In vitro Activities of Colistin and Ampicillin/Sulbactam against *Acinetobacter baumannii*

Warunee Punpanich MD, PhD*, Anurak Munsrichoom BSc, MSc**,
Suchada Srisarang BSc (Statistics)***, Vipa Treeratweeraphong BSc, MSc**

** Department of Pediatrics, Queen Sirikit National Institute of Child Health, College of Medicine, Rangsit University, Bangkok, Thailand*
*** Division of Microbiology, Queen Sirikit National Institute of Child Health, Bangkok, Thailand*
**** Pediatric Infectious Disease Research Unit, Queen Sirikit National Institute of Child Health, Bangkok, Thailand*

Objective: *The aim of the present study was to examine the in vitro antimicrobial activity of colistin, and ampicillin/sulbactam against A. baumannii isolated from pediatric patients and to compare the susceptibility testing using disc diffusion with minimal inhibitory concentration (MIC) E-test method.*
Material and Method: *One hundred strains of A. baumannii from various clinical isolates were included in the present study. Antimicrobial susceptibilities of A. baumannii to colistin, ampicillin/sulbactam were determined by disc diffusion and minimal inhibitory concentration (MIC) using E-test method. The analysis was stratified by carbapenem resistance status. Sensitivity and specificity of the disc diffusion test compared to the MIC E-test were estimated.*
Results: *Ninety-seven strains of all isolates (97%) were sensitive to colistin using both disc diffusion and E-test methods. In contrast, 41% and 34% of the isolates were sensitive to ampicillin/sulbactam by disc diffusion and MIC E-test, respectively. The colistin MIC_{50} and MIC_{90} for A. baumannii were 0.38 and 1 μg/mL, respectively. The ampicillin/sulbactam MIC_{50} and MIC_{90} were 16 and 89.6 μg/mL, respectively. Based on the results of MIC E-test, ninety-eight (n = 49) and six (n = 3) percent of carbapenem-resistant A. baumannii (n = 50) were susceptible to colistin and ampicillin/sulbactam, respectively. Sensitivity and specificity of disc diffusion test compared to MIC E-test were 99% and 66.7% for colistin and 80.5% and 98.3% for ampicillin/sulbactam, respectively.*
Conclusion: *The antimicrobial activities of colistin against A. baumannii isolates remained high for both cabapenem-susceptible and -resistant strains. However, the in vitro activity of ampicillin/sulbactam against A. baumannii was low. Thus, a combination, rather than monotherapy, of ampicillin/sulbactam with other antibiotics is strongly recommended when dealing with A. baumannii infection. In addition, disc diffusion test appeared to be a useful screening method for susceptibility testing for colistin and ampicillin/sulbactam against A. baumanii.*

Keywords: *In vitro susceptibility, Acinetobacter baumannii, Multi-drug resistance, Colistin, Ampicillin/Sulbactam*

J Med Assoc Thai 2011; 94 (Suppl. 3): S95-S100
Full text. e-Journal: *http://www.mat.or.th/journal*

During the recent decades, there has been a growing concern regarding the increased incidence of infections caused by multi-drug and/or pan-drug resistant (MDR/PDR) gram negative bacteria especially *Acinetobacter baumannii*. A systematic review has demonstrated the significant negative impact of MDR gram negative bacterial infections on mortality, length of stay, treatment cost and outcome. The additional hospital cost and length of stay attributable to hospital-acquired drug resistant gram negative pathogen were estimated to be 30% and 24% higher than those caused by non drug-resistant gram negative bacteria, respectively[1]. MDR Acinetobacter infection has been reported to be associated with a two-fold increased risk of extended hospital and intensive care unit stay compared with susceptible Acinetobacter infection odds ratio (OR) 2.5, 95% confidence interval (CI) 1.2-5.2 and OR 2.1, 95% CI 1.0-4.3, respectively[2]. Given the absence of novel therapeutic agents, polymyxin antibiotics such as colistin, despite its potential renal toxicity, have re-emerged as a potential last-resort treatment option against MDR *A. baumannii*. However, *A. baumannii* can acquire resistance to polymyxin antibiotics by loss of the binding target, the lipid A component of lipopolysaccharide[3].

Correspondence to:
Punpanich W, Department of Pediatrics, Queen Sirikit National Institute of Child Health, Bangkok 10400, Thailand.
Phone: 0-2354-8345
E-mail: warunee@vandepitte.eu

In addition, due to the intrinsic activity of sulbactam, ampicillin/sulbactam has been reported to have *in vitro* activity against[4,5] and be effective for the treatment of[6-11], MDR/PDR *A. baumannii* infection especially when used in combination with other antibiotics[12-15]. At Queen Sirikit National Institute of Child Health (QSNICH), the largest urban children's health center in Bangkok, Thailand, the authors have experienced an increasing number of infections caused by MDR gram negative bacteria especially *A. baumannii* during the past decade. Colistin and ampicillin/sulbactam have been the mainstay antimicrobial agents to combat with this infection at QSNICH since 2007. A previous study conducted at QSNICH indicated that percent susceptibility of cephalosporin-resistant *A. baumannii* to colisin was 95.2% by E-test with MIC_{90} of 1.4 μg/mL[16]. Given the high incidence and limited treatment options of this infection, it is imperative to monitor the antimicrobial activity of the existing available antimicrobial agents. The aim of the present study was to evaluate the current antimicrobial activity of colistin and ampicillin/sulbactam against *A baumannii* using disc diffusion tests and minimum inhibitory concentration (MIC) E-test method.

Material and Method

During the period of October 2008 and September 2010, *A. baumannii* isolated from clinical specimens (both normally sterile and non-sterile sites) from pediatric patients aged 0-18 years receiving care at QSNICH were tested for colistin and ampicillin/sulbactam using both disc diffusion and MIC using E-test. Identification of the bacteria was performed by conventional microbiological methods. Percent susceptibility and level of MIC_{50} and MIC_{90} were evaluated for both antimicrobial agents. The antimicrobial activities were also compared between those with and without carbapenem resistance. Carbapenem-resistance was defined as: isolates resistant to imipenem or meropenem by conventional disc diffusion test.

The results of disc zone sizes were interpreted based on the Clinical and Laboratory Standards Institute (CLSI) criteria for colistin (resistant < 11 mm and susceptible ≥ 11 mm) and ampicillin/sulbactam (resistant ≤ 11 mm and susceptible ≥ 15 mm)[17]. MICs were determined by E-test method according to the manufacturer's guidelines (AB bioMerieux, Solna, Sweden). MICs of E-test were rounded up to the next higher twofold dilution. For E-test methods, MICs ≤ 2 μg/mL for colistin and ≤ 8/4 μg/mL for ampicillin/sulbactam (≤ 8 μg/mL for ampicillin and ≤ 4 μg/mL for sulbactam) were the breakpoints to designate susceptible strains[17]. In the present study, the susceptibility test of the combined ampicillin/sulbactam using MIC E-test was focused on the cut-point of ≤ 4 μg/mL of sulbactam rather than that of ampicillin.

Results

A total of 100 *A. baumannii* isolates obtained from 100 patients receiving care at QSNICH from October 2008 to September 2010 were included in the present study. Fifty (50%) isolates were identified as being resistant to both imipenem and meropenem by disc diffusion test based on routine susceptibility testing. According to the breakpoints for disc diffusion test by CLSI, 97 (97%) and 41 (41%) isolates were susceptible to colistin and ampicillin/sulbactam, respectively. According to the breakpoints for MIC E-test by CLSI, 97 (97%) and 34 (34%) isolates were susceptible to colistin and sulbactam, respectively. Among carbapenem resistant strains (n = 50), 49 (98%) and 8 (16 %) isolates were susceptible to colistin and ampicillin/sulbactam, respectively by disc diffusion test as compared to 49 (98%) and 3 (6%) isolates were susceptible to colistin and sulbactam, respectively by E-test.

The sensitivity and specificity of disc diffusion test for the identification of *in vitro* susceptibility of *A. baumannii* of these two antibiotics were also investigated. The results indicated that the *in vitro* activity of both antimicrobial agents against *A. baumannii* were comparable between the two methods. For colistin, the sensitivity and specificity of disc diffusion test as compared to MIC E-test were estimated to be 99% and 67%, respectively. The sensitivity and specificity of disc diffusion test as compared to MIC E-test for sulbactam against *A. baumannii* were 80.5% and 98.3%, respectively. The colistin MIC_{50} and MIC_{90} were 0.38 and 1 μg/mL, respectively. The ampicillin/sulbactam MIC_{50} and MIC_{90} were 16 and 89.6 μg/mL, respectively.

Discussion

Gram negative bacteria belonging to the genus Acinetobacter was recognized as a potential nosocomial pathogen since early 1970's[18]. *A. baumannii* and its closely related unnamed genomic species 3 and 13 sensu Tjernberg and Ursing (13TU) are the most clinically relevant pathogen accounting for the vast majority of nosocomial infections and outbreaks involving *Acinetobacter* spp[19]. During the

Table 1. *In vitro* activity of colistin against *A. baumannii* by carbapenem susceptibility

Carbapenem susceptibility	Disc diffusion		MIC E-test	
	Susceptible n (%)	Non-susceptible n (%)	Susceptible n (%)	Non-susceptible n (%)
Susceptible	48 (96)	2 (4)	48 (96)	2 (4)
Non-susceptible	49 (98)	1 (2)	49 (98)	1 (2)
Total	97 (97)	3 (3)	97 (97)	3 (3)

Table 2. *In vitro* activity of ampicillin/sulbactam against *A. baumannii* by carbapenem susceptibility

Carbapenem susceptibility	Disc diffusion		MIC E-test	
	Susceptible n (%)	Non-susceptible n (%)	Susceptible* n (%)	Non-susceptible n (%)
Susceptible	33 (66)	17 (34)	31 (62)	19 (38)
Non-susceptible	8 (16)	42 (84)	3 (6)	47 (94)
Total	41 (41)	59 (59)	34 (34)	66 (66)

*Using MIC ≤ 4 μg/mL for sulbactam as a cut-point for susceptibility designation

Table 3. Sensitivity and specificity of disc diffusion test for the identification of *in vitro* susceptibility testing of *A. baumannii*

Disc diffusion	MIC E-test			
	Colistin		Sulbactam	
	Susceptible n (%)	Non-susceptible n (%)	Susceptible* n (%)	Non-susceptible n (%)
Susceptible	96 (99)	1 (1)	33 (80.5)	8 (19.5)
Non-susceptible	1 (33)	2 (66.7)	1 (1.7)	58 (98.3)
Total	97 (97)	3 (3)	34 (34)	66 (66)

*Using MIC ≤ 4 μg/mL for sulbactam as a cut-point for susceptibility designation

early period, it was generally susceptible and relatively easy to be treated by commonly used antibiotic such as ampicillin, aminoglycoside, and nalidixic acid[20]. Recently, parallel with its increased in incidence, infection with MDR *A. baumannii* has recently gained particular attentions in medical community worldwide. Of particular concern is its ability to resist virtually all available antimicrobial agents especially carbapenems which has been a major threat to successful treatment and thus patients' survival. The two major resistance mechanisms against carbapenem were mediated by the production of class D oxacillinase or OXA carbapenemases and metallo-beta-lactamase enzyme[21] as a consequence of extensive use of potent broad-spectrum antimicrobial agents especially in hospital settings. Potential alternative therapeutic options for MDR *A. baumannii* include colistin, ampicillin-sulbactam, tigecycline, and combination of these drugs with carbapenem[22,23]. Previous reports indicated that treatment with ampicillin/sulbactam provided a similar[24] or more favorable treatment outcome[25] when compared to colistin for MDR *A. baumannii* infections. Nevertheless, the incidence of nephrotoxicity was generally higher among those who received colistin

treatment[24]. The present study aimed to evaluate the current commonly used antimicrobial therapy against these pathogens at QSNICH *i.e.* colistin and ampicillin/sulbactam. The authors' findings indicated that 97% of *A. baumannii* remained susceptible to colistin despite the wide use of this drug in QSNICH since 2007 whereas only 34% were susceptible to sulbactam. The percent of susceptibility of colistin was rather similar to the results reported in 2008 (95.2%) at the same center[16]. However, the authors did not have existing data of percent susceptibility test of sulbactam against *A. baumannii* during the earlier period when it was first introduced in July 2008 to compare with the finding from the present study. As a result, the authors strongly recommend against the use of sulbactam or ampicillin/sulbactam as a sole agent, but rather as a combination therapy when combating this infection. Existing literature indicated that, despite the *in vitro* non-susceptibility, the use of sulbactam as a combination therapy with other antibiotic such as imipenem, meropenem, colistin had resulted in favorable treatment outcomes both in animal models[26] and clinical settings[12,14]. A recent study in Thailand indicated that a triple combination of meropenem/sulbactam/colistin exhibited a synergistic effect against 97% of MDR *A. baumannii*[15]. In addition, the time-kill study demonstrated a better killing effect by the triple combination (meropenem/colistin/sulbactam) than any of the double combinations, *i.e.* meropenem/sulbactam, meropenem/colistin, or colistin/sulbactam. Thus, it seemed that the addition of sulbactam to meropenem and colistin may further improve their antibacterial activity against this pathogen[15].

The results of susceptibility testing using disc susceptibility were found to be comparable with those obtained from E-test for both antibiotics with a particularly high sensitivity of disc diffusion test to predict *A. baumannii* susceptibility for colistin. This is in agreement with a recent work by Behera et al that evaluated the different susceptibility testing methods for polymyxins B and E (colistin) against gram-negative bacteria using the new CLSI guidelines which showed that the disk diffusion method can be useful for initial screening in diagnostic laboratories[27]. In addition, E-test demonstrated good concordance with the reference method (broth microdilution test).

Conclusion

The results of the present study highlighted that colistin has maintained a satisfactory *in vitro* antimicrobial activity against *A. baumannii* since the introduction of its use at QSNICH in June 2007. In contrast, the current antimicrobial activity of ampicillin/sulbactam was rather low. Therefore, the authors recommend against using sulbactam as a sole agent when dealing with *A. baumannii* infection.

Potential conflicts of interest

None.

References

1. Shorr AF. Review of studies of the impact on Gram-negative bacterial resistance on outcomes in the intensive care unit. Crit Care Med 2009; 37: 1463-9.
2. Sunenshine RH, Wright MO, Maragakis LL, Harris AD, Song X, Hebden J, et al. Multidrug-resistant Acinetobacter infection mortality rate and length of hospitalization. Emerg Infect Dis 2007; 13: 97-103.
3. Moffatt JH, Harper M, Harrison P, Hale JD, Vinogradov E, Seemann T, et al. Colistin resistance in *Acinetobacter baumannii* is mediated by complete loss of lipopolysaccharide production. Antimicrob Agents Chemother 2010; 54: 4971-7.
4. Higgins PG, Wisplinghoff H, Stefanik D, Seifert H. *In vitro* activities of the beta-lactamase inhibitors clavulanic acid, sulbactam, and tazobactam alone or in combination with beta-lactams against epidemiologically characterized multidrug-resistant *Acinetobacter baumannii* strains. Antimicrob Agents Chemother 2004; 48: 1586-92.
5. Pandey A, Kapil A, Sood S, Goel V, Das B, Seth P. *In vitro* activities of ampicillin-sulbactam and amoxicillin-clavulanic acid against *Acinetobacter baumannii*. J Clin Microbiol 1998; 36: 3415-6.
6. Corbella X, Ariza J, Ardanuy C, Vuelta M, Tubau F, Sora M, et al. Efficacy of sulbactam alone and in combination with ampicillin in nosocomial infections caused by multiresistant *Acinetobacter baumannii*. J Antimicrob Chemother 1998; 42: 793-802.
7. Levin AS, Levy CE, Manrique AE, Medeiros EA, Costa SF. Severe nosocomial infections with imipenem-resistant *Acinetobacter baumannii* treated with ampicillin/sulbactam. Int J Antimicrob Agents 2003; 21: 58-62.
8. Sayin KS, Sacar S, Suzer T, Cevahir N, Okke D, Dirgen CS, et al. Successful treatment of a patient with multidrug resistant *Acinetobacter baumannii* meningitis with high dose ampicillin-sulbactam. Mikrobiyol Bul 2008; 42: 353-8.
9. Smolyakov R, Borer A, Riesenberg K, Schlaeffer F,

Alkan M, Porath A, et al. Nosocomial multi-drug resistant *Acinetobacter baumannii* bloodstream infection: risk factors and outcome with ampicillin-sulbactam treatment. J Hosp Infect 2003; 54: 32-8.
10. Takahashi N, Shimada T, Tanabe K, Sato M, Kitamura J, Sato H, et al. A case of unforeseen intractable severe bacteremia due to *Acinetobacter baumannii*-an efficacy of sulbactam. Jpn J Infect Dis 2009; 62: 461-3.
11. Wood GC, Hanes SD, Croce MA, Fabian TC, Boucher BA. Comparison of ampicillin-sulbactam and imipenem-cilastatin for the treatment of acinetobacter ventilator-associated pneumonia. Clin Infect Dis 2002; 34: 1425-30.
12. Kuo LC, Lai CC, Liao CH, Hsu CK, Chang YL, Chang CY, et al. Multidrug-resistant *Acinetobacter baumannii* bacteraemia: clinical features, antimicrobial therapy and outcome. Clin Microbiol Infect 2007; 13: 196-8.
13. Lee CH, Tang YF, Su LH, Chien CC, Liu JW. Antimicrobial effects of varied combinations of meropenem, sulbactam, and colistin on a multidrug-resistant *Acinetobacter baumannii* isolate that caused meningitis and bacteremia. Microb Drug Resist 2008; 14: 233-7.
14. Lee NY, Wang CL, Chuang YC, Yu WL, Lee HC, Chang CM, et al. Combination carbapenem-sulbactam therapy for critically ill patients with multidrug-resistant *Acinetobacter baumannii* bacteremia: four case reports and an *in vitro* combination synergy study. Pharmacotherapy 2007; 27: 1506-11.
15. Pongpech P, Amornnopparattanakul S, Panapakdee S, Fungwithaya S, Nannha P, Dhiraputra C, et al. Antibacterial activity of carbapenem-based combinations againts multidrug-resistant *Acinetobacter baumannii*. J Med Assoc Thai 2010; 93: 161-71.
16. Punpanich W, Tantichattanon W, Wongwatcharapaiboon S, Treeratweeraphong V. *In vitro* susceptibility pattern of cephalosporin-resistant Gram-negative bacteria. J Med Assoc Thai 2008; 91 (Suppl 3): S21-7.
17. Cockerill F, Wikler M, Bush K, Dudley M, Eliopoulos G, Hardy D, et al. Clinical and Laboratory Standards Institute performance standards for antimicrobial susceptibility testing: Twentieth informational supplement M100-S20. Wayne, Pennsylvania: CLSI; 2010.
18. Towner KJ. Acinetobacter: an old friend, but a new enemy. J Hosp Infect 2009; 73: 355-63.
19. Van Looveren M, Goossens H. Antimicrobial resistance of *Acinetobacter* spp. in Europe. Clin Microbiol Infect 2004; 10: 684-704.
20. Bergogne-Berezin E. Resistance of *Acinetobacter* spp. to antimicrobials overview of clinical resistance patterns and therapeutic problems. In: Bergogne-Berezin E, Joly-Guillou M, Towner K, editors. Acinetobacter, microbiology, epidemiology, infections, management. Boca Raton: CRC Press; 1996: 133-83.
21. Gordon NC, Wareham DW. Multidrug-resistant *Acinetobacter baumannii*: mechanisms of virulence and resistance. Int J Antimicrob Agents 2010; 35: 219-26.
22. Bassetti M, Righi E, Esposito S, Petrosillo N, Nicolini L. Drug treatment for multidrug-resistant *Acinetobacter baumannii* infections. Future Microbiol 2008; 3: 649-60.
23. Jain R, Danziger LH. Multidrug-resistant Acinetobacter infections: an emerging challenge to clinicians. Ann Pharmacother 2004; 38: 1449-59.
24. Betrosian AP, Frantzeskaki F, Xanthaki A, Douzinas EE. Efficacy and safety of high-dose ampicillin/sulbactam vs. colistin as monotherapy for the treatment of multidrug resistant *Acinetobacter baumannii* ventilator-associated pneumonia. J Infect 2008; 56: 432-6.
25. Oliveira MS, Prado GV, Costa SF, Grinbaum RS, Levin AS. Ampicillin/sulbactam compared with polymyxins for the treatment of infections caused by carbapenem-resistant *Acinetobacter* spp. J Antimicrob Chemother 2008; 61: 1369-75.
26. Ko WC, Lee HC, Chiang SR, Yan JJ, Wu JJ, Lu CL, et al. *In vitro* and *in vivo* activity of meropenem and sulbactam against a multidrug-resistant *Acinetobacter baumannii* strain. J Antimicrob Chemother 2004; 53: 393-5.
27. Behera B, Mathur P, Das A, Kapil A, Gupta B, Bhoi S, et al. Evaluation of susceptibility testing methods for polymyxin. Int J Infect Dis 2010; 14: e596-601.

ความไวต่อยาปฏิชีวนะของเชื้อ A. baumannii ต่อยา colistin และ ampicillin/sulbactam

วารุณี พรรณพานิช, อนุรักษ์ มันศรีชุม, สุชาดา ศรีศรัง, วิภา ตรีรัตนวีรพงษ์

วัตถุประสงค์: *เพื่อประเมินประสิทธิภาพทางห้องปฏิบัติการของยาต้านจุลชีพ colistin และ ampicilin/sulbactam ต่อเชื้อ Acinetobacter baumannii ที่เพาะเชื้อขึ้นจากผู้ป่วยเด็ก โดยเปรียบเทียบระหว่างผลการตรวจด้วยวิธี disc diffusion test กับ minimal inhibitory concentration (MIC) E-Test*

วัสดุและวิธีการ: *จำนวนเชื้อ A. baumannii ทั้งหมด 100 สายพันธุ์ ได้รับการทดสอบความไวต่อยาปฏิชีวนะ colistin และ ampicillin/sulbactam โดยวิธี disc diffusion และ MIC E-Test. การวิเคราะห์ข้อมูลจะแยกเป็นกลุ่มที่ไวและกลุ่มที่ไม่ไวต่อยากลุ่ม carbapenem และผลที่ได้ นำมาคำนวณหาความไวและความจำเพาะของการตรวจโดยวิธี disc diffusion test เมื่อเปรียบเทียบกับวิธี MIC E-Test*

ผลการศึกษา: *พบว่า 97% ของเชื้อ A. baumannii ทั้งหมด ไวต่อยา colistin จากการตรวจด้วยวิธี disc diffusion และ MIC E-test ในขณะที่เชื้อดังกล่าวนี้มีความไวต่อยา ampicillin/sulbactam เท่ากับ 41% และ 34% เมื่อตรวจโดยวิธี disc diffusion และ MIC ตามลำดับ MIC_{50} และ MIC_{90} สำหรับ colistin เท่ากับ 0.38 และ 1 μg/mL ตามลำดับ ในขณะที่ MIC_{50} และ MIC_{90} ของ ampicillin/sulbactam เท่ากับ 16 และ 89.6 μg/mL ตามลำดับ จากการตรวจโดยวิธี MIC E-Test พบว่า 98% (n = 49) และ 6% (n = 3) ของ carbapenem-resistant A. baumannii (n = 50) ไวต่อ colistin และ ampicillin/sulbactam ตามลำดับ ความไวและความจำเพาะของการตรวจโดยวิธี disc diffusion เมื่อเปรียบเทียบกับ MIC E-Test เท่ากับ99% และ 66.7% สำหรับ colistin และ80.5% และ 98.3% สำหรับ ampicillin/sulbactam ตามลำดับ*

สรุป: *ยา colistin ยังมีประสิทธิภาพทางห้องปฏิบัติการต่อเชื้อ A. baumannii ทั้งสายพันธุ์ที่ดื้อและไม่ดื้อต่อยา carbapenem ค่อนข้างสูง ในขณะที่ความไวต่อยา ampicillin/sulbactam จากการตรวจทางห้องปฏิบัติการของเชื้อ A. baumannii ค่อนข้างต่ำ ดังนั้นการพิจารณาใช้ยา ampicillin/sulbactam ในการรักษาการติดเชื้อ A. baumannii ไม่ควรใช้ในรูปของยาเดี่ยว จากผลการประเมินความไวและความจำเพาะของการตรวจด้วยวิธี disc diffusion พบว่าการตรวจด้วยวิธีนี้มีประโยชน์ในการคัดกรองในห้องปฏิบัติการในการประเมินรูปแบบความไวต่อยาทั้ง 2 ชนิดต่อเชื้อ A. baumannii*