

## Interaction of Imipenem and Tigecycline against Carbapenem-Resistant *Acinetobacter baumannii*: The Relativity of Synergism

(Interaksi Imipenem dan Tigecycline terhadap Rintang-Carbapenem

*Acinetobacter baumannii*: Kerelatifan Sinergisme)

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### ABSTRACT

*Acinetobacter baumannii* is an opportunistic bacterium that causes widespread nosocomial infections and tends to be multi-resistant to most of antibiotics. Tigecycline is a well-known antibiotic that possesses a wide-range of activities and is very active in vitro towards a variety of resistant pathogens, including *A. baumannii*. The aim of this study was to evaluate the effects of the imipenem and tigecycline combination against carbapenem-resistant *A. baumannii* (CRAB). The minimal inhibitory concentration (MIC) was determined using the E-test method. The microbroth checkerboard technique was employed to determine the effects of the imipenem and tigecycline interaction by obtaining the fractional inhibitory concentration (FIC) index value. A time kill (TK) study was performed to identify any synergistic effects when both imipenem and tigecycline were used against CRAB strains. The result demonstrated that all *A. baumannii* strains exhibited imipenem MIC values of 32 µg/mL. The combination of imipenem and tigecycline demonstrated an additive effect (FIC > 0.5-4) against all of the strains and synergistic activity (decrement of > 2 log<sub>10</sub> CFU/mL) towards AC 34/07 and AC 32/06 strains. The use of imipenem in combination with tigecycline resulted in a more efficient activity and an increased capability to control CRAB infections. This effect showed potential combination and may be of importance in the development or modification of antimicrobial agents for the treatment of CRAB infections.

**Keywords:** *Acinetobacter baumannii*; carbapenem-resistant; imipenem; tigecycline

### ABSTRAK

*Acinetobacter baumannii* adalah bakteria oportunistik yang menyebabkan jangkitan nosokomial secara meluas dan cenderung untuk menjadi multirintang terhadap kebanyakan antibiotik. Tigecycline adalah antibiotik yang mempunyai pelbagai aktiviti dan sangat aktif secara in vitro terhadap pelbagai patogen rintang antibiotik, termasuklah *A. baumannii*. Tujuan kajian ini adalah untuk menilai kesan dan gabungan antibiotik imipenem dan tigecycline terhadap *A. baumannii* rintang-karbapenem (CRAB). Kepekatan perencatan minimum (MIC) ditentukan dengan menggunakan kaedah E-test. Teknik papan penyamak mikrokaldu digunakan untuk menentukan kesan imipenem dan interaksinya dengan tigecycline dengan mendapatkan nilai indeks kepekatan rencatan berperingkat (FIC). Ujian masa membunuh (TK) telah dijalankan untuk mengenal pasti kesan sinergi apabila kedua-dua imipenem dan tigecycline digunakan terhadap strain CRAB. Hasil ujian menunjukkan bahawa semua strain *A. baumannii* memberi nilai imipenem MIC sebanyak 32 µg/mL. Gabungan imipenem dan tigecycline menunjukkan kesan tambahan (FIC > 0,5-4) terhadap semua strain yang diuji dan aktiviti sinergi (pengurangan sebanyak > 2 log<sub>10</sub> CFU/mL) terhadap strain AC 34/07 dan AC 32/06. Penggunaan kombinasi imipenem dan tigecycline menghasilkan aktiviti yang lebih cekap dan meningkatkan kemampuan untuk mengawal jangkitan CRAB. Kesan ini menunjukkan kombinasi yang berpotensi dan mungkin penting dalam pembangunan atau pengubahsuaian agen antimikrob untuk rawatan jangkitan CRAB.

**Kata kunci:** *Acinetobacter baumannii*; imipenem I; rintang-karbapenem; tigecycline

### INTRODUCTION

*A. baumannii* is an opportunistic pathogen and associated with a variety of nosocomial infections worldwide. These nosocomial infections include pneumonia, urinary tract infection and septicemia which occur most frequently in patients who are being treated in the intensive care unit (ICU) (Al-Dabaibah et al. 2012; Chaaria et al. 2013; Tsakiridou et al. 2014). *A. baumannii* causes serious bacteremia infections and an increased mortality rate of 49% when resistance towards the main standard antibiotic

carbapenem is present. These strains are referred to as carbapenem-resistant *A. baumannii* (CRAB) and they exhibit resistance towards most drugs, including β-lactams, aminoglycosides and fluoroquinolones (Bonomo & Szabo 2006).

The management of an *A. baumannii* multi-resistant infection is medical concern because of limited therapeutic options available and can be complicated, due to the emergence of carbapenem-resistant isolates (Bou et al. 2000). Treatment of CRAB poses a problem for modern

medicine worldwide and is challenging to the physicians. It has been proposed that combinations of different antibiotics should be administered to create an active treatment and prevent the emergence of resistance (Peleg et al. 2008). The activity of an antimicrobial agent can sometimes be enhanced by its use in combination with another agent with a different mode of action. Various antibiotic combinations such as polymyxin and imipenem, rifampicin or azithromycin, as well as other combinations, have been studied to determine whether they exhibit a synergistic effect against CRAB (Lim et al. 2011; Wareham & Bean 2006; Yoon et al. 2004). Some studies have shown that the *in vitro* synergy of antibiotic combinations may be strain dependent (Lim et al. 2011; Wareham & Beam 2006). However, the use of antibiotic combination for the practical treatment of CRAB infections is still controversial.

Tigecycline has been shown to possess a wide spectrum of antibiotic activity against many pathogens *in vitro*, including *A. baumannii* (Castanheira et al. 2008). The combination of tigecycline with other antibiotics such as imipenem, has shown synergistic antibacterial activity and could be valuable for the treatment of infectious CRAB. Thus, in this study, tigecycline is investigated to determine its potential activity against CRAB strains when combined with imipenem.

## MATERIALS AND METHODS

### BACTERIAL ISOLATES

Fifty clinical isolates of CRAB were obtained from the Microbiology and Immunology Laboratory, Universiti Kebangsaan Malaysia Medical Centre (UKMMC) in the year 2006. The isolates were identified using the API 20NE system (Bio-Merieux, Marcy l'Etoile France). The isolates were stored at  $-80^{\circ}\text{C}$  in Mueller-Hinton broth containing 15% glycerol.

### ANTIMICROBIAL AGENTS

Antimicrobial agents were purchased from companies as standard reference powders for laboratory use. The following antimicrobial agents were tested: Imipenem (Sigma-Aldrich, German) and tigecycline (Sigma-Aldrich, UK).

### SUSCEPTIBILITY TESTING

The MICs of the 50 bacterial isolates for both imipenem and tigecycline were determined by the E-test method, using commercially available E-test strips (Bio-Merieux, France). Bacterial suspensions containing approximately  $10^5$  colony-forming units (CFU) were inoculated onto Mueller-Hinton agar plates. Then, E-test strips were placed on top of the inoculated agar plates. The plates were then incubated at  $35\pm 2^{\circ}\text{C}$  for 18-20 h. The MIC was defined as the lowest concentration of antimicrobial agent that completely inhibited the bacterial growth. The

concentrations of imipenem and tigecycline that were tested against all of the strains ranged from 0.002 to 32  $\mu\text{g}/\text{mL}$  and 0.016 to 256  $\mu\text{g}/\text{mL}$ , respectively. *Escherichia coli* ATCC 25922 were used as a control for each test. The breakpoint for imipenem susceptibility was interpreted to be  $\leq 4$   $\mu\text{g}/\text{mL}$ , 8  $\mu\text{g}/\text{mL}$  and  $\geq 16$   $\mu\text{g}/\text{mL}$  for susceptible, intermediate and resistant, respectively (CLSI 2011). Because there is no approved standard for classifying *A. baumannii* as susceptible or resistant to tigecycline, the provisional MIC breakpoints used for this agent were  $\leq 2$   $\mu\text{g}/\text{mL}$ , 4  $\mu\text{g}/\text{mL}$  and  $\geq 8$   $\mu\text{g}/\text{mL}$ , which designated as susceptible, intermediate and resistant, respectively (Wyeth Research, personal communication).

### CHECKERBOARD ANALYSIS

The checkerboard method was used to assess the activity of the antimicrobial combinations (Petersen et al. 2006). Imipenem and tigecycline solution were combined in the form of a checkerboard in a 96-well microtiter plate. Each antimicrobial agent was prepared in a fixed volume of 50  $\mu\text{L}$  (a total volume of 100  $\mu\text{L}$  for two antimicrobial agents) and 100  $\mu\text{L}$  of bacterial suspension was then added to each well. The final concentration of the bacterial suspension was approximately  $5 \times 10^5$  CFU/mL in a total volume of 200  $\mu\text{L}$  in each well. The microtiter plate was then incubated at  $35\pm 2^{\circ}\text{C}$  for 18-24 h. After the overnight incubation, the MIC values were determined. Positive (with bacteria) and negative controls (without bacteria) were analyzed simultaneously. The fractional inhibitory concentration (FIC) index for each antibiotic was determined by dividing the concentration of a particular antibiotic necessary to inhibit growth in a given row by the MIC of the antibiotic alone. The FIC index was calculated by adding the separate FICs for each of the drugs present in a particular well. Interpretation of the FIC index was as follows: FIC index  $\leq 0.5$ , synergy; FIC index  $> 0.5-4$ , additive; FIC index  $> 4.0$ ; antagonistic (Eliopoulos & Moellering 1991).

### TIME-KILL ASSAY

A time-kill study was done to assess the synergistic combinations of the drugs (Petersen et al. 2006). The antimicrobial agents were tested at concentrations of 1, 1/2 and 1/4  $\times$  MIC (sub-inhibitory concentrations) with a starting inoculum of  $5 \times 10^5$  CFU/mL prepared in Mueller-Hinton broth. Sample aliquots (10  $\mu\text{L}$ ) were removed from cultures at 0, 2, 4, 8 and 24 h. Antimicrobial activity was ascertained by serial dilution (10- to 10,000-fold) of plated samples. Growth control wells for each organism were prepared without antimicrobial agent and performed in parallel to the antimicrobial test tubes. Synergy of a combination of antibiotics was defined as a decrease of at least 2  $\log_{10}$  CFU/mL at 24 h compared to the single most active drug in combination (Domaracki et al. 2000). An antibiotic or its combination was considered bactericidal when it produced a reduction of at least 3  $\log_{10}$  CFU/mL from starting inoculum at 4 or 24 h post incubation (Rand & Houck 2004).

## RESULTS

## SUSCEPTIBILITY TESTING

The MIC values for imipenem and tigecycline were shown in Table 1. All isolates were resistant to imipenem (MIC at 32 µg/mL). As for tigecycline, 45 isolates were susceptible with MIC values ranging from 2 to 4 µg/mL and 5 isolates were intermediate with MIC value of 6 µg/mL. The CRAB isolates (AC 04/07, AC 34/07, AC 32/06 and AC 09/07 strains) that exhibited the lowest MIC value (2 µg/mL) for tigecycline were subsequently analyzed by the checkerboard method and the time-kill assay to determine their sensitivity to synergistic effects of the tigecycline and imipenem combination.

TABLE 1. Susceptibility of the 50 CRAB isolates towards imipenem and tigecycline antibiotics

Antibiotic	MIC (µg/mL)	No. of isolates (%)
Imipenem	32	50 (100)
Tigecycline	2	4 (8)
	3	21 (42)
	4	20 (40)
	6	5 (10)

## CHECKERBOARD ANALYSIS

The results from the checkerboard analysis of the imipenem/tigecycline combination are shown in Table 2. All four isolates demonstrated an additive sensitivity with FIC > 0.5-4. No antagonistic interaction (FIC ≥ 4) was observed.

## TIME-KILL ANALYSIS

In the time-kill assay (Table 3), the imipenem and tigecycline combination at 0.25 × MIC for imipenem and 1 × MIC for tigecycline produced a synergistic activity towards strain AC 34/07 at the 24-h time point. Likewise, the combination of the imipenem and tigecycline exhibited a synergistic effect against strain AC 32/06 at the 2-, 4- and 6-h time points, at a concentration of 0.5 × MIC for imipenem and 0.25 × MIC for tigecycline. The synergistic effect was demonstrated by a reduction in the bacterial colony count of ≥ 2 log<sub>10</sub> CFU/mL. However, this

antimicrobial combination exhibited no synergistic effects toward strain AC 04/07 at 0.5 × MIC for imipenem and 0.25 × MIC for tigecycline, or toward strain AC 09/07 at 0.25 × MIC for imipenem and 0.5 × MIC for tigecycline.

## DISCUSSION

Infections caused by CRAB have increased tremendously and the struggle to treat these infections has been complicated by the development of resistance towards a variety of antibiotics, including β-lactams, aminoglycosides and fluoroquinolones, by various isolates (Fishbain & Peleg 2010; Garnacho-Montero & Amaya-Villar 2010). Aggressive treatment with two antimicrobial agents possessing synergistic activity has been shown to increase positive outcomes in the treatment of immune-compromised patients with CRAB-related infections (Kuo et al. 2008; Lee et al. 2007). The results of this study showed the presence of synergistic activity between imipenem and tigecycline. Tigecycline is a bacteriostatic agent (Pachon-Ibanez et al. 2004) used to treat *A. baumannii* infections, including CRAB (Henwood et al. 2002). In this study, tigecycline is more active than imipenem against CRAB. Tigecycline possesses wide-spectrum antibiotic activity and is very active *in vitro* against almost all pathogens, including *A. baumannii* (Castanheira et al. 2008; Fritsche et al. 2005). Due to the bacteriostatic activity of tigecycline against CRAB, it is appropriate to combine this drug with another antimicrobial agent, such as imipenem (Pachon-Ibanez et al. 2004; Scheetz et al. 2007), to improve the outcome of a patient's treatment. The *in vitro* results of this study have demonstrated that the imipenem and tigecycline combination is effective against CRAB and that a maximum synergistic effect can be observed over time using this combination treatment.

The checkerboard method and time-kill analysis have been used globally to investigate the *in vitro* activity of an antimicrobial combination against various types of resistant pathogens (Lee et al. 2007; Petersen et al. 2006; Scheetz et al. 2007). However, there was a slight difference observed in the results that were obtained by both methods; all four of the isolates exhibited an additive effect according to the checkerboard method, but demonstrated synergistic activity according to the time-kill analysis. Based on these observations, it was postulated

TABLE 2. Fractional inhibitory concentration (FIC) values for the imipenem/tigecycline combination towards CRAB isolates

<i>A. baumannii</i> isolates	Combination MIC (µg/mL)		Individual FIC values		FIC index
	Imipenem (I)	Tigecycline (T)	FIC(I)	FIC(T)	FIC(I) + FIC(T)
AC 04/07	16	0.5	0.5	0.25	0.75 (Ad)
AC 34/07	8	3	0.25	1	1.25 (Ad)
AC 32/06	16	1	0.5	0.25	0.75 (Ad)
AC 09/07	8	3	0.25	0.5	0.75 (Ad)

Ad = additive interaction

TABLE 3. Time-kill analysis of the imipenem/tigecycline combination towards CRAB isolates

<i>A. baumannii</i> isolates	Combination concentration		Log <sub>10</sub> CFU/mL (time of sampling, hour)					
	Imipenem	Tigecycline	0	2	4	6	8	24
AC 04/07	0.5 x MIC	0.25 x MIC	0.14	0.86	0.77	0.69	0.29	0.01
AC 34/07	0.25 x MIC	1 x MIC	0.45	0.70	0.42	0.31	0.70	2.70
AC 32/06	0.5 x MIC	0.25 x MIC	0.38	2.12	3.58	2.49	1.25	0.11
AC 09/07	0.25 x MIC	0.5 x MIC	0.51	0.24	0.68	0.76	0.53	0.50

that the time-kill method was more sensitive than the checkerboard method in detecting synergy. The additive interactions that were observed were not affected by the strains' individual susceptibility to tigecycline. However, these interactions resulted in an increase in imipenem activity from antagonistic effect to an additive effect. The end result for the antimicrobial combinations against all four of the CRAB isolates indicated that the MIC value for imipenem decreases from its individual MIC value of  $\geq 32$  to 8  $\mu\text{g}/\text{mL}$  and 16  $\mu\text{g}/\text{mL}$  when it is used in combination with tigecycline. This outcome proves that in the presence of another type of antimicrobial agent, such as tigecycline, the susceptibility of CRAB to  $\beta$ -lactam antibiotic increases.

The development of resistance to tigecycline among CRAB has been reported with resistance rates ranging between 9.5 and 66% (Lolans et al. 2006; Navon-Venezia et al. 2007). This was usually observed when tigecycline was used as monotherapy for treatment of CRAB infections (Karageorgopoulos et al. 2008). Combination therapy with several classes of antibiotics used for the treatment of carbapenemase-producing Gram-negative infections has shown greater activity and delayed development of resistance (Rahal et al. 2002). As shown in this study, combination therapy is more effective against CRAB. However, the synergistic effect of each antibiotic is specific to each clinical isolates as shown by 2 out of 4 strains tested. This shows that the combination treatment efficacy may vary for every single strain (Carmeli et al. 2010). Combination therapy should be the preferred choice of treatment as it improves the possibility of achieving a synergistic effect and helps to overcome the development of resistance.

In conclusion, this *in vitro* study reporting that the combination of tigecycline with other antimicrobial agents can be used to achieve a better treatment of infections caused by CRAB strains. In addition, the imipenem and tigecycline combination promises to be useful for the treatment of CRAB infections.

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