In Vitro Activity of Polymyxin B Against Carbapenem-Resistant *Acinetobacter baumannii*

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Objective: *To determine in vitro activity of polymyxin B against carbapenem-resistant Acinetobacter baumannii.*
Material and Method: *The activity of polymyxin B was determined against 217 strains of carbapenem-resistant A. baumannii collected from different patients by standard agar dilution method and disk diffusion test using polymyxin B disk (300 units). The control strains were E. coli ATCC 25922 and P. aeruginosa ATCC 27853.*
Results: *The MIC values and inhibition zone diameters of polymyxin B against the quality control bacteria were within the acceptable range. The MIC_{50} and MIC_{90} values of polymyxin B against 217 strains of carbapenem-resistant A. baumannii were 0.5 and 1 mg/l, respectively. If the susceptible MIC breakpoint of polymyxin B was ≤2 mg/l, 98.2% of carbapenem-resistant A. baumannii strains were susceptible to polymyxin B. If the susceptible MIC breakpoint of polymyxin B was ≤2 mg/l, the sensitivity and the specificity of the inhibition zone diameter of >12 mm were 100% and 75%, respectively. The aforementioned diagnostic parameters gave positive predictive value of 99.5% and negative predictive value of 100% for predicting susceptibility of carbapenem-resistant A. baumannii to polymyxin B by disk diffusion test.*
Conclusion: *Polymyxin B was very active against carbapenem-resistant A. baumannii. The inhibition zone diameters of >12 mm was accurate enough to determine susceptibility of carbapenem-resistant A. baumannii to polymyxin B. Polymyxin B can be an alternative to or more preferable than colistin for therapy of carbapenem-resistant A. baumannii infections.*

Keywords: *Polymyxin B, In vitro activity, Acinetobacter baumannii*

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Infections caused by carbapenem-resistant *Acinetobacter baumannii* have become a serious health problem in Asia including Thailand[1-3]. Up to 85% of *A. baumannii* isolated from patients who had hospital-acquired *A. baumannii* infectons at Siriraj Hospital were resistant to carbapenems. Polymyxins (polymyxin B and polymyxin E or colistin) have been reintroduced for clinical use for therapy of multidrug-resistant (MDR) Gram-negative bacterial infections especially those caused by carbapenem-resistant Gram-negative bacteria over the past decade[4]. Only colistin has been available in Thailand since 2007. Carbapenem-resistant *A. baumannii* infection had a high mortality and its mortality was associated with inappropriate initial antimicrobial therapy[2]. Efficacy of colistin for therapy of carbapenem-resistant *A. baumannii* and *P. aeruginosa* in Thai patients was modest[5]. Colistin is administered parenterally as colistimethate sodium (CMS), an inactive prodrug that is converted into colistin, which exerts the antibacterial activity[6]. The extent of conversion of CMS to colistin is low, only 20% to 25% of CMS is converted to colistin in patients with normal kidney function whereas most of CMS is predominantly cleared by renal excretion[7]. The plasma concentrations of formed colistin rise slowly after CMS is given even with a loading dose of CMS at the initiation of therapy and it may take several hours to achieve sufficient plasma colistin concentrations that may be effective. An increase of the dose of CMS to compensate for the low conversion of CMS to colistin is limited by an increase in incidence of nephrotoxicity due to more intrarenal conversion of CMS to colistin resulting in toxic effect upon renal tubular cells[7]. This might explain the modest efficacy and a common nephrotoxicity in using CMS for therapy of carbapenem-resistant *A. baumannii* infections.

Polymyxin B is different from colistin by just one amino acid in the peptide ring[8]. Polymyxin B is administered as its sulfate salt meaning that the active antibacterial compound is directly given to patients. Polymyxin B is subject to extensive renal tubular reabsorption and it is eliminated mainly by non-renal

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clearance mechanism resulting in relatively low urinary concentrations[9-11]. Therefore, an adjustment of polymyxin B dosing in patients with renal impairment may not be needed[11]. A small comparative study revealed that the efficacy of colistin and polymyxin B for the treatment of serious infections caused by carbapenem-resistant *Acinetobacter* spp. were comparable[12]. Polymyxin B might be less nephrotoxic than colistin[13,14].

The objective of the study was to determine in vitro activity of polymyxin B against carbapenem-resistant *A. baumannii* isolated from the hospitalized patients at Siriraj Hospital.

Material and Method

Study bacteria

They were 217 strains of *A. baumannii* isolated from different patients with pneumonia, blood stream infections, urinary tract infections and skin and soft tissue infections. All strains were resistant to carbapenem.

Antimicrobial agents

The reference standard powder of polymyxin B sulfate was purchased from Sigma-Aldrich, USA. The stock solutions were prepared using sterile distilled water as a solvent, and they were kept frozen at -80°C until used.

Antimicrobial susceptibility test

The minimum inhibitory concentration (MIC) was determined by standard agar dilution method according to the Clinical and Laboratory Standards Institute (CLSI) 2014[15]. Mueller Hinton agar (MHA) (Oxoid, UK) and cation adjusted MHA (CAMHB) (BBL, Becton Dickinson, USA) were used as the media and the diluents. *A. baumannii* inoculum preparation was made by broth method, adjusted to 0.5 McFarland turbidity and then diluted the bacterial suspension with CAMHB to 10^6 CFU/ml. Final inocula of approximately 10^4 CFU/spot were inoculated on a series of polymyxin B agar containing polymyxin B concentrations from 0.16 mg/l to 8 mg/l by multipoints spot inoculators. The inoculated agars were incubated at 35°C for 20-24 hours in ambient air. The MIC was defined as the lowest concentration of antimicrobial agent that inhibited visible growth of bacteria on agar. The susceptible and resistant breakpoints of polymyxin B were the MIC of ≤2 and >2 mg/l, respectively[16]. The disk diffusion test of polymyxin B (300 units/disk) (Oxoid, UK) was determined by standard disk diffusion method according to CLSI 2014[16]. The medium used for the test was MHA (Oxoid, UK). *A. baumannii* inoculum preparation was made by broth method, adjusted to 0.5 McFarland turbidity and it was spread on MHA plate. The polymyxin B disk was placed on the inoculated MHA plate and the plate was then incubated at 35°C for 20-24 hours in ambient air. The quality control strains were *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853.

Data analysis

The in vitro susceptibility test results were described by descriptive statistics, and the sensitivity and the specificity of the best cut-off value of the inhibition zone diameter were computed.

Results

The MIC values of polymyxin B against *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were 0.5 and 1 mg/l, respectively. These MIC values of the quality control strains were within the acceptable range. The distribution of MIC values of polymyxin B against 217 strains of carbapenem-resistant *A. baumannii* is shown in Table 1. The MIC_{50} and MIC_{90} values of polymyxin B against 217 strains of carbapenem-resistant *Acinetobacter baumannii* were 0.5 and 1 mg/l, respectively. If the susceptible and resistant MIC breakpoints of polymyxin B were ≤2 and >2 mg/l, respectively, 98.2% of carbapenem-resistant *A. baumannii* strains were susceptible to polymyxin B. The distribution of inhibition zone diameters of polymyxin B against 217 strains of carbapenem-resistant *A. baumannii* is shown in Table 2. Most of the isolates had inhibition zone diameter 14 mm. The correlation of MIC values and inhibition zone diameters of polymyxin B against 217 strains of carbapenem-resistant *A. baumannii* is shown in Table 3. If the susceptible MIC breakpoint of polymyxin B was ≤2 mg/l, the sensitivity and the specificity of the inhibition zone diameter of >12 mm was 100% (213/213) and 75% (3/4), respectively. Therefore, if the inhibition zone diameter was >12 mm, the probability of carbapenem-resistant *A. baumannii* to be susceptible to polymyxin B was 99.5%. If the inhibition zone diameter was ≤12 mm, the probability of carbapenem-resistant *A. baumannii* to be resistant to polymyxin B was 100%.

Discussion

The carbapenem-resistant *A. baumannii* isolates collected from hospitalized patients at Siriraj

Hospital were very susceptible to polymyxin B with the MIC_{50} and MIC_{90} 0.5 and 1 mg/l, respectively. Polymyxin B seemed to be somewhat more active than colistin because the in vitro activity of colistin against the same 217 isolates of carbapenem-resistant *A. baumannii* revealed that the MIC_{50} and MIC_{90} values of colistin were 0.5 and 2 mg/l, respectively. However, the study methods used to test in vitro activity of colistin against many isolates of carbapenem-resistant *A. baumannii* were broth microdilution and E-test in addition to agar dilution method. The previous study on evaluation of susceptibility testing methods for polymyxins was done by comparing broth microdilution, agar dilution, E-test, and disk diffusion against MDR Gram-negative bacteria and revealed that the susceptibility results from Etest and agar dilution methods showed good concordance with broth microdilution method[16]. Given that polymyxin B is an active compound with more favorable pharmacokinetics and probably more active and less nephrotoxic than colistin, polymyxin B can be an alternative to or more preferable than colistin for therapy of carbapenem-resistant *A. baumannii* infections in Thai patients.

Table 1. The distribution of MIC values of polymyxin B against 217 strains of carbapenem-resistant *A. baumannii*

MIC (mg/l)	Number of strain (%)
0.25	1 (0.5)
0.5	156 (71.9)
1.0	55 (25.3)
2.0	1 (0.5)
4.0	0
8.0	3 (1.4)
>8.0	1 (0.5)

MIC = minimum inhibitory concentration

Table 2. The distribution of inhibition zone diameters of polymyxin B against 217 strains of carbapenem-resistant *A. baumannii*

Inhibition zone diameter (mm)	Number of strain (%)
10	1 (0.5)
11	0
12	2 (0.9)
13	15 (6.9)
14	163 (75.1)
15	35 (16.1)
16	1 (0.5)

Table 3. The correlation of MIC values and inhibition zone diameters of polymyxin B against 217 strains of carbapenem-resistant *A. baumannii*

	MIC ≤2 mg/l	MIC >2 mg/l	Total
Inhibition zone diameter >12 mm	213	1	214
Inhibition zone diameter ≤12 mm	0	3	3
Total	213	4	217

What is already known on this topic?

Polymyxin B is used for therapy of antibiotic resistant gram negative bacterial infections.

What this study adds?

Since polymyxin B is not available in Thailand, the results of this study indicated that nearly all isolates of carbapenem-resistant *A. baumannii* are susceptible to polymyxin B.

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Potential conflicts of interest

None.

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ฤทธิ์ของ polymyxin B ต่อเชื้อ Acinetobacter baumannii ที่ดื้อ carbapenem

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วัตถุประสงค์: เพื่อทราบฤทธิ์ของ polymyxin B ต่อเชื้อ Acinetobacter baumannii ที่ดื้อ carbapenem
วัสดุและวิธีการ: ทดสอบฤทธิ์ของ polymyxin B ต่อเชื้อ A. baumannii ที่ดื้อ carbapenem จำนวน 217 สายพันธุ์ ที่แยกได้จากผู้ป่วยไทยต่างคนกันด้วยวิธีมาตรฐาน agar dilution และ disk diffusion ด้วย polymyxin B disk ขนาด 300 หน่วย เชื้อที่ใช้เป็นเชื้อควบคุมมาตรฐานคือ E. coli ATCC 25922 และ P. aeruginosa ATCC 27853
ผลการศึกษา: MIC และ inhibition zone diameter ของยา polymyxin B ต่อเชื้อควบคุมมาตรฐานอยู่ในเกณฑ์ที่กำหนด ค่า MIC_{50} และ MIC_{90} ของยา polymyxin B ต่อเชื้อ A. baumannii ที่ดื้อ carbapenem จำนวน 217 สายพันธุ์ คือ 0.5 และ 1 มิลลิกรัม/ลิตร ตามลำดับ หากค่า MIC ของ polymyxin B ที่แสดงว่าเชื้อ A. baumannii ที่ดื้อ carbapenem ไวต่อยา polymyxin B คือ ≤2 มิลลิกรัม/ลิตร เชื้อ A. baumannii ที่ดื้อ carbapenem ร้อยละ 98.2 ไวต่อยา polymyxin B ขนาดของ inhibition zone >12 มิลลิเมตร ในการวินิจฉัย MIC ของ polymyxin B ≤2 มิลลิกรัม/ลิตร มีความไวและความจำเพาะร้อยละ 100 และ 75 ตามลำดับ โดยมีค่า positive predictive ร้อยละ 99.5 และค่า negative predictive ร้อยละ 100
สรุป: polymyxin B มีฤทธิ์ดีมากต่อเชื้อ A. baumannii ที่ดื้อ carbapenem ขนาดของ inhibition zone >12 มิลลิเมตร มีความแม่นยำมากในการวินิจฉัยว่าเชื้อ A. baumannii ที่ดื้อ carbapenem ไวต่อยา polymyxin B ดังนั้น polymyxin B น่าจะใช้แทน colistin หรือ ใช้เป็นยาลำดับแรกในการรักษาการติดเชื้อที่เกิดจาก A. baumannii ที่ดื้อ carbapenem