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In vitro Effects of Tigecycline in Combination with Other Antimicrobials against Multidrug-Resistant *Acinetobacter baumannii* Isolates

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Acinetobacter **is a group of bacteria commonly found in soil and water and prefer to colonize aquatic environments. Moreover there are many species of *Acinetobacter* and all can be opportunistic human pathogens, around eighty percent of human infections are caused by *A. baumannii.* Resistance isolates of *A. baumannii* are very common and have increased dramatically over the last 30 years. Most isolates are resistant to older antimicrobial agents including extended spectrum penicillins, 1st and 2nd generation cephalosporins, and aminoglycosides. Due to high rates of resistance associated with *A. baumannii*, combination therapy is typically employed for the treatment of infections. This study is aimed to evaluate the in *vitro* antimicrobial activities of drug combination regimens against Saudi isolates of *A. baumannii* by using minimum inhibitory concentration determination, checkerboard synergy and time-kill assays for tigecycline, imipenem, Amikacin, piperacillin/tazobactam, Cefepime, colistin, ciprofloxacin and Ampicillin/ Sulbactam and were determined according to the Clinical and Laboratory Standards Institute guideline. The result of the presented *in vitro* study revealed that the double combination of tigecycline with colistin, imipenem and amikacin could be a promising alternative for the treatment of infections due to multi-drug resistance *A. baumannii* strains. Further investigations required to validate the role of these combinations for the treatment of infections due to current isolates found in Saudi Arabia.**

Key words: *Acinetobacter baumannii,* Tigecycline, Drug combination, MDR.

Acinetobacter is a group of bacteria commonly found in soil and water and prefer to colonize aquatic environments. Moreover there are many species of Acinetobacter and all can be opportunistic human pathogens, around eighty percent of human infections are caused by *A. baumannii*. *A. baumannii* is a non-fermenter, strictly aerobic and Gram-negative pleomorphic bacillus (Falagas *et al*., 2007, Peleg *et al*., 2008, Peymani *et al*., 2011). *A. baumannii* is commonly isolated from the hospital setting, often isolated from hospitalized patients as it commonly colonizes irrigating solutions and intravenous solutions. *Acinetobacter* species have low virulence but are opportunistic pathogens that are capable of causing infection (Qi *et al*., 2004). Outbreaks of *A. baumannii* infections usually occur in Intensive care units (ICU) and various other healthcare units housing seriously ill and immunocompromised patients. Infections rarely occur outside of the hospital environment (Cerqueira *et al*., 2011).

Resistance isolates of *A. baumannii* are very common and have increased dramatically over the last 30 years. Most isolates are resistant to older antimicrobial agents including extended spectrum penicillins, 1st and 2nd generation

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cephalosporins, and aminoglycosides (Peleg *et al*., 2008, Bassetti *et al*., 2011).

A. baumannii is characterized by its potential to acquire antimicrobial resistance genes rapidly, resulting in multidrug resistance. Moreover, the genetic flexibility of this microorganism has resulted in the rapid and global emergence of multidrug resistance. Recently, strains displaying resistance to all available antimicrobial agents have been reported, making the treatment of these infections very difficult (Davies and Davies, 2010).

Due to high rates of resistance associated with *A. baumannii*, combination therapy is typically employed for the treatment of infections. In this regard, a combination of carbapenem and colistin or sulbactam is the preferred choice (Babinchak *et al*., 2005, Batirel *et al*., 2014). The role of combination antimicrobial therapy for *A. baumannii* has been studied, for possibly synergistic effects, but this type of study has not been described for clinical isolates of *A. baumannii* in Saudi Arabia. This study is aimed to evaluate the *in vitro* antimicrobial activities of drug combination regimens against Saudi isolates of *A. baumannii* by using minimum inhibitory concentration (MIC) determination, checkerboard synergy and time-kill assays.

MATERIALS AND METHODS

Bacterial strains

In this particular study, 18 *A. baumannii* strains were screened for multi-drug resistance; all were collected from different patients at Ohad Hospital, Almadinah in Saudi Arabia between May and December 2013. Bacterial identities were confirmed using Vitek II (bioMérieux, France).

Antimicrobials susceptibility testing

Susceptibility testing was performed using the disk diffusion method, according to Clinical and Laboratory Standards Institute (CLSI,) guidelines (CLSI, 2012). A strain was classified to be MDR when it was resistant to three or more of the tested agents (Manchanda *et al*., 2010). The susceptibility patterns for the selected MDR isolates are displayed in Table 1. *A. baumannii* strains (ATCC 25922) were used in this study as a control. MIC values of Tigecycline, imipenem, Amikacin, piperacillin/tazobactam, Cefepime, colistin, ciprofloxacin and Ampicillin/ Sulbactam were determined for the MDR strains using dilution method according to the CLSI guideline (CLSI, 2012).

Checkerboard assay for synergism testing

The synergistic effect for tigecycline with imipenem, amikacin, piperacillin/tazobactam, cefepime, colistin, ciprofloxacin and ampicillin/ sulbactam were determined by the checkerboard method, performed according to the approved Guidelines and were determined after 24 hrs of growth. The fractional inhibitory concentration indexes (FICI) were considered as the sum of the fractional inhibitory concentrations (FICs) of either drug. The results were interpreted as synergy, indifference and antagonism if the FICI were ≤ 0.5, $0.5 < - \leq 4$ and > 4 respectively. All experiments were repeated at least three times, and the results of a representative experiment are presented (CLSI, 2012, Manchanda *et al*., 2010, White *et al*., 1996).

Time–kill studies

In vitro drug combination effects were evaluated using time-kill methods according to CLSI guideline for selected strains to confirm the effect. Bacteria were grown until exponential phase then diluted to approximately $5x10^5$ CFU/ml in media containing antimicrobial agents alone or in combination, and left incubated for 24 hrs at 37°C. The concentration of each agent used in this study was selected based on the MIC results. Synergy was considered when there is a ≥ 2 log10 decrease in CFU/ml between the two drug combination and its most active drug. Antagonism was considered when there is a ≥ 2 log10 increase in CFU/ml between the two drug combination and its most active drug. All experiments were repeated at least three times, and the results of a representative experiment are presented CLSI, 2012, Manchanda *et al*., 2010, White *et al*., 1996).

RESULTS

The Antibiotic resistance profile, were preliminarily determined for the all the isolates. The susceptibility patterns for the isolates are displayed in Table 1. Eight isolates were MDR strains. The susceptibility patterns for the selected MDR isolates are displayed in Table 2.

Checkerboard analysis performed for the MDR stains with all antimicrobials agents in combination with tigecycline showed 7.14% synergy, 83.94% indifference, and 8.92% antagonism. Tigecycline showed a synergistic activity with colistin (in 2 isolates), imipenem and amikacin (1 isolate each). Antagonistic interactions were detected when tigecycline combined with piperacillin-tazobactam (in 3 isolates), or Cefepime (in 1 isolate). The Checkerboard results for the isolates are displayed in Table 3.

All synergistic combinations showed by the chequerboard methods were retested using a kinetic time-kill method. Time-kill graphs for synergistic combinations are shown in Figure 1. All four synergistic combinations were confirmed.

Table 1. Antibiotic resistance profile of the *A. baumannii* isolates

Antimicrobial drugs	IPM	TGC	AMK	TZP	FEP	CS	CIP	SAM
Number of resistant strains out of 18	6 (33.33%)	9 (50.00%)	8 (44.44%)	12 (66.66%)	9 (50.00%)	3 (16.66%)	15 (83.33%)	7 (38.88%)

Tigecycline; TGC, Imipenem: IPM, Amikacin; AN. Piperacillin/tazobactam; TZP, Cefepime; FEP, Colistin; CS, Ciprofloxacin; CIP and Ampicillin/ Sulbactam; SAM.

Table 2. Antibiotic resistance profile of MDR *A. baumannii* isolates

MDR Strains	Antibiotic resistance profile
Ab3	TGC, IPM, AN, TZP, FEP, CS, SAM
Ab5	TGC, AN, TZP, FEP, CS, CIP
Ab6	IPM, AN, TZP, CIP
Ab8	TGC, IPM, AN, TZP, CS, CIP
Ab10	TGC ,IPM, TZP, FEP, CIP,
Ab11	TZP, FEP, CIP, SAM
Ab15	TGC, IPM, TZP, FEP, CIP,
Ab17	TGC, IPM, AN, TZP, FEP,CIP

Tigecycline; TGC, Imipenem: IPM, Amikacin; AN. Piperacillin/tazobactam; TZP, Cefepime; FEP, Colistin; CS, Ciprofloxacin; CIP and Ampicillin/ Sulbactam; SAM, A. baumannii; Ab.

DISCUSSION

A. baumannii has emerged as the most significant nosocomial and opportunistic pathogen, often affecting ICU patients. *A. baumannii* is increasingly becoming resistant to almost all antibiotics and presents a significant problem in health care settings and is severely limiting the antimicrobials therapeutic options (Sopirala *et al*., 2010, Dijkshoorn *et al*., 2007). Due to the high rate of MDR incidents among *A. baumannii*, infection management is usually difficult (Sydnor *et al*., 2011). Carbapenems have been considered the most active agent against MDR pathogens. However, carbapenem resistant

Table 3. Checkerboard results for Tigecycline in combination with seven antibiotics for eight MDR strains of *A. baumannii*.

MDR Strains	IPM	AMK	TZP	FEP	CS	CIP	SAM
Ab3	Synergy	Indifference	Indifference	Indifference	Indifference	Indifference	Indifference
Ab5	Indifference	Indifference	Indifference	Indifference	Synergy	Indifference	Indifference
Ab6	Indifference	Indifference	antagonism	Indifference	Indifference	Indifference	Indifference
Ab8	Indifference	Indifference	antagonism	Indifference	Synergy	Indifference	Indifference
Ab10	Indifference	Synergy	Indifference	Indifference	Indifference	Indifference	Indifference
Ab11	Indifference	Indifference	antagonism	antagonism	Indifference	Indifference	Indifference
Ab15	Indifference	Indifference	Indifference	Indifference	Indifference	Indifference	Indifference
Ab17	Indifference	Indifference	Indifference	Synergy	Indifference	Indifference	Indifference

Tigecycline; TGC, Imipenem: IPM, Amikacin; AN. Piperacillin/tazobactam; TZP, Cefepime; FEP, Colistin; CS, Ciprofloxacin; CIP and Ampicillin/ Sulbactam; SAM. A. *baumannii*; Ab.

isolates are increasing worldwide (Kanj *et al*., 2011). In recent years, carbapenem resistance rates were reported to be up to 92% in ICUs (Dijkshoorn *et al*., 2007). Colistin has been prescribed as the last resort. Nevertheless, the extensive use of colistin and the absence of an optimum dosage could lead to resistance. The potential problem with colistin is monotherapy. In recent years, many studies have investigated combination therapy with other agents to increase treatment success and to hold back the emerging resistance to colistin (Li *et al*., 2006, Hawley *et al*., 2007).

A. baumannii isolates that are resistant to a broad spectrum of antimicrobial agents have been reported and support the necessity to search for new combinations of synergistic agents.

In this study, we examined the synergy effects against eight clinical isolates of MDR *A.*

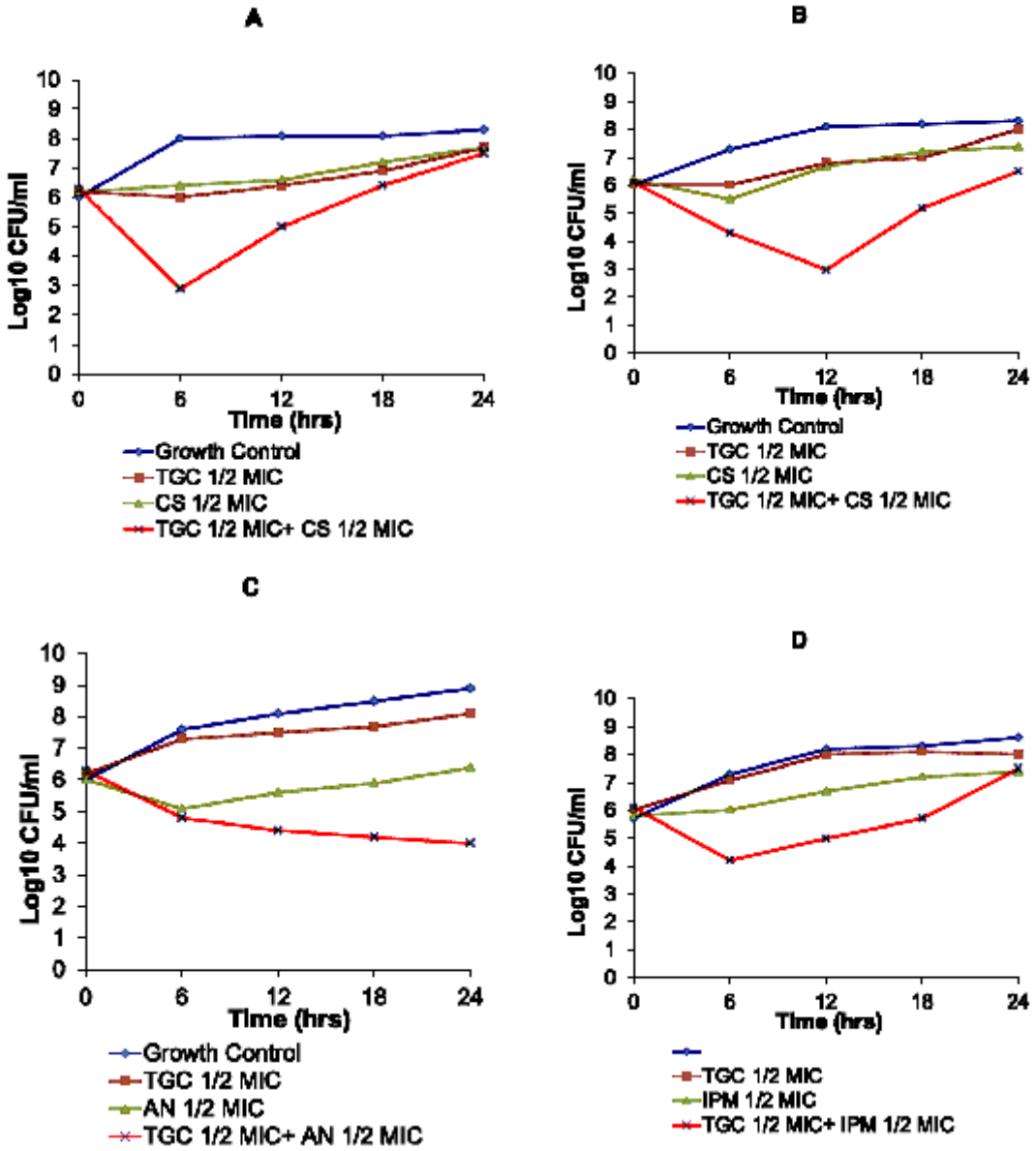

Fig. 1. Time-kill results for the confirmed synergistic combinations. (A) TGC/CS- isolate Ab5. (B) TGC/CS- isolate Ab8. (C) TGC/AN-isolate Ab10. (D) TIG/IPM-isolate Ab3

baumannii isolated from Saudi Arabia using checkerboard and time-kill methods for tigecycline combined with imipenem, amikacin, piperacillin/ tazobactam, cefepime, colistin, ciprofloxacin and ampicillin/ sulbactam.

Synergy with tigecycline using the checkerboard test ranged from 25% for colistin to 12.5% for imipenem and amikacin. Overall the synergy, indifference and antagonism were detected as 7.14%, and 83.94%, 8.92% respectively. These findings are comparable to other researchers reporting synergy against *A. baumannii* using the checkerboard test. Synergy was detected with the checkerboard and further confirmed by time-kill methods using isolates that are resistant to one or both antimicrobial agents (Pongpech *et al*., 2010).

In conclusion, the result of the presented *in vitro* study revealed that the double combination of tigecycline with colistin, imipenem and amikacin could be a promising alternative for the treatment of infections due to MDR *A. baumannii* strains. The encouraging result of the presented *in vitro* study calls for further investigations in other combinations therapy to be trialled. As the antimicrobial concentrations in this study may not completely presents *in vivo* conditions, such as virulence factors and the host immune system, further investigations required including clinical trials to validate the role of these combinations for the treatment of infections due to the current isolates found in Saudi Arabia.

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