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Antibiotics susceptibility of *Burkholderia* species of Sarawak origin

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ABSTRACT

Aims: The *Burkholderia* species is comprised of more than 70 members which co-exist in the same ecological niche including *Burkholderia pseudomallei,* which causes fatal melioidosis infections in humans and animals. Many of the members of the *Burkholderia* species share similarities in their biochemical and morphological profiles. *B. pseudomallei* is intrinsically resistant to a myriad of antibiotics and hence, the treatment of melioidosis involves various types of antibiotics with prolonged prescription. Apart from *B. pseudomallei* which has been widely described due to its clinical importance, little is known about the antibiotics mechanisms and susceptibility profile of *Burkholderia* species. This leads to the question of whether the antibiotics susceptibility profile of the *Burkholderia* species is similar to that of *B. pseudomallei*.
Methodology and results: In this study, *Burkholderia* species isolated from environmental samples were tested for their susceptibility against gentamicin, ceftazidime, cotrimoxazole (trimethoprim/sulfamethoxazole) and azithromycin using the disk diffusion test method. The antibiogram profiles *Burkholderia* species isolates tested in this study suggested that the antibiogram profile of *Burkholderia* spp. resembles that of *B. pseudomallei* for some antibiotics while totally different for other antibiotics.
Conclusion, significance and impact of study: The actual mechanisms which render these observations and whether the interaction of these subspecies within the same ecological niche attribute to these observations warrant further investigation.

Keywords: Antibiotics susceptibility, *Burkholderia*, *Burkholderia pseudomallei*

INTRODUCTION

Burkholderia species is a Gram-negative bacilli bacterium with over 70 types of subspecies found in a wide range of ecological niches, many of which share similarities in their biochemical and morphological characteristics (Coenye and Vandamme, 2006). Some of the *Burkholderia* spp. are pathogenic to plants (Stoyanova *et al*., 2007), while others such as *Burkholderia pseudomallei* which causes melioidosis and *Burkholderia mallei* which causes glanders, are both pathogenic and potentially fatal to humans and animals (Wiersinga *et al*., 2012). Apart from that, *Burkholderia cepacia* has been reported to cause opportunistic infections in individuals suffering from cystic fibrosis and chronic granulomatous disease (Coenye and Vandamme, 2006).

Burkholderia pseudomallei is inherently resistant to myriad antibiotics such as β-lactams, aminoglycosides and macrolides, conferred through various mechanisms including inactivating enzymes, cell exclusion, and broad-range efflux pumps (Dance *et al.*, 1989; Simpson *et al.*, 1999; Jenney *et al.*, 2001). However, unlike what has been reported in other melioidosis endemic countries, over 80% *B. pseudomallei* strains in Sarawak were found to be susceptible towards aminoglycosides and macrolides (Podin *et al.*, 2014). Apart from a previous report of *B. cepacia* being resistant to tobramycin (Kennedy *et al.*, 2015), there is little knowledge on the antibiotics susceptibility profile of other *Burkholderia* spp.

It has been previously established that recombination and lateral gene transfer may occur between *B. pseudomallei* and other *Burkholderia* spp. that are sharing the same ecological niche which have led to greater intra-species diversity (Kim *et al.*, 2005; Tuanyok *et al.*, 2007). This brings about the question of whether such intra-species interactions include the antibiotics susceptibility mechanisms and whether there are similarities of antibiotics susceptibility profile between *B. pseudomallei* and other *Burkholderia* spp. Hence, the objective of this study is to investigate the antibiotics susceptibility profile of *Burkholderia* spp. isolates of Sarawak origin.

MATERIALS AND METHODS

Bacterial strains

Burkholderia spp. isolates, some of which were archival from a previous study, were used for this project. The

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strains were isolated from water and soil samples collected from various melioidosis-endemic districts in Sarawak and were identified by the previously described *recA* gene DNA sequencing method (Payne *et al.*, 2005).

Antibiotics susceptibility testing

Antibiotics susceptibility testing was performed using the standard disk diffusion test method using disks purchased from Liofilchem (Liofilchem, Italy). The antibiotics selected for this study were clinically relevant to *B. pseudomallei* in Sarawak namely, gentamicin, azithromycin, cotrimoxazole (trimethoprim/sulfamethoxazole) and ceftazidime. The minimal inhibitory concentration (MIC) breakpoints were determined based on the Clinical and Laboratory Standard Institute (CLSI) guidelines (CLSI, USA).

RESULTS AND DISCUSSION

As shown in Table 1, 81 *Burkholderia* spp. isolates of eight types of subspecies commonly found in the environment in Sarawak were subjected to disk diffusion test. A majority of the isolates belong to the subspecies *Burkholderia pyrrocinia*, *B. thailandensis* and *B. ubonensis*.

Aminoglycoside and macrolide susceptibility

Figure 1 depicts the antibiotics susceptibility of the tested isolates against azithromycin. Of the 20 *B. thailandensis* isolates tested, 65% appeared to be resistant against azithromycin. Five out of 7 isolates belonging to the *B. cepacia* complex group were resistant towards azithromycin. *Burkholderia ubonensis* on the other hand, were mostly susceptible to azithromycin with 79% of 14 isolates, while *B. pyrrocinia* had almost equal number of isolates that belonged to the susceptible and intermediate category.

The antibiotics susceptibility of *Burkholderia* spp. against gentamicin is shown in Figure 2. Almost all the subspecies tested were predominantly resistant against gentamicin. The only subspecies that has equal number of isolates resistant and susceptible to gentamicin was *B. ubonensis*. *Burkholderia cepacia* on the other hand had equal number of isolates that were intermediately susceptible and resistant towards gentamicin.

Azithromycin belongs to the macrolide drug group while gentamicin is an aminoglycoside, both of which have been previously described as antibiotics that *B. pseudomallei* were intrinsically resistant against (Simpson *et al.*, 1999; Coenye and Vandamme, 2006). However, the strains from central Sarawak have been shown to be more than 80% susceptible to both aminoglycosides and macrolides due to a nonsynonymous mutation within amrB which encodes an essential component of the AmrAB-OprA multidrug efflux pump (Podin *et al.*, 2014). While most of the *Burkholderia* spp. tested in this study were resistant towards azithromycin and gentamicin, 79% *B. ubonensis* had been shown to be susceptible to azithromycin and 42% susceptible to gentamicin. This observation suggests that *B. ubonensis* may possibly share the same mechanism as the majority of *B. pseudomallei* from central Sarawak, namely the AmrAB-OprA multidrug efflux pump, which conferred susceptibility towards aminoglycosides and macrolides. Nonetheless, this observation can only be confirmed by further studies to elucidate the antibiotics susceptibility mechanism in the *B. ubonensis* isolates tested in this study.

Table 1: List of isolates subjected to antibiotics susceptibility testing.

Type of species	No. of isolates
Burkholderia cepacian	5
*Burkholderia cepacia C*omplex	7
Burkholderia diffusa	1
Burkholderia pyrrocinia	31
Burkholderia multivorans	1
Burkholderia thailandensis	20
Burkholderia ubonensis	14
Burkholderia species	2
Total	81

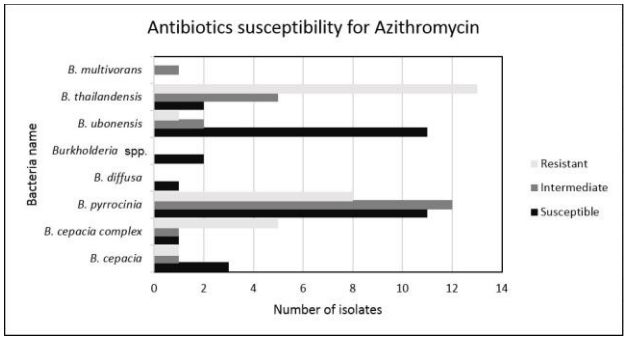

Figure 1: Antibiotics susceptibility of *Burkholderia* spp. against azithromycin.

Cotrimoxazole susceptibility

As shown in Figure 3, a majority of all the subspecies were susceptible to cotrimoxazole except for *B. pyrrocinia* where 17/31 (54%) were intermediately susceptible towards the antibiotics. In addition to that, 29% of the *B. pyrrocinia* tested were resistant towards cotrimoxazole.

A majority of the *Burkholderia* spp. isolates tested in the present study were susceptible to cotrimoxazole, which is consistent with what was observed previously in *B. pseudomallei* (Dance *et al.*, 1989; Jenney *et al.*, 2001). Cotrimoxazole is one of the important antibiotics prescribed in the eradication phase of melioidosis treatment regime which lasts for up to 20 weeks (Wiersinga *et al*., 2012). Although there have been previous reports of cotrimoxazole resistance (Saiprom *et al.*, 2015), primary resistance is rare and most resistance occurred due to contraindication with amoxicillin/clavulanic acid (Wiersinga *et al*., 2012). That 40% *B. thailandensis* and 30% of *B. pyrrocinia* tested in

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this study were resistant towards cotrimoxazole indicated that these two subspecies have different antibiogram profiles to that of *B. pseudomallei*. Certainly, this observation needs to be further confirmed using E-test which is more accurate especially when cotrimoxazole is a combination of trimethoprim/sulfamethoxazole. A previous work has described the bpeEF-oprC efflux pump as one of the mechanisms in conferring cotrimoxazole resistance in a strain of *B. pseudomallei* (Schweizer, 2012). Whether this mechanism is shared by *B. thailandensis* and *B. pyrrocinia* remains to be determined in future studies. Notably, *B. thailandensis*, which has over 90% genomic similarities with *B. pseudomallei* is avirulent (Smith *et al.*, 1997).

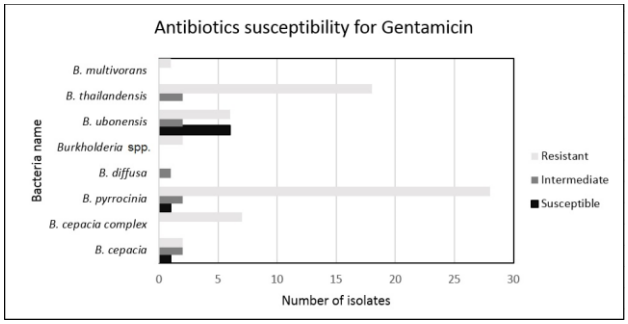

Figure 2: Antibiotics susceptibility of *Burkholderia* spp. against gentamicin.

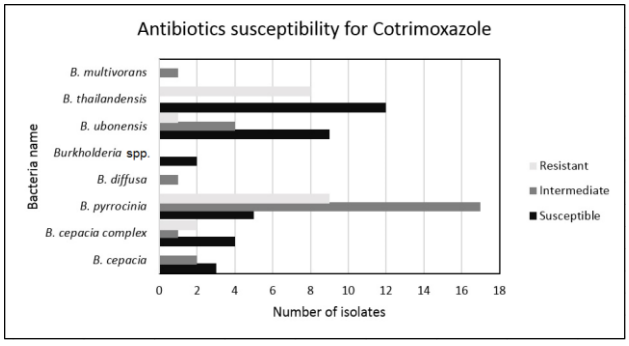

Figure 3: Antibiotics susceptibility of *Burkholderia* spp. against cotrimoxazole.

Ceftazidime susceptibility

All the *Burkholderia* spp. tested in the study were susceptible towards ceftazidime as shown in Figure 4. This result is very reassuring considering that ceftazidime, a β-lactam, is one of the most important drugs in the intravenous phase treatment of melioidosis (Wiersinga *et al*., 2012). While resistance against ceftazidime is rare, amino acid changes in the highly conserved class A β-lactam, PenA, has been attributed as one of the mechanisms for the resistance of β-lactams in *B. pseudomallei* (Sarovich *et al.*, 2012).

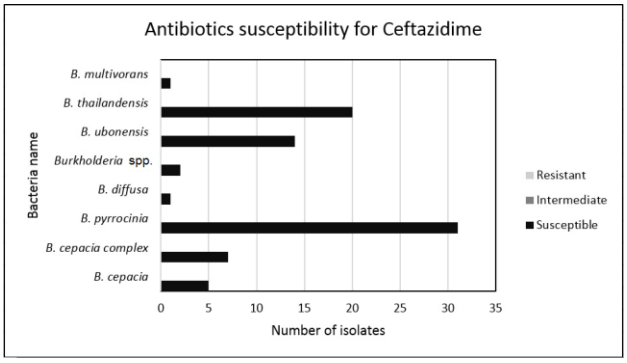

Figure 4: Antibiotics susceptibility of *Burkholderia* spp. against ceftazidime.

CONCLUSION

More than half of the *B. pyrrocinia* and *B. thailandensis* tested in this study were shown to be either intermediately susceptible or resistant towards cotrimoxazole; different from what was observed in *B. pseudomallei*. When tested against aminoglycoside and macrolide, *B. ubonensis* exhibited similar profile with the unique strains of *B. pseudomallei* from the central region of Sarawak as described previously by Podin *et al.* (2014), while the rest of the *Burkholderia* spp. were similar to the wildtype *B. pseudomallei* which make predominantly resistant to these two drug groups. Interestingly, all *Burkholderia* spp. tested were susceptible to ceftazidime, an important antibiotic in the treatment of melioidosis. It is apparent from the findings of this study that the antibiogram profile of *Burkholderia* spp. resembles that of *B. pseudomallei* for some antibiotics while totally different for other antibiotics. The mechanisms which render these observations and whether the interaction of these subspecies within the same ecological niche attribute to these observations warrant further investigation.

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