Antibacterial Activity of Carbapenem-Based Combinations Againts Multidrug-Resistant *Acinetobacter baumannii*

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Background: *Multidrug-resistant (MDR) Acinetobacter baumannii are increasingly encountered and frequently susceptible only to colistin with their MIC values close to resistance breakpoint. Antibacterial activity of two carbapenem-based combinations were explored in order to overcome the bacterial resistance.*
Material and Method: *Thirty clinical isolates of MDR A. baumannii were employed to assess in vitro antibacterial activity of two carbapenem-based regimens. Imipenem combined with colistin and meropenem combined with colistin and sulbactam were the first and second regimens, respectively. All isolates were resistant to imipenem (MIC range: 8-128 μg/ml) and meropenem (MIC range: 64-256 μg/ml) but still susceptible to colistin (MIC range: 0.5-2 μg/ml). The MIC range of sulbactam was 4-64 μg/ml. None of the isolates produced metallo-β-lactamase.*
Results: *Synergistic antibacterial effect of imipenem combined with colistin was observed against 100 percent of A. baumannii isolates by the checkerboard microdilution panel method. In a subsequent time kill study, the most active concentration of this regimen was the combination of imipenem at the fixed concentration of 32 μg/ml and colistin at the 1/4 of the MIC values of each isolate that exerted significantly higher bactericidal activity than imipenem at 32 μg/ml alone and colistin alone at the 1/4 of the MIC values. The scanning electron micrographs demonstrated major cell morphological change and cell wall destruction after 2-hour exposure to this combination. The triple combinations of meropenem, sulbactam and colistin showed synergy against 96.7 percent of MDR A. baumannii while double combinations of either meropenem and sulbactam, meropenem and colistin, and sulbactam and colistin showed synergy effects of 70%, 73.3% and 53.3%, respectively. The time kill study using ten isolates also showed better killing effect by the triple combination than any of the double combinations.*
Conclusion: *Antibacterial activity against MDR A. baumannii of imipenem plus colistin was superior over any single of the two agents. The addition of sulbactam to meropenem and colistin may further improve their antibacterial activity. The double or triple carbapenem-based combinations offer promising alternatives in the treatment of infections due to MDR A. baumannii.*

Keywords: *Acinetobacter baumannii, Imipenem, Meropenem, Colistin, Sulbactam, Antimicrobial combination, Multidrug-resistance, Synergy*

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Multi-drug resistant (MDR) *Acinetobacter baumannii* currently causes significant nosocomial infections[1], particularly in intensive care unit (ICUs) and often leaves clinicians with colistin and tigecycline to be chosen as empiric antimicrobials[2]. However, reports of therapeutic failures with monotherapy or higher adverse drug reactions[3,4] have been reported with the use of either antimicrobial.

At present, carbapenem still plays a crucial role in the treatment of various nosocomial infections though rising carbapenem resistance among the gram-negative isolates has been observed[5]. *A. baumannii* are the ones among the most resistant gram-negative nosocomial isolates and exhibit low to moderate carbapenem resistance, most of which do not produce

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metallo-beta-lactamase. It is anticipated that the addition of other active antimicrobials to carbapenem may save its role as a core antimicrobial to fight against infections due to MDR *A. baumannii*. In this regard, colistin is a preferred choice due to its broad-spectrum activities against many resistant gram-negative bacilli. Sulbactam is another interesting agent that has intrinsic activity against *A. baumannii.* The role of combination antimicrobial therapy for MDR *Acinetobacter baumannii* with carbapenem and colistin or sulbactam has been studied *in vitro* or in animals, for possibly synergistic effect[6-11] but to the authors' knowledge, this type of study has not been described for clinical isolates of MDR *A. baumannii* in Thailand. The authors aimed to evaluate the *in vitro* antibacterial activities of double and triple combination regimens against Thai MDR *A. baumannii* isolates by using MICs determination, synergy and time-kill studies, as well as the demonstration of bacterial damage using scanning electron microscopy.

Material and Method

Microorganisms

Thirty clinical isolates of *A. baumannii*, all identified by API20NE strips (BioMerieux Inc, France), were collected from different patients at Siriraj Hospital between January and December 2006. The molecular typing of the strains was performed using a fingerprint patterns obtained from Randomly Amplified Polymorphic DNA analysis. *Escherichia coli* ATCC 25922 was used as quality control strain.

Susceptibility testing

Susceptibility testing was performed using cefepime (30 μg), ceftazidime (30 μg), ciprofloxacin (5 μg), rifampin (5 μg), piperacillin/tazobactam (100 μg), gentamicin (10 μg), tobramycin (10 μg), amikacin (30 μg), imipenem (10 μg), and colistin (10 μg) [BBL chemical, USA] by the disk diffusion method, according to NCCLS (2004) guidelines[12].

MIC values of imipenem, meropenem, colistin and sulbactam were determined by agar dilution method according to NCCLS (2004)[12]. Standard powders of imipenem/cilastatin for injection [MSD Ltd, Thailand] with potency 463 μg of imipenem/463 μg of cilastatin/mg, meropenem [Siam Bheasach Co, Ltd, Thailand] with potency 717.3 μg/mg, colistin [Atlantic Pharmaceutical Co., Ltd, Thailand] with potency 437.8 μg/mg, and sulbactam [Siam Bheasach Co, Ltd, Thailand] with potency 891.5 μg/mg were used. Working antimicrobial agents solutions (ranged from 0.03-256 μg/ml) were prepared immediately prior to use, as specified by the manufacturers.

Multidrug resistant strains (MDR) detection

The MIC values for imipenem, ciprofloxacin, piperacillin/tazobactam, cefepime and amikacin [AB Biodisk, Solna, Sweden] were determined by E-test according to CLSI (2006)[13]. A strain was classified to be MDR when it was resistant to three or more of the agents tested[14].

Synergism testing

The synergistic effect was determined for double combinations (imipenem and colistin, meropenem and sulbactam, meropenem and colistin, sulbactam and colistin) and triple combination (meropenem, colistin, and sulbactam) by the checker-board method modified from Eliopoulos and Moellering[15] and Yoon et al[7]. The MICs and fractional inhibitory concentrations (FICs) were determined after 24 hours of growth. The MIC was defined as the lowest drug combination at which no visible growth was observed in the well in the microtiter plate. The following formulas were used to calculate the FIC and FIC index:

FIC of imipenem = MIC of imipenem in combination/ MIC of imipenem alone

FIC of colistin = MIC of colistin in combination/MIC of colistin alone

FIC of meropenem = MIC of meropenem in combination/ MIC of meropenem alone

FIC of sulbactam = MIC of sulbactam in combination/ MIC of sulbactam alone

FIC index (FICI) = Sum of the FICs of each antimicrobial agent

The results were interpreted as synergy, additive and antagonism if the FICI were less than 1.0, 1.0, and over1.0, respectively.

Time-kill study

In vitro bactericidal activities were evaluated using time kill technique according to NCCLS (1999)[16]. The concentration of each agent used in the time-kill studies was selected according to the average achiev-able serum concentration in human with standard dosing: imipenem 32 μg/ml, meropenem 50 mg/mL, sulbactam 30 mg/mL, while colistin concentrations combined with imipenem were 1/16 and 1/4 of the MIC values (average MIC value against 15 strains of MDR *A. baumannii* that previously showed the synergistic effect with imipenem), and when combined with meropenem and sulbactam, was 0.5 mg/mL. These strains

were not clonally related by genotypic method (data not shown here). Fifteen isolates which demon-strated synergistic effects, were included in the studies for double combination and 10 selected isolates for triple combination.

Bactericidal activity was defined as a decrease of at least 3 $\log_{10}$ CFU/mL in the viable cell counts with respect to the original inoculum (99.9% killing) and bacteriostatic activity was defined as a decrease of less than 3 $\log_{10}$ CFU/mL in viable cell counts (90-99% killing). The regrowth was defined as an increase of pathogen by at least 2 $\log_{10}$ CFU/ml after at least 6 hours of incubation[17,18].

Morphological change

Scanning electron microscopy was chosen to examine the morphological changes in the selected MDR *A. baumannii* strain no. 29 which was cultured in media containing imipenem 32 μg/ml, colistin at 1/4 of the MIC value and the combination of the two drugs for 2 hours. The cultures were collected, fixed with a graded series of ethanol, allowed to dry and then coated with gold. Bacterial cell morphology were observed under a scanning electron microscope [JEOL, model JSM-5410LV][19].

Metallo β-lactamase(MBL) producton

The detection of MBL production was performed using double disks (ceftazidime/EDTA disk and ceftazidime disk) diffusion test[12,20].

Statistical analysis

ANOVA was used to compare the BA_{24} (Bacteriolytic area for 24 hours). Any value of P below 0.05 was significant.

Results

Susceptibility of the tested strains

The MIC_{50} and MIC_{90} values of imipenem, meropenem, colistin and sulbactam were 32, 64; 128, 128; 1, 2; 32, 32 ug/mL, respectively. The MIC values and percentage of MIC distributions of each anti-microbial against all *A. baumannii* strains are shown in Table 1. The molecular typing of the present study strains revealed eight different genotypic strains.

MDR strains

Using E-test method, the MIC_{50} and MIC_{90} values of imipenem, ciprofloxacin, piperacillin/tazobactam, cefepime and amikacin against 30 isolates of *A. baumannii* were 32, 64, > 32, > 32, > 256, > 256, 32, > 256, 24, 256 ug/mL, respectively. Hence, all strains were resistant to imipenem, ciprofloxacin and piperacillin/tazobactam. Only 3.3 and 36.7 percents of the isolates were susceptible to cefepime and amikacin but all isolates were susceptible to colistin. In addition, resistance to ceftazidime, rifampicin and tobramycin were found in 76.7, 56.7 and 70 percents respectively (data not shown). The MIC determination study indicated these isolates were all MDR strains.

Metallo-β-lactamase producers

All 30 strains of carbapenem-resistant *A. baumannii* did not produce metallo-β-lactamase.

Synergistic effect

The results of the checkerboard synergy study of the double and triple combinations for synergistic, additive and antagonistic effects among the 30 strains of *A. baumannii* are shown in Table 2. For double combination, the synergistic effects were achieved with imipenem plus colistin, meropenem plus sulbactam, meropenem plus colistin, sulbactam plus colistin in 100, 70, 73.3 and 53.3 percent, respectively whereas triple combination (meropenem, sulbactam and colistin) produced synergy in 96.7 percent. Antagonism was only detected with the combinations of meropenem and colistin, sulbactam and colistin in two (6.7%) and four (13.3%) isolates, respectively.

Time-kill study and morphology changes

Imipenem alone at the concentration of 32 μg/ml was able to express bactericidal effect in only one strain and regrowth after 24 hours of incubation was detected in 7 strains. The sub-MICs of colistin alone were unable to produce bacteriostatic or bactericidal activity during the present study time and regrowth of all 15 strains were found at 24th hour of colistin incubation. The combination of imipenem at 32 μg/ml and colistin at 1/16 of the MIC expressed bactericidal activity in two strains (13.3%) at the 8th hour and 4 strains (26.7%) at the 24th hour of incubation. However, bactericidal activity occurred faster and greater when 1/4 MIC of colistin was substituted for 1/16 MIC. This combination expressed bactericidal at 2 hours of growth in 1 strain (6.7%) and at 4th, 6th, 8th and 24th hour of growth in 1(6.7%), 4 (26.7%), 4 (26.7%), and 10 strains (66.7%), respectively. Regrowth was found in four strains incubated with the combination of imipenem and colistin at 1/16 MIC and 2 strains with the combination of imipenem and colistin 1/4 MIC. The amounts of bacteria killed by the combination of

Table 1. Distribution of minimal inhibitory concentration (MIC) of MDR *A. baumannii* (n = 30) for imipenem, meropenem, colistin and sulbactam with MIC_{50} and MIC_{90} of each antimicrobial determined by agar dilution technique

Imipenem		Meropenem		Colistin		Sulbactam	
MIC (ug/ml)	%	MIC (ug/ml)	%	MIC (ug/ml)	%	MIC (ug/ml)	%
0.03	0	0.03	0	0.03	0	0.03	0
0.06	0	0.06	0	0.06	0	0.06	0
0.12	0	0.12	0	0.12	0	0.12	0
0.25	0	0.25	0	0.25	0	0.25	0
0.5	0	0.5	0	0.5	3.3	0.5	0
1	0	1	0	1	53.3	1	0
2	0	2	0	2	43.3	2	0
4	0	4	0	4	0	4	3.3
8	3.3	8	0	8	0	8	16.7
16	13.3	16	0	16	0	16	16.7
32	66.7	32	0	32	0	32	60
64	13.3	64	40	64	0	64	3.3
128	3.3	128	56.7	128	0	128	0
256	0	256	3.3	256	0	256	0
MIC_{50} = 32 MIC_{90} = 64		MIC_{50} = 128 MIC_{90} = 128		MIC_{50} = 1 MIC_{90} = 2		MIC_{50} = 32 MIC_{90} = 32	

Table 2. Antibacterial effects of double and triple combinations of imipenem, meropenem, colistin, and sulbactam against 30 strains of MDR *A. baumannii*

Antimicrobial combinations	Synergy (ΣFIC < 1)	Additive (ΣFIC = 1)	Antagonism (ΣFIC > 1)
Imipenem + colistin	30/30 (100%)	0/30 (0%)	0/30 (0%)
Meropenem + sulbactam	21/30 (70%)	9/30 (30%)	0/30 (0%)
Meropenem + colistin	22/30 (73.3%)	6/30 (20%)	2/30 (6.7%)
Sulbactam + colistin	16/30 (53.3%)	10/30 (33.3%)	4/30 (13.3%)
Meropenem + sulbactam + colistin	29/30 (96.7%)	1/30 (3.3%)	0/30 (0%)

imipenem 32 μg/ml plus colistin at 1/16 of the MIC were significantly higher than those killed by colistin alone at 1/16 and 1/4 MICs but not significantly higher than those killed by imipenem alone at 32 μg/ml while the amounts of bacteria killed by the combination of imipenem 32 μg/ml plus colistin at 1/4 MIC were significantly higher than those killed by colistin alone at 1/16 and 1/4 of the MICs and imipenem alone at 32 μg/ml. The details of extent of bacterial killing, the mean $\log_{10}$ decrease of viable cell count and bacteriolytic area for 24-hour incubation (BA_{24}) at each interval are shown in Tables 3, 4 and Fig. 1. Morphology change at two hours after exposure to imipenem at 32 μg/ml, colistin at 1/4 MIC and combination of imipenem at 32 μg/ml and colistin at 1/4 MIC revealed outpouchings of spherical surface and numerous protrusions on the cell surface that caused significant damage of the bacteria (Fig. 3C, 3D).

Similarly, colistin alone at 0.5 μg/ml reduced the initial inoculums during the first 8 hours in some strains but the bacteriostatic or bactericidal effects were subsequently abolished after 24 hours of incubation and regrowth was detected in 8 strains. However, colistin at 0.5 μg/ml expressed greater antibacterial activity than either meropenem at 50 μg/ml or sulbactam at 30 μg/ml. Bactericidal and bacteriostatic activities exerted by meropenem plus sulbactam during the first 8 hours of incubation were also not sustainable for most strains after 24 hours and regrowth was detected in 8 strains. The combinations of meropenem and sul-bactam or sulbactam and colistin exerted antibacterial activity significantly

Table 3. Extent of bacterial killing exerted by imipenem, colistin and the combinations over time against 15 selected strains of MDR *A. baumannii*

Antimicrobial agent and concentration (μg/mL)	No. of strains killed* at each time point																
	2 hours			4 hours			6 hours			8 hours				24 hours			
	-1	-2	-3	-1	-2	-3	-1	-2	-3	-1	-2	-3	R**	-1	-2	-3	R**
Imipenem 32 μg/ml	1	1	-	6	5	-	3	6	-	-	7	1	-	2	3	1	7
Colistin 1/16 MIC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	15
Colistin 1/4 MIC	-	-	-	1	-	-	2	-	-	1	-	-	-	-	-	-	15
Imipenem 32 μg/ml + colistin 1/16 MIC	5	3	-	7	6	-	6	7	-	4	7	2	-	2	4	4	4
Imipenem 32 μg/ml + colistin 1/4 MIC	9	2	1	7	7	1	2	9	4	2	8	4	-	1	2	10	2

* -1 = 90% of viable cell reduction versus initial inoculum; -2 = 99% of viable cell reduction versus initial inoculum; -3 = 99.9% of viable cell reduction versus initial inoculum
** R= regrowth

Table 4. Mean log change of viable counts at various time intervals, $AUBKC_{0-24}$ and BA_{24} after exposure to imipenem, colistin and the combination in 15 selected strains of MDR *A. baumannii*

Condition	Mean (± SD) log change of viable cell counts					Mean (± SD) $AUBKC_{0-24}$	Mean (± SD) BA_{24}
	Δ2	Δ4	Δ6	Δ8	Δ24		
Control	1.40 ± 0.59	2.74 ± 0.95	3.61 ± 0.99	4.34 ± 0.92	9.24 ± 1.29	304.08 ± 24.63	-
Imi 32 μg/ml	-0.91 ± 0.60	-1.31 ± 1.11	-1.15 ± 0.76	-1.05 ± 1.99	2.23 ± 4.89	176.42 ± 64.94	127.66 ± 58.61
Col 1/16 MIC	0.82 ± 0.64	2.34 ± 0.77	3.09 ± 0.76	3.48 ± 0.83	8.24 ± 1.15	284.38 ± 19.03	19.70 ± 10.75
Col 1/4 MIC	0.58 ± 0.82	1.40 ± 1.23	2.14 ± 1.56	2.69 ± 1.54	7.31 ± 2.36	265.82 ± 44.18	38.27 ± 33.74
Imi 32 μg/ml + Col 1/16MIC	-1.32 ± 0.77	-1.61 ± 0.95	-1.79 ± 1.28	-1.89 ± 1.66	-0.40 ± 4.65	145.99 ± 61.67	158.12 ± 55.12[b,c]
Imi 32 μg/ml + Col 1/4 MIC	-1.54 ± 0.74	-2.12 ± 0.81	-2.63 ± 0.80	-2.87 ± 0.98	-3.34 ± 2.71	110.36 ± 35.07	193.72 ± 36.81[a,b,c]

[a] = $p < 0.05$ compared to activity of imipenem 32 μg/ml alone
[b] = $p < 0.05$ compared to activity of colistin 1/16 MIC alone
[c] = $p < 0.05$ compared to activity of colistin 1/4 MIC alone
Δ = Mean log change viable cell counts at 2, 4, 6, 8 and 24 hours, respectively
$AUBKC_{0-24}$ = Area under bacterial killing and regrowth curves for 24 hours
BA_{24} = Bacteriolytic area for 24 hours
Imi = imipenem, Col = colistin
MIC = minimal inhibitory concentration

greater than meropenem alone. In addition, the combinations of meropenem and colistin or meropenem, colistin and sulbactam exerted antibacterial activity significantly greater than either sulbactam or meropenem alone. Maximal bactericidal effect was observed with the three combinations: meropenem plus colistin, sulbactam plus colistin, and meropenem plus colistin and sulbactam. Details of extent of bacterial killing, the mean $\log_{10}$ decrease of viable cell count and bacteriolytic area for 24-hour incubation (BA_{24}) after exposure to each antimicrobial and their combinations at each interval are shown in Tables 5 and 6. Cellular disruptions and release of intra-cellular materials after exposure to meropenem plus colistin (Fig. 4A), and meropenem plus colistin and sulbactam (Fig. 4B) are clearly shown.

Hence, the present study confirmed that colistin plus imipenem or meropenem was more active

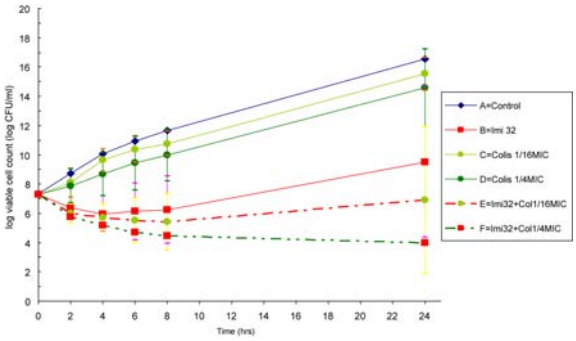

Fig. 1 Average time-kill curve showing the antibacterial activity of the combinations of imipenem and colistin against 15 strains of MDR *A. baumannii*, Data are means ± SD (error bars)
Abbreviation: Imi 32 = imipenem at 32 μg/ml; colis 1/16MIC = colistin at concentration of 1/16 of MIC value; colis 1/4MIC = colistin at concentration of 1/4 of MIC value

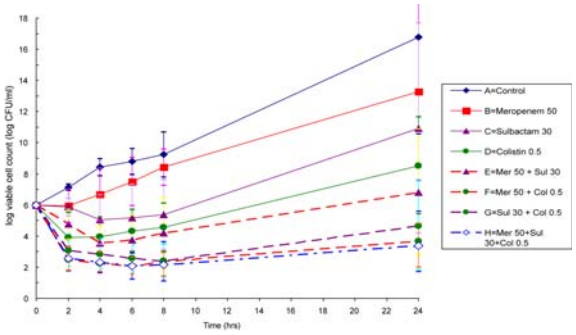

Fig. 3 Average time-kill curve showing the antibacterial activity of the combinations of meropenem, colistin, and sulbactam against 10 selected strains of MDR *A. baumannii*. Data are means ± SD (error bars).
Abbreviation: Mer 50 = meropenem 50 μg/ml; col 0.5 = colistin 0.5 μg/ml; sul 30 = sulbactam 30 μg/ml

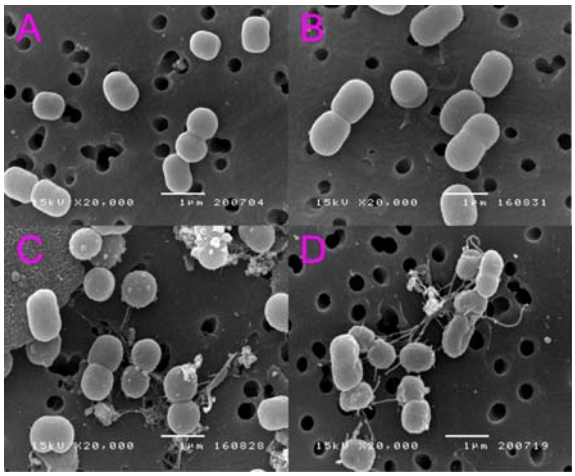

Fig. 2 Scanning electron micrographs of *A. baumannii* strain no. 29 after 2 hours exposure to (A) no antibiotic, (B) colistin 1/4 MIC [0.25 μg/ml], (C) imipenem [32 μg/ml] and (D) combination of imipenem and colistin. Each bar indicates 1 μm

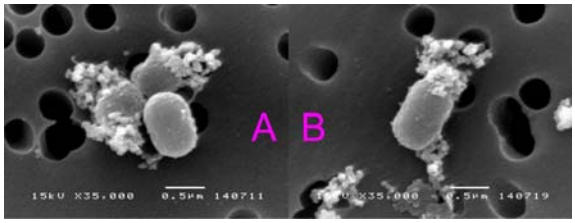

Fig 4. Scanning electron micrographs of *A. baumannii* strain no.29 after 2 hours exposure to double combination of meropenem 50 μg/ml + colistin 0.5 μg/ml (A) and triple combination of meropenem 50 μg/ml, sulbactam 30 μg/ml and colistin 0.5 □g/ml (B) showed cellular disruption and release of intra-cellular materials

than colistin alone or meropenem plus sulbactam against MDR *A. baumannii*. The addition of sulbactam to carbapenem plus colistin may offer little benefit in bacterial killing or might prevent development of resistant strain after prolonged exposure to the combination.

Discussion

The global emergence of MDR *A. baumannii* and other gram-negative bacilli is a frightening reality and has spurred interest in finding a treatment strategy that leads to a more effective therapy. Infections caused by MDR *A. baumannii* tend to occur in immuno-suppressed patients, in patients with serious underlying diseases, and in those subjected to invasive procedures and treated with broad-spectrum antibiotics and are associated with high mortality rate. Combination antimicrobial therapy with bactericidal activity is a common strategy often employed in an attempt to ensure reliable synergy or additive effects for the treatment of MDR *A. baumannii* infections and may reduce emergence of resistant strains during treatment. Colistin stands out as a reliable antimicrobial since its recent use was effective and still safe for the treatment of patients infected with MDR gram-negative bacteria[21-24]. However, report of resistance emergence

Table 5. Extent of bacterial killing exerted by several antimicrobial agents and their combinations over time against 10 selected strains of MDR *A. baumannii*

Antimicrobial agent and concentration (μg/mL)	No. of strains* killed at each time point																
	2 hours			4 hours			6 hours			8 hours				24 hours			
	-1	-2	-3	-1	-2	-3	-1	-2	-3	-1	-2	-3	R	-1	-2	-3	R**
Meropenem 50 μg/ml	3	-	-	2	-	-	1	-	-	-	-	-	-	-	-	-	5
Sulbactam 30 μg/ml	-	-	-	4	3	-	2	4	-	-	2	2	-	-	-	-	9
Colistin 0.5 μg/ml	3	1	3	3	1	4	2	2	2	3	3	1	-	-	-	-	8
Meropenem 50 μg/ml + sulbactam 30 μg/ml	4	3	-	-	4	4	-	4	4	1	1	4	-	-	-	2	8
Meropenem 50 μg/ml + colistin 0.5 μg/ml	-	4	7	-	1	9	1	-	9	1	-	8	1	1	1	5	4
Sulbactam 30 μg/ml + colistin 0.5 μg/ml	2	3	5	2	2	6	1	1	8	-	-	9	-	1	1	3	6
Meropenem 50 μg/ml + sulbactam 30 μg/ml + colistin 0.5 μg/ml	-	3	7	-	2	8	-	2	8	1	1	8	-	1	3	3	2

* -1 = 90% of viable reduction versus initial inoculum; -2 = 99% of viable reduction versus initial inoculum; -3 = 99.9% of viable reduction versus initial inoculum
** R= regrowth

Table 6. Mean log change of viable counts at various time intervals, $AUBKC_{0-24}$ and BA_{24} after exposure to meropenem, sulbactam, colistin and their combinations in 10 selected strains of MDR *A. baumannii*

Condition	Mean (± SD) log change of viable cell counts					Mean (± SD) $AUBKC_{0-24}$	Mean (± SD) BA_{24}
	Δ2	Δ4	Δ6	Δ8	Δ24		
Control	1.14 ± 0.35	2.43 ± 0.65	2.80 ± 0.91	3.25 ± 1.49	10.73 ± 6.32	271.36 ± 59.67	
Mer 50 μg/ml	-0.05 ± 1.18	0.69 ± 1.76	1.52 ± 1.61	2.43 ± 1.22	7.29 ± 5.72	228.03 ± 56.94	45.72 ± 40.25
Sul 30 μg/ml	-0.13 ± 0.58	-0.93 ± 1.63	-0.93 ± 1.63	-0.61 ± 2.26	4.94 ± 6.76	173.85 ± 79.62	113.10 ± 63.39
Col 0.5 μg/ml	-2.06 ± 1.45	-2.04 ± 1.19	-2.04 ± 1.19	-1.42 ± 1.44	2.55 ± 3.12	139.70 ± 38.08	121.29 ± 73.25
Mer 50 μg/ml + Sul 30 μg/ml	-1.23 ± 1.00	-2.42 ± 1.16	-2.42 ± 1.16	-1.80 ± 2.34	0.84 ± 4.10	122.31 ± 57.19	160.29 ± 60.45[a]
Mer 50 μg/ml + Col 0.5 μg/ml	-3.48 ± 0.69	-3.74 ± 0.62	-3.74 ± 0.62	-3.61 ± 1.31	-2.32 ± 2.05	70.19 ± 26.73	204.18 ± 53.70[a,b]
Sul 30 μg/ml + Col 0.5 μg/ml	-2.91 ± 0.96	-3.11 ± 0.90	-3.11 ± 0.90	-3.56 ± 1.11	-1.33 ± 2.88	81.99 ± 35.15	192.20 ± 56.97[a]
Mer 50 μg/ml + Sul 30 μg/ml + Col 0.5 μg/ml	-3.41 ± 0.73	-3.64 ± 0.63	-3.64 ± 0.63	-3.81 ± 0.84	-2.59 ± 1.51	66.68 ± 17.67	204.68 ± 57.86[a,b]

[a] = $p < 0.05$ compared to activity of meropenem alone
[b] = $p < 0.05$ compared to activity of sulbactam alone
Δ = Mean log change viable cell counts at 2, 4, 6, 8 and 24 hours, respectively
$AUBKC_{0-24}$ = Area under bacterial killing and regrowth curves for 24 hours
BA_{24} = Bacteriolytic area for 24 hours
Mer = meropenem, Col = colistin, Sul = sulbactam

during colistin treatment and its potential toxicity lead to the use of combination antimicrobial rather than elevation of the dosage of colistin alone. Antimicrobial frequently used for this purpose is a carbapenem such as imipenem, meropenem or doripenem which still has assumed an important antibiotic niche for

therapy of various MDR gram-negative infections with good safety profile. Sulbactam is another interesting antimicrobial that possesses intrinsic antibacterial activity against *A. baumannii* with minimal side effect.

The present study revealed that monotherapy of either imipenem, meropenem, colistin or sulbactam for MDR *A. baumannii* bacteremia or infection is probably inadequate. Unsustainable antibacterial activity and regrowth at 24 hours after incubation had been found with either single agent for most MDR strains though drug concentrations at or close to therapeutic levels of each drug were used in the present study. The present study result supported previous finding of possibly synergy effect between colistin and meropenem against MDR *A. baumannii*[11]. The synergistic effect may be related to subsequent weakening of cell wall or membrane due to actions of carbapenem and colistin and results in numerous protrusions seen on electron microscopy. The addition of imipenem or meropenem to colistin dramatically improves the bactericidal effect against most MDR strains as demonstrated by the checkerboard synergy study of the double and triple combinations and morphology damage demonstrated with scanning electron microscopy. The authors believe that either imipenem, meropenem or doripenem can be used as an additional carbapenem to colistin for an effective combination since most MDR *A. baumannii* do not produce metallo-beta-lactamase[25]. Development of resistant mutant during antimicrobial therapy might also be prevented or reduced by carbapenem addition since regrowth after 24 hours of incubation was seen less with the combinations. The authors' finding implies that maximal dose of imipenem or meropenem must be used with colistin to achieve the sustainable bactericidal activity against MDR *A. baumannii* for 24 hours. The authors prefer to keep colistin used at standard dosage and optimize carbapenem dosing by using maximal therapeutic dose coupled with prolonged (4-hour) infusion time to combat these higher-MIC gram-negative organisms[26]. The prolonged infusion of carbapenem should be a conventional administration in the current era of antimicrobial resistance. The combination of anti-pseudomonas carbapenem and colistin could also be used to treat infections due to ESBL-producing enterobacteria or resistant *Pseudomonas aeruginosa.*

The addition of sulbactam to the meropenem-colistin combination is an attempt to search for more effective alternative but the present study result did not show significant superior activity to the meropenem-colistin combination. Ko WC et al demon-strated synergistic bactericidal effects in the time-kill study when meropenem at a concentration of half of the MIC (4 μg/ml) was combined with sulbactam at concentration equivalent to the MIC (8 μg/ml) and there was at least a 5 log10 reduction in bacterial colony counts after 48 hours, compared with either drug alone[27]. The authors' tested isolates were more resistant and need higher doses of meropenem and sulbactam. However, the antibacterial effect of triple combi-nation of meropenem, colistin and sulbactam was not inferior to the double combination of meropenem or imipenem and colistin. In practice, sulbactam could be added to the carbapenem-colistin combination when infection due to MDR *A. baumannii* is highly suspected either by clinical setting or gram-stain of clinical specimen. For those who are strongly allergic to beta-lactam or carbapenem, sulbactam could be substituted for carbapenem without compromising the antibacterial activity derived from the carbapenem-colistin combination. At least, the addition of sulbactam did not antagonize the bactericidal action of colistin or carbapenem plus colistin. Other combinations such as rifampicin plus imipenem or colistin would also be synergistic against *A. baumannii*[28] but their antibacterial spectrums may be inadequate to cover other resistant gram-negative bacteria.

The present study had several limitations. The result is not applicable to MDR *A. baumannii* that produces metallo-beta-lactamase. It should point out that the MDR *A. baumannii* isolates in the present study are moderately resistant to carbapenem and may behave differently to the combinations if their MICs to carbapenem are lower or fall to more susceptible range. The authors also remind that result from the in vitro study, although important, may not guide and predict successful therapy with antimicrobial combination in patients until proven in a prospective clinical trial. However, the authors do not think that such a trial is likely to be done soon. Given the ever increasing resistance of *A. baumannii* causing bacteremia and other life-threatening infections and the devastating consequences of inadequate therapy during the first 48 hours of treatment of sepsis, it is prudent to use the in vitro data to treat most of our patients with the carbapenem-based combination options discussed in order to guarantee that the patient receives at least one effective antimicrobial from the outset and that there is a reasonable likelihood of an added advantage with combination therapy.

In conclusion, the result of the presented *in vitro* study revealed the double combination of imipenem, meropenem and possible doripenem plus colistin could be a promising alternative for the treatment of infections due to MDR *A. baumannii* strains. Without elevation of colistin dosage, the combination with maximal dose of anti-pseudomonas carbapenem enhances the bactericidal effect and such approach may improve the therapeutic outcome in patients with MDR *A. baumannii* infections as well as reduceingthe toxicity of colistin. The addition of sulbactam to the carbapenem-colistin combination may further improve the strength of antimicrobial activity against MDR *A. baumannii*. The combination is also useful to treat other infections due to MDR-gram-negative bacteria. The encouraging result of the presented in vitro study supports further investigations of double or triple combinations for therapy of MDR-*A. baumannii* though a large clinical trial is needed to finally validate the role of carbapenem-based combination for the treatment of infections due to current MDR *A. baumannii.*

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ฤทธิ์ของยาต้านจุลชีพร่วมกันที่มีคาร์บาพีเนมเป็นฐานต่อเชื้อ *Acinetobacter baumannii* ที่ดื้อยาหลายขนาน

พิณทิพย์ พงษ์เพ็ชร, สุภรักษ์ อมรนพรัตนกุล, สกุลทิพย์ พนาภักดี, ศิริภรณ์ ฟุ้งวิทยา, เพ็ญพรรณ แน่นหนา, เชิดศักดิ์ ธีระบุตร, อมร ลีลารัศมี

ภูมิหลัง: *เชื้อ Acinetobacter baumannii ที่ดื้อยาต้านจุลชีพหลายขนานพบว่า ก่อโรคบ่อยขึ้น และมักจะไวต่อยาโคลิสตินขนานเดียว แต่มีค่าความเข้มข้นต่ำสุดของยาที่สามารถยับยั้งเชื้อได้ใกล้กับค่า MIC breakpoint ที่ใช้ตัดสินว่าเชื้อดื้อต่อยาขนานนี้ ดังนั้นจึงสนใจที่จะการศึกษาฤทธิ์ของยาร่วมที่มีคาร์บาพีเนมเป็นฐานในการต่อต้านเชื้อชนิดนี้ที่ดื้อยาหลายขนาน*

วัสดุและวิธีการ: *ได้ศึกษาในหลอดทดลองถึงฤทธิ์การยับยั้งเชื้อ Acinetobacter baumannii ที่ดื้อยาหลายขนาน ซึ่งแยกได้จากผู้ป่วยจำนวน 30 ราย โดยใช้อิมิพีเนมร่วมกับโคลิสติน และเมอโรพีเนมร่วมกับโคลิสติน และซัลแบคแทมเชื้อจากผู้ป่วยทั้ง 30 ราย ดื้อยาอิมิพีเนม (MIC อยู่ระหว่าง 8-128 ไมโครกรัมต่อมิลลิลิตร) และเมอโรพีเนม (MIC อยู่ระหว่าง 64-256 ไมโครกรัมต่อมิลลิลิตร) แต่ยังคงไวต่อโคลิสติน (MIC อยู่ระหว่าง 0.5-2 ไมโครกรัมต่อมิลลิลิตร) นอกจากนี้พบว่า MIC ของซัลแบคแทม อยู่ระหว่าง 4-64 ไมโครกรัมต่อมิลลิลิตร เชื้อทดสอบทั้งหมดไม่สร้างเอนไซม์เมทาโรเบตาแลคทาเมส*

ผลการศึกษา: *เมื่อให้ยาร่วมกัน 2 ขนานคือ อิมิพีเนม และโคลิสติน การทดสอบด้วยวิธี checkerboard microdilution panel พบฤทธิ์เสริมกันในการยับยั้งเชื้อที่นำมาทดสอบทั้งหมด ผลการศึกษาด้วยวิธี time kill พบว่าขนาดยารวมกันที่เหมาะสมคืออิมิพีเนมที่ความเข้มข้น 32 ไมโครกรัมต่อมิลลิลิตร และโคลิสติน ที่ความเข้มข้นเท่ากับ 1/4 ของค่า MIC ของเชื้อแต่ละตัว ภาพจากกล้องจุลทัศน์อิเลคตรอนแสดงในเห็นการเปลี่ยนแปลงสัณฐานของเซลล์อย่างชัดเจนเมื่อเชื้อสัมผัสกับยาอิมิพีเนมและโคลิสตินเป็นเวลานาน 2 ชั่วโมง เมื่อให้ยาร่วมกัน 3 ขนาน คือ เมอโรพีเนมซัลแบคแทมและโคลิสติน การทดสอบด้วยวิธี checkerboard microdilution panel พบฤทธิ์เสริมกันในการยับยั้งเชื้อร้อยละ 96.7 ในขณะที่การให้ยาร่วมกันเพียง 2 ขนาน คือ เมอโรพีเนมร่วมกับซัลแบคแทม เมอโรพีเนมร่วมกับโคลิสตินและซัลแบคแทมร่วมกับโคลิสติน เกิดการเสริมฤทธิ์กัน ในการยับยั้งเชื้อร้อยละ 70, 73.3 และ 53.3 ของเชื้อตามลำดับทั้งหมด ผลจากการศึกษาด้วยวิธี time kill กับเชื้อ 10 ตัว พบว่าฤทธิ์ในการฆ่าเชื้อของยาที่ให้ร่วมกัน 3 ขนาน สูงกว่าการให้ยาร่วมกันเพียงสองขนาน ภาพจากกล้อง จุลทัศน์อิเลคตรอนแสดงในเห็นการแตก ของเซลล์แบคทีเรียอย่างชัดเจน เมื่อเชื้อสัมผัสกับยาเมอโรพีเนม 50 ไมโครกรัม/มิลลิลิตร ร่วมกับซัลแบคแทม 30 ไมโครกรัม/มิลลิลิตร และโคลิสติน 0.5 ไมโครกรัม/มิลลิลิตร เป็นเวลานาน 2 ชั่วโมง*

สรุป: *ฤทธิ์ต้านเชื้อ A. baumannii ที่ดื้อยาหลายขนานของยาอิมิพีเนมที่ให้ร่วมกับโคลิสติน สูงกว่าฤทธิ์ที่เกิดจากยาเดี่ยวแต่ละขนาน เมื่อใช้ยาเมอโรพีเนม โคลิสติน และซัลแบคแทมร่วมกันสามขนาน อาจจะเพิ่มฤทธิ์ในการต้านเชื้อได้ดีกว่ายาร่วมกันสองขนาน ดังนั้นการใช้ยาสองหรือสามขนานร่วมกันที่มียาคาร์บาพีเนมเป็นฐานจะเป็นทางเลือกทางหนึ่งในการรักษาโรคติดเชื้อ A. baumannii ที่ดื้อยาหลายขนาน*