Research Article
The In Vitro Antibiotic Susceptibility of Malaysian Isolates of <i>Burkholderia pseudomallei</i>

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Acute melioidosis may present as localised or septicaemic infections and can be fatal if left untreated. <i>Burkholderia pseudomallei</i> resistant to antibiotics used for the treatment of melioidosis had been reported. The aim of this study was to determine the in vitro antibiotic susceptibility patterns of <i>Burkholderia pseudomallei</i> isolated in Malaysia to a panel of antibiotics used for the treatment of melioidosis and also to potential alternative antibiotics such as tigecycline, ampicillin/sulbactam, and piperacillin/tazobactam. A total of 170 <i>Burkholderia pseudomallei</i> isolates were subjected to minimum inhibitory concentration determination using <i>E</i>-test method to eleven antibiotics. All isolates were sensitive to meropenem and piperacillin/tazobactam. For ceftazidime, imipenem, amoxicillin/clavulanic acid, and doxycycline resistance was observed in 1 isolate (0.6%) for each of the antibiotics. Trimethoprim/sulfamethoxazole resistance was observed in 17 (10%) isolates. For other antibiotics, ampicillin/sulbactam, chloramphenicol, tigecycline, and ciprofloxacin resistance were observed in 1 (0.6%), 6 (3.5%), 60 (35.3%) and 98 (57.7%) isolates respectively. One isolate B170/06 exhibited resistance to 4 antibiotics, namely, ciprofloxacin, chloramphenicol, trimethoprim/sulfamethoxazole, and tigecycline. In conclusion, the Malaysian isolates were highly susceptible to the current antibiotics used in the treatment of melioidosis in Malaysia. Multiple resistances to the antibiotics used in the maintenance therapy are the cause for a concern.

1. Introduction

The causative agent of melioidosis, <i>Burkholderia pseudomallei</i>, is endemic in the Northern part of Australia and South-east Asia including Malaysia. Acute melioidosis may present as localized or septicaemic infections and can be fatal if left untreated. It was the common cause of community-acquired pneumonia in Northeastern Thailand and was attributed as the cause of fatal community-acquired bacteremic pneumonia in Northern Australia [1, 2]. Latent infection may remain asymptomatic for years only to be reactivated from a latent focus when the host is immunocompromised. Therefore, it is important to treat melioidosis with prolonged course of antibiotics so as to avoid disease relapses which are commonly associated with short courses of antibiotics. Some patients may default treatment or take improper dosage of antibiotics because of the long duration of treatment, and this may contribute to the relapse or the development of resistance.

<i>Burkholderia pseudomallei</i> is intrinsically resistant to a wide range of antibiotics which include some β-lactam antibiotics, aminoglycosides, and macrolides. The antibiotics that are currently being used for the therapy of melioidosis are ceftazidime, imipenem, meropenem, amoxicillin/clavulanate, cefoperazone/sulbactam, trimethoprim-sulfamethoxazole (TMP-SMX), doxycycline, and chloramphenicol. The antibiotic regime for the treatment of melioidosis varies from one country to another. In Malaysia, ceftazidime alone, or in combination with TMP-SMX or cefoperazone/sulbactam alone or in combination with TMP-SMX or imipenem, is the recommended antibiotic of choice for the intensive phase of treatment followed by oral TMP-SMX plus doxycycline or amoxicillin/clavulanate in the maintenance phase [3]. The development of resistance of <i>Burkholderia pseudomallei</i> to some of these antibiotics has been reported in neighbouring countries such as Singapore and Thailand [4, 5]. Reports on the antibiotic susceptibility of <i>Burkholderia pseudomallei</i> in
Malaysia have been limited to a few selected antibiotics and a smaller number of tested strains and from restricted demographic areas [6, 7].

Therefore, the aim of the study was to determine the in vitro antibiotic susceptibility patterns of clinical isolates of *Burkholderia pseudomallei* to a panel of antibiotics used for the treatment of melioidosis and also to the potential alternative antibiotics such as tigecycline, ampicillin/sulbactam, and piperacillin/tazobactam.

### 2. Materials and Methods

#### 2.1. Bacterial Isolates

A total of 170 *Burkholderia pseudomallei* nonrepeat clinical isolates were collected from the year 2001 until the year 2009, from the microbiology laboratories of 29 government hospitals situated in 11 out of 14 states in Malaysia (Table 1). These isolates were sent from these hospitals for the confirmation of identification at the Bacteriology Unit, Institute for Medical Research. Species identification was carried out by Gram-staining, motility, API 20NE (bioMérieux), and polymerase chain reaction technique using specific 16rRNA primers as described by Brook et al. in 1997 [8]. The strains were stored at −80°C in 20% glycerol and were revived by subculturing onto blood agar plates before being further used.

#### 2.2. Antibiotic Susceptibility Testing

Minimum inhibitory concentrations (MIC) of the antibiotics were determined by E-test (bioMérieux) following the manufacturer’s instructions. Eleven antibiotics were tested, namely, ceftazidime, imipenem, meropenem, amoxicillin/clavulanic acid, TMP-SMX, ciprofloxacin, doxycycline, chloramphenicol, piperacillin/tazobactam, ampicillin/sulbactam, and tigecycline.

A 0.5 McFarland suspension was made for each bacterial isolate and then inoculated on Mueller-Hinton (MH) (BD) agar. The E-test strips of each antibiotic were placed on the MH agar and incubated overnight at 37°C. The zones of inhibition were noted after 18 hours of incubation. The MIC (µg/mL) interpretation for susceptible (s), intermediate (i), and resistant (r) for amoxicillin-clavulanic acid (s ≤ 8/4, i 16/8, r ≥ 32/16), ceftazidime (s ≤ 8, i 16, r ≥ 32), imipenem (s ≤ 4, i 8, r ≥ 16), doxycycline (s ≤ 4, i 8, r ≥ 16), and TMP-SMX (s ≤ 2/38, i ≤ 4/76) was determined following the CLSI approved guideline M45-A2 [9]. For ciprofloxacin (s ≤ 1, i 2, r ≥ 4), chloramphenicol (s ≤ 8, i 16, r ≥ 32), piperacillin/tazobactam (s ≤ 16/4, i 32/4−64/4, r ≥ 128/4), and amoxicillin/sulbactam (s ≤ 8, i 16/8, r ≥ 32/16), the MIC (µg/mL) for Enterobacteriaceae was referred [10]. For tigecycline, the US FDA approved breakpoints for Enterobacteriaceae were used (s ≤ 2, i 4, r ≥ 8). For meropenem, the interpretative criteria outlined by the E-test manufacturer for aerobes were followed (s ≤ 4, i 8, r ≥ 16). Any values which were in between the sensitive and resistant breakpoints were interpreted as intermediates. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as controls.

### 3. Results

All strains were sensitive to meropenem and piperacillin/tazobactam. Sensitivity to ceftazidime, imipenem, amoxicillin/clavulanic acid, ampicillin/sulbactam, and doxycycline was noted in 169 (99.4%) of the isolates. One isolate was shown to have heterogenous population with ceftazidime susceptibility of 8µg/mL and ceftazidime resistance of ≥256µg/mL. This strain, however, remained susceptible to other antibiotics. The isolate that was immediately resistant to imipenem (MIC 6µg/mL) was also resistant to amoxicillin/clavulanic acid and ampicillin/sulbactam. For chloramphenicol and TMP-SMX, 164 (96.5%) and 153 (90%) of the strains were susceptible, respectively. Only 72 (42.3%) isolates were susceptible to ciprofloxacin while 94 (55.3%) isolates showed intermediate resistance with MIC of 1.5-3.0µg/mL. Susceptibility to tigecycline was observed in 110 isolates (64.7%) while intermediate resistance was noted in 59 (34.7%) of the isolates. One isolate was resistant to tigecycline at the MIC of 8.0µg/mL.

The minimum concentration of antibiotics that inhibited 50% (MIC50) and 90% (MIC90) of the isolates and the range of MICs of the tested antibiotics were shown in Table 2. The MIC50 of most of the antibiotics were within the range of 0.38 to 4.0µg/mL. Chloramphenicol was shown to have higher MIC50 and MIC90 values than the other antibiotics (MIC50, 6.0µg/mL; MIC90, 8.0µg/mL). Imipenem (MIC50, 0.38µg/mL; MIC90, 0.38µg/mL) was more active towards these strains than meropenem (MIC50, 0.75µg/mL and MIC90, 1.0µg/mL).

The resistance pattern observed among these strains was mainly monoresistance where 7 strains showed resistance to a single antibiotic, either to ceftazidime (1 strain), ciprofloxacin (1 strain), or trimethoprim/sulfamethoxazole (5 strains). Two strains showed resistance to 2 different types of antibiotics (1 isolate to ciprofloxacin & trimethoprim/sulfamethoxazole; 1 strain to amoxicillin/clavulanic acid and ampicillin/sulbactam). One strain showed multiple resistances to 4 antibiotics namely ciprofloxacin, chloramphenicol, trimethoprim/sulfamethoxazole, and tigecycline and intermediate resistance to doxycycline.

### 4. Discussion

This study showed that the local strains were highly susceptible to meropenem and piperacillin/tazobactam. For ceftazidime, imipenem, amoxicillin/clavulanic acid, and doxycycline, only 0.6% of the isolate was resistant to these antibiotics. A highly resistant subpopulation of ceftazidime resistance was detected in one of the strain. This was a blood isolate from a patient who had no past record of melioidosis and had passed away a day after admission before the culture result was obtained. Primary resistance to ceftazidime is rare and studies had documented that the development of resistance emerge mainly during treatment [11, 12].

The susceptibility to trimethoprim/sulfamethoxazole is slightly lower compared to 97.5% in the another local study on 80 *Burkholderia pseudomallei* [6]. Only 4.0% of our isolates was resistant to trimethoprim/sulfamethoxazole compared...
| Lab ID   | Year isolated | State         | Sex | Source       |
|---------|---------------|---------------|-----|--------------|
| RZ 14/01| 2001          | Negeri Sembilan | m   | wound swab   |
| RZ 16/01| 2001          | Negeri Sembilan | m   | urine        |
| RZ 21/01| 2001          | Perak          | f   | blood        |
| RZ 31/01| 2001          | Selangor       | f   | NA           |
| RZ 37/01| 2001          | Perak          | m   | sputum       |
| RZ 116/01| 2001        | Kedah          | m   | pus          |
| RZ 166/01| 2001        | Perak          | m   | blood        |
| RZ 169/01| 2001        | Perak          | m   | blood        |
| RZ 191/01| 2001        | Perak          | f   | blood        |
| RZ 12/02| 2002          | Negeri Sembilan | f   | pus          |
| RZ 50/02| 2002          | Johor          | m   | blood        |
| RZ 51/02| 2002          | Johor          | m   | swab         |
| RZ 69/02| 2002          | Perak          | m   | blood        |
| RZ 94/02| 2002          | Perak          | m   | blood        |
| RZ 95/02| 2002          | Perak          | m   | blood        |
| RZ 107/02| 2002        | Perak          | m   | blood        |
| RZ 130/02| 2002        | Sarawak        | m   | blood        |
| RZ 143/02| 2002        | Perak          | m   | blood        |
| RZ 144/02| 2002        | Perak          | m   | blood        |
| RZ 145/02| 2002        | Perak          | m   | blood        |
| RZ 176/02| 2002        | Selangor       | f   | blood        |
| RZ 194/02| 2002        | Pahang         | f   | blood        |
| RZ 203/02| 2002        | Perak          | m   | blood        |
| RZ 207/02| 2002        | Penang         | m   | pus          |
| RZ 15/03| 2003          | Perak          | m   | urine        |
| RZ 51/03| 2003          | Perak          | f   | blood        |
| RZ 58/03| 2003          | Penang         | f   | blood        |
| RZ 76/03| 2003          | Penang         | m   | blood        |
| RZ 4/05 | 2005          | Johor          | m   | NA           |
| RZ 5/05 | 2005          | Pahang         | m   | NA           |
| RZ 7/05 | 2005          | Pahang         | f   | NA           |
| RZ 11/05| 2005          | Sarawak        | m   | NA           |
| RZ 14/05| 2005          | Sarawak        | m   | NA           |
| RZ 15/05| 2005          | Sarawak        | m   | NA           |
| RZ 18/05| 2005          | Johor          | m   | NA           |
| RZ 46/05| 2005          | Sarawak        | m   | NA           |
| RZ 49/05| 2005          | Perlis         | m   | NA           |
| RZ 50/05| 2005          | Sarawak        | m   | NA           |
| RZ 52/05| 2005          | Perak          | f   | NA           |
| RZ 61/05| 2005          | Sabah          | f   | NA           |
| RZ 76/05| 2005          | Johor          | f   | NA           |
| RZ 77/05| 2005          | Perak          | m   | NA           |
| RZ 85/05| 2005          | Johor          | m   | NA           |
| RZ 86/05| 2005          | Johor          | m   | NA           |
| RZ 87/05| 2005          | Pahang         | m   | NA           |
| RZ 88/05| 2005          | Sabah          | f   | NA           |
| RZ 77/06| 2006          | Johor          | m   | blood        |
| RZ 97/06| 2006          | Selangor       | m   | blood        |
| Lab ID   | Year isolated | State | Sex | Source |
|---------|---------------|-------|-----|--------|
| RZ102/06 | 2006          | Johor | m   | Blood  |
| RZ125/06 | 2006          | Johor | m   | Blood  |
| B 146/06 | 2006          | Pahang| m   | Blood  |
| B 149/06 | 2006          | Pahang| m   | Blood  |
| B 150/06 | 2006          | Pahang| m   | Pus    |
| B 151/06 | 2006          | Pahang| m   | Blood  |
| B 152/06 | 2006          | Pahang| m   | Blood  |
| B 153/06 | 2006          | Kedah | m   | Blood  |
| B 154/06 | 2006          | Kedah | m   | Blood  |
| B 155/06 | 2006          | Kedah | f   | Blood  |
| B 156/06 | 2006          | Kedah | f   | Pus    |
| B 158/06 | 2006          | Pahang| m   | Blood  |
| B 159/06 | 2006          | Pahang| f   | Blood  |
| B 160/06 | 2006          | Pahang| m   | Blood  |
| B 161/06 | 2006          | Pahang| m   | Blood  |
| B 162/06 | 2006          | Pahang| m   | Blood  |
| B 164/06 | 2006          | Pahang| m   | Blood  |
| B 168/06 | 2006          | Perak | m   | Blood  |
| B 169/06 | 2006          | Pahang| m   | Blood  |
| B 170/06 | 2006          | Kedah | f   | Pus    |
| B 171/06 | 2006          | Kedah | m   | Blood  |
| B 172/06 | 2006          | Pahang| m   | Blood  |
| B 174/06 | 2006          | Perak | m   | Blood  |
| B 175/06 | 2006          | Perak | m   | Pus    |
| B 176/06 | 2006          | Perak | m   | Urine  |
| B 177/06 | 2006          | Kedah | m   | Blood  |
| B 178/06 | 2006          | Kedah | f   | Blood  |
| B 179/06 | 2006          | Kedah | m   | Aspirate |
| B 180/06 | 2006          | Kedah | m   | Blood  |
| B 181/06 | 2006          | Kedah | m   | Blood  |
| B 183/06 | 2006          | Kedah | m   | Blood  |
| B 184/06 | 2006          | Kedah | m   | Pus    |
| RZ 3/07  | 2007          | Pahang| m   | Blood  |
| RZ 7/07  | 2007          | Penang| m   | Blood  |
| RZ 8/07  | 2007          | Penang| m   | Blood  |
| RZ 9/07  | 2007          | Penang| m   | Blood  |
| RZ 19/07 | 2007          | Johor | m   | Blood  |
| RZ 57/07 | 2007          | Selangor| f | NA     |
| RZ 162/07| 2007         | Sarawak| f | Blood  |
| RZ 191/07| 2007         | Selangor| m | Blood  |
| RZ 355/07| 2007         | Johor | m   | Blood  |
| B 185/07 | 2007          | Kedah | m   | Blood  |
| B 186/07 | 2007          | Kedah | m   | Blood  |
| B 187/07 | 2007          | Kedah | m   | Blood  |
| B 191/07 | 2007          | Penang| m   | Blood  |
| B 192/07 | 2007          | Kedah | m   | Blood  |
| B 193/07 | 2007          | Selangor| m | NA     |
| B 194/07 | 2007          | Selangor| f | NA     |
| B 195/07 | 2007          | Johor | f   | Blood  |
| B 196/07 | 2007          | Johor | m   | Blood  |
| B 197/07 | 2007          | Johor | m   | Blood  |
| Lab ID | Year isolated | State       | Sex | Source          |
|--------|---------------|-------------|-----|-----------------|
| B198/07  | 2007          | Perak       | m   | Blood           |
| B199/07  | 2007          | Perak       | m   | Blood           |
| B200/07  | 2007          | Perak       | m   | Blood           |
| B201/07  | 2007          | Perak       | m   | Blood           |
| B202/07  | 2007          | Johor       | m   | Blood           |
| RZ27/08  | 2008          | Sarawak     | m   | Blood           |
| RZ61/08  | 2008          | Sarawak     | m   | Blood           |
| RZ64/08  | 2008          | Sarawak     | m   | Blood           |
| RZ72/08  | 2008          | Selangor    | m   | Blood           |
| RZ76/08  | 2008          | Sarawak     | m   | knee aspirate   |
| RZ77/08  | 2008          | Sarawak     | f   | Sputum          |
| RZ91/08  | 2008          | Selangor    | m   | Blood           |
| RZ96/08  | 2008          | Pahang      | f   | Blood           |
| RZ107/08 | 2008          | Selangor    | f   | Peritoneal fluid|
| RZ115/08 | 2008          | Sarawak     | m   | Abscess         |
| RZ116/08 | 2008          | Sarawak     | m   | Blood           |
| RZ117/08 | 2008          | Sarawak     | m   | Blood           |
| RZ118/08 | 2008          | Sarawak     | m   | Blood           |
| RZ120/08 | 2008          | Sarawak     | f   | Blood           |
| RZ160/08 | 2008          | Sarawak     | m   | Blood           |
| RZ170/08 | 2008          | Sarawak     | m   | Blood           |
| RZ176/08 | 2008          | Sarawak     | m   | Blood           |
| RZ190/08 | 2008          | Sarawak     | m   | Blood           |
| RZ193/08 | 2008          | Sarawak     | f   | Blood           |
| RZ197/08 | 2008          | Sarawak     | m   | Blood           |
| RZ207/08 | 2008          | Sarawak     | m   | Urine           |
| RZ209/08 | 2008          | Sarawak     | f   | Sputum          |
| RZ210/08 | 2008          | Sarawak     | m   | Blood           |
| RZ267/08 | 2009          | Sarawak     | m   | Liver aspirate  |
| RZ269/08 | 2009          | Johor       | f   | Blood           |
| RZ276/08 | 2009          | Sarawak     | m   | Blood           |
| RZ299/08 | 2009          | Sarawak     | m   | Blood           |
| RZ305/08 | 2009          | Sarawak     | m   | Blood           |
| RZ307/08 | 2009          | Johor       | m   | Tissue          |
| RZ365/08 | 2009          | Sarawak     | m   | Blood           |
| HB1/09   | 2009          | Pahang      | m   | Abscess         |
| RZ9/09   | 2009          | Selangor    | m   | Blood           |
| RZ43/09  | 2009          | Sarawak     | f   | Blood           |
| RZ116/09 | 2009          | Wilayah Persekutuan | f | Blood |
| RZ117/09 | 2009          | Wilayah Persekutuan | f | Blood |
| RZ118/09 | 2009          | Wilayah Persekutuan | f | Blood |
| RZ167/09 | 2009          | Sarawak     | m   | Urine           |
| RZ168/09 | 2009          | Sarawak     | f   | Sputum          |
| RZ169/09 | 2009          | Sarawak     | m   | Blood           |
| RZ170/09 | 2009          | Sarawak     | m   | Blood           |
| RZ193/09 | 2009          | Sarawak     | m   | Blood           |
| RZ197/09 | 2009          | Selangor    | m   | Urine           |
| RZ207/09 | 2009          | Selangor    | m   | Sputum          |
| RZ267/09 | 2009          | Pahang      | m   | Blood           |
| RZ267/09 | 2009          | Selangor    | m   | Liver aspirate  |
| RZ367/09 | 2009          | Johor       | m   | Blood           |
| RZ369/09 | 2009          | Sarawak     | f   | Blood           |
| RZ370/09 | 2009          | Sarawak     | m   | Blood           |
| RZ465/09 | 2009          | Sarawak     | m   | Blood           |
| RZ466/09 | 2009          | Sarawak     | m   | Blood           |
| MZ7/10   | 2010          | Wilayah Persekutuan | m | Abscess |
| RZ60/10  | 2010          | Sarawak     | f   | Blood           |
| RZ61/10  | 2010          | Sarawak     | f   | Blood           |
| RZ97/10  | 2010          | Selangor    | m   | Pus             |
Table 1: Continued.

| Lab ID | Year isolated | State   | Sex | Source |
|--------|---------------|---------|-----|--------|
| RZ 104/10 | 2010          | Sarawak | f   | Blood  |
| RZ 154/10 | 2010          | Johor   | m   | Blood  |
| RZ 158/10 | 2010          | Sarawak | m   | Blood  |
| RZ 161/10 | 2010          | Johor   | m   | Blood  |
| RZ 162/10 | 2010          | Johor   | f   | Blood  |
| RZ 164/10 | 2010          | Sarawak | f   | Pus    |
| RZ 181/10 | 2010          | Selangor| m   | Blood  |
| RZ 193/10 | 2010          | Sarawak | m   | Blood  |
| RZ 205/10 | 2010          | Johor   | f   | Blood  |
| RZ 206/10 | 2010          | Pahang  | m   | Blood  |
| RZ 207/10 | 2010          | Pahang  | m   | Blood  |
| RZ 208/10 | 2010          | Pahang  | m   | Blood  |
| RZ 209/10 | 2010          | Sarawak | m   | Blood  |
| RZ 210/10 | 2010          | Selangor| m   | Blood  |
| RZ 229/10 | 2010          | Sarawak | m   | pus    |
| RZ 236/10 | 2010          | Perlis  | m   | blood  |
| RZ 265/10 | 2010          | Sarawak | m   | blood  |
| RZ 272/10 | 2010          | Wilayah Persekutuan | m | blood  |
| RZ 273/10 | 2010          | Sarawak | m   | blood  |
| MZ 17/10   | 2010          | Selangor| m   | knee aspirate |
| MZ 24/10   | 2010          | Pahang  | m   | pus    |

NA: not available.

Table 2: The MIC of antibiotics against 170 *Burkholderia pseudomallei* isolates.

| Antibiotic | No. of isolates with the MIC (µg/mL) of | MIC (µg/mL) | %S |
|------------|----------------------------------------|-------------|----|
|            | ≤0.25 0.38 0.5 0.75 1.0 1.5 2.0 3.0 4.0 6.0 8.0 12.0 16 128 ≥256 | AMC             | CAZ             | CIP             | CHL             | SXT             | DOX             | IMI             | MEM             | TZP             | SAM             | TIG             | |
| AMC        | 39 118 9 3 | 1 | 2.0 3.0 | 99.4 |
| CAZ        | 1 11 75 65 15 2 | 1 1 | 1.5 2.0 | 99.4 |
| CIP        | 7 23 42 56 28 10 2 1 1 | 1 | 1.5 2.0 | 42.3 |
| CHL        | 1 14 37 81 31 4 1 1 | 1 | 6.0 8.0 | 96.5 |
| SXT        | 18 14 19 16 28 24 34 10 5 2 | 1 | 1.0 2.0 | 90 |
| DOX        | 1 | 10 11 26 48 48 20 4 1 1 | 1 | 1.0 2.0 | 99.4 |
| IMI        | 48 | 114 7 1 | 0.38 | 0.38 | 99.4 |
| MEM        | 2 12 98 49 8 1 | 1 | 0.75 | 1.0 | 100 |
| TZP        | 2 | 50 99 16 1 2 | 1.5 | 2.0 | 100 |
| SAM        | 1 | 47 99 20 1 1 | 1 | 3.0 4.0 | 99.4 |
| TIG        | 5 | 15 | 13 32 39 36 19 4 1 | 1.5 | 4.0 | 64.7 |

AMC: amoxicillin/clavulanic acid; CAZ: ceftazidime; CIP: ciprofloxacin; CHL: chloramphenicol; SXT: trimethoprim-sulfamethoxazole; DOX: doxycycline; IMI: imipenem; MEM: meropenem; TZP: piperacillin/tazobactam; SAM: ampicillin/sulbactam; TIG: tigecycline.

to 13% resistance rate in Thailand [13]. Our local strains were also highly susceptible to chloramphenicol. An open label study in Thailand had shown that the combination of chloramphenicol, TMP-SMX, and doxycycline was associated with higher adverse effect compared to TMP-SMX and doxycycline only [14]. Chloramphenicol was not included in the maintenance therapy of melioidosis in Malaysia [3].

The antibiotic susceptibility rate of ciprofloxacin was low at 42.3%, and 90% of the isolates were inhibited at the intermediate MIC value of 2 µg/mL, which implied that ciprofloxacin was less effective towards these strains. Fluoroquinolones have been shown to be less effective clinically [15,16].

A study on the activity of tigecycline against *Burkholderia pseudomallei* in Thailand showed low MIC<sub>50</sub> and MIC<sub>90</sub> (0.5 µg/mL and 2.0 µg/mL) [17]. This finding is similar to another study in Malaysia [7]. In contrast, the isolates tested in this study were less susceptible to tigecycline, where higher MIC<sub>50</sub> and MIC<sub>90</sub> values (0.75 µg/mL and 4.0 µg/mL) were observed. The susceptibility of tigecycline was only 64.7%, and 34.7% of the strains were inhibited at the intermediate range of MIC of 3.0–6.0 µg/mL. This is in concordance with another study in Australia, where 85.5% of the isolates were inhibited at an intermediate MIC of 4.0 µg/mL with lower susceptibility rate of 14.5% [18].
The isolate that was intermediate resistant to imipenem with MIC 6 μg/mL, also had co-resistance to amoxicillin/clavulanic acid and ampicillin/sulbactam. This isolate was susceptible to meropenem at MIC 1.5 μg/mL. The MIC<sub>50</sub> and MIC<sub>90</sub> of meropenem were noted to be higher than those in imipenem; however, meropenem is still effective against all the strains tested. In Malaysia, meropenem or imipenem has been used for severe infection or in the event of treatment failure with ceftazidime. All the multiply resistant strains were still susceptible to ceftazidime but some of these strains were resistant to the antibiotics used in the maintenance therapy. The mechanism of resistance of these isolates will be further studied.

In conclusion, the *Burkholderia pseudomallei* isolates from Malaysia were highly susceptible to the antibiotics used in the treatment of melioidosis namely ceftazidime, trimethoprim/sulfamethoxazole, imipenem, meropenem, amoxicillin-clavulinate, and doxycycline. Ciprofloxacin and tigecycline were not active in vitro against these isolates. The presence of isolates that were resistant to the antibiotics used for maintenance therapy is of concern because this could affect the treatment outcome and may lead to the relapse of infection.

**Conflict of Interests**

The authors have no conflict of interests to declare.

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