Comparision Between the Antimicrobial Susceptibility of *Burkholderia pseudomallei* to Trimethoprim-Sulfamethoxazole by Standard Disk Diffusion Method and by Minimal Inhibitory Concentration Determination

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Abstract

Melioidosis, an infection caused *by Burkholderia pseudomallei*, usually occurs in immunocompromised patients and requires prolonged antibiotic therapy. Previously, oral trimethoprim-sulfamethoxazole (TM/SM), an inexpensive and effective drug has been used as a maintenance therapy. The susceptibility of *B. pseudomallei* to TM/SM by the standard disk diffusion method is very low. However, some patients who were treated with TM/SM as a maintenance therapy despite the *in vitro* resistance showed good clinical responses. There were no data comparing the susceptibility of *B. pseudomallei* by the standard disk diffusion method with other quantitative susceptibility tests. The objective of this study was to determine the agreement between the antimicrobial susceptibility of *B. pseudomallei* to TM/SM by standard disk diffusion and minimal inhibitory concentration determination (MIC).

We performed the susceptibility test of 144 strains of *B. pseudomallei* to TM/SM by both the standard disk diffusion and microbroth dilution MIC. The sensitivity results were 53.5 per cent and 84.0 per cent respectively. The agreement between the 2 tests was very poor (Kappa = 0.14 ; 95% CI = -0.01 to 0.29). The false resistant rate by the standard disk diffusion test was 67.9 per cent. Further *in vitro* susceptibility and clinical study are needed to define the interpretive criteria that correlate with clinical response.

Key word : Susceptibility, *B. pseudomallei*, TM/SM, Disk Diffusion, MIC

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Melioidosis, an infection caused *by Burkholderia pseudomallei*, is endemic in Thailand, other parts of Southeast Asia and northern Australia[1]. More than half of the patients had underlying conditions, particularly renal diseases and diabetes. Severe melioidosis had high mortality and high relapse rates, so long term oral maintenance therapy is recommended[2]. Trimethoprim-sulfamethoxazole (TM/SM), usually in combination with chloramphenicol and doxycycline is one of the recommended oral maintenance therapies for melioidosis because of its low cost, good efficacy and lower relapse rate when compared to amoxycillin-clavulanic acid[3]. The antimicrobial susceptibility of *B. pseudomallei* to TM/SM by the standard disk diffusion method in one study from Thailand showed a high resistant rate of 81.4 per cent[4]. However, in some of our patients, TM/SM was used as an oral maintenance therapy for melioidosis with successful outcome despite the *vitro* resistance by the standard disk diffusion method[5]. Whether the interpretive criteria used to define the susceptibility of *B. pseudomallei* to TM/SM is reliable or not, is not known. Further more, there has been no study that compared the susceptibility results of TM/SM against *B. pseudomallei* by the standard disk diffusion method; the method routinely performed in most clinical microbiological laboratories and by the minimal inhibitory concentration (MIC) determination.

The objective of this study was to determine the agreement of the antimicrobial susceptibility of *B. pseudomallei* to TM/SM by the standard disk diffusion method and MIC determination.

MATERIAL AND METHOD

Bacterial strains: One hundred and forty-six clinical isolates of *B. pseudomallei* recovered from patients treated at Srinagarind Hospital, Khon Kaen University from 1989 to 1994 which were stored at -70°C were studied.

Antimicrobial susceptibility: Disk diffusion tests were performed by standard method with Mueller–Hinton agar (MHA) (Diffco Laboratories, Detroit, MI, USA.)[6]. Briefly, four to five colonies from a culture agar plate were suspended in NSS to match the turbidity of 0.5 Mc Farland standard (approximately 10^8 CFU/ml). A sterile cotton swab was dipped into a standardized suspension and streaked evenly in three directions over the surface of the MHA plate (not supplemented with thymidine phosphorylase). Control strain E.coli ATCC 25922 was included on each set of plates and exhibited an inhibition zone of 24-32mm[6]. The MIC determination was done by a broth microdilution method[7]. TM was dissolved in 0.1 N HCl and SM was dissolved in 4 per cent NaOH. Because thymidine presented in Mueller–Hinton broth can inhibit the antimicrobial activity of TM/SM, an enzyme, thymidine phosphorylase was added to the medium to reduce the amount of thymidine. The final concentration of thymidine phosphorylase in Mueller–Hinton broth was 0.1 ug/ml. TM and SM stock solutions were mixed to yield a solution of TM/SM with concentration of each drug equal to 64 ug and 1,280 ug per ml respectively. The microtiter trays were freshly prepared by serial two-fold dilution with TM/SM concentration in each well ranging from 32/640 ug/ml to 0.036/0.625 ug/ml. The inoculum was applied to each well by multi-channel micropipette to yield a final inoculum of approximately $5*10^5$ CFU/ml. *E. coli* ATCC 25922 was included on each set of plates as control. The MIC was defined as the lowest concentration of antibiotic that inhibited visible bacterial growth after overnight incubation. The MIC of TM/SM for the control strain was <, = 0.5/9.5ug/ml[6]. Since interpretive criteria have not been established for *B. pseudomallei*, National Committee for Clinical Laboratory Standard (NCCLS) criteria for organisms other than *Haemophilus*[8] were used to define susceptibility in both tests. The criteria were as follows : disk diffusion test, inhibition zone diameter >, = 16 mm. = sensitive, 11-16 mm. = intermediate, =, < 10 mm. = resistant ; MIC =, < 2/40 ug/ml. = sensitive, 4/80 ug/ml. = intermediate, >, = 8/160 ug/ml. = resistant.

Antibiotics and enzyme were obtained from commercial suppliers as follows : TM/SM susceptibility test disk (1.25ug/23.75ug) (Difco Laboratories, Detroit, MI, USA.); USP Reference standard powder of TM and SM (USP. Co. Inc., Rockville, USA.) ; thymidine phosphorylase (Sigma, St. Louis, Mo, USA.)

Statistical analysis: Susceptibility results of each method were reported in number and percentage. Kappa statistics were used to determine the agreement between the two methods[9].

RESULTS

The 142 *B. pseudomallei* strains were collected from pus (40.8%), blood (31.7%), sputum

Table 1. Minimal inhibitory concentration (MIC) of trimethoprim-sulfamethoxazole (TM/SM) against 146 strains of *Burkholderia pseudomallei.*

MIC (ug/ml)	No.	Cummulative No.	Cummulative%
0.125	1	1	0.7
0.25	3	4	2.7
0.5	2	6	4.1
1	23	29	19.8
2	94	123	84.2
4	20	143	97.9
8	1	144	98.6
32	2	146	100

(15.5%), urine (2.8%) and other body fluids (9.2%). The sources of collection could not be identified in 4 strains. The MIC results of 146 strains are shown in Table 1. The disk diffusion test was done for only 144 strains. Comparison of results obtained by the standard disk diffusion method and microdilution MIC for 144 strains are shown in Table 2. The susceptibility of *B. pseudomallei* to TM/SM by MIC determination was much higher than the standard disk diffusion method (84.0% *vs* 53.5%). The agreement between these 2 methods was 54.2 per cent { (74+2+2) * 100 /144 }. The chance corrected agreement, Kappa, was = 0.14 (95% CI = -0.01 to 0.29), suggesting a very poor agreement. The false resistant rate by standard disk diffusion method was 67.9 per cent (38/56).

DISCUSSION

The correlation between zone diameter and MICs for various antimicrobial agents including TM/SM against *B. pseudomallei* has not been established[4,8]. The interpretive criteria of NCCLS for organisms other than *Haemophilus* were used to define the susceptibility of *B. pseudomallei* arbitarily. Our results showed that the agreement of these 2 methods based on NCCLS criteria was only 54.2 per cent. Eventhough this was a small study, it suggested that a new interpretive zone should be defined for *B. pseudomallei* because this organism behaves very differently from other bacteria in the family that it previously belonged to[10]. On the other hand, the standard disk diffusion method might not be a reliable method to determine the susceptibility of *B. pseudomallei* to TM/SM. Further MIC study particularly by E- test which is more practical, is needed to define the zone diameter interpretive standard and equivalent MIC breakpoints for *B. pseudomallei.* In addition, a long term follow- up study of the patients to evaluate the clinical efficacy of the drug is also important to determine the reliability of the interpretive criteria.

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Table 2. Agreement between results of susceptibility *of* 144 strains of *Burkholderia pseudomallei* to TM/SM by standard disk diffusion and microdilution MIC.

Disk diffusion	MIC			Total	
	Sensitive	Intermediate	Resistant		
Sensitive	74	2	1	77	(53.5%)
Intermediate	9	2	0	11	(7.6%)
Resistant	38	16	2	56	(38.9%)
Total	121 (84.0%)	20 (13.9%)	3 (2.1%)	144	

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ความไวของเชื้อต่อยาไตรเมโธพริม-ซัลฟามีซอกซาโซล (ทีเอ็ม/เอสเอ็ม) โดยการทดสอบวิธี standard disk diffusion และการหาระดับยาต่ำสุดที่ยับยั้งเชื้อได้

ผกากรอง ลุมพิกานนท์, พ.บ., อัญชลี ตัดตะวะศาสตร์, ปร.ด.**, เพลินจันทร์ เชษฐ์โชติศักดิ์, พ.บ.***, สุรศักดิ์ วงศ์รัตนชีวิน, ปร.ด.**, บัณฑิต ถิ่นคำรพ, M.P.H.*****

เมลิออยโดสิส เป็นโรคติดเชื้อที่เกิดจากเชื้อ *B. pseudomallei* ซึ่งมักเป็นในผู้ป่วยที่มีโรคเดิมอยู่ก่อนและต้องให้ยารักษาเป็นเวลานาน TM/SM เป็นยาที่ใช้รักษาต่อเนื่องเมื่อผู้ป่วยมีอาการดีขึ้นเนื่องจากเป็นยารับประทานที่มีราคาถูกและเคยใช้ได้ผลดี ในปัจจุบันเชื้อ *B. pseudomallei* ดื้อต่อยา TM/SM โดยการทดสอบด้วย standard disk diffusion ค่อนข้างมากโดยที่ผู้ป่วยที่เชื้อดื้อยาก็ตอบสนองต่อการรักษาด้วย TM/SM ได้ดีและไม่มีการกลับเป็นซ้ำ การศึกษานี้ต้องการเปรียบเทียบความไวของเชื้อ *B. pseudomallei* ซึ่งทดสอบด้วย standard disk diffusion และการทดสอบหาระดับยาต่ำสุดที่ยับยั้งเชื้อได้ (minimal inhibitory concentration, MIC) ว่ามีความสอดคล้องกันหรือไม่

ผลการทดสอบความไวของเชื้อ *B. pseudomallei* จำนวน 144 ตัวอย่างโดยวิธี standard disk diffusion และ microbroth dilution เพื่อหา MIC พบว่าเชื้อไวต่อยาร้อยละ 53.5 และ 84.0 ตามลำดับ การทดสอบทั้ง 2 วิธีมีความสอดคล้องกันต่ำมาก (Kappa = 0.14 และ 95% CI = -0.01 ถึง 0.29) การทดสอบวิธี standard disk diffusion ให้ผลดื้อยาลวง 67.9% ควรต้องมีการศึกษาเปรียบเทียบความไวของเชื้อ *B. pseudomallei* ต่อยา TM/SM โดยวิธีอื่นและศึกษาผลการรักษาในผู้ป่วยเพื่อหามาตรฐานการทดสอบที่เหมาะสมต่อไป

คำสำคัญ : ความไวต่อยา, *B. pseudomallei*, TM/SM, disk diffusion, MIC

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