ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF *BURKHOLDERIA PSEUDOMALLEI* AMONG MELIOIDOSIS CASES IN KEDAH, MALAYSIA

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Abstract. *Burkholderia pseudomallei*, the causative agent of melioidosis is an important cause of morbidity and mortality particularly among diabetics. We evaluated 228 isolates of *B.pseudomallei* for antimicrobial sensitivity during 2005-2010 using the disc diffusion technique, of which 144 were obtained from blood culture. More than 90% of the strains were susceptible to cefoperazone, ceftazidime, chloramphenicol and imipenem. Eighty-two percent of the isolates were susceptible to tetracycline and amoxicillin/clavulanate. The susceptibilities to ciprofloxacin was 78% and to trimethoprim-sulfamethoxezole was 47%. The susceptibilities to aminoglycoside antibiotics were low (21% to gentamicin and 6% to amikacin). The susceptibilities were similar between isolates from females and males, bacteremic and abacteremic cases, diabetics and non-diabetics, pneumonia and non-pneumonia cases and between those who died and those who survived. Our findings show antibiotic susceptibility patterns are not a major factor in determining outcomes of *B. pseudomallei* infection. Monitoring the drug susceptibilities among *B. pseudomallei* isolates needs to be conducted regularly to guide empiric therapy for melioidosis, as it causes high mortality, especially among diabetic cases.

Keywords: *Burkholderia pseudomallei*, antimicrobial susceptibility, melioidosis, Malaysia

INTRODUCTION

Burkholderia pseudomallei, formerly known as *Pseudomonas pseudomallei* is an environmental gram-negative saprophytic bacillus, that causes melioidosis, a disease with a high mortality rate. *B. pseudomallei* has inherent resistance to many antimicrobials in clinical use and has the propensity to cause relapses in spite of successful initial and maintenance therapy (Leelerasamee, 1998; White, 2003; Estes *et al*, 2010). This bacterium has the potential of being a bio-weapon and it

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causes infections with serious consequences among individuals with diabetes, chronic renal failure, alcoholism and immunosuppression (Cheng and Currie, 2005; Puthucheary, 2009; Estes *et al*, 2010; Hassan *et al*, 2010; Limmathurotsakul and Peacock, 2011; Whitlock *et al*, 2011). The expanding presence of this organism has resulted in sporadic cases and outbreaks in a variety of geographic areas worldwide (Cheng and Currie, 2005; Currie *et al*, 2008; Puthucheary, 2009; Estes *et al*, 2010; Limmathurotsakul and Peacock, 2011). It is important to periodically monitor the antibiotic susceptibility patterns of *B.pseudomallei* to guide initial empiric therapy. In this paper, we report the antibiotic susceptibility patterns of 228 isolates of *B.pseudomallei* obtained from cases of melioidosis during 2005-2010 at Hospital Sultanah Bahiyah, Alor Setar, Kedah State, northern Malaysia.

MATERIALS AND METHODS

Data for this study were obtained from the Melioidosis Registry for the State of Kedah, Malaysia, at Clinical Research Centre (CRC), Hospital Sultanah Bahiyah, Alor Setar, Kedah (Hassan *et al*, 2010). The cases of melioidosis were diagnosed by culture identified by Analytical Profile Index 20 Non-enterobacteria (API 20 NE) or with Indirect Fluorescent Antibody method using a cutoff titer of 1:80. Four hundred cases of melioidosis were reported during 2005-2010, of these 228 cases had *B.pseudomallei* isolated in culture and sensitivity. The specimens were obtained from blood, sputum, pus and urine. The isolates were tested for susceptibility to antimicrobials using a standard disk diffusion method on Mueller-Hinton agar (Bauer *et al*, 1966). The antimicrobials tested were amikacin, amoxicillin/clavulanate, trimethoprim-sulfamethoxazole, cefoperazone, ceftazidime, chloramphenicol, ciprofloxacin, gentamicin, imipenem and tetracycline.

This study was approved by the CRC, KL and the Malaysian Research Ethics Committee (MREC). The project was registered under the National Medical Research Registry (NMRR) of Malaysia.

Statistical analysis

Data was entered into and analyzed with SPSS (Version 11.0, Chicago, IL). The Fisher's exact test and chi-square tests were used to compare proportions and sensitivities among groups. The chi-square test with a linear trend was used to analyze the sensitivity patterns from 2005 to 2010.

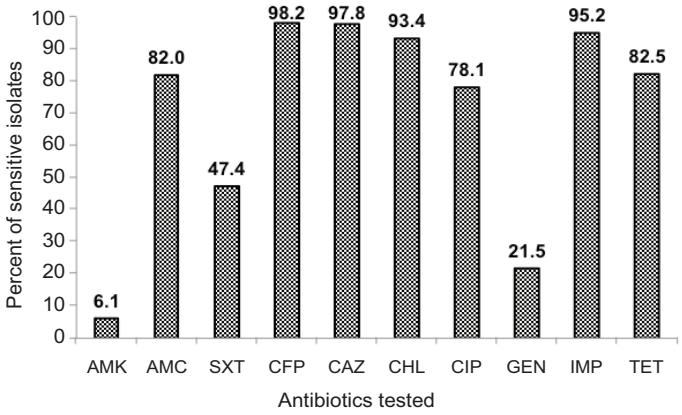
RESULTS

The susceptibilities of the 228 isolates tested are shown in Fig 1. More than 95% of the isolates were susceptible to ceftazidime, imipenem and cefoperazone. Eighty-two percent of the isolates were susceptible to tetracycline and amoxicillin/clavulanate. Seventy-eight percent of the isolates were susceptible to ciprofloxacin and 47.4% were susceptible to trimethoprim-sulfamethoxazole. Twenty-one point five percent of isolates were susceptible to gentamicin and 6.1% were susceptible to amikacin. The sensitivities by year for 2005 to 2010 are shown in Table 1. *B. pseudomallei* remained sensitive to ceftazidime and imipenem throughout the study period. A trend of increasing susceptibility to gentamicin, trimethoprim-sulfamethoxazole and ciprofloxacin and decreasing susceptibility to amoxicillin/clavulanate was seen during the study period (Table 1).

Of the 228 isolates, 144 were obtained

Table 1
Susceptibilities of *B.pseudomallei* isolates to different antibiotics by year.

Antibiotics	Year						Chi-square for linear trend	*p*-value	Trend direction
	2005 (*n*=12)	2006 (*n*=32)	2007 (*n*=25)	2008 (*n*=28)	2009 (*n*=90)	2010 (*n*=41)			
	Percentage sensitive								
Amikacin	33.3	0.0	0.0	10.7	4.4	7.3	0.99	0.319	None
Amoxicillin/clavulanate	83.3	96.9	100.0	92.9	70.0	78.0	11.27	<0.001	Downward
Trimethoprim-sulfamethoxazole	66.7	25.0	12.0	42.9	56.7	63.4	10.96	<0.001	Upward
Cefoperazone	100.0	96.9	100.0	96.4	98.9	97.6	0.05	0.819	None
Ceftazidime	100.0	96.9	100.0	100.0	96.7	97.6	0.56	0.454	None
Chloramphenicol	100.0	93.8	92.0	96.4	93.3	90.2	0.94	0.333	None
Ciprofloxacin	66.7	62.5	68.0	82.1	88.9	73.2	5.47	0.019	Upward
Gentamicin	8.3	0.0	0.0	42.9	25.6	31.7	14.63	<0.001	Upward
Imipenem	100.0	90.6	100.0	100.0	95.6	90.2	0.76	0.381	None
Tetracycline	83.3	75.0	96.0	82.1	82.2	80.5	0.02	0.883	None



AMK, amikacin; AMC, amoxicillin/clavulanate; SXT, trimethoprim-sulfamethoxazole; CFP, cefoperazone; CAZ, ceftazidime; CHL, chloramphenicol; CIP, ciprofloxacin; GEN, gentamicin; IMP, imipenem; TET, tetracycline.

Fig 1–Susceptibilities of 228 isolates of *B.pseudomallei* to various antibiotics.

from the blood and the rest (84) were obtained from other specimens. There were no differences in sensitivities to antimicrobials by the type of specimen except for gentamicin, where isolates from the blood were less suceptible to gentamicin than isolates from other specimens (Table 2).

Susceptibilities were similar between females (*n*=52) and males (*n*=176) except for trimethoprim-sulfamethoxazole in which isolates from males were more susceptible than isolates from females (Table 3). Isolates from diabetics (*n*=133) had the same susceptibilities as isolates from non-diabetics (*n*=95) except isolates in diabetics were significantly more susceptible to amoxicillin/clavulanate and significantly less susceptible to gentamicin (Table 4). The isolates from pneumonia (*n*=79) and non-pneomonia (*n*=149) cases had the same susceptibilities except isolates from pneumonia cases were more susceptible to amoxicillin/clavulanate (Table 5). The clinical outcome for 29 of the 228 cases were not available because they were discharged against medical advice or transferred elsewhere as per family request. Of the remaining 199 cases for which an outcome was available, 118 survived and 81 (40.70%) died. There was no significant difference in the susceptibilities of isolates between those who survived and those who died (Table 6).

DISCUSSION

Melioidosis is a major public health problem in parts of Thailand, Malaysia and Australia, causes sporadic cases and outbreaks in other parts of the world which may be endemic but have not yet been studied, such as Vietnam, Myanmar, Cambodia, Taiwan and India (Hsueh *et al*, 2001; Cheng and Currie, 2005; Aung and Mar, 2008; Overtoom *et al*, 2008; Phuong *et al*, 2008; Saravu *et al*, 2008;); or are non-endemic, such as Brazil, Venezuela and New Caledonia (Currie, 2008; Limmathurotsakul and Peacock, 2011). Melioidosis cases may also be reported after natural disasters, such as the 2004 Tsunami, where cases were reported from Indonesia (Currie *et al*, 2008; Limmathurotsakul and Peacock, 2011) and after the Haitang Typhoon in Taiwan (Limmathurotsakul and Peacock, 2011). Melioidosis may also be easier to diagnose in laboratories with enhanced facilities to evaluate blood cultures (Peacock and Newton, 2008). Global warming and other geo-climatic and environmental changes may also increase the number of cases and spread to newer areas, where conditions are favorable for survival of the organisms (Dance, 1991; Currie *et al*, 1994). Therefore, it is important that the distribution of this organism be mapped

Tabel 2
Comparison of susceptibilities of isolates between bacteremic cases and abacteremic cases.

Antibiotics	Isolates from blood (*n*=144)	Isolates from other specimens (*n*=84)	Comparison between blood and other isolates (chi-square value)	*p*-value
	% sensitive			
Amikacin	7.6	3.6	1.52	0.217
Amoxicillin/clavulanate	83.3	79.8	0.46	0.498
Trimethoprim-sulfamethoxazole	45.8	50.0	0.37	0.543
Cefoperazone	97.9	98.8	0.25	0.620
Ceftazidime	98.6	96.4	1.18	0.278
Chloramphenicol	92.4	95.2	0.71	0.398
Ciprofloxacin	74.3	84.5	3.24	0.072
Gentamicin	17.4	28.6	3.95	0.047
Imipenem	93.8	97.6	1.73	0.188
Tetracycline	80.6	85.7	0.98	0.323

Table 3
Comparison of susceptibilities of isolates between male and female patients.

Antibiotics	Isolates from male (*n*=176)	Isolates from female (*n*=52)	Comparison between isolates from males and females (chi-square value)	*p*-value
	% sensitive			
Amikacin	5.7	7.7	0.28	0.596
Amoxicillin/clavulanate	83.0	88.5	0.92	0.339
Trimethoprim-sulfamethoxazole	47.7	28.9	5.82	0.016
Cefoperazone	98.3	100.0	0.9	0.343
Ceftazidime	97.2	100.0	1.51	0.219
Chloramphenicol	92.6	96.2	0.82	0.366
Ciprofloxacin	76.1	84.6	1.69	0.194
Gentamicin	94.3	100.0	3.09	0.079
Imipenem	83.5	78.9	0.61	0.436
Tetracycline	5.7	7.7	0.28	0.596

out (Limmathurotsakul and Peacock, 2011) and the antimicrobial susceptibility patterns determined periodically to guide initial empirical treatment.

In this study, *B. pseudomallei* was susceptible to ceftazidime and imipenem throughout the study period from 2005 to 2010. Our findings are similar to

Table 4
Comparison of susceptibilities of isolates between diabetic and non-diabetic patients.

Antibiotics	Isolates from diabetics (*n*=133)	Isolates from non-diabetics (*n*=95)	Comparison between isolates from diabetics and non-diabetics (chi-square value)	*p*-value
	% sensitive			
Amikacin	6.0	4.5	0.01	0.926
Amoxicillin/clavulanate	87.2	53.4	5.85	0.015
Trimethoprim-sulfamethoxazole	44.4	36.8	3.28	0.070
Cefoperazone	97.7	70.7	0.47	0.495
Ceftazidime	98.5	69.2	0.71	0.400
Chloramphenicol	94.0	66.2	0.17	0.684
Ciprofloxacin	77.4	56.4	0.07	0.787
Gentamicin	15.8	21.1	6.15	0.013
Imipenem	97.0	66.2	2.3	0.130
Tetracycline	84.0	57.9	0.87	0.769

Table 5
Comparison of susceptibilities of isolates between cases with and without pneumonia.

Antibiotics	Isolates from pneumonia cases (*n*=79)	Isolates from non-pneumonia cases (*n*=149)	Comparison between isolates from pneumonia and non-pneumonia cases (chi-square value)	*p*-value
	% sensitive			
Amikacin	3.8	7.4	1.15	0.283
Amoxicillin/clavulanate	89.9	77.9	5.06	0.025
Trimethoprim-sulfamethoxazole	41.8	50.3	1.52	0.218
Cefoperazone	98.7	98.0	0.17	0.682
Ceftazidime	97.5	98.0	0.06	0.799
Chloramphenicol	92.4	94.0	1.23	0.266
Ciprofloxacin	81.0	76.5	0.61	0.434
Gentamicin	15.2	24.8	2.84	0.092
Imipenem	94.9	95.3	0.02	0.903
Tetracycline	82.3	82.6	0	0.959

other studies worldwide (Jenny *et al*, 2001; Thibault *et al*, 2004; Sivalingam *et al*, 2006; Karunakaran and Puthucheary, 2007; Raja, 2008; Tan and Tan, 2008). The susceptibilities were similar between isolates from females and males, bacteremic and abacteremic cases, diabetics and non-diabetics, pneumonia and non-pneumonia

Table 6
Comparison of susceptibilities of isolates between cases who died and who survived.

Antibiotics	Isolates from cases who died (*n*=81)	Isolates from cases who survived (*n*=118)[a]	Comparison between isolates from cases who died and those who survived (chi-square value)	*p*-value
	% sensitive			
Amikacin	9.9	4.2	1.66	0.197
Amoxicillin/clavulanate	86.4	85.6	0.03	0.869
Trimethoprim-sulfamethoxazole	45.7	36.4	1.34	0.246
Cefoperazone	96.3	100.0	FET[b]	0.066
Ceftazidime	97.5	97.5	FET	1.000
Chloramphenicol	93.8	94.9	0.00	0.988
Ciprofloxacin	80.2	76.3	0.24	0.624
Gentamicin	16.0	23.7	1.29	0.255
Imipenem	93.8	98.3	FET	0.123
Tetracycline	82.7	84.7	0.04	0.852

[a]In 29 patients, the outcome is not known; [b]Fisher's exact test.

cases and between those who died and those who survived. Our findings show antibiotic susceptibility patterns are not a major factor in determining outcomes.

Susceptibility to trimethoprim-sulfamethoxazole in our study ranged from 12% to 66.7% throughout the study but another study from Thailand and Australia found susceptibilities ranging from 84 to 97% (Estes *et al*, 2010). Susceptibilities of *B. pseudomallei* to trimethoprim-sulfamethoxazole vary widely in different areas (Estes *et al*, 2010). These results may also be influenced by the type of test used: the disk diffusion test or the E-test. Of the two, the E-test reflects the susceptibility with better accuracy than the disk diffusion test (Tan and Tan, 2008). However, the E-test may not be feasible for resource restricted settings (Limmathurotsakul and Peacock, 2011). One large study from Thailand, determined that if the isolates did not grow right up to the disk, it could be classified as "probably susceptible", although further investigation is needed (Limmathurotsakul and Peacock, 2011).

Although *B. pseudomallei* resistance to ceftazidime is low, (0.05 to 0.6%) (Estes *et al*, 2010; Wuthiekanun *et al*, 2011), there is still a cause for concern. The wide spread use of ceftazidime and imipenem to treat *P. aeruginosa* can result in resistance (Taneja *et al*, 2003; Mukhopadhyay *et al*, 2008) not only by *P. aeruginosa* but also *B. pseudomallei* (Sam *et al*, 2009; Kung *et al*, 2010; Chantratita *et al*, 2011; Behera *et al*, 2012; Sarovich *et al*, 2012 a,b). In many tropical countries a number of common infections are clinically indistinguishable from melioidosis (Limmathurotsakul and Peacock, 2011), such as enteric fever (Valsalan *et al*, 2008); therefore, a high index of suspicion is needed to diagnose melioidosis.

Studies of recurrent melioidosis have found increasing resistance to ceftazidime due to its frequent use (Estes *et al*, 2010;

Hayden *et al*, 2012; Sarovich *et al*, 2012b). One study found resistance to ceftazidime in a patient with an isolate of *B. pseudomallei* resistant to amoxicillin/clavulanate (Sam *et al*, 2009). Because of the heavy reliance on ceftazidime as a first line of treatment for melioidosis, resistance to ceftazidime is likely to pose a significant challenge in the treatment of melioidosis in the future (Sarovich *et al*, 2012a,b). Therefore, monitoring the drug susceptibilities among *B.pseudomallei* isolates needs to be conducted regularly to guide empiric therapy for melioidosis (Peacock and Newton, 2008). Repositories of different strains of *B.pseudomallei* need to be established for different geographical regions to monitor drug resistance.

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