International Journal of Antimicrobial Agents 53 (2019) 34–39

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

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

An alternative strategy for combination therapy: Interactions between polymyxin B and non-antibiotics

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ARTICLE INFO

Article history:
Received 5 July 2018
Accepted 10 September 2018

Editor: Jean-Marc Rolain

Keywords:
Polymyxin B
Checkerboard assay
Antibiotic/non-antibiotic combinations
Screening test
Drug synergy
Antibiotic resistance

ABSTRACT

Antimicrobial resistance is increasing and few new antibiotics are in the development pipeline. Alternative strategies to treat infectious diseases, such as combination therapy, are urgently needed. Polymyxin B is a neglected and disused antibiotic with moderate antibacterial activity. In this study, we aimed to find synergistic interactions between polymyxin B and a wide range of non-antibiotics (non-ABs) to improve its efficacy. Thirty non-AB compounds from various drug classes were screened for synergistic potential with sub-minimum inhibitory concentrations (MICs) of polymyxin B in an agar diffusion assay against *Acinetobacter baumannii, Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (3 isolates per species). Potential candidates were further studied in in vitro checkerboard assays, up to 5 isolates per species, using optical density to assess growth. Interactions were assessed with fractional inhibitory concentration index (FIC_i) analysis and surface response analysis with Loewe, Bliss and Highest Single Agent analysis using the Combenefit program. Twenty non-ABs enhanced polymyxin B activity in the agar diffusion test in one or more species. Of these, three showed a consistent synergistic effect ($FIC_i \leq 0.5$) in the checkerboard assay for at least one species: citalopram, sertraline and spironolactone. Surface response analyses were largely in concordance, and further assessment showed only spironolactone was synergistic with polymyxin B at clinically relevant levels. The screening strategy used showed consistent synergism in vitro between polymyxin B and some non-ABs for *A. baumannii, E. coli* and *K. pneumoniae.* The synergistic interactions found merit further exploration as alternative strategies for difficult-to-treat infections.

1. Introduction

The emergence of antibiotic resistance is an important issue. Annual reports by the European Centre for Disease Prevention and Control show that an increasing percentage of infections is caused by resistant pathogens, rendering current drug therapies increasingly ineffective [1]. A September 2017 report by the World Health Organization (WHO) [2] shows the discovery and development of new antibiotics and antibiotic classes has slowed down substantially. Alternative strategies are necessary to ensure bacterial infections remain treatable. Combination therapy is commonly used to treat bacterial infections, but the increasing incidence of resistance mechanisms inherently limits the options for antibiotic combinations.

An alternative strategy is to search for drugs that are already available to treat other diseases but have never been studied for antimicrobial effects. A limited number of studies [3,4] have shown that various non-antibiotics (non-ABs) demonstrate an antimicrobial effect besides their intended use. Although toxicity may be an issue, synergistic effects of non-ABs in combination with antibiotics may be of significant value, particularly those antibiotics that have become neglected and disused because of moderate activity (ND-ABs) [5].

Polymyxin B has received increased interest [6] as an alternative to colistin. However, polymyxin B is only moderately active at clinical concentrations and resistant strains have emerged [7,8]. Combinations with other drugs would therefore appear to be a potential solution to enhance its in vivo effectiveness. In this study, we selected 30 non-AB compounds from various drug classes because of their potential antimicrobial effects. These were screened

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https://doi.org/10.1016/j.ijantimicag.2018.09.003
0924-8579/

Table 1
Characteristics of Gram-negative isolates used in this study.

Isolate number	Species	Resistance mechanisms	MIC polymyxin B (mg/L)
109	*Acinetobacter baumannii*	OXA-23-like (plasmid) + OXA-51-like (chromosomal)	2
110	*Acinetobacter baumannii*	OXA-23-like (plasmid) + OXA-51-like (chromosomal)	1
111	*Acinetobacter baumannii*	OXA-23-24-like (plasmid)+ OXA-51-like (chromosomal)	2
112	*Acinetobacter baumannii*	OXA-23-24-like (plasmid)+ OXA-51-like (chromosomal)	1
113	*Acinetobacter baumannii*	OXA-23-24-like (plasmid)+ OXA-51-like (chromosomal)	1
ATCC25922	*Escherichia coli*	-	1
15	*Escherichia coli*	CTX-M 15	1
51	*Escherichia coli*	OXA-1, CTX-M 15	1
107	*Escherichia coli*	VIM	1
108	*Escherichia coli*	KPC-2, TEM-1	1
17	*Klebsiella pneumoniae*	SHV-1, OXA-1, CTX-M 15	1
100	*Klebsiella pneumoniae*	KPC-2	2
104	*Klebsiella pneumoniae*	KPC-3, OmpK35red, OmpK36red	2
58	*Klebsiella pneumoniae*	TEM-84, SHV-11	2
74	*Klebsiella pneumoniae*	CTX-M 1	2
ATCC27853	*Pseudomonas aeruginosa*	-	1
85	*Pseudomonas aeruginosa*	nitrocefinase activity '+++'; AmpC transcript overexpressed; β-lactamase genotype: blaampC, blapoxB; class A-, class B-	1
89	*Pseudomonas aeruginosa*	OprD-, AmpCind, Class A-, Class B-	2
87	*Pseudomonas aeruginosa*	nitrocefinase activity '++++'; AmpC transcript overexpressed; β-lactamase genotype: blaampC, blapoxB; class A-, class B-	2
95	*Pseudomonas aeruginosa*	OprD-, AmpCcon, Class A-, Class B-	2

MIC values for polymyxin B were determined in triplicate by broth microdilution. Median value is shown.

for interaction with polymyxin B against a range of bacterial isolates with various resistance mechanisms, including several that were recently published by the WHO as high priority pathogens, such as *Acinetobacter baumannii* [9].

2. Methods

2.1. Compounds

Polymyxin B sulphate salt (Sigma-Aldrich, Zwijndrecht, The Netherlands [NL]) was dissolved in sterile water to a stock solution of 1280 mg/L and stored at -80°C. Further dilutions were prepared in Mueller-Hinton II (MH-II) broth (BD, Vianen, NL). Thirty non-AB compounds representing various classes of therapeutic drugs were selected to screen for interaction with polymyxin B (for details, see **Table A.1**). Aqueous stock solutions of 1280 mg/L were prepared and stored at -80°C with the exception of glibenclamide, containing 512 mg/L, and loperamide, which was readily available as a 200 mg/L aqueous solution.

2.2. Bacterial isolates

Twenty Gram-negative bacterial strains with a wide array of resistance mechanisms were used (Table 1). These comprised four species: *A. baumannii, Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, 5 strains per species. All isolates were of clinical origin except for two ATCC strains (*E. coli* ATCC25922 and *P. aeruginosa* ATCC27853). Strains were grown overnight on Trypticase Soy Agar with 5% sheep blood (BD, Vianen, NL) before use.

2.3. MICs and agar screening test

MICs for all compounds were determined by ISO [10] compliant methods for broth microdilution, using a range of 0.25 to 128 mg/L for all non-ABs except loperamide (range: 0.125–64 mg/L). MICs of polymyxin B were determined over 0.03 to 16 mg/L.

We screened for interaction between polymyxin B and non-ABs by an adapted agar diffusion method designed for this purpose. For each species, 3 strains were selected. 50 µL bacterial suspension (0.5 McFarland standard; 1-2 $\times 10^8$ CFU/mL) was spread on an MH-II agar (BD) plate containing 0.5 x MIC polymyxin B. Non-ABs were added in wells with a diameter of 5 mm, using 100 µL stock solution of each non-AB. Plates were incubated overnight at 37°C. Inhibition zones around each well were measured.

2.4. Checkerboard assays

Combinations that showed interaction in the screening assay were further studied in a checkerboard assay as described by Berkhout et al. [11]. If growth inhibition was observed for two or three isolates per species in the screening assay, the checkerboard assay for that combination was performed for all five isolates of that species. When growth inhibition was observed for one isolate per species, the checkerboard assay was performed for that isolate only. If no growth inhibition was observed, the combination was not tested any further. In an 8×6 checkerboard design, 50 µL of both polymyxin B solution and non-AB solution was added to each well. Final polymyxin B concentrations ranged from 0.063 to 4 mg/L and non-AB concentrations ranged from 4 to 64 mg/L except loperamide (2–32 mg/L).

The 5 combinations per species that showed the highest synergistic effect in surface response analysis were subsequently studied in an 8×12 checkerboard design. Final polymyxin B concentrations remained unchanged; final non-AB concentrations ranged from 0.031 to 32 mg/L to include clinically relevant concentrations. Freshly prepared trays were stored at -20°C and used within 7 days. Every plate included a drug-free growth control. 100 µL bacterial suspension (final inoculum 2-8 $\times 10^5$ CFU/mL) was added to each well, and plates were incubated at 37°C for 16–20 h. Each set of checkerboard assays included a negative control and positive control *E. coli* ATCC25922.

The optical density (OD) of each well was measured on an Epoch 2 microplate reader (Biotek, Winooski, Vermont, USA) at OD=600 nm, using Gen5 Microplate Reader and Imager Software (Biotek). ODs were converted to relative OD compared with the growth control after subtracting background OD.

2.5. Analysis

Analysis was performed using the Fractional Inhibitory Concentration index (FIC_i) [12] and various surface response

Table 2
Number of isolates per species for which non-ABs showed growth inhibition in combination with polymyxin B in agar screening assay.

Drug class	Non-AB	*A. baumannii* (*n*=3)	*E. coli* (*n*=3)	*K. pneumoniae* (*n*=3)	*P. aeruginosa* (*n*=3)
Analgesic	Acetylsalicylic acid	0	0	0	0
	Diclofenac sodium	0	0	0	0
	Ibuprofen	0	3	3	0
	Paracetamol	0	0	0	0
Antidepressants	Amitriptyline	3	3	3	3
	Citalopram	3	3	3	0
	Imipramine	3	3	3	3
	Sertraline	3	3	3	3
Antidiabetic	Glibenclamide	0	0	0	0
Antidiarrhoeal	Loperamide	3	2	1	0
Antihypertensive	Nifedipine	0	0	0	0
	Propranolol	3	3	3	2
	Verapamil	3	3	2	0
Antimuscarinics	Mebeverine	3	0	2	0
Antiplatelets	Clopidogrel	1	0	0	0
Antipsychotics	Chlorpromazine	3	3	3	3
	Clonazepam	3	3	3	3
	Diazepam	1	1	0	0
	Haloperidol	3	3	3	3
	Levomepromazine	3	3	3	2
	Promethazine	3	3	3	3
	Zuclopenthixol	2	2	2	0
Barbiturates	Phenobarbital	0	0	0	0
Diuretic	Spironolactone	1	2	1	0
Local anaesthetics	Lidocaine	0	0	0	0
Mucolytic agent	N-acetylcysteine	0	0	0	0
Pump inhibitors	Esomeprazole	0	0	0	0
	Omeprazole	0	0	0	0
Statin	Atorvastatin	1	0	0	0
	Simvastatin	3	0	0	0

Non-AB: non-antibiotic.

models (Loewe; Bliss; Highest Single Agent) [13]. The FIC_i was calculated for wells without growth (≤10% relative OD) adjacent to wells with growth (>10% relative OD). The FIC_i was calculated as follows: $\Sigma FIC = \frac{Ca}{MICa} + \frac{Cb}{MICb}$ with C_a and C_b the concentrations of polymyxin B and the non-AB in the combination, respectively, and MIC_a and MIC_b the MICs of drug A (polymyxin B) and B (the non-AB), respectively. If the MIC of a non-AB was >128 mg/L for any isolate, ΣFIC was calculated with an MIC_b of 256 mg/L.

F_{min} was defined as the lowest ΣFIC-value and F_{max} as the highest ΣFIC-value. Additivity range was set at 0.5<ΣFIC<2. ΣFIC≤0.5 indicates synergism, and ΣFIC>2 indicates antagonism [12]. If F_{max} >2, the combination is defined as antagonistic, regardless of F_{min}.

Surface response analysis was performed using the Combenefit program [13]. The program performs Loewe, Bliss and Highest Single Agent (HSA) analyses and provides various output parameters. The parameter that was considered most important for interpretation of interaction was the summary parameter describing both synergistic and antagonistic effects (SUM_SYN_ANT). A separate analysis was performed for each checkerboard result.

3. Results

3.1. Susceptibility testing

MICs of polymyxin B are shown in Table 1. Median (range) MICs (mg/L) of polymyxin B per species were: *A. baumannii* 1 (1–2); *E. coli* 1 (1–1); *K. pneumoniae* 2 (1–2) and *P. aeruginosa* 2 (1–2). MICs of non-ABs were 128 mg/L or higher for all tested isolates, with a few exceptions of 32 mg/L and 64 mg/L (Table A.1). Ranges did not deviate more than one two-fold dilution step from median MICs for any compound.

3.2. Agar screening test

Results of the agar screening test are shown in Table 2. The table shows the number of isolates per species per non-AB drug where an inhibition zone was detected around the well with the corresponding non-AB in the presence of 0.5 x MIC of polymyxin B. Inhibition zones were found for seven non-ABs for all tested isolates: amitriptyline, imipramine, sertraline, chlorpromazine, clonazepam, haloperidol and promethazine. Propranolol and levomepromazine showed consistent inhibition zones for all species except *P. aeruginosa*. Citalopram, loperamide, verapamil, zuclopenthixol and spironolactone showed inhibition zones only for one or more *A. baumannii, E. coli* and *K. pneumoniae* isolates. Ten non-ABs showed no inhibition for any tested isolate.

3.2.1. 8 × 6 Checkerboard assays – FIC analysis

The results of the 8 × 6 checkerboard assays can be found in Table A.2. Citalopram, sertraline and levomepromazine showed a synergistic effect with polymyxin B for all *A. baumannii, E. coli* and *K. pneumoniae* isolates. In addition, amitriptyline, chlorpromazine and simvastatin displayed synergism for all tested *A. baumannii* isolates; amitriptyline also showed synergism for all *E. coli* isolates and 3 of 5 *K. pneumoniae* isolates. For *P. aeruginosa*, only chlorpromazine showed a synergistic effect for 3 isolates, whereas amitriptyline, sertraline, chlorpromazine and promethazine showed a synergistic effect for ≤3 isolates. Six non-ABs showed no synergism for any isolate in combination with polymyxin B.

3.2.2. 8 × 6 Checkerboard assays – Surface response analysis

As the assays were read by spectrophotometer, we were able to apply a more detailed analysis of interaction using the Combenefit program. In general, the program predicts the response surface of no-interaction based on single drug responses using each of the

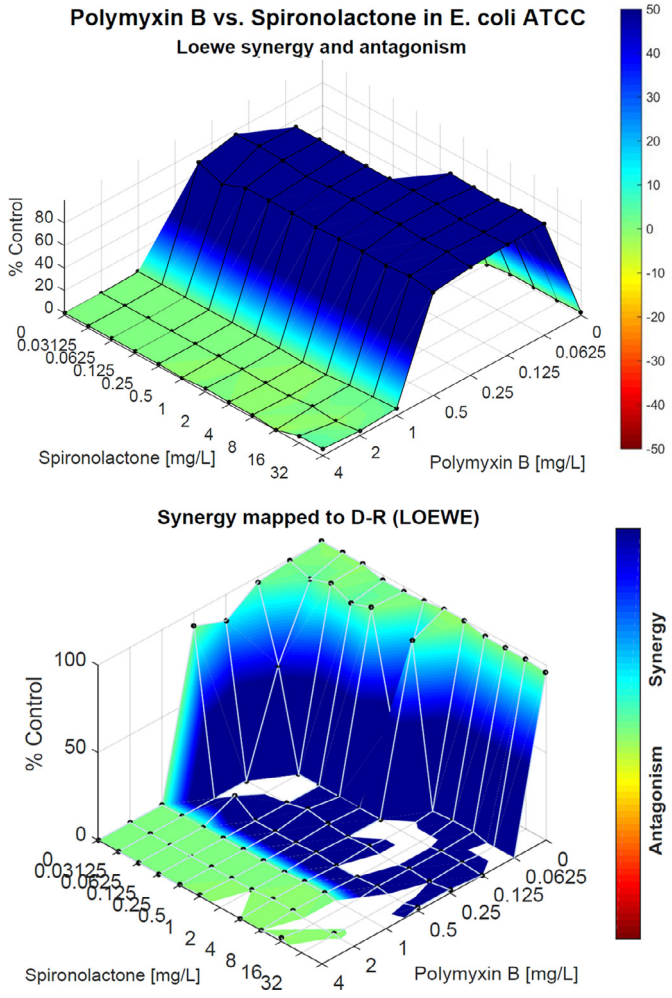

Fig. 1. Example of a response surface plot using the Loewe model for polymyxin B and spironolactone. Top panel displays the amount of interaction. Bottom panel displays the concentration response surface. In both panels the degree of synergism is indicated by different colours.

different models and compares this with the measured response. An example is shown in Figure 1, representing the surface response of polymyxin B and spironolactone for an *E. coli* isolate in an 8 × 12 checkerboard design. The coloured areas represent the degree of synergism. Table A.3 shows the degree of interaction using a measure of interaction that summarises the overall interaction, both areas of synergism and areas of antagonism for the Loewe model. The results of Bliss and HSA interactions, and those of maximum synergism and antagonism can be found in Table B.1. In general, there was a good concordance between each of the three methods used in that the degree of synergism was in a similar range, supporting the overall conclusions for each combination. Citalopram, levomepromazine, amitriptyline and promethazine showed high degrees of synergism for at least three species. Most combinations were found to be synergistic against *A. baumannii*. Most compounds showed similar degrees of synergism for *E. coli* and *K. pneumoniae* isolates. Interactions for *P. aeruginosa* were generally less synergistic. Clonazepam was found to be antagonistic against all species, and haloperidol for all species except *A. baumannii*. The 5 most synergistic combinations per species were selected for a more detailed interaction study using 8 × 12 checkerboard assays.

3.2.3. 8 × 12 Checkerboard assays – FIC analysis

The results of the 8 × 12 checkerboard assays for the top 5 non-ABs are summarised in Table 3. Median F_{min} values were higher for all combinations for all species, except for spironolactone, which was lower for both *E. coli* and *A. baumannii* isolates, and levomepromazine, which was lower for *P. aeruginosa* isolates. Levomepromazine was the only drug to display synergism for 3 of 5 tested *P. aeruginosa* isolates. Citalopram showed a synergistic effect for all *A. baumannii* and *K. pneumoniae* isolates, as well as >3 *E. coli* isolates. Half the increased median F_{min} values per species was found to correlate to a lower observed MIC value for polymyxin B compared with the 8 × 6 checkerboard assays (data not shown). The other half correlated to higher concentrations of polymyxin B or non-AB found for the corresponding F_{min}.

3.2.4. 8 × 12 Checkerboard assays – Surface response analysis

Data resulting from the three models was largely in concordance (Table C.1). We found varying levels of synergism and antagonism at lower non-AB concentrations (Table 3). Citalopram showed high degrees of synergism against *A. baumannii, E. coli* and *K. pneumoniae* isolates. Spironolactone showed the highest amount of synergism against *E. coli* isolates. Levomepromazine was synergistic for both *K. pneumoniae* and *P. aeruginosa* isolates. However, indifference and antagonism was found to be prevalent at lower concentrations for various compounds, most notably atorvastatin and promethazine.

3.2.5. Assessment of clinical relevance

To assess the clinical relevance of the interactions found in this study, we compared our data to known therapeutic and toxic blood-plasma concentrations of relevant drugs [14]. Therapeutic concentrations were not reported for atorvastatin, and toxic concentrations were not reported for atorvastatin, simvastatin and spironolactone. Reported concentrations were compared with the minimum concentration of the relevant non-AB where the measure of synergy according to the Loewe surface response model was higher than 25 (Table 4). Only spironolactone was found to be consistently synergistic with polymyxin B against *E. coli* isolates at non-toxic levels. However, concentrations were close to the therapeutic range, and the diuretic effect of the drug should be considered. Citalopram, sertraline and promethazine displayed synergism at (sub-)therapeutic levels for some *K. pneumoniae* isolates. For all other combinations, the minimum concentration for synergy with polymyxin B exceeded the toxic dose of the non-AB.

4. Discussion

In this study, we aimed to find combinations of polymyxin B and non-ABs that show a synergistic interaction against some of the priority pathogens marked by the WHO [9]. In this study we were able to identify several non-ABs that showed consistent synergistic effects in vitro. Surface response analysis was in concordance with FIC analysis in identifying synergistic combinations. Of 30 tested non-ABs, 20 showed a synergistic effect with polymyxin B for at least 1 isolate in the agar diffusion assay; 14 of these combinations displayed synergism in the 8 × 6 checkerboard assay, and all of the 9 selected combinations showed synergistic effects against at least 1 isolate in the 8 × 12 checkerboard assay. Only spironolactone displayed synergism at clinically relevant levels. In all experiments, most synergism was found against *A. baumannii*, albeit at relatively high concentrations. Combinations were often synergistic for both *E. coli* and *K. pneumoniae*, and few combinations were synergistic for *P. aeruginosa* isolates. Most synergistic effects were found for combinations with antidepressants.

Although synergistic combinations of polymyxin B with other antibiotics have been described for all 4 species [15,16], non-ABs have received limited attention. Synergistic combinations between polymyxin B and two cystic fibrosis drugs, ivacaftor and lumacaftor [17], showed increased killing activity against polymyxin-resistant

Table 3
FICs and the SUM_SYN-ANT estimates based on the Loewe model of polymyxin B combined with various non-AB from the 8 × 12 checkerboard assay for four species tested.

Non-AB	*A. baumannii*		*E. coli*		*K. pneumoniae*		*P. aeruginosa*	
	Median F_{min} (range)	Median SUM_SYN_ ANT (range)	Median F_{min} (range)	Median SUM_SYN_ ANT (range)	Median F_{min} (range)	Median SUM_ SYN_ANT (range)	Median F_{min} (range)	Median SUM_SYN_ ANT (range)
Amitriptyline	0.63 (0.38–0.75)	40.1 (17.1–64)	0.50 (0.38–0.63)	26.9 (−79.1–42.8)	0.63 (0.50–0.75)	18.3 (3.1–64.3)	0.75 (0.50–0.75)	12.9 (−14.8–36.6)
Citalopram	0.31 (0.19–0.31)	64.7 (36.5–111.6)	0.38 (0.31–0.56)	36.5 (−24–72.3)	0.31 (0.25–0.50)	43.7 (−22.5–159)	–	–
Sertraline	–	–	–	–	0.31 (0.25–0.50)	36.1 (16.5–90.1)	0.53 (0.28–0.56)	30.7 (26.5–49.6)
Chlorpromazine	–	–	–	–	–	–	0.63 (0.50–0.75)	19.8 (−4.2–74.3)
Levomepromazine	–	–	0.56 (0.50–0.63)	28.6 (−68.4–36.6)	0.50 (0.38–0.63)	36.1 (5.9–38.1)	0.50 (0.50–0.75)	31.8 (−11.6–69.6)
Promethazine	–	–	0.56 (0.50–1.00)	0.9 (−53.3–51.5)	1.00 (0.50–1.00)	6.7 (−34.7–27.2)	1.00 (1.00–1.00)	−17.5 (−46.8–32.8)
Spironolactone	0.28	51.1	0.26 (0.09–0.50)	113.9 (21.5–281)	–	–	–	–
Atorvastatin	0.52	2.5	–	–	–	–	–	–
Simvastatin	0.38 (0.25–0.63)	57.2 (30.7–60.8)	–	–	–	–	–	–

Values shown are the median followed by range, n=5. If n=1, no range is noted. FIC: fractional inhibitory concentration. Non-AB: non-antibiotic. Synergistic interactions found by FIC analysis are underlined.

Table 4
Comparison of clinically relevant concentrations of non-AB to observed concentration range for synergy with polymyxin B.

Non-AB	Therapeutic plasma concentration (mg/L)	Toxic plasma concentration (mg/L)	*A. baumannii*, synergistic minimum (mg/L)	*E. coli*, synergistic minimum (mg/L)	*K. pneumoniae*, synergistic minimum (mg/L)	*P. aeruginosa*, synergistic minimum (mg/L)
Amitriptyline	0.05–0.3	0.5–0.6	4 (0.03125–8)	8 (4–8)	16 (1–16)	8 (8–16)
Citalopram	0.05–0.11	0.22	0.5 (0.03125–4)	2 (0.25–4)	0.5 (0.03125–8)	–
Sertraline	0.01–0.25	0.29	–	–	2 (0.0125–4)	2 (0.5–4)
Chlorpromazine	0.03–0.1	1–2	–	–	–	4 (0.03125–8)
Levomepromazine	0.005–0.025	0.4	–	4 (2–8)	4 (0.03125–8)	8 (0.0625–8)
Promethazine	0.05–0.2	1–2	–	2 (0.03125–32)	32 (0.03125–32)	32
Spironolactone	0.05–0.5	–	2	0.03125 (0.03125–0.0625)	–	–
Atorvastatin	–	–	8	–	–	–
Simvastatin	0.0027–0.0056	–	1 (0.125–4)	–	–	–

Concentrations are given in mg/L. Toxic and therapeutic data were taken from Schulz et al, 2012 [14]. Non-AB: non-antibiotic.

P. aeruginosa isolates. Combinations of polymyxin B with selective oestrogen receptor modulators, tamoxifen, raloxifene and toremifene [18], displayed synergistic killing for polymyxin-resistant *A. baumannii, K. pneumoniae* and *P. aeruginosa*.

Ejim et al. [19] screened a large number of combinations of both antibiotics and non-ABs against non-clinical isolates with a focus on minocycline. They also tested polymyxin B in combination with loperamide and found some synergism for *E. coli*; however, we could not reproduce this finding. Schneider et al. [20] have reviewed studies of combinations of antimicrobials and other antibiotics with non-ABs, demonstrating a wide variety of potentially synergistic combinations with possible clinical relevance. Together with our results, this shows that potentially synergistic non-AB–antibiotic combinations that are active against clinical isolates are promising for further study.

We aimed to use a three-step strategy that would enable us to effectively screen for synergistic interactions against various clinical strains from different species. By screening for interaction prior to performing checkerboard assays for a limited number of strains, the number of combinations to test in the checkerboard assays was significantly reduced. However, potential synergistic interactions can remain undetected. If synergism is present for some strains only, there is a risk of overlooking the inhibitory effect of potential non-AB–antibiotic combinations. Three strains per species was considered a reasonable compromise and in case only one strain was positive, that isolate was still included in the checkerboard assay. Another potential limitation is that we screened at relatively high concentrations to determine any positive interaction and synergism at lower concentrations was overlooked. However, as we used a diffusion assay, synergistic activity would have been indicated by a double ring (an inner zone of growth and an outer zone of no growth).

The concentrations of polymyxin where synergism appeared to be highest were all in the physiological range. However, this was not true for the majority of non-ABs. Synergism was mostly observed at relatively high concentrations that exceeded clinically relevant plasma concentrations, with the exception of spironolactone. The lowest concentrations used in our assay were already in the therapeutic region for most drugs and a further detailed study could lead to more useful synergistic concentrations. In addition, tissue concentrations of these compounds at the infection site are unknown and could be higher. Further investigation of these combinations by in vitro time kill kinetic assays will provide more information on the potential clinical relevance. In addition, synergy studies performed against intracellular microorganisms may provide additional Information on the activity and clinical relevance of these synergistic non-AB–antibiotic combinations. Finally, modifications of the molecules may enhance antimicrobial activity. This may be particularly true for spironolactone, where synergism was clearly present, but alterations of the molecule may reduce its activity as a diuretic.

The analysis of interactions between an active and non-active drug is challenging, particularly for mechanistic models. In those models, one would assume the mechanism of action for both compounds to build a proper model for analysing those interactions. By contrast, empirical mechanism-free models like the ones used in the present study can be easily used for analysing such combinations. The same effect (growth inhibition) was assessed for both drugs alone and in combination without assuming any particular mechanism of action. As the non-AB drug was inactive, the nature of interaction was mainly defined by the enhancement of the action of the antibiotic drug. This explains the similar results obtained with three models used in the present study as the effect of a non-interactive combination is similar for all three

models. Furthermore, the three response surface models that were used in the present study assessed interactions at the whole drug-concentration range as opposed to the FIC index, which assesses only interactions at the MIC level. As drug concentrations fluctuate in vivo, interactions at sub-MIC effects may also be important.

Synergistic effects were observed for 3 of 4 studied antidepressants, 3 chemically similar antipsychotics belonging to the phenothiazine family, 1 diuretic and 2 statins. The mechanism by which this occurs remains unclear, and can only be speculated on without further study. Possibly there are molecular structures present in Gram-negative bacteria that are structurally similar to the respective drug targets, such as bacterial efflux pumps and mammalian receptors. Bacteria have developed various mechanisms of resistance to polymyxins. Next to LPS-Lipid A modification, it is known that bacteria can enlist efflux pumps to confer resistance [21,22]. One possible mechanism of undermining this resistance for non-ABs is by interacting and inhibiting these pumps.

To conclude, we have been able to positively identify synergism between polymyxin B and several non-AB compounds for one or more species. The non-ABs are already used clinically and these interactions warrant further investigations. This screening approach poses a solid model for the investigation of synergistic combinations between ND-ABs and non-ABs for the treatment of bacterial infections.

Conflict of interest statement

J.W. Mouton has received research funding from Adenium, Astra-Zeneca, Basilea, Cubist, Polyphor, Roche, Eumedica, Basilea, VenatorX, AiCuris, Gilead and Wockhardt. J. Meletiadis has received research funding from Astellas, Gilead, MSD and Pfizer. All others: nothing to declare.

Funding

This study was jointly supported by the Joint Programme Initiative on Antimicrobial Resistance (JPIAMR) and ZonMw under research grant “CO-ACTION”.

Ethical approval

Not required.

Acknowledgements

We thank Sandra Franch-Arroyo for discussions. This study was jointly supported by the Joint Programme Initiative on Antimicrobial Resistance (JPIAMR) and ZonMw under research grant “CO-ACTION”.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2018.09.003.

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