Journal of Global Antimicrobial Resistance 18 (2019) 37–44

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

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

Colistin plus meropenem combination is synergistic in vitro against extensively drug-resistant *Pseudomonas aeruginosa*, including high-risk clones

María M. Montero[a,*], Sandra Domene Ochoa[a], Carla López-Causapé[b], Brian VanScoy[c], Sonia Luque[d], Luisa Sorlí[a], Núria Campillo[d], Eduardo Padilla[e], Núria Prim[e], Concepción Segura[e], Virginia Pomar[f], Alba Rivera[f,g], Santiago Grau[d], Paul G. Ambrose[c], Antonio Oliver[b], Juan P. Horcajada[a,*]

[a] *Infectious Diseases Service, Hospital del Mar, Infectious Pathology and Antimicrobials Research Group (IPAR), Institut Hospital del Mar d'Investigacions Mèdiques (IMIM), Universitat Autònoma de Barcelona (UAB), CEXS-Universitat Pompeu Fabra Barcelona, Spain*
[b] *Servicio de Microbiología y Unidad de Investigación, Hospital Son Espases, IdISBa, Palma de Mallorca, Spain*
[c] *Institute for Clinical Pharmacodynamics, Schenectady, NY, USA*
[d] *Pharmacy Service, Hospital del Mar, Barcelona, Spain*
[e] *Laboratori de Referència de Catalunya, Barcelona, Spain*
[f] *Infectious Diseases Unit, Department of Internal Medicine, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain*
[g] *Department of Clinical Microbiology, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain*

ARTICLE INFO

Article history:
Received 7 January 2019
Received in revised form 29 March 2019
Accepted 24 April 2019
Available online 30 May 2019

Keywords:
Colistin
Meropenem
Combination therapy
Pseudomonas aeruginosa
Synergy

ABSTRACT

Background: Extensively drug-resistant (XDR) *Pseudomonas aeruginosa* (*P. aeruginosa*) and particularly *P. aeruginosa* high-risk clones, are of growing concern because treatment options are limited. For years, colistin monotherapy has been the only available treatment, but is well known that is not an optimal treatment. A combination of colistin with another antibiotic could be a possible therapeutic option.
Objectives: This study aimed to investigate effective antibiotic combinations against 20 XDR *P. aeruginosa* isolates obtained in a Spanish multicentre study (2015).
Methods: Forty-five checkerboards with six antipseudomonal antibiotics (amikacin, aztreonam, ceftazidime, meropenem, colistin, and ceftolozane/tazobactam) were performed to determine whether combinations were synergic or additive by fractional inhibitory concentration indices. On average, 15 different regimens were evaluated in duplicate against the three most prevalent high-risk clones (ST175, ST235, ST111) by time-kill analyses over 24 h. The combination showing synergism in the three high-risk clones was validated in all studied XDR isolates.
Results: In time-kill curves, the untreated control failed, as did each study regimen when administered alone. Two combinations were synergistic in the three high-risk clones that were initially studied: amikacin plus ceftazidime and colistin plus meropenem, with the second being the most effective combination. The efficacy of colistin plus meropenem was then tested in all 20 isolates. A synergistic bacterial density reduction for the duration of the study occurred in 80% of the entire XDR collection.
Conclusions: These data suggest that colistin plus meropenem may be a useful combination for the treatment of infections due to XDR *P. aeruginosa*, including high-risk clones, which warrants evaluation in a clinical trial.

1. Introduction

The world has faced a dramatic increase in antimicrobial resistance of Gram-negative bacteria in recent years. One representative microorganism with an extraordinary capacity to develop resistance is *Pseudomonas aeruginosa* (*P. aeruginosa*) [1,2]. Extensively drug-resistant (XDR) *P. aeruginosa* isolates, and

* Corresponding authors.
E-mail addresses: 95422@parcdesalutmar.cat (M.M. Montero), jhorcajada@psmar.cat (J.P. Horcajada).

https://doi.org/10.1016/j.jgar.2019.04.012
2213-7165/

particularly those recently designated as 'high-risk clones', are disseminated in hospitals around the world and have been related to very-difficult-to-treat infections [3–5]. The currently available therapeutic options for these infections yield suboptimal results, with concerning toxicity rates and a very narrow therapeutic window [6,7]. Without new therapeutic options, the outcome of patients with many types of infectious diseases will be compromised. Another worrisome feature of *P. aeruginosa* infection is the high risk for selection of resistant isolates during monotherapy [7]. These factors argue in favour of using combined therapy [5], which could broaden the spectrum of coverage, achieve an additive or synergistic antibacterial effect, and suppress emerging resistance [7].

Pseudomonas aeruginosa has a non-clonal epidemic population structure, in which a small number of widespread clones are selected from a background of a large number of rare and unrelated genotypes that recombine at a high rate [1]. In addition to classical molecular epidemiology and phenotypically-targeted assessment of resistance mechanisms, recent whole genome sequencing studies have provided relevant information regarding the complex resistome of multidrug-resistant (MDR)/XDR high-risk clones [8–14]. The most prevalent *P. aeruginosa* high-risk clones are thought to be ST111, ST175 and ST235 [1]. In a recent report, the current group analysed 150 XDR *P. aeruginosa* isolates from nine Spanish hospitals. Most of the isolates belonged to ST175 (67.3%), although ST244 (10.7%), ST235 (5.3%), and ST111 (1.3%) were also found. The remaining clones were less common and also less widely disseminated; these included ST253, ST313, ST179, ST274, ST395, ST455, ST2221, and four recently described STs: ST2533, ST2534, ST2535, and ST2536 [15].

Patients with infection caused by XDR *P. aeruginosa* high-risk clones are mainly treated with polymyxins or aminoglycosides [16]. Colistin use is hampered by the associated side effects (particularly nephrotoxicity) and difficulty in establishing an optimal dose and reaching therapeutic levels [6,16,17]. Polymyxin monotherapy may result in treatment failure (as reliably effective plasma exposure is not always attained), mainly relates to colistimethate, and bacterial resistance may emerge [18]. One solution for the scarcity of therapeutic options is to actively search for new strategies related to dosing, combining existing antibiotics, and developing new molecules. There is some hope in this line, as new molecules with antipseudomonal activity and new combinations with β-lactamase inhibitors are being developed. The clinical and microbiological impact of these new approaches against XDR *P. aeruginosa* high-risk clones is currently unknown; hence, clinical studies focusing on the treatment of these infections are urgently needed [19]. Furthermore, development of resistance to these new β-lactams has been recently reported [20].

Combination antibiotic therapy for XDR *P. aeruginosa* is generating interest because of the potential severity of the infection and the high risk of resistance selection with monotherapies. Several studies have examined in vitro interactions between various antipseudomonal antibiotics (e.g. carbapenems, colistin and polymyxin B, fosfomycin, aminoglycosides, and quinolones), using a variety of methods such as synergy testing using the microdilution checkerboard technique, gradient diffusion (Etest), and time-kill curve assays [7]. Nonetheless, no clear recommendations for clinical practice have emerged from these studies, and consensus is lacking as to which antibiotic combinations should be used against these complex infections to improve the therapeutic response and reduce selection of resistant mutants [21].

The primary aim of this study was to evaluate various antipseudomonal antibiotics alone and in combination for the three most prevalent XDR *P. aeruginosa* high-risk clones (ST175, ST111 and ST235) and to validate the most effective combination via checkerboard and time-kill curves in a collection of XDR isolates containing 20 representative isolates from a multicentre study.

2. Material and methods

2.1. Bacterial isolates

Twenty XDR *P. aeruginosa* clinical isolates were studied; they had been recovered in a recent study (COLIMERO study) in which 150 XDR *P. aeruginosa* isolates from nine Spanish hospitals were analysed using pulsed field gel electrophoresis (PFGE), multilocus sequence typing (MLST), and whole-genome sequencing [15]. The 20 selected isolates were considered to provide a representative profile of all the clones and resistance mechanisms detected in the multicentre study.

2.2. Antibiotics

The antipseudomonal antibiotics used in the experiments were amikacin, aztreonam, ceftazidime, meropenem, colistin obtained from Sigma-Aldrich, and ceftolozane/tazobactam obtained from MSD (Merck Sharp & Dohme). Antibiotic solutions were prepared according to the Clinical and Laboratory Standards Institute (CLSI) guidelines, using their corresponding solvent and dissolvent [22]. The doses stipulated for each antibiotic corresponded to the high doses used in clinical practice for the treatment of several infections. Antibiotic concentrations for time-kill experiments were chosen based on the area under the curve (AUC) serum levels: amikacin 1 g q24 h, AUC_{24}196 μg*h/mL [23,24]; aztreonam 2 g q8h, AUC_{24}1050 μg*h/mL [25]; ceftazidime 2 g q8h, AUC_{24} 800 μg*h/mL [26,27]; meropenem 2 g q8h, AUC_{24} 425 μg*h/mL [28]; colistin 4.5 MIU q12 h, AUC_{24} 50 μg*h/mL [29,30]; and ceftolozane/tazobactam 2/1 g q8h, AUC_{24} 912/150 μg*h/mL [31]. Colistin and meropenem concentrations in time-kill curves were validated by high performance liquid chromatography.

2.3. Susceptibility studies and resistance mechanisms

The susceptibility profiles and the β-lactam resistance mechanisms of the studied XDR isolates were obtained from a previous Spanish multicentre study [15]. The isolates accounted for the most prevalent and relevant resistance mechanisms, which included chromosomal mutations (AmpC hyperproduction and OprD inactivation) and horizontally acquired enzymes, including several metallo-β-lactamases (MBLs).

Antimicrobial susceptibility was performed according to the CLSI guidelines [22] for broth microdilution and agar dilution methods utilising cation-adjusted Mueller-Hinton broth (CAMHB).

2.4. Checkerboard experiments

Checkerboard studies were performed in 96-well microplates. The antibiotic values to be tested should include broad values ranging from 4–8 times the value of the expected MIC to at least 1/8 to 1/16 of it; 50 μL of CAMHB was distributed into each well. The first antibiotic solution was serially diluted and dispensed along the ordinate. The second antibiotic solution was diluted and dispensed along the abscissa. The bacteria inoculum equal to a 0.5 McFarland was prepared and 100 μL were distributed in each well. Plates were incubated at 35 °C for 48 h [32]. The fractional inhibitory concentration indices (FICIs) were calculated according to the following formula: FICI = $(IC_{A+B}/IC_A) + (IC_{A+B}/IC_B)$. The interaction was considered synergistic when FICI was ≤ 0.5,

additive when FICI was > 0.5 to ≤ 1, antagonistic when FICI was ≥ 4, and indifferent for intermediate values [33,34]. The experiments were performed in triplicate. Fifteen checkerboards were performed for each chosen strain. A representative strain of each of the three most prevalent high-risk clones — ST175, ST111 and ST235 — was evaluated.

2.5. Time-kill experiments

Time-kill studies were conducted with the six selected antibiotics alone and in combinations at clinically achievable drug concentrations (when maximum indicated clinical doses were used). Time-kill curves were performed with all the resulting combinations of the checkerboards, in each of the three isolates (ST175, ST111 and ST235). The most synergistic combination was then validated in the entire collection of XDR *P. aeruginosa* isolates, consisting of 20 isolates. All experiments were performed in duplicate. Study flow is represented in Fig. 1.

An overnight culture of isolate was diluted with CAMHB and further incubated at 35 °C to reach early log-phase growth. The bacterial suspension was diluted with CAMHB, according to its absorbance at 630 nm; 50 mL sterile conical flasks were used with 30 mL CAMHB. The final concentration of the bacterial suspension in each flask was approximately 7–8 $\log_{10}$ cfu/mL. Flasks were incubated in a shaker water bath at 35 °C for 24 h. Samples were collected from each flask at 0, 2, 4, 8, 12, and 24 h. The extracted broth samples (1 mL) were centrifuged twice at 5000 g for 5 min and then reconstituted with sterile saline solution to their original volumes to minimise drug carryover. Ten-fold serial dilutions were performed with CAMHB, 200 μL was plated on Muller Hinton E agar (MHE) plates, and total bacterial count was quantified for each sample. The inoculated plates were incubated in a humidified incubator (35 °C) for 18–24 h, bacterial colonies were visually counted, and the original bacterial density from the original sample was calculated based on the dilution factor. The limit of quantification (LOQ) was 400 CFU/mL (equivalent to 20 colonies per plate).

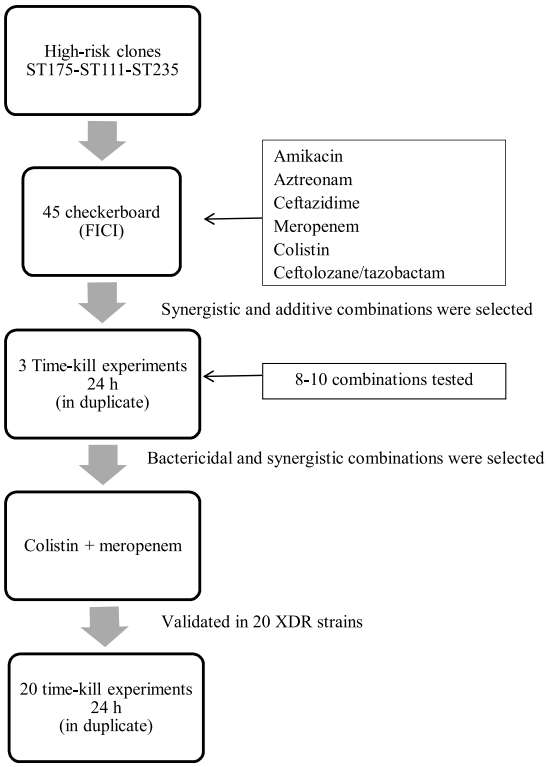

Fig. 1. Study flow.
Forty-five checkerboard screens with six selected antibiotics and with the three most prevalent *Pseudomonas aeruginosa* high-risk clones (ST175, ST111 and ST235) were conducted to identify additive and synergistic combinations. To ultimately identify 'the best combination', time-kill curves with the same clones were performed using an average of nine combinations previously detected on checkboard therapy. The combination selected was colistin plus meropenem, and this combination was validated in the entire collection (20 isolates) of extremely drug resistant bacteria.
FICI, fractional inhibitory concentration indice.

2.6. Pharmacodynamic checkpoints

Bactericidal activity was defined as a ≥ 3 $\log_{10}$ cfu/mL reduction in colony count at 24 h. Synergy was defined as a ≥ 2 $\log_{10}$ cfu/mL reduction in colony count at 24 h, with the combination as compared with the most active single drug. The combination was established as indifferent when there was a ≤ 2 $\log_{10}$ cfu/mL change at 24 h. Antagonism was defined as ≥ 1 $\log_{10}$ cfu/mL regrowth, with the combination as compared with the least active component [35].

3. Results

3.1. In vitro antimicrobial susceptibility testing and resistance mechanisms

The susceptibility profiles and resistance mechanisms are shown in the Table 1. The polymorphisms/mutations found in the genes related to colistin resistance in the 20 isolates of XDR *P. aeruginosa* are shown in the Table S1. All strains were resistant to meropenem, but three of them were intermediate (MIC 8 mg/L) according to CLSI definitions. ST111 (10-009) was resistant to colistin (MIC 4 mg/L), likely due to a 4-bp deletion within parR. All the other strains were susceptible (MIC ≤ 2 mg/L), and although amino acid polymorphisms within the main genes related with polymyxin resistance were detected in several strains [15], their effect, if any, would need to be specifically determined.

3.2. Checkerboard studies

In the checkerboard experiments, all selected combinations had a FICI within the range of 0.5–1; that is, only combinations that were synergistic (FICI ≤ 0.5) or additive (FICI > 0.5 ≤ 1). The combination colistin-meropenem was additive for the three most prevalent high-risk clones – ST175, ST111 and ST235 – in these experiments. These data are shown in the Table S2.

3.3. Time-kill studies

In the time-kill studies, growth in the untreated controls reached 9–10 $\log_{10}$ cfu/mL by the 24 h time point for all regimens. All isolates treated with single antibiotics (ceftazidime, aztreonam, meropenem, colistin, amikacin, or ceftolozane/tazobactam) did not show bactericidal effect after 24 h. The time-kill curves indicated that two combinations were synergistic in the three most prevalent high-risk isolates (ST175, ST111 and ST235): amikacin plus ceftazidime, and colistin plus meropenem, with the second being more effective (Table 2). When the colistin-meropenem combination was validated in time-kill studies including all 20 isolates, it was synergistic in 80% (Table 3, Fig. 2). Colistin-meropenem was not synergistic in four isolates, which surprisingly included three isolates with low MICs for meropenem (8 mg/L) — ST395/10-017, ST2534/06-025, and ST2535/06-027 — in which the monotherapy regimens showed results similar to those of the combination (Table 3, Fig. 2).

Table 1
Susceptibility profiles and resistance mechanisms of the 20 studied extremely drug resistant *Pseudomonas aeruginosa* isolates.

Isolate	ST	Beta -lactamases	AmpC hyper-production	OprD deficiency	Polymyxin resistance mechanisms	TOL/TZ	MER	CAZ	AZT	AMI	COL
04–025	175	–	Yes	Yes		2//4	16	32	16	4	1
10–009	111	VIM-2	Yes	Yes	*parR*- nt621Δ4	<64/4	<32	<64	<128	32	4
06–042	235	VIM-47	No	No		<64/4	<32	64	32	64	2
12–012	175	VIM-20, OXA-2	No	Yes		<64/4	<32	16	8	16	2
12–003	244	–	Yes	Yes		4/4	32	64	32	8	2
07–004	235	GES-19, OXA-2	No	Yes		<64/4	<32	<64	128	128	2
04–017	111	OXA-46	Yes	No		8/4	32	64	64	4	2
01–008	253	VIM-1	No	Yes		<64/4	<32	<64	4	8	2
10–023	175	–	Yes	Yes		2/4	16	32	16	4	2
07–016	175	GES-5	No	Yes		16/4	>32	32	16	16	2
09–007	313	–	Yes	Yes		4/4	16	64	32	8	2
06-035	455		Yes	No		4/4	>32	32	64	<2	0.5
06–014	179	OXA-10	Yes	Yes		4/4	32	16	16	8	2
10-019	2221		Yes	Yes		8/4	32	64	64	<2	2
10–021	2533		Yes	Yes		8/4	32	64	64	<2	1
06–001	2536		Yes	Yes		4/4	32	>64	64	8	2
09–011	274		Yes	Yes		8/4	32	64	64	128	1
06–025	2534		Yes	Yes		4/4	8	64	64	<2	2
06–027	2535		Yes	No		4/4	8	64	32	8	2
10–017	395		Yes	No		1/4	8	32	32	4	2

Minimal inhibitory concentrations (MICs) (mg/L) of the various antibiotics tested in this study: TOL/TZ, ceftolozane/tazobactam; MER, meropenem; CAZ, ceftazidime; AZT, aztreonam; AMI, amikacin; COL, colistin.

Table 2
Time-kill experiments performed against the three most prevalent extremely drug resistant *Pseudomonas aeruginosa* high-risk clones. A summary of mean bacterial concentrations (log_{10} CFU/mL) at 8, 12, and 24 h is shown for each strain and antibiotic treatment.

Isolate	ST	Inoculum	Antibiotic	8 h	12 h	SD12 h	24 h	SD24 h	Δ24 h	Synergy
04-025	**ST175**	7.19	Control	9.25	9.37	1.54	9.57	1.68	2.38	
			Amikacin	3.50	3.37	2.70	6.42	0.54	-0.77	
			Meropenem	3.21	3.83	2.37	5.18	1.42	-2.01	
			Ceftazidime	4.99	5.35	1.30	6.36	0.59	-0.83	
			Aztreonam	4.33	4.67	1.78	5.60	1.13	-1.59	
			Colistin	2.63	2.53	3.29	6.32	0.61	-0.87	
			Ceftolozane/tazobactam	4.65	4.79	1.70	5.28	1.35	-1.91	
			Amikacin + meropenem	2.90	2.97	2.99	4.03	2.23	-3.16	-1.15
			Amikacin + ceftazidime	2.76	2.65	3.21	3.37	2.70	-3.82	-3.05
			Amikacin + aztreonam	2.46	3.15	2.86	3.98	2.27	-3.21	-1.62
			Amikacin + ceftolozane/tazobactam	3.34	2.98	2.98	4.74	1.73	-2.45	-0.54
			Ceftolozane/tazobactam + meropenem	3.68	3.58	2.55	4.21	2.11	-2.98	-0.97
			Ceftolozane/tazobactam + ceftazidime	4.57	4.74	1.74	5.49	1.20	-1.70	0.21
			Ceftolozane/tazobactam + aztreonam	4.13	4.41	1.96	5.38	1.28	-1.81	0.10
			Ceftazidime + colistin	0.00	0.00	5.08	2.75	3.14	-4.44	-3.58
			Ceftazidime + aztreonam	4.46	4.92	1.61	5.70	1.06	-1.49	0.10
			Colistin + meropenem	0.00	0.00	5.08	2.03	3.65	-5.16	-3.16
10-009	**ST111**	6.94	Control	9.74	9.89	2.08	10.02	2.18	3.08	
			Amikacin	7.65	9.47	1.79	9.98	2.15	3.04	
			Meropenem	9.69	10.20	2.31	10.01	2.17	3.07	
			Ceftazidime	6.80	7.18	0.17	9.81	2.03	2.87	
			Aztreonam	6.97	7.20	0.18	7.41	0.33	0.47	
			Colistin	3.42	3.34	2.54	5.81	0.80	-1.13	
			Ceftolozane/tazobactam	9.60	10.03	2.18	10.00	2.16	3.06	
			Amikacin + meropenem	7.07	8.36	1.01	10.14	2.26	3.20	0.16
			Amikacin + ceftazidime	4.36	4.30	1.87	6.09	0.60	-0.85	-3.72
			Amikacin + aztreonam	3.69	4.44	1.76	4.15	1.97	-2.79	-3.26
			Amikacin + ceftolozane/tazobactam	5.47	6.40	0.38	10.22	2.32	3.28	0.24
			Ceftolozane/tazobactam + meropenem	8.91	9.52	1.82	9.93	2.11	2.99	-0.07
			Ceftolozane/tazobactam + ceftazidime	6.03	6.57	0.26	9.82	2.04	2.88	0.01
			Ceftazidime + colistin	1.34	2.16	3.38	2.49	3.15	-4.45	-3.32
			Colistin + meropenem	1.02	2.00	3.49	2.10	3.42	-4.84	-3.71
			Aztreonam + colistin	1.65	3.05	2.75	5.14	1.27	-1.80	-0.67
06-042	**ST235**	7.07	Control	9.70	8.84	1.25	9.88	1.98	2.81	
			Amikacin	9.54	9.91	2.01	9.97	2.05	2.90	
			Meropenem	4.07	4.84	1.58	9.98	2.06	2.91	
			Ceftazidime	7.50	7.83	0.54	10.03	2.09	2.96	
			Aztreonam	5.31	5.62	1.02	6.21	0.61	-0.86	
			Colistin	2.56	2.95	2.92	4.27	1.98	-2.80	
			Ceftolozane/tazobactam	9.43	9.58	1.77	10.11	2.15	3.04	
			Amikacin + meropenem	4.24	4.28	1.97	5.82	0.88	-1.25	-4.15
			Amikacin + ceftazidime	5.00	5.32	1.24	6.14	0.66	-0.93	-3.83
			Amikacin + aztreonam	4.75	5.12	1.38	5.34	1.23	-1.73	-0.87
			Amikacin + ceftolozane/tazobactam	7.20	7.30	0.16	9.90	2.00	2.83	-0.07
			Ceftolozane/tazobactam + meropenem	3.55	4.32	1.95	8.87	1.27	1.80	-1.11
			Ceftolozane/tazobactam + ceftazidime	6.57	7.82	0.53	10.09	2.14	3.02	0.07
			Ceftazidime + colistin	4.32	5.03	2.05	5.20	1.20	-1.70	0.93
			Colistin + meropenem	2.13	2.13	3.49	2.14	3.48	-4.93	-2.13

The standard deviation (SD) at 12 and 24 h, and change (**Δ**) in bacterial concentration in log_{10} CFU/mL at 24 h compared with the starting inoculum are shown. Bactericidal effect and synergy ($\geq$3 log_{10} reduction in CFU/mL after 24 h and $\geq$ 2 log_{10} reduction in CFU/mL at 24 h with the combination as compared with the most active single drug, respectively) are highlighted in orange and yellow, respectively.

Table 3
Time-kill experiments performed against 20 extremely drug resistant *Pseudomonas aeruginosa* strains. Summary of mean bacterial concentration (log_{10}CFU/mL) at 8, 12 and 24 h is shown for each strain and antibiotic treatment.

Isolate	ST	Inoculum	Antibiotic	8 h	12 h	SD12 h	24 h	SD24 h	Δ24 h	Synergy
04-025	**175**	7.19	Control	9.25	9.37	1.54	9.57	1.68	2.38	
			Colistin	2.63	2.53	3.30	6.31	0.62	-0.88	
			Meropenem	3.21	3.83	2.38	5.18	1.42	-2.01	
			Colistin + meropenem	0	0	5.08	2.03	3.65	-5.16	-4.28
10-009	**111**	6.94	Control	9.74	9.89	2.08	10.02	2.18	3.08	
			Colistin	3.42	3.34	2.55	5.81	0.80	-1.13	
			Meropenem	9.69	10.2	2.31	10.01	2.17	3.07	
			Colistin + meropenem	1.02	2	3.49	2.1	3.42	-4.84	-3.71
06-042	**235**	7.07	Control	9.70	8.84	1.25	9.88	1.98	2.81	
			Colistin	2.56	2.95	2.91	4.27	1.98	-2.80	
			Meropenem	4.07	4.84	1.58	9.98	2.06	2.91	
			Colistin + meropenem	2.13	2.13	3.49	2.14	3.49	-4.93	-2.13
12-012	**175**	6.94	Control	9.82	10.14	2.26	9.81	2.02	2.87	
			Colistin	2.48	3.63	2.34	6.5	0.31	-0.44	
			Meropenem	9.44	9.76	1.99	9.88	2.08	2.94	
			Colistin + meropenem	1.75	1.69	3.71	3.6	2.36	-3.34	-2.9
12-003	**244**	6.99	Control	10.03	10.24	2.29	9.89	2.05	2.9	
			Colistin	5.28	6.4	0.42	6.9	0.06	-0.09	
			Meropenem	9.42	7.82	0.59	9.66	1.89	2.67	
			Colistin + meropenem	2.35	3.47	2.49	2.55	3.14	-4.44	-4.35
07-004	**235**	6.49	Control	9.69	10.04	2.51	10.05	2.52	3.56	
			Colistin	2.97	3.08	2.41	5.6	0.63	-0.89	
			Meropenem	9.61	10.02	2.50	9.86	2.38	3.37	
			Colistin + meropenem	2.12	2.87	2.56	3.26	2.28	-3.23	-2.34
04-017	**111**	7.03	Control	10.24	9.77	1.94	9.76	1.93	2.73	
			Colistin	2.32	3.22	2.69	5.51	1.07	-1.52	
			Meropenem	5.86	5.76	0.90	9.56	1.79	2.53	
			Colistin + meropenem	1.42	2.73	3.04	2.53	3.18	-4.5	-2.98
01-008	**253**	6.93	Control	9.79	9.82	2.04	10.15	2.27	3.22	
			Colistin	2.9	3.52	2.41	5.95	0.69	-0.98	
			Meropenem	9.78	9.86	2.07	10.15	2.28	3.22	
			Colistin + meropenem	2.21	1.95	3.52	3.8	2.21	-3.13	-2.15
10-023	**175**	7.0	Control	9.87	10.12	2.20	9.78	1.96	2.78	
			Colistin	3.69	4.06	2.08	8.09	0.77	1.09	
			Meropenem	4.5	4.54	1.74	7.93	0.66	0.93	
			Colistin + meropenem	2.84	3.07	2.78	4.35	1.87	-2.65	-3.58
07-016	**175**	6.92	Control	9.76	9.32	1.69	9.32	1.69	2.4	
			Colistin	3.25	4.56	1.67	6.55	0.26	-0.37	
			Meropenem	3.44	4.39	1.79	5.26	6.40	-1.66	
			Colistin + meropenem	2.51	2.09	3.42	2.88	2.86	-4.04	-3.67
09-007	**313**	6.9	Control	10.03	10.10	2.26	9.99	2.18	3.09	
			Colistin	3.56	3.31	2.54	7.68	0.55	0.78	
			Meropenem	3.85	3.79	2.20	5.39	1.07	-1.51	
			Colistin + meropenem	3.3	2.59	3.05	3.11	2.68	-3.79	-2.28
06-035	**455**	7.04	Control	9.96	10.02	2.11	9.86	1.99	2.82	
			Colistin	2.64	3.42	2.56	8.64	1.13	1.6	
			Meropenem	3.9	3.8	2.29	6	0.74	-1.04	
			Colistin + meropenem	2.28	3.24	2.69	2.96	2.88	-4.08	-3.04
06-014	**179**	7.02	Control	9.71	8.68	1.17	10.13	2.19	3.11	
			Colistin	1.85	2.83	2.96	4.82	1.56	-2.20	
			Meropenem	5.86	7.33	0.22	10.27	2.30	3.25	
			Colistin + meropenem	2.02	2.67	3.08	2.77	3.01	-4.25	-2.05
10-019	**2221**	7.16	Control	8.96	10.01	2.01	9.82	1.88	2.66	
			Colistin	2.44	4.21	2.09	6.35	0.57	-0.81	
			Meropenem	5.51	5.67	1.05	9.91	1.94	2.75	
			Colistin + meropenem	2.29	3.62	2.50	3.3	2.73	-3.86	-3.05
10-021	**2533**	7.22	Control	9.92	10.15	2.07	9.96	1.93	2.74	
			Colistin	3.48	3.23	2.82	5.13	1.48	-2.09	
			Meropenem	7.45	8.96	1.23	9.43	1.56	2.21	
			Colistin + meropenem	3.06	3.64	2.53	3.13	2.89	-4.09	-2
06-001	**2536**	6.8	Control	9.70	9.68	2.03	9.97	2.24	3.17	
			Colistin	3.94	4.02	1.97	6.89	0.06	0.09	
			Meropenem	5.77	6.75	0.04	9.56	1.95	2.76	
			Colistin + meropenem	3.14	3.58	2.28	3.28	2.49	-3.54	-3.61
09-011	**274**	7.13	Control	8.83	9.98	2.01	10.03	2.05	2.9	
			Colistin	1.71	3.05	2.88	3.8	2.35	-3.33	
			Meropenem	5.57	6.37	0.54	9.8	1.89	2.67	
			Colistin + meropenem	2.23	2.63	3.18	2.35	3.38	-4.78	-1.45
06-025	**2534**	7.07	Control	9.69	9.47	1.69	10.18	2.19	3.11	
			Colistin	3.66	4.29	1.97	5.54	1.08	-1.53	
			Meropenem	4.15	4.4	1.89	4.05	2.14	-3.02	
			Colistin + meropenem	3.77	4.27	1.98	2.78	3.03	-4.29	-1.27
06-027	**2535**	6.74	Control	10.08	9.61	2.02	9.82	2.17	3.08	
			Colistin	3.92	4.37	1.68	4.17	1.82	-2.57	
			Meropenem	3.94	4.72	1.43	2.8	2.79	-3.94	
			Colistin + meropenem	3.66	4.19	1.80	3.05	2.61	-3.69	0.25
10-017	**395**	6.92	Control	9.93	10.02	2.19	10.08	2.23	3.16	
			Colistin	3.15	3.62	2.33	4.5	1.71	-2.42	
			Meropenem	3.95	5.04	1.33	9.81	2.04	2.89	
			Colistin + meropenem	1.93	2.97	2.79	3.32	2.55	-3.6	-1.18

The standard deviation (SD) at 12 and 24 h, and the change (**Δ**) in bacterial concentration in log_{10} CFU/mL at 24 h compared with the starting inoculum are shown. Bactericidal effect and synergy (≥ 3 log_{10} reduction in CFU/mL after 24 h and ≥ 2 log_{10} reduction in CFU/mL at 24 h with the combination as compared with the most active single drug, respectively) are highlighted in orange and yellow, respectively.

4. Discussion

This study evaluated the activity of antipseudomonal antibiotics used in clinical practice, alone and in combination, against representative XDR *P. aeruginosa* high-risk clones, with the aim of identifying the most effective antimicrobial combinations. The results open a new perspective in this line compared with the findings in previous studies. The combination with the greatest efficacy — colistin plus meropenem — showed a synergistic effect against 80% of the 20 strains that were studied, including those producing MBLs, one of which additionally showed colistin resistance and, therefore, panresistance.

Colistin-meropenem was not synergistic in four isolates, which surprisingly included three isolates with low MICs for meropenem. In two of three of the MIC of 8 to meropenem isolates the lacks of synergy appears to be due to the inability to improve markedly over meropenem monotherapy or are the results only valid when using drugs with beta lactams with compromised PK / PD that would be considered resistant. For the fourth isolate, the combination was not shown to be synergistic because of less than the required level of activity compared with colistin alone.

In previous studies, polymyxin-carbapenem combinations have been proposed for use in MDR Gram-negative infections to enhance the therapeutic response and minimise potential polymyxin resistance [7]. In the case of MDR *P. aeruginosa*, previous in vitro studies have found that colistin plus doripenem combination therapy is synergistic [7,8,18]. Other combinations with reported synergy against MDR *P. aeruginosa* include colistin-ceftazidime [36], colistin-rifampin [37,38], meropenem-levofloxacin [39], and colistin-imipenem [40]. The efficacy of the colistin-meropenem combination against XDR *P. aeruginosa* high-risk clones in the current study indicates that it may be a good option for infections, due to these difficult-to-treat bacteria. Although another synergistic combination – ceftazidime-amikacin – was found; it was decided to use the colistin-meropenem combination to test all 20 study isolates because the synergy values were better, and because the synergy between betalactamics and aminoglycosides has previously been studied more. As these infections often occur in patients with multiple conditions, including a risk of renal failure, the colistin-meropenem combination would be more useful in clinical practice because of its theoretically higher efficacy and lower risk of nephrotoxicity.

The mechanism of action of this combination is thought to be based on the combined effect of the two molecules on bacterial cells. Colistin acts against the lipopolysaccharide of the outer bacterial membrane, causing local disturbance, permeability changes, osmotic imbalance, and, usually, cell death [41]. Meropenem has to enter into the periplasmic space to the acetylate penicillin-binding proteins (PBPs), and interferes with the formation of peptidoglycan in the cell wall [42]. Mechanistically, colistin interferes with the outer membrane, changing its permeability, which in turn allows meropenem to enter the bacteria in higher amounts. Higher concentrations of meropenem in the periplasmic space could reduce the effect of resistance mechanisms, thereby rendering meropenem active against resistant bacteria. The classical mechanism of action of this combination (based on the permeability effect of colistin) has recently been complemented by new data from metabolomic studies in multidrug-resistant *Acinetobacter baumannii* treated with colistin

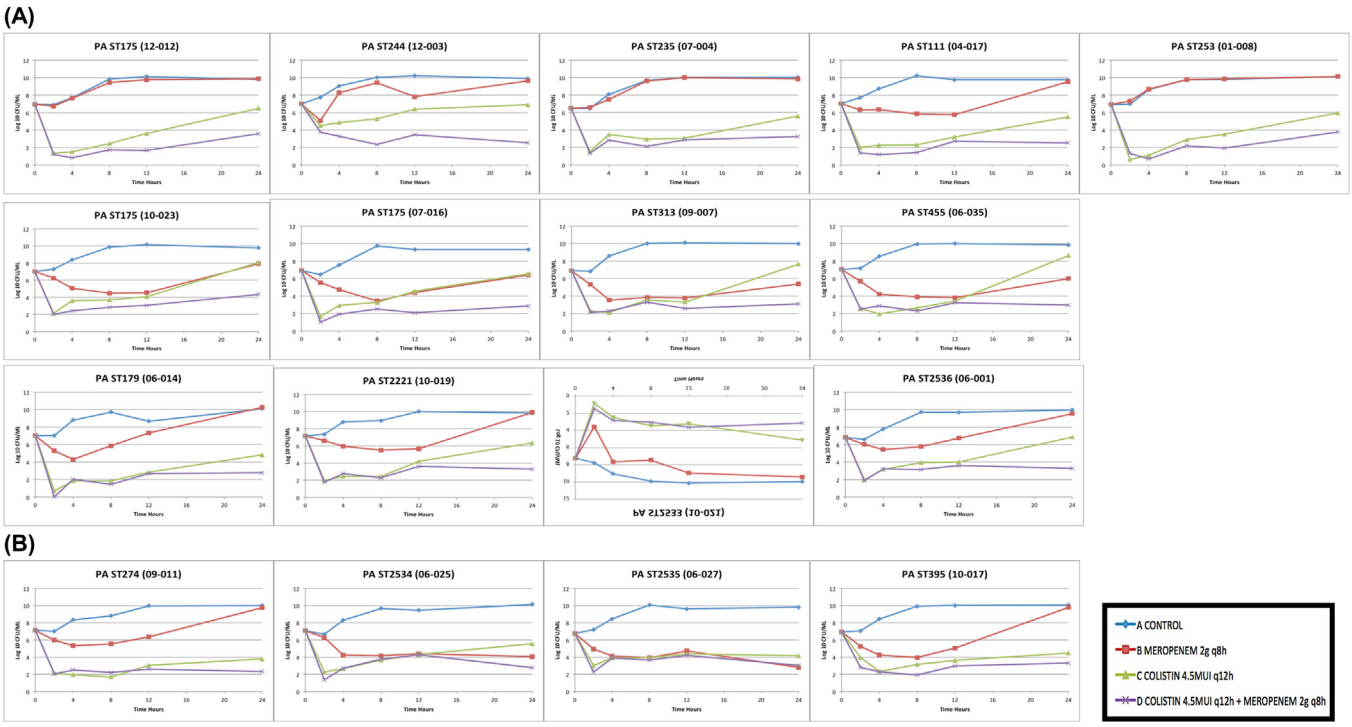

Fig. 2. Time-kill experiments displaying the activity of colistin and meropenem alone and in combination against the remainder (17/20) extremely drug resistant *Pseudomonas aeruginosa* strains. (A) Time-kill curve experiments for the 80% of the strains in which the combination showed a synergistic effect. (B) Time-kill curve experiments for the strains in which there was no synergistic effect.

plus doripenem [43]. Polymyxins and doripenem both interfere with key bacterial metabolic pathways in a time-dependent manner. In the reported experiments, colistin led to prompt inhibition of metabolic pathways (15 min–1 h), which was followed by the metabolic effects of doripenem at 4 h. This could explain the synergistic effect. Specifically, significant metabolic changes via disorganisation of membrane lipids and depletion of nucleotides, energy, and amino sugar metabolites were evident following treatment with colistin alone, and clearly enhanced by combining this drug with doripenem [43].

The phenomenon of bacterial regrowth shown in these single-drug experiments could be either due to a loss of functionality of these antibiotics or selection of resistant isolates. Presumably, the latter could include selection of pre-existing resistant subpopulations, de novo mutations, adaptive resistance, or formation of persistent cells [44]. Further studies would be required in order to evaluate these possibilities. It should be noted that bacterial regrowth is much more common in *P. aeruginosa* than in other Gram-negative bacteria when antimicrobial monotherapy is used. In this sense, combination therapy would not only enhance the antimicrobial effect, but also prevent the selection of resistant isolates [8], as was shown in the current study with the colistin-meropenem combination. Studies investigating resistance development with colistin monotherapy compared with combination therapy have shown suppression or delay of colistin resistance when combination therapy is used [7]. This is a part of the apparent success of combination therapy, and should be considered another argument in favour of using combinations in multidrug-resistant *P. aeruginosa* infection.

There are no clinical studies investigating colistin-meropenem in XDR *P. aeruginosa*. In a recently published clinical trial, the performance of the colistin-meropenem combination did not differ from that of colistin monotherapy against carbapenem-resistant Gram-negative bacteria. However, most of the infections that were included were due to *Acinetobacter baumannii* and no conclusions were obtained for MDR *P. aeruginosa* infections. [45]]. The current results indicate that clinical studies with MDR *P. aeruginosa* infection could be warranted to evaluate the colistin-meropenem combination.

This study had several limitations. Checkerboard studies were used only as screening, since it is a model with a fixed time and concentration and with low reproducibility [33,46]. Results provided by time-kill assays are more precise and sensitive for identifying possible synergies with combination regimens than checkerboard studies. Nevertheless, due to the differences in methodology and specific factors, it is hard to compare the different methods. Apart from that, antibiotic combinations were studied using fixed concentrations in time-kill studies. Since the interaction between antibiotics is dynamic and concentration-dependent [42], the results could vary if other concentrations were analysed. Furthermore, considering the usual posology in clinical practice, samples were obtained at different time points up to 12 h. Additionally, the curves were lengthened to 24 h to verify bacterial eradication, although the data obtained at that point cannot be considered relevant since they are not representative of the clinical administration guidelines for most antibiotics [33].

In summary, this study shows that the colistin-meropenem combination is bactericidal and synergistic against representative isolates of XDR *P. aeruginosa*. These results suggest that this therapy could be a potential option in severe infections caused by high-risk clones such as ST175, ST111, and ST235, including carbapenemase-producing and even panresistant isolates. Thus, this combination should be considered in future in vitro dynamic bi-compartmental studies and in clinical practice.

Funding

This study was partially supported by the Ministerio de Economía y Competitividad of Spain, Instituto de Salud Carlos III. FEDER (PI16/00669).

Competing interests

The authors declare no conflicts of interest.

Ethical approval

Not required.

Acknowledgements

We thank The Institute for Clinical Pharmacodynamics (ICPD), Schenectady, NY, and the Infectious Pathology and Antimicrobials Research Group (IPAR), Institute Hospital del Mar d' Investigacions Mèdiques (IMIM), for their support.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jgar.2019.04.012.

References

[1] Oliver A, Mulet X, López-Causapé C, Juan C. The increasing threat of *Pseudomonas aeruginosa* a high-risk clones. Drug Resist Updat 2015;21–22:41–59, doi:http://dx.doi.org/10.1016/j.drup.2015.08.002.
[2] Gellatly SL, Hancock REW. *Pseudomonas aeruginosa*: new insights into pathogenesis and host defenses. Pathog Dis 2013;67:159–73, doi:http://dx.doi.org/10.1111/2049-632X.12033.
[3] Loveday HP, Wilson JA, Kerr K, Pitchers R, Walker JT, Browne J. Association between healthcare water systems and *Pseudomonas aeruginosa* infections: a rapid systematic review. J Hosp Infect 2014;86:7–15, doi:http://dx.doi.org/10.1016/j.jhin.2013.09.010.
[4] El Zowalaty ME, Al Thani AA, Webster TJ, et al. *Pseudomonas aeruginosa*: arsenal of resistance mechanisms, decades of changing resistance profiles, and future antimicrobial therapies. Future Microbiol 2015;10:1683–706, doi:http://dx.doi.org/10.2217/fmb.15.48.
[5] Drusano GL, Louie A, Macgowan A, Hope W. Suppression of emergence of resistance in pathogenic bacteria: keeping our powder dry, part 1 how did we get into this situation? Antimicrob Agents Chemother 2016;60:1194–201, doi:http://dx.doi.org/10.1128/AAC.02177-15.
[6] Horcajada JP, Sorlí L, Luque S, Benito N, Segura C, Campillo N, et al. Validation of a colistin plasma concentration breakpoint as a predictor of nephrotoxicity in patients treated with colistin methanesulfonate. Int J Antimicrob Agents 2016;48:725–7, doi:http://dx.doi.org/10.1016/j.ijantimicag.2016.08.020.
[7] Zusman O, Avni T, Leibovici L, Adler A, Friberg L, Stergiopoulou T, et al. Systematic review and meta-analysis of in vitro synergy of polymyxins and carbapenems. Antimicrob Agents Chemother 2013;57:5104–11, doi:http://dx.doi.org/10.1128/AAC.01230-13.
[8] Ly NS, Bulitta JB, Rao GG, Landersdorfer CB, Holden PN, Forrest A, et al. Colistin and doripenem combinations against *Pseudomonas aeruginosa*: profiling the time course of synergistic killing and prevention of resistance. J Antimicrob Chemother 2015;70:1434–42, doi:http://dx.doi.org/10.1093/jac/dku567.
[9] Cabot G, López-Causapé C, Ocampo-Sosa AA, Sommer LM, Domínguez MÁ, et al. Deciphering the resistome of the widespread *Pseudomonas aeruginosa* sequence type 175 international high-risk clone through whole-genome sequencing. Antimicrob Agents Chemother 2016;60:7415–23, doi:http://dx.doi.org/10.1128/AAC.01720-16.
[10] Jeukens J, Freschi L, Kukavica-Ibrulj I, Emond-Rheault J-G, Tucker NP, Levesque RC. Genomics of antibiotic-resistance prediction in *Pseudomonas aeruginosa*. Ann N Y Acad Sci 2017, doi:http://dx.doi.org/10.1111/nyas.13358.
[11] Treepong P, Kos VN, Guyeux C, Blanc DS, Bertrand X, Valot B, et al. Global emergence of the widespread *Pseudomonas aeruginosa* ST235 clone. Clin Microbiol Infect 2018;24:258–66, doi:http://dx.doi.org/10.1016/j.cmi.2017.06.018.
[12] Jaillard M, van Belkum A, Cady KC, Creely D, Shortridge D, et al. Correlation between phenotypic antibiotic susceptibility and the resistome in *Pseudomonas aeruginosa*. Int J Antimicrob Agents 2017;50:210–8, doi:http://dx.doi.org/10.1016/j.ijantimicag.2017.02.026.
[13] Kos VN, Déraspe M, McLaughlin RE, Whiteaker JD, Roy PH, Alm RA, et al. The resistome of *Pseudomonas aeruginosa* in relationship to phenotypic susceptibility. Antimicrob Agents Chemother 2015;59:427–36, doi:http://dx.doi.org/10.1128/AAC.03954-14.
[14] Turton JF, Wright L, Underwood A, Witney AA, Chan Y-T, et al. High-resolution analysis by whole-genome sequencing of an international lineage (sequence type 111) of *Pseudomonas aeruginosa* associated with metallo-carbapenemases in the United Kingdom. J Clin Microbiol 2015;53:2622–31, doi:http://dx.doi.org/10.1128/JCM.00505-15.
[15] del Barrio-Tofiño E, López-Causapé C, Cabot G, Rivera A, Benito N, et al. Genomics and susceptibility profiles of extensively drug-resistant *Pseudomonas aeruginosa* isolates from Spain. Antimicrob Agents Chemother 2017;61:e01589–17, doi:http://dx.doi.org/10.1128/AAC.01589-17.
[16] Montero M, Horcajada JP, Sorlí L, Alvarez-Lerma F, Grau S, Riu M, et al. Effectiveness and safety of colistin for the treatment of multidrug-resistant *Pseudomonas aeruginosa* infections. Infection 2009;37:461–5, doi:http://dx.doi.org/10.1007/s15010-009-8342-x.
[17] Sorlí L, Luque S, Segura C, Campillo N, Montero M, Esteve E, et al. Impact of colistin plasma levels on the clinical outcome of patients with infections caused by extremely drug-resistant *Pseudomonas aeruginosa*. BMC Infect Dis 2017;17:11, doi:http://dx.doi.org/10.1186/s12879-016-2117-7.
[18] Bergen PJ, Tsuji BT, Bulitta JB, Forrest A, Jacob J, Sidjabat HE, et al. Synergistic killing of multidrug-resistant *Pseudomonas aeruginosa* at multiple inocula by colistin combined with doripenem in an in vitro pharmacokinetic/pharmacodynamic model. Antimicrob Agents Chemother 2011;55:5685–95, doi:http://dx.doi.org/10.1128/AAC.05298-11.
[19] Montero M, VanScoy BD, López-Causapé C, Conde H, Adams J, Segura C, et al. Evaluation of ceftolozane-tazobactam in combination with meropenem against *Pseudomonas aeruginosa* ST175 in a hollow-fiber infection model. Antimicrob Agents Chemother 2018AAC.00026-18, doi:http://dx.doi.org/10.1128/AAC.00026-18.
[20] Fraile-Ribot PA, Cabot G, Mulet X, Periañez L, Martín-Pena ML, Juan C, et al. Mechanisms leading to in vivo ceftolozane/tazobactam resistance development during the treatment of infections caused by MDR *Pseudomonas aeruginosa*. J Antimicrob Chemother 2018;73:658–63, doi:http://dx.doi.org/10.1093/jac/dkx424.
[21] Petrosillo N, Ioannidou E, Falagas ME. Colistin monotherapy vs. combination therapy: evidence from microbiological, animal and clinical studies. Clin Microbiol Infect 2008;14:816–27, doi:http://dx.doi.org/10.1111/j.1469-0691.2008.02061.x.
[22] CLSI. Performance Standards for Antimicrobial Susceptibility Testing: Twentieth Informational Supplement. CLSI document. Wayne, PA: Clinical and Laboratory Standards Institute. n.d.
[23] Mahmoudi L, Mohammadpour AH, Ahmadi A, Niknam R, Mojtahedzadeh M. Influence of sepsis on higher daily dose of amikacin pharmacokinetics in critically ill patients. Eur Rev Med Pharmacol Sci 2013;17:285–91.
[24] de Montmollin E, Bouadma L, Gault N, Mourvillier B, Mariotte E, et al. Predictors of insufficient amikacin peak concentration in critically ill patients receiving a 25 mg/kg total body weight regimen. Intensive Care Med 2014;40:998–1005, doi:http://dx.doi.org/10.1007/s00134-014-3276-x.
[25] Smith PF, Ballow CH, Booker BM, Forrest A, Schentag JJ. Pharmacokinetics and pharmacodynamics of aztreonam and tobramycin in hospitalized patients. Clin Ther 2001;23:1231–44.
[26] Bakker-Woudenberg IAJM, ten Kate MT, Goessens WHF, Mouton JW. Effect of treatment duration on pharmacokinetic/pharmacodynamic indices correlating with therapeutic efficacy of ceftazidime in experimental *Klebsiella pneumoniae* lung infection. Antimicrob Agents Chemother 2006;50:2919–25, doi:http://dx.doi.org/10.1128/AAC.00859-05.
[27] Ljungberg B, Nilsson-Ehle I. Comparative pharmacokinetics of ceftazidime in young, healthy and elderly, acutely ill males. Eur J Clin Pharmacol 1988;34:179–86.
[28] Tam VH, Nikolaou M. A novel approach to pharmacodynamic assessment of antimicrobial agents: new insights to dosing regimen design. PLoS Comput Biol 2011;7:e1001043, doi:http://dx.doi.org/10.1371/journal.pcbi.1001043.
[29] Plachouras D, Karvanen M, Friberg LE, Papadomichelakis E, Antoniadou A, Tsangaris I, et al. Population pharmacokinetic analysis of colistin methanesulfonate and colistin after intravenous administration in critically ill patients with infections caused by gram-negative bacteria. Antimicrob Agents Chemother 2009;53:3430–6, doi:http://dx.doi.org/10.1128/AAC.01361-08.
[30] Garonzik SM, Li J, Thamlikitkul V, Paterson DL, Shoham S, Jacob J, et al. Population pharmacokinetics of colistin methanesulfonate and formed colistin in critically ill patients from a multicenter study provide dosing suggestions for various categories of patients. Antimicrob Agents Chemother 2011;55:3284–94, doi:http://dx.doi.org/10.1128/AAC.01733-10.
[31] FDA, Zerbaxa (Ceftolozane/Tazobactam) For Injection Package Insert. Lexington, MA, USA: Cubist Pharmaceuticals U.S., 2014. n.d. https://www.accessdata.fda.gov/drugsatfda_docs/label/2014/206829lbl.pdf. [Accessed 15 March 2018].
[32] Orhan G, Bayram A, Zer Y, Balci I. Synergy tests by E test and checkerboard methods of antimicrobial combinations against *Brucella melitensis*. J Clin Microbiol 2005;43:140–3, doi:http://dx.doi.org/10.1128/JCM.43.1.140-143.2005.
[33] Tängdén T, Karvanen M, Friberg LE, Odenholt I, Cars O. Assessment of early combination effects of colistin and meropenem against *Pseudomonas aeruginosa* and *Acinetobacter baumannii* in dynamic time-kill experiments. Infect Dis (Auckl) 2017;49:521–7, doi:http://dx.doi.org/10.1080/23744235.2017.1296183.
[34] White RL, Burgess DS, Manduru M, Bosso JA. Comparison of three different in vitro methods of detecting synergy: time-kill, checkerboard, and E test. Antimicrob Agents Chemother 1996;40(8):1914–8.
[35] Lim T-P, Cai Y, Hong Y, Chan ECY, Suranthran S, Teo JQ-M, et al. In vitro pharmacodynamics of various antibiotics in combination against extensively drug-resistant *Klebsiella pneumoniae*. Antimicrob Agents Chemother 2015;59:2515–24, doi:http://dx.doi.org/10.1128/AAC.03639-14.
[36] Gunderson BW, Ibrahim KH, Hovde LB, Fromm TL, Reed MD, Rotschafer JC. Synergistic activity of colistin and ceftazidime against multiantibiotic-resistant

Pseudomonas aeruginosa in an in vitro pharmacodynamic model. Antimicrob Agents Chemother 2003;47:905–9.

[37] Timurkaynak F, Can F, Azap O, Demirbilek M, Arslan H, Karaman S. In vitro activities of non-traditional antimicrobials alone or in combination against multidrug-resistant strains of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolated from intensive care units. Int J Antimicrob Agents 2006;27:224–8, doi:http://dx.doi.org/10.1016/j.ijantimicag.2005.10.012.

[38] Cai Y, Yang D, Wang J, Wang R. Activity of colistin alone or in combination with rifampicin or meropenem in a carbapenem-resistant bioluminescent *Pseudomonas aeruginosa* intraperitoneal murine infection model. J Antimicrob Chemother 2018;73:456–61, doi:http://dx.doi.org/10.1093/jac/dkx399.

[39] Louie A, Grasso C, Bahniuk N, Van Scoy B, Brown DL, et al. The combination of meropenem and levofloxacin is synergistic with respect to both *Pseudomonas aeruginosa* kill rate and resistance suppression. Antimicrob Agents Chemother 2010;54:2646–54, doi:http://dx.doi.org/10.1128/AAC.00065-10.

[40] Bergen PJ, Forrest A, Bulitta JB, Tsuji BT, Sidjabat HE, Paterson DL, et al. Clinically relevant plasma concentrations of colistin in combination with imipenem enhance pharmacodynamic activity against multidrug-resistant *Pseudomonas aeruginosa* at multiple inocula. Antimicrob Agents Chemother 2011;55:5134–42, doi:http://dx.doi.org/10.1128/AAC.05028-11.

[41] Trimble MJ, Mlynárčik P, Kolář M, Hancock REW. Polymyxin: alternative mechanisms of action and resistance. Cold Spring Harb Perspect Med 2016;6:a025288, doi:http://dx.doi.org/10.1101/cshperspect.a025288.

[42] Mohamed AF, Kristoffersson AN, Karvanen M, Nielsen EI, Cars O, Friberg LE. Dynamic interaction of colistin and meropenem on a WT and a resistant strain of *Pseudomonas aeruginosa* as quantified in a PK/PD model. J Antimicrob Chemother 2016;71:1279–90, doi:http://dx.doi.org/10.1093/jac/dkv488.

[43] Maifiah MHM, Creek DJ, Nation RL, Forrest A, Tsuji BT, Velkov T, et al. Untargeted metabolomics analysis reveals key pathways responsible for the synergistic killing of colistin and doripenem combination against *Acinetobacter baumannii*. Sci Rep 2017;7:45527, doi:http://dx.doi.org/10.1038/srep45527.

[44] Fernández L, Breidenstein EBM, Hancock REW. Creeping baselines and adaptive resistance to antibiotics. Drug Resist Updat 2011;14:1–21, doi:http://dx.doi.org/10.1016/j.drup.2011.01.001.

[45] Paul M, Daikos GL, Durante-Mangoni E, Yahav D, Carmeli Y, et al. Colistin alone versus colistin plus meropenem for treatment of severe infections caused by carbapenem-resistant Gram-negative bacteria: an open-label, randomised controlled trial. Lancet Infect Dis 2018;18:391–400, doi:http://dx.doi.org/10.1016/S1473-3099(18)30099-9.

[46] Soudeiha MAH, Dahdouh EA, Azar E, Sarkis DK, Daoud Z. In vitro evaluation of the colistin-carbapenem combination in clinical isolates of*A. baumannii* using the checkerboard, Etest, and time-kill curve techniques. Front Cell Infect Microbiol 2017;7:209, doi:http://dx.doi.org/10.3389/fcimb.2017.00209.