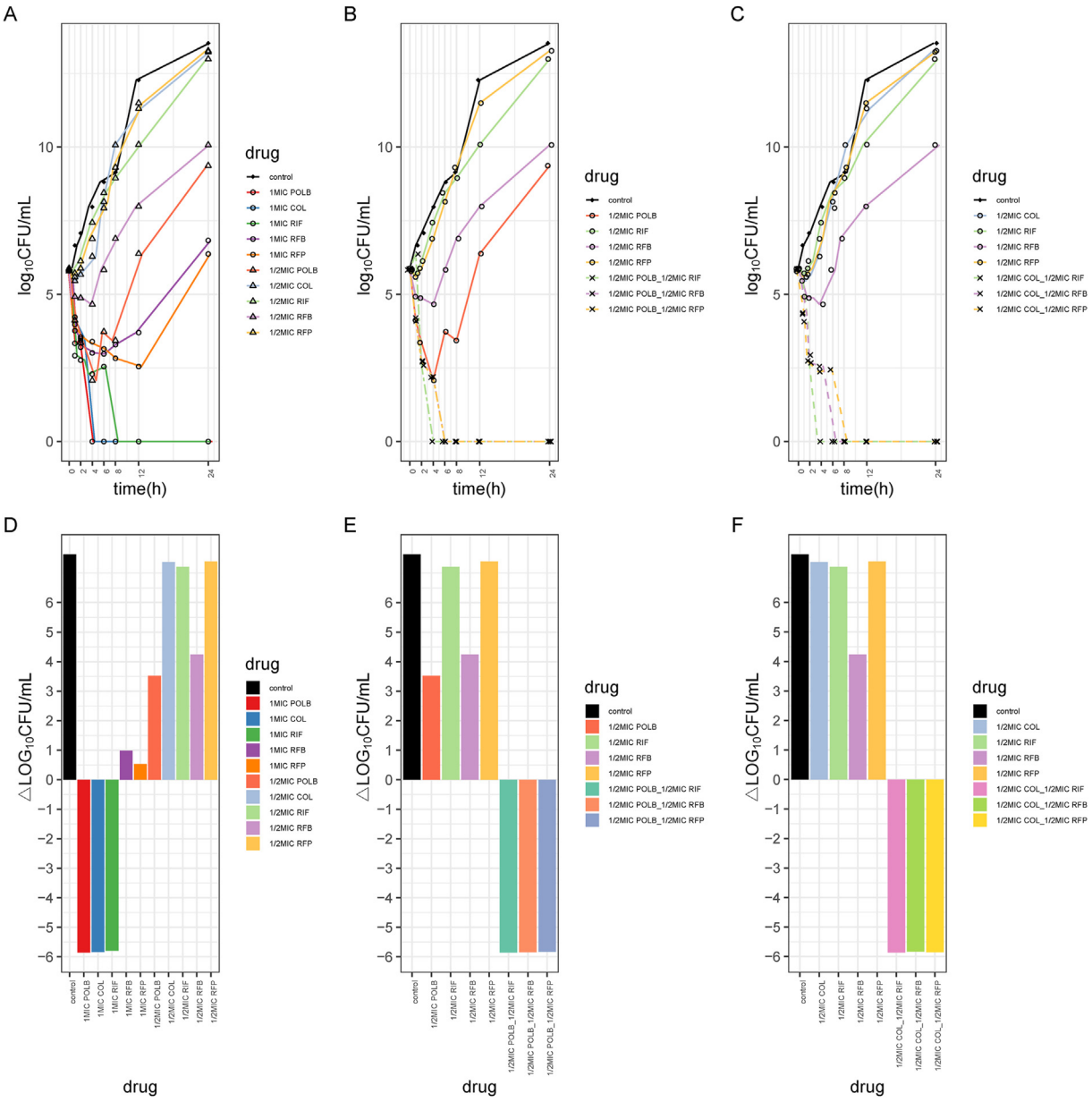

Figure 3. *In vitro* time-kill assays using 1 MIC or 1/2 MIC POLB/COL and rifamycin (RIF, RFB, and RFP). (A, D) 1 MIC and 1/2 MIC POLB, COL RIF, RFB, RFP alone. (B, E) 1/2 MIC RIF, RFB, RFP alone and in combination with 1/2 MIC POLB. (C, F) 1/2 MIC RIF, RFB, RFP alone and in combination with 1/2 MIC COL. Abbreviations: POLB, polymyxin B; COL, colistin; RIF, rifampicin; RFB, rifabutin; RFP, rifapentine; MIC, minimum inhibitory concentration.

lower for all isolates than those for each antimicrobial alone. This may suggest rapid permeabilization of the outer membrane by polymyxin facilitating entry of additional drugs.[20,21] However, some drugs, including FOS, FEP, VAN, TEC, SAM, and AMK, only displayed synergy with POLB/COL at very high concentrations. A number of recent studies highlighted a potential benefit of treating MDR-AB pneumonia with polymyxins in combination with high-dose SAM, FOS.[22] However, further studies are needed to confirm the clinical efficacy of these combination schemes.

In a meta-analysis, the overall synergistic rates with polymyxin and carbapenem combinations were 32% for *A. baumannii* in the checkerboard studies.[23] This result was coincident with ours (20%—30%). This study demonstrated that POLB/COL showed the greatest synergy against MDR-AB when combined with rifamycin class drugs. RFB, RIF, and RFP showed synergy levels with POLB/COL of 90%/96%, 60%/78% and 56%/76%, respectively. Furthermore, RFB, RFP, and RIF showed synergy at very low antibiotic concentrations demonstrated by chequerboard and time-kill assays, observations in keeping with previous study.[24] Like RIF, RFB and RFP are both rifamycin class antibiotics, drugs targeting the bacterial RNA polymerase. Few studies have addressed RFB and RFP combinations to treat

infections caused by MDR-AB. In our study, RFB displayed greater synergistic rates with polymyxins than RIF and RFP. Similarly, Brian et al.[25] found that RFB was more potent against MDR-AB than RIF *in vitro* and *in vivo* mice experiments. A randomized, controlled clinical trial demonstrated significantly improved microbiological eradication but no significant improvement of clinical cure when using RIF and COL combination for MDR-AB infections.[26] Given that RIF had some effect clinically, we believe that RFB and polymyxin combination could be a therapeutic option for MDR-AB infections. Thus, studies are in progress to evaluate the clinical utility of RFB for the treatment of MDR-AB infections for which there are currently limited treatment options.

POLB and COL have very similar chemical structures, differing by only one amino acid in the peptide loop. Studies have suggested that the difference between POLB and COL may manifest when used clinically.[27] POLB is administered as a sulfate salt, meaning that active drug is directly administered to the patient. COL, however, is administered in the form of colistimethate sodium (CMS), an inactive prodrug, which is converted to COL *in vivo*. COL is used as a sulfate salt in susceptibility testing, obscuring the complex effect of converting CMS to COL during therapy. The SENTRY surveillance project found that POLB and COL had similar *in vitro* activities (MIC 90, $\leq$0.5—1 mg/L) against *P. aeruginosa*, *Klebsiella pneumoniae* and *A. baumannii*,[28] but there is a lack of experimental data comparing combinations between POLB/COL and other antibiotics. This study found that when combined with other antibacterial agents, POLB and COL showed no significant difference in the *in vitro* antibacterial activity.

There are some limitations of the present study that require consideration. This study only tested the synergy effect between polymyxin B or colistin in combination with 16 antimicrobial agents against MDR-AB *in vitro*, lacking clinically relevant data. Second, unlike the findings in prior studies,[29—32] the strain for Time-kill assays in this study showed no regrowth at 1MIC POLB/COL/RIF/RFB/RFP in 24 h. In previous studies,[29—32] regrowth was mainly due to the heterogeneity of susceptibility to polymyxin. To confirm the character of antimicrobial susceptibility of this strain, PAP was carried out, and the strain had shown a homogeneous response to POLB/COL/RIF/RFB/RFP. Therefore, this study did not take into account the impact of combination therapy on heterogeneous resistant strains of *A. baumannii*. Besides, the MDR-AB strains in this study are highly susceptible to polymyxins and rifamycins. The synergistic effect of polymyxins/rifamycin class combinations in non-susceptible strains is not certain. These problems need to be highlighted and explored in the future study.

Facing with the challenge of multiple or extensive resistant *A. baumannii* infections, polymyxins are emerging as candidates for treatment of these infections in the way of combining with other agents. Our data demonstrate nearly no antagonism between POLB/COL in combination with other antimicrobials against MDR-AB. The combination of POLB/COL with RFB, RIF, and RFP showed the greatest synergy, with RFB being the most effective.

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Availability of data and materials

The datasets generated and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

All authors made substantial contributions to conception and design, acquisition of data, analysis and interpretation of data, and drafting and revision of the article. All authors agreed on the journal submission, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Ethics approval and consent to participate

Not required.

Patient consent for publication

Not applicable.

Declaration of competing interest

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmii.2024.01.009.