IN VITRO ACTIVITY OF COLISTIN AGAINST MULTIDRUG-RESISTANT *PSEUDOMONAS AERUGINOSA* ISOLATES FROM PATIENTS IN SONGKLANAGARIND HOSPITAL, THAILAND

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Abstract. Multidrug-resistant *Pseudomonas aeruginosa* (MDR-PA) infection creates problems for therapy. Previous studies have found MDR-PA is susceptible to colistin. We studied the *in vitro* susceptibility of MDR-PA to colistin and determined the minimum inhibitory concentration (MIC). One hundred MDR-PA isolates were obtained from patients at Songklanagarind Hospital, in southern Thailand, during January 2008-March 2011. Antimicrobial susceptibilities to amikacin (AK), ceftazidime (CAZ), ciprofloxacin (CIP), imipenem (IMP) and colistin (CO) were tested by standard disk diffusion method. The antimicrobial susceptibility to colistin and the MIC were determined with the E-test. The MDR-PA isolates were susceptible to ceftazidime, ciprofloxacin, amikacin and imipenem in 1, 5, 11 and 32%, respectively. There were 5 antimicrobial resistance patterns of MDR-PA: AK-CAZ-CIP-IMP (50%), AK-CAZ-CIP (32%), CAZ-CIP-IMP (11%), AK-CAZ-IMP (6%) and AK-CIP-IMP (1%). Colistin had good efficacy against MDR-PA (98% susceptibility rate). The MIC_{50} and MIC_{90} for colistin were 1.0 and 1.5 μg/ml, respectively. Only 2 MDR-PA isolates were resistant to colistin with the MICs of 3 and 12 μg/ml, respectively. The majority of MDR-PA isolates remained susceptible to colistin; therefore, colistin is a good option for treatment of MDR-PA.

Keywords: *Pseudomonas aeruginosa*, multidrug-resistant, colistin, Thailand

INTRODUCTION

Pseudomonas aeruginosa is a known nosocomial pathogen and cause of pneumonia, bacteremia and urinary tract infection. It may be resistant to various antimicrobial agents utilizing strategies such as impermeability, an intrinsic resistance to many antimicrobials and it has an efflux pump, which is associated with elevated MICs with penicillin, cephalosporins, quinolones, tetracyclines, chloramphenicol, metallo-β-lactamases and later carbapenems (Ho *et al*, 2002; Lombardi *et al*, 2002; Lagatolla *et al*, 2004; Landman *et al*, 2005; Pankey and Ashcraft, 2005).

The resistance of *P. aeruginosa* to β-lactams, quinolones, aminoglycosides and carbapenems, especially imipenem has steady increased (Ho *et al*, 2002; Gunder-

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son *et al*, 2003; Landman *et al*, 2005; Pankey and Ashcraft, 2005). Various reports from Asian countries, such as Thailand, Taiwan and Korea, have shown 19-24% of isolates resistant to ceftazidime and 15-20% of isolates resistant to imipenem (Hsueh *et al*, 2001; Girlich *et al*, 2002; Lee *et al*, 2006). In 2009, data from Songklanagarind Hospital in Songkhla Province, Thailand, revealed 16% of *P. aeruginosa* isolates were resistant to amikacin, 24% to ceftazidime, 21% to ciprofloxacin and 18% to imipenem (Songklanagarind Hospital, 2009).

Multidrug-resistant *P. aeruginosa* (MDR-PA) is defined as *P. aeruginosa* resistant to at least 3 groups of antimicrobials, including aminoglycosides, cephalosporins, fluoroquinolones and carbapenems (Obritsch *et al*, 2005). MDR-PA infections are increasing worldwide (Hachem *et al*, 2007). Studies in the United States have shown 15% of *P. aeruginosa* isolates during 2001-2002 in intensive care units were MDR-PA and 14% of blood stream isolates during 2005-2007 were MDR-PA (Obritsch *et al*, 2004; Tam *et al*, 2010).

Colistin (polymyxin E) is a cationic polypeptide antibiotic produced by *Bacillus polymyxa* (Koyama *et al*, 1950). The drug was first used in Japan in 1949, toxicity to the kidneys and nervous system was reported (Brown *et al*, 1970; Koch-Weser *et al*, 1970; Kumazawa and Yagisawa, 2002). Colistin was abandoned in the 1980s; however, increasing drug resistant gram-negative bacteria, particularly *P. aeruginosa* and *Acinetobacter baumannii* have led to the use of colistin (Falagas and Michalopoulos, 2006). Colistin is bactericidal to gram-negative bacteria, including *Acinetobacter* sp, *P. aeruginosa*, *Klebsiella* sp and *Escherichia coli*. Colistin targets the cell membrane by binding to anionic lipopolysaccharide molecules to replace Ca^{2+} and Mg^{2+} in the outer cell membrane of gram-negative bacteria, leading to leaking of the cytoplasmic membrane and cell death (Gales *et al*, 2001).

A previous study reported *P. aeruginosa* to be more susceptible to colistin than imipenem and ceftazidime (100, 78.8 and 68.8%, respectively) and the MIC_{90} of colistin, imipenem and ceftazidime were ≤1 µg/ml, >16 µg/ml and >32 µg/ml, respectively (Gales *et al*, 2001). Between 2001 and 2003, all MDR-PA isolates from Siriraj Hospital, Bangkok, Thailand were susceptible to colistin (Tribuddharat *et al*, 2003). The objective of this study was to investigate the *in vitro* susceptibility of MDR-PA to colistin and determine its minimum inhibitory concentration (MIC).

MATERIALS AND METHODS

Bacterial strains

One hundred isolates of MDR-*P. aeruginosa* obtained from patients at Songklanagarind Hospital, Songkhla Province, Thailand during January 2008 -March 2011 were included in this study. Bacterial isolation and identification were performed using standard laboratory methods (Giligan, 1995). This study was approved by the Ethics Committee of the Faculty of Medicine, Prince of Songkla University, Thailand.

Antimicrobial susceptibility testing and MIC determination

Antimicrobial susceptibilities to amikacin (30 µg, AK), ceftazidime (30 µg, CAZ), ciprofloxacin (5 µg, CIP) and imipenem (10 µg, IMP) (Oxoid, Basingstoke, Hampshire, England) were determined by the disk diffusion method. Colistin (10 µg; 0.016-256 µg/ml, CO) (Oxoid, Basingstoke, Hampshire, England; AB Biodisk,

Table 1
In vitro activities of 5 antimicrobial agents against 100 isolates of MDR-*P. aeruginosa*.

Antimicrobial agent	Number of *Pseudomonas aeruginosa* isolates (*n*=100)					
	Disk diffusion test[a]			E-test[b]		
	Susceptible	Intermediately resistant	Resistant	Susceptible	Intermediately resistant	Resistant
Amikacin	11	11	78	ND	ND	ND
Ceftazidime	1	4	95	ND	ND	ND
Ciprofloxacin	5	6	89	ND	ND	ND
Imipenem	32	11	57	ND	ND	ND
Colistin	99	0	1	98	1	1

[a]Zone diameter used for *P. aeruginosa*: amikacin (30 μg): susceptible ≥17 mm, intermediately resistant 15-16 mm, resistant ≤14 mm; ceftazidime (30 μg): susceptible ≥18 mm, intermediately resistant 15-17 mm, resistant ≤14 mm; ciprofloxacin (5 μg): susceptible ≥21 mm, intermediately resistant 16-20 mm, resistant ≤15 mm; imipenem (10 μg): susceptible ≥16 mm, intermediately resistant 14-15 mm, resistant ≤13 mm; colistin (10 μg): susceptible ≥11 mm, resistant ≤10 mm (CLSI, 2011).
[b]E-test, MIC breakpoints for colistin (0.016-256 μg/ml): susceptible ≤2 μg/ml, intermediately resistant 4 μg/ml, resistant ≥8 μg/ml (CLSI, 2011).
ND, not determined.

Solna, Sweden), was tested by both the disk diffusion method and the E-test. The minimal inhibitory concentration (MIC) of colistin was determined with the E-test; a MIC ≤2 μg/ml was considered susceptible. The antimicrobial susceptibility and MIC breakpoints for *P. aeruginosa* followed the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2011). *P. aeruginosa* ATCC 27853 was used as a control strain. For colistin susceptibility testing, errors were ranked as follows: "very major error" was susceptible by disk diffusion but resistant by E-test; "major error" was resistant by disk diffusion but susceptible by E-test; "minor error" was intermediately resistant by one method and susceptible or resistant by the other method. Unacceptable levels were >1.5% for "very major errors", >3% for "major errors" and 10% for "minor errors" (Morosini *et al*, 2005; Galani *et al*, 2008).

RESULTS

Antimicrobial susceptibility and antimicrobial resistance pattern

One hundred MDR-PA isolates were collected from 75 male and 25 female patients. The majority of MDR-PA isolates (43%) came from sputum, followed by urine (24%), pus (14%), tissue (10%), bodily fluid (5%) and blood (4%). One isolate (1%) was susceptible to ceftazidime (CAZ), 5% were susceptible to ciprofloxacin (CIP), 11% were susceptible to amikacin (AK) and 32% susceptible to imipenem (IMP) (Table 1).

The MDR-PA isolates had five antimicrobial resistance patterns; one pattern was resistant to four antimicrobials and four patterns were resistant to three antimicrobials (Table 2). The most common antimicrobial resistance pattern was AK-CAZ-CIP-IMP resistance (50%), followed

Table 2
Antimicrobial patterns and *in vitro* activities of colistin against 100 isolates of MDR-*P. aeruginosa*.

Antimicrobial patterns	Number of Isolates	Antimicrobial resistance patterns				Susceptibility to colistin			
		AK	CAZ	CIP	IMP	MIC (µg/ml) Median (IQR 25-75)	Susceptible (≤2 µg/ml)	Intermediately resistant (4 µg/ml)	Resistant (≥8 µg/ml)
1	50	R	R	R	R	1.00 (0.75-1.50)	49	1[a]	0
2	32	R	R	R	S	1.00 (0.75-1.00)	31	0	1[b]
3	6	R	R	S	R	1.25 (0.87-2.00)	6	0	0
4	1	R	S	R	R	1.00 (1.00-1.00)	1	0	0
5	11	S	R	R	R	0.75 (0.50-1.50)	11	0	0
Total							98	1	1

Antimicrobial resistance patterns: 1, resistant to amikacin, ceftazidime, ciprofloxacin and imipenem; 2, resistant to amikacin, ceftazidime and ciprofloxacin; 3, resistant to amikacin, ceftazidime and imipenem; 4, amikacin, ciprofloxacin and imipenem; 5, ceftazidime, ciprofloxacin and imipenem. S, Susceptible; R, intermediately resistant (I) or Resistant (R); [a]$MIC_{colistin}$ = 3 µg/ml; [b]$MIC_{colistin}$ = 12 µg/ml

by AK-CAZ-CIP (32%), CAZ-CIP-IMP (11%), AK-CAZ-IMP (6%) and AK-CIP-IMP (1%). The susceptibilities to colistin among the 100 MDR-PA isolates are shown in Table 1. According to the E-test, 98% of isolates were susceptible to colistin, 1% had intermediate resistance and 1% was resistant. The MIC_{50} and MIC_{90} for colistin were 1.0 and 1.5 µg/ml, respectively.

All MDR-PA isolates that exhibited the three antimicrobial resistance patterns (CAZ-CIP-IMP, AK- CAZ-IMP and AK-CIP-IMP) were susceptible to colistin except for one isolate with the AK-CAZ-CIP pattern. In total, only two isolates were resistant, 1 of the 32 (3%) isolates with the AK-CAZ-CIP resistance pattern showed resistance to colistin (MIC 12 µg/ml), and 1 of the 50 (2%) isolates with the AK-CAZ-CIP-IMP pattern exhibited intermediate resistance to colistin (MIC 3 µg/ml). The median MICS for colistin among the isolates with the five antimicrobial resistance patterns were not significantly different from each other ($p = 0.64$).

Comparison of disk diffusion method and E-test for colistin

The comparison between the disk diffusion method and the E-test for colistin is shown in Fig 1. Using the disk diffusion method, 99 of 100 (99%) of the MDR-PA isolates were susceptible to colistin (zone diameter=10 mm). With the E-test method, 98 of 100 (98%) of the MDR-PA isolates were susceptible to colistin (MIC range 0.25-2.0 µg/ml); 1 isolate (1%) had intermediate resistant (MIC 3 µg/ml) and 1 isolate (1%) was resistant (MIC 12 µg/ml) (Tables 1 and 2). The MDR-PA isolates that had intermedi-

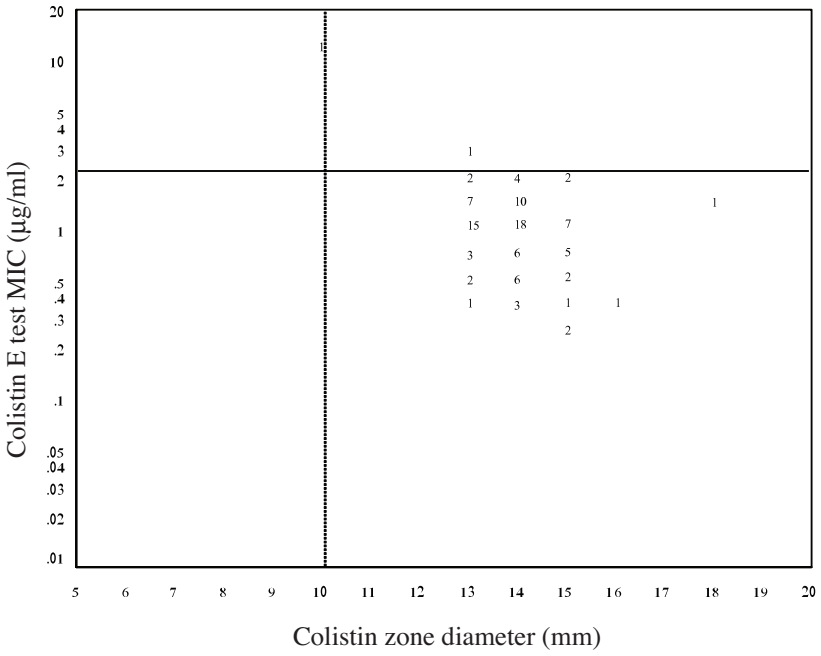

Fig 1-Scattergram showing MIC with the E-test and zone diameter with the disk diffusion method in 100 isolates of MDR-*P. aeruginosa*. The solid lines show the breakpoints for colistin with the E-test (MICs, S ≤2 µg/ml, I=4 µg/ml, R ≥8 µg/ml) and dotted lines showed the breakpoints for colistin with the disk diffusion method (S ≥11 mm, R ≤10 mm) as recommended by CLSI (2011); the numbers represent *P. aeruginosa*.

ate resistance on the E-test (MIC 3 µg/ml) was sensitive on the disk diffusion method (13 mm zone diameter).

DISCUSSION

Colistin had good activity against MDR-PA isolates (98% susceptibility rate with low MIC_{50} and MIC_{90}). Tam *et al* (2010) found 95% of MDR-PA isolates were susceptible to colistin with a MIC_{50} and MIC_{90} for colistin of 1.5 and 2 µg/ml, respectively. One of the 19 isolates in this study (5%) was colistin resistant, with a MIC of 3 µg/ml. Galani *et al* (2008) found 93.5% of 124 MDR-PA isolates were susceptible to colistin with a MIC_{50} and MIC_{90} for colistin of 1 and 2 µg/ml, respectively. Walkty *et al* (2009), evaluated colistin susceptibility with the broth micro-dilution method, found all 76 MDR-PA isolates were susceptible to colistin (MIC ranges of 0.5-2 µg/ml, and MIC_{50} and MIC_{90} both 2 µg/ml).

There have been reports of *P. aeruginosa* resistance to colistin (Landman *et al*, 2005; Li *et al*, 2005; Zapantis *et al*, 2007; Johansen *et al*, 2008; Tam *et al*, 2010). The emergence of colistin resistant *P. aeruginosa* has increased due to the use of colistin in the treatment of *P. aeruginosa* being on the rise (Landman *et al*, 2005; Johansen *et al*, 2008; Tam *et al*, 2010). This study found two colistin resistant MDR-PA isolates from two patients who had no previous history of colistin therapy. However, both patients had received other antimicrobials; one patient had received ceftriaxone and imipenem+cilastatin, and the other had received ciprofloxacin. Souli *et al* (2008) found 10 MDR-PA isolates from 10 patients who had been treated previously with ciprofloxacin were resistant to colistin. The colistin resistant MDR-PA isolates in our study were obtained from two patients with a history of urinary tract infection (UTI). One patient had been hospitalized for 2 months and the other for 3 weeks. We assume the colistin resistant

isolates were hospital acquired.

The antimicrobial resistance among MDR-PA isolates showed various patterns; about half the isolates exhibited the AK-CAZ-CIP-IMP resistance pattern. Colistin was effective in treating these resistant infections. Thirty-two percent of isolates had the AK-CAZ-CIP resistance pattern. This finding is similar to 20-30% reported by Dejsirilert *et al* (2009).

In our study one of the isolates was susceptible to colistin on the disk diffusion test but showed intermediate resistance on the E-test. Galani *et al* (2008) reported a similar mismatch between the disk diffusion method and the E-test among 2 of 124 isolates (1.6%). One of the 124 isolates (0.8%) was susceptible to colistin (zone diameter=13 mm) with the disk diffusion method, but was intermediately resistant to colistin (MIC 4 µg/ml) with the E-test. The other isolate (0.8%) was susceptible to colistin (zone diameter=12 mm) with the disk diffusion method but resistant to colistin (MIC 8 µg/ml) with the E-test. One explanation for this is that colistin diffuse poorly on agar (Galani *et al*, 2008; Behera *et al*, 2010). Tan and Ng (2006) observed a 5-11% discordant rate between the disk diffusion method and the dilution test (reference method) when the effect of colistin against gram-negative bacteria. Gales *et al* (2001) also found a 3.5-6% discordant rate for colistin against gram negative bacteria when comparing the disk diffusion method with the dilution test. Many of the errors with the disk diffusion method occur with a zone diameter between 12 and 13 mm as occurred in our study. Zone diameters of 12-13 mm with the disk diffusion method should be confirmed with the E-test or broth dilution method when evaluating colistin (Galani *et al*, 2008).

In summary, we found colistin had excellent antimicrobial activity against MDR-PA isolates. Most MDR-PA isolates were susceptible to colistin and had low MIC_{50} and MIC_{90} values. There were 2 colistin resistant MDR-PA isolates in this study. Constant surveillance of antimicrobial resistance is important. If the zone diameter is 12-13 mm with the disk diffusion test for colistin the MIC value should be used for colistin to dismiss if there is resistance or not.

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