In Vitro Activity of Colistin plus Sulbactam against Extensive-Drug-Resistant *Acinetobacter baumannii* by Checkerboard Method

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Objective: *To determine in vitro activity of colistin plus sulbactam against extensive-drug-resistant (XDR) Acinetobacter baumannii.*

Material and Method: *Checkerboard method was used to determine in vitro activity of colistin plus sulbactam against 11 clinical isolates of XDR A. baumannii. The concentrations of colistin and sulbactam used in the study were 0.025 to 128 mg/l and 4 to 256 mg/l, respectively. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of colistin, sulbactam and colistin plus sulbactam at various concentrations were determined. Fractional inhibitory concentration index (FICI) was calculated. The antibiotic combination is considered synergistic if FICI <0.5, indifferent if FICI 0.5 to 4.0, and antagonistic if FICI >4.0.*

Results: *Ten isolates of XDR A. baumannii were susceptible to colistin (MIC <2 mg/l) and one isolate was resistant to colistin (MIC 8 mg/l). There were no antagonistic effects of colistin plus sulbactam against all study isolates. For 10 isolates of colistin-susceptible XDR A. baumannii, some MIC values of the combinations were lower than those of single antibiotics. However, no synergistic effect of colistin and sulbactam was observed in colistin-susceptible XDR A. baumannii isolates. The synergistic effect of colistin and sulbactam was detected in some concentrations of colistin and sulbactam against colistin-resistant XDR A. baumannii isolate.*

Conclusion: *The combination of colistin and sulbactam showed an indifferent effect against colistin-susceptible XDR A. baumannii. The combination of colistin and sulbactam showed synergistic effect at some concentrations of colistin and sulbactam against a clinical isolate of colistin-resistant XDR A. baumannii. In vitro time-kill method should be performed to confirm the aforementioned observations.*

Keywords: *Colistin, Sulbactam, Synergy, A. baumannii*

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Extensive-drug-resistant (XDR) *Acinetobacter baumannii* is defined as *A. baumannii* that is resistant to all but 1 or 2 antimicrobial agents including colistin and tigecycline[1]. The prevalence of XDR *A. baumannii* in Thailand has been increasing over the past decade. The prevalence of XDR *A. baumannii* in Thai patients with nosocomial pneumonia due to *A. baumannii* was 82%[2]. Colistin has been an important key antibiotic therapy for infections caused by XDR *A.baumannii* in Thailand over the past 8 years. Treatment of infections due to XDR non-fermenters at Siriraj Hospital in Bangkok, Thailand observed that the mortality of the patients who received colistin was still high, 46%[3]. One of the strategies to enhance the outcomes of therapy of infections with XDR *A. baumannii* is to use colistin in combination with other antibiotics. A review of various studies examining colistin alone versus combinations of colistin with rifampicin or carbapenem for *Pseudomonas aeruginosa* or *A. baumannii* was reported[4]. *In vitro* synergistic effect was detected in all studies examining the combination of colistin and rifampicin, whereas carbapenem exhibited a synergistic effect in two of three studies. Most of the animal studies examined colistin monotherapy versus combinations with rifampicin, carbenicillin, piperacillin and imipenem for treatment of *P. aeruginosa*, *A. baumannii* or *Escherichia coli* infections. Mortality rates were significantly lower in

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the combination treatment arm in three of six relevant studies. However, the data from the small number of relevant human studies suggested non-inferiority of colistin monotherapy as compared with combination therapy. Sulbactam, a beta-lactamase inhibitor, is found to contain intrinsic activity against some isolates of *Acinetobacter spp*.[5].

The objective of the present study is to determine *in vitro* activity of colistin in combination with sulbactam against colistin-susceptible and colistin-resistant XDR *A. baumannii* by checkerboard method.

Material and Method

Study bacteria

They were 11 clinical isolates of XDR *A. baumannii* and *E.coli* ATCC 25922 as a control isolate.

Study procedures[6]

Concentrations of antibiotics

The concentrations of each antibiotic to be used for synergy study are shown in Table 1. Many antibiotic concentrations used in this study covered achievable plasma concentrations of colistin and sulbactam in infected patients after receiving regular doses of both antibiotics. However, some concentrations of colistin and sulbactam used in this study were higher than those achievable in the plasma of the patients receiving the recommended dosages of colistin and sulbactam.

Antibiotic preparation

Two-fold serial dilutions of each antibiotic were prepared in Mueller Hinton broth (MHB) to obtain 4-time of the final concentration as shown in Table 2. In sterile 96-well U-bottom microtiter plate, 50 µl of the two-fold serial dilutions of colistin from 512 to 0.5 mg/L were put into columns 1 to 11 of the plate. The two-fold serial dilutions of sulbactam from 1,024 to 16 mg/L were dropped into row A to G of the plate. Rows H1 to H11 of the plate were colistin alone and columns 12A to 12G of the plate were sulbactam alone. Well H12 (or 12H) of the plate was the control well which was filled with 100 µl of MHB.

Table 1. Concentrations of each antibiotic used in synergy study

Antibiotic	Concentration (mg/l)
Colistin	0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128
Sulbactam	4, 8, 16, 32, 64, 128, 256

Inoculum preparation

Each studied organism was subcultured on blood agar and was kept at 35°C overnight. The inoculum was prepared by growth method in MHB and adjusted to 0.5 McFarland standards in sterile normal saline, then diluted in MHB to achieve a concentration of 10^6 CFU/ml. Each well was inoculated with 100 µl of bacterial solution, so that the final concentration of the inoculum was $5x10^4$ CFU/well. The plate was incubated at 35° C overnight.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determination

MIC was the lowest concentration of the well which was clear as judged by naked eyes. MBC was determined by culturing the fluids from the wells that were equal or higher than MIC on brain-heart infusion agars and they were incubated at 35°C overnight.

The MBC was the lowest concentration of the well which killed 99.95% of inoculated bacteria from the original inoculum size.

Interpretation of synergy test

Fractional Inhibitory Concentration Index (FICI) is calculated from the following equation.

FICI = [(MIC of colistin in combination)/(MIC of colistin alone)] + [(MIC of sulbactam in combination)/(MIC of sulbactam alone)]

The antibiotic combination is considered synergistic if FICI <0.5, indifferent if FICI 0.5 to 4.0, and antagonistic if FICI >4.0.

Results

The MICs and MBCs of colistin and sulbactam against 11 isolates of XDR *A. baumannii* and *E.coli* ATCC 25922 are shown in Table 3. Ten isolates were susceptible to colistin (MIC <2 mg/l) and one isolate was resistant to colistin (MIC = 8 mg/l**)**. There were no antagonistic effects for a combination of colistin and sulbactam against all study isolates. The selected lowest MIC values of colistin and sulbactam for antibiotic combination, and FICI of colistin and sulbactam combination against a control isolate and 11 isolates of XDR *A. baumannii* are shown in Table 4. For 10 isolates of colistin-susceptible XDR *A. baumannii* (strain 01 to 10), some MIC values of the combinations were lower than those of single antibiotics. However, no synergistic effect of colistin and sulbactam was observed in colistin-susceptible XDR *A. baumannii* isolates. The synergistic effect of

Table 2. Final antibiotic concentrations of colistin (italic) and sulbactam (bold) in each well of a 96-well microtiter plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	*128*	*64*	*32*	*16*	*8*	*4*	*2*	*1*	*0.5*	*0.25*	*0.125*	*0*
	256	**256**	**256**	**256**	**256**	**256**	**256**	**256**	**256**	**256**	**256**	**256**
B	*128*	*64*	*32*	*16*	*8*	*4*	*2*	*1*	*0.5*	*0.25*	*0.125*	*0*
	128	**128**	**128**	**128**	**128**	**128**	**128**	**128**	**128**	**128**	**128**	**128**
C	*128*	*64*	*32*	*16*	*8*	*4*	*2*	*1*	*0.5*	*0.25*	*0.125*	*0*
	64	**64**	**64**	**64**	**64**	**64**	**64**	**64**	**64**	**64**	**64**	**64**
D	*128*	*64*	*32*	*16*	*8*	*4*	*2*	*1*	*0.5*	*0.25*	*0.125*	*0*
	32	**32**	**32**	**32**	**32**	**32**	**32**	**32**	**32**	**32**	**32**	**32**
E	*128*	*64*	*32*	*16*	*8*	*4*	*2*	*1*	*0.5*	*0.25*	*0.125*	*0*
	16	**16**	**16**	**16**	**16**	**16**	**16**	**16**	**16**	**16**	**16**	**16**
F	*128*	*64*	*32*	*16*	*8*	*4*	*2*	*1*	*0.5*	*0.25*	*0.125*	*0*
	8	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**
G	*128*	*64*	*32*	*16*	*8*	*4*	*2*	*1*	*0.5*	*0.25*	*0.125*	*0*
	4	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
H	*128*	*64*	*32*	*16*	*8*	*4*	*2*	*1*	*0.5*	*0.25*	*0.125*	*Control*
	0	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	

NB = The unit of the number in each cell is mg/l

Table 3. MICs and MBCs of colistin and sulbactam against 11 study isolates of XDR *A. baumannii*

	Colistin (mg/l)		Sulbactam (mg/l)	
	MIC	MBC	MIC	MBC
XDR *A. baumannii* 01	0.5	0.5	64	128
XDR *A. baumannii* 02	0.5	0.5	32	32
XDR *A. baumannii* 03	0.5	0.5	32	32
XDR *A. baumannii* 04	1	1	32	64
XDR *A. baumannii* 05	1	1	64	128
XDR *A. baumannii* 06	1	1	64	128
XDR *A. baumannii* 07	1	1	64	128
XDR *A. baumannii* 08	1	1	64	64
XDR *A. baumannii* 09	1	1	64	128
XDR *A. baumannii* 10	2	2	64	128
XDR *A. baumannii* 11	8	8	32	32
E.coli ATCC 25922	0.5	0.5	32	32

colistin and sulbactam was, however, detected in some concentrations of colistin and sulbactam against colistin-resistant XDR *A. baumannii* isolate (strain 11).

Discussion

The main findings of the present study were that a combination of colistin and sulbactam had indifferent effects against all isolates of colistin-susceptible XDR *A.baumannii* whereas such combination had synergistic effect against colistin-resistant XDR *A. baumannii.* The previous study of in vitro activity of colistin plus sulbactam against 8 isolates colistin-susceptible carbapenem-resistant *A.baumannii* also showed no synergistic effect[7] whereas another study observed synergistic effect in 5 out of 10 study isolates of *A. baumannii*[8]. All isolates of *A. baumannii* in both aforementioned studies were susceptible to colistin. The concentrations of colistin (0.5 to 1 mg/l) and sulbactam (4 to 8 mg/l) that showed synergistic effects against colistin-resistant XDR *A. baumannii* are those that could be achieved in plasma after treatment with regular doses of colistin and sulbactam. The mechanism of synergistic effect of colistin plus sulbactam against colistin-resistant XDR

Table 4. MICs and MBCs of colistin and sulbactam, and the selected lowest MICs of colistin and sulbactam for antibiotic combination, and the FICI of colistin and sulbactam combination against a control isolate (*E.coli* ATCC 25922) and 11 isolates of XDR *A.baumannii*

	Colistin		Sulbactam		Antibiotic Combination		
	MIC (mg/l)	MBC (mg/l)	MIC (mg/l)	MBC (mg/l)	Colistin MIC (mg/l)	Sulbactam MIC (mg/l)	FICI
Control Isolate							
E.coli ATCC 25922	0.5	0.5	32	32	0.125	32	1.25
Study isolate							
XDR *A.baumannii* 01	0.5	0.5	64	128	0.5	16	1.25
				0.125	64	1.25	
XDR *A.baumannii* 02	0.5	0.5	32	32	0.5	4	1.25
				0.125	32	1.25	
XDR *A.baumannii* 03	0.5	0.5	32	32	0.5	8	1.25
				0.125	32	1.25	
XDR *A.baumannii* 04	1	1	32	64	1	4	1.125
				0.5	16	1	
				0.5	32	1.5	
XDR *A.baumannii* 05	1	1	64	128	1	4	1.06
				0.5	16	0.75	
				0.5	32	1	
XDR *A.baumannii* 06	1	1	64	128	1	4	1.06
				0.5	16	0.75	
				0.5	32	1	
XDR *A.baumannii* 07	1	1	64	128	1	4	1.06
				0.5	16	0.75	
				0.5	32	1	
XDR *A.baumannii* 08	1	1	64	64	1	4	1.06
				0.5	16	0.75	
				0.5	32	1	
XDR *A.baumannii* 09	1	1	64	128	1	4	1.06
				0.5	16	0.75	
				0.5	32	1	
XDR *A.baumannii* 10	2	2	64	128	1	4	0.56
				1	16	0.75	
				0.5	32	0.75	
				0.125	64	1.06	
XDR *A.baumannii* 11	8	8	32	32	1	4	0.25
				0.5	8	0.31	
				1	8	0.38	
				0.5	16	0.56	
				0.125	32	1.02	

FICI <0.5 = synergy, FICI 0.5 to 4.0 = indifference, FICI >4.0 = antagonistic

A. baumannii is unknown and it should be explored. Since FICI is only a preliminary indicator of antibiotic synergy, the time-kill method, which is a more accurate method, should be performed especially for the combination of colistin and sulbactam at the concentrations that showed FICI be near 0.5 against colistin-susceptible XDR *A. baumannii* isolates and for more isolates of colistin-resistant XDR *A. baumannii*. This preliminary observation suggests that a combination of colistin and sulbactam might be an effective therapy of infection caused by colistin-resistant XDR *A. baumannii*.

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Potential conflicts of interest

None.

References

1. Falagas ME, Karageorgopoulos DE. Pandrug resistance (PDR), extensive drug resistance (XDR), and multidrug resistance (MDR) among Gram-negative bacilli: need for international harmonization in terminology. Clin Infect Dis 2008; 46: 1121-2.
2. Chung DR, Song JH, Kim SH, Thamlikitkul V, Huang SG, Wang H, et al. High prevalence of multidrug-resistant nonfermenters in hospital-acquired pneumonia in Asia. Am J Respir Crit Care Med 2011; 184: 1409-17.
3. Koomanachai P, Tiengrim S, Kiratisin P, Thamlikitkul V. Efficacy and safety of colistin (colistimethate sodium) for therapy of infections caused by multidrug-resistant Pseudomonas aeruginosa and Acinetobacter baumannii in Siriraj Hospital, Bangkok, Thailand. Int J Infect Dis 2007; 11: 402-6.
4. Petrosillo N, Ioannidou E, Falagas ME. Colistin monotherapy vs. combination therapy: evidence from microbiological, animal and clinical studies. Clin Microbiol Infect 2008; 14: 816-27.
5. Michalopoulos A, Falagas ME. Treatment of Acinetobacter infections. Expert Opin Pharmacother 2010; 11: 779-88.
6. Lorain V. Antibiotics in laboratory medicine. 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2005.
7. Santimaleeworagun W, Wongpoowarak P, Chayakul P, Pattharachayakul S, Tansakul P, Garey KW. In vitro activity of colistin or sulbactam in combination with fosfomycin or imipenem against clinical isolates of carbapenem-resistant Acinetobacter baumannii producing OXA-23 carbapenemases. Southeast Asian J Trop Med Public Health 2011; 42: 890-900.
8. Deveci A, Coban AY, Acicbe O, Tanyel E, Yaman G, Durupinar B. In vitro effects of sulbactam combinations with different antibiotic groups against clinical Acinetobacter baumannii isolates. J Chemother 2012; 24: 247-52.

การทดสอบฤทธิ์ของ colistin ร่วมกับ sulbactam ต่อเชื้อ Acinetobacter baumannii ที่ดื้อยาต้านจุลชีพหลาย ๆ ขนานด้วยวิธี checkerboard

วิษณุ ธรรมลิขิตกุล, สุรภี เทียนกริม

วัตถุประสงค์: เพื่อทราบฤทธิ์ของยา colistin ร่วมกับยา sulbactam ต่อเชื้อ Acinetobacter baumannii ที่ดื้อยาต้านจุลชีพหลาย ๆ ขนาน

วัสดุและวิธีการ: ใช้วิธี checkerboard ในการทดสอบเชื้อ Acinetobacter baumannii ที่ดื้อยาต้านจุลชีพหลาย ๆ ขนานจำนวน 11 สายพันธุ์ ความเข้มข้นของยา colistin และ sulbactam ที่ใช้ คือ 0.025 ถึง 128 มก./ล. และ 4 ถึง 256 มก./ล. ตามลำดับ โดยวัด minimum inhibitory concentration และ minimum bactericidal concentration ของยา colistin, sulbactam และ colistin ร่วมกับ sulbactam แล้วนำผลมาคำนวณค่า fractional inhibitory concentration index (FICI) หากค่า FICI <0.5 แสดงว่ายาสองขนานออกฤทธิ์เสริมกัน หาก FICI มีค่า 0.5-4 แสดงว่า ยาสองขนานออกฤทธิ์ไม่แตกต่างจากยาขนานเดียวและหาก FICI >4.0 แสดงว่ายาสองขนานออกฤทธิ์ต้านกัน

ผลการศึกษา: เชื้อ Acinetobacter baumannii ที่ดื้อยาต้านจุลชีพหลาย ๆ ขนาน 10 สายพันธุ์ไวต่อยา colistin (MIC ≤2 มก./ล.) ส่วนอีก 1 สายพันธุ์ดื้อยา colistin (MIC = 8 มก./ล.) ไม่พบว่ายาทั้งสองขนานออกฤทธิ์ต้านกันในเชื้อทุกสายพันธุ์ เชื้อ 10 สายพันธุ์ ที่ไวต่อยา colistin พบ MICs บางค่าของยาสองขนานนี้ร่วมกันที่ต่ำกว่าค่า MICs ของยาขนานเดียว โดยยาทั้งสองขนานนี้ร่วมกันออกฤทธิ์ไม่แตกต่างจากยาขนานเดียวในเชื้อทุกสายพันธุ์ที่ไวต่อยา colistin แต่ยาทั้งสองขนานนี้ร่วมกันออกฤทธิ์เสริมกันในเชื้อสายพันธุ์ที่ดื้อยา colistin

สรุป: ยา colistin ร่วมกับยา sulbactam ออกฤทธิ์ไม่แตกต่างจากยา colistin หรือยา sulbactam ขนานเดียวต่อเชื้อ A. baumannii ที่ดื้อยาต้านจุลชีพ หลาย ๆ ขนานแต่ไวต่อยา colistin แต่ยา colistin ร่วมกับยา sulbactam ออกฤทธิ์เสริมกันต่อเชื้อ A. baumannii ที่ดื้อยาต้านจุลชีพหลาย ๆ ขนานที่ดื้อยา colistin ด้วย ควรทดสอบการออกฤทธิ์เสริมกันของยา colistin และยา sulbactam ด้วยวิธี time-kill เพื่อยืนยันผลที่พบจากการศึกษานี้