Published in final edited form as:

Int J Antimicrob Agents. 2016 September ; 48(3): 331–336. doi:10.1016/j.ijantimicag.2016.06.006.

Combinatorial pharmacodynamics of polymyxin B and tigecycline against heteroresistant *Acinetobacter baumannii*

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Abstract

The prevalence of heteroresistant *Acinetobacter baumannii* is increasing. Infections due to these resistant pathogens pose a global treatment challenge. Here, the pharmacodynamic activities of polymyxin B (PMB) (2–20 mg/L) and tigecycline (0.15–4 mg/L) were evaluated as monotherapy and in combination using a 4 × 4 concentration array against two carbapenem-resistant and polymyxin-heteroresistant *A. baumannii* isolates. Time Kill Experiments was employed at starting inocula of 10^6 and 10^8 CFU/mL over 48 h. Clinically relevant combinations of PMB (2 mg/L) and tigecycline (0.90 mg/L) resulted in greater reductions in the bacterial population compared with polymyxin alone by 8 h (ATCC 19606, −6.38 vs. −3.43 $\log^{10}$ CFU/mL; FADDI AB115, −1.38 vs. 2.08 $\log_{10}$ CFU/mL). At 10× the clinically achievable concentration (PMB 20 mg/L in combination with tigecycline 0.90 mg/L), there was bactericidal activity against FADDI AB115 by 4 h that was sustained until 32 h, and against ATCC 19606 that was sustained for 48 h. These studies show that aggressive polymyxin-based dosing in combination with clinically achievable tigecycline concentrations results in early synergistic activity that is not sustained beyond 8 h,

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Competing interests: None declared.

Ethical approval: Not required

whereas combinations with higher tigecycline concentrations result in sustained bactericidal activity against both isolates at both inocula. These results indicate a need for optimised front-loaded polymyxin-based combination regimens that utilise high polymyxin doses at the onset of treatment to achieve good pharmacodynamic activity whilst minimising adverse events.

Keywords

Polymyxins; Polymyxin B; Tigecycline; Pharmacodynamics; Combinations; *Acinetobacter baumannii*

1. Introduction

The intrinsic impermeability of the outer membrane of *Acinetobacter baumannii* has allowed it to acquire highly effective resistance determinants, making it a reservoir of resistance genes. The emergence of multidrug-resistant (MDR) *A. baumannii* strains resistant to nearly all commercially available antibiotics has compromised treatment options against these pathogens. Owing to their nephrotoxicity, old antibiotics such as polymyxins have been abandoned in favour of newer agents such as tigecycline. However, both of these antibiotics are attracting attention as two of the remaining effective antibiotics against MDR *A. baumannii*.

Polymyxin B (PMB) is a polypeptide polymyxin that has not undergone the rigour of modern drug development but is increasingly being utilised as a drug of last resort. In contrast, tigecycline is a newer broad-spectrum glycylcycline antibiotic that can evade tetracycline efflux pumps (TetA–E) in MDR *A. baumannii* [1]. The emergence of polymyxin heteroresistance has been reported among *A. baumannii* strains and is associated with clinical failure. Amplification of existing resistant subpopulations among heteroresistant *A. baumannii* in response to polymyxin monotherapy may be the main driver responsible for promoting resistance, especially when there is high bacterial density. The substantial increase in resistant subpopulations observed upon exposure to polymyxin suggests that sufficiently high drug pressure is required at the start of therapy to eradicate these resistant subpopulations. However, PMB monotherapy is not usually a clinical option, since dose escalation to achieve sufficiently high concentrations with currently recommended dosing protocols risks rapid-onset nephrotoxicity [2]. Furthermore, tigecycline is not recommended for severe difficult-to-treat MDR *A. baumannii* infections, despite their susceptibility to tigecycline, owing to the reported clinical failures, poor outcomes and disappointing bacteriostatic activity [3].

Consequently, clinicians faced with limited treatment options are resorting to combination therapy to treat patients infected with MDR *A. baumannii*. Optimisation and validation of polymyxin-based combinations would therefore be of considerable clinical benefit. The pharmacodynamics of combination antimicrobial therapy with PMB and tigecycline is poorly defined, especially in situations of high bacterial density and pre-existing polymyxin heteroresistance. The objective of the current study was to systemically investigate the in vitro pharmacodynamics of PMB and tigecycline at clinically achievable and higher

concentrations against polymyxin-heteroresistant *A. baumannii* isolates and to quantify their activity profiles for possible clinical translation.

2. Materials and methods

Two carbapenem-resistant and polymyxin-heteroresistant *A. baumannii* isolates were used: a PMB-susceptible (PB^S) reference strain ATCC 19606 [minimum inhibitory concentrations (MICs): PMB, 0.5 mg/L; tigecycline, 2.0 mg/L; and meropenem, 16 mg/L] and a PMB-resistant (PB^R) clinical isolate FADDI AB115 (MICs: PMB, >8.0 mg/L; tigecycline, 2.0 mg/L; and meropenem 16 mg/L). The MICs for tigecycline and PMB were determined in quadruplicate by broth dilution [4]. Colistin heteroresistance was defined as the presence of bacterial subpopulations able to grow on agar containing >2.0 mg/L colistin when the MIC was ≤2.0 mg/L [5]. PMB (Sigma-Aldrich, St Louis, MO) and tigecycline (Pfizer Inc., New York, NY) were dissolved in sterile water and saline, respectively, immediately prior to each experiment, and fresh cation-adjusted Mueller–Hinton broth (Ca^{2+} at 25.0 mg/L and Mg^{2+} at 12.5 mg/L; Difco, Detroit, MI) prepared prior to each experiment was used for all in vitro experiments and for susceptibility testing. Static time–kill experiments were conducted to characterise the pharmacodynamics of PMB and tigecycline alone and in combination [6]. A 4 × 4 concentration array of PMB (2, 8, 16 and 20 mg/L) and tigecycline (0.15, 0.90, 2 and 4 mg/L) [7] against low inocula (10^6 CFU/mL) and high inocula (10^8 CFU/mL) was evaluated. Serial cultures were obtained over 48 h. Bacterial counts were determined based on quantitative cultures on Mueller–Hinton agar plates after 24 h of incubation at 37 °C.

The pharmacodynamic analysis was performed by evaluating microbiological responses to monotherapy and combination therapy by determining the $\log_{10}$ change, calculating the change in bacterial density at 4, 8, 24 and 48 h from baseline (0 h) to characterise early and late pharmacodynamic activities for monotherapy and combination therapy. Bactericidal activity was defined as a ≥3 $\log_{10}$ CFU/mL reduction. Regimens were categorised based on the reduction in the initial bacterial inoculum, where activity was defined as at least 1 $\log_{10}$ CFU/mL reduction compared with the initial inoculum. Additivity and synergy were defined as 1.0 to <2 $\log_{10}$ CFU/mL and ≥2 $\log_{10}$ CFU/mL reduction by the PMB + tigecycline combination compared with the most active single agent in the combination, respectively [8].

3. Results

The results of time–kill studies for PMB and tigecycline alone and in combination against low and high inocula of both isolates are summarised in Figs 1 and 2. At the lower inoculum of 10^6 CFU/mL, PMB monotherapy achieved early bactericidal activity, and sustained activity was seen with the higher PMB concentrations of 16 mg/L and 20 mg/L against the PB^S isolate [Fig. 1(A1)]. Tigecycline monotherapy at 0.15 mg/L [Fig. 1(A2)] and 0.90 mg/L [Fig. 1(A3)] performed no different from the growth control against the PB^S isolate. Monotherapy at the higher tigecycline concentrations of 2 mg/L [Fig. 1(A4)] and 4 mg/L [Fig. 1(A5)] resulted in >1 $\log_{10}$ reduction by 8 h followed by re-growth. All tigecycline concentrations in combination with the clinically achievable PMB concentration of 2 mg/L resulted in early bactericidal activity followed by re-growth beyond 8 h, whereas in

combination with the higher PMB concentrations of 16 mg/L and 20 mg/L, it resulted in sustained bactericidal activity against the PB^S isolate [Fig. 1(A2–A5); Table 1].

Against the lower inoculum of the PB^R isolate, monotherapy with tigecycline and PMB was no different from the growth control [Fig. 1(B1–B5); Table 1]. The clinically achievable tigecycline concentration of 0.15 mg/L in combination with all PMB concentrations did not result in appreciable sustained activity [Fig. 1(B2); Table 1]. Comparatively, the higher tigecycline concentration of 0.90 mg/L in combination with PMB concentrations of 2 mg/L and 8 mg/L resulted in early >1 $\log_{10}$ reduction of the PB^R inoculum up to 8 h, whilst combination with higher PMB concentrations of 16 mg/L and 20 mg/L resulted in sustained bactericidal activity up to 24 h [Fig. 1(B3); Table 1]. Tigecycline 2 mg/L and 4 mg/L in combination with PMB 8, 16 and 20 mg/L demonstrated synergy and resulted in sustained bactericidal activity up to 48 h, whilst combination with PMB 2 mg/L showed a lack of activity beyond 8 h against the PB^R isolate [Fig. 1(B4 and B5); Table 1].

Monotherapy with either PMB or tigecycline against the higher inoculum of the PB^S isolate demonstrated a lack of activity at 48 h with the exception of tigecycline 4 mg/L resulting in a 1.30 $\log_{10}$ reduction [Fig. 2(A1–A5); Table 1]. Similarly, no activity was seen with clinically achievable concentrations of tigecycline 0.15 mg/L and 0.90 mg/L in combination with PMB 2 mg/L. Tigecycline 0.15 mg/L in combination with PMB 20 mg/L and tigecycline 0.90 mg/L in combination with PMB 16 mg/L and 20 mg/L resulted in >3 $\log_{10}$ reduction beyond 24 h [Fig. 2(A2 and A3); Table 1]. Tigecycline 2 mg/L and 4 mg/L in combination with PMB 2 mg/L resulted in >2 $\log_{10}$ reduction by 8 h, whereas combinations with PMB concentrations >2 mg/L were synergistic and resulted in sustained bactericidal activity against the PB^S isolate beyond 8 h [Fig. 2(A4 and A5); Table 1].

Similar to the high inoculum of the PB^S isolate, against the high inoculum of the PB^R isolate monotherapy with either PMB or tigecycline demonstrated attenuated killing [Fig. 2(B1–B5)]. Tigecycline 0.15 mg/L in combination with PMB was similar to monotherapy with respect to its killing activity [Fig. 2(B2); Table 1]. Tigecycline 0.90 mg/L in combination with PMB concentrations of 8, 16 and 20 mg/L resulted in an early reduction of >1 $\log_{10}$ by 8 h that was followed by re-growth [Fig. 2(B3); Table 1]. Tigecycline 2 mg/L and 4 mg/L in combination with PMB 2 mg/L resulted in >1 $\log_{10}$ reduction of the initial high inoculum of the PB^R isolate. These higher tigecycline concentrations in combination with PMB 8, 16 and 20 mg/L were synergistic, resulting in sustained killing activity of the PB^R isolate [Fig. 2(B4 and B5); Table 1].

4. Discussion

Treatment of infections caused by *A. baumannii* is a growing clinical problem due to its intrinsic ability to acquire antibiotic resistance genes via multiple mechanisms and to develop resistance [9]. A number of previous studies have explored polymyxin and tigecycline combinations, with outcomes ranging from indifference to synergy [10–12]. In the current study, at the lower inoculum, a clinically achievable PMB concentration of 2 mg/L combined with tigecycline 0.90 mg/L resulted in a 6.38 log10 reduction by 8 h, which was synergistic by 24 h against the PB^S reference strain, followed by re-growth by 48 h.

These observations from the current study are consistent with the findings of Yilmaz et al [12] who showed that colistin in combination with tigecycline against *A. baumannii* isolates in a time–kill study resulted in early bactericidal activity by 4 h at concentrations of 4× MIC that was sustained for 24 h (colistin MIC, 0.25 mg/L; tigecycline MIC, 1.0 mg/L). The authors attributed the attenuated in vivo activity to the lack of knowledge about colistin pharmacokinetics precluding accurate determination of the optimum colistin dose in rats. Furthermore, the 48 h endpoint does not accurately reflect the true clinical picture in patients, in which there is a time lag before observable colistin plasma concentrations are achieved. A loading dose of colistin would therefore be beneficial to reduce this time lag in practice [13,14].

Hagihara et al observed that PMB was responsible for the majority of the antibacterial effect against four carbapenem-resistant *A. baumannii* isolates tested with PMB (MIC of 1 mg/L) and tigecycline (MICs ranging from 1 mg/L to 4 mg/L) [10]. Similar to our observations, there was a lack of appreciable activity with tigecycline monotherapy. As the area under the concentration–time curve of the free (unbound) drug (*f*AUC)/MIC ratio was the pharmacodynamic parameter of interest, tigecycline dosing regimens of ≥200 mg daily were required to achieve *f*AUC/MIC exposures of 2.17 and 8.78 for 1 and 2 $\log_{10}$ reductions in bacterial density, respectively. This tigecycline regimen of 200 mg resulted in a mean change in bacterial density of 0.80 ± 0.59 $\log_{10}$ CFU/mL at 24 h with an initial inoculum of 10^6 CFU/mL. Simulated PMB monotherapy regimens of 1 mg/kg resulted in a mean change of −2.05 ± 0.68 $\log_{10}$ CFU/mL at 24 h with an initial inoculum of 10^6 CFU/mL. Combination therapy resulted in bactericidal activity with a reduction of 3.31 ± 0.71 $\log_{10}$ CFU/mL at 24 h [10].

In vitro models of infection may be useful for identifying potential synergy between different tigecycline and polymyxin concentrations, evaluated against the more susceptible isolates, at certain time points during the time course of the experiment. However, studies conducted over 24 h were unable to ascertain whether the suppression of resistant bacterial subpopulations is sustained beyond 24 h at the studied concentrations. In an attempt to optimise tigecycline regimens, Xie et al concluded that the clinical effectiveness of current standard tigecycline dosing was less than adequate given the continued resistance and reduced AUC in vivo against MDR *A. baumannii* strains with enhanced MICs [15]. Therefore, bacterial density and duration of in vitro studies are important to consider when evaluating these polymyxin combinations. Recently, tigecycline in combination with colistin for the treatment of bacteraemia due to extensively drug-resistant *A. baumannii* was shown to be associated with decreased bacteriological clearance and increased 14-day mortality [16]. In this study (55 patients met the inclusion criteria: 29 received colistin in combination with tigecycline and 26 received colistin with a carbapenem), the crude 14-day mortality was markedly higher in the colistin–tigecycline group compared with the colistin–carbapenem group (35% vs. 15%; $P = 0.105$); breakthrough bacteraemia due to *A. baumannii* was also 18% higher in the colistin + tigecycline group. Furthermore, over the duration of the study, 10 (71.4%) of the 14 non-surviving patients received colistin–tigecycline ($P = 0.010$). The findings from the abovementioned study are similar to the findings of the current study indicating that pre-existing reduced tigecycline susceptibilities among carbapenem-resistant *A. baumannii* isolates and pretreatment tigecycline MIC ≥ 2

mg/L may be indicative of poor clinical outcomes even when tigecycline is used in combination. This could be attributed to the fact that tigecycline is bacteriostatic and is widely distributed in the tissues resulting in low serum concentrations.

Taken together with the current data, it is prudent to conclude that administering either tigecycline or PMB as monotherapy or even in a traditional manner in combination against *A. baumannii* strains with reduced tigecycline susceptibilities is not recommended, particularly in infections with heterogeneous susceptibility and markedly higher bacterial density [2]. However, against *A. baumannii* strains susceptible to tigecycline with MICs ≤ 2 mg/L, at infection sites where tigecycline can achieve adequately high concentrations, these data may hold particular relevance in the selection of treatment. As it relates to the clinical use of tigecycline, tigecycline-based therapy has been compared with colistin-based therapy for the treatment of MDR *A. baumannii* infections [16,17]. Colistin-based therapy has been shown to be associated with lower in-hospital mortality and higher microbial eradication rates. The resulting suboptimal drug concentrations in serum and epithelial lining fluids with tigecycline makes physicians favour the use of high-dose tigecycline combination regimens for the treatment of MDR infections [18].

5. Conclusions

These in vitro results are indicative that against highly resistant strains with high inoculum, tigecycline in combination with PMB results only in a transitory effect with clinically achievable PMB and tigecycline concentrations. Furthermore, outcomes for patients with infections due to MDR *A. baumannii* are poor and are associated with high mortality, highlighting the importance of exploring non-traditional dosing and combinatorial approaches. Hence, the results presented here are indicative of the urgent need for newer antimicrobial agents with novel targets to help address the lack of viable treatment options against such hard-to-treat MDR pathogens.

Acknowledgments

Funding: Research reported in this publication was supported by the National Institute of Allergy and Infectious Diseases (NIAID) of the (National Institutes of Health (NIH) [award no. R01AI111990]. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. J. L. is an NHMRC Senior Research Fellow.

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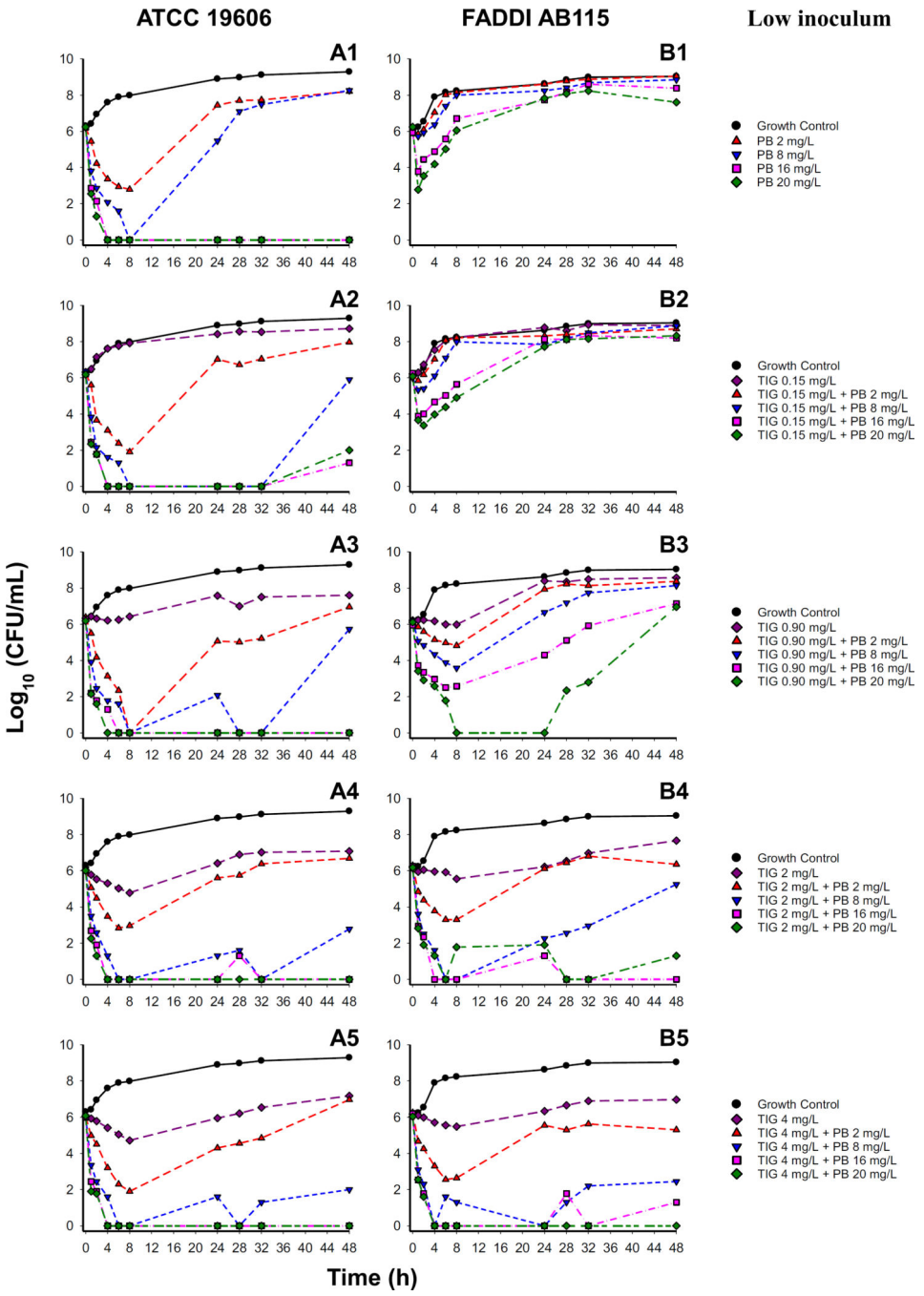

Fig. 1.

Time–kill experiments with polymyxin B (PB) monotherapy (A1, B1) and tigecycline (TIG) 0.15 mg/L (A2, B2), TIG 0.90 mg/L (A3, B3), TIG 2 mg/L (A4, B4) and TIG 4 mg/L (A5, B5) alone and in combination against an initial low inoculum of 10^6 CFU/mL of *Acinetobacter baumannii* reference strain ATCC 19606 (A) and clinical isolate FADDI AB115 (B).

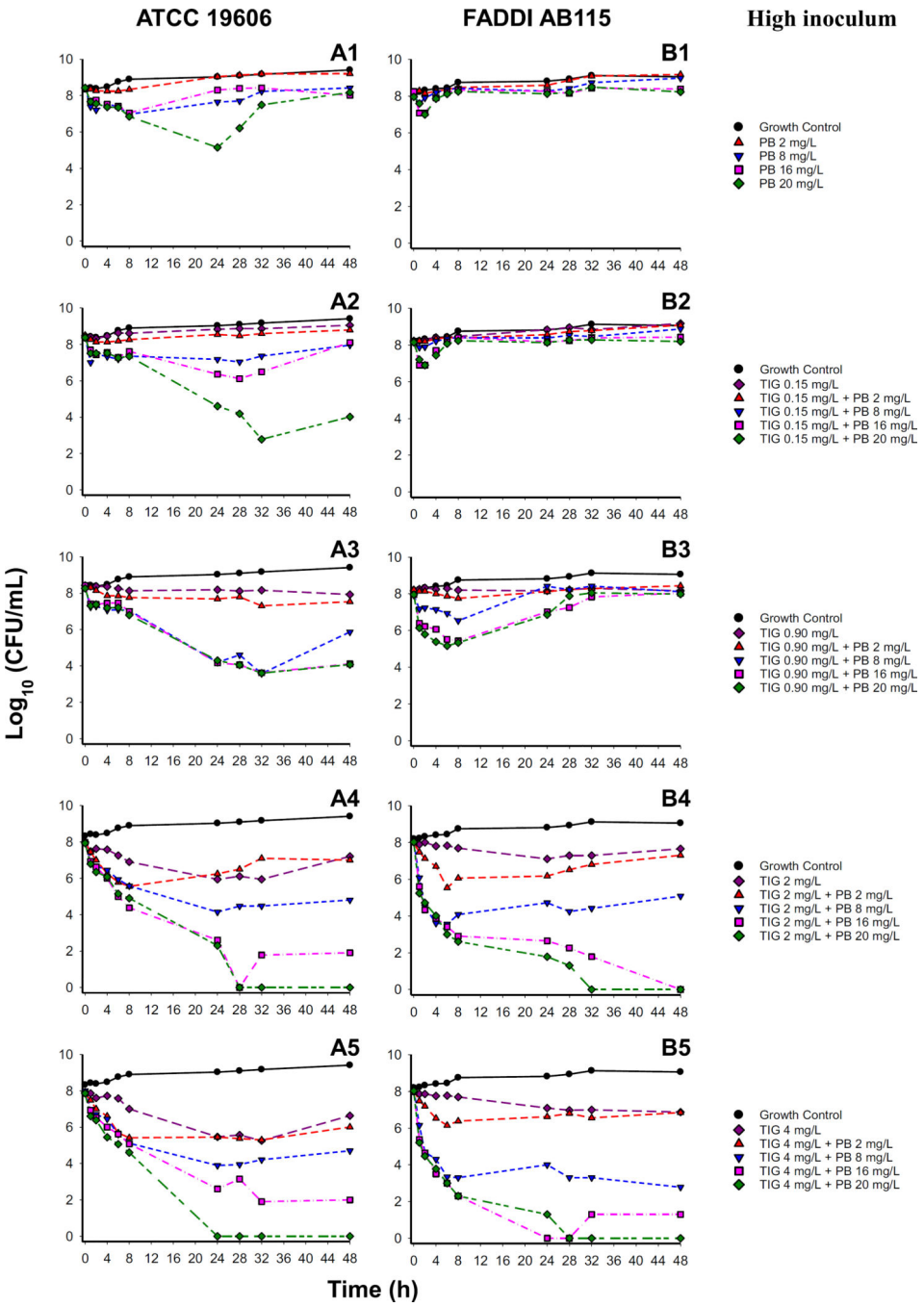

Fig. 2.

Time–kill experiments with polymyxin B (PB) monotherapy (A1, B1) and tigecycline (TIG) 0.15 mg/L (A2, B2), TIG 0.90 mg/L (A3, B3), TIG 2 mg/L (A4, B4) and TIG 4 mg/L (A5, B5) alone and in combination against an initial high inoculum of 10^8 CFU/mL of *Acinetobacter baumannii* reference strain ATCC 19606 (A) and clinical isolate FADDI AB115 (B).

Table 1

Changes in bacterial density (log_{10} CFU/mL) at 4, 8, 24 and 48 h compared with the initial inoculum (0 h) for polymyxin B (PMB) and tigecycline (TIG) combination therapy against the initial inocula of 10^6 CFU/mL and 10^8 CFU/mL of heteroresistant *Acinetobacter baumannii* reference strain ATCC 19606 and clinical isolate FADDI AB115.

Time (h)	TIG 0.15 mg/L+ PMB (mg/L)				TIG 0.90 mg/L+ PMB (mg/L)				TIG 2 mg/L+ PMB (mg/L)				TIG 4 mg/L+ PMB (mg/L)			
	2	8	16	20	2	8	16	20	2	8	16	20	2	8	16	20
ATCC 19606 at low inoculum (10^6 CFU/mL)																
4	**−3.14**	**−4.76**	**−6.20**	**−6.18**	**−3.25**	**−4.48**	**−4.98**	**−6.19**	−2.61	**−4.82**	**−6.01**	**−6.01**	−2.81	**−4.44**	**−5.99**	**−6.07**
8	**−4.33**	**−6.37**	**−6.20**	**−6.18**	**−6.38**	**−6.26**	**−6.28**	**−6.19**	**−3.12**	**−6.12**	**−6.01**	**−6.01**	**−4.11**	**−6.04**	**−5.99**	**−6.07**
24	0.78	**−6.37**	**−6.20**	**−6.18**	−1.32	**−4.18**	**−6.28**	**−6.19**	−0.48	**−4.82**	**−6.01**	**−6.01**	−1.72	**−4.44**	**−5.99**	**−6.07**
48	1.73	−0.47	**−4.90**	**−4.18**	0.57	−0.53	**−6.28**	**−6.19**	0.59	**−3.34**	**−6.01**	**−6.01**	0.95	**−4.04**	**−5.99**	**−6.07**
FADDI AB115 at low inoculum (10^6 CFU/mL)																
4	0.88	0.11	−1.59	−2.11	−1.05	−1.88	−2.99	**−3.53**	−2.51	**−4.53**	**−6.25**	**−4.88**	−2.94	**−6.15**	**−6.19**	**−6.03**
8	2.06	1.99	−0.61	−1.18	−1.38	−2.64	**−3.37**	**−6.11**	−2.99	**−6.13**	**−6.25**	**−4.40**	**−3.60**	**−4.85**	**−6.19**	**−6.03**
24	2.16	1.85	1.88	1.64	1.73	0.44	−1.65	**−6.11**	−0.17	**−3.87**	**−4.94**	**−4.27**	−0.70	**−6.15**	**−6.19**	**−6.03**
48	2.55	2.88	1.94	2.24	2.17	1.92	1.19	0.85	0.07	−0.88	**−6.25**	**−4.88**	−0.94	**−3.70**	**−4.89**	**−6.03**
ATCC 19606 at high inoculum (10^8 CFU/mL)																
4	−0.37	−1.09	−0.83	−0.85	−0.55	−1.24	−0.96	−1.07	−1.72	−1.49	−1.97	−1.86	−1.26	−1.55	−1.86	−2.41
8	−0.24	−1.06	−0.78	−1.03	−0.65	−1.25	−1.42	−1.47	−2.42	−2.35	**−3.59**	**−3.03**	−2.44	−2.89	−2.78	**−3.26**
24	0.04	−1.24	−2.03	**−3.77**	−0.73	**−4.11**	**−4.25**	**−3.97**	−1.73	**−3.79**	**−5.37**	**−5.63**	−2.41	**−4.11**	**−5.26**	**−7.86**
48	0.29	−0.46	−0.29	**−4.36**	−0.88	−2.44	**−4.31**	**−4.19**	−0.97	**−3.13**	**−6.07**	**−7.93**	−1.85	**−3.30**	**−5.86**	**−7.86**
FADDI AB115 at high inoculum (10^8 CFU/mL)																
4	0.14	−0.05	−0.47	−0.71	−0.23	−0.78	−1.89	−2.57	−1.27	**−4.43**	**−4.15**	**−4.02**	−1.55	**−3.79**	**−4.48**	**−4.25**
8	0.20	0.14	0.23	0.09	−0.48	−1.39	−2.52	−2.62	−1.92	**−3.95**	**−5.13**	**−5.42**	−1.70	**−4.79**	**−5.68**	**−5.72**
24	0.38	0.12	0.01	−0.02	−0.11	0.49	−0.94	−1.10	−1.80	**−3.32**	**−5.39**	**−6.25**	−1.46	**−4.09**	**−7.98**	**−6.72**
48	0.89	0.61	0.26	0.05	0.21	0.19	0.09	0.03	−0.67	−2.95	**−8.03**	**−8.03**	−1.23	**−5.32**	**−6.68**	**−8.03**

Regimens were categorised based on the reduction in the initial bacterial inoculum, where grey shading highlights activity (at least 1 log_{10} CFU/mL reduction), orange shading indicates additivity (at least 1.0 to <2 log_{10} CFU/mL reduction) and green shading indicates synergy (at least ≥2 log_{10} CFU/mL reduction) by the combination of PMB and TIG compared with the most active single agent in the combination. Bold type indicates bactericidal activity (≥3 log_{10} CFU/mL reduction).