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In vitro evidence of the synergistic interaction of ceftopibrole and other antibiotics against multidrug-resistant Gram-negative isolates

Ausilia Aprile [a], Carla Caio [a], Floriana Gona [b], Stefania Stefani [a], Maria Lina Mezzatesta [a,*]

[a] Department of Biomedical and Biotechnological Sciences, section of Microbiology, University of Catania, Italy
[b] Emerging Bacterial Pathogens Unit, Division of Immunology, Transplantation and Infectious Diseases, IRCCS San Raffaele Scientific Institute, Milan, Italy

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ABSTRACT

The purpose of the present study was to investigate the *in vitro* activity of ceftobiprole in combination with other antimicrobials against 27 selected Gram-negative isolates, including ESBL-producing *E. coli* and KPC-OXA-48-producing *K. pneumoniae*. Ceftobiprole activity in combination with amikacin, colistin, levofloxacin, piperacillin/tazobactam and rifampin was evaluated by time-kill curves and gradient-cross method (except colistin). Among the 27 strains tested with gradient strips most were resistant to ceftobiprole. Synergy was observed in some cases with piperacillin/tazobactam. There was at least one synergistic combination towards 9 isolates belonging to different species. No antagonism was observed with any of the antibiotic tested. In time-kill curves, performed for 12 selected isolates, synergistic interaction was more frequent, occurring with 32/60 combinations. The most interesting results of our study are the bactericidal effects of ceftobiprole in combination with colistin or piperacillin/tazobactam tested against Gram-negative isolates, including KPC and OXA-48-producing *K. pneumoniae*.

1. Introduction

Gram-negative bacteria are important causes of both community-acquired pneumonia (CAP) and hospital-acquired pneumonia (HAP) (American Thoracic Society 2005; Mandell et al. 2007). While *Staphylococcus aureus* is the single most frequently isolated microorganism in patients hospitalized with pneumonia, Gram-negative bacteria predominate overall and are increasingly resistant to multiple classes of antimicrobial agents (Falcone et al. 2009; Falcone et al. 2010; Jones 2010). The emergence of carbapenemase-producing nosocomial strains of *Klebsiella pneumoniae* is currently of particular concern (Pitout et al. 2015).

Ceftobiprole is an advanced-generation broad-spectrum cephalosporin with potent activity against both Gram-positive bacteria (including methicillin-resistant staphylococci) and Gram-negative bacteria, including those expressing several classes of β-lactamases to which it is relatively refractory (Barboura et al. 2009; Davies et al. 2007; Marti et al. 2009; Pfaller et al. 2018).

The prodrug ceftobiprole medocaril has been approved in the EU and a number of other countries for the treatment of HAP, excluding ventilator-associated pneumonia (VAP), and CAP caused by susceptible Gram-positive and Gram-negative pathogens (Syed 2014).

Ceftobiprole has potent inhibitory and bactericidal activity against susceptible Gram-negative isolates (Farrell et al. 2014; Issa et al. 2004; Rossolini et al. 2011; Waltky et al. 2011).

However, most strains that produce extended spectrum β-lactamases (ESBL) or carbapenemases are resistant to ceftobiprole (Queenan et al. 2007).

Combination therapy, often with a β-lactam agent plus another antimicrobial, may be indicated for initial empiric or extended treatment of CAP and HAP, depending on the underlying disease, patient factors, the infecting bacteria, and local resistance patterns (Campanile et al. 2019; Jean et al. 2016).

The current study investigated the activity/potency of ceftobiprole with various other antimicrobial agents against selected Gram-negative isolates, including ceftobiprole-resistant bacteria, in order to provide new possible future therapeutic options for clinicians against difficult-to-treat strains.

2. Materials and methods

2.1. Bacterial strains

A total of 27 gram-negative bacteria were isolated, between 2010 and 2015, from clinical sources, including sputum (8), blood (4), urine (3), wounds (3), rectal swabs (3), pharyngeal swabs (3), peritoneal fluid (1), an abdominal drain (1), and a tracheostomy swab (1). They were tested and were identified by conventional methods at the

* Corresponding author. Tel.: +39-095-4781240.
E-mail address: mezzate@unict.it (M.L. Mezzatesta).

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Table 1
Activities of ceftobiprole and comparators tested by gradient test and broth microdilution method against Gram negative isolates.

Species (number) Antimicrobial agent	MIC (mg/L)						% Resistance[d]
	Range		MIC_{50}		MIC_{90}		EUCAST
	GT	BMD	GT	BMD	GT	BMD	
Escherichia coli (5)[a]							
Ceftobiprole	6–>32	16–128	>32	64	>32	64	100
Amikacin	1.5–4	1–8	3	4	4	8	0
Colistin	-	≤0.25	-	≤0.25		≤0.25	0
Levofloxacin	0.38–>32	0.5–64	>32	16	>32	16	80
Piperacillin/tazobactam	2–>256	2–>512	8	32	16	64	20
Rifampin	6–12	0.5–16	8	2	8	2	NA
Klebsiella pneumoniae (19)[b]							
Ceftobiprole	0.016–>32	0.015–128	>32	128	>32	128	84
Amikacin	1–128	0.03–4096	24	16	96	64	37
Colistin	-	≤0.25–256	-	32	-	256	53
Levofloxacin	0.06–>32	≤0.5–256	>32	64	>32	256	89
Piperacillin/tazobactam	0.5–>256	2–4096	>256	>512	>256	>512	84
Rifampin	8–>32	2–1024	>32	16	>32	512	NA
Pseudomonas aeruginosa (3)[c]							
Ceftobiprole	0.75–>32	1–128	>32	64	-	-	NA
Amikacin	3–32	0.5–16	12	8	-	-	0
Colistin	-	≤0.25–0.25	-	0.25	-	-	0
Levofloxacin	1–>32	0.25–64	>32	32	-	-	67
Piperacillin/tazobactam	3–>256	8–512	>256	256	-	-	67
Rifampin	>32	4–16	>32	8	-	-	NA

MIC, minimum inhibitory concentration; MIC50/90, MIC for 50% and 90% of the microorganisms, respectively. GT, gradient test; BMD, broth microdilution method.
[a] All ESBL producers.
[b] Including 4 ESBL producers, 2 OXA-48 producers, and 10 KPC producers.
[c] Including 1 isolate resistant to ceftazidime and meropenem.
[d] Based on broth microdilution MICs. NA, not available.

Department of Microbiology, University of Catania (Italy). They comprised *K. pneumoniae* strains (n.19), most of them expressing extended spectrum β-lactamases (ESBL) or carbapenemases, including KPC and OXA-48 (Cascio et al. 2014; Gona et al. 2011; Gugliandolo et al. 2017); ESBL-producing *Escherichia coli* (n.5); and *Pseudomonas aeruginosa* strains (n.3), including one resistant strain to ceftazidime and meropenem.

2.2. Antimicrobial susceptibility testing

MICs of ceftobiprole and comparator agents were determined by broth microdilution method (BMD) and gradient-test (GT) in cation-adjusted Mueller-Hinton broth (CAMHB) and Mueller Hinton agar respectively as described by CLSI guidelines (Clinical and Laboratory Standards Institute 2015).

Ceftobiprole was supplied by Basilea Pharmaceutica International Ltd. (Basel, Switzerland). Commercial products were used for all other antibiotics: amikacin, colistin, levofloxacin, piperacillin/tazobactam and rifampin. MIC test strips were purchased from Liofilchem, Pescara, Italy. Colistin MIC determination was only performed by BMD as EUCAST and CLSI recommend.

E. coli ATCC 25922 was used as the quality control strain (CLSI, 2018). Antibiotic breakpoints for the interpretative criteria for clinical isolates were used according to the EUCAST guidelines v.8.0, for isolates of Enterobacteriaceae (http://www.eucast.org.) (The European Committee on Antimicrobial Susceptibility Testing 2018).

2.3. Combination testing by gradient diffusion method

For combination studies by gradient diffusion method, strips of the two agents were placed at a 90° angle, crossing at the previously determined MICs of the two agents when tested alone. When the MIC exceeded the concentration range on one or both of the gradient strips, the strips were crossed at the highest concentration present on the respective test strip. The plates were incubated for 18 h at 35 °C. The fractional inhibitory concentration index (FIC index) was used to interpret the gradient test as follows: synergy, FIC index ≤0.5; additivity, FIC index >0.5 to 1; indifference, FIC index >1 to 4; antagonism, FIC index >4 (White et al. 1996).

The following agents were tested in combination with ceftobiprole: amikacin, levofloxacin, piperacillin/tazobactam and rifampin.

Table 2
Interpretation of gradient test combinations against Gram negative isolates.

Species (number)	BPR + AK	BPR + LEV	BPR + RIF	BPR + TZP
Escherichia coli ESBL (5)	5 IND	5 IND	5 IND	4 SYN 1 ADD
Klebsiella pneumoniae (3)	1 SYN 1 ADD 1 IND	1 SYN 1 ADD 1 IND	1 SYN 2 IND	2 SYN 1 ADD
K. pneumoniae ESBL (4)	4 IND	4 IND	4 IND	2 SYN 1 ADD 1 IND
K. pneumoniae KPC COL-S (1)	IND	IND	IND	IND
K. pneumoniae KPC COL-R (9)	9 IND	9 IND	9 IND	9 IND
K. pneumoniae OXA-48 (2)	2 IND	2 IND	2 IND	2 IND
Pseudomonas aeruginosa (2)	1 ADD 1 IND	1 SYN 1 IND	2 IND	1 ADD 1 IND
P. aeruginosa (1)[a]	IND	IND	IND	IND

SYN, synergy; ADD, additivity; IND, indifference; ANT, antagonism; BPR, ceftobiprole; AK, amikacin; LEV, levofloxacin; RIF, rifampin; TZP, piperacillin/tazobactam; COL-S, colistin-susceptible; COL-R, colistin-resistant.
[a] Resistant to ceftazidime and meropenem.

Table 3
Bactericidal activity of ceftobiprole alone and in combination with other antibacterial agents against n.12 selected isolates.

Strains Antibiotic	Ceftobiprole		Antibiotic		
	MIC[a]	Kill[b]	MIC[a]	Kill alone[b]	Kill in combination
Escherichia coli ESBL – CT00023	64	−1.8 (4×)			
Amikacin			4	+3.9	No effect
Colistin			0.03	−6.1	SYN 2×, 4×
Levofloxacin			0.5	−2.6	ANT
Piperacillin/tazobactam			8	+3.9	SYN 1×
Rifampin			16	+3.9	SYN 1×
K. pneumoniae – CT0004	0.015	−3.2 (2×)			
Amikacin			1	+2.3	No effect
Colistin			0.03	+3.8	SYN 1×
Levofloxacin			0.06	+3.3	SYN 1×
Piperacillin/tazobactam			2	+1.6	SYN 1×
Rifampin			8	+2.0	No effect
K. pneumoniae ESBL – CT00012	64	−3.6 (4×)			
Amikacin			1	+3.5	No effect
Colistin			0.12	+3.0	SYN 2×, 4×
Levofloxacin			8	+2.8	No effect
Piperacillin/tazobactam			32	+3.3	SYN 1×
Rifampin			4	+4.0	No effect
K. pneumoniae KPC/COL-R – CT00016	128	−6.5 (4×)			
Amikacin			64	+3.2	No effect
Colistin			32	+3.9	No effect
Levofloxacin			64	+1.3	SYN 2×
Piperacillin/tazobactam			2048	−6.5	No effect
Rifampin			64	+1.7	No effect
K. pneumoniae KPC/COL-R – CT00018	32	−6.5 (2×)			
Amikacin			16	+3.9	SYN 1×
Colistin			256	−1.0	ANT 1×
Levofloxacin			256	+0.7	No effect
Piperacillin/tazobactam			2048	−6.1	ANT 1×
Rifampin			512	−1.1	No effect
K. pneumoniae KPC/COL-R – CT00019	16	−6.2 (2×)			
Amikacin			16	+4.0	SYN 1×
Colistin			128	+3.1	SYN 1×
Levofloxacin			256	0.0	No effect
Piperacillin/tazobactam			2048	+2.8	SYN 1×
Rifampin			512	−0.8	ANT 1×, 2×, 4×
K. pneumoniae KPC/COL-S – CT00020	128	−6.4 (4×)			
Amikacin			1	+3.6	No effect
Colistin			1	+1.1	SYN 2× (rapid 4× at 4 h)
Levofloxacin			64	+1.6	No effect
Piperacillin/tazobactam			4096	−6.4	SYN 2× (rapid 4× at 4 h)
Rifampin			16	+0.6	SYN 2×
K. pneumoniae KPC/COL-R – CT0009	64	−6.1 (4×)			
Amikacin			32	+3.8	SYN 1×
Colistin			16	+3.2	No effect
Levofloxacin			64	−6.1	ANT
Piperacillin/tazobactam			1024	+3.4	SYN 1×
Rifampin			16	+2.1	No effect
K. pneumoniae OXA-48/COL-R – CT00021	128	−3.0 (2×)			
Amikacin			4096	+3.3	ANT
Colistin			8	+3.9	SYN 2×; >rapid 4×
Levofloxacin			32	+3.0	No effect
Piperacillin/tazobactam			4096	+2.3	SYN 2×; >rapid 4×
Rifampin			8	+2.8	SYN 2×; >rapid 4×
K. pneumoniae OXA-48/COL-R – CT00022	128	−6.2 (4×)			
Amikacin			2	+3.9	SYN 2×
Colistin			32	+3.3	SYN 2×
Levofloxacin			32	+3.5	SYN 2×
Piperacillin/tazobactam			512	+3.2	SYN 2×
Rifampin			1024	−6.2	SYN 1×
P. aeruginosa CT0002	1	−0.4 (4×)			
Amikacin			0.5	+4.0	SYN 4×
Colistin			0.25	+2.0	SYN 2×, 4×
Levofloxacin			0.25	+2.2	SYN 2×, 4×
Piperacillin/tazobactam			8	+1.9	SYN 1×, 2×, 4×
Rifampin			4	+3.6	SYN 2×, 4×
P. aeruginosa CAZ/MEM-R – CT0003	128	−6.3 (2×)			
Amikacin			16	+1.8	No effect
Colistin			0.25	+2.0	SYN 1×
Levofloxacin			64	+0.7	No effect
Piperacillin/tazobactam			512	+3.9	No effect
Rifampin			16	+1.8	No effect

2.4. Synergy testing by time-kill curves

Twelve strains were selected for time-kill curves conducted by CLSI guidelines (Eliopoulos and Moellering 1991). They were performed in triplicate using a starting inoculum of 1–6 × 10^6 CFU/mL in tubes containing 20 mL of CAMHB, either with each antibiotic at 1 × MIC concentration or 1×, 2× or 4× MIC of ceftobiprole combined with 1× MIC of the other agent tested. Aliquots were removed at 0, 4, 8 and 24 h and plated in duplicate on MHA for determination of viable counts. The total bacterial $\log_{10}$ CFU/mL was determined after 18 h of incubation at 35 °C.

Bactericidal activity was defined as a ≥ 3 $\log_{10}$ decrease in CFU/mL by 24 h. Synergy was defined as a ≥ 2 $\log_{10}$ decrease in CFU/mL at 24 h by the drug combination when compared with its most active constituent. Antagonism was defined as a ≥ 2-$\log_{10}$ increase in colony count at 24 h with the combination compared with the most active single drug (Mezzatesta et al. 2016).

3. Results

3.1. Antimicrobial susceptibility testing

In the current study, 27 clinical isolates of Gram-negative bacteria were tested including 19 *K. pneumoniae*, 5 *E. coli* and 3 *P. aeruginosa*. MICs of antibiotics determined by using gradient strips and broth microdilution method are shown in Table 1. Similar MICs were obtained with the two methods for the majority of the microorganisms. Most of the 24 *Enterobacteriaceae* strains were multidrug-resistant (MDR), including ceftobiprole. All ESBL-producing *E. coli* strains were resistant to ceftobiprole (MICs range 16–128 mg/L) and 84% of *K. pneumoniae* (based on the broth microdilution MICs). Ceftobiprole susceptibility breakpoints were not been established for non-fermenters; however, the lowest broth microdilution MIC for the 3 *P. aeruginosa* isolates tested was 1 mg/L; many of the isolates were resistant to other antimicrobials; susceptibility was highest to colistin and amikacin among the agents tested.

3.2. Gradient test combination study

The interpretation of the interactions is summarized in Table 2. The majority of combinations (113/135, 84%) showed indifference. No antagonism was observed with any agent. There were 12 combinations that showed synergy: ceftobiprole with piperacillin/tazobactam against 4 of 5 *E. coli* ESBL isolates and against 4 of 19 *K. pneumoniae* isolates (2 of which were susceptible to both agents, whereas the other 2 were resistant to ceftobiprole and also to piperacillin/tazobactam). Against one of the 2 ceftobiprole-susceptible *K. pneumoniae* strains with ceftobiprole-piperacillin/tazobactam synergy there was also synergy between ceftobiprole and amikacin, levofloxacin and rifampin. Synergy between ceftobiprole and levofloxacin was observed with one isolate of *P. aeruginosa* while all combinations were indifferent against the ceftazidime-resistant isolate. Additivity was observed in 10 instances: with amikacin in one isolate each of *K. pneumoniae* and *P. aeruginosa*; with levofloxacin in one *K. pneumoniae*; with piperacillin/tazobactam in 2 strains of *K. pneumoniae* and one each of *E. coli* and *P. aeruginosa*.

3.3. Bactericidal activity

Time-kill curves were performed with 12 of the isolates selected based on their resistance profile: 1 ESBL *E. coli,* 9 *K. pneumoniae* (including KPC and OXA-48 producing); and 2 *P. aeruginosa* (CAZ-S and CAZ/MEM-R).

Concentrations of 1×, 2× and 4× the broth microdilution MIC of ceftobiprole, alone and in combination with 1× the MIC of the comparator agents, which were also tested alone. The results are summarized in Table 3.

Ceftobiprole, at 1 × MIC concentration, showed bacteriostatic activity in all isolates within 8 h but re-growth was observed after 24 h. Bactericidal activity was always achieved at 2× or 4× MIC of ceftobiprole.

Overall, there were 32 synergistic combinations out of 60 (53%) and 6 instances of antagonism (10%). Synergy was most frequently observed at one or more concentrations of ceftobiprole with colistin (in *E. coli*, 6 *K. pneumoniae* and both *P. aeruginosa* isolates) and with piperacillin/tazobactam (n.1 *E. coli*, n.7 *K. pneumoniae*, and n.1 *P. aeruginosa*). Colistin and piperacillin/tazobactam each showed antagonism against only one KPC COL-R strain. When ceftobiprole was combined with levofloxacin, synergism was observed against 3 *K. pneumoniae* and one *P. aeruginosa* strain and antagonism against ESBL *E. coli* and 1 KPC COL-R *K. pneumoniae* strain. Amikacin showed a more rapid bactericidal activity at 4 h against CT00016 *K. pneumoniae* isolate but re-growth was observed at 24 h. Synergy with rifampin was seen in 3 *K. pneumoniae* strains, a more rapid bactericidal activity in combination with 1× and 2× the MIC of ceftobiprole in another *K. pneumoniae* strain, and antagonism in 1 *K. pneumoniae* isolate.

Although it is resistant to ceftobiprole (MIC 128 mg/L), $bla_{\text{OXA-48}}$ *K. pneumoniae* CT00022 was killed by ceftobiprole tested alone at 4× the MIC at 4 h, maintaining its activity until 24 h. Synergy with 2× the MIC of ceftobiprole (256 mg/L) was demonstrated by amikacin, colistin, levofloxacin and piperacillin/tazobactam. Rifampin produced a more rapid bactericidal effect in combination with 1× and 2× the MIC of ceftobiprole.

With the *E. coli* strain, against which levofloxacin alone was bactericidal, the activity of this agent was antagonized by ceftobiprole; in the gradient test combination study, indifference was seen with these combinations against all 5 *E. coli* strains.

Time-kill curves for 2 ceftobiprole-resistant of the isolates for which some synergy was observed, ESBL *E. coli* and KPC/COL-R *K. pneumoniae* strains are shown in Figs. 1 and 2, respectively. Against the *E. coli* strain, ceftobiprole at 2 and 4× MIC produced a 2-log decrease in CFU/mL at 24 h compared with the starting inoculum (Fig. 1); while at 1× MIC, there was regrowth at 24 h. Against this isolate with ceftobiprole, the combination of ceftobiprole MIC of 64 mg/L with rifampin (MIC 16 mg/L) and piperacillin/tazobactam (MIC 8 mg/L) exhibited bactericidal synergism. Against this *E. coli* strain, colistin also demonstrated synergy with 2× and 4× MIC of ceftobiprole (Table 3; data not shown in Fig. 1). Fig. 2 shows the bactericidal activities against the CT00019 colistin-resistant $bla_{\text{-KPC}}$ *K. pneumoniae* of ceftobiprole at 2× and 4× the MIC (32 and 64 mg/L) alone and at 1× the MIC in combination with colistin, amikacin and piperacillin/tazobactam. These 3 combinations were synergistic. The data for levofloxacin, which had no effect on ceftobiprole activity against this strain, and for rifampin, which showed antagonism, are also not shown in Fig. 2 but are summarized in Table 3.

4. Discussion

The current study focused on the interaction between ceftobiprole and several other antimicrobial agents, when tested in combination against a number of Gram-negative isolates with different mechanisms of resistances. Among the 27 isolates included, only 3 were susceptible

SYN, synergy (at least 2 $\log_{10}$ decrease in CFU/mL with combination); ANT, antagonism (at least 2 $\log_{10}$ increase in CFU); 1×, 2×, 4×, multiple of ceftobiprole MIC at which observed; CAZ/MEM-R, ceftazidime and meropenem-resistant.

[a] mg/L, broth microdilution.

[b] Mean change ($\log_{10}$ CFU/mL) from starting inoculum at 24 h with the antibiotics tested as single agents and in combinations by time–kill assays (positive and negative values indicate growth and reduction, respectively).

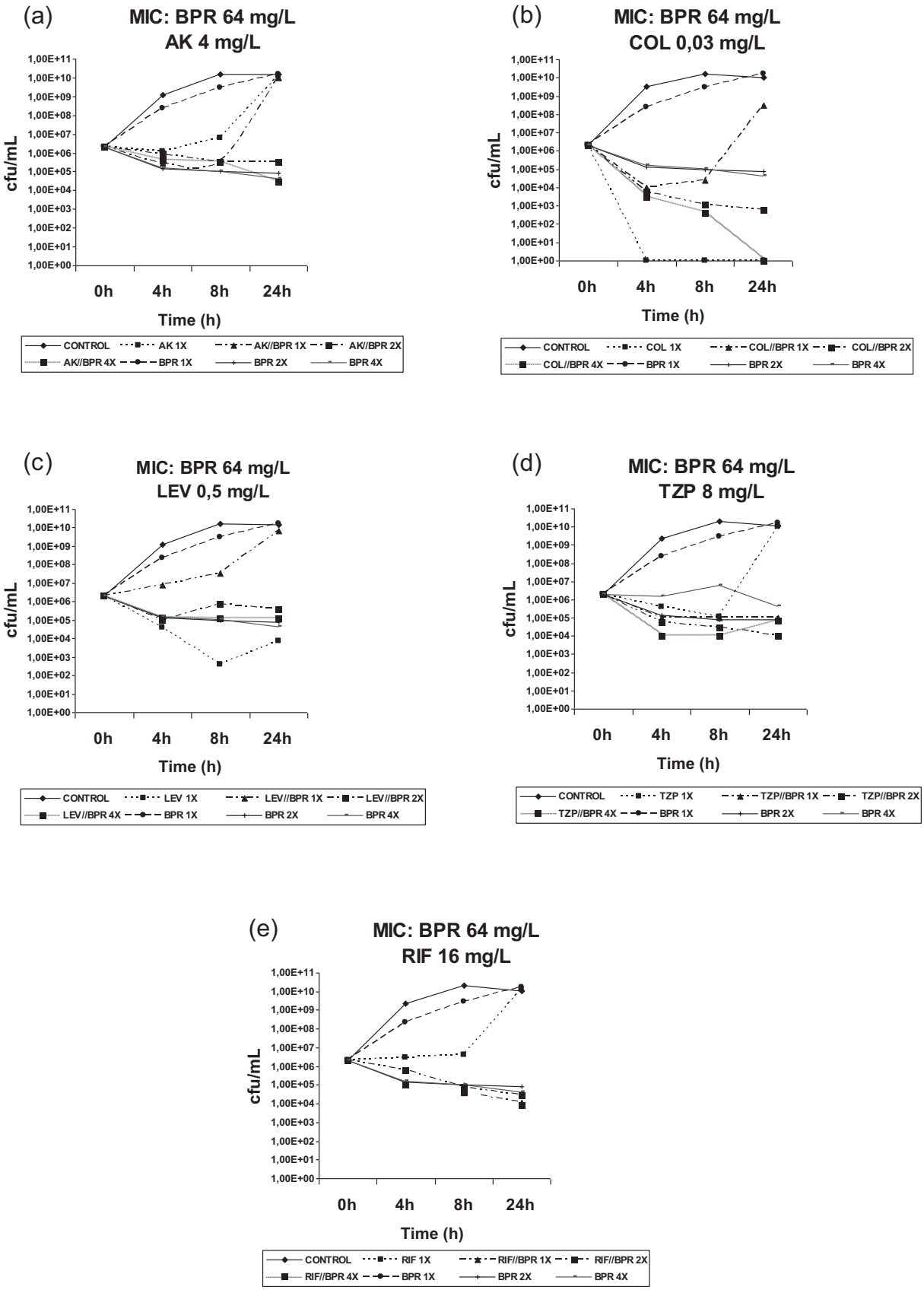

Fig. 1. Time-kill curves of ceftobiprole (1×, 2×, and 4× MIC) alone and in combination with amikacin (a); colistin (b); levofloxacin (c); piperacillin/tazobactam (d) and rifampin (e) (1× MIC) against ESBL *E. coli* CT00023. BPR, ceftobiprole; AK, amikacin; COL, colistin; LEV, levofloxacin; TZP, piperacillin/tazobactam; RIF, rifampin.

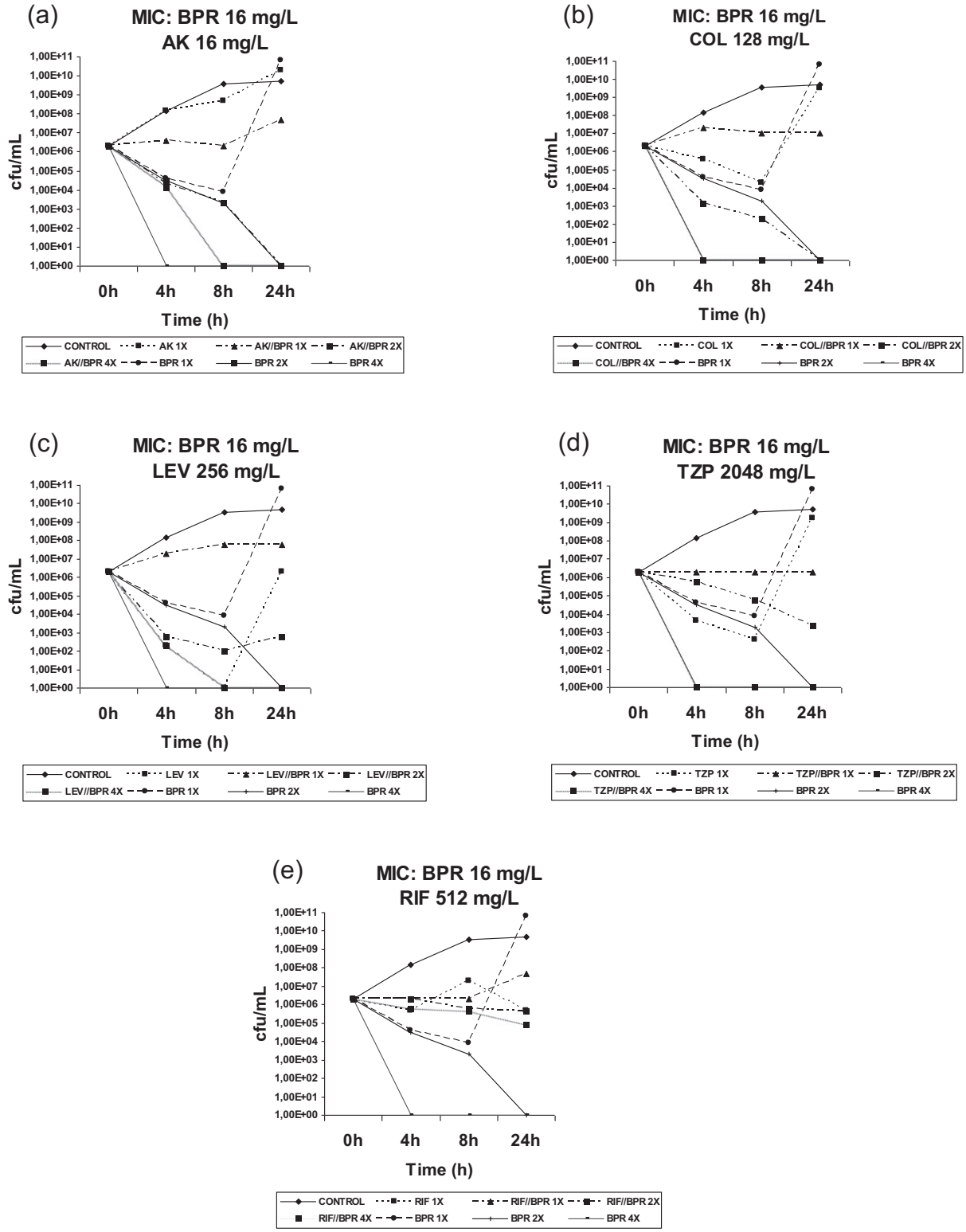

Fig. 2. Time-kill curves of ceftobiprole (1×, 2×, and 4× MIC) alone and in combination with amikacin (a); colistin (b); levofloxacin (c); piperacillin/tazobactam (d) and rifampin (e) (1× MIC) against *K. pneumoniae* KPC CT00019. BPR, ceftobiprole; AK, amikacin; COL, colistin; LEV, levofloxacin; TZP, piperacillin/tazobactam; RIF, rifampin.

to ceftobiprole. Nevertheless, synergy was observed with some combinations even in resistant microorganisms, albeit at very high antibiotic concentrations (64–128 mg/L), although such concentrations are hardly achievable in serum: the maximum drug concentration (Cmax) for ceftobiprole with a 500 mg i.v. dose delivered over 2 h is reported to be 29.2 mg/L with an area under the concentration–time curve from 0 h to 8 h of 90.0 mg h/L (Torres et al. 2016).

In the gradient strip test, inhibitory synergy between ceftobiprole and piperacillin/tazobactam was observed with 2 of the 3 ceftobiprole-susceptible *K. pneumoniae* strains and additivity with the

third strain; all 3 were susceptible to piperacillin/tazobactam. Interestingly, synergy between these two agents was also observed with 4 of 5 ESBL-expressing *E. coli* (and additivity against the fifth isolate, which was additionally resistant to piperacillin/tazobactam) and with 2 *K. pneumoniae* ESBL-positive strains (both of them resistant to piperacillin/tazobactam by EUCAST criteria). No synergistic interactions were seen in the 12 *K. pneumoniae* KPC or OXA-48 isolates. With the gradient strip test there was no clear pattern of synergistic interactions with the other agents tested.

Twelve strains, including 9 *K. pneumoniae* of various resistance phenotypes, one ESBL-producing *E. coli*, and 2 *P. aeruginosa* were selected for time-kill curves in which 1×, 2× and 4× the respective MICs of ceftobiprole were combined with 1× the MIC of the antibiotics tested. Ceftobiprole showed bactericidal activity at 2x and 4× MIC concentrations against 10 isolates including ceftobiprole-resistant strains (MIC 16–128 mg/L). Among the 10 *Enterobacteriaceae* strains tested, only one *K. pneumoniae* was ceftobiprole-susceptible (MIC ≤0.25 mg/L); the ceftobiprole MIC for one of the 2 *P. aeruginosa* strains was relatively low (1 mg/L), although there is no breakpoint for this organism. Synergy were observed with a larger number of combinations in the kill-curves than in the gradient strip test; this may be in part due to the exposure to 3 different concentrations of ceftobiprole and in part to the fact that the kill-curve is a more stringent test that provides more quantitative data. Synergy between ceftobiprole and multiple other agents was clearly evident in the two most susceptible isolates tested, although it was also seen with multiple agents against the two OXA-48 strains and less commonly in some other non-susceptible isolates.

Killing-curves confirmed superior activity of ceftobiprole plus amikacin against KPC-producing *K. pneumoniae* isolates. Bactericidal synergy was previously reported (only in one study) with ceftobiprole plus amikacin or levofloxacin against strains of *P. aeruginosa* with ceftobiprole MICs of ≤8 mg/L (Kresken et al. 2011).

Interactions, which in some strains appear to be synergistic or antagonistic at very high, physiologically unattainable concentrations, may be of limited relevance.

To our knowledge this is the first study evaluating the efficacy of double combinations of ceftobiprole with other antibiotics against MDR Enterobacteriaceae strains.

5. Conclusions

The main conclusion of the *in vitro* studies was that the interaction of ceftobiprole with other antimicrobials produced primarily a synergistic response and very rarely showed antagonism. In particular, synergism was observed when ceftobiprole was combined with colistin or piperacillin/tazobactam against most of the isolates. These combinations have a clinical relevance; as the microorganisms they target belonged to multidrug-resistant pathogens. *In vivo* and clinical data are needed before they can be recommended to be used in clinical practice.

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Competing interests

The authors declare that they have no conflicts of interest.

Ethical approval

Not required.

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