

In Vitro Activity of Colistin, Fosfomycin, and Piperacillin/tazobactam Against *Acinetobacter baumannii* and *Pseudomonas aeruginosa* in Songklanagarind Hospital, Thailand

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ABSTRACT

A total of 118 isolates of Gram-negative, non-fermentative bacilli including *Pseudomonas aeruginosa* (60 isolates) and *Acinetobacter baumannii* (58 isolates) were tested for susceptibility to colistin, fosfomycin, and piperacillin/tazobactam. The minimal inhibitory concentration (MIC) of fosfomycin and piperacillin/tazobactam was determined using the Epsilon (E)-test method. The susceptibility of colistin was determined by both disc diffusion and E-test methods. *A. baumannii*, and *P. aeruginosa* were 61.7 percent and 50 percent susceptible to fosfomycin, respectively; and 65 percent and 100 percent susceptible to piperacillin/tazobactam. The inhibition zone of colistin against all isolates was 11-14 mm with 100 percent susceptibility within the MIC range of 0.19-2 mg/L. In conclusion, *A. baumannii*, and *P. aeruginosa* showed little resistance to colistin, and piperacillin/tazobactam was very active against *P. aeruginosa*. The MIC data did not support stand-alone usage of fosfomycin. (*J Infect Dis Antimicrob Agents* 2009;26:91-6.)

INTRODUCTION

Acinetobacter baumannii and *Pseudomonas aeruginosa* are important nosocomial pathogens.

Carbapenems and other beta-lactam antibiotics have been used in recent years for treating these infections and are potent agents for treating infections caused by

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these two pathogens. Recently, the resistance to these drugs has been increasing reported mostly due to inactivation by bacterial beta-lactamase enzymes.¹

Colistin is a polypeptide antibiotic belonging to the polymyxin group, and is being increasingly used for the treatment of multidrug-resistant Gram-negative bacterial infections.² In vitro, colistin has shown an excellent activity against a variety of Gram-negative bacilli (GNB), and in vivo has not shown serious toxicity after prolonged intravenous administration.³

Fosfomycin is a bactericidal antibiotic with a broad-spectrum activity against both Gram-positive and Gram-negative bacteria.⁴ It has been used for the treatment of methicillin-resistant *Staphylococcus aureus* (MRSA), but fosfomycin also seems to be effective in the treatment of multidrug-resistant *P. aeruginosa* by acting synergistically with beta-lactam antibiotics.⁵

Piperacillin/tazobactam is a broad-spectrum antibiotic which is stable to most beta-lactamases, and covers a large number of pathogens including multidrug-resistant pathogens.⁶

Much of the data on the antimicrobial activity of colistin, fosfomycin, and piperacillin/tazobactam have been derived from previous studies, but knowledge of local antibiotic susceptibility profiles is an important prerequisite for the appropriate use of antibiotics. The objective of this study was to assess the in vitro activity of colistin, fosfomycin, and piperacillin/tazobactam against *P. aeruginosa* and *A. baumannii* in Songklanagarind Hospital, Songkhla, South Thailand.

MATERIALS AND METHODS

Bacterial isolates

All clinical isolates of *P. aeruginosa* (60 isolates), *A. baumannii* (58 isolates) were collected from patients

hospitalized at Songklanagarind Hospital between 2006 and 2007. They were identified by the standard microbiological methods. These isolates were stored in 10 percent glycerine tryptic soy broth, then frozen at 80°C prior to susceptibility testing.

Susceptibility testing

Three different antimicrobial agents were tested. The minimal inhibitory concentration (MIC) of fosfomycin and piperacillin/tazobactam was tested using the Epsilon (E)-test method, (E-test^R fosfomycin with glucose-6-phosphate). The susceptibility of colistin was determined by the disk diffusion test and MIC testing was performed using the E-test method. Quality control testing was performed using *Escherichia coli* ATCC 25922, ATCC 35218 (for piperacillin/tazobactam) and *P. aeruginosa* ATCC 27853. The MIC of each drug was reported as an MIC range, MIC₅₀, and MIC₉₀. The MIC₅₀ and MIC₉₀ values are the MIC values when 50 and 90 percent of bacterial growth are inhibited.

RESULTS

The clinical isolates of *A. baumannii* were obtained from the sterile sites (38 isolates: 32 blood, 4 tissue, and 2 body fluid specimens) and non-sterile sites (20 isolates: 12 sputum and 8 purulent discharge specimens). *P. aeruginosa* was isolated from 22 sterile sites including 14 blood, 5 body fluid, and 3 tissue specimens, as well as 38 non-sterile sites including 19 sputum, 11 purulent discharge, and 8 urine specimens.

The susceptibility, MIC ranges, MIC₅₀, and MIC₉₀ of fosfomycin and piperacillin/tazobactam against *A. baumannii*, and *P. aeruginosa* are presented in Table 1. The inhibition zone of colistin against all isolates was 11-14 mm with 100 percent susceptibility of MIC range of 0.19-2 mg/L (at breakpoint of ≤ 2 mg/L).⁷

Table 1. The minimal inhibitory concentration (MIC) of fosfomycin and piperacillin/tazobactam against *Acinetobacter baumannii* and *Pseudomonas aeruginosa* using the Epsilon (E)-test.

Antimicrobial agents/bacteria (N)	MIC (mg/L)				
	Range	MIC ₅₀	MIC ₉₀	% susceptibility	B-P
Fosfomycin					≤ 64
<i>Acinetobacter baumannii</i> (58)	24-512	64	128	61.7	
Imipenem-susceptible (34)	24-256	64	96	51.5	
Imipenem-resistant (24)	32-512	64	96	75	
<i>Pseudomonas aeruginosa</i> (60)	2->1,024	64	>1,024	50	
Ceftazidime-susceptible (32)	2->1,024	64	128	32	
Ceftazidime-resistant (28)	16->1,024	>1,024	>1,024	62.5	
Piperacillin/tazobactam					≤ 16/4
<i>Acinetobacter baumannii</i> (58)	0.016->256	2	128	65	
Imipenem-susceptible (34)	0.016-64	0.016	12	91.4	
Imipenem-resistant (24)	0.016->256	128	>256	16.7	
<i>Pseudomonas aeruginosa</i> (60)	0.5-64	4	48	100	≤ 64/4
Ceftazidime-susceptible (32)	0.5-64	2	4	100	
Ceftazidime-resistant (28)	0.5-64	12	48	100	

B-P: break-point value according to the Clinical and Laboratory Standards Institute (CLSI) 2007

(Table 2). Piperacillin/tazobactam showed high activity with a MIC₉₀ of 4 mg/L against ceftazidime-susceptible *P. aeruginosa* and 100 percent susceptibility against all isolates of *P. aeruginosa*.

DISCUSSION

In our setting data, we considered the suscep-

tibility breakpoint of ≤ 64 mg/L for fosfomycin. However, there are different breakpoints to determine the resistance, such as ≥ 256 mg/L according to the Clinical and Laboratory Standards Institute and > 128 mg/L according to British Society for Antimicrobial Chemotherapy.⁸ Barry reported the MIC₅₀ and MIC₉₀ of fosfomycin against *Acinetobacter* spp. and *P.*

Table 2. Inhibition zone of colistin disk diffusion and minimal inhibitory concentration (MIC) value using the Epsilon (E)-test against *Acinetobacter baumannii* and *Pseudomonas aeruginosa*.

Microorganisms (N)	Zone size							
	≥ 11 mm	9-10 mm	≤ 8 mm	Range	MIC ₅₀ (mg/L)	MIC ₉₀ (mg/L)	% susceptibility	B-P (mg/L)
<i>Acinetobacter baumannii</i>								
All (58)				0.19-1.5	0.38	0.38	100	≤ 2
Imipenem-susceptible (34)	34/34	-	-	0.19-0.5	0.25	0.38	100	
Imipenem-resistant (24)	23/24	2/24	-	0.19-1.5	0.25	0.38	100	
<i>Pseudomonas aeruginosa</i>								
All (60)				0.5-2	1	1.5	100	≤ 2
Ceftazidime-susceptible (32)	32/32	-	-	0.5-2	1.5	1.5	100	
Ceftazidime-resistant (28)	28/28	-	-	1-2	1.5	1.5	100	
<i>E. coli</i> ATCC 25922	11-13	-	-	0.5-1	-	-		
<i>P. aeruginosa</i> ATCC27853	11-13	-	-	1-1.5	-	-		

B-P: break-point value according to the Clinical and Laboratory Standards Institute (CLSI) 2007

aeruginosa as 128, 512 and 32, 64 mg/L, respectively. In our study, the MIC₅₀ and MIC₉₀ of fosfomycin against *A. baumannii* were 64 and 128, and against *P. aeruginosa* were 64 and > 1,024 mg/L, respectively. The very high MIC of fosfomycin against *A. baumannii* and *P. aeruginosa* implies the ineffective clinical use of fosfomycin alone. However, the treatment of multidrug-resistant *A. baumannii* and *P. aeruginosa* infections is usually based on a combination of various antimicrobial agents.⁹ Fosfomycin is usually one of antimicrobial agents that possess anti-pseudomonal activity, as part of combination use.¹⁰

Colistin is an antibiotic in which there has had a revived interest recently for treating infections caused by multidrug-resistant *A. baumannii* and *P. aeruginosa*.¹⁰⁻¹¹ Berlana and colleagues reported the efficacy and safety of colistin in the treatment of patients infected with *A. baumannii* and *P. aeruginosa* infections. Our in vitro study showed that all the culture isolates were susceptible to colistin.¹³ Therefore, our results were in consistent with other studies which showed high susceptibility rates to colistin among these organisms.

Piperacillin/tazobactam had an excellent activity against *P. aeruginosa*. A surveillance study from the United States published in 2003 showed the susceptibility of *P. aeruginosa* to piperacillin/tazobactam to be 70-90 percent and *A. baumannii* to be 58-65 percent.¹⁴ The results of our study in Thailand may suggest an increasing frequency of clinical use of piperacillin/tazobactam.

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