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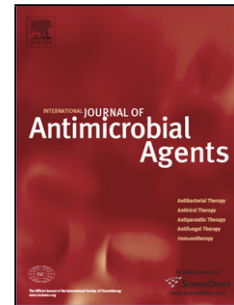
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In vitro activities of ceftobiprole combined with amikacin or levofloxacin against *Pseudomonas aeruginosa*: evidence of a synergistic effect using time–kill methodology

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ABSTRACT

Ceftobiprole is an investigational intravenous broad-spectrum cephalosporin with in vitro activity against Gram-positive and Gram-negative pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa*. *Pseudomonas aeruginosa* is a frequent nosocomial pathogen, increasingly associated with complicated skin and skin-structure infections. Combination antimicrobial therapy is recommended as empirical therapy for serious infections where *P. aeruginosa* is suspected. Therefore, in this study the interaction of ceftobiprole with two other antipseudomonal agents (amikacin and levofloxacin) was investigated. Time–kill studies were performed for each single agent and for the combination of ceftobiprole 4 mg/L with either amikacin or levofloxacin at 0.5 \times , 1 \times and 2 \times the minimum inhibitory concentration. Five clinical isolates of *P. aeruginosa* as well as the *P. aeruginosa* ATCC 27853 reference strain were tested at initial inocula of 5×10^5 colony-forming units (CFU)/mL (low inoculum) or 5×10^7 CFU/mL (high inoculum). Synergy was defined as a decrease of $\geq 2 \log_{10}$ CFU/mL with the combination compared with the most active single drug at 6 h and 24 h. At low inoculum with ceftobiprole as a single agent, viable counts were decreased by 1.5–2 \log_{10} at 6 h. Addition of either amikacin or levofloxacin resulted in synergistic bactericidal activity at 24 h. At high inoculum the combination of ceftobiprole with amikacin or levofloxacin demonstrated synergism in one of three and three of five strains, respectively. This study demonstrated that the combination of ceftobiprole at a clinically achievable concentration of 4 mg/L with amikacin or levofloxacin exhibited synergistic activity against *P. aeruginosa*. There was no evidence of antagonism for either combination.

1. Introduction

Pseudomonas aeruginosa is a highly drug-resistant and opportunistic pathogen [1]. It has become a leading cause of Gram-negative infection both in hospitals and the community and it has been reported as one of the principal causes of nosocomial infection, particularly among immunocompromised patients [1]. Although Gram-positive organisms, including *Staphylococcus aureus*, are the most common pathogens in skin infections, Gram-negative bacilli, and particularly *P. aeruginosa*, are also recovered from complicated infections [2].

Guidelines recommend combination therapy with two drugs to cover *P. aeruginosa* for treating patients with serious infections or when drug-resistant organisms are suspected [3]. Empirical initial combination therapy is superior to monotherapy for *P. aeruginosa*-associated bacteraemia and ventilator-associated pneumonia [4]. Combination therapy, frequently given empirically for severe *P. aeruginosa*, usually comprises an adequate β -lactam and an aminoglycoside or fluoroquinolone [4].

Ceftobiprole is a broad-spectrum investigational cephalosporin with activity against Gram-positive and Gram-negative pathogens, including clinical isolates of methicillin-resistant *S. aureus* (MRSA), drug-resistant pneumococci, Enterobacteriaceae and *P. aeruginosa* [2,5]. The in vitro activity of ceftobiprole, together with clinical data, suggest that it may be useful as empirical monotherapy for complicated skin and skin-structure infections (cSSSIs) and pneumonia, in combination with other antimicrobials if additional coverage is needed in lower respiratory tract infections [6]. For the treatment of patients with cSSSI, two phase III studies have shown that ceftobiprole is as effective as vancomycin in treating Gram-positive infections and as

effective as vancomycin plus ceftazidime in treating infections due to Gram-positive and Gram-negative bacteria [7,8].

Although there are data demonstrating synergistic activity between cephalosporins and both aminoglycosides and fluoroquinolones against *P. aeruginosa* [9–11], evidence of synergism involving ceftobiprole in the same setting is, to our knowledge, non-existent. A single study has shown synergism of ceftobiprole and aminoglycosides against *Enterococcus faecalis* [12]. The objective of this study was to evaluate the interaction of ceftobiprole with amikacin or levofloxacin against *P. aeruginosa* using time–kill methodology. Here we demonstrate that ceftobiprole at clinically achievable levels exhibits synergistic activity against *P. aeruginosa* when combined with amikacin or levofloxacin.

2. Materials and methods

2.1. Bacterial strains

Five clinical isolates of *P. aeruginosa* as well as the *P. aeruginosa* ATCC 27853 reference strain were evaluated. Strains were selected based on minimum inhibitory concentration (MIC) results to provide a range of susceptibilities to the different antimicrobial agents tested.

2.2. Antibiotics

Ceftobiprole was provided by Johnson & Johnson Pharmaceutical Research & Development, L.L.C. (Raritan, NJ). Levofloxacin was provided by Sanofi-Aventis

Deutschland GmbH (Berlin, Germany). Amikacin was purchased from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany).

2.3. Susceptibility testing

MICs were determined by the broth microdilution method with geometric two-fold serial dilutions in cation-adjusted Mueller–Hinton broth (CA-MHB) (Becton Dickinson, Heidelberg, Germany) according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI). Broth microdilution studies were performed for individual antibiotics. Interpretive criteria applied to amikacin and levofloxacin were those published by the CLSI.

2.4. Time–kill assays

Time–kill assays were performed in glass flasks containing 20 mL of CA-MHB, either with each compound alone or with ceftobiprole in combination with amikacin or levofloxacin at starting inocula of ca. 5×10^5 colony-forming units (CFU)/mL (low inoculum) or 5×10^7 CFU/mL (high inoculum). The antibacterial activity of ceftobiprole was measured at 4 mg/L to simulate free drug concentrations that were previously achieved for 40–50% of an 8-h dosing interval when ceftobiprole was administered at 500 mg intravenously over 2 h to 150 subjects enrolled in phase I/II trials. The same dosing regimen was selected for a phase III trial comparing ceftobiprole with vancomycin plus ceftazidime for the treatment of patients with cSSSI caused by Gram-positive and Gram-negative pathogens [7]. Amikacin and levofloxacin were tested at concentrations corresponding to the MIC, one dilution below the MIC ($0.5 \times$ MIC) and one dilution above the MIC ($2 \times$ MIC) for each

organism. *Pseudomonas aeruginosa* test organisms were incubated at 35 °C with vigorous shaking over 24 h. The combination of ceftobiprole and amikacin was tested against two clinical isolates (PEG-01-14 and PEG-02-54) as well as the reference strain. The combination of ceftobiprole and levofloxacin was tested against four clinical isolates (PEG-01-14, PEG-02-33, P-03-29 and P-04-03) as well as the reference strain.

Briefly, 200 μ L samples were removed from each culture flask at 0, 1, 2, 3, 6 and 24 h after inoculation and were added to 1.8 mL of saline. These suspensions were then serially diluted using 10-fold dilutions, with 50 μ L aliquots subsequently plated onto tryptic soy agar plates (Fluka, Buchs, Switzerland) and incubated for 24 h at 37 °C. Plates showing growth of 10–100 CFU were counted and time–kill curves were plotted as \log_{10} CFU/mL over time. The limit of detection was 1.3 \log_{10} CFU/mL (or 2×10^1 CFU/mL). Antibiotic carryover was controlled by dilution. All experiments were performed at least in duplicate with results presented as the mean of all experiments. In each experiment, one flask containing inoculated CA-MHB without an antimicrobial agent served as a growth control.

A bactericidal effect was defined as a $\geq 3 \log_{10}$ CFU/mL decrease in the viable count both at 6 h and 24 h compared with the starting inoculum. Synergism was defined as a decrease in viable count of $\geq 2 \log_{10}$ CFU/mL with the combination compared with the most active single drug after 6 h and 24 h. Antagonism was defined as an increase in viable counts of $\geq 2 \log_{10}$ CFU/mL with the combination compared with the most active single drug.

3. Results

3.1. Minimum inhibitory concentrations

MICs of the study drugs for the five clinical *P. aeruginosa* isolates and the *P. aeruginosa* reference strain ATCC 27853 are shown in Table 1. None of the organisms were resistant to either amikacin or levofloxacin based on CLSI interpretive criteria [13], but two isolates (PEG-02-33 and P-03-29) demonstrated intermediate susceptibility to levofloxacin. MICs for ceftobiprole ranged from 2 mg/L to 8 mg/L.

3.2. Time–kill studies

The findings of time–kill studies for each of the six *P. aeruginosa* strains during exposure to single-agent ceftobiprole 4 mg/L or to amikacin or levofloxacin (0.5×, 1× and 2× MIC) are summarised in Table 2i. At low inoculum with ceftobiprole at 4 mg/L, viable *P. aeruginosa* counts were decreased by 1.5–2 log₁₀ at 6 h, with an increase in viable counts relative to the initial inoculum subsequently observed for all six strains at 24 h. With high inoculum, ceftobiprole at 4 mg/L exhibited bacteriostatic activity at 6 h, but re-growth was seen with all strains at 24 h.

Amikacin and levofloxacin both exhibited a concentration-dependent effect when tested as single agents at concentrations of 0.5×, 1× and 2× MIC, as expected. At 2× MIC, bactericidal activity was nearly always achieved at 6 h. Thereafter, amikacin allowed for re-growth of the three strains evaluated, whereas levofloxacin maintained its activity in three of five *P. aeruginosa* strains.

When ceftobiprole was combined with amikacin, synergism was noted in all three strains at 24 h at low inoculum (Table 2ii). Synergism was observed for ceftobiprole at 4 mg/L combined with amikacin at $0.5\times$ MIC, whilst bactericidal synergy was noted when ceftobiprole was combined with amikacin at concentrations $\geq 1\times$ MIC. At high inoculum, antimicrobial activity was at least bacteriostatic for the combination of ceftobiprole 4 mg/L and amikacin at concentrations $\geq 1\times$ MIC. Antagonism was not observed for any of the strains.

When ceftobiprole was combined with levofloxacin, synergistic activity was observed at low inoculum in all five *P. aeruginosa* strains at 24 h (Table 2iii). Against four strains with ceftobiprole MICs of 2–4 mg/L, the combination of ceftobiprole 4 mg/L and levofloxacin at $0.5\times$ MIC exhibited bactericidal synergism. Against the fifth strain (P-03-29) for which MICs for ceftobiprole and levofloxacin were 8 mg/L and 4 mg/L, respectively, bactericidal synergism occurred for ceftobiprole 4 mg/L in combination with levofloxacin $\geq 1\times$ MIC. At high inoculum, addition of levofloxacin at $0.5\times$, $1\times$ and $2\times$ MIC to ceftobiprole at 4 mg/L resulted in bactericidal synergism for strain ATCC 27853 (ceftobiprole MIC = 2 mg/L), PEG-02-33 (ceftobiprole MIC = 2 mg/L) and P-04-03 (ceftobiprole MIC = 4 mg/L), respectively. Antagonism was not observed in any of the strains.

Fig. 1 shows representative time–kill curves for ceftobiprole alone and in combination with amikacin or levofloxacin against *P. aeruginosa* ATCC 27853, PEG-02-54 for which the amikacin MIC was 8 mg/L, and P-03-29 for which the levofloxacin MIC was 4 mg/L.

4. Discussion

Ceftobiprole is a first-in-class anti-MRSA broad-spectrum cephalosporin with activity against other prominent Gram-positive and Gram-negative pathogens, including *P. aeruginosa*. The activity of ceftobiprole against *P. aeruginosa* has been attributed to its affinity for essential targets, namely penicillin-binding proteins 1a and 3 [14]. In this in vitro study, it was demonstrated for the first time that combinations of ceftobiprole at a clinically achievable concentration of 4 mg/L with amikacin or levofloxacin exhibited synergistic activity against *P. aeruginosa*. At low inoculum, the combination of ceftobiprole and either amikacin or levofloxacin at 0.5× MIC demonstrated synergism against each of the *P. aeruginosa* strains tested, with evidence of bactericidal synergism at amikacin concentrations of ≥1× MIC and at levofloxacin concentrations of ≥0.5× MIC. At high inoculum, the combination of ceftobiprole and levofloxacin resulted in bactericidal synergism against three of the five strains evaluated, whereas the activity of the combination of ceftobiprole and amikacin demonstrated bactericidal synergism against one of the three strains tested. Antagonism was not observed with either combination. Consistent with other in vitro studies investigating antimicrobial synergism [1,11,12], time–kill methodology was employed rather than disk diffusion or checkerboard titration methods. Time–kill assays have previously been shown to discriminate synergistic activity better than other in vitro methods [15].

The reason for re-growth of the test organisms observed for the low inoculum at concentrations of ≥1× MIC in the experiments performed with the single agents is unknown. Most probably, MICs of the strains would be one dilution higher if the MICs were determined in a larger volume of growth medium (i.e. in glass flasks containing

20 mL). However, it is also possible that it was due to selection of resistant mutants or, in the case of ceftobiprole, due to degradation of the drug in the growth medium.

In vitro antimicrobial synergism has previously been reported against *P. aeruginosa* [1,9–11]. Fish et al. [11] reported synergistic activity for various fluoroquinolones against 10 clinical isolates of *P. aeruginosa* when tested in combination with ceftazidime or cefepime. Synergy was observed particularly when the bacterial strains were resistant to one or both agents in the combination, whereas no synergy was observed in two strains susceptible to all drugs. In both inhibitory and bactericidal testing, Meyer and Liu [9] observed that the combination of ciprofloxacin with a β -lactam was more likely to show a synergistic effect and lack of antagonism against multidrug-resistant *P. aeruginosa* than a combination of ciprofloxacin and an aminoglycoside.

As a single agent, ceftobiprole has been compared favourably in vitro with ceftazidime and cefepime, which are third- and fourth-generation cephalosporins, respectively, with activity against *P. aeruginosa* [5,16]. As such, a ceftobiprole-resistant isolate is most likely also to have reduced susceptibility to ceftazidime and cefepime, but the relative incidence of such isolates is unknown. Baum et al. [17] demonstrated differential efflux effects among the cephalosporins. Ceftobiprole has a similar activity profile to cefepime in that it is affected by increased expression of MexXY by resistant pseudomonal isolates.

Among 419 clinical isolates of *P. aeruginosa* collected as part of the Canadian Intensive Care Unit study, the MIC distributions for ceftobiprole and cefepime were

similar, irrespective of specimen source [5]. In the SENTRY Antimicrobial Surveillance Program (2005–2006), which included a large number of isolates from North America, Latin America and Europe, ceftobiprole was equal in potency to ceftazidime against 2239 *P. aeruginosa* isolates and two-fold more potent than cefepime [16]. Furthermore, ceftobiprole was broadly active against *Streptococcus pneumoniae*, β -haemolytic and viridans group streptococci, and *E. faecalis*, and was similar in potency to third- and fourth-generation cephalosporins for all tested Enterobacteriaceae. Unlike cefepime and ceftazidime, in addition to meticillin-susceptible strains ceftobiprole had potent activity against MRSA and meticillin-resistant coagulase-negative staphylococci, with MIC₅₀ and MIC₉₀ values (MICs for 50% and 90% of the organisms, respectively) ranging from 0.25–1 mg/L and 0.5–2 mg/L, respectively [16].

Among 52 *P. aeruginosa* isolates identified at baseline in two phase III trials comparing ceftobiprole with vancomycin alone or vancomycin plus ceftazidime in 1271 patients with cSSSI, ceftobiprole had an MIC₉₀ of >64 mg/L and a modal MIC of 2 mg/L [2]. Moreover, 26 (86.7%) of 30 patients in whom *P. aeruginosa* was isolated and who were treated with ceftobiprole compared with 9 (100%) of 9 patients treated with vancomycin plus ceftazidime in one of these phase III studies achieved a clinical cure (difference –13.3%; 95% confidence interval –30.2% to 18.5%) [7]. Thus, ceftobiprole was shown to achieve equivalent clinical efficacy to ceftazidime, which in the same study had an MIC₉₀ of >128 mg/L and an equivalent modal MIC [2,7].

Pseudomonas aeruginosa was isolated as the sole pathogen at baseline in 12 of 30 patients assigned to ceftobiprole. Of these 12 patients, 9 (75%) had a clinical cure, including 6 patients with isolates having MICs \leq 4 mg/L. All three patients who did not

achieve cure had isolates with MICs ≥ 8 mg/L [7]. It is possible that these three patients would have benefited from combination therapy with ceftobiprole and either a fluoroquinolone or an aminoglycoside.

The continued emergence of antimicrobial resistance in commonly occurring pathogens highlights the need for novel broad-spectrum agents able to be used successfully as empirical therapy [16]. In serious *P. aeruginosa* infections, the pathogen often exhibits high-level resistance to many antimicrobials or develops resistance during therapy. In these cases, initial use of combination therapy is recommended as it significantly reduces the likelihood of inappropriate therapy compared with monotherapy [3,4,11]. In this regard, ceftobiprole offers a number of advantages in terms of potency, spectrum and β -lactamase stability compared with current extended-spectrum β -lactams. Its unique antibacterial profile may not only make it suitable for empirical use in suspected *P. aeruginosa* and MRSA infections, but its use as monotherapy may also be possible for patients where combination therapy would be problematic [16]. Furthermore, the findings of the present in vitro study lend support for its use in combination with either an aminoglycoside or a fluoroquinolone based on evidence of synergism and absence of antagonism.

In conclusion, in an era in which MRSA and now *P. aeruginosa* are increasingly implicated in cSSSIs in both the hospital and community settings, the ability to provide initial empirical therapy for severely ill patients with a broad-spectrum agent that offers potent activity against both organisms will determine the standard of care. The current findings show that at clinically achievable concentrations, combinations of ceftobiprole and either amikacin or levofloxacin were synergistic against *P.*

aeruginosa. This potentially increased activity of ceftobiprole in combination with other antipseudomonal drugs warrants further investigation in vivo.

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Competing interests

MK is a partner and CEO of Antiinfectives Intelligence GmbH, a research organisation providing services to pharmaceutical companies. BK-I is an employee of Antiinfectives Intelligence GmbH. JML is an employee of Johnson & Johnson and owns stock in that company. TD is an employee of Johnson & Johnson Pharmaceutical Research & Development and owns stock in Johnson & Johnson. SD-B is an employee of Janssen-Cilag, Germany.

Ethical approval

Not required.

References

- [1] Sakharkar MK, Jayaraman P, Soe WM, Chow VT, Sing LC, Sakharkar KR. In vitro combinations of antibiotics and phytochemicals against *Pseudomonas aeruginosa*. J Microbiol Immunol Infect 2009;42:364–70.
- [2] Amsler KM, Davies TA, Shang W, Jacobs MR, Bush K. In vitro activity of ceftobiprole against pathogens from two phase 3 complicated skin and skin structure infection clinical trials. Antimicrob Agents Chemother 2008;52:3418–23.
- [3] American Thoracic Society; Infectious Diseases Society of America. Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. Am J Respir Crit Care Med 2005;171:388–416.
- [4] Garnacho-Montero J, Sa-Borges M, Sole-Violan J, Barcenilla F, Escosca-Ortega A, Ochoa M, et al. Optimal management therapy for *Pseudomonas aeruginosa* ventilator-associated pneumonia: an observational, multicenter study comparing monotherapy with combination antibiotic therapy. Crit Care Med 2007;35:1888–95.
- [5] Walkty A, Decorby M, Nichol K, Karlowsky JA, Hoban DJ, Zhanel GG. In vitro activity of ceftobiprole against clinical isolates of *Pseudomonas aeruginosa* obtained from Canadian Intensive Care Unit (ICU) patients as part of the CAN-ICU Study. J Antimicrob Chemother 2008;62:206–8.
- [6] Schirmer PL, Deresinski SC. Ceftobiprole: a new cephalosporin for the treatment of skin and skin structure infections. Expert Rev Anti Infect Ther 2009;7:777–91.
- [7] Noel GJ, Bush K, Bagchi P, Ianus J, Strauss RS. A randomized, double-blind trial comparing ceftobiprole medocaril with vancomycin plus ceftazidime for the

- treatment of patients with complicated skin and skin-structure infections. *Clin Infect Dis* 2008;46:647–55.
- [8] Noel GJ, Strauss RS, Amsler K, Heep M, Pypstra R, Solomkin JS. Results of a double-blind, randomized trial of ceftobiprole treatment of complicated skin and skin structure infections caused by Gram-positive bacteria. *Antimicrob Agents Chemother* 2008;52:37–44.
- [9] Meyer RD, Liu S. In vitro synergy studies with ciprofloxacin and selected β -lactam agents and aminoglycosides against multidrug-resistant *Pseudomonas aeruginosa*. *Diagn Microbiol Infect Dis* 1988;11:151–7.
- [10] Isenberg HD, Alperstein P, France K. In vitro activity of ciprofloxacin, levofloxacin, and trovafloxacin, alone and in combination with β -lactams, against clinical isolates of *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, and *Burkholderia cepacia*. *Diagn Microbiol Infect Dis* 1999;33:81–6.
- [11] Fish DN, Choi MK, Jung R. Synergic activity of cephalosporins plus fluoroquinolones against *Pseudomonas aeruginosa* with resistance to one or both drugs. *J Antimicrob Chemother* 2002;50:1045–9.
- [12] Arias CA, Singh KV, Panesso D, Murray BE. Time–kill and synergism studies of ceftobiprole against *Enterococcus faecalis*, including β -lactamase-producing and vancomycin-resistant isolates. *Antimicrob Agents Chemother* 2007;51:2043–7.
- [13] Clinical and Laboratory Standards Institute. *Performance standards for antimicrobial susceptibility testing; 18th informational supplement*. Document M100-S18. Wayne, PA: CLSI; 2008.
- [14] Davies TA, Page MG, Shang W, Andrew T, Kania M, Bush K. Binding of ceftobiprole and comparators to the penicillin-binding proteins of *Escherichia coli*,

Pseudomonas aeruginosa, *Staphylococcus aureus* and *Streptococcus pneumoniae*. *Antimicrob Agents Chemother* 2007;51:2621–4.

- [15] Cappelletty DM, Rybak MJ. Comparison of methodologies for synergism testing of drug combinations against resistant strains of *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 1996;40:677–83.
- [16] Fritsche TR, Sader HS, Jones RN. Antimicrobial activity of ceftobiprole, a novel anti-methicillin-resistant *Staphylococcus aureus* cephalosporin, tested against contemporary pathogens: results from the SENTRY Antimicrobial Surveillance Program (2005–2006). *Diagn Microbiol Infect Dis* 2008;61:86–95.
- [17] Baum EZ, Crespo-Carbone SM, Morrow BJ, Davies TA, Foleno BD, He W, et al. Effect of MexXY overexpression on ceftobiprole susceptibility in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2009;53:2785–90.

Fig. 1. In vitro time–kill against (A,B) *Pseudomonas aeruginosa* ATCC 27853, (C) PEG-02-54 and (D) PEG-03-29 strains at low inoculum for ceftobiprole (BPR) 4 mg/L alone as well as BPR 4 mg/L plus either amikacin (AMK) (A,C) or levofloxacin (LVX) (B,D) at 0.5×, 1× and 2× the minimum inhibitory concentration (MIC). CFU, colony-forming units.

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Table 1

Minimum inhibitory concentrations (MICs) of ceftobiprole, amikacin and levofloxacin against *Pseudomonas aeruginosa* test strains

<i>P. aeruginosa</i> strain	MIC (mg/L)		
	Ceftobiprole	Amikacin ^a	Levofloxacin ^b
ATCC 27853	2	4 (S)	2 (S)
PEG-01-14	4	4 (S)	0.5 (S)
PEG-02-33	2	N/D	4 (I)
PEG-02-54	2	8 (S)	N/D
P-03-29	8	N/D	4 (I)
P-04-03	4	N/D	1 (S)

S, susceptible; I, intermediate; N/D, not determined; CLSI, Clinical and Laboratory Standards Institute.

^a Interpretive criteria based on CLSI recommendations: S, ≤ 16 mg/L; I, 32 mg/L; and R, ≥ 64 mg/L.

^b Interpretive criteria based on CLSI recommendations: S, ≤ 2 mg/L; I, 4 mg/L; and R, ≥ 8 mg/L.

Table 2Change in viable counts (\log_{10} CFU/mL) of *Pseudomonas aeruginosa* strains

<i>P. aeruginosa</i> strain	Drug	Drug concentration (\times MIC)	\log_{10} difference in CFU/mL from inoculum				
			Low inoculum ^a		High inoculum ^b		
			6 h	24 h	6 h	24 h	
(i) After exposure to ceftobiprole, amikacin or levofloxacin for 6 h and 24 h							
ATCC 27853	Ceftobiprole	4 mg/L (2 \times)	-1.55	0.68	-0.87	1.47	
		Amikacin	2 mg/L (0.5 \times)	-1.42	3.84	0.13	1.75
		4 mg/L (1 \times)	-3.06	3.56	-1.90	1.54	
		8 mg/L (2 \times)	-4.10a	1.27	-2.41	1.34	
	Levofloxacin	1 mg/L (0.5 \times)	-1.82	2.12	-2.97	0.50	
		2 mg/L (1 \times)	-3.48	1.30	-4.10	-0.71	
4 mg/L (2 \times)		-4.21^c	-4.21^c	-6.36^c	-3.05		
PEG-01-14	Ceftobiprole	4 mg/L (1 \times)	-1.79	3.61	0.33	1.72	
		Amikacin	2 mg/L (0.5 \times)	-0.23	3.80	-0.72	1.79
		4 mg/L (1 \times)	-2.63	2.35	-3.01	0.29	
		8 mg/L (2 \times)	-4.27	0.56	-3.72	-0.86	
	Levofloxacin	0.25 mg/L (0.5 \times)	-1.88	2.45	-1.46	-0.19	
		0.5 mg/L (1 \times)	-3.46	1.58	-3.41	0.35	
1 mg/L (2 \times)		-4.31^c	-2.32	-4.26	-0.62		

PEG-02-33	Ceftobiprole	4 mg/L (2×)	-1.95	0.57	-0.49	1.78
	Levofloxacin	2 mg/L (0.5×)	-0.04	0.87	-0.72	0.36
		4 mg/L (1×)	-2.41	0.79	-3.16	-1.97
		8 mg/L (2×)	-4.19^c	-4.19^c	-5.78	-5.79
PEG-02-54	Ceftobiprole	4 mg/L (2×)	-2.08	1.77	-0.77	1.06
	Amikacin	4 mg/L (0.5×)	-1.24	3.13	-1.95	1.33
		8 mg/L (1×)	-3.53	3.08	-3.50	1.25
		16 mg/L (2×)	-4.56^c	1.21	-4.75	1.56
P-03-29	Ceftobiprole	4 mg/L (0.5×)	-1.66	3.49	0.03	1.49
	Levofloxacin	2 mg/L (0.5)	0.79	2.21	-1.03	0.63
		4 mg/L (1)	-1.39	-0.49	-2.32	-2.53
		8 mg/L (2×)	-2.84	-4.28^c	-4.00	-4.93
P-04-03	Ceftobiprole	4 mg/L (1×)	-1.47	3.09	0.16	1.68
	Levofloxacin	0.5 mg/L (0.5×)	-1.40	2.86	-2.24	0.25
		1 mg/L (1×)	-3.89	2.28	-3.98	0.66
		2 mg/L (2×)	-4.32^c	-4.32^c	-5.05	0.46
(ii) After exposure to ceftobiprole 4 mg/L plus amikacin (0.5×, 1× or 2× MIC) for 6 h and 24 h						
<i>P. aeruginosa</i> strain	Amikacin (×MIC)	Log ₁₀ difference in CFU/mL from inoculum				
		Low inoculum		High inoculum		
		6 h	24 h	6 h	24 h	

ATCC 27853	0.5x	-3.06	-2.11 ^d	-2.29	0.75
	1x	-3.55	-3.27 ^d	-2.52	0.03
	2x	-4.10 ^a	-4.10 ^{c,d}	-2.85	-0.89
PEG-01-14	0.5x	-3.10	-2.00 ^d	-2.85 ^d	0.20
	1x	-3.43	-3.95 ^d	-3.28	-1.29
	2x	-4.27 ^a	-4.27 ^{c,d}	-4.16	-4.11 ^d
PEG-02-54	0.5x	-4.02	-2.17 ^d	-3.66	1.10
	1x	-4.56 ^a	-4.56 ^c	-4.30	0.01
	2x	-4.56 ^a	-4.56 ^c	-5.41	-1.76

(iii) After exposure to ceftobiprole 4 mg/L plus levofloxacin (0.5x, 1x or 2x MIC) for 6 h and 24 h

<i>P. aeruginosa</i> strain	Levofloxacin (xMIC)	Log ₁₀ difference in CFU/mL from inoculum			
		Low inoculum		High inoculum	
		6 h	24 h	6 h	24 h
ATCC 27853	0.5x	-2.14	-4.21 ^{c,d}	-3.42	-3.34 ^d
	1x	-3.24	-4.12 ^d	-4.45	-6.21 ^d
	2x	-4.10 ^a	-4.21 ^c	-6.36 ^c	-6.36 ^{c,d}
PEG-01-14	0.5x	-3.04	-3.42 ^d	-1.61	-0.32
	1x	-3.96	-4.31 ^a	-3.46	-0.08
	2x	-4.31 ^a	-4.31 ^a	-4.51	-2.21
PEG-02-33	0.5x	-3.61	-4.19 ^{a,b}	-1.80	-2.32

	1×	-3.84	-4.19 ^{a,b}	-4.76	-5.98 ^{a,b}
	2×	-4.19 ^a	-4.19 ^a	-5.98 ^a	-5.98 ^a
P-03-29	0.5×	-2.87	-2.32 ^b	-1.13	0.54
	1×	-2.42	-4.28 ^{a,b}	-2.18	-2.03
	2×	-3.02	-4.28 ^a	-4.22	-5.67
P-04-03	0.5×	-3.37	-3.33 ^b	-3.81	0.79
	1×	-3.97	-4.32 ^{a,b}	-4.68	-0.94
	2×	-4.32 ^a	-4.32 ^a	-5.26	-6.35 ^{a,b}

CFU, colony-forming units.

^a Approximately 5×10^5 CFU/mL.

^b Approximately 5×10^7 CFU/mL.

^c Below the limit of detection.

^d Synergy achieved at 24 h.

Numbers in bold indicate a bactericidal effect.

Fig. 1.

(A)

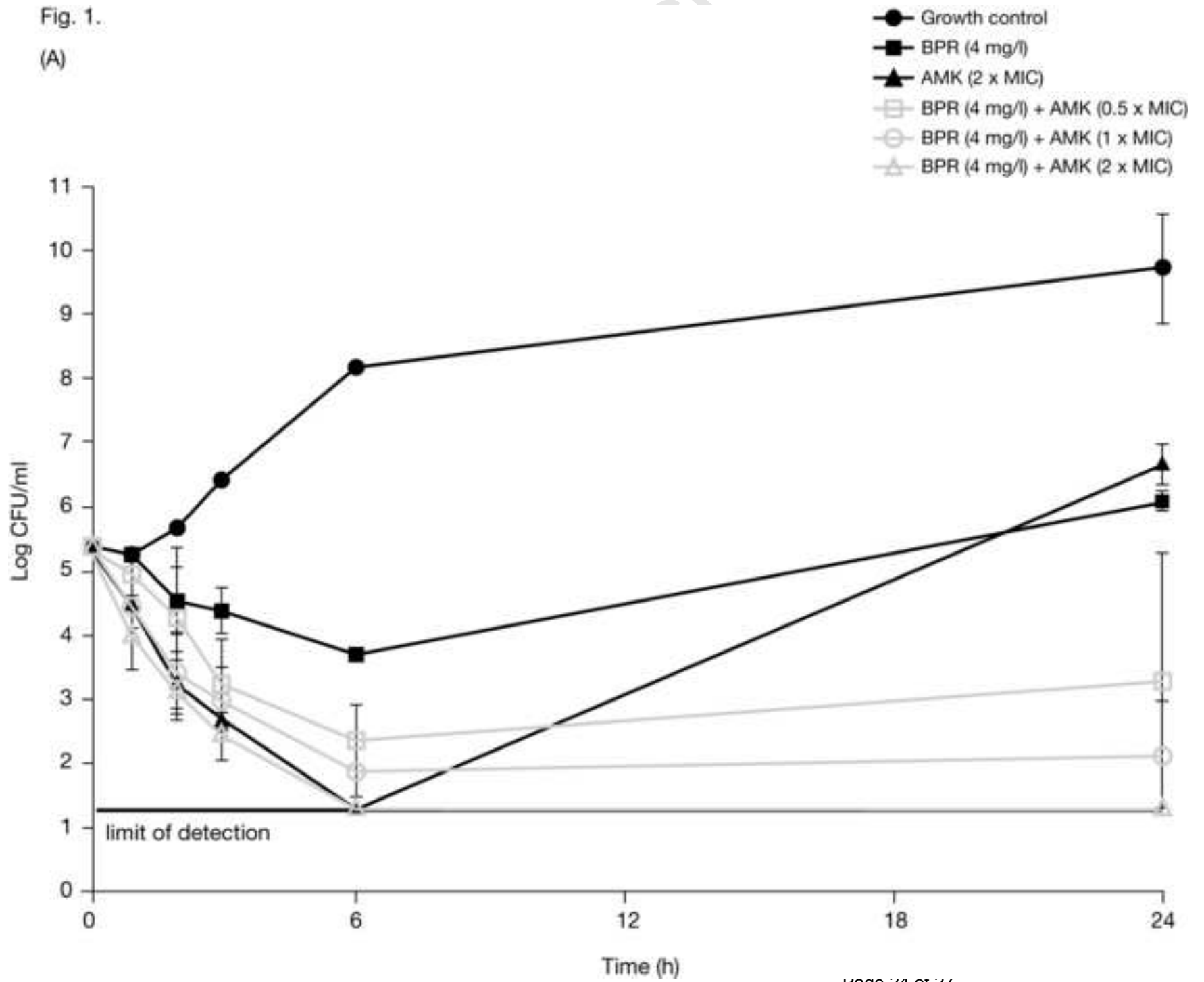


Fig. 1b.

(B)

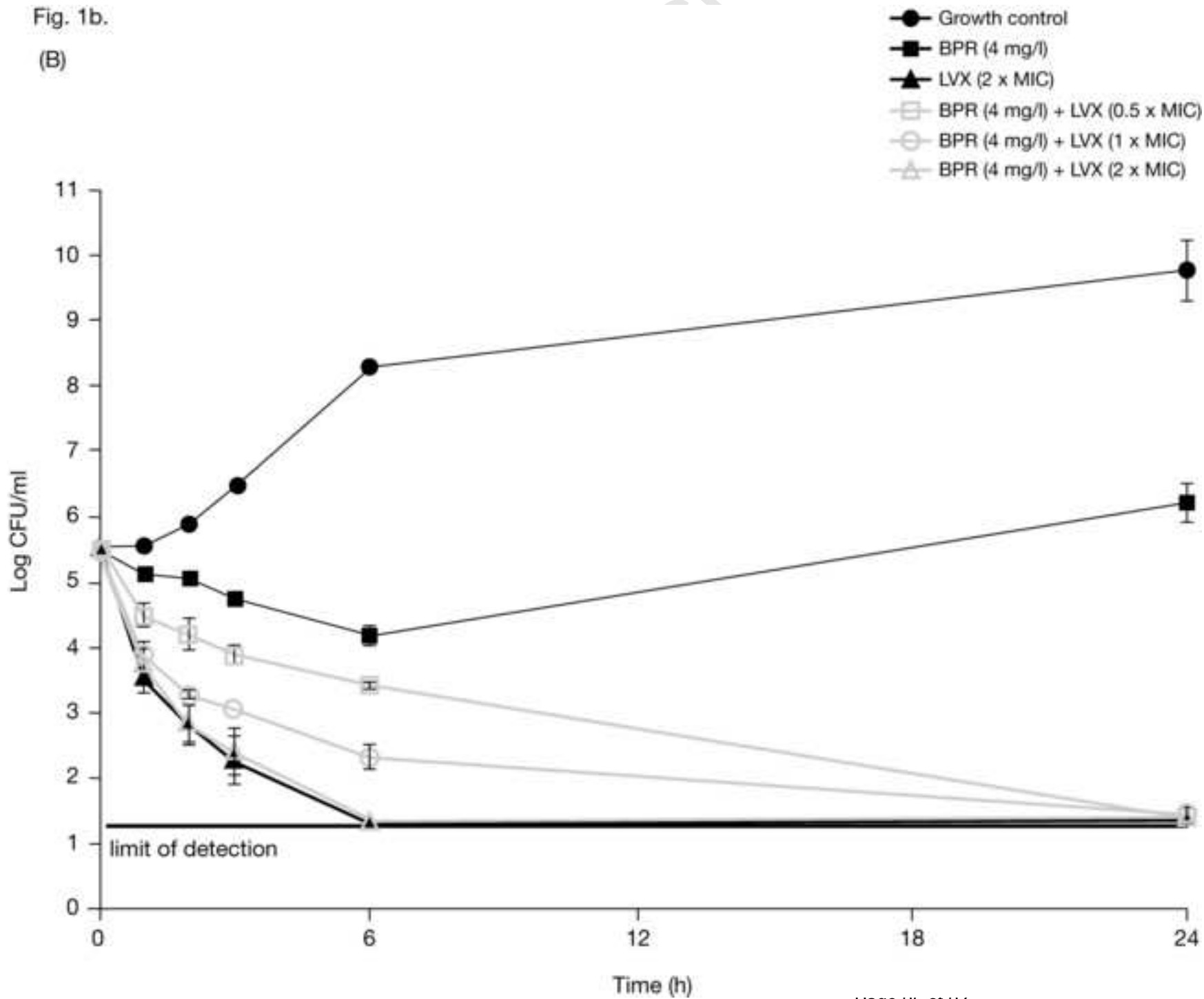


Fig. 1c

(C)

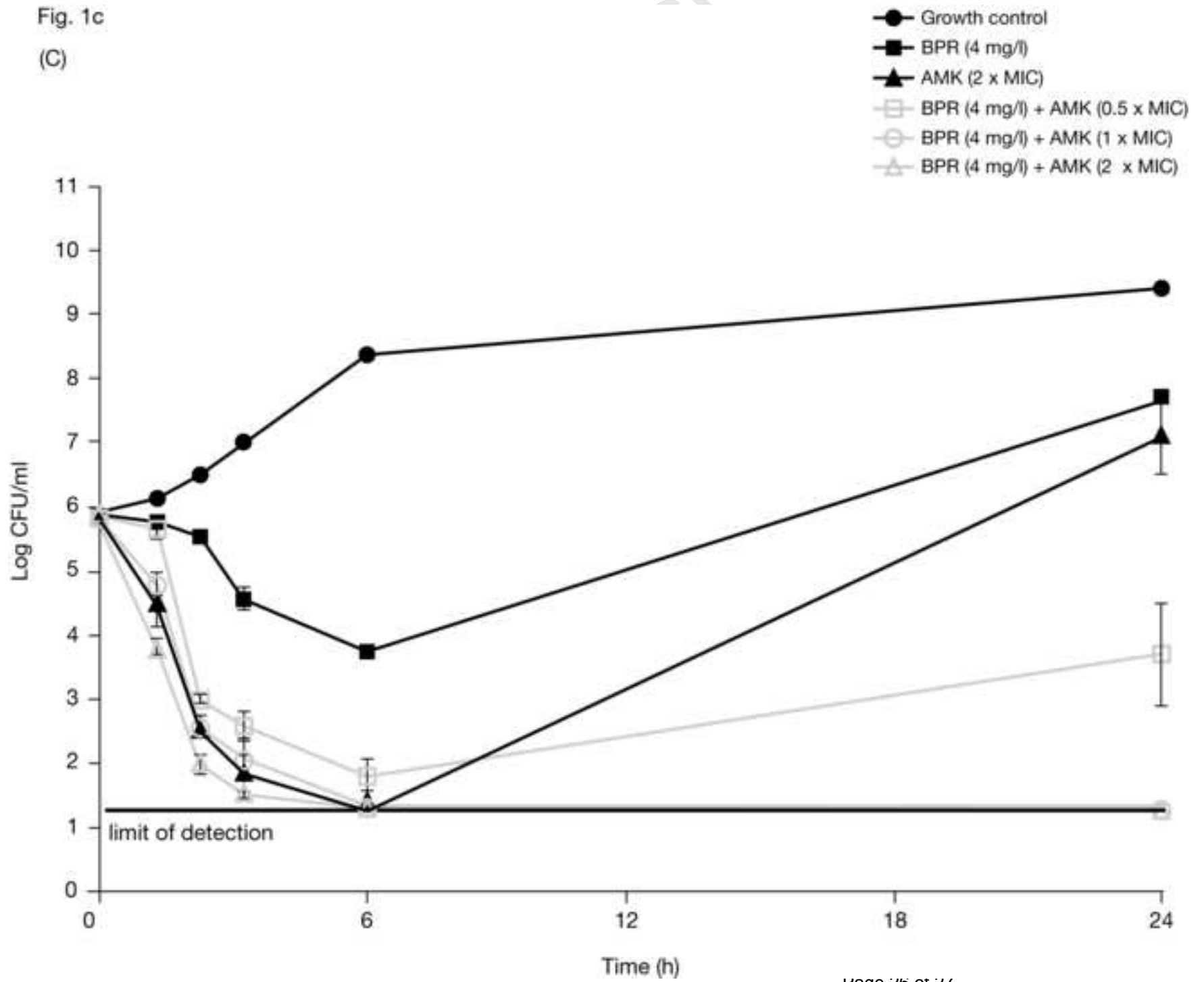


Fig. 1d

(D)

