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In vitro antimicrobial activities of meropenem, ciprofloxacin, colistin alone and in combinations against meropenem-resistant *Pseudomonas aeruginosa*

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Abstract

Determinations of *in vitro* antimicrobial activities of meropenem, ciprofloxacin, colistin alone and in combinations against 20 clinical isolates of *P. aeruginosa* have shown that 16 isolates (80%) were multidrug-resistant (MDR) isolates. All isolates were resistant to meropenem (MIC_{90}=32 μg/mL) whereas 4 out of 20 isolates (20%) were susceptible to ciprofloxacin (MIC_{90}=64 μg/mL). Furthermore, all isolates were susceptible to colistin (MIC_{90}=2 μg/mL). With checkerboard method, it has been shown that meropenem in combination with ciprofloxacin or colistin and ciprofloxacin in combination with colistin had synergistic effects with drug concentration within the therapeutic serum level against 3, 6 and 4 isolates, respectively. For triple combination of meropenem, ciprofloxacin and colistin, the synergistic effect was observed in 5 isolates. Taken these results into account, meropenem in combination with colistin may be optimal alternative treatment for meropenem-resistant *P. aeruginosa* infections.

Keywords: *Pseudomonas aeruginosa*, Meropenem, Resistant

Introduction

P. aeruginosa is one of the major causes of nosocomial infections. Empirical therapies are used for *P. aeruginosa* infections and combination therapy is preferred (1). However, increasing of MDR *P. aeruginosa* has been a problem worldwide (2). Carbapenems are drugs of choice for the treatment of MDR pseudomonal infection. However, carbapenem-resistant *P. aeruginosa* is also emerging and increasing (2). Therefore, new therapies are needed urgently. Previous studies reported the synergistic effects between carbapenems with aminoglycosides and/or fluoroquinolones against *P. aeruginosa*. Nevertheless, resistances to these agents have also been documented which limit therapeutic options (1-3). Recent study has shown that polymyxins (old class of antibiotics) which were abandoned earlier because of their toxicities may be the only available active agents existing against MDR *P. aeruginosa* (3). Therefore, this study was aimed to investigate *in vitro* activities of meropenem, ciprofloxacin, colistin and the combinations against meropenem-resistant *P. aeruginosa.*

Materials and methods

Bacterial isolates

Twenty isolates of *P. aeruginosa* were clinically isolated from the patients at Siriraj Hospital between years 2006-2008. *Escheriachia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used as quality control strains.

Antimicrobial agents

Antimicrobial disks were amikacin, ciprofloxacin (BBL™: Beckton Dickinson, USA), cefepime, meropenem (BBL™: BENEX Limited, USA) and piperacillin/tazobactam (Oxoid: Oxoid, Basingstoke, Hants, England). Standard laboratory powders were meropenem and sodium

carbonate and ciprofloxacin HCL (Siam Bhesach CO., Ltd., Bangkok, Thailand). Colistin sulfate (Sigma Aldrich, St Louis, MO).

Antimicrobial susceptibility testing

Susceptibility to each 5 board-spectrum antimicrobial agents (amikacin, ciprofloxacin, cefepime, meropenem, piperacillin/tazobactam) against all 20 *P. aeruginosa* isolates were determined by disk diffusion method according to CLSI (4). An isolate was considered to be MDR isolate when it resists to 3 or more of 5 broad-spectrum antimicrobial agents as described previously. Agar dilution method (5) was performed to determine MICs of meropenem, ciprofloxacin and colistin. The susceptibility were interpreted by referring to CLSI (6)

Evaluation of the combination effects

The combination effects of meropenem, ciprofloxacin and colistin were determined by checkerboard microdilution method, which modified from previous study (7). After incubated at 37 ^{o}C for 18 hours, FIC index (ΣFIC) was calculated according to the following formula: FIC index = (MIC of drug A in combination/MIC of drug A alone) + (MIC of drug B in combination/MIC of drug B alone) + (MIC of drug C in combination/MIC of drug C alone). FIC index < 1.0, 1.0 and > 1.0 was used to define synergy, additive and antagonism, respectively according to previous study (7).

Results

Antimicrobials susceptibility

From disk diffusion method, all isolates were resistant to meropenem whereas 80%, 65%, 70% and 30% of the tested isolates were resistant to ciprofloxacin, amikacin, cefepime and piperacillin/tazobactam, respectively. Sixteen isolates (80%) were MDR *P. aeruginosa*. MICs showed that most of the tested isolates were resistant to both meropenem and ciprofloxacin (40%), whereas all tested isolates were susceptible to colistin (Table 1).

Table 1 Antimicrobial susceptibility, MIC_{50} and MIC_{90} of meropenem, ciprofloxacin and colistin against 20 clinical isolates of *P. aeruginosa*

Susceptibility	Meropenem		Ciprofloxacin		Colistin	
	No. of isolates	%	No. of isolates	%	No. of isolates	%
Susceptible (S)	0	0	4	20	20	100
Intermediate (I)	8	40	0	0	0	0
Resistant (R)	12	60	16	80	0	0
Range (µg/mL)	8-32		0.5-128		1-2	
MIC_{50} (µg/mL)	16		64		1	
MIC_{90} (µg/mL)	32		64		2	

Susceptibility breakpoint (CLSI 2007) ; Meropenem : (S) ≤ 4 µg/mL, (I) = 8 µg/mL, (R) ≥ 16 µg/mL ;
Ciprofloxacin : (S) ≤ 1 µg/mL, (I) = 2 µg/mL, (R) ≥ 4 µg/mL ; Colistin : (S) ≤ 2 µg/mL, (I) = 4 µg/mL, (R) ≥ 8 µg/mL.

Combination effects

The combination effects between meropenem, ciprofloxacin and colistin against 20 clinical isolates of *P. aeru*ginosa were shown in Table 2

Table 2 Combination effects of meropenem, ciprofloxacin and colistin against 20 clinical isolates of *P. aeruginosa*

Antimicrobial combinations	FICI range	[a]Synergy (ΣFIC < 1)	Additive (ΣFIC = 1)	Antagonism (ΣFIC > 1)	[b]No. of isolates showed synergy within therapeutic serum level
		No. of isolates (%)	No. of isolates (%)	No. of isolates (%)	
MEM + CIP	0.75-1.063	6(30)	10(50)	4(20)	3
MEM + COL	0.563-1.060	6(30)	4(20)	10(50)	6
CIP + COL	0.560-1.060	6(30)	4(20)	10(50)	4
MEM + CIP + COL	0.623-1.060	13(65)	1(5)	6(30)	5

Abbreviations; MEM, meropenem; CIP, ciprofloxacin; COL, colistin; FICI, fraction inhibitory concentration index.
[a]Synergy : 1) MEM + CIP at concentration range from 4-16 and 0.25-64 µg/mL, respectively.
2) MEM + COL at concentration range from 1-8 and 1 µg/mL, respectively. 3) CIP + COL at concentration range from 0.06-16 and 1 µg/mL, respectively. 4) MEM + CIP + COL at concentration range from 1-16, 0.06-64 and 0.06-1 µg/mL, respectively.
[b]Therapeutic serum level : MEM ≈ 25 µg/mL (500 mg IV infusion q 8 hr), CIP ≈ 4 µg/mL (400 mg IV infusion q 8-12 hr)

Discussion and conclusion

The present study observed low level of meropenem resistance (MIC_{90}=32 µg/mL) . This may caused by loss of OprD porin in conjunction with overexpression of multidrug efflux pump (1,8). Moreover, high level of resistance of ciprofloxacin (MIC_{90}=64 µg/mL) were also observed. This may caused by overexpression of multidrug efflux pump together with the target sites mutation (1,2). In combination study, although, antagonism (FIC index > 1) was observed, there was no change in MIC values when tested with drug alone and drug in combinations. Triple combination of meropenem, ciprofloxacin and colistin showed the highest synergistic effect against *P. aeruginosa* (13 out of 20 isolates). When, the therapeutic serum concentration level is taken into account, meropenem in combination with colistin may be the best alternative treatment of meropenem-resistant *P. aeruginosa* infection (Table 2). Colistin may disturb bacterial membrane and increase cell's permeability which resulting in the enhancement of penetration of the antibiotic into cell (7). However, further *in vitro* and *in vivo* studies are needed prior to its application in the clinical setting.

References

1. Mesaros N, Nordmann P, Plésiat P, Roussel-Delvallez M, Van Eldere J, Glupczynski Y, et al. *Pseudomonas aeruginosa*: resistance and therapeutic options at the turn of the new millennium. Clin Microbiol Infect 2007; 13(6): 560-78.
2. McGowan, JE. Jr. Resistance in Nonfermenting Gram-Negative Bacteria : Multidrug Resistance to The Maximum. The American Journal of Medicine 2006; 119(6): S29-36.
3. Arnold TM, Forrest GN, and Messmer KJ. Polymyxin antibiotics for gram-negative infections. Am J Health Syst Pharm 2007; 64: 819-26
4. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk susceptibility tests, approved standard M2-A9. 9th ed. Wayne, PA: CLSI. 2006
5. Clinical and Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard M7-A7. 7th ed. Wayne, PA: CLSI. 2006
6. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing, 17th Informational supplement M100-S17. Wayne, PA: CLSI. 2007
7. Yoon J, Urban C, Terzian C, Mariano N, and Rahal JJ. *In vitro* double and triple synergistic activities of polymyxin B, imipenem, and rifampin against multidrug-resistant *Acinetobacter baumannii*. Antimicrob Agents Chemother 2004; 48(3): 753-75
8. Livermore DM. Of *Pseudomonas***,** porin**s,** pump**s** and carbapenems. J Antimicrob Chemother. 2001; 47: 247-50