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In vitro activities of carbapenem/sulbactam combination, colistin, colistin/rifampicin combination and tigecycline against carbapenem-resistant *Acinetobacter baumannii*

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***Objectives*: To determine the *in vitro* activities and interactions of imipenem, colistin and tigecycline with old antibacterial agents against carbapenem-resistant *Acinetobacter baumannii*.**

***Methods*: Forty-three carbapenem-resistant *A. baumannii* isolates from the intensive care unit of a university hospital were collected and their MICs of imipenem, colistin and tigecycline were determined. With eight randomly selected carbapenem-resistant isolates, an *in vitro* time–kill study was performed for the evaluation of antibacterial activity of colistin, tigecycline, imipenem/sulbactam and colistin/rifampicin.**

***Results*: The time–kill study of colistin demonstrated bactericidal activity against *A. baumannii* at concentrations of 4× MIC and 8× MIC, whereas tigecycline showed bacteriostatic activity at all concentrations. The combination regimens of imipenem/sulbactam and colistin/rifampicin were synergistic and bactericidal at 1× MIC.**

***Conclusions*: Imipenem/sulbactam combination, colistin and tigecycline showed good *in vitro* activities against carbapenem-resistant *A. baumannii* isolates. Even though colistin is bactericidal against carbapenem-resistant *A. baumannii*, the colistin/rifampicin combination is more warranted in order to be certain.**

Keywords: *A. baumannii*, synergy, time–kill assay

Introduction

Acinetobacter baumannii has emerged as one of the most important nosocomial pathogens, especially in patients admitted to an intensive care unit (ICU). *A. baumannii* can colonize multiple body sites of hospitalized patients and survive for a long time on inanimate surfaces.[1,2] Both these aforementioned characteristics may have contributed to the prominent role of *A. baumannii* in nosocomial infections.

Fluoroquinolones, ceftazidime, trimethoprim/sulfamethoxazole and carbapenems have been considered active against nosocomial *A. baumannii* strains. However, treatment of this organism has become a medical challenge because of the emergence of multidrug-resistant isolates, including resistance to carbapenems, with increasing frequency.[3,4] Therefore, new agents or combination regimens of old agents known to be effective against multidrug-resistant *A. baumannii* are attracting more attention. Meropenem/sulbactam combinations have shown synergistic effects in multidrug-resistant strains of *A. baumannii*.[5,6] An old drug, colistin, which was abandoned since the 1960s due to nephrotoxicity, gained new interest for its activity against *A. baumannii* and one study, although small in sample size, showed a good outcome with a combination regimen of colistin and rifampicin.[7,8] Tigecycline showed good *in vitro* bacteriostatic activity against *A. baumannii* strains that showed different susceptibilities to imipenem.[9]

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Table 1. PCR primers, conditions and product sizes for the detection of carbapenemases among *A. baumannii* strains

Target gene	Primers	Reaction conditions	Product size (bp)
$bla_{\text{IMP-1}}$	5′-ACC GCA GCA GAG TCT TTG CC-3′ 5′-ACA ACC AGT TTT GCC TTA CC-3′	annealing at 59°C	587
$bla_{\text{IMP-2}}$	5′-GTT TTA TGT GTA TGC TTC C-3′ 5′-AGC CTG TTC CCA TGT AC-3′	annealing at 55°C	678
$bla_{\text{VIM-1}}$	5′-GGG AGC CGA GTG GTG AGT-3′ 5′-GGC ACA ACC ACC GTA TAG-3′	annealing at 55°C	519
$bla_{\text{VIM-2}}$	5′-ATG TTC AAA CTT TTG AGT AAG-3′ 5′-CTA CTC AAC GAC TGA GCG-3′	annealing at 56°C	801
OXA-23	5′-GAT CGG ATT GGA GAA CCA GA-3′ 5′-ATT TCT GAC CGC ATT TCC AT-3′	annealing at 52°C	501
OXA-24	5′-GGT TAG TTG GCC CCC TTA AA-3′ 5′-AGT TGA GCG AAA AGG GGA TT-3′	annealing at 52°C	246
OXA-51	5′-TAA TGC TTT GAT CGG CCT TG-3′5 ′-TGG ATT GCA CTT CAT CTT GG-3′	annealing at 52°C	353
OXA-58	5′-AAG TAT TGG GGC TTG TGC TG-3′ 5′-CCC CTC TGC GCT CTA CAT AC-3′	annealing at 52°C	599

The objective of the present study was to demonstrate *in vitro* activities of carbapenem/sulbactam combination, colistin, colistin/rifampicin combination and tigecycline against carbapenem-resistant *A. baumannii*.

Materials and methods

Forty-three clinical isolates of carbapenem-resistant *A. baumannii* were collected from routine clinical isolates from patients admitted to the ICU during the period of January 2002 to June 2006. Isolates were selected randomly from different patients.

For the 43 isolates, MICs of imipenem, colistin (colistimate sodium), tigecycline, sulbactam and rifampicin were determined by a broth microdilution method in Mueller–Hinton broth by geometric 2-fold serial dilutions. According to CLSI (formerly NCCLS) standards, strains were considered resistant to carbapenems if the MICs of imipenem were $\geq$32 mg/L.[10] The following concentrations were considered as the susceptibility breakpoints of other tested antimicrobials: colistin, 4 mg/L; tigecycline, 4 mg/L; sulbactam, 8 mg/L; rifampicin, 2 mg/L.[10–12]

In vitro bactericidal activities and synergistic effects were evaluated using time–kill assays. Eight imipenem-resistant isolates were randomly selected from the 43 clinical isolates.

Aliquots of *A. baumannii* strains were cultured at 35°C in tubes containing Trypticase soy broth, bacterial inoculum at an approximate size of 10^5 cfu/mL and antibiotics to be tested. The concentrations used for colistin and tigecycline were 1×, 4× and 8× MIC. For the evaluation of synergistic effects, imipenem and sulbactam were combined at 1× MIC each and colistin and rifampicin at 1× MIC each. At 0, 4, 8 and 24 h of incubation at 35°C, aliquots of 100 μL were obtained from each tube and 10-fold dilutions were made and cultured on Trypticase soy agar plates for 24 h at 35°C. The number of colonies formed was counted. Bactericidal activity was defined as a 3 $\log_{10}$ decrease in cfu/mL, and synergy was defined as a $\geq$2 $\log_{10}$ decrease in cfu/mL between the combination and its most active constituent.[10,13]

Antibiotic powders were obtained from their respective manufacturers: colistin methanesulphonate (X-gen Pharmaceuticals Inc., NY, USA), imipenem cilastatin (Choong-Wae Pharm, Seoul, Korea), sulbactam pivoxil (Keun-hwa Pharm, Seoul, Korea), tigecycline (Wyeth Co., MA, USA) and rifampicin (Donginbiotech Co, Seoul, Korea).

As for the above eight imipenem-resistant isolates, PCR was carried out to detect Ambler class B carbapenemase $bla_{\text{IMP-1}}$, $bla_{\text{IMP-2}}$, $bla_{\text{VIM-1}}$ and $bla_{\text{VIM-2}}$ and Ambler class D OXA-type carbapenemases. The primers used in this study are shown in Table 1. Each reaction mixture (20 μL) contained 1 μL of genomic DNA, 10 pmol of each primer, 1 U of *Taq* DNA polymerase, 0.25 mM dNTP, 10 mM Tris–HCl (pH 9.0), 40 mM KCl and 1.5 mM $MgCl_2$. For Ambler class B carbapenemases, the mixture was heated to 94°C for 5 min and then subjected to 30 cycles of 94°C for 30 s, at each specific annealing temperature (Table 1) for 1 min and 72°C for 1 min, followed by a final extension for 7 min at 72°C. PCR reactions for OXA-type carbapenemases were carried out with heating to 94°C for 5 min, followed by 30 cycles of 94°C for 30 s, 52°C for 40 s and 72°C for 50 s, and final extension for 7 min at 72°C.

Results

MICs of imipenem, colistin, tigecycline, sulbactam and rifampicin are shown in Table 2. All the isolates were resistant to imipenem but susceptible to colistin and tigecycline.

In time–kill studies, colistin showed bactericidal activity against all carbapenem-resistant strains at 4× and 8× MIC concentrations, usually beginning at 8 h after inoculation (Table 3). In comparison, tigecycline was not bactericidal, but showed good bacteriostatic activity against all carbapenem-resistant strains at any tested concentration (Table 4).

According to the time–kill synergy results, the combination of imipenem and sulbactam, both at a concentration of 1× MIC, appeared to be synergistic against most of the carbapenem-resistant strains after 4 h, excluding the second strain which

Table 2. Susceptibilities of 43 *A. baumannii* isolates

Antibiotics	MIC_{50} (mg/L)	MIC_{90} (mg/L)	Range (mg/L)	Susceptibility (%)		
				R	*I*	*S*
Imipenem[a]	32	32	0.5–64	100	0	0
Colistin[b]	1	1	0.5–4	0	0	100
Tigecycline[c]	2	4	1–4	0	44	56
Sulbactam[d]	16	32	8–128	30	70	0
Rifampicin[e]	4	4	2–32	95	0	5

[a]Susceptible, ≤4 mg/L; resistant, ≥16 mg/L by NCCLS.
[b]Susceptible, ≤4 mg/L; resistant, ≥8 mg/L by BSAC.
[c]Susceptible, ≤2 mg/L; resistant, ≥8 mg/L by Wyeth Research.
[d]Susceptible, ≤8 mg/L; intermediate, 16 mg/L; resistant, ≥32 mg/L by NCCLS.
[e]Susceptible, ≤2 mg/L; resistant, ≥4 mg/L by working party report of the BSAC.

failed to show synergism (Figure 1). Moreover, a bactericidal effect of imipenem/sulbactam was shown after 8 h. There was sustained synergistic inhibitory activity lasting for more than 24 h; bacterial colony counts were reduced by $\geq 2\ \log_{10}$ cfu/mL when compared with imipenem alone. Likewise, the combination of colistin and rifampicin was synergistic and bactericidal with a mean decrease of $4.29 \pm 1.47\ \log_{10}$ cfu/mL after 8 h (Figure 2).

According to the PCR results for carbapenemases, the $bla_{OXA\text{-}51}$ β-lactamase gene was detected in all eight strains, but

Table 3. Time–kill results of colistin against carbapenem-resistant *A. baumannii* strains

Strains	Concentration of colistin	Log_{10} cfu at each time after inoculation			
		0 h	4 h	8 h	24 h
1	1 × MIC	6.04	7.89	10.38	12.71
	4 × MIC	6.17	5.25	4.98	1
	8 × MIC	6.05	4.54	1	1
2	1 × MIC	6.04	6.93	7.41	12.77
	4 × MIC	6.05	1	1	1
	8 × MIC	6.11	1	1	1
3	1 × MIC	6.04	6.75	8.42	12.92
	4 × MIC	6.08	5.58	1	1
	8 × MIC	6.08	5.79	1	1
4	1 × MIC	6.02	6.11	7.22	12.46
	4 × MIC	5.91	1	1	1
	8 × MIC	5.9	1	1	1
5	1 × MIC	6.02	6.49	7.81	12.87
	4 × MIC	6	5.88	1	1
	8 × MIC	6.13	6.15	1	1
6	1 × MIC	5.99	5.79	8.39	12.55
	4 × MIC	5.87	4	1	1
	8 × MIC	5.82	1	1	1
7	1 × MIC	6.02	7.44	9.53	12.79
	4 × MIC	6.02	5.42	1	1
	8 × MIC	6	5.55	1	1
8	1 × MIC	6	7.15	9.13	12.56
	4 × MIC	5.21	4.35	1	1
	8 × MIC	5.29	4.62	1	1

Table 4. Time–kill results of tigecycline against carbapenem-resistant *A. baumannii* strains

Strains	Concentration of tigecycline	Log_{10} cfu at each time after inoculation			
		0 h	4 h	8 h	24 h
1	1 × MIC	5.97	5.59	5.35	4.18
	4 × MIC	5.97	5.75	5.46	4.54
	8 × MIC	5.97	5.55	5.28	4.3
2	1 × MIC	5.99	5.75	5.75	4.39
	4 × MIC	6	5.83	5.56	4.85
	8 × MIC	5.95	5.76	5.62	4.69
3	1 × MIC	6.06	6.08	5.88	4
	4 × MIC	6.06	6.01	5.85	4.3
	8 × MIC	6.06	5.98	5.63	4.39
4	1 × MIC	6.04	6.06	5.96	5.11
	4 × MIC	6.04	6.15	6.02	5.07
	8 × MIC	6.04	6.09	6.01	5
5	1 × MIC	6	5.96	5.31	4.1
	4 × MIC	6.02	5.79	5.63	4.39
	8 × MIC	6.04	5.89	5.69	4.3
6	1 × MIC	5.98	5.76	5.71	4.48
	4 × MIC	6	5.73	5.55	4.54
	8 × MIC	6.02	5.75	5.46	4.47
7	1 × MIC	6.06	6.04	5.98	5.26
	4 × MIC	6	5.95	5.86	5.46
	8 × MIC	6.05	5.99	5.88	5.51
8	1 × MIC	5.93	5.82	5.19	4
	4 × MIC	5.96	5.98	5.61	4
	8 × MIC	5.97	5.88	5.71	4

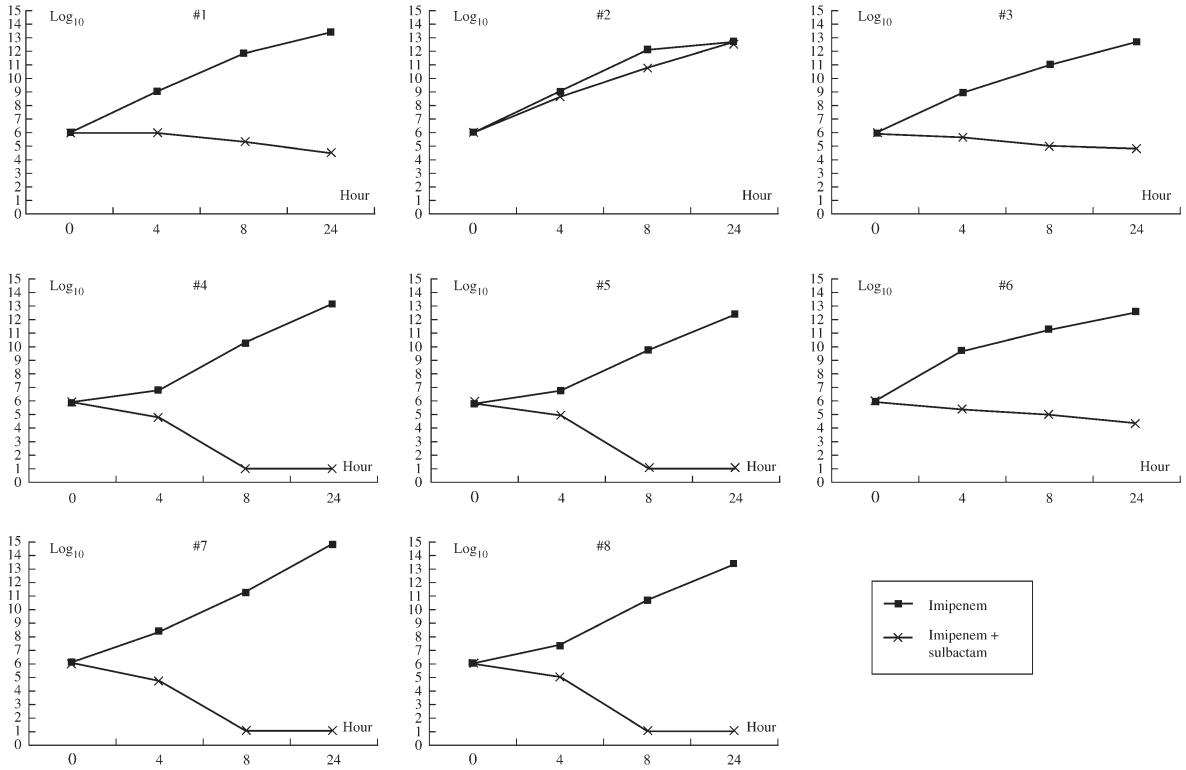

Figure 1. Time–kill curves showing synergistic effect of imipenem/sulbactam combination compared with imipenem alone against carbapenem-resistant *A. baumannii* strains; viable bacterial counts were reduced by at least ≥ 2 $\log_{10}$ cfu/mL compared with imipenem alone.

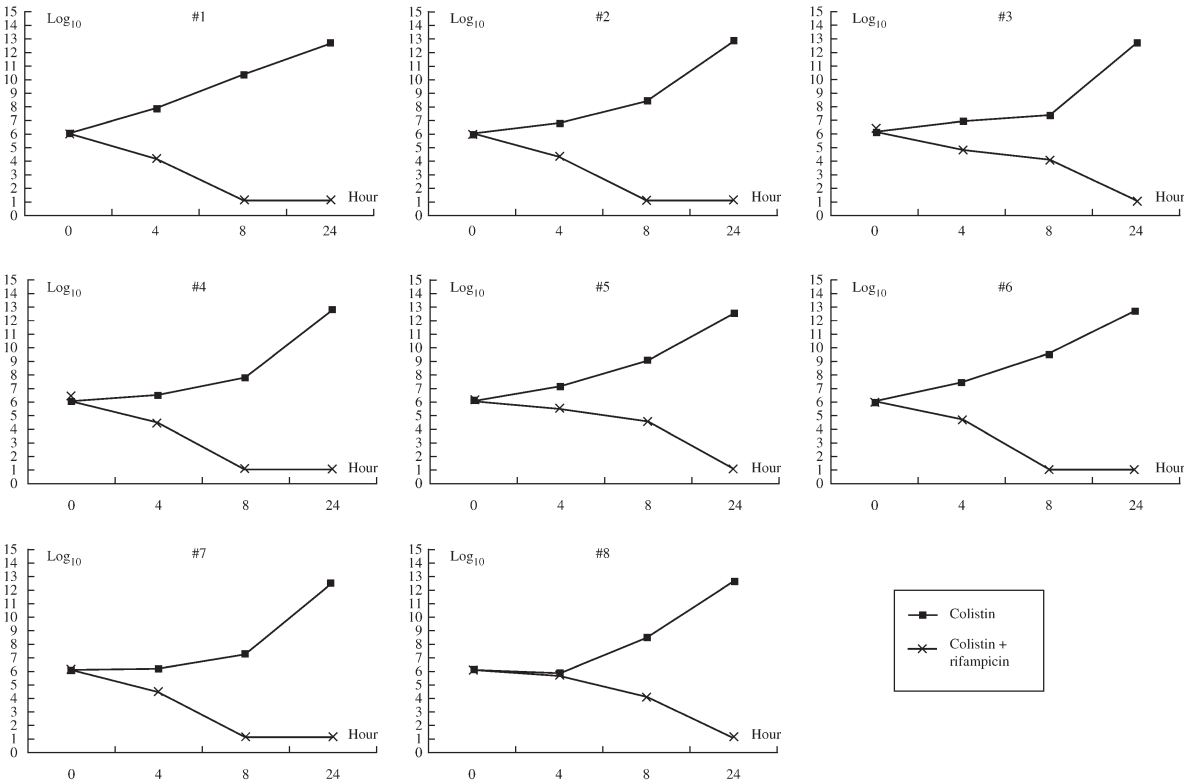

Figure 2. Time–kill curves showing synergistic effect of colistin/rifampicin combination compared with colistin alone against carbapenem-resistant *A. baumannii* strains; viable bacterial counts were reduced by at least ≥ 2 $\log_{10}$ cfu/mL compared with colistin alone.

PCR results for other OXA β-lactamase (OXA-23, -24 and -58) and Ambler class B carbapenemase (bla_{IMP-1}, bla_{IMP-2}, bla_{VIM-1} and bla_{VIM-2}) genes were all negative.

Discussion

Nowadays, the rate of carbapenem resistance among nosocomial *A. baumannii* isolates is high, particularly in ICUs. Increasing rates of carbapenem resistance have led to widespread use of colistin for the treatment of diverse infectious disease due to *A. baumannii*. However, colistin-resistant *A. baumannii* has been reported recently, and heterogeneous colistin resistance among multidrug-resistant isolates has been reported.[14] Moreover, according to the results of this study, colistin showed a bactericidal effect only at a high concentration, above 4× MIC, which is clinically important considering the dose-dependent nephrotoxicity of colistin.[15] Although some studies have reported clinical efficacy of colistin, the effects of combined antimicrobial agents or antimicrobial susceptibility were not considered in those studies.[16,17] In contrast, Motaouakkil *et al.*[18] reported a favourable clinical response to a colistin- and rifampicin-combined regimen against multidrug-resistant *A. baumannii*. Colistin is a concentration-dependent antimicrobial agent, and it is known to show only a modest post-antibiotic effect against *A. baumannii* at high concentrations.[15] In this study, we could see the synergistic and bactericidal effects of the colistin- and rifampicin-combined regimen just at the MIC level, which was sustained for ≥24 h.

Synergistic activity of imipenem and sulbactam against *A. baumannii* has been reported previously, and included two imipenem-resistant strains.[5] In the present study, we again showed the synergistic and bactericidal effects of this regimen even against carbapenem-resistant strains. This synergistic activity might be related to the concentration of bacterial penicillin-binding protein 2.[5] However, there is a chance that those effects can differ according to the resistance mechanisms; serine-β-lactamase, metallo-β-lactamase (IMP and VIM type), efflux pumps and outer membrane protein change.[19] Especially, VIM-2 β-lactamase in *A. baumannii* from Korea showed significantly high level resistance to carbapenem. Actually, the imipenem/sulbactam combination failed to show synergism against one of the carbapenem-resistant strains in this study, which had a higher MIC of imipenem (≥64 mg/L) when compared with the strains. Likewise, colistin did not show a bactericidal effect in some strains (strains 1, 3, 5, 7 and 8) at 4× and 8× MIC concentrations after 4 h from bacterial inoculation. Although we could not find a difference in carbapenemase production among study strains, the therapeutic effectiveness of antibiotic regimens might differ according to the diverse mechanisms of antimicrobial resistance; further studies are required.

Regarding tigecycline, it is very active against *Acinetobacter* spp. (generally >90% susceptibility, with MIC <8 mg/L).[12] Previously, Pachon-Ibanez *et al.*[9] reported that tigecycline has good *in vitro* bacteriostatic activity against *A. baumannii*, including one imipenem-intermediate and one imipenem-resistant strain. Likewise in this study, tigecycline showed favourable bacteriostatic activity against all carbapenem-resistant strains even at the MIC level, which was sustained for more than 24 h. Although tigecycline has not been registered in many countries yet, it is thought to be clinically useful, having good tissue penetration. Tigecycline-based combination regimens are expected to be investigated and applied clinically.

In conclusion, imipenem/sulbactam combination, colistin and tigecycline showed good *in vitro* activities against carbapenem-resistant *A. baumannii* isolates. Even though colistin is bactericidal against carbapenem-resistant *Acinetobacter* spp., the colistin/rifampicin combination is more warranted in order to be certain.

Transparency declarations

None to declare.

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